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Research Report

Transplantation of autologous bone marrow mesenchymal stem cells in the treatment of complete and chronic cervical spinal cord injury

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ARTICLE INFO

Article history:
Accepted 7 August 2013
Available online 12 August 2013

Keywords:
Bone marrow mesenchymal stem cells
Spinal cord injury
Stem cell transplantation

ABSTRACT

Neuronal injuries have been a challenging problem for treatment, especially in the case of complete and chronic cervical spinal cord injury (SCI). Recently, particular attention is paid to the potential of stem cell in treating SCI, but there are only few clinical studies and insufficient data. This study explored the efficacy of autologous bone marrow mesenchymal stem cells (BMMSCs) transplantation in the treatment of SCI. Forty patients with complete and chronic cervical SCI were selected and randomly assigned to one of the two experimental groups, treatment group and control group. The treatment group received BMMSCs transplantation to the area surrounding injury, while the control group was not treated with any cell transplantation. Both the transplant recipients and the control group were followed up to 6 months, postoperatively. Preoperative and postoperative neurological functions were evaluated with AIS grading, ASIA score, residual urine volume and neurophysiological examination. Results showed that in the treatment group 10 patients had a significant clinical improvement in terms of motor, light touch, pin prick sensory and residual urine volume, while nine patients showed changes in AIS grade. Neurophysiological examination was consistent with clinical observations. No sign of tumor was evident until 6 months postoperatively. In the control group, no improvement was observed in any of the neurological functions specified above. BMMSCs transplantation improves neurological function in patients with complete and chronic cervical SCI, providing valuable information on applications of BMMSCs for the treatment of SCI.

1. Introduction

Spinal cord injury (SCI) often occurs due to the high-energy trauma during sports or traffic accidents (Barnabe-Heider and Frisen, 2008). The annual incidence of SCI is about 15–40/million worldwide and most of the patients are found to be 10–40 years old at the time of injury (Barnabe-Heider and Frisen, 2008). Although complete transection of spinal cord

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0006-8993/$ - see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.brainres.2013.08.016

rarely occurs, during the early period of the injury, the spine fracture squeeze as well as inflammatory edema often cause total loss of nerve functions. As a result, neuronal cells die in the span of first 12 h to a few weeks (McDonald et al., 2004). There might be a possibility that some neural functions could be restored if treated in time. However, the conventional treatments such as medication and rehabilitation exercises produce no significant effects on neurological dysfunctions, including motor control, sensory input and urine control in chronic SCI. In general, vast majority of patients suffer life-long disability and their quality of life is seriously affected. Previously, it was thought that nerve injuries cannot be repaired or nerve tissue lacks the regenerating ability, unlike other tissues in the body. In recent years, the transplantation of stem cell (SC) from various sources has shown promise in nerve regeneration after SCI. Its efficacy in animal experiments has been widely recognized (Barnabe-Heider and Frisen, 2008) and the safety has also been partially verified (Deda et al., 2008). A small number of clinical trials (Curt et al., 2008; Geffner et al., 2008) have demonstrated partial recovery of nerve function, which brings hope for rehabilitation to the patients with SCI. The current research on the mechanism of stem cell transplantation suggest that transplanted bone marrow mesenchymal stem cells (BMMSCs) are capable of surviving in the region of injury and differentiate into nerve cells (neurons, astrocytes and oligodendrocytes) (Alberti et al., 2009; Syková et al., 2006). Additionally, they are involved in axon regeneration and remyelination (Peru et al., 2008; Tysseling-Mattiace et al., 2008), neurotrophic effect (Alexanian et al., 2008; Saha et al., 2008; Xuan et al., 2008), neovascularization (Hess and Borlongan, 2008; Oz Oyar et al., 2009; Sasaki et al., 2009), directed migration of endogenous neural stem cells (NSCs) (Walker T.L. et al., 2008) and regulation of local inflammation (Walker P.A. et al., 2008). Although embryonic stem cells display a distinct advantage, their source and ethical concerns limit clinical utility. On the other hand, autologous BMMSCs avoid ethical controversy, immune rejection (Beachy et al., 2004) and can be easily obtained through repeated harvests. Hence, BMMSCs has become an important source of seed cells for the treatment of a wide variety of nervous diseases (Parr et al., 2007).

According to American Spinal Injury Association (ASIA), chronic and complete cervical SCI is defined as the course of disease that persist more than one year after the injury with ASIA impairment scale (AIS) A (Savic et al., 2007). This group of patients represents severe cervical SCI, because no efficacy is observed when treated with the conventional treatment. Although the use of stem cell in animal model of cervical SCI produced satisfactory results, it cannot be equalized with the clinical trials. Moreover, in few of the reported treatments of SCI using bone marrow stem cells, the efficacy has been controversial. In the present study, we selected 40 patients with chronic and complete cervical SCI for BMMSCs transplantation and followed them up for 6 months to assess the feasibility and clinical efficacy.

2. Results

2.1. Clinical manifestations

Treatment groups: Ten patients (50%) showed clinical improvement. Among these, one patient had improvement in motor function alone, two had both motor and sensory improvement, one had improvement in sensory and urinary function and six patients had improvement in both motor, sensory and bladder functions. In the control group, none of the cases showed improvement in any of motor, sensory, or urinary functions.

2.1.1. AIS grading

In the treatment group, changes in AIS grade were observed in nine patients (45%) with an improvement from A to B. In the control group, no significant change was observed in AIS grading.

2.1.2. ASIA score

As shown in Table 1, comparisons within the groups were as follows: in the treatment group, motor scores before operation and 6 months after operation were 5.95 ± 4.50 and 6.85 ± 4.96; respectively ($P < 0.01$); pain scores before operation and 6 months after operation were 12.80 ± 2.82 and 18.00 ± 8.25; respectively ($P < 0.01$); light touch score before operation and 6 months after operation were 12.85 ± 2.76 and 18.25 ± 8.74; respectively ($P < 0.01$); and ASIA total score were 31.60 ± 9.82 and 43.10 ± 19.32; respectively ($P < 0.01$). In the control group, there was no significant difference between scores at the first examination (preoperative) and the score after 6 months in motor, pin prick, light touch score and ASIA

Table 1 – Comparison of ASIA scores before and after the operation.

Clinical manifestations	N		Treatment group		Control group	
			Mean ± S.D.	P value	Mean ± S.D.	P value
Motor score	20	Prior	5.95 ± 4.5		5.8 ± 4.63	
		After	6.85 ± 4.96	0.001	5.9 ± 4.71	0.163
Sensory pin prick score	20	Prior	12.8 ± 2.82		13.45 ± 3.52	
		After	18 ± 8.25	0.008	13.55 ± 3.53	0.163
Sensory light touch score	20	Prior	12.85 ± 2.76		13.5 ± 3.49	
		After	18.25 ± 8.74	0.008	13.6 ± 6.55	0.163
ASIA score	20	Prior	31.6 ± 9.82		32.75 ± 11.38	
		After	43.1 ± 19.32	0.005	33.2 ± 11.56	0.056

N: number of the patients; Prior: prior to transplantation; After: after transplantation.
$P < 0.05$ was considered statistically significant.

total score ($P>0.05$). As shown in Table 2, comparisons between the two groups were as follows: in the treatment group, the differences between before and after operation in motor, pain, light touch and ASIA total score were 0.9±1.07, 5.2±7.78, 5.4±8.22 and 11.5±17.07; respectively. Whereas in the control group, movement, light touch, pin prick score and total ASIA score were 0.10±0.31, 0.25±0.44, 0.10±0.31 and 0.45±1.06; respectively. The comparison between two groups showed significant differences ($P<0.01$).

2.1.3. *Residual urine volume*

As shown in Table 2, the residual urine volumes in the treatment and control groups were 61.6±77.4 ml and 0.3±13.7 ml; respectively, which was significantly different ($P<0.01$).

As shown in Table 3, in the treatment group, residual urine volume changed significantly from preoperative volume 235.4±54.0 ml to postoperative volume 173.9±75.8 ml ($P<0.01$). Whereas, in the control group, the amount of residual urine was changed from preoperative volume 236.1±52.5 ml to 6th month postoperative volume 235.9±56.5 ml ($P=0.94$).

2.2. *Electrophysiological findings*

2.2.1. *Electromyography (EMG)*

Among the 20 patients from the treatment group, one patient (5.0%) failed to elicit preoperative wave, but showed slight wave postoperatively. Postoperative EMG showed that in eight patients (40.0%), part of the affected muscle contracted with improved wave pattern and the increased potential in the number of motor units and other 11 patients (55.0%) did not show any change. Patients in the control group exhibited no change at all.

2.2.2. *Paraspinal somatosensory evoked potential (PSSEP) examination*

Among the 20 patients from the treatment group, the sensory levels went down in nine patients (45.0%) by an average of 1.8±2.4. Furthermore, 11 patients (55.0%) had some degree of change, but no sensory levels went down. None of the 20 patients in the control group showed any change.

2.3. *Spinal cord MRI findings*

Changes in the MRI findings for the patients with treatment are given in Fig. 1. There was no increase in the diameter of the spinal cord at the cell transplantation site and no other significant changes such as tumor formation of the transplanted BMMSCs were found.

2.4. *Adverse reactions*

In the treatment group, no signs of adverse events such as wound infection, incision leakage of cerebrospinal fluid, intracranial infection, or any deteriorating complications occurred. No sign of tumor was evident at the transplant site, 6 months after BMMSC transplantation. However, there were a few mild, adverse reactions reported by the patients. Within 24 h, non-inflammatory fever was observed in two patients (10%), which was transient and did not exceed 38.5 °C. After giving physical cooling and symptomatic treatment, patients recovered within 72 h. One patient (5%) reported headache and dizziness, which was mild and posture-related, with no evidence of meningeal irritation. After rapid intravenous saline injection, the symptoms disappeared within 72 h. Two patients (10%) complained pain and numbness in spinal cord dominant area, but relieved spontaneously in 24 h or disappeared within 72 h after administration of analgesics, dehydration and hormone treatment.

3. Discussion

In this study, we applied BMMSCs therapy by local injection to 20 patients with complete and chronic cervical SCI. The clinical symptoms were improved in 10 patients with total effective rate 50%. The two sets of AIS grades, ASIA

Table 2 – Differences at two time points (Δ) in ASIA score and residual urine volume (mean±S.D.).

Groups	Motor	Sensory pin prick score	Sensory light touch score	ASIA score	Residual urine volume (ml)
Treatment group	0.9±1.07	5.2±7.78	5.4±8.22	11.5±17.07	61.55±77.43
Control group	0.1±0.31	0.25±0.44	0.1±0.31	0.45±1.06	0.25±13.66
P value	0.004	0.008	0.01	0.006	0.001

$P<0.05$ was considered statistically significant.

Table 3 – Comparison of residual urine volume between the treatment group and the control group.

Bladder function	N		Treatment group		Control group	
			Mean±S.D.	P value	Mean±S.D.	P value
Residual urine volume	20	Prior to Transplant	235.40±54.02		236.10±52.51	
		After Transplant	173.85±75.79	0.001*	235.85±56.46	0.94

N: number of the patients; Transplant: transplantation.
$P<0.05$ was considered statistically significant.

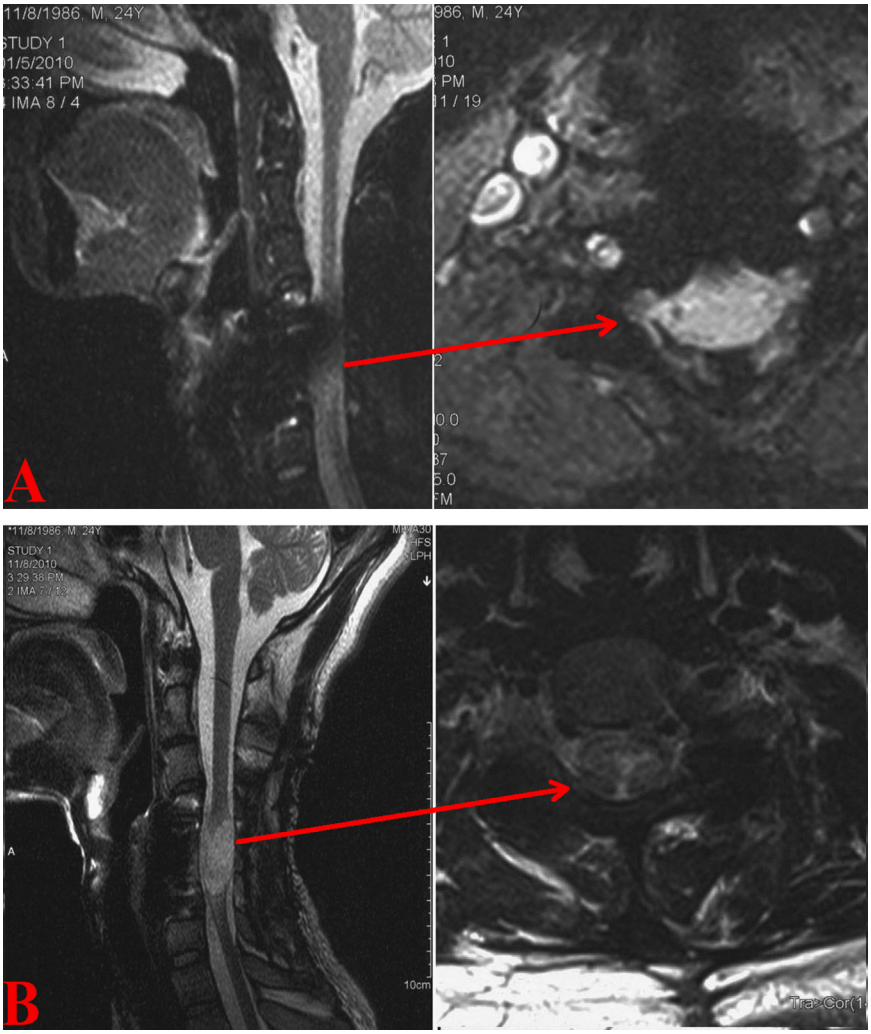

Fig. 1 – Follow up spinal cord magnetic resonance imaging (MRI) findings (T1 weighted image, T1WI). Preoperative initial (A) and postoperative follow-up (B) MRI at 6 months showed that there is no increase in the diameter of the spinal cord at the cell transplantation site and no sign of tumor was evident at the transplant site.

scores, residual urine volume, EMG, and PSSEP from the two groups of patients confirmed that BMMSCs can effectively improve the neurologic dysfunction associated with complete and chronic cervical SCI.

The treatment group showed significant improvement in motor function, ASIA score and EMG. Motor function in some patients was improved in two aspects. First, we evaulated the recovery of the completely lost muscle strength, Before transplantation, 18 patients in the treatment group showed no muscle strength according to ASIA motor score and after treatment, bilateral brachial muscle displayed slight contraction and muscle grade increased by one point. Although some of the patients showed slight recovery in motor function which was insufficient to impact the ASIA scores, in these patients slight raised EMG wave were observed. Secondly, the muscle strength was improved from weak to strong. Furthermore, physical flexibility and coordination were improved. EMG showed wave amplitude increase from the affected muscle contraction.

Significant differences were found between the treatment group and the control group in sensory function, ASIA score and PSSEP. Some patients in the treatment group showed the improvement of sensory function in two aspects. Firstly, sensory level went down in nine patients (45%), which was confirmed by PSSEP. The patients had an average of 1.8 ± 2.4 downward sensory level and the maximum downward sensory levels of eight. Secondly, before cell transplantation, some patients felt paresthesia above the original sensory level, such as hyperalgesia. However, the majority of patients showed insensitivity. The 11th patient in the treatment group recovered from sensory abnormality.

There are a few relevant reports evaluating the improvement in urine control after stem cell therapy. Geffner et al. (2008) reported that patients with both acute and chronic SCI recovered bladder function, with the acute patients recovered better than chronic ones. Kishk et al. compared bladder function of the patient before and after the treatment and they found significant improvement, however, no significant difference was observed between the treatment group and the control group (Kishk et al., 2010). In our study, there was a significant improvement with respect to the amount of residual urine in the treatment group. However, patients

who received transplant showed no significant improvement including urine perception, urine control after BMMSCs transplantation.

Seung hwan Yoon and his colleagues treated complete and chronic cervical spinal cord injury patients using bone marrow cells transplantation and GM-CSF administration, the results demonstrated that 29.5% of patients in the acute (<2 weeks) treatment group showed neurologic improvement from AIS A to B or C. In the subacute (3–8 weeks) treatment group, 33.3% of patients improved to AIS B or C. However, the patients in the chronic (>8 weeks) treatment group did not show any changes in the neurologic status (Yoon et al., 2007a). Liu et al. (2013) used umbilical cord mesenchymal stem cells (UC-MSCs) in treating spinal cord injury (SCI) by intrathecal injection and six patients with complete SCI, all had an ineffective treatment response. Both the above results were contrary to ours. There were three possible reasons. Firstly, BMMSC have more advantages than bone marrow cells in terms of differentiation, immune regulation, paracrine more cytokines and histocompatibility. Secondly, cell transplantation approach is different. There are three commonly used cell transplantation approaches: local transplantation into the lesion area (Chen et al., 2010), subarachnoid transplantation (Cizkova et al., 2011) and intravenous infusion (Hong et al., 2009). Local transplantation into the lesion area is the most commonly used technique and is considered the most effective approach (Takahashi et al., 2011) for treating SCI by SC transplantation. Thirdly, the volume of cell suspensions may influence the results. Seung hwan Yoon and his colleagues used 300 μl, but the doses we used were 25 μl. The larger volume of transplantation may result in more edema, which will increase the risk of secondary spinal cord injury.

After transplantation, patients did not experience any adverse reactions including wound infection, cerebrospinal fluid leakage from incision, intracranial infection, or deteriorating symptoms, suggesting that surgery was safe. At graft site, no tumor was evident on MRI until 6 months after cell transplantation, indicating the relative safety of BMMSCs transplantation, but the long-term tumorigenicity still needs further observation. However, some patients experienced varying degrees of adverse reactions. Among these reactions, fever occurred within 24 h after surgery and can be considered as a normal reaction after surgery. All fevers were transient, with a maximum temperature below 38.5 °C. After physical cooling and other symptomatic treatments, the fever was reduced within 72 h. Some patients experienced headache and dizziness after transplantation. In the most severe conditions, this reaction was accompanied by severe nausea and vomiting. However, meningeal irritation signs were negative. In addition, the severity of the symptoms has a clear relationship with the patient's position. The symptoms were mitigated in the head-down position and deteriorated in the head-up position. The symptoms disappeared within 1–3 days after ordering bed rest and rapid intravenous administration of saline. Because headache and dizziness are types of low intracranial pressure responses, this type of response in the open surgical transplantation group is likely caused by the excessive outflow of cerebrospinal fluid after cutting open the dura. The occurrence of this kind of response in the CT-guided transplantation group may be related to chronic cerebrospinal fluid leakage after puncturing the dura with the puncture needle. Comparing the incidence rates of the low intracranial pressure responses between the two groups of patients, we believe that the CT-guided transplantation led to a significantly reduced risk of cerebrospinal fluid leakage than the open surgical transplantation. The two groups of patients showed the same incidence of nerve radicular pain. These symptoms mainly include electric-like pain and a feeling of numbness and swelling in the body or limbs controlled by the corresponding spinal segment after surgery, and these symptoms may be caused by the stimulation of the spinal cord by the puncture needle and the cell suspension. The symptoms can be relieved within 72 h after treating patients with dehydration or steroid therapy.

In conclusion, based on the results of this clinical study, we consider that BMMSCs transplantation has a clear role in promoting neurological rehabilitation for the complete and chronic cervical spinal cord injury. The improvements were not only presented as enhanced motor, sensory and urinary functions, but also objectively evaluated with neuronal electrophysiological examination and measurement of residual urine volume.

4. Experimental procedures

4.1. *General information*

The present study was carried out during the period from December 2008 to December 2010. All the procedures were approved by the ethics committee of our hospital and written informed consent was obtained from all patients. Through physical examination and cervical spinal cord magnetic resonance imaging (MRI), the patients diagnosed with cervical SCI were selected. Forty patients with complete and chronic cervical SCI were selected and randomly assigned to one of the two experimental groups, treatment group and control group. The treatment group received BMMSC transplantation to the area surrounding injury, while the control group was not treated with cell transplantation. The treatment group consisted of 20 patients, 14 males and six females with average age of 34.7±8.9 years (range: 22–54 years) and the average time interval between the injury and stem cell therapy was 51.9±18.3 months (range: 18–74 months). The SCI were categorized as AIS grade A. Four cases had C4-6 injury, C4-5 injury in four cases, C5-6 injury in one, C4 injury in three, C5 injury in five, and C6 injury in three cases. The control group consisted of 20 patients, 14 males and six females with average age of 35.1±8.0 years (range: 24–52 years old) and the average time interval between the injury and visit to our hospital was 43.2±15.3 months (average: 19–68 months). Preoperative AIS grade were A. Four cases had C4-6 injury, C4-5 injury in five cases, C5-6 injury in two, C4 injury in three, C5 injury in three and C6 injury in three cases. The inclusion criteria were as follows: (1) complete cervical SCI with post-operative or post-traumatic period more than one year and (2) the site of injury within the cervical spinal cord C3–C7 excluding C3 and C7. The following cases were excluded: patients with (1) incomplete spinal cord injury;

(2) neurological function with tendency to recover; (3) adhesion, syringomyelia, dural vascular malformations or significant compression in spinal cord, requiring surgical intervention; (4) unexplained nerve dysfunction; (5) intended surgical site with inflammation or skin ulceration; (6) with bleeding tendency or coagulation disorders; (7) poor general condition or other organ dysfunction intolerant of surgery; and (8) disagreement with the research protocol.

4.2. *Treatment procedure*

4.2.1. *BMMSCs isolation*

Bone marrow puncture was performed under local anesthesia in a sterile operating room. A volume of 90 ml bone marrow was collected from unilateral or bilateral posterior iliac spine. The BMMSC was separated, allowed for adhesion and proliferation in GMP laboratory. After passaging every 5–7 days, the fourth generation cells were collected for transplantation. Inverted phase contrast microscopy and flow cytometry were used for the evaluation.

4.2.2. *Stem cell preparation*

BMMSCs were digested by trypsin, collected from culture flasks, washed with saline and prepared aliquots of 25 μl concentrated cell suspensions (8×10^5 cells/μl).

4.2.3. *BMMSC transplantation*

All patients were treated in the prone position under general anesthesia. Through minimally invasive surgery, the injured site was approached from the rear segment of injury site. Using high-speed burr, injury site on the spinal cord was completely exposed. Through midline incision in the dura mater, spinal cord injury site and its adjacent normal spinal cord tissue was visualized under a microscope. Using OT needle, aliquots of 25 μl cell suspension (8×10^5 cells/μl) was slowly injected to a depth of 3 mm at multiple sites in the central dorsal area across the junction of injured and normal spinal cord. Special care was exercised to avoiding puncturing of adjacent blood vessels. After administering cell suspension, the dura mater and arachnoid were sutured and subsequently the muscle and skin layers were opposed in routine fashion.

4.2.4. *Postoperative treatment and exercise*

To avoid the influence of drugs on the neurological rehabilitation by nourishing the nerves and improving the microcirculation, all patients did not receive treatments with these drugs. To exclude the effect of rehabilitation exercises on neurological rehabilitation, all patients received formal rehabilitation exercises at the same hospital during the observation period.

4.3. *Examination of neurological and neurophysiological functions*

Preoperative and postoperative examination for nerve functional assessment, neurophysiological examination and estimation of residual urine volume using abdominal B-mode ultrasound were undertaken in both groups. All the evaluation and inspection procedures were single-blinded. The nerve function evaluation criteria were applied according to AIS grade, ASIA scale. The neurophysiological examinations included electromyography (EMG) and the paravetebral somatosensory evoked potentials (PSSEP) (Yoon et al., 2007b).

4.4. *Postoperative follow-up*

In the treatment group, all the patients were followed up to 6 months after undergoing stem cell transplantation. The control group patients were visited within 6 months after enrollment.

4.5. *Statistical analysis*

All data were processed using SPSS11.5 package. Numerical data were expressed as mean ± standard deviation (S.D.). The scores before and after surgery were compared with independent-samples *t*-test, *P* value of less than 0.05 was considered statistically significant.

Approval of clinical application

The present clinical study was registered at chictr.org (registration number: ChiCTR-TNRC-12002477).

Acknowledgments

This study was supported by the National Natural Science Foundation of China (30571916), Director Foundation of Ministry of Science and Technology of China (30840082) and the Military Key Medicine and Health Program during the 12th Five-Year Plan Period (BWS11J002).

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