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Transplantation of human bone marrow stromal cell-derived neuroregenrative cells promotes functional recovery after spinal cord injury in mice

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Transplantation of bone marrow stromal cells (BMSCs) for spinal cord injury (SCI) has been shown to improve functional outcome. BMSCs can be easily obtained from bone marrow aspirate and have fewer problems in the clinical application for human SCI from the ethical and legal points of view. Recently, we produced cells with neural stem and/or progenitor cell property and neural regeneration supporting capacity from human bone marrow stromal cells (human bone marrow stromal cell-derived neuroregenerative cells: hBMSC-NRs). The aim of the present study was to clarify the effectiveness of transplantation of hBMSC-NRs to injured spinal cord of severe combined immunodeficiency (NOD/SCID) mice. Neurite outgrowth assay of PC-12 cells was performed. One week after a T9-level contusion SCI, hBMSCs or hBMSC-NRs were transplanted into the spinal cord. After the transplantation, functional and histological examinations were performed. Conditioned media of hBMSC-NRs significantly promoted the neurite outgrowth of PC-12 cells *in vitro*. Transplanted hBMSC-NRs survived in the injured spinal cord 8 weeks after SCI. Immunohistochemistry revealed that the density of serotonin-positive fibers of the transplanted group was significantly higher than that of the control group at the epicenter and caudal segment to the injured site. The recovery of hind limb function of the hBMSC-NRs group was significantly better than that of the control group. In conclusion, hBMSC-NRs can be one of the realistic candidates for cell transplantation therapy for human SCI.

Key words: bone marrow stromal cell, NOD/SCID mice, spinal cord injury, transplantation

INTRODUCTION

It has been widely believed that injured adult mammalian spinal cord cannot regenerate. Recent advance of stem cell research is gradually converting that dogma of neuroscience. Repair of injured spinal cord can be achieved by various kinds of cell therapies (Garbossa et al. 2012, Mothe and Tator 2012), including embryonic stem cells (McDonald et al. 1999, Keirstead et al. 2005), fetal neural stem cells (Ogawa et al. 2002) and induced pluripotent stem cells (Tsuji et al. 2010, Nori et al. 2011, Fujimoto et al. 2012), etc. Major obstacles to utilise those cells for clinics are the immunological rejection, the ethical problems raised by the use of fetal tissue and the safety issue (Aznar and Sánchez 2011, Thomas and Moon 2011).

Transplantation of bone marrow stromal cells (BMSCs) has been also shown to improve functional outcome after SCI (Chopp et al. 2000, Quetainmont et al. 2012, Ritfeld et al. 2012, Drela et al. 2013). BMSCs can be easily obtained from bone marrow aspirates and be expanded in a large scale for autotransplantation. Therefore BMSCs have less difficulty for clinical application to human SCI from the ethical point of view.

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We recently reported that cells with neural stem and/or progenitor cell property could be induced from BMSCs (BMSC-neuroregenerative cells: BMSC-NRs) *in vitro* and transplanted those cells effectively attenuated abnormal behavior of Parkinson's disease in rat (Dezawa et al. 2004). In addition we previously reported that transplanted BMSC-NRs promoted functional recovery in rat spinal cord transection (Aizawa-Kohama et al. 2013). To make further step forward to clinical application, we used human BMSC-NRs (hBMSC-NRs) and clinically relevant spinal cord contusion injury model.

The aim of the present study was to clarify the effectiveness of transplantation of hBMSC-NRs to injured spinal cord of severe combined immunodeficiency (NOD/SCID) mice.

METHODS

Production of hBMSC-NRs

hBMSC-NRs were produced as we previously described (Dezawa et al. 2004) with slight modifications. hBMSCs were taken from human bone marrow aspirates of healthy adult volunteers, were cultured for 2 weeks until confluent and were expanded for 2 to 3 passages. hBMSCs were used for transplanatation experiments between passage 2 to 3. hBMSC-NRs were generated from hBMSCs by overnight transfection with the intracellular domain (NICD) of the human Notch1 gene (Kindly provided from Dr. Kawaichi of the Nara Institute of Science and Technology, NICD comprises a sequence coding for a small extracellular domain portion, the transmembrane region and the entire intracellular domain of Notch 1 gene) subcloned into pCI-neo mammalian expression vector containing CMV promoter (Promega, Madison, WI) with Lipofectamine (Invitrogen, Carlsbad, CA). The transfection efficacy was approximately 70%. Stable transfectants were selected by resistance for G418 (200μg/ml). hBMSC-NRs were preserved in liquid nitrogen until usage. Thawed hBMSC-NRs were cultured in 60–70% confluency with α-MEM (Sigma, St Louis, MO) containing 10% fetal bovine serum (FBS; Thermo Trace, Melbourne, Australia) in 5% CO_2 at 37°C and were used within 2 passages after being thawed. The proliferation rate of hBMSC-NRs was same as non-induced hBMSCs and there was no apparent cytotoxicity of NICD gene introduction for hBMSCs.

Neurite outgrowth assay

To confirm neurite outgrowth-promoting potential of soluble factors secreted by hBMSC-NRs,

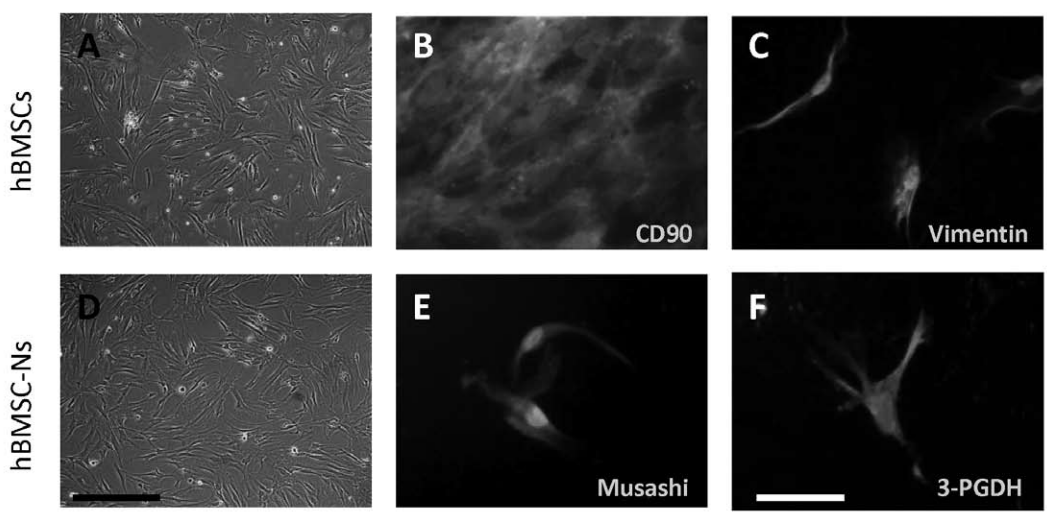

Fig. 1. hBMSC-NRs expressed neural stem cell markers. Phase-contrast microscopic image of human bone marrow stromal cells (hBMSCs, A) and human BMSCs derived cells with neural stem and/or progenitor property (hBMSC-NRs, D). Phase-contrast microscopic image of hBMSC-NRs were morphologically similar to hBMSCs (A, D). Immunocytochemistry revealed that hBMSCs were positive for CD90 (B) and vimentin (C), both of which are markers of mesenchymal cells. After induction, hBMSC-NRs were positive for Musashi (E) and 3-phosphoglycerate dehydrogenase (3-PGDH, F), both of which are markers for neural stem cells. Nuclei were stained with DAPI. Scale bars are 200 μm (D) and 50 μm (F).

neurite outgrowth assay using rat adrenal phaeochromocytoma cells (PC-12; ECACC, Salisbury, UK) was performed. The conditioned media of hBMSC-NRs was collected after 48 hours incubation in α-MEM without FBS and frozen in liquid nitrogen. PC-12 cells were cultured in RPMI 1640 medium (Sigma) containing 10% FBS on collagen typeIV-coated chamber slides (Nalge Nunc International) in 5% CO_2 at 37°C. After 2 days of incubation, culture media was changed to RPMI 1640 media supplemented with 50 ng/ml NGF (Wako Pure Chemical Industries, Osaka, Japan) and 2% horse serum (Invitrogen). Then the conditioned media of hBMSC-NRs was added. After 48 hours incubation, PC-12 cells on the chamber slides were stained with anti-neuronal class III β-Tubulin (Tuj1, 1:400; Covance, San Diego, CA) and length of their neurites was measured using Scion Image software (Scion Corporation, Frederick, MD).

Cytokine assay of the conditioned media of hBMSC-NRs

To detect cytokines and growth factors secreted by hBMSC-NRs, antibody array analysis was performed. hBMSC-NRs were cultured in α-MEM without FBS in 5% CO_2 at 37°C for 24 hours and the conditioned media was collected. Cytokines were investigated using Human Cytokine Antibody Array (RayBiotech, Inc., Norcross, GA) according to the manufacturer's instruction.

Spinal cord injury and transplantation

We used adult female NOD/SCID mice (20–25 g body weight; Central Institute for Experimental Animals, Kawasaki, Japan). All the aspects of animal care and treatment were carried out in accordance with the guidelines of the experimental animal care committee of Chiba University Graduate School of Medicine. Mice were anesthetized with 1–1.2% inhaled Halothane in 0.5 l/min oxygen and were laminectomized at vertebral level T9. The Infinite Horizon Impactor (Precision Systems, Lexington, KY) was used to produce a spinal cord contusion injury (60-kilodyne). Mice received subcutaneous injection of 1 ml saline containing Cefmetazole Sodium (1 mg/ml) up to 3 days (once a day) to prevent infection and dehydration. Cotrimoxazole [Sulfamethoxazole (1.6 mg/ml) and Trimetoprim (0.32 mg/ml)] was dissolved in drinking water. One week after injury, mice were randomized to receive hBMSC-NRs (hBMSC-NRs group, n=10) or sterile phosphate-buffered saline (PBS; control group, n=9). For the transplantation surgery, mice were anesthetized and the SCI site was re-exposed. Under microscopic

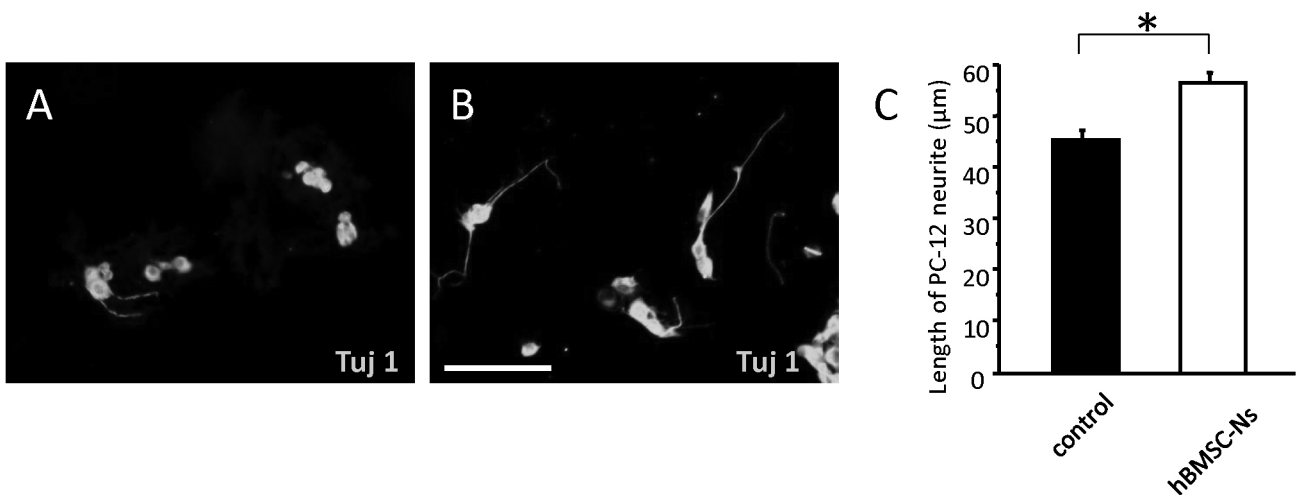

Fig. 2. Conditioned media of hBMSC-NRs promoted neurite outgrowth of PC-12 cells. Conditioned media collected from hBMSC-NRs were added to the culture medium of pre-differentiated PC12 cells. Neurites were stained with anti-neuronal class III β-tubulin (Tuj1) antibody and their length was measured. Length of neurites of the PC-12 cells cultured with conditioned media of hBMSC-NRs (B)(C, open column) was significantly longer than that of the control group (A) (C, closed column). Scale bar is 100 μm (B). Values are mean ± SD. *P<0.01 (C).

visualization, hBMSCs or hBMSC-NRs were injected into the injury site at a concentration of 150 000 cells/3 µl (suspended in PBS) using a glass micropipette connected to a Hamilton Syringe (Hamilton Company, Reno, NV). The tip of glass micropipette in the injured spinal cord was kept in the same place for 4 minutes after the injection. Three-µl PBS was injected in the same way as for the control.

Tissue processing

Mice were euthanized by intraperitoneal injection of pentobarbital overdose and perfused intracardially with 50 ml of 4% paraformaldehyde/PBS 8 weeks after SCI. Spinal cords were dissected 1 cm long including the injured site and post-fixed for overnight in same fixative at 4°C. Then they were equilibrated in 20% sucrose/PBS at 4°C, embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan) and frozen at −80°C. All specimens were cut into the 12-µm thickness sagittal sections by cryostat and the sections were mounted onto poly-L-lysine-coated glass slides (Matsunami Glass, Tokyo, Japan). Every fifth section of the central portion of the spinal cords was serially mounted. At least 4 samples, each 60 µm interval within a 240 µm width centered of the lesion site, were mounted on a slide and picked up from each animal.

Immunocytochemistry and immunohistochemistry

Cultured hBMSC-NRs on chamber slides were washed in PBS 3 times, fixed in 4% paraformaldehyde/PBS for 10 minutes. After 3 wash in PBS, the slides were permeabilized with 0.3% TritonX-100/PBS for 10 minutes. Histological sections were rehydrated with 0.3% TritonX-100/PBS for 1 hour. After that, all the slides were washed with PBS for 3 times and were incubated with blocking solution [0.05 M Tris HCl, pH 7.6, 1% bovine serum albumin, Block Ace (Yukijirusi, Sapporo, Japan), 0.15 M NaCl, 0.1% Tween20] for 1 hour at room temperature. After blocking, slides were incubated with primary antibodies overnight at 4°C. Slides were then washed in PBS 3 times and incubated with secondary antibodies for 30 minutes at room temperature. Slides were then washed in PBS 3 times, were coverslipped and analyzed by fluorescence microscopy (Nikon Instruments Company, Kawasaki, Japan). Primary antibodies used in the present study were Tuj1 (1:400; Covance) to identify neurons, anti-Musashi (1:400; Chemicon), anti-3-phosphoglycerate dehydrogenase (3-PGDH; 1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and anti-human nestin (1:200; Chemicon), anti-Vimentin (1:200; Dako Cytomation, Denmark) and anti-CD90 (1:400; BD Biosciences Pharmingen, San Jose, CA) to identify bone marrow stromal cells, anti-human mitochondria (1:200; Chemicon), anti-glial fibrillary acidic protein (GFAP, 1:400; Sigma) and anti-serotonin (1:800; Sigma). Cell nuclei were stained with DAPI (Invitrogen). Alexa Fluor®-488-conjugated anti-mouse, anti-goat or anti-rabbit IgG and Alexa Fluor® 594-conjugated anti rabbit IgG (Invitrogen) were used as secondary antibodies.

Quantification of injured area

The injured area of spinal cord in mice is occupied with the inflammatory cells. Those inflammatory cells are delineated by GFAP-positive reactive astro-

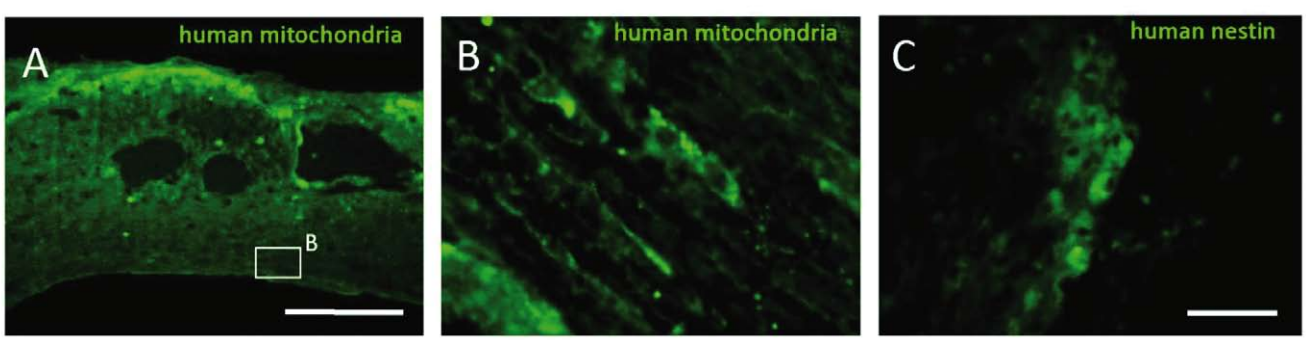

Fig. 3. Transplanted hBMSC-NRs survived in spinal cord after 8 weeks SCI. Survived hBMSC-NRs were located around the epicenter and in the white matter of ventral side of spinal cord (A). hBMSC-NRs were stained with anti-human mitochondria antibody . Magnified image of the box B in the white matter of ventral side of spinal cord on (A). Most of transplanted hBMSC-NRs expressed human-specific nestin (C). Scale bars are 1 mm (A) and 50 µm (B,C).

cytes. Thus we defined the area which was surrounded by GFAP-positive astrocytes as the injured area in this study. Size of the injured area in sagittal plane was measured using Scion Image and figured out the ratio of the injured area to the total spinal cord area.

Serotonin-positive fiber count

To confirm the axonal regeneration-promoting property of hBMSCs and hBMSC-NRs, we performed immunohistochemistry for serotonin, one of the descending motor fibers which are important for locomotor control (Shapovalov 1975, Bregman et al. 1993, Deumens et al. 2005) and counted the number of serotonin-positive fibers. We drew the imaginary lines perpendicular to the long axis of the spinal cord at the epicenter, 500 μm and 1000 μm rostral and caudal to the epicenter. Then we counted the number of serotonin-positive fibers which traversed the each line and figured out the density of serotonin-positive fibers.

Assessment of hind limb locomotor function

Hind limb locomotor function of mice was assessed with Basso Mouse Scale (BMS, Basso et al. 2006) on 1, 3 days and every week until 8 weeks after SCI. To investigate the effect of selective ablation of engrafted hBMSC-NRs on locomotor recovery, two mice in the hBMSC-NRs group whose BMS score were 4 points or above were randomly selected 8 weeks after injury and they received ip injection of diphtheria toxin (DT; 50 μg/kg) twice 24 hours apart. They were reassessed on the BMS 1 week later. Because murine cells are 100 000 times less sensitive to DT than are human-derived cells, DT can selectively ablates human-derived cells (Cummings et al. 2005).

Statistical analysis

The ratio of injured area to the spinal cord and the density of serotonin-positive fibers in the control, hBMSCs and hBMSCNRs groups were subjected to the Tukey-Kramer HSD-test. Points of BMS were subjected to repeated-measures ANOVA followed by *post hoc* test using the Tukey-Kramer HSD-test. The ideal sample size was calculated with power analysis. Data are presented as mean values ± SD. Values of $P<0.05$ were considered statistically significant. All the statistical analyzes were performed with statistical software JMP (v.10, SAS Institute Japan, Tokyo, Japan)

RESULTS

hBMSC-NRs expressed neural stem cell markers *in vitro*

Firstly, we compared between hBMSCs and hBMSC-NRs in their morphology under the phase microscopy. Cultured hBMSC-NRs showed fibroblast-like morphology, which was similar to that of hBMSCs (Fig. 1A,D). Next, we performed immunocytochemistry to examine the phenotype of hBMSCs and hBMSC-NRs. Before the transfection of NICD, hBMSCs were positive for vimentin and CD90 (positive ratio was ~90%), both of which are expressed in mesenchymal cells (Fig. 1B,C). After induction, hBMSC-NRs were positive for Musashi and 3-PGDH (Fig. 1E,F, ~90%), both of which have

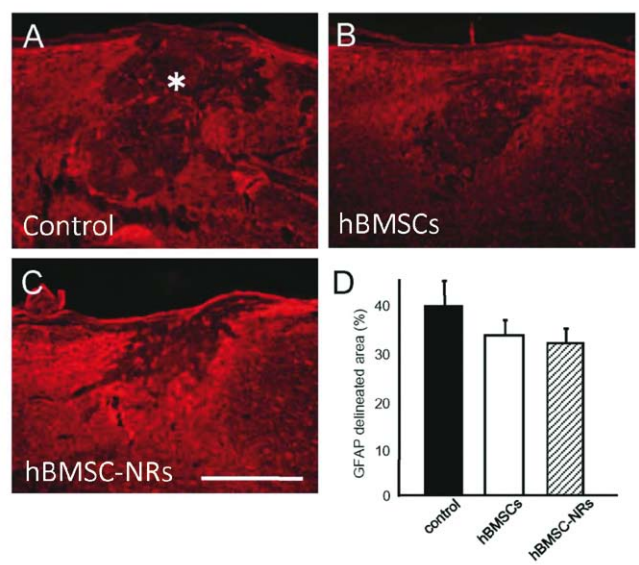

Fig. 4. Transplantation of hBMSC-NRs tended to decrease the injured area. Injured area of spinal cord is delineated GFAP-positive reactive astrocytes. Thus we used anti-GFAP antibody to measure the area of injured site in sagittal plane and figured out the ratio of GFAP-delineated area to the spinal cord (the control group: A, asterisk, the hBMSC group: B, the hBMSC-NRs group: C). Though the ratio of GFAP delineated area of the hBMSC-NRs group (A–C, D, hatched column) tended to be lower than that of the control group (D, closed column), the difference was not significant ($P=0.15$). Scale bar is 1 mm (A). Values are mean ± SD (B).

been found to be expressed in NSCs (Yamasaki et al. 2001, Okano et al. 2005) and they lost expression of CD90 and vimentin. Approximately ~10% of hBMSCs showed expression of Musashi and 3-PGDH (data not shown).

Conditioned media of hBMSC-NRs promoted neurite outgrowth of PC-12 cells

Lengths of neurites with or without conditioned media of hBMSC-NRs were 53.82±9.84 μm and 42.47±8.06 μm respectively. Neurites of PC-12 cells cultured with conditioned media of hBMSC-NRs were significantly longer than those cultured without hBMSC-NRs conditioned media (P<0.05, Fig. 2 A–C), indicating that soluble factors secreted by hBMSC-NRs had neurotrophic property.

Cytokines secreted by hBMSC-NRs

Cytokines in the hBMSC-NRs were analyzed using cytokine antibody array. Listed cytokines were detected as shown in Table I.

hBMSC-NRs survived in injured spinal cord

Immunohistochemistry for human mitochondria 8 weeks after SCI revealed that transplanted hBMSC-NRs

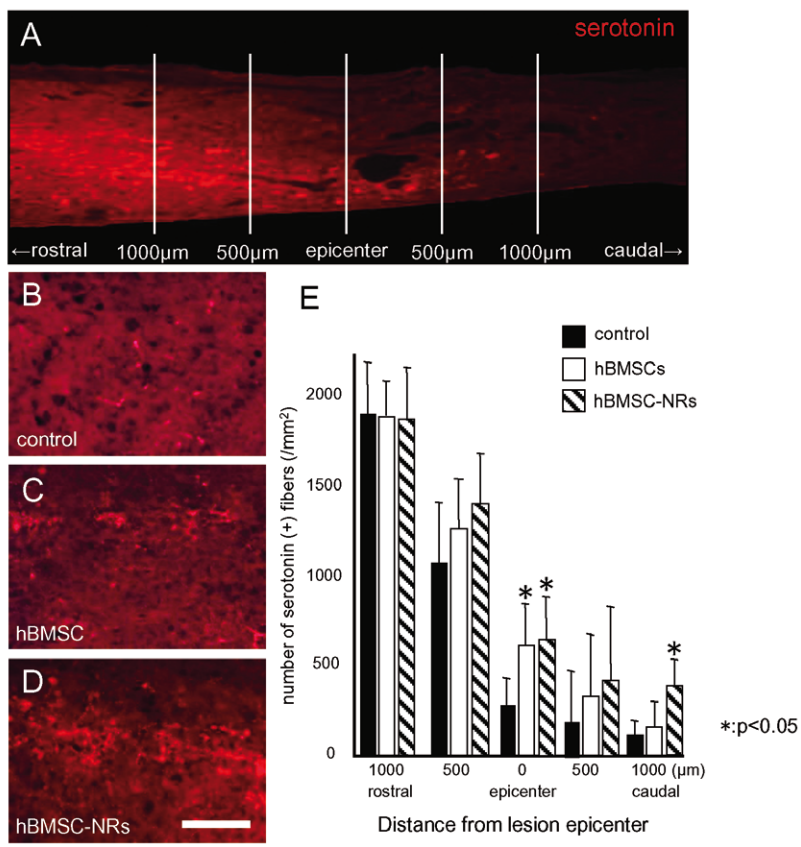

Fig. 5. Transplantation of hBMSC-NRs promoted regeneration/sparing of serotonin-positive fibers. Imaginary lines which were perpendicular to the axis of sagittal segment of spinal cord were drawn and serotonin-positive fibers were counted at each line (A). Serotonin-positive fibers decreased as the level went down from rostral to caudal (E, closed column). Representative images of the spinal cord 1000 μm caudal to the epicenter in the control (B), the hBMSC group (C) and hBMSC-NRs (D) groups. Only a few serotonin-positive fibers were identified in the control group (B) whereas more serotonin-positive fibers were identified in the hBMSC group (C) and the hBMSC-NRs group (D). The density of serotonin-positive fibers of the hBMSC-NRs group (E, hatched column) was significantly higher than that of the control group (E, closed column) and the hBMSC group (E, open column) at the epicenter. There was significant difference between the hBMSC-NRs group (E, hatched column) and the control group (E, closed column) in the number of serotonergic fiber at 1000 μm caudal level. Scale bar is 100 μm. Values are mean ± SD. *P<0.05 (E).

Table I

Cytokines and growth factors detected in the conditioned media of hBMSC-NRs			
BDNF	BMP-4	Fas/TNFRSF6	G-CSF
IGFBP-2, 4	IL-6	MCP-1	NT3
TIMP-1, 2	TNF-β	uPAR	VEGF

hBMSC-NRs expressed various kinds of growth factors and cytokines. hBMSC-NRs were cultured in α-MEM without FBS for 24 hours and the conditioned media was collected. Cytokines in the hBMSC-NRs media were analyzed using cytokine antibody array. hBMSC-NRs secreted some cytokines which have been shown to have neurotrophic effects.

survived within the injured spinal cord of NOD/SCID mice. Survived hBMSC-NRs were located around the epicenter and in the white matter of ventral side of spinal cord (Fig. 3A,B). Most of survived hBMSC-NRs were positive for human specific nestin (Fig. 3C). To check the side effects such as tumor formation, hBMSC-NRs were transplanted into the normal brain and spinal cord of NOD/SCID mice (n=2). Those hBMSC-NRs showed no tumor formation after 6 months after SCI (data not shown).

hBMSC-NRs had a tendency to reduce injured area

We used immunohistochemistry for GFAP to measure the area of injured site (Fig. 4A–C). The ratio of GFAP-delineated area to the spinal cord were 30.06±10.02% in the hBMSC-NRs group, 32.40±12.24% in the hBMSC group and 38.53±8.20% in thecontrol group (Fig. 4D) There was no significant difference between the control, hBMSC and hBMSC-NRs groups in GFAP-delineated area.

hBMSC-NRs promoted sparing and/or regeneration of serotonergic fibers

We counted the serotoin-positive fibers around the injury site (Fig. 5A). The density of serotonin-positive fibers gradually decreased as the levels went down from rostral to caudal levels, indicating that SCI caused loss of descending serotonin-positive fibers especially in the caudal level (Fig. 5E, closed columns). The density of serotonin-positive fibers of the hBMSC (Fig. 5E, open columns) and hBMSC-NRs (Fig. 5E, hatched columns) groups was significantly higher than that of the control group (Fig. 5E, closed columns) at the epicenter level. At 1000 μm caudal level, the hBMSC-NRs group showed significant larger number of serotonin-positive fibers compared with the control and hBMSC groups (Fig. 5B–D, P<0.05, Fig. 5E, hatched columns).

hBMSC-NRs promoted functional recovery of hind limb after SCI

Locomotor assessment revealed that there was statistically significant difference between the control and the hBMSC-NRs groups (P=0.033, repeated measures ANOVA), whereas the hBMSCs group failed to show significant recovery compared with the control group. There was no significant differ-

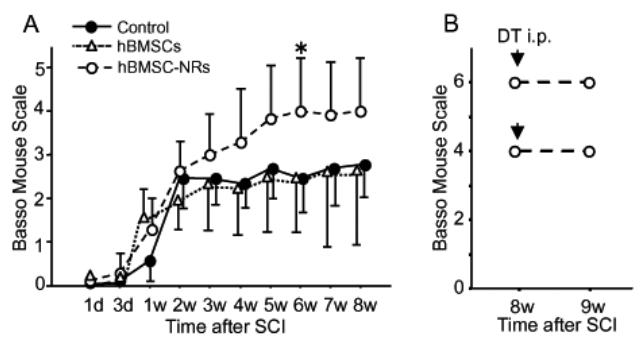

Fig. 6. Transplantation of hBMSC-NRs promoted recovery of hind limb locomotor function. Locomotor function was assessed using Basso Mouse Scale (BMS). Repeated measures ANOVA and *Post-hoc* Tukey-Kramer test showed better functional recovery in the hBMSC-NRs group (A, open circle, dashed line) than the control group (A, closed circle, line). Two hBMSC-NRs transplanted mice received diphtheria toxin (DT) injection at 8 weeks after SCI. These mice showed no deterioration of motor function assessed by BMS one week after DT injection (B). Values are mean ± SD. *P<0.05 (A).

ence between the hBMSCs and hBMSC-NRs groups in hindlimb locomotor recovery. *Post-hoc* Tukey-Kramer test revealed that BMS score of the hBMSC-NRs group was significantly higher than that of the control group at 6 weeks after SCI (P=0.039, Fig. 6A). BMS of the hBMSC-NRs group tended to be higher than that in the hBMSCs group at 6 weeks after SCI (P=0.069). There was no significant difference between the control and the hBMSCs groups in BMS score at 6 weeks after SCI. The average score of BMS at 6 weeks after SCI in the hBMSC-NRs group was 4.00±1.5, indicating occasional plantar stepping whereas the average recovery score in the hBMSC group was 2.64±1.5 and that in the control group was 2.78±0.70, indicating only extensive ankle movement or plantar placing of the paw without plantar stepping. The statistical power was 0.56 calculated by power analysis, indicating that the sample size was slightly small to obtain sufficient statistical power.

Two mice which received DT injection were re-assessed one week after DT injection, however, no deterioration of BMS score was observed in those mice (Fig. 6B). Transplanted hBMSC-NRs disappeared in those mice after DT injection (data not shown), showing that ablation of human-derived cells could be efficiently performed by DT treatment.

DISCUSSION

It has been reported that NSCs and BMSCs exert therapeutic potential for SCI by secretion of several kinds of neurotrophic factors (Lu et al. 2003, Chen et al. 2005, Sahni and Lessler 2010, Tohda and Kuboyama 2011). BMSC-NRs might also have capability to secrete neurotrophic factors. To confirm this, we performed neurite outgrowth assay and cytokine assay in the present study. The results showed that conditioned media of hBMSC-NRs significantly promoted neurite extension of PC-12 cells *in vitro,* suggesting that hBMSC-NRs secreted neurotrophic factors and stimulated neurite extension. We performed cytokine assay of the conditioned media of hBMSC-NRs to detect secreted neurotrophic factors. The assay revealed that hBMSC-NRs secreted many cytokines and growth factors (Table I). Among the factors detected, several kinds of cytokines and growth factors are well known to have neurotrophic property. Those factors are as followings; brain derived neurotrophic factor, granulocyte colony-stimulating factor, interleukin (IL)-6, neurotrophin (NT)-3 and vascular endothelial growth factor (VEGF). The results of the neurite outgrowth assay and the cytokine assay showed that hBMSC-NRs have neurotrophic effects including promotion of neurite extension. The density of serotonin-positive raphe-spinal fibers, one of the major descending fibers which contribute to motor function, were significantly higher in the hBMSC and hBMSC-NRs groups than that in the control group, indicating that hBMSC and hBMSC-NRs have serotonergic fiber sparing and/or regeneration promoting effects. Especially in the caudal to the lesion epicenter, the number of spared/regenerated serotonergic fiber was significantly larger in the hBMSC-NRs group than that of the hBMSC group. This suggests the possible superiority of hBMSC-NRs compared with non-induced hBMSC in the viewpoint of axonal sparing/regeneration promoting effect *in vivo.*

The functional recovery of hind limb was significantly better in the hBMSC-NRs group than in the control group, shown by the assessment using BMS. *Post-hoc* analysis showed that BMS at 6 weeks after SCI in the hBMSC-NRs group was significantly higher than that in the control group and tended to be higher than that in the hBMSCs group. The result of locomotor assessment was consistent with the results of immunohistochemistry for serotonin, suggesting that axon sparing/regeneration promoting effect is one of the possible mechanisms of hBMSC-NRs to promote functional recovery after SCI.

DT injection to two hBMSC-NRs transplanted mice at 8 weeks after SCI did not affect their motor function though hBMSC-NRs were ablated by DT injection in the present study. This result suggests that survived hBMSC-NRs have only little contribution to motor function at 8 weeks after SCI, in contrast to the previous report by Cummings and coworkers (2005), which showed the DT ablation-mediated loss of recovered function. The possible explanations of the difference between the results of DT ablation in the present study and the previous one were as followings, the soluble factor secreted from hBMSC-NRs promoted axonal elongation in the earlier time points and protected spinal cord tissue from damage, contributing to motor function recovery.

hBMSC-NRs have great advantage that they can be transplanted autologously. Auto-transplantation is more desirable than allograft in clinical applica-

tion to avoid the immunological and ethical problems. However, hBMSC-NRs have specific disadvantage for clinical use. It takes 6–7 weeks to prepare hBMSC-NRs for transplantation. Therefore, to shorten the time to produce hBMSC-NRs is necessary.

CONCLUSION

The present study showed that the transplantation of hBMSC-NRs promoted sparing and/or regeneration of serotonin-positive fibers and functional recovery after SCI. These lines of evidence make hBMSC-NRs as one of the possible candidates for source of human SCI cell therapy.

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