BIOMEDICAL AND ENVIRONMENTAL SCIENCES **20**, 242-249 (2007)

Induction of Functional Recovery by Co-transplantation of Neural Stem Cells and Schwann Cells in a Rat Spinal Cord Contusion Injury Model[1]

JIN LI[※], CHONG-RAN SUN[※], HAN ZHANG[※], KAM-SZE TSANG[#], JUN-HUA LI[※], SHAO-DONG ZHANG[※], AND YI-HUA AN[※,2]

[※]*Beijing Neurosurgical Institute, Capital University of Medical Science, Beijing 100050, China;*
[#]*Blood and Marrow Transplantation, Department of Anatomical and Cellular Pathology, the Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China*

Objective To study the transplantation efficacy of neural stem cells (NSCs) and Schwann cells (SC) in a rat model of spinal cord contusion injury. **Methods** Multipotent neural stem cells (NSCs) and Schwann cells were harvested from the spinal cords of embryonic rats at 16 days post coitus and sciatic nerves of newborn rats, respectively. The differential characteristics of NSCs *in vitro* induced by either serum-based culture or co-culture with SC were analyzed by immunofluorescence. NSCs and SCs were co-transplanted into adult rats having undergone spinal cord contusion at T9 level. The animals were weekly monitored using the Basso-Beattie-Bresnahan locomotor rating system to evaluate functional recovery from contusion-induced spinal cord injury. Migration and differentiation of transplanted NSCs were studied in tissue sections using immunohistochemical staining. **Results** Embryonic spinal cord-derived NSCs differentiated into a large number of oligodendrocytes in serum-based culture upon the withdrawal of mitogens. In cocultures with SCs, NSCs differentiated into neuron more readily. Rats with spinal cord contusion injury which had undergone transplantation of NSCs and SCs into the intraspinal cavity demonstrated a moderate improvement in motor functions. **Conclusions** SC may contribute to neuronal differentiation of NSCs *in vitro* and *in vivo*. Transplantation of NSCs and SCs into the affected area may be a feasible approach to promoting motor recovery in patients after spinal cord injury.

Key words: Spinal cord injury; Transplantation; Neural stem cells; Schwann cells; Cell differentiation

INTRODUCTION

Traumatic spinal cord injury (SCI) accounts for a large number of physical injuries to the central nervous system (CNS). Patients with severe SCI may lose their locomotor and sensory function completely underneath the affected level. The natural repairing of traumatic spinal cord injury is very limited and there is currently no effective treatment for SCI.

Advances in experimental regeneration research have led to several transplantation strategies that promote axonal regrowth and partial functional recovery in animal models of SCI[1-3]. Schwann cells (SCs), which provide an environment for permissive peripheral nerve regeneration, have been proven to be an axonal regeneration promoter in CNS[4]. A recent study has shown that SC can induce neural stem cells (NSCs) to differentiate into neurons more readily *in vitro*[5].

Taking spinal cord as a non-neurogenic tissue where neuronal differentiation is impermissive[6], and considering the fact that inflammatory cytokines generated *in situ* in the traumatic site elicit negatively regulatory effects on the survival, migration, and neuronal differentiation of implanted NSCs, we transplanted SCs along with NSCs into the injured site of adult rat spinal cord. Our aim was to study whether SCs negate the inhibitory regulators *in situ* and enhance the survival, migration and differentiation of implanted NSCs.

MATERIALS AND METHODS

Animals

Eighty-six female adult Sprague-Dawley rats weighing 200-220 g were obtained from the Laboratory Animal Center, The Chinese Academy of

[1]This research was supported by the National Natural Science Foundation of China (No. 30371452).
[2]Correspondence should be addressed to Prof. Yi-Hua AN, Beijing Neurosurgical Institute, 6# Tiantan Xili, Chongwen District, Beijing 100050, China. Tel: 86-10-67028785. Fax: 86-10-67028785. E-mail: riveran@163.com

Biographical note of the first author: Jin LI, male, born in 1977, Ph. M., research assistant of Beijing Neurosurgical Institute, Capital Medical University, majoring in regeneration medicine of central nervous system injury. Tel: 86-13691367124. E-mail: flintli@gmail.com (E-mail address for requesting reprints).

0895-3988/2007
CN 11-2816/Q

Medical Sciences. Animal treatments were carried out to minimize pain or discomfort in accordance with current protocols in compliance with the current institutional guidelines and related laws.

Induction of Spinal Cord Injury

The protocol of spinal cord contusion described by von Euler and his co-workers with slight modification was used[7]. Briefly, rats were anesthetized with an intraperitoneal injection of chloral hydrate (Beijing Chemical Reagents Company, China) at 0.35 g per kg body weight. A laminectomy was performed at the T9 level under surgical microscope. The exposed spinal cord was transiently compressed by a modified aneurysm clip applied vertically with a closing pressure of 150 g for five minutes. The force of the clip for spinal cord compression was standardized in accordance with the method of Eugen J Dolan[8]. The body temperature of animals was maintained at 37℃ during the operation. Thereafter the clip was released. The skin incision was sutured and the rats were allowed to gain access to food and water at adlibitum in isolated cages. Bladders of animals were emptied manually twice a day.

Culture of Rat NSCs and SC

The techniques used in the study were described previously[9-10]. Spinal cords of embryonic rats at 16 days post coitus were dissected and mechanically dissociated into single cells in DMEM/F12 culture medium (Gibco, Carlsbad, CA). The cell viability was assessed by trypan blue dye exclusion. Viable cells at 1×10^5/mL DMEM/F12 (1:1) supplemented with B27 (Gibco), 20 ng/mL each of basic fibroblast growth factor (Promega, Madison, WI) and epidermal growth factor (Promega) were seeded onto 25 mm^2 culture flasks (Corning, Corning, NY) and kept at 37℃ in a humidified incubator containing 5% CO_2. After the floating neurosphere was formed, cells were subsequently passaged weekly.

The method described by Morrissey was used to isolate SC[11]. Basically, sciatic nerves of new born Sprague-Dawley rats were removed and placed into Dulbecco's modified Eagle's medium (DMEM, Gibco) and divested of the epineurium and connective tissues. They were then cut into 1 mm^3 pieces and then plated onto 25 mm^2 culture flasks containing DMEM supplemented with 10% fetal bovine serum (FBS, Gibco), 2 μmol/L forskolin (Gibco) and 20 μg/mL bovine pituitary extract (Gibco). Upon 80% confluent growth, cultures were rinsed twice with calcium- and magnesium-free Hank's balanced salt solution (HBSS; Gibco), and briefly treated with 0.25% trypsin (GIBCO/BRL). Reaction was stopped by adding and washing with DMEM containing 10% FBS. Cultures were passaged in purification culture medium of serum-free DMEM. Pure cultures up to 98 % of SCs were obtained upon passages for four to six weeks.

Co-culture of NSC and SC

The co-culture has been elsewhere[5]. Neurospheres derived from the third passage were triturated into single cells and seeded onto SCs feeder layer preestablished on 60-mm^2 culture dishes at 50% confluence. The co-culture medium was DMEM/F12 supplemented with 1×B27 supplement and 5% FBS. Spent culture medium was demi-discarded and supplemented with fresh medium every three days. NSCs propagated without SCs feeder support were taken as controls. Cultures having grown for a week were fixed in 4% paraformaldehyde (Beijing Chemical Reagents Company, China).

Characterization of NSC Culture

Cells in selected areas of *in vitro* cultures were visualized by nuclear dye Hoechst 33342 (Sigma). Cells expressing the specific antigens, nestin, neuron specific enolase (NSE), microtubule-associated protein 2 (Map-2), galactocerebroside (GalC) and glial fibrillary acidic protein (GFAP) were counted in 10 randomly selected microscopical fields. The proportion of differentiated progenies derived from NSCs was enumerated.

Cell Preparation for Transplant

Neurospheres derived from the third passage and SC were labeled with 5 μmol/L bromodeoxyuridine (BrdU; Roche, Basel, Switzerland) in the supplemented culture medium two days prior to transplantation to the injured animals for *in vivo* study. Cells were then washed three times with HBSS and adjusted to 1×10^7 viable cells per mL HBSS.

Transplantation

NSCs and SCs were implanted into animals nine days after spinal cord compression. Rats were anesthetized with 0.35 g chloral hydrate per kg body weight. Under a surgical microscope, the traumatic site of the spinal cord was re-exposed. Five microliters of BrdU-labelled cells (50% each of NSCs and SCs) with a total amount of 5×10^4 cells was injected into the intraspinal cavity of 33 rats using a microsyringe. Thirty-three rats in another arm were transplanted with 5 μL BrdU-labeled NSCs, whereas 20 rats of the control arm were injected with 5 μL of HBSS.

Behavioral Analysis

Locomotor behavior of SCI rats transplanted with both NSC and SC (n=33) and NSC (n=33) and 20 control rats were assessed weekly up to eight weeks. The 21-point rating scale of Basso, Beattie, Bresnahan (BBB) open field rating system was used to evaluate the various degrees of locomotor function; a score of 21 represents normal movement whereas 0 denotes complete paralysis of the hind limbs[12].

Immunofluorescent and Immunohistochemical Staining

Immunofluorescent stainings of nestin, NSE, Map-2, GalC and GFAP were performed on NSCs derived from serum-based medium and co-cultures with SC. Basically monoclonal antibody against nestin (1:400, Chemicon, Temecula, CA), polyclonal anti-neuron specific enolase (NSE, 1:300, Chemicon), polyclonal anti-microtubule associated protein-2 (Map-2, 1:800, Chemicon), polyclonal anti-galactocerebroside (GalC, 1:1000, Chemicon) and anti-glial fibrillary acidic protein (GFAP, 1:800, Chemicon) were used. Cell cultures were rinsed with HBSS, blocked in 10 % normal sheep serum and 0.2 % Triton X-100 in PBS for 30 minutes before incubation with the primary antibody overnight at 4℃. Specific cellular markers were detected by incubation for 30 minutes with the pertinent secondary antibodies, including FITC-conjugated donkey anti-rabbit IgG, TRITC-conjugated donkey anti-rabbit IgG, TRITC-conjugated goat anti-mouse IgG (Zymed, London, UK) at 1:100 dilution.

Upon completion of the functional assessments rats were anesthetized. Thoraxes were cut open and animals were transcardially infused with physiologic saline and 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB, pH 7.35) solution. Spinal cords of transplanted animals were removed and segmented. Spinal cord segments containing the transplant were fixed in 4% paraformaldehyde overnight, embedded in paraffin and longitudinally sectioned at 5 μm. After deparaffinization, the spinal cord sections were treated according to the protocol described previously[5]. In brief, sections in citrate buffer (pH 6.0) were baked for 15 minutes in a microwave oven at 98℃ and then immersed in 3% hydrogen peroxide-methanol for another 15 minutes. Sections were blocked in 1% normal sheep serum for 30 minutes before incubation with the primary antibody overnight at 4℃. The primary antibodies used are monoclonal anti-BrdU (1:800, SIGMA), monoclonal anti-Nestin (1:400, CHEMICON), polyclonal anti-NSE (1:300, CHEMICON), polyclonal anti-GFAP (1:800, CHEMICON), monoclonal anti-CGRP (1:1500, CHEMICON). The subsequent immunohistochemical double stain method was in accordance with the manual provided by the producer (ZYMED, Histostain-Plus kits) using LAB-SA system except for BrdU detection in which the TRITC-conjugated goat anti-mouse IgG (Zymed, London, UK) was used. Tissue sections were visualized with either diaminobenzidine (DAB), BCIP/NBT or AEC according to the need.

Statistical Analysis

BBB open field locomotor rating scores were reckoned and expressed as $\bar{x} \pm s$ for each arm of animals at different time points. Data derived from the three arms of rodents in each week were compared using one-way ANOV statistical analysis. The survival rates of three arms of SCI rats receiving NSC transplant, NSC and SC transplant and normal saline were evaluated by Kaplan-Meier analysis. The statistic software SPSS version 13.0 was used. Differences between groups were regarded as significant if $P \leqslant 0.05$.

RESULTS

In vitro Assays

Neurosphere-like cell clusters were noted in *in vitro* cultures for five to seven days. The morphology of free floating spheres resembled neurospheres derived from the embryonic cortex. After cultured in serum-based medium for seven days, they differentiated into microglia-like cells accounting for about 71% of the total cells (Fig. 1A). Immunofluorescent staining demonstrated GalC expression suggesting the presence of oligodendrocytic progenies (Fig. 1B). In addition, $GFAP^+$ and NSE^+ cells accounted for 20.2% and 14.8% (Fig. 1C), respectively. $Nestin^+$ cells accounted for majority of the total cells (Fig. 1D), and some of the cells exhibited typical neuron shape with asymmetric appearance, multiple dendritic processes, and a long single axonal projection, while the others displayed astrocytic or oligodendrocytic features (Fig. 1D).

In co-cultures for seven days, a comparatively higher proportion of 20% of mature neurons was noted (Fig. 2A). However, a large population of progeny was still GalC-positive oligodendrocytes (Fig. 2B).

In vivo Assays

The weighting force in opening the limbs of the aneurysm clip was 150 g, while it was 136 g when the two limbs of the clip opened to one degree. A total of 86 rats were subjected to spinal cord contusion injury.

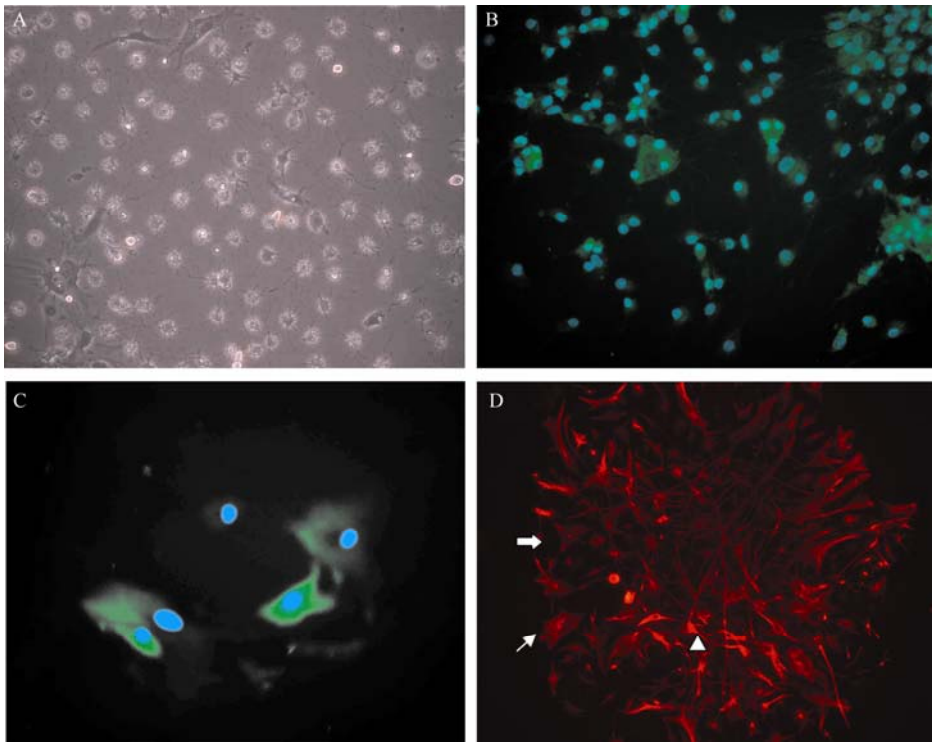

FIG. 1. E16 spinal cord derived NSCs induced *in vitro* by serum for 7 days: A: under the phase contrast microscopy, most progenies were small glial cells with multiple thin process which was a typical microglia morphology. B: Specific antigen GalC Immunofluorescent staining and double-stained by Hoechst 33342 illustrating about 71% of the total progenies were oligodendrocyes. C: NSE-expressing neurons were also detected (accounting for 14.8%). D: The majority of the differentiated cells were also nestin-positive which had typical morphology of neurons (△), astrocytes (→), and oligodendrocytes (⇨).

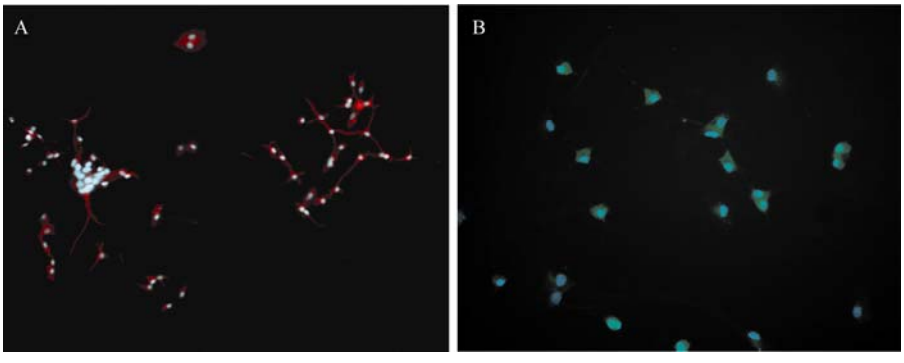

FIG. 2. E16 spinal cord derived NSCs co-cultured with SC *in vitro* for 7 days: A: NSCs differenting into neurons in a comparatively larger number of mature neurons (20%). B: a large population of progeny being still GalC-positive oligodendrocytes, and arrowhead showing a schwann cell in a spindle-like shape and a ellipse nuclear co-cultured with NSCs (B).

Upon completion of the induction of SCI, three rats with BBB rating scores more than five points in the first week of assessment were excluded. Thirty-five rats survived until the 8th week. The overall survival rate was 42.2% (35/83). The overall survival rate of three arms of rodents was 46.9% (15/32, co-transplantation group), 43.8% (14/32, NSCs group) and 31.6% (6/19, control group), respectively. The means and medians for survival time of the three arms are displayed in Table 1. Survival curves for the three arms of rats were evaluated with the Kaplan-Meier method (Fig. 3A). A separate Kaplan-Meier statistic was performed to evaluate if there were statistically significant differences in the survival rates for the

co-transplanted arm versus the NSCs transplanted arm (Fig. 3B) (log-rank test, P=0.932; Tarone Ware test, P=0.995; and Breslow test, P=0.949). Another separate Kaplan-Meier evaluation for co-transplanted arm versus the control arm (Fig. 3C) showed no statistically significant differences in the survival rate (log-rank test, P=0.257; Tarone Ware test, P=0.260; and Breslow test, P=0.269). A separate Kaplan-Meier evaluation for NSCs transplanted arm versus the control arm (Fig. 3D) also had no statistically significant differences in the survival rate (log-rank test, P=0.290; Tarone Ware test, P=0.263; and Breslow test, P=0.248).

TABLE 1

Means and Medians for Survival Time

Treatment	Mean[a]				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
Co-transplantation	30.813	4.297	22.390	39.235	18.000	.	.	.
NSCs	30.969	4.164	22.807	39.130	23.000	19.092	0.000	60.420
Control	23.000	5.285	12.642	33.358	10.000	3.627	2.890	17.110
Overall	29.084	2.630	23.929	34.239	18.000	10.122	0.000	37.839

Note. [a]Estimation is limited to the largest survival time if it is censored.

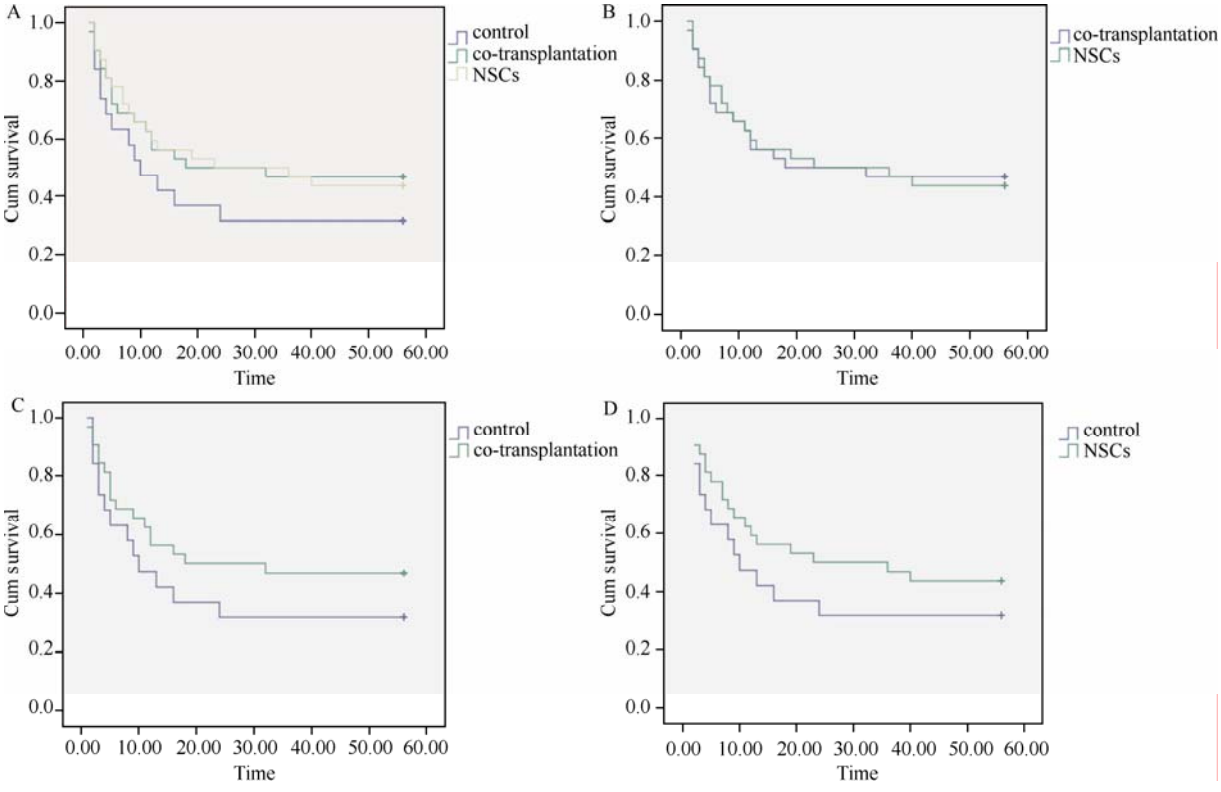

FIG. 3. Kaplan-Meier evaluation for the 3 arms of rats (A), co-transplanted arm versus NSCtransplanted arm (B), co-tansplanted arm versus control arm (C), and NSC transplanted arm versus control arm (D).

Figure 4 shows the BBB rating scores of three arms of rats over eight weeks. Data were in line with the Gaussian distribution. The $\bar{x} \pm s$ of BBB scores at the first week was 0 to 1, suggesting none to little locomotor function of the hind limbs. An explicit recovery was noted among the rodents during the second to third week but the progress slowed down in the fourth week. Scores beyond nine points were generally not evident up to the end of eighth week. In the 4th week, higher BBB scores were noted in two arms of transplanted rats compared to the control group (BBB scores: 8.4167±0.5149 of 16 rats transplanted with NSC and SC *vs*. 5.7500±1.7321 of 6 control rats; $P<0.05$, 7.8333±1.2247 of 16 rats

transplanted with NSC *vs*. 5.7500±1.7321 of 6 control rats; $P<0.05$). The recovery of locomotor function in traumatized rats undergone NSC and SC transplant was better than the counterparts transplanted with NSC, but there was no statistically significant difference (BBB scores: 8.5833±1.0814 of 16 rats transplanted with NSC and SC *vs*. 8.2222±1.0035 of 16 rats transplanted with NSC; $P>0.05$). The same results were observed at the following time points.

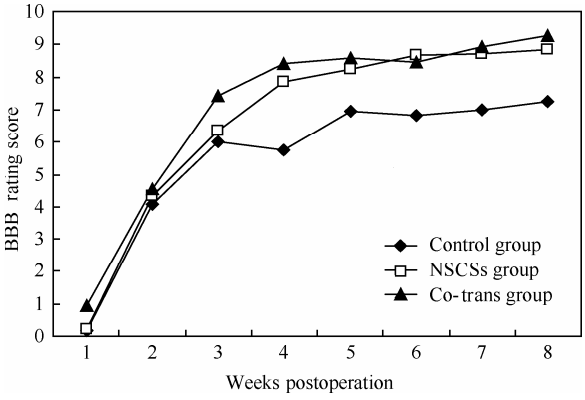

FIG. 4. BBB open field locomotor rating scores recorded postoperationally.

Immunoflurescent staining of BrdU in spinal cord sections from the sacrificed animals having undergone co-transplantation of NSC and SC for 56 days revealed that a significant number of $BrdU^+$ cells migrated to the gray matter adjacent to the injection site (Fig. 5A), while few scattered to the white matter. The migration of $BrdU^+$ cells was also evident in the rostral and caudal region 1 cm to the injection site. In addition, $nestin^+$ cells were noted localizing mainly at the central cannule and the peripheral white matter, while $GFAP^+$ cells scattered to the vicinity of the traumatic cavity. The closer to the cavity, the stronger GFAP immmunoreactivity of cells was. In contrast to the localization of $BrdU^+$ $GFAP^+$ cells in the grey matter of the spinal cords in both arms of rats undergone transplant, $BrdU^+$ NSE^+ and $BrdU^+$ $CGRP^+$ cells were only detected in rats receiving co-transplant of NSCs and SC (Figs. 5B and 5C).

DISCUSSION

Patients may experience long-term or permanent neurological deficits after SCI. Currently, no effective treatment is available. Medical and scientific professionals are poised to begin translating promising

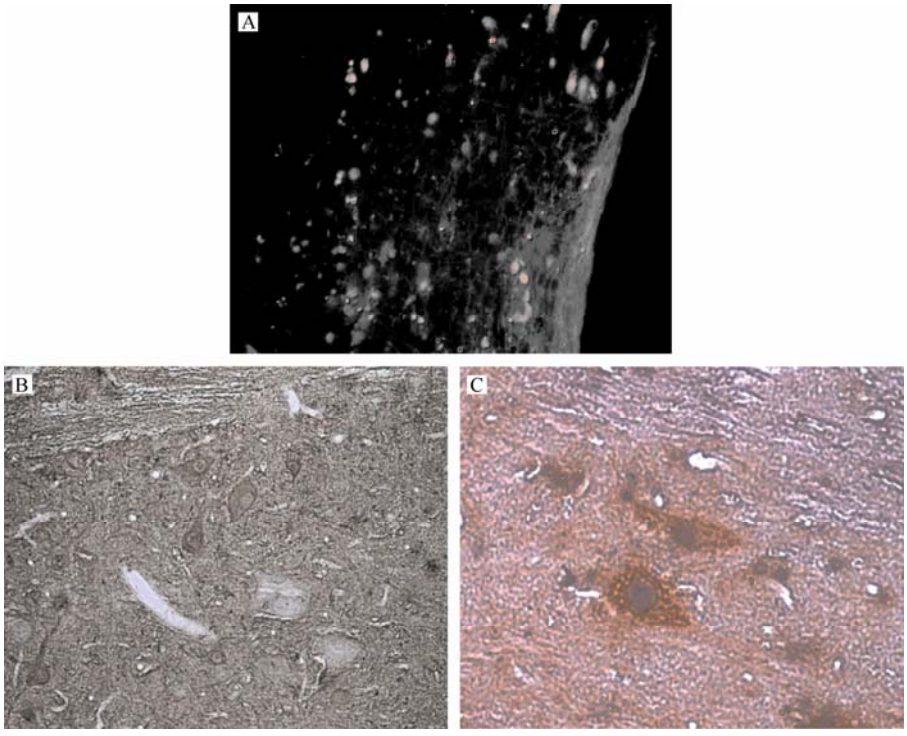

FIG. 5. Immunoflurescent and immunohistochemical staining of spinal cord sections showing $BrdU^+$ cells (A), $BrdU^+$ NSE^+ cells $BrdU^+$ NSE^+ cells (B) and $BrdU^+$ $CGRP^+$ cells $BrdU^+$ $CGRP^+$ cells (C) in gray matter of rats.

new experimental findings into treatments for patients suffering from SCI. In this study, we investigated the efficacy of transplantation of NSCs and SCs into the intraspinal cavity in a rat spinal cord contusion injury model. The novel modality enhanced the recovery of locomotor function. It was safe and had no adverse reactions. In addition, no local tumor development was observed in the tissue samples from the sacrificed animals. Our data are in line with those derived from other animal studies, suggesting that transplantation strategies are feasible for partial recovery of locomotor functions.

A number of studies have illustrated that NSCs derived from different anatomical sites or developmental stages respond differently to growth factors, in particular epidermal growth factor and basic fibroblast growth factor[15], suggesting that NSCs may be intrinsically different in their biological functions and characteristics. In this study, the majority of NSCs derived from embryonic spinal cord 16 days post coitus were able to differentiate *in vitro* into oligodendrocytic progenies, which may be due to the *in vitro* culturing condition and the intrinsic potential for oligodendrocyte formation of the E16 rodent embryonic spinal cord[14]. Fibroblast growth factor-enriched culture medium may activate *Olig2* gene through MAP-dependent signal transduction pathway and induce NSCs to differentiate into oligodendrocytes[15].

Nestin is an intermediate filament protein and has long been used as a marker of NSCs, and nestin can also be expressed by other cell types including reactive astrocytes[16], immature oligodendrocytes and neurons[17]. These data may explain why the majority of the differentiated progenies in NSCs are nestin positive. CGRP is the marker of inter-neurons in the hind horn. $BrdU^+$ $CGRP^+$ cells were noted adjacent to the intraspinal cavity, suggesting that the implanted NSCs can differentiate into comparatively mature neurons that may innervate in the spinal cord network.

SCs are the peripheral nerve-ensheathing cells, which can produce a variety of extracellular matrices and adherent molecules including laminin and collagen. They can also secrete a number of soluble factors such as nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF), which have been implicated in a series of normal and pathological process related to neural development and regeneration. In the culture of NSCs, growth medium *in vitro* could provide an environment vulnerable to autocrines and paracrines of NSCs, suggesting that NSCs in cultures can modulate their proliferation, differentiation and survival. In the co-culture system, SCs may provide additional benefits and favorable environments which may not be readily available in the routine culture. In this study, SC-derived biometabolites could elicit a differentiating potential for NSC *in situ*.

It was recently reported that NSCs transplanted into spinal cord are restricted to glial commitment and NSC-derived neurons are only evident in the brain parenchyma with active neurogenesis[18]. In contrast, this study showed that NSCs derived from embryonic spinal cord were able to differentiate into neurons after transplantation into the cavity of injured spinal cord, which is in line with the reported findings[19]. The scenarios may be attributed to the relatively high number of transplanted NSCs where autocrines and paracrines affect the differentiation, the timelines of transplantation after SCI at which the concentration of inflammatory and toxic cytokines may diminish dramatically and present a more favorable environment for NSCs survival and differentiation, and SC-derived cytokines and extracellular matrices which may ameliorate the adverse local environment and induce the neuronal differentiation of implanted NSCs *in vivo*.

The transplanted cells are not a homogeneous population of NSCs, but a heterogeneous population of NSCs, neural progenitors and mature neurons. Hence, methods for propagation and purification of NSC should be adopted. In this study, the neuronal differentiation was not evident in the arm of rats receiving NSC transplant compared to the other arm of rats transplanted with NSCs and SCs, which excluded the likelihood of mature neurons in the graft.

On the basis of Kaplan-Meier survival rate evaluation, we did not find statistically significant differences in the survival rates of three arms of traumatized rats 56 days after spinal cord injury, implying that the derived cell products have no adverse or life-threatening risks for transplanted rodents and host rats tolerate well with the transplanted cells. The survival rate of the transplanted arms was better than that of the control arm, although no statistically significant difference was observed, suggesting that cell transplantation could prolong the survival of injured rats.

In the fourth week, there was a detectable difference in locomotive function of the hind limbs, in terms of the BBB scores between the control and transplanted groups, which may be due to the amelioration of the local toxic microenvironment by soluble neurotrophins produced by the implanted NSCs or SCs, and remyelination of the spared processes and sprouting to reinnervate the circuitry underneath the traumatic region of the spinal cord. Conversely, it would be unlikely that neuronal

differentiation of the implanted NSCs played an important role in reconnecting the truncated nerve fibers in such a short period of time, since the injured intraspinal cavity disrupting the connection of rostral to caudal stump of the spinal cord remained in the eighth week. There was no significant difference in BBB scores between the two arms of rats receiving co-transplant of NSCs and SC and the arm of rats transplanted with NSCs, which may be due to the small cohort of animals available for analyses. The relatively high mortality observed in this study is in concordance with previous reports[20].

In conclusion, SCs can enhance neuronal differentiation of NSCs *in vitro* and *in vivo*. The novel therapeutic regimen may be used clinical practice to tackle the long-term or permanent locomotor impairments.

REFERENCES

1. Ogawa Y, Sawamoto K, Miyata T, *et al*. (2002). 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* **69**, 925-933.
2. McDonald J W, Liu X Z, Qu Y, *et al*. (1999). Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nat Med* **12**, 1410-1412.
3. Nakamura M, Okano H, Toyama Y, *et al*. (2005). Transplantation of embryonic spinal cord-derived neurospheres support growth of supraspinal projections and functional recovery after spinal cord injury in the neonatal rat. *J Neurosci Res* **81**, 457-468.
4. Xu X M, Zhang S X, Li H, *et al*. (1999). Regrowth of axons into the distal spinal cord through a Schwann cell seeded wini channel implanted into hemisected adult rat spinal cord. *Eur J Neurosci* **11**, 1723-1740.
5. An Y H, Wan H, Zhang Z S, *et al*. (2003). Effect of rat Schwann cell secretion on proliferation and differation of human neural stem cells. *Biomed Environ Sci* **16**, 90-94.
6. Cao Q L, Zhang Y P, Howard R M, *et al*. (2001). Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to a glial lineage. *Exp Neurol* **167**, 48-58.
7. Von Euler M, Seiger A, Sundstrom E (1997). Clip compression injury in the spinal cord: a correlative study of neurological and morphological alterations. *Exp Neurol* **145**, 502-510.
8. Eugen j dolan (1979). A new method for testing the force of clips for aneurysms or experimental spinal cord compression. *J Neurosurg* **51**, 229-233.
9. An Y H (2004). Neural stem cells transplantation improved the neurological function of cerebral ischemic rat. *J Neurochemistry* **88**(supple 1), 35-34.
10. Fu S L, Ma Z W, Yin L, *et al*. (2003). Isolation and cultivation of neural stem cells from the embryonic rat brain and spinal cord. *Sheng Li Xue Bao* **55**, 278-283. (In Chinese)
11. Morrissey T K, Kleitman N, Bunge R P (1991). Isolation and functional characterization of Schwann cells derived from adult peripheral nerve. *J Neurosci* **11**, 2433-2442.
12. Basso D M, Beattie M S, Bresnahan J C (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrau* **12**, 1-21.
13. Represa A, Shimazaki T, Simmonds M, *et al*. (2001). EGF-responsive neural stem cells are a transient population in the developing mouse spinal cord. *Eur J Neurosci* **14**, 452-462.
14. Warf B C, Fok-Seang J, Miller R H (1991). Evidence for the ventral origin of oligodendrocyte precursors in the rat spinal cord. *J Neurosci* **11**, 2477-2488.
15. Chandran S, Kato H, Gerreli D, *et al*. (2003). FGF-dependent generation of oligodendro- cytes by a hedgehog-independent pathway. *Development* **130**, 6599- 6609.
16. Clarkem S R, Shettym A K, Bradley J L, *et al*. (1994). Reactive astrocytes express the embryonic intermediate neurofilament nestin. *Neuroreport* **5**, 1885-1888.
17. Ernst C, Christie B R (2006). The putative neural stem cell marker, nestin, is expressed in heterogeneous cell types in the adult rat neocortex. *Neuroscience* **138**, 183-188.
18. Englund U, Fricker-Gates R A, Lundberg C, *et al*. (2002). Ransplantation of human neural progenitor cells into the neonatal rat brain: extensive migration and differentiation with long-distance axonal projections. *Exp Neurol* **173**, 1-21.
19. Chow S Y, Moul J, Tobias C A, *et al*. (2000). Characterization and intraspinal grafting of EGF/bFGF-dependent neurospheres derived from embryonic rat spinal cord. *Brain Res* **874**, 87-106.
20. Rivlin AS, Tator CH (1978). Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. *Surg Neurol* **10** (1), 38-43.

(*Received September* 14, 2006 *Accepted March* 18, 2007)