ORIGINAL ARTICLE

Transplantation of a combination of autologous neural differentiated and undifferentiated mesenchymal stem cells into injured spinal cord of rats

MS Pedram¹, MM Dehghan^{1,2}, M Soleimani³, D Sharifi^{1,2}, SH Marjanmehr⁴ and Z Nasiri⁵

¹Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; ²Center of Excellence for Studies of Animal Stem Cells, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; ³Department of Hematology, School of Medical Science, Tarbiat Modares University, Tehran, Iran; ⁴Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran and ⁵Department of Animal Physiology, School of Science, University of Tehran, Tehran, Iran

Study design: The use of stem cells for functional recovery after spinal cord injury.

Objective: The aim of this study was to evaluate the effects of a combination of autologous undifferentiated and neural-induced bone marrow mesenchymal stem cells (MSCs) on behavioral improvement in rats after inducing spinal cord injury and comparing with transplantation of undifferentiated and neural-induced MSCs alone.

Setting: The study was conducted at the department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Methods: The spinal cord was injured by contusion using a Fogarty embolectomy catheter at the T8–T9 level of the spinal cord, and autologous MSCs were transplanted into the center of the developing lesion cavity, 3 mm cranial and 3 mm caudal to the cavity, at 7 days after induction of spinal cord compression injury.

Results: At 5 weeks after transplantation, the presence of transplanted cells was detected in the spinal cord parenchyma using immunohistochemistry analysis. In all treatment groups (differentiated, undifferentiated and mix), there was less cavitation than lesion sites in the control group. The Basso–Beattie–Bresnahan (BBB) score was significantly higher in rats transplanted with a combination of cells and in rats transplanted with neural-induced MSCs alone than in undifferentiated and control rats.

Conclusion: Pre-differentiation of MSCs to neuron-like cells has a very important role in achieving the best results for functional improvement.

Spinal Cord (2010) **48**, 457–463; doi:10.1038/sc.2009.153; published online 15 December 2009

Keywords: bone marrow; mesenchymal stem cell; spinal cord injury; transplantation; behavioral improvement

Introduction

Numerous studies have evaluated the strategies that attempt to promote axon regeneration in spinal cord injuries (SCIs). Among these strategies, cell transplantation is at present considered to be the most effective way of repairing SCIs.¹ So far, several different kinds of cells have been used as transplants for spinal cord regeneration, including Schwann cells,² embryonic spinal cord stem cells,³ olfactory ensheathing cells,⁴ macrophages,⁵ choroids plexus ependymal cells,⁶ neural stem cells⁷ and bone marrow stromal cells.¹ SCI does not need to be cured completely for function to return.

Partial recovery can enormously improve a patient's life,⁸ and cell therapy can achieve this goal.

Among the different kinds of cells for the treatment of spinal cord injury, embryonic neural stem cells have been most enthusiastically studied.⁹ However, several difficulties, including ethical issues and clinical complications such as immune reactions and teratoma formation, make it impossible to use human fetal tissue as a practical and immediate source for therapeutic treatment.¹⁰

In this study, we studied the effect of bone marrow undifferentiated mesenchymal stem cells (MSCs), neural differentiated MSCs and a combination of them transplanted directly into the lesion site for the repair of injured rat spinal cord. This study was carried out to determine which kinds of stem cells (undifferentiated, differentiated or a combination of them) have more beneficial effects on the behavioral recovery of acute spinal cord injured rat.

Correspondence: Dr MM Dehghan, Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Azadi Street, Gharib Street, P.O. Box 14155-6453, Tehran, Iran.

E-mail: mdehghan@ut.ac.ir

Received 27 January 2009; revised and accepted 13 October 2009; published online 15 December 2009

Materials and methods

Isolation of mesenchymal stem cells (MSCs)

For elimination of the problem of rejection, bone marrow was obtained from femoral bones of rats, harvested, and transplanted into the same rat. Collection, isolation and propagation of MSCs were performed as described by Wakitani.¹¹ In brief, MSCs were isolated from bone marrow aspirates taken from the femur. Rats were anesthetized with ketamine (75 mg kg⁻¹) and xylazine (10 mg kg⁻¹) intramuscularly. A small hole (1–1.5 mm) in the femur was made with a burr micromotor drill after skin incision (5 mm), and 0.5–1 ml of bone marrow was aspirated using a 2-ml syringe with a 21-G needle supplemented with 500–750 IU heparin. The samples were diluted in L-15 medium (2 ml) containing 3 ml Ficoll. The cells in the mononuclear layer were collected after centrifugation (2000 r.p.m., for 15 min) and were resuspended in 2 ml serum-free medium. After centrifugation (2000 r.p.m., for 15 min), the cells were suspended in 1 ml neural progenitor maintenance medium.

To identify the nature of the MSCs of isolated cells, cells were treated with osteogenic Dulbecco's modified Eagle's medium composed of 50 mg ml⁻¹ ascorbic acid 2-phosphate, 10 nM dexamethasone and 10 mM β -glycerophosphate, and adipogenic (Dulbecco's modified Eagle's medium supplemented with 50 mg ml⁻¹ indomethacine and 100 nM dexamethasone (Sigma Chemical, St Louis, MO, USA)) medium for 21 days.¹²

For cell-surface antigen characterization, MSC antigens were measured by flow cytometry using fluorescein isothiocyanate-conjugated mouse anti-rat CD31 (Abd Serotec, Oxford, UK), CD34 (Cedarlane, Hornby, Canada), CD29 (Becton Dickinson, Mountain View, CA, USA), Phycoerythrin-conjugated mouse anti-rat CD45 (Biolegend, San Diego, CA, USA), CD90 1.1 (eBioscience, San Diego, CA, USA) and CD11b (BD Biosciences, San Jose, CA, USA).

Differentiation of mesenchymal stem cells (MSCs) into neurocyte cells

Cells were induced to differentiate into neuron-like cells in serum-withdrawal medium in 2 weeks using a multi-step protocol. Cells that were seeded at a density of 3000–4000 cells cm⁻² were treated with the first-step medium containing Dulbecco's modified Eagle's medium/F12 (Gibco, Gaithersburg, MD, USA) supplemented with 1% insulin/transferrin/selenium supplement (Gibco), 2% B27 supplement (Gibco), 10 ng ml⁻¹ basic fibroblast growth factor (Peprotech, Rocky Hill, NJ, USA) and 5 μ M retinoic acid (Sigma). After 1 week, the medium was changed to a medium containing insulin/transferrin/selenium and B27 supplements with 1 μ M dibutyryl cyclic adenosine monophosphate (Sigma) and 100 μ M ascorbic acid (Sigma), and cells were treated for 4 days. The third step in the treatment of cells was performed in a medium containing insulin/transferrin/selenium and B27 with 10 μ M forskolin (Sigma), 0.1 μ M isobutylmethylxanthine (Sigma) and 100 μ M ascorbic acid for 3 days. All culture media were changed every 2–3 days.

Immunocytochemistry analysis

Cells were fixed with 4% paraformaldehyde on the third day of differentiation and incubated for 1 h in 4 °C and then permeabilized with Triton X-100 (0.2%) and processed for immunocytochemistry using primary antibodies to β -tubulin III in a 1:50 dilution (mouse monoclonal; Chemicon, Temecula, CA, USA), and NFM in a 1:500 dilution (mouse monoclonal; Sigma). For fluorescence, fluorescein isothiocyanate-conjugated (Sigma) anti-mouse secondary antibody at a dilution of 1:500 was applied. Neuron-specific immunostaining against neural-specific enolase and β -tubulin III also confirmed the neural differentiation of MSCs.

RNA isolation and reverse transcriptase (RT)-PCR analysis

For RT-PCR analysis, total cellular RNA was extracted using TRI-reagent BD (Sigma). Synthesis of complementary DNA was carried out with M-MuLV reverse transcriptase (RT) and random hexamer as primer, according to the manufacturer's instructions (Fermentas, Vilnius, Lithuania). PCR amplification was performed using a standard procedure with Taq DNA polymerase (Fermentas) with denaturation at 94 °C for 15 s; annealing at 55 °C or 60 °C for 30 s according to primers; and extension at 72 °C for 45 s. The number of cycles varied between 30 and 40, depending on the abundance of particular mRNA. The primers and product lengths are listed in Figure 3. Using RT-PCR, we defined the expression of several neural-specific genes, including neuron-specific enolase, Nestin, β -tubulin and microtubule-associated protein 2, 3 days after induction of differentiation.

Labeling by bromodeoxyuridine (BrdU)

To enable the detection of transplanted cells *in vivo*, cells were labeled with bromodeoxyuridine (BrdU, Sigma) 24 h before implantation. For transplantation, cells were washed with phosphate-buffered saline, trypsinized, washed with phosphate-buffered saline again, counted and then resuspended in serum-free culture medium.

Animal groups

Male Fischer-344 Wistar rats ($n = 24$), 8–12 weeks of age, were used in this experiment. To minimize variations in the size of the spinal canal, care was taken to include only animals with body weights of 300–350 g at the beginning of the experiment. All animals were housed separately in a large, well-lit laboratory controlled for temperature (21 °C) and maintained with a daily photoperiod of 12 h of light. Each animal had *ad libitum* access to food and water. Spinal cord injured rats were randomly divided into four groups: control group (injured animals that received no treatments; $n = 5$), differentiated group (injured animals that received differentiated neural cells; $n = 6$), undifferentiated group (injured animals that received undifferentiated MSCs; $n = 6$) and mix group (injured animals that received undifferentiated MSCs and differentiated neural cells; $n = 7$). All experiments followed the guidelines of the Iran Animal Care Committee and were approved by the faculty of veterinary medicine animal care committee.

Spinal cord injury and transplantation of mesenchymal stem cells (MSCs)

Paraplegia was induced in rats according to the procedure by Vanicky *et al.*¹³ Briefly, rats were anesthetized. Under sterile conditions, a 2-cm midline incision was made over the T10–L1 spinous processes. Both soft tissue and spinous processes of vertebrae T10–T11 were removed. Under a surgical microscope, a small hole (1.5 mm diameter) was made in the vertebral arch of T10 using a micromotor. A groove was drilled into the midline on the dorsal surface of the T11 vertebral lamina to guide the insertion of the catheter and hold it positioned at the midline. A 2-French Fogarty catheter (Perouse Medical, Ivry Le Temple, France) was filled with saline and connected to an airtight 50- μ l Hamilton syringe (Hamilton Co., Reno, NV, USA) held in a precise sampling device. The catheter was inserted into the epidural space and advanced cranially for 1 cm, so that the center of the balloon rested at the T8–T9 level of the spinal cord (Figure 1). The balloon was rapidly inflated with 20 μ l volume of saline for 5 min. The catheter was then deflated and removed. Soft tissues and skin were sutured in anatomical layers. Manual bladder expression was performed at least twice daily until reflex bladder activity was established, and antibiotic therapy with enrofloxacin (10 mg kg⁻¹, every 24 h) was carried out for 1 week. All rats were paraplegic after injury, with no signs of functional recovery.

At 7 days after induction of spinal cord compression injury, rats were anesthetized as above. A small hole was made in the vertebral arches of T8 and/or T9 using a micromotor. The tip of a 20- μ l Hamilton syringe was inserted through the intact dura into the center of the developing lesion cavity, 3 mm cranial and 3 mm caudal to the cavity (penetration depth of 1.0 mm at an angle of 40–45° past perpendicular). In the undifferentiated group, 1×10^6 autologous undifferentiated MSCs were injected into the rats at three points (5 μ l at each site of injection). In the differentiated group, 1×10^6 autologous differentiated neural cells were injected at three points (5 μ l at each site of injection). In the mix group, 0.5×10^6 autologous undifferentiated MSCs + 0.5×10^6 autologous differentiated neural cells were injected into rats at three points (5 μ l at each site of injection) and in the control group, medium alone (without donor cell administration) was injected. Transplants were performed incrementally over a 1-min period at each site of injection and the spinal cord surface was examined microscopically. To minimize cellular reflux, the needle was left in

Figure 1 Schematic drawing showing the induction of spinal cord injury (SCI). The catheter is inserted into the vertebral canal in T10 through laminotomy and the balloon center is advanced to the T8–T9 level.¹⁸

place for 60 s after the completion of injections. Muscles and skin were closed in layers and the rats received postsurgery care as described previously.

Behavioral analyses

After the surgeries, behavioral evaluation was assessed using the Basso–Beattie–Bresnahan (BBB) locomotor rating scale.¹⁴ Scores from 0 (complete paralysis) to 21 (normal gait) were recorded once a week for a total of 6 weeks. For BBB assessment, rats were placed in an open field (radius = 90 cm). Hindlimb motor function was scored simultaneously by two observers who were blind to the transplantation status of the animals. Significant difference was observed with one-way analysis of variance using SPSS (SPSS Inc., Chicago, IL, USA).

Histological preparation of tissues

Animals were deeply anesthetized by intraperitoneal injection of pentobarbital sodium (100 mg kg⁻¹), and then perfused transcardially with 200 ml 0.1 M phosphate buffer, pH 7.4, followed by 300 ml 4% phosphate-buffered saline (pH 7.4) containing 1% glutaraldehyde and 4% paraformaldehyde.

Spinal cords were sectioned transversely from T7 to T10 and histological observations after immunohistochemical staining were made.

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers/animals were followed during the course of this research.

Results

Cell culture and characterization of mesenchymal stem cells (MSCs)

Fibroblast-like cells from bone marrow cells were isolated by adhesion of these cells to the surface of plastic culture dishes. After 2 weeks, fibroblast-like cells with a spindle-shaped morphology appeared on culture dishes.

To confirm their mesenchymal nature, fibroblast-like cells were treated with appropriate osteo-, chondro- and adipogenic media,¹⁵ and their differentiation was confirmed through appropriate staining, including alizarin red (for osteogenic differentiation), alcian blue (for chondrogenic differentiation) and oil red (for adipogenic differentiation) staining (Figure 2). Furthermore, flow cytometry analysis showed that the expression of cell-surface markers such as CD90(Thy) and CD29 was positive, but the expression of CD34, CD45, CD11b and CD31 was negative (Figure 3).

Neurogenic differentiation of mesenchymal stem cells (MSCs)

Undifferentiated and differentiated stem cells were cultured for 14 days and 16 days, respectively, before transplantation into the spinal cord lesion site. RT-PCR analysis indicated that differentiated cells expressed neuron-specific enolase, Nestin, β -tubulin and microtubule-associated protein 2 (Figure 4).

Figure 2 *In vitro* differentiation of mesenchymal stem cells: the cells have differentiated into (a) mineralizing cells stained with alizarin red; (b) adipocytes stained with oil red O; and (c) chondrocytes stained with alcian blue (magnification $\times 20$).

Figure 3 Flow cytometry analysis of surface markers of mesenchymal stem cells.

Immunocytochemistry analysis

In addition to a morphological evaluation of differentiated cells, to prove the neural differentiation of cells, they were processed for immunocytochemistry using primary antibodies to neuron-specific enolase (Figure 5a) and β -tubulin III (Figure 5b). Results showed that neuron-specific enolase and β -tubulin III proteins were expressed in cells that were treated with neurogenic medium for 7 days.

Behavioral analysis

All groups showed hindlimb dysfunction that was maximal during the first few days after the first surgery, with a BBB score of 0 at day 1 after injury (Figure 6). All groups showed an equal BBB score (without any significant improvement in locomotion) 7 days after injury. In the undifferentiated group, the BBB score increased to 7.2 on day 49 after injury. In the differentiated group, the BBB score increased to 14

and in the mix group to 15 on day 49 after injury. In the control group, the BBB score remained unchanged for the entire period of study.

Statistical analysis revealed that there were significant differences in the BBB score between the control group and the other three groups. In addition, the differences between the undifferentiated group and the other two groups (the differentiated group and the mix group) were significant ($P < 0.05$, t , d.f. = 1.82, 2, two-way analysis of variance). There were no significant differences in the BBB score between the differentiated group and the mix group.

Figure 4 Reverse transcription-polymerase chain reaction analysis of neuron-specific markers (neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), microtubule-associated protein 2 (MAP2), β-tubulin and β-actin) and a neural progenitor marker (Nestin). Mesenchymal stem cells (MSCs) treated by neural induction protocol for 3 days express neuron-specific markers.

Immunohistochemistry analysis

Immunohistochemistry against BrdU showed a variable number of cells, thus supporting the presence of MSCs after intralesional administration. Indeed, histological examination and immunohistochemistry for BrdU-labeled cells at 5 weeks after transplantation disclosed that, in all groups, transplanted cells survived, partially filled the lesion cavity at the injury site, did not gross impinge upon host tissue and integrated into the host spinal cord tissue (Figure 7). In addition, BrdU-labeled stem cells integrated into the parenchyma of the spinal cord, showing that cells were able to migrate at least short distances within the host tissue (Figure 8).

Discussion

This study has shown that local transplantation of MSCs can improve locomotion in the injured rat spinal cord and revealed that pre-differentiating of MSCs to neuron-like cells has a very important role in achieving the best results in hindlimb locomotion.

Several experimental strategies to treat SCI are being investigated, and cell therapy may have the best results for improving the clinical condition of a paralytic patient.¹⁶ Among the different kinds of cells used for this purpose, bone marrow MSCs seem to be one of the best candidates, because they are relatively easy to isolate and can be expanded rapidly *in vitro* and differentiated into multiple cell types such as neuron-like cells.¹⁷

Different weight-drop techniques are used to induce spinal cord compression injury in rat spinal cord studies.¹⁸ These methods are time consuming, require custom-built lesion-making devices and cause severe trauma to soft tissues and vertebrae that makes it difficult to inject cells later at the site of injury. In this study, a catheter (Fogarty embolectomy catheter) is used to induce spinal cord injury. This method is very consistent and there is minimal damage to surrounding tissues.¹³ In this method, spinal dura remained intact at the site of the lesion.

In this study, a purified population of fibroblast-like cells with high proliferation and differentiation potential into mesenchymal lineages was obtained. Osteoblastic, chondrogenic and adipogenic differentiations were shown by alizarin red, alcian blue and oil red staining, respectively. Further-

Figure 5 *In vitro* differentiation of mesenchymal stem cells (MSCs) into neuron-like cells 3 days after induction of differentiation. Differentiated cells showed positive immunoreactivity for neuron-specific markers (a) neuron-specific enolase (NSE) and (b) β-tubulin III.

Figure 6 The effect of cell transplantation on the Basso–Beattie–Bresnahan (BBB) score measurements after a delay of seven days after injury. There are significant differences between undifferentiated, differentiated and mix groups when compared with the control group. The difference between differentiated and mix groups with the undifferentiated group is significant ($P < 0.01$).

Figure 7 Brown bromodeoxyuridine (BrdU) + nuclei (grafted mesenchymal stem cells (MSCs)) integrated into the host tissue.

more, flow cytometry analysis showed that the expression of cell-surface markers, such as CD90(Thy) and CD29, was positive, but the expression of CD34, CD45, CD11b and CD31 was negative. This piece of evidence, together with the fibroblastic morphology of cells, allowed us to conclude that these cells were MSCs.

Neuronal differentiation of MSCs may require activation by specific exogenous factors,¹⁹ as they were not observed expressing a neuronal phenotype after intraspinal transplantation and they probably changed to glial cells.²⁰ Thus, neuronal replacement in the spinal cord is feasible but requires grafting pre-differentiated stem cells or neuronal precursor cells.²¹ It seems that cell transplantation at 7 days after SCI may have the best result.

In this study, MSCs were cotransplanted with neural-induced MSCs. We did not observe significant differences in functional improvement in the co-transplantation of neural-induced/undifferentiated MSCs, and neural-induced MSCs, but there were significant differences between these two groups and undifferentiated MSCs. These results showed that pre-differentiation of these cells to neuron-like cells has

Figure 8 Short-distance migration of grafted mesenchymal stem cells (MSCs) within the host tissue.

a very important role in achieving the best results. It has been shown that when differentiated cells are transplanted into injured spinal cord, the grafted cell processes always made synaptic connections with the processes of endogenous neurons.²² Other short-term studies have noted neuronal differentiation, synapse formation and electrophysiological connectivity after transplantation of neural precursor cells.²³ Beside these synaptic connections, secretion of neurotrophic factors, including transforming growth factor- α and brain-derived neurotrophic factor, from transplanted differentiated cells can contribute to functional recovery.²⁴

Transplantation of neural-induced MSCs alone or in combination with undifferentiated MSCs has a significantly better effect in comparison with transplantation of MSCs alone. However, there was no significant difference between mix and differentiated cells. Understanding the mechanisms that control the fate of different kinds of cells after transplantation into injured spinal cord must be carefully delineated before their clinical use can be contemplated.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Dr M Abarkar and Dr M Abdi for surgical assistance and Dr M Nasiri and Miss Haghighi for technical assistance in immunohistochemistry. This work was supported by a grant from The Center of Excellence for Studies of Animal Stem Cells, University of Tehran.

References

- Ohta M, Suzuki Y, Noda T, Ejiri Y, Dezawa M, Kataoka K *et al*. Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. *Exp Neurol* 2004; **187**: 266–278.

- 2 Martin D, Robe P, Franzen R, Delree P, Schoenen J, Stevenaert A *et al*. Effects of Schwann cell transplantation in a contusion model of rat spinal cord injury. *J Neurosci Res* 1996; **45**: 588–597.
- 3 Iwashita Y, Kawaguchi S, Murata M. Restoration of function by replacement of spinal cord segments in the rat. *Nature* 1994; **367**: 167–170.
- 4 Doucette R. Olfactory ensheathing cells: potential for glial cell transplantation into areas of CNS injury. *Histol Histopathol* 1995; **10**: 503–507.
- 5 Rapalino O, Lazarov-Spiegler O, Agranov E, Velan GJ, Yoles E, Fraidakis M *et al*. Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. *Nat Med* 1998; **4**: 814–821.
- 6 Ide C, Kitada M, Chakraborty S, Taketomi M, Matsumoto N, Kikukawa S *et al*. Grafting of choroid plexus ependymal cells promotes the growth of regenerating axons in the dorsal funiculus of rat spinal cord: a preliminary report. *Exp Neurol* 2001; **167**: 242–251.
- 7 Ogawa Y, Sawamoto K, Miyata T, Miyao S, Watanabe M, Nakamura M *et al*. Transplantation of *in vitro*-expanded fetal neural progenitor cells results in neurogenesis and functional recovery after spinal cord contusion injury in adult rats. *J Neurosci Res* 2002; **69**: 925–933.
- 8 Myckatyn TM, Mackinnon SE, McDonald JW. Stem cell transplantation and other novel techniques for promoting recovery from spinal cord injury. *Transpl Immunol* 2004; **12**: 343–358.
- 9 McDonald JW, Liu XZ, Qu Y, Liu S, Mickey SK, Turetsky D *et al*. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nat Med* 1999; **5**: 1410–1412.
- 10 Bjorklund A, Lindvall O. Cell replacement therapies for central nervous system disorders. *Nat Neurosci* 2000; **3**: 537–544.
- 11 Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve* 1995; **18**: 1417–1426.
- 12 Nadri S, Soleimani M, Kiani J, Atashi A, Izadpanah R. Multipotent mesenchymal stem cells from adult human eye conjunctiva stromal cells. *Differentiation* 2008; **76**: 223–231.
- 13 Vanicky I, Urdzikova L, Saganova K, Cizkova D, Galik J. A simple and reproducible model of spinal cord injury induced by epidural balloon inflation in the rat. *J Neurotrauma* 2001; **18**: 1399–1407.
- 14 Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 1995; **12**: 1–21.
- 15 Nadri S, Soleimani M, Mobarra Z, Amini S. Expression of dopamine-associated genes on conjunctiva stromal-derived human mesenchymal stem cells. *Biochem Biophys Res Commun* 2008; **377**: 423–428.
- 16 McKerracher L. Spinal cord repair: strategies to promote axon regeneration. *Neurobiol Dis* 2001; **8**: 11–18.
- 17 Deng W, Obrocka M, Fischer I, Prockop DJ. *In vitro* differentiation of human marrow stromal cells into early progenitors of neural cells by conditions that increase intracellular cyclic AMP. *Biochem Biophys Res Commun* 2001; **282**: 148–152.
- 18 Gruner JA. A monitored contusion model of spinal cord injury in the rat. *J Neurotrauma* 1992; **9**: 123–126; discussion 126–128.
- 19 Munoz-Elias G, Woodbury D, Black IB. Marrow stromal cells, mitosis, and neuronal differentiation: stem cell and precursor functions. *Stem Cells* 2003; **21**: 437–448.
- 20 Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K *et al*. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* 2003; **425**: 968–973.
- 21 Vescovi AL, Parati EA, Gritti A, Poulin P, Ferrario M, Wanke E *et al*. Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. *Exp Neurol* 1999; **156**: 71–83.
- 22 Lepore AC, Neuhuber B, Connors TM, Han SSW, Liu Y, Daniels MP *et al*. Long-term fate of neural precursor cells following transplantation into developing and adult CNS. *Neuroscience* 2006; **139**: 513–530.
- 23 Auerbach JM, Eiden MV, McKay RD. Transplanted CNS stem cells form functional synapses *in vivo*. *Eur J Neurosci* 2000; **12**: 1696–1704.
- 24 Kerr DA, Llado J, Shamblott MJ, Maragakis NJ, Irani DN, Crawford TO *et al*. Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuse motor neuron injury. *J Neurosci* 2003; **23**: 5131–5140.