

# BDNF-hypersecreting human umbilical cord blood mesenchymal stem cells promote erectile function in a rat model of cavernous nerve electrocautery injury

Lujie Song<sup>1</sup> · Jianqiang Zhu<sup>2</sup> · Xiong Zhang<sup>3</sup> · Zhiqiang Cui<sup>1,4</sup> · Qiang Fu<sup>1</sup> · Jianwen Huang<sup>1</sup> · Hongkai Lu<sup>4</sup>

Received: 22 June 2015 / Accepted: 30 October 2015  
© Springer Science+Business Media Dordrecht 2015

## Abstract

**Purpose** Erectile dysfunction (ED) continues to be a significant problem for men following radical prostatectomy. We hypothesize that intracavernous injection of BDNF-hypersecreting human umbilical cord blood mesenchymal stem cells (hUCB-MSCs) can ameliorate ED in a rat model of cavernous nerve electrocautery injury (CNEI).

**Methods** Forty-two male Sprague–Dawley rats were randomly divided into four groups: sham + PBS ( $n = 6$ ), CNEI + PBS ( $n = 12$ ), CNEI + hUCB-MSCs ( $n = 12$ ) and CNEI + BDNF-hUCB-MSCs ( $n = 12$ ). At day 28 post-surgery, erectile function was examined and specimens were harvested for histology. Immunofluorescence staining, Masson's trichrome staining and transmission electron microscopy were performed to determine the structural changes in corpus cavernosum. Cells that are injected into penis were labeled by BrdU and tracked by immunofluorescence staining. Three days post-surgery, the

concentration of BDNF protein in penile tissues was measured by Western blotting.

**Results** Rats intracavernosally injected with BDNF-hUCB-MSCs showed the most significant improvement in the ratio of maximal ICP to MAP (ICP/MAP). Histological examinations showed moderate recovery of nNOS-positive nerve fibers, ratio of smooth muscle to collagen and smooth muscle content in the CNEI + hUCB-MSCs group and remarkable recovery in the CNEI + BDNF-hUCB-MSCs group compared to the CNEI + PBS group. By TEM examination, atrophy of myelinated and non-myelinated nerve fibers was noted in CNEI + PBS group and significant recovery was observed in two treated groups. There were more BrdU-positive cells in the BDNF-hUCB-MSCs group than in the hUCB-MSCs group both in the penis and in the MPG. Three days post-surgery, the concentration of BDNF protein in penile tissues in BDNF-hUCB-MSCs group was much higher than in other groups.

**Conclusions** Intracavernous injection of BDNF-hypersecreting hUCB-MSCs can enhance the recovery of erectile function, promote the CNs regeneration and inhibit corpus cavernosum fibrosis after CNEI in a rat model.

Lujie Song and Jianqiang Zhu have contributed equally to this work and are considered as co-first authors.

✉ Xiong Zhang  
zhangxiongsh@foxmail.com

<sup>1</sup> Department of Urology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China

<sup>2</sup> Department of Urology, the Second Hospital of Tianjin Medical University, Tianjin Institute of Urology, Tianjin 300211, China

<sup>3</sup> Department of Burn and Plastic Surgery, Shanghai Jiao Tong University School of Medicine Affiliated Ruijin Hospital, 197 Ruijin Second Road, Shanghai 200025, China

<sup>4</sup> Department of Urology, Weifang People's Hospital, Weifang 261042, Shandong, China

**Keywords** Electrocautery injury · Erectile dysfunction (ED) · Radical prostatectomy (RP) · Brain-derived neurotrophic factor (BDNF) · Human umbilical cord blood mesenchymal stem cells (hUCB-MSCs)

## Introduction

Erectile dysfunction (ED) continues to be a significant problem for many men following radical prostatectomy (RP) even if the operation is performed with the nerve-sparing surgical technique. Previous reports have shown

that about 25–90 % of men undergoing RP experience postoperative ED, depending upon the studies reviewed [1]. It is postulated that the development of post-RP ED is due to a combination of cavernous nerve (CN) injuries including traction, dissection, crush or transection. Besides, during RP, electrocautery is often used to achieve hemostasis and facilitate dissection and electrocautery injury to CNs cannot be ignored as a cause of ED.

During recent years, mesenchymal stem cells (MSCs) and growth factors have emerged as a new treatment for post-RP ED in experimental studies. Studies in a rat model of CN crush injury [2, 3] have yielded promising results, showing that adipose tissue and bone marrow-derived MSCs can recover erectile function when injected intracavernosally. It was suggested that these effects were mediated by a broad range of trophic factors secreted by the transplanted MSCs or by stimulation of their endogenous production. The related trophic factors include brain-derived neurotrophic factor (BDNF), vascular endothelial growth factors (VEGF), basic fibroblast growth factor (bFGF), neurotrophic factor 3 (NT3), nerve growth factor (NGF) and insulin-like growth factor 1 (IGF-1) [4, 5]. Among the various growth factors, BDNF has gained particular interest because it can activate Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway in Schwann cells which is believed to be important in mediating neuroregenerative effects in the major pelvic ganglion (MPG) after CN injury [4, 5].

An alternative source of MSCs is umbilical cord blood (UCB). Several studies have demonstrated that hUCB-MSCs possess a significant potential of neural differentiation and these cells have been used for treatment of various neurodegenerative diseases such as brain injury, spinal cord injury and stroke [6–9]. In 2010, the first clinical trial of intracavernous transplantation of hUCB-MSCs was reported to improve erectile function (regain of morning erections) and decrease blood glucose levels in patients with diabetic ED [10].

In a previous study, we established a rat model with CN electrocautery injury (CNEI) and found that CNEI was accompanied by reduced intracavernous pressure (ICP), reduced number of nerve fibers in the dorsal penile nerve and increased fibrosis in the corpus cavernosum [11]. Although previous studies had shown promising results of application of MSCs and BDNF for the treatment of ED resulting from CN injury, the effects of hUCB-MSCs combined with BDNF for the treatment of CNEI have never been investigated. Therefore, in the current study, we transfected hUCB-MSCs with the BDNF gene and evaluated whether BDNF-hypersecreting hUCB-MSCs could promote erectile function in a rat model of CNEI.

## Methods

### Animals

Forty-two age-matched male Sprague–Dawley rats (300–350 g) were randomly divided into four groups. group A: sham operation rats intracavernosally injected with PBS (sham + PBS,  $n = 6$ ); group B: bilateral CNEI rats intracavernosally injected with PBS (CNEI + PBS,  $n = 12$ ); group C: bilateral CNEI rats intracavernosally injected with hUCB-MSCs (CNEI + hUCB-MSCs,  $n = 12$ ); and group D: bilateral CNEI rats intracavernosally injected with BDNF-hUCB-MSCs (CNEI + BDNF-hUCB-MSCs,  $n = 12$ ). The Animal Experiment Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital approved the present study, which was conducted in accordance with the ethical standards of the responsible Institutional Committee on Animal Experimentation.

### BDNF gene delivery to hUCB-MSCs

Adeno-associated virus carrying a human BDNF cDNA was constructed by Cyagen Biosciences Co. Ltd. (Guangzhou, China) as described previously [12]. Briefly, BDNF-cDNA fused to the 3' end of signal peptide of Ig coding sequence was constructed by PCR and digested and subcloned into shuttle plasmid pSNAV to obtain a recombinant plasmid pSNAV-Ig-BDNF. Then, the plasmid encoding fusion protein was transfected into HEK293 cell lines (Shanghai Cell Bank of Type Culture Collection of China), the virus was collected from supernatant and infected hUCB-MSCs, and the stably transfected clones were selected. hUCB-MSCs were provided by Cyagen Biosciences Co. Ltd. and cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO, USA) containing 15 % fetal bovine serum (GIBCO, USA), 2 mM L-glutamine and 1 % antibiotics/antimycotics comprising 100 U/ml penicillin, 100 lg/ml streptomycin and 25 lg/ml amphotericin B. hUCB-MSCs were seeded at a density of  $2 \times 10^6$  per 15 cm plate and incubated overnight at 37 °C. hUCB-MSCs were exposed to the infectious viral particles for 60 min after which the medium was then removed and the cells were washed twice with DMEM and then cultured with normal medium for 24 h. The BDNF secretion in vitro was detected by enzyme-linked immunosorbent assay (ELISA; Boster, China).

### BrdU labeling of the hUCB-MSCs

When 70 % confluence was reached, the hUCB-MSCs and BDNF-hUCB-MSCs were incubated with 20  $\mu\text{mol/L}$  and 5-bromo-2-deoxyuridine, respectively (BrdU; Sigma-Aldrich, USA) for 48 h before injection.

### *Surgical procedure and intracavernous injection*

After anesthesia with sodium pentobarbital (40 mg/kg) by an intraperitoneal injection, a lower midline abdominal incision exposed the prostate gland. The major pelvic ganglia (MPG) and their inflows (hypogastric and pelvic nerves) and outflow (CNs) were identified on both sides of the prostate using an operating microscope (10× magnification). In group A, the prostatic fascia and outside fat were dissected to expose the CNs and no additional surgical manipulation was performed. For groups B, C and D, the CNEI was induced by applying a monopolar electrocautery tip connected to an electrocautery generator (Chunguang, HR-302, Wuhan, China) on the CNs 5 mm distal to the MPG at both sides of prostate. The monopolar electrocautery was performed for 0.5-s duration at 350 kHz 15 W.

After cutting the skin of the penis, the corpora cavernosa were exposed. An elastic band was placed at the base of the penis before injection and was removed 5 min after injection. Using a 25-gauge needle, the rats received 0.2 ml PBS (groups A and B), 2 million hUCB-MSCs in 0.2 ml PBS (group C) and 2 million BDNF-hUCB-MSCs in 0.2 ml PBS (group D) into the corpus cavernosum. The wound was closed in layers, and the rats were closely monitored for up to 4 weeks.

### *Erectile functional assessment*

Four weeks after operation, the animals were anesthetized again for the non-survival surgery. A stainless steel electrode connected to an electrical pulse stimulator (PowerLab, AD Instruments) was placed around the CNs at 1–2 mm proximal to the injury site, and the ICP was measured and recorded using a fine butterfly needle (23 gauge) with heparin (250 U/ml) that was attached to PE-50 tubing placed at the left crus of the penis. The electrical stimulations (1.5 mA with a pulse width of 5 ms 20 Hz and 60 s) were conducted on either side separately. Systemic mean arterial pressure (MAP) was monitored via a PE-50 tubing placed in the left carotid artery. The catheters were connected to pressure transducers for ICP and MAP monitoring. The ratio of maximal ICP to MAP was calculated to normalize variations between animals.

### **Immunofluorescence and Masson's trichrome staining**

After ICP examination, the rats were euthanized. The middle part of the penile shafts was harvested and fixed 24 h in 10 % neutrally buffered formalin and dehydrated and embedded in paraffin for histology. Five-micrometer sections were cut and mounted. For histological evaluation,

immunofluorescent staining was done for rabbit anti-neuronal nitric oxide synthase (nNOS; ab5583, diluted 1:400; Abcam) in the dorsal penile nerves and for  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA, diluted 1:200, sc-53142, Santa Cruz) in the corpus cavernosum. Masson's trichrome staining was performed to assess smooth-muscle-to-collagen ratio. Sections stained with nNOS,  $\alpha$ -SMA and Masson's trichrome were evaluated by two independent and blinded observers using a fluorescent microscope (Nikon Eclipse 80i, Japan). Image analysis was performed by computerized densitometry using Image-Pro Plus imaging software (Media Cybernetics Inc., MD, USA) in 10 separate fields for each tissue.

### **Transmission electron microscopy (TEM)**

Using an operating microscope, 3-mm segment of the CN distal to the site of electrocautery injury was harvested and placed in 2.5 % glutaraldehyde for 4 h. After that, the CN was post-fixed in 1 %  $\text{OsO}_4$  for 3 h dehydrated and embedded in plastic. These sections were cut and double-stained with 3 % acetic acid uranium-nitrate lead. CN morphology was examined by an electron microscope (H-7500, Hitachi Corp., Tokyo, Japan).

### **Cell tracing in the penis and MPG**

Immunofluorescence with the monoclonal antibody against BrdU (1:40; rat monoclonal IgG, ab6326; Abcam) was used to identify the injected cells in the penis and MPG at 28-days post-intracavernous injection.

### **Western blot analysis**

The penile tissues were collected from rat three days post-surgery and homogenized for protein preparation using a TissueLyser (Scientz, Zhejiang, China). The protein concentration was measured by lowry kit (Solarbio, Beijing, China). Equal amounts of proteins (60  $\mu\text{g}$ ) were separated on 10 % SDS-PAGE gels and transferred onto the polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5 % skim milk powder in TBST at room temperature. Then the membranes were incubated with BDNF antibody (1:200 dilution, Abcam) at 4 °C with overnight shaking. After TBST washing, the membranes were incubated with secondary antibody (1:2000 dilutions) for 1 h at room temperature. After washing, the signals were detected using a Bio-Rad chemiluminescence system.

### **Statistical analysis**

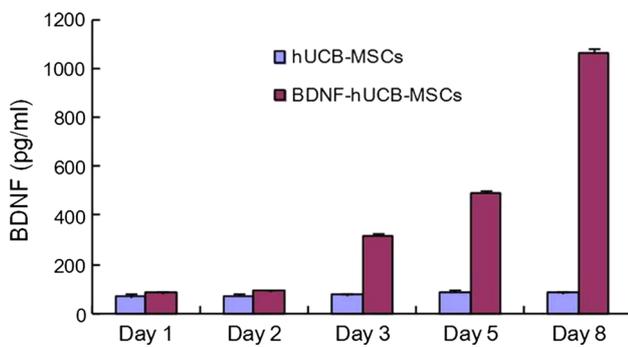
All measurements were reported as mean  $\pm$  standard deviation (SD). Differences between groups were evaluated by analysis of variance (ANOVA) tests followed by

a Student–Newman–Keuls post hoc test for multiple comparisons. Values of  $P < 0.05$  were considered statistically significant.

## Results

### BDNF expression in vitro

As shown in Fig. 1, the secretion of BDNF of the gene-transfected hUCB-MSCs gradually increased from day 1 to day 8. At day 8, the value of BDNF ( $1061.6 \pm 19.5$  pg/ml) is greater than 12-fold compared with non-transfected hUCB-MSCs ( $83.5 \pm 8.6$  pg/ml) ( $P < 0.001$ ).



**Fig. 1** ELISA analysis on the BDNF secretion in vitro. BDNF secretion by the cultured hUCB-MSCs with BDNF gene transfection at day 1, day 2, day 3, day 5 and day 8 was significantly higher than hUCB-MSCs without BDNF-gene transfection ( $P < 0.001$ )

### ICP/MAP ratio

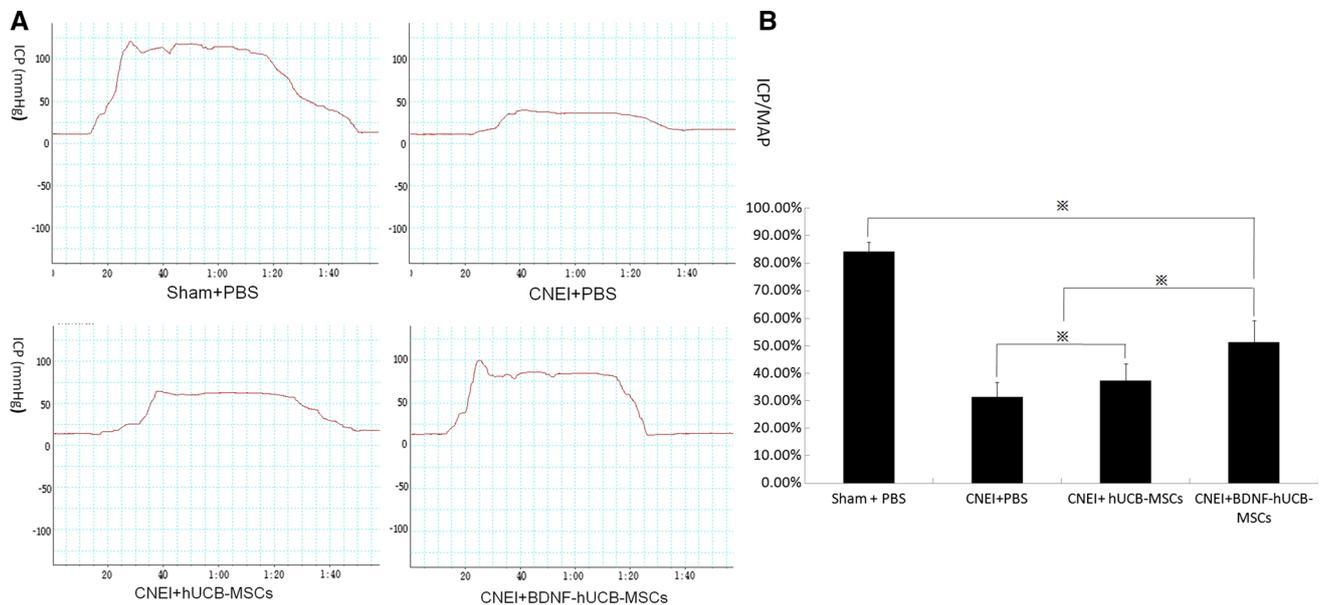
At week 4 after treatment, intracavernous injection of hUCB-MSCs and BDNF-hUCB-MSCs significantly improved the ICP (Fig. 2a) and the value of ICP/MAP ( $37.4 \pm 6.2/51.3 \pm 7.8$  %) compared with the CNEI + PBS group ( $31.4 \pm 5.2$  %) ( $P = 0.02$  and  $P < 0.001$ ). The value of ICP/MAP in CNEI + BDNF-hUCB-MSCs group was also significantly higher than in the CNEI + hUCB-MSCs group ( $P < 0.001$ ) but still lower than in the sham + PBS group ( $P < 0.001$ ) (Fig. 2b).

### nNOS-positive nerve fibers in the dorsal penile nerves

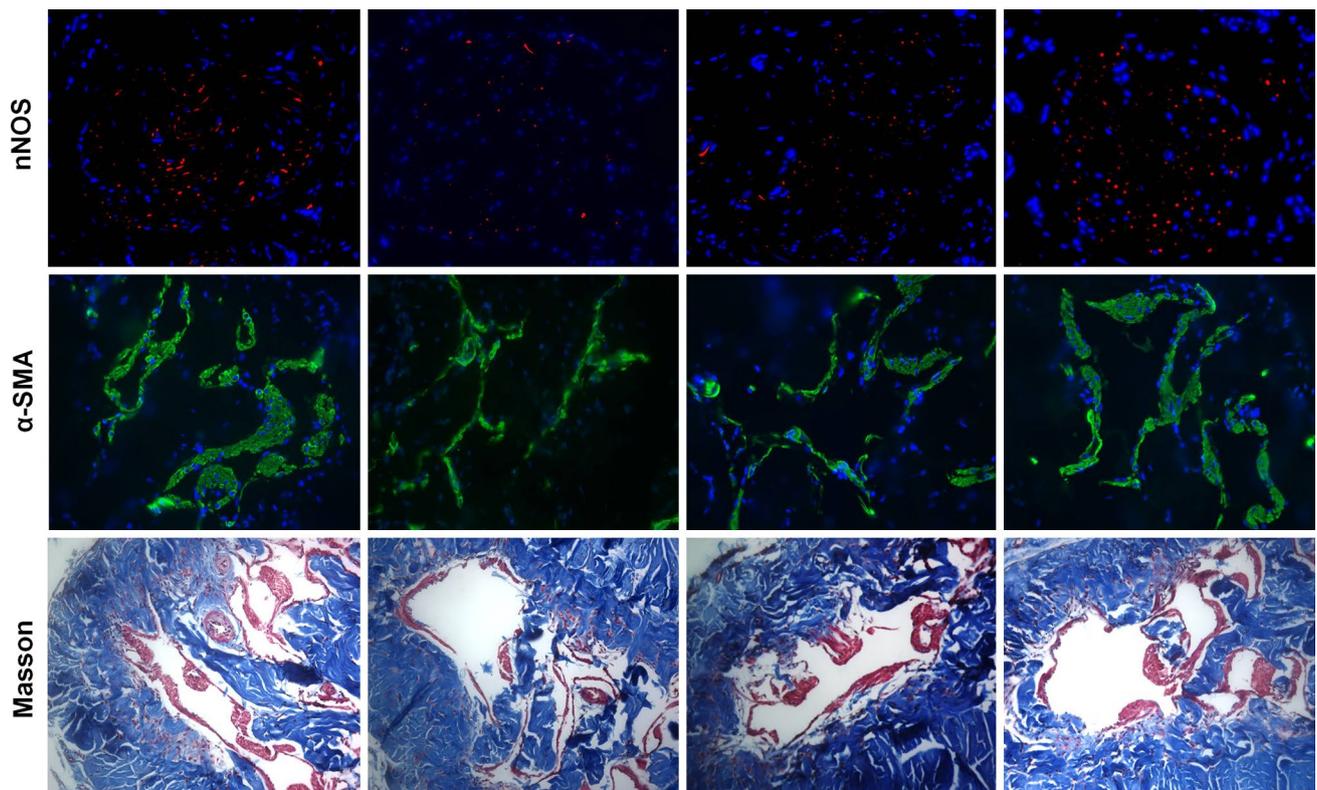
Immunofluorescence examination showed a reduction in nNOS-positive nerve fibers in the dorsal nerves at week 4 in the CNEI + PBS group. Moderate and obvious recovery of nNOS-positive nerve fibers was noted in the CNEI + hUCB-MSCs group and CNEI + BDNF-hUCB-MSCs group (Fig. 3), and the differences were statistically significant among the four groups on computer-assisted image analysis ( $P < 0.05$ ) (Table 1).

### Smooth-muscle-to-collagen ratio and smooth muscle content in the corpus cavernosum

CNEI reduced the ratio of smooth muscle to collagen as estimated by Masson's trichrome staining and quantitative image analysis (Fig. 3). The ratio of smooth muscle to collagen revealed statistically significant difference between the CNEI + PBS ( $6.2 \pm 0.7$  %), CNEI + hUCB-MSCs ( $7.3 \pm 0.8$  %) and CNEI + BDNF-hUCB-MSCs



**Fig. 2** ICP examination and ratio of maximal ICP to MAP (ICP/MAP) in each group at 4 weeks. **a** Representative recording of ICP to electrostimulation of CN. **b** ICP/MAP in each group. \*The differences were statistically significant between the four groups ( $P < 0.05$ )



**Fig. 3** Results of immunofluorescence and Masson's trichrome staining. The *top line*: representative immunofluorescence staining for nNOS (red) in the dorsal nerves of penis. Cell nucleus was stained by 4'6-diamidino-2-phenylindole (DAPI, blue) (original magnification  $\times 400$ ). The *middle line*: representative immunofluorescence for

the  $\alpha$ -smooth muscle actin (SMA, green). Cell nucleus was stained by 4'6-diamidino-2-phenylindole (DAPI, blue) (original magnification  $\times 400$ ). The *bottom line*: representative Masson's trichrome staining. Smooth muscles were stained in red collagen fibers in blue (original magnification  $\times 200$ )

**Table 1** Image analysis for nNOS and  $\alpha$ -SMA immunofluorescence, and Masson's trichrome staining

Group	N	nNOS-positive nerve fibers in the dorsal nerves of penis (%)	$\alpha$ -SMA positive contents in the penis (%)	Smooth muscle to collagen ratio
Sham + PBS	6	1.35 $\pm$ 0.08	5.82 $\pm$ 0.41	0.091 $\pm$ 0.011
CNEI + PBS	12	0.81 $\pm$ 0.11	3.40 $\pm$ 0.64	0.062 $\pm$ 0.007
CNEI + hUCB-MSCs	12	0.95 $\pm$ 0.08*	4.69 $\pm$ 0.99*	0.073 $\pm$ 0.008*
CNEI + BDNF-hUCB-MSCs	12	1.18 $\pm$ 0.09*.*#	5.05 $\pm$ 0.71*.*.#	0.087 $\pm$ 0.003*.*#

\* Statistical difference compared with CNEI + PBS group ( $P < 0.05$ )

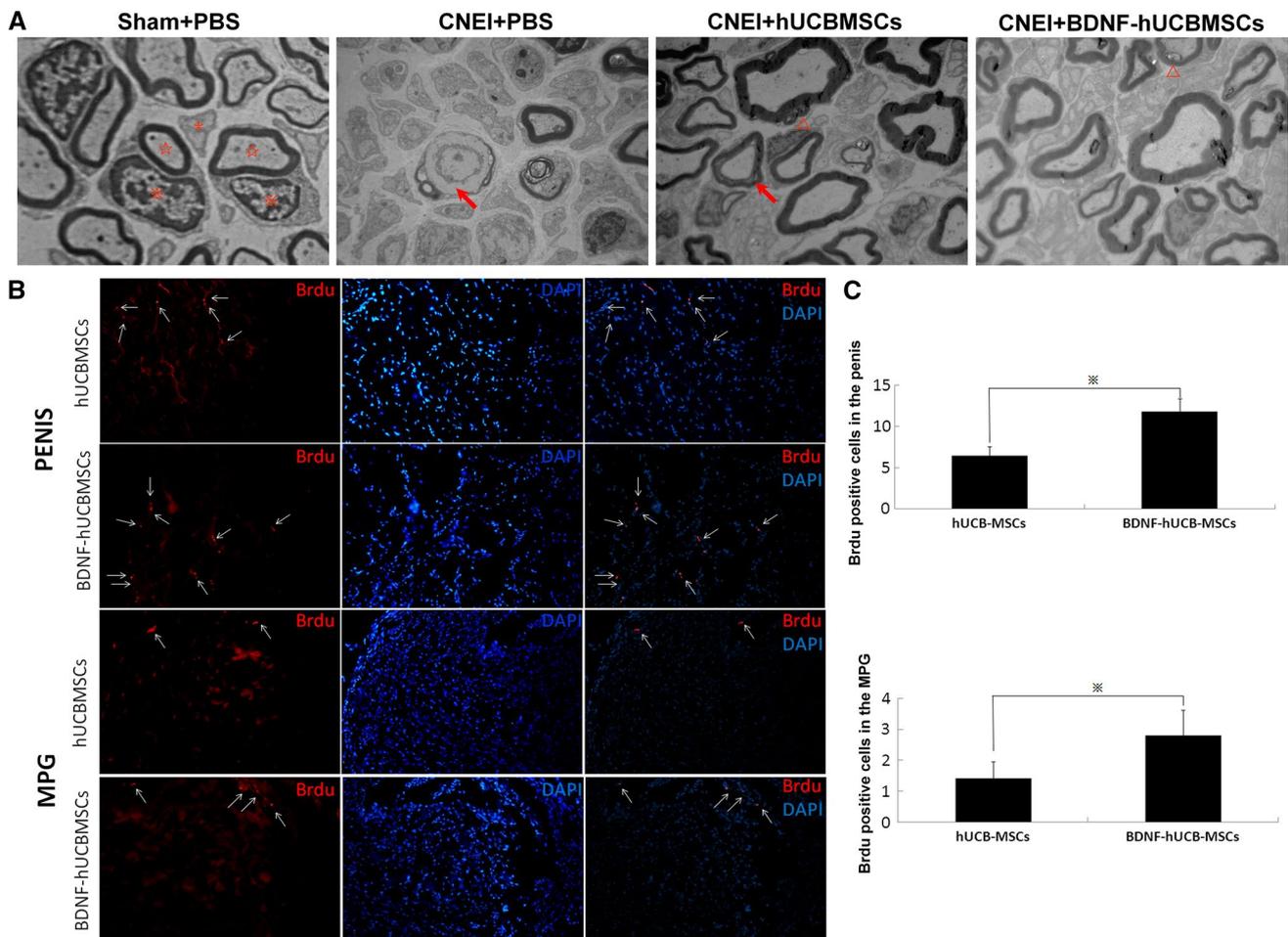
\* Statistical difference compared with CNEI + hUCB-MSCs group ( $P < 0.05$ )

# Statistical difference compared with sham + PBS group ( $P < 0.05$ )

( $8.7 \pm 0.3$  %) groups 4 weeks after injection ( $P < 0.05$ ). No statistical difference was found between the CNEI + BDNF-hUCB-MSCs and sham + PBS ( $9.1 \pm 1.1$  %) groups ( $P = 0.35$ ). The percentage of smooth muscle in the CNEI + hUCB-MSCs and CNEI + BDNF-hUCB-MSCs groups was significantly higher than in the CNEI + PBS group ( $P < 0.001$ ). There was statistically significant difference between CNEI + hUCB-MSCs and CNEI + BDNF-hUCB-MSCs groups ( $P = 0.04$ ) (Table 1).

### TEM examination

TEM revealed normal ultrastructures of myelinated fibers, non-myelinated fibers and Schwann cell nucleus in the sham + PBS group (Fig. 4a). In the CNEI + PBS group, abundant breakdowns of myelin sheaths and axonal degeneration were observed which lead to significant reduction in the number of myelinated fibers. The hUCB-MSCs-treated rats show significant regeneration of myelinated fibers. However, there



**Fig. 4 a** Representative transmission electron microscopy (TEM) graphs corresponding to four groups at week 4 after intracavernous injection. Sham + PBS: TEM of CN showed normal myelinated and non-myelinated fibers and Schwann cells. The nuclei of Schwann cells are seen occasionally near the nerve fibers (☆: myelinated fiber, \*: non-myelinated fiber, †: nuclei of Schwann cells); CNEI + PBS: abundant breakdowns of myelin in myelinated fibers and axonal degeneration (arrows); CNEI + hUCB-MSCs: significant regeneration of myelinated fibers in the CNEI + hUCB-MSCs group by comparison to the CNEI + PBS group larger fibers with thick myelin sheaths were noted. A small amount of dissolved and separated

myelin (arrows) and myelin ovoid corpuscle (△); CNEI + BDNF-hUCB-MSCs: the regeneration of myelinated fibers was significantly larger fibers with thick myelin sheaths were also noted. There was no myelin separation, but the a few myelin ovoids still remained (△) (original magnification  $\times 5000$ ). **b** Representative immunofluorescence examination on the BrdU-labeled cells in the penis and MPG at week 4 (white arrow). BrdU-labeled cell nucleus (red) and 4'6-diamidino-2-phenylindole-labeled cell nucleus (DAPI, blue) (original magnification  $\times 200$ ). **c** The number of BrdU-positive cells in the penis and MPG, respectively. \*Statistically significant difference

were some myelin lesions such as separated myelin sheaths, dissolved myelins and myelin ovoid formation. BDNF-hUCB-MSCs treatment resulted in a more obvious recovery compared with the hUCB-MSCs treatment. There was no myelin separation, but few myelin ovoids still remained.

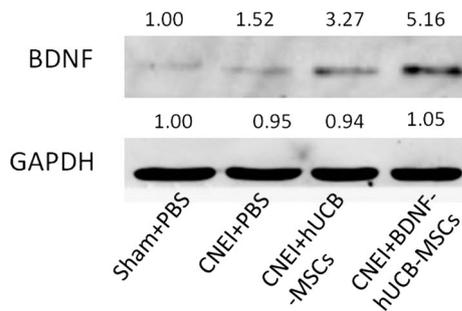
#### Tracking of the injective hUCB-MSCs in the penis and MPG

In sections from both CNEI + hUCB-MSCs and CNEI + BDNF-hUCB-MSCs groups 4 weeks after injection, few BrdU-labeled hUCB-MSCs were found in the

penis and MPG. There were more BrdU-positive cells in the BDNF-hUCB-MSCs group than in the hUCB-MSCs group both in the penis and in the MPG ( $P < 0.05$ ) (Fig. 4b, c).

#### The concentration of BDNF protein in penile tissues

The concentration of BDNF protein in penile tissues was slightly elevated after CN injury, and it was significantly elevated after hUCB-MSCs and BDNF-hUCB-MSCs injection. There are much more BDNF proteins in penile tissues in BDNF-hUCB-MSCs group than in hUCB-MSCs group (Fig. 5).



**Fig. 5** Western blot analysis on the concentration of BDNF protein in penile tissues in each group at three days post-surgery

## Discussion

The etiology of ED following RP is multifactorial, but neurogenic factors seem to play a major role [13]. Among the various kinds of damage to the CN during RP and which result in ED, CNEI is one of the most common damages [11, 14]. We have previously demonstrated [11] and confirm in the present study that CNEI will lead to the irreversible structural alterations in cavernous tissue such as degeneration of nNOS-containing nerves and reduced smooth muscle content with an exaggerated deposition of collagen deposition. These pathological changes are similar to those caused by CN crush injury [15, 16]. In the present study, we have shown that BDNF-hypersecreting hUCB-MSCs injected intracavernosally may be an efficient method for enhancing the recovery of erectile function after CNEI, promoting CNs regeneration and inhibiting corpus cavernosum fibrosis.

Several animal studies have demonstrated the efficacy of stem cell therapy in CN injury models using cells derived from different sources, e.g., bone marrow, skeletal muscle and fat [4, 5, 12]. Human umbilical cord blood is easily accessible without any invasive procedures and ethical problems. hUCB-MSCs are multipotent and can differentiate into various cell types including osteoblasts, adipocytes, chondrocytes, hepatocytes and neural cells [17]. Recently, Divya et al. [6] suggested hUCB-MSCs harbor a unique population of cells that possess an inherent neurogenic potential and are responsible for instantaneous neuronal differentiation. Many studies have shown that hUCB-MSCs have very low immunogenicity and immunomodulatory properties compared to cells from other sources [18] which may explain why we observed no rejection after hUCB-MSCs xenogeneic transplantation in the present investigation.

Exogenous BDNF intervention can promote the survival of injured CNs [19]. However, BDNF has a low molecular weight and a short half-life, making it susceptible to metabolism in vitro [20]. Therefore, a method for effective

and sustained BDNF release should be established. In this study, hUCB-MSCs were transfected with BDNF gene to release BDNF continuously, and the BDNF level at day 8 was about 12 times of the non-transfected hUCB-MSCs which was demonstrated by the ELISA examination in vitro. Three days post-surgery, BDNF protein concentration in penile tissues was measured by Western blotting and the highest BDNF protein concentration in penile tissues in BDNF-hUCB-MSCs group was observed, and the BDNF-hUCB-MSCs group presented the most noticeable improvement. All of these support our hypothesis that BDNF is an important neurotrophic factor that can be used for treatment of CNEI.

There has been an ongoing debate about the mechanisms responsible for stem cell therapy for ED. In the early studies, most results supported the theory that stem cells differentiate to penile cavernosum cells in vivo [21–23]. However, more recent studies have suggested that paracrine effects may constitute the primary mechanism responsible for the positive effects of stem cell therapy in ED. Kendirci et al. [3] showed that transplantation of BMSCs isolated with p75 NGF receptor into the rat penis could rescue erectile function at week 4 following cavernous nerve injury. These effects were thought to be mediated by FGF, NGF, BDNF, VEGF and IGF-1 secreted by the stem cells. Albersen et al. [2] reported beneficial functional effects of adipose-derived stem cells (ADSCs) therapy on erectile function after CN crush injury. The involvement of paracrine effects was supported by the data showing that the number of nNOS-positive nerve fibers in the rats treated with the lysate of ADSCs was similar to that in animals treated with ADSCs. Fandel et al. [24] demonstrated that ADSCs migrated out of the corpus cavernosum in a time-dependent manner (up to 28 days) and there was transient homing of ADSCs to the MPG after CN crush injury. Elevated stromal cell-derived factor-1 (SDF-1) secretion at the MPG appeared to be responsible for this observation.

In the current study, intracavernous injection of hUCB-MSCs or BDNF-hUCB-MSCs significantly promoted the regeneration of nNOS-positive nerve fibers which was also verified by the TEM observations that the electrocautery injury caused significant changes in myelinated and non-myelinated nerves in the CNs and that hUCB-MSCs or BDNF-hUCB-MSCs treatment stimulated the growth and myelination of nerve sprouts.

It can be assumed that the mechanisms by which hUCB-MSCs or BDNF-hUCB-MSCs improved erectile function in our CNEI model are multiple. The homing results with hUCB-MSCs were in accordance with those of previous studies with ADSCs injected intracavernosally [4], showing that few engrafted hUCB-MSCs were found in the corpus cavernosum and MPG after 4 weeks. Given their low number, the mechanism for the observed significant histological

and functional recovery was not likely due to the hUCB-MSCs differentiation, and our results may be interpreted as supporting the concept of paracrine effect from the grafted hUCB-MSCs. Interestingly, we found that there were more traced BrdU-positive cells in the BDNF-hUCB-MSCs group than in the hUCB-MSCs group whether in the penis or in the MPG. The exact mechanism is unclear; however, BDNF-hypersecreting can be hypothesized to make the difference.

As a proof of concept, our study has some limitations. The exact homing mechanism after hUCB-MSCs intracavernous injection was not fully identified. Future studies will be needed to identify the specific recruiting factors for stem cells homing after CNEI and to improve the local retention and regulation of the targeted migration of the grafted hUCB-MSCs in vivo. In addition, long-term investigations with multiple time points of observation will be required to elucidate the efficacy and safety of hUCB-MSCs or BDNF-hUCB-MSCs therapy for CNEI-related ED.

**Acknowledgments** This study was funded by the National Natural Science Foundation of China (30901489); Scientific Research Foundation for Returned Scholars Ministry of Education of China; Shanghai Jiaotong University Biomedical Engineering cross research foundation (YG2014MS15); Shandong science and technology development plan (2011GGH21813).

#### Compliance with ethical standards

**Conflict of interest** All authors declare no competing financial interests.

## References

- Resnick MJ, Koyama T, Fan KH, Albertsen PC, Goodman M, Hamilton AS, Hoffman RM, Potosky AL, Stanford JL, Stroup AM, Van Horn RL, Penson DF (2013) Long-term functional outcomes after treatment for localized prostate cancer. *N Engl J Med* 368(5):436–445. doi:10.1056/NEJMoa1209978
- Albersen M, Fandel TM, Lin G, Wang G, Banie L, Lin CS, Lue TF (2010) Injections of adipose tissue-derived stem cells and stem cell lysate improve recovery of erectile function in a rat model of cavernous nerve injury. *J Sex Med* 7(10):3331–3340. doi:10.1111/j.1743-6109.2010.01875.x
- Kendirci M, Trost L, Bakondi B, Whitney MJ, Hellstrom WJ, Spees JL (2010) Transplantation of nonhematopoietic adult bone marrow stem/progenitor cells isolated by p75 nerve growth factor receptor into the penis rescues erectile function in a rat model of cavernous nerve injury. *J Urol* 184(4):1560–1566. doi:10.1016/j.juro.2010.05.088
- Albersen M, Kendirci M, Van der Aa F, Hellstrom WJ, Lue TF, Spees JL (2012) Multipotent stromal cell therapy for cavernous nerve injury-induced erectile dysfunction. *J Sex Med* 9(2):385–403. doi:10.1111/j.1743-6109.2011.02556.x
- Lin CS, Xin ZC, Wang Z, Deng C, Huang YC, Lin G, Lue TF (2012) Stem cell therapy for erectile dysfunction: a critical review. *Stem Cells Dev* 21(3):343–351. doi:10.1089/scd.2011.0303
- Divya MS, Roshin GE, Divya TS, Rasheed VA, Santhoshkumar TR, Elizabeth KE, James J, Pillai RM (2012) Umbilical cord blood-derived mesenchymal stem cells consist of a unique population of progenitors co-expressing mesenchymal stem cell and neuronal markers capable of instantaneous neuronal differentiation. *Stem Cell Res Ther* 3(6):57. doi:10.1186/scrt148
- Park DH, Lee JH, Borlongan CV, Sanberg PR, Chung YG, Cho TH (2011) Transplantation of umbilical cord blood stem cells for treating spinal cord injury. *Stem Cell Rev* 7(1):181–194. doi:10.1007/s12015-010-9163-0
- Mothe AJ, Tator CH (2012) Advances in stem cell therapy for spinal cord injury. *J Clin Invest* 122(11):3824–3834. doi:10.1172/JCI64124
- Roh DH, Seo MS, Choi HS, Park SB, Han HJ, Beitz AJ, Kang KS, Lee JH (2013) Transplantation of human umbilical cord blood or amniotic epithelial stem cells alleviates mechanical allodynia after spinal cord injury in rats. *Cell Transplant* 22(9):1577–1590. doi:10.3727/096368912X659907
- Bahk JY, Jung JH, Han H, Min SK, Lee YS (2010) Treatment of diabetic impotence with umbilical cord blood stem cell intracavernosal transplant: preliminary report of 7 cases. *Exp Clin Transplant* 8(2):150–160
- Song LJ, Zhu JQ, Xie MK, Wang YC, Li HB, Cui ZQ, Lu HK, Xu YM (2014) Electrocautery-induced cavernous nerve injury in rats that mimics radical prostatectomy in humans. *BJU Int* 114(1):133–139. doi:10.1111/bju.12348
- Li H, Qiu W, Wang F, Wei F, Chen X, Wu X, Huang Q (2008) Construction and expression of recombinant adeno-associated virus expressing brain-derived neurotrophic factor. *Sheng Wu Gong Cheng Xue Bao* 24(2):328–332
- Lin CS, Xin Z, Dai J, Huang YC, Lue TF (2013) Stem-cell therapy for erectile dysfunction. *Expert Opin Biol Therapy* 13(11):1585–1597. doi:10.1517/14712598.2013.847085
- Ong AM, Su LM, Varkarakis I, Inagaki T, Link RE, Bhayani SB, Patriciu A, Crain B, Walsh PC (2004) Nerve sparing radical prostatectomy: effects of hemostatic energy sources on the recovery of cavernous nerve function in a canine model. *J Urol* 172(4 Pt 1):1318–1322
- Chung E, De Young L, Brock GB (2011) Investigative models in erectile dysfunction: a state-of-the-art review of current animal models. *J Sex Med* 8(12):3291–3305. doi:10.1111/j.1743-6109.2011.02505.x
- Canguven O, Burnett A (2008) Cavernous nerve injury using rodent animal models. *J Sex Med* 5(8):1776–1785. doi:10.1111/j.1743-6109
- Mennan C, Wright K, Bhattacharjee A, Balain B, Richardson J, Roberts S (2013) Isolation and characterisation of mesenchymal stem cells from different regions of the human umbilical cord. *Biomed Res Int*. doi:10.1155/2013/916136
- Lee M, Jeong SY, Ha J, Kim M, Jin HJ, Kwon SJ, Chang JW, Choi SJ, Oh W, Yang YS, Kim JS, Jeon HB (2014) Low immunogenicity of allogeneic human umbilical cord blood-derived mesenchymal stem cells in vitro and in vivo. *Biochem Biophys Res Commun* 446(4):983–989. doi:10.1016/j.bbrc.2014.03.051
- Chen KC, Minor TX, Rahman NU, Ho HC, Nunes L, Lue TF (2005) The additive erectile recovery effect of brain-derived neurotrophic factor combined with vascular endothelial growth factor in a rat model of neurogenic impotence. *BJU Int* 95(7):1077–1080
- Yuan Y, Pan S, Sun Z, Dan Q, Liu J (2014) Brain-derived neurotrophic factor-modified umbilical cord mesenchymal stem cell transplantation improves neurological deficits in rats with traumatic brain injury. *Int J Neurosci* 124(7):524–531. doi:10.3109/0207454.2013.859144
- Song YS, Lee HJ, Park IH, Kim WK, Ku JH, Kim SU (2007) Potential differentiation of human mesenchymal stem cell transplanted in rat corpus cavernosum toward endothelial or smooth muscle cells. *Int J Impot Res* 19(4):378–385

22. Bivalacqua TJ, Deng W, Kendirci M, Usta MF, Robinson C, Taylor BK, Murthy SN, Champion HC, Hellstrom WJ, Kadowitz PJ (2007) Mesenchymal stem cells alone or ex vivo gene modified with endothelial nitric oxide synthase reverse age-associated erectile dysfunction. *Am J Physiol Heart Circ Physiol* 292(3):H1278–H1290
23. Nolzco G, Kovanecz I, Vernet D, Gelfand RA, Tsao J, Ferrini MG, Magee T, Rajfer J, Gonzalez-Cadavid NF (2008) Effect of muscle-derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *BJU Int* 101(9):1156–1164. doi:[10.1111/j.1464-410X.2008.07507.x](https://doi.org/10.1111/j.1464-410X.2008.07507.x)
24. Fandel TM, Albersen M, Lin G, Qiu X, Ning H, Banie L, Lue TF, Lin CS (2012) Recruitment of intracavernously injected adipose-derived stem cells to the major pelvic ganglion improves erectile function in a rat model of cavernous nerve injury. *Eur Urol* 61(1):201–210. doi:[10.1016/j.eururo.2011.07.061](https://doi.org/10.1016/j.eururo.2011.07.061)