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Neural precursor cells differentiated from mouse embryonic stem cells relieve symptomatic motor behavior in a rat model of Parkinson's disease

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Abstract

Pluripotent embryonic stem (ES) cells are the most versatile cells, with the potential to differentiate into all types of cell lineages including neural precursor cells (NPCs), which can be expanded in large numbers for significant periods of time to provide a reliable cell source for transplantation in neurodegenerative disorders such as Parkinson's disease (PD). In the present study, we used the MESPU35 mouse ES cell line, which expresses enhanced green fluorescent protein that enables one to distinguish between transplanted cells and cells of host origin. Embryoid bodies (EBs) were formed and were induced to NPCs in N2 selection medium plus fibronectin. Praxiology and immunohistochemistry methods were used to observe the survival, differentiation, and therapeutic effect of NPCs after grafted into the striatum of PD rats. We found that mouse ESc were differentiated into nestin-positive NPCs 6 days after the EBs formed and cultured in the N2 selection medium. The number of survival NPCs was increased significantly by fibronectin. About $23.76 \pm 2.29\%$ of remaining cells were tyrosine hydroxylase (TH)-positive 12 days after NPCs were cultured in N2 selective medium. The survival rates of NPCs were $2.10 \pm 0.41\%$ and about $90.90 \pm 3.00\%$ of the engrafted NPCs were TH-positive 6 weeks after transplantation into the striatum of PD rats. The rotation of PD rats was relieved 3 weeks after the NPCs transplantation and this effect was kept for at least 6 weeks. It suggests that most of the survival NPCs derived from ES cells differentiated into TH-positive neurons after grafted into the striatum of PD rats, which produces therapeutic effect on PD.

Keywords: Embryonic stem cell; Neural precursor cell; Parkinson's disease; Transplantation

Mammalian embryonic stem (ES) cell lines are derived from the inner cell mass of the blastocyst prior to its implantation in the uterus [1,2]. Clonal ES cell lines have been established for several mammals, including mice and humans [3–7]. ES cells are capable of forming many specialized cell types of the organism, including cells that constitute all three embryonic germ layers, and as such, they are termed pluripotent [8–10]. ES cells are continuously growing stem cell lines of embryonic origin. Their distinguishing features are a capacity to be maintained in culture indefinitely in an undifferentiated state and an ability to develop into multilineage cells [8–10]. Further, stem cells, including ES cells, can respond to environmental cues by differentiating into unique cell types [11,12]. Currently, ES cells have been demonstrated to be differentiated and reconstructed according to the environmental cues after being transplanted into the brain [13,14]. As a result, ES cells are thought to be an optimal cell source for cell-replacement

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therapy. It is expected that the use of ES cells will overcome some of the problems encountered in Parkinson's disease (PD) treatment, such as the difficulty of obtaining sufficient donor cells and the controversial ethical and legal issues raised by the use of human fetal allografts [15,16].

Obviously, ES cells would be candidates for cell replacement therapies for degenerative diseases, but direct transplantation of ES cells may result in teratoma [5,17]. To reduce this risk, several protocols were developed to generate neurons from ES cells including treatments with retinoic acid, ascorbic acid, and BMP-2. However, the reported percentile of cells expressing neuronal markers is usually low and does not exceed 30% [18]. Generation of specific neuronal populations in sufficient quantities for transplantation represents a significant practical problem. Besides, neurons and glial cells are post-mitotic cells and they lost the ability of proliferation when grafted to the brain, and neurons cannot differentiate according to the environmental cues or migrate to the injury areas of brain. Thus, the neural precursor cells (NPCs) with the properties of neural stem cells differentiated from ES cells would be suitable candidates for cell replacement [19]. Transplantation in a rat model of myelin disease shows that the ES cell-derived precursors interact with host neurons and efficiently myelinization around axons in brain and spinal cord [20]. It suggested that ES cells serve as a valuable source of cell type-specific somatic precursors for neural transplantation.

In this work we used the MESPU35 ES cell line [21,22], which expresses enhanced green fluorescent protein (EGFP) that enables one to distinguish between transplanted cells and cells of host origin. Praxiology and immunohistochemistry methods were used to observe the survival, differentiation, and therapeutic effect of NPCs differentiated from the ES cells after grafted in the striatum of PD rats.

Materials and methods

MEF preparation. Mouse embryonic fibroblasts (MEFs) were isolated from 13.5d mouse embryos provided by Animal Breeding Center of The Third Military Medical University (Chongqing, China). MEFs were expanded in Dulbecco's modified Eagle's medium (DMEM) with high glucose (Gibco) supplemented with 10% fetal calf serum (FCS) (Hyclone). MEFs of passages 2–5 were used as feeders. MEF cultures were treated with 10 mg/L Mitomycin C (Sigma) for 60 min to arrest cell division, trypsinized, and plated at $2 \times 10^4/cm^2$ in culture medium overnight. Feeders were washed five times with PBS and then incubated in ES cell medium for at least 1 h before plating ES cells. ES cells plated on top of MEF feeders were cultured at 37 °C in the atmosphere of 5% CO_2 within a humidified tissue culture incubator from Thermo Forma.

ES cell culture. Mouse ES cell line MESPU35, which expresses EGFP that enables one to distinguish between transplanted cells and cells of host origin, was obtained from College of Life Sciences, Peking University (Beijing, China), and cultured according to their instructions [21,22]. Briefly, cells were cultured on MEF feeders in ES cell medium consisting of DMEM with high glucose (Gibco), 15% FCS (Hyclone), 1000 U/ml human leukemia inhibitory factor (hLIF, Chemicon), and 100 μM β-mercaptoethanol (Sigma). ES cells plated on top of MEF feeders were cultured at 37 °C in the atmosphere of 5% CO_2 within a humidified tissue culture incubator from Thermo Forma.

EB differentiation. To induce EB formation, the ES cells were dissociated into a single-cell suspension by 0.25% trypsin and 0.04% EDTA in PBS, and plated onto unattached 70 mm bacteriological dishes at a density of $2–2.5 \times 10^4$ cells/cm^2 in the medium consisting of DMEM with high glucose, 15% FCS. The EBs were formed for 4 days and then plated onto adhesive tissue culture surface precoated with poly-L-ornithine (15 μg/ml, Sigma), fibronectin(10 μg/ml, Sigma), and laminin (5 μg/ml, Sigma) in the medium consisting of DMEM with high glucose, 10% FCS, and 100 μM β-mercaptoethanol. After 24 h of culture, selection of NPCs was initiated by replacing the medium by the serum-free defined culture medium—DMEM/F12 (Gibco) plus N2 supplement (Gibco) and fibronectin (5 μg/ml, Sigma) [23]. After 5–7 days of selection, cell expansion was initiated. The cells were dissociated by 0.25% trypsin/0.04% EDTA, plated on tissue culture plastic or glass coverslips at a concentration of $1.5–2 \times 10^5$ cells/cm^2 in DMEM/F12 (Gibco) medium, and supplemented with 20 ng/ml basic fibroblast growth factor (bFGF) (Sigma), B27 (2%, Gibco), and epidermal growth factor (EGF, human recombinant, 20 ng/ml, Sigma). Before cell plating, dishes and coverslips were precoated with poly-L-ornithine (15 μg/ml) and laminin (5 μg/ml, Sigma). Nestin-positive cells were expanded for 6 days. The medium was changed every 2 days. According to the method of Okabe with modifications [37], NPCs were kept in DMEM/F12 plus N2 supplement and fibronectin (5 μg/ml), but without bFGF for another 7 days to induce DA neurons.

Neural precursor cells detection and quantification. At the end of the selection stage, cells were dispersed, sieved, and cultured on GFR-Matrigel-coated coverslips (1×10^6 cells/coverslip) overnight to allow attachment. Cells were then fixed and immunostained for monoclonal anti-nestin antibody (1:1500, Pharmergen) for 48 h, and then incubated with goat anti-mouse IgG (1:500, DAKO) antibody overnight, and Cy3-ABC (1:500, DAKO) for 2 h at RT for signal detection.To determine the effect of fobronectin on NPCs cell density after EBs were cultured in N2 selective medium, we cultured single EB in 24-well plates. The average number of plated NPCs/single EB was obtained by counting plated 10 EBs of each group at the same time point and cultured in N2 selective medium from 3 to 6 days after plating.

Parkinson's disease model. Adult Sprague–Dawley rats (200–250 g) from Animal Breeding Center of The Third Military Medical University were housed in temperature-controlled conditions under a 14-h light and 10-h dark light-cycle (lights on at 06:00 h) with food and water supplied ad libitum. All experiments were performed in accordance with approved principles of laboratory animal care. Animals were anesthetized with sodium pentobarbital (40 mg/kg in 0.9% saline i.p.) and fixed in stereotaxic apparatus (Shanghai, China). Rats were lesioned with 6-OHDA as described [24,25]. Each rat was given two stereotaxic injection of 2 μl of 6-OHDA (Sigma, 4 μg/μl in 0.2 mg/ml L-ascorbate saline) unilaterally into the left ascending nigrostriatal dopaminergic pathway using 10 μl Hamilton syringe at the following coordinates [26] (in mm with respect to bregma) (i) 2 μl at AP −5.0, L 1.7, V 7.6; (ii) 2 μl at AP −4.6, L 0.9, V 7.5. The injection rate was 1 μl/min over a 5 min period with the help of auto-injector. Additional 5 min were allowed prior to retraction of needle (1 mm/min). Rats injected with 2 μl of 0.2 mg/ml L-ascorbate saline in identical manner were taken as sham operated control. Four weeks post-6-OHDA lesion animals were given 0.5 mg/kg apomorphine (Sigma) injection (in 0.9% saline, i.p.) and rotational scores were observed for 30 min [27]. Only animals exhibiting a mean net ipsilateral rotational score of seven full body turns/minute were chosen for further study.

NPCs transplantation and calculation of survival rates and differentiation ratios. FACS-sorted NPCs (approx. 5×10^6 cells/4μl of differentiation medium [28], $n = 9$) or vehicle medium PBS (sham surgery, 4 μl, $n = 9$) were placed at the two sites in the striatum ipsilateral to the lesion (AP +1.0 mm, L +3.0 mm, V −5.0 mm; AP ±0 mm, L +3.0 mm, V −5.0 mm) at the speed of 1 μl/min. There was no significant difference in rotation speed between the two groups (15.2 ± 2.1 r/min vs. 14.3 ± 1.8 r/min, $P > 0.05$) before transplantation. All NPCs transplant recipients and control group were given cyclosporine A (10 mg/kg p.o., Novartis Pharma) daily. At 3, 4, 5, and 6 weeks post-transplantation, the efficacy of transplantation was assessed in all rats by testing them for rotational behavior and recording it on a video tape after injection of apomorphine (0.5 mg/kg, i.p.).

Transplanted cell counts were done under green fluorescence-field and red fluorescence-field illumination on every fourth section with a microscope connected to a neuron tracing system (NTS; Eutectic). Cells displaying an intense EGFP staining and TH staining, a well-preserved cellular structure, were counted as TH-positive cells differentiated from grafted cells. According to the methods of Höglinger et al. [29], the survival rates of engrafted NPCs in the striatum of PD rats were calculated by comparing the number of NPCs in the grafts with the mean number of NPCs cultured in N2 selective medium of a corresponding culture time. The differentiation ratios of NPCs to TH-positive cells were obtained by calculating the TH-positive cells and EGFP staining cells in the same visual field.

Immunohistochemistry. Animals were transcardially perfused, under terminal barbiturate anesthesia, with 100 ml PBS (pH 7.4) followed by 250 ml of 4% paraformaldehyde (in PBS, pH 7.4) 4 weeks after 6-OHDA injection or 6 weeks after NPCs transplantation. Brains were left in 4% paraformaldehyde overnight, transferred to 25% sucrose until they sank, and brain sections (30 μm) were cut on a Leica Vibratome VT1000 S (Leica Instruments) and after incubating with rabbit polyclonal anti-TH antibody (Chemicon, 1:1500) for 48 h, with biotin-conjugated goat anti-rabbit IgG (1:500, DAKO) overnight, and then incubated with an ABC kit (Vector) [30]. For immunofluorescence staining, sections and coverslips were incubated with rabbit polyclonal anti-TH antibody (Chemicon, 1:1500), and then incubated with goat anti-rabbit IgG (1:500, DAKO) antibody overnight, and Cy3-ABC (1:500, DAKO) for 2 h at RT for signal detection.

Statistical analyses. Rotation speeds are given as means ± SEM, the number of turns in the apomorphine test was compared using nonparametric ANOVA test followed by Dunn's post hoc analysis. Treatment differences were considered significant at $P < 0.05$. The average number of plated NPCs/single EB is given as mean ± SEM. Differences between means were determined by one-way ANOVA followed by Fisher's protected least-significant difference (PLSD) post hoc test. A P value <0.05 was considered statistically significant.

Results

Rotational behavior testing and TH-immunohistochemistry

No apomorphine-induced rotation was recorded 1 week after 6-OHDA injection, while obvious rotation appeared 2 weeks after the 6-OHDA injection and the rats reached the peak rotational rate in the fourth week. TH-immunohistochemistry revealed severe loss of dopaminergic cells in the substantia nigra pars compacta in the PD rats model 4 weeks after 6-OHDA administration. The loss of dopaminergic cells on the lesioned side was significant compared to contralateral side (Figs. 1A and B).

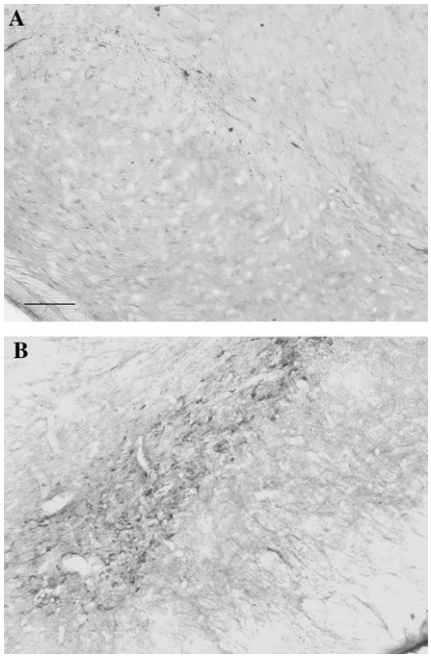
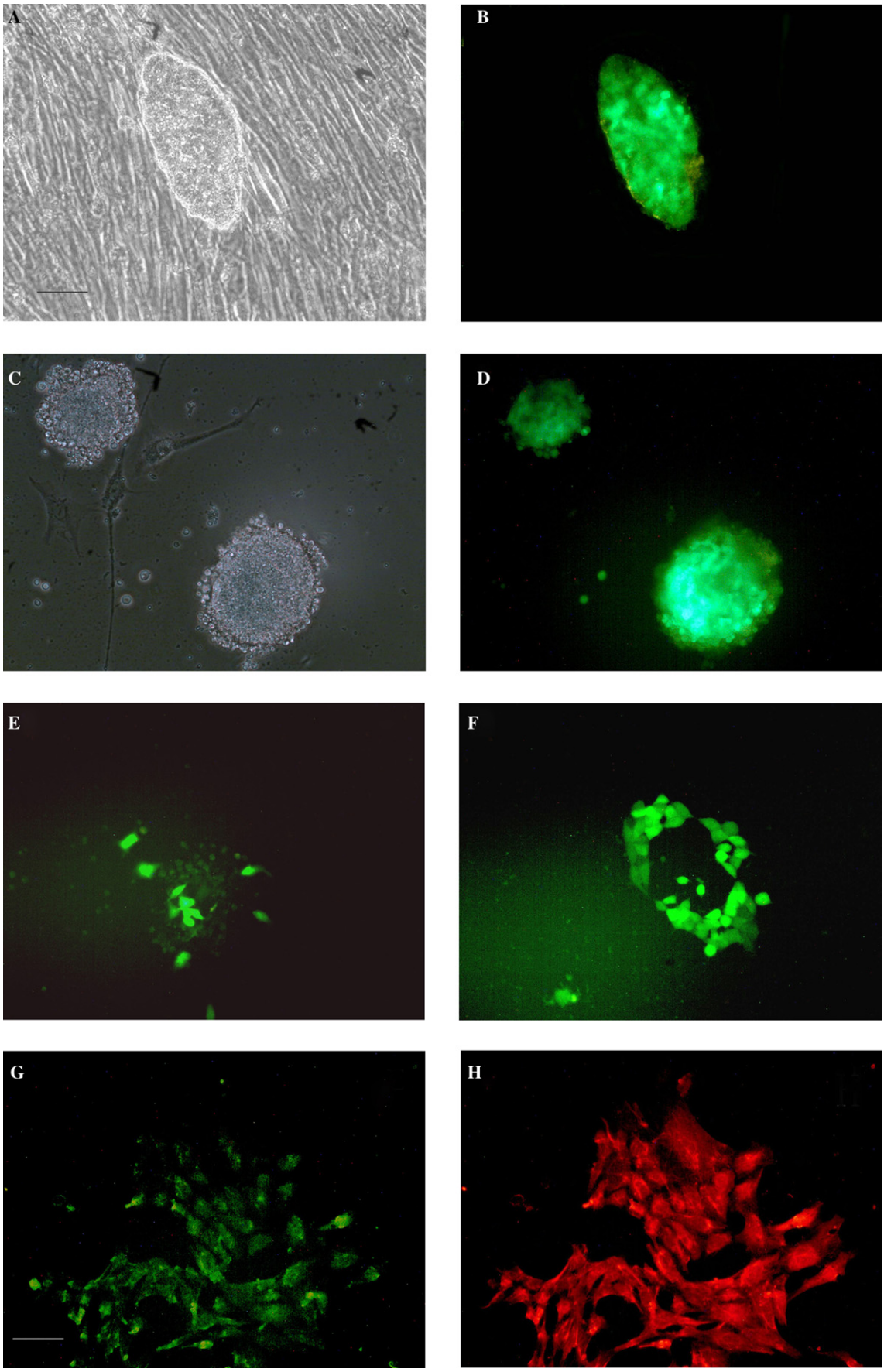


Fig. 1. TH-immunoreactivity in a representative 6-OHDA-treated animal. Sections were photographed from the substantia nigra pars compacta from both the lesioned (A) and the contralateral sides (B) 4 weeks after 6-OHDA administration. Scale bar = 100 μm (A,B).

In vitro differentiation of mouse ES cells to NPCs

We formed EBs from the ES cells expressing EGFP (Figs. 2A and B) by withdrawing LIF and culturing ES cells in 70 mm bacteriological dishes with a non-adhesive substratum. The EBs formed in dishes 4 days later still expressed EGFP (Figs. 2C and D). EBs on the 5th day were collected and plated to a tissue culture dish treated with poly-L-ornithine (15 μg/ml), laminin (5 μg/ml), and fibronectin (10 μg/ml). One day after plating, EBs were attached and proliferating cells spread and expanded. The medium was changed with serum-free DMEM/F12 medium supplement with N2 and fibronectin (5 μg/ml). During the first 72 h in N2 plus fibronectin medium, a large proportion of the cells were detached from the plate and lysed. The remaining cells changed their morphology from tightly packed epithelial cells to small elongated cells. We observed the average number of plated NPCs/EB by counting 10 EBs 3–6 days after selection and found that the fibronectin (5 μg/ml) added in the DMEM/F12 medium supplement with N2 increased the number of survival cells significantly ($P < 0.01$) (Figs. 2E, F, and 3) . The cells cultured in N2 medium plus fibronectin for 96 h still expressed EGFP (Fig. 2G) and were nestin-positive when tested by immunochemistry (Fig. 2H). No color was seen in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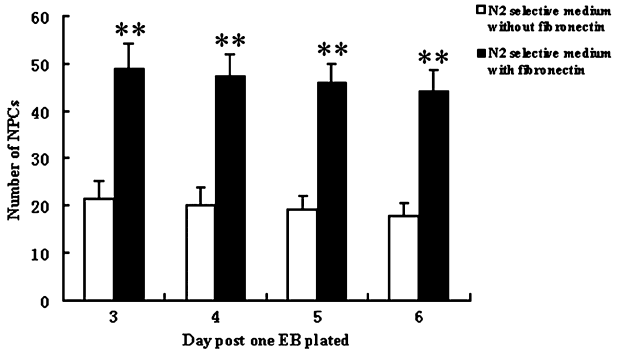

Fig. 3. The number of survival cells in the N2 selective medium (empty bars) and N2 selective medium plus fibronectin (solid bars). Bars represent mean ± SEM ($n = 10$). The asterisk indicates a significant difference from N2 selective medium without fibronectin ($P < 0.01$).

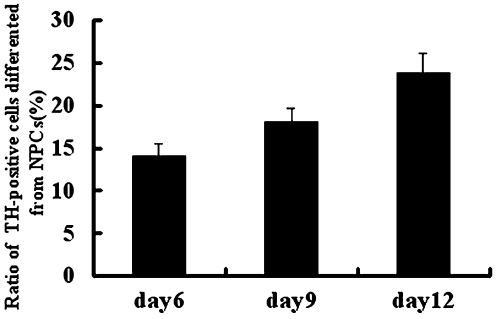

Fig. 4. In vitro differentiation of mouse TH neurons from NPCs. Percentage of TH-positive cells at 6, 9, and 12 days after the NPCs were cultured in the N2 selective medium plus fibronectin. Bars represent mean ± SEM. ($n = 5$).

the negative control, in which no nestin antibody was added. NPCs were kept in DMEM/F12 plus N2 supplement and fibronectin (5 μg/ml), but without bFGF for another 8 days to induce DA neurons. TH-positive cells were detected as early as 6 days after NPCs were cultured in N2 selective medium, about 14.06 ± 1.44% of the plated cells was TH-positive. The differentiation ratio was increased to 23.76 ± 2.29% 12 days after NPCs were cultured in N2 selective medium without bFGF (Fig. 4).

Functional recovery of apomorphine-induced motor asymmetry in a rat model of PD when grafting NPCs

6-OHDA-lesioned animals were selected for transplantation by quantification of rotational behavior in

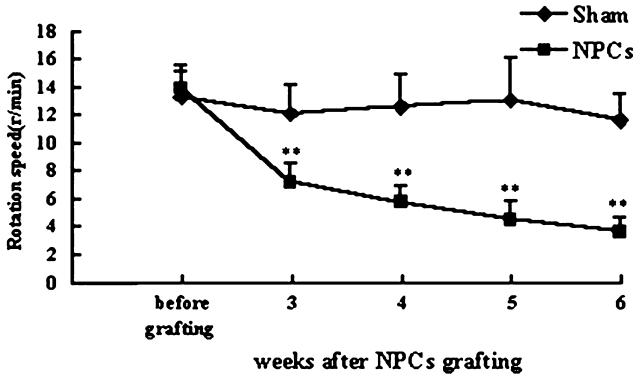

Fig. 5. Rotational behavior in response to apomorphine (0.5 mg/kg) was tested before grafting and at 3, 4, 5, and 6 weeks post-grafting. A significant decrease in rotation speed of apomorphine-induced turning was seen in animals with NPCs grafts in the striatum ($n = 9$) compared with control animals that received sham surgery ($n = 9$). Animals with sham surgery showed no change in rotational score over time ($P > 0.05$). In contrast, animals receiving ES cell-derived NPCs grafts showed a significant reduction in rotations over time ($P < 0.01$).

response to apomorphine. The rotational response to apomorphine was examined at 3, 4, 5, and 6 weeks post-transplantation of ES cell-derived NPCs in the striatum of rats. Animals with NPCs ($n = 9$) showed recovery over time from apomorphine-induced turning behavior, whereas control (sham surgery) animals ($n = 9$) did not. Importantly, animals with NPCs grafted showed a significant decrease in rotation speed from sham surgery values at 3 weeks (14.0 ± 1.6 r/min vs. 7.2 ± 1.42 r/min, $P < 0.01$). And the decrease in rotational scores was gradual and the effect lasted for at least 6 weeks while the experiment was continued (Fig. 5).

NPCs differentiate into dopaminergic-like phenotypes after transplantation to the adult brain

The presence of the implanted NPCs within striatum of PD rats was examined by immunohistochemistry under immunofluorescence microscope 6 weeks after transplantation. In contrast, brain sections of sham-grafted animals showed a complete absence of TH and EGFP immunoreactivity within ipsilateral striatum while strong and deep staining was observed within striatum of NPCs-grafted animals 6 weeks post-grafting in the majority of these transplants. In the transplantation site, the striatum of the PD rats, grafted cells expressed both EGFP (Fig. 6A) and TH (Fig. 6B). The survival rates of

Fig. 2. Enhanced green fluorescent protein (EGFP) expressing mouse embryonic stem cells (MESPU35) and their neural precursor cells (NPCs) differentiation in vitro. (A) Phase morphology of established MESPU35 cell clone. (B) The same cultured MESPU35 cell clone expressed EGFP. (C) Phase morphology of EBs derived from ES cells with light microscopy. (D) The same EBs derived from ES cells expressed EGFP. (E) Cells cultured in the N2 supplement serum-free media for 3 days. (F) Cells cultured in the N2 supplement serum-free media plus fibronectin (5 μg/ml) for 3 days. Showing the effect of fibronectin on the number of survival cells. (G) NPCs cultured 96 h in the N2 serum-free medium expressed EGFP (green). (H) The NPCs are stained for nestin (red). Scale bar = 100 μm (A–F). Scale bar = 25 μm (G–H). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

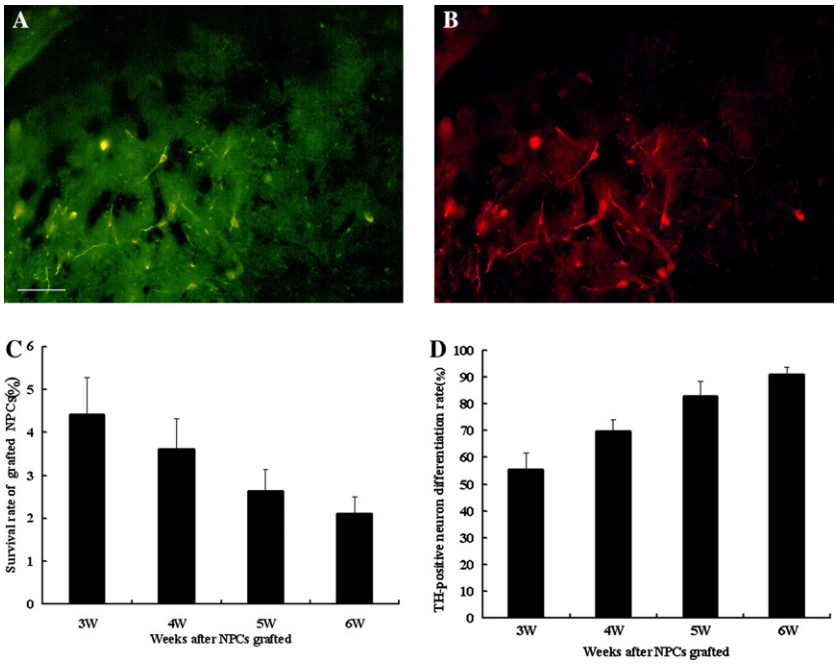

Fig. 6. NPCs differentiate into dopaminergic-like phenotypes 6 weeks after transplantation in the striatum in a rat model of Parkinson's disease (A,B). In the transplantation site, grafted cells expressing both EGFP (A) and TH (B) can be seen in the striatum. Scale bar = 100 μm. (A–B). The survival rates of NPCs 3, 4, 5, and 6 weeks after being transplantated in the striatum of PD rats. Bars represent means ± SEM ($n = 9$) (C). The differentiation ratio to TH-positive cells of the engrafted NPCs 3, 4, 5, and 6 weeks after being transplanted in the striatum of PD rats. Bars represent means ± SEM ($n = 9$) (D).

NPCs following transplantation were calculated by comparing the number of NPCs in the grafts with the mean number of NPCs cultured in N2 selective medium of a corresponding culture time. Three weeks after transplantation, the survival rates of NPCs were $4.41 \pm 0.88\%$ and decreased to $2.10 \pm 0.41\%$ 3 more weeks later (Fig. 6C). About $55.50 \pm 6.13\%$ of the engrafted NPCs express the TH 3 weeks after transplantation and this ratio was increased to $90.90 \pm 3.00\%$ another 3 weeks later (Fig. 6D). Of the nine animals which received NPCs as well as nine sham surgery animals which received vehicle medium PBS, no tumor formation was detected by light microscopic observation (data not shown).

Discussion

In the present study, we used cells from a mouse ES cell-derived population as allografts for a unilateral rat model of Parkinson's disease. We induced mouse ES cells to NPCs, and observed the survival, differentiation, and therapeutic effect of NPCs after transplanted in the striatum of PD rats. The presence of dopamine neurons in the grafts is a critical factor for the improvement of rotational behavior in PD model rats. We demonstrated that some strong and deep staining TH positive cells were founded in the striatum of PD rats and confirmed they were derived from the grafted NPCs. As expected, a significant reduction in rotational behavior was observed 3 weeks after transplantation in all rats which received NPCs grafts and this effect was kept for at least 6 weeks. Further, it showed steady reduction of rotational behavior at every observation point after transplantation. Therefore, we speculated that NPCs from an undifferentiated cell population could be used as grafts for the treatment of PD model rats.

Obviously, ES cells have many characteristics required for an optimal cell source for cell-replacement therapy [31,32]. Evidences have demonstrated that undifferentiated ES cells may develop into a terminally differentiated cell type in implanted sites in the body, however, the high risk of ES cell-derived tumor development cannot be avoided as long as native or insufficiently differentiated lineage-unrestricted ES cells are used as grafts [33–35]. To reduce the risk of teratomas, cell differentiation should take place in vitro and not after transplant into brain. Lee et al. [11] have used a progressive expansion, selection, and differentiation strategy to convert mouse ES cells to a mixed population of mature neurons in tissue culture with 30% having the characteristics of dopamine cells. To increase the fraction of neural cells, Kawasaki et al. [28] continued the differentiation process in neuron-selective media. This strategy of transplantation of ES cell-derived DA neurons reduced the risk of tumor formation activity and should be useful for cellular and molecular studies of DA neurons and for clinical application in the

treatment of Parkinson's disease [36]. However, neurons are post-mitotic cells and lose the ability of proliferation, the survival, and integration ability of neurons is lower than NPCs when grafted to the brain. NPCs are regarded as suitable and practical cell sources from ES cells. The NPCs of high purity from ES cells are good transplantation materials. ES cells can generate proliferating NPCs which can be expanded and differentiate efficiently to synaptically connected neurons and glial cells by serum-free culture [37]. These ES cell-derived NPCs can be used for nervous system repair. Since ES cells can be maintained and expanded in an undifferentiated state [38], it is possible to generate virtually unlimited number of cells for transplantation. A successful transplantation in a rat model of myelin disease with ES cell-derived precursors was acquired. In this model, the following steps are included: ES cells—embryoid bodies—NPCs—neurons/glia [20]. In our experiment, we noted no signs of tumor growth or non-neural tissue in the transplant recipients. Compared with ES cells and mature neural cells, NPCs can be expanded in large numbers for significant periods of time and have plasticity which allows them differentiation according to the environmental cues of recipient brain. NPCs provide a reliable source of cells for transplantation in neurodegenerative disorders such as PD, which is typified by a progressive degeneration of a single type of dopaminergic neurons located within substantia nigra compacta.

It was reported that fibronectin increases the neural differentiation of ES cells [18]. And the fibronectin offers a tool for growing neural stem cells (NSCs) as a monolayer without impairing their differentiation potential and for generating specific differentiated progeny for cell transplantation [39]. We found that the number of survival cells was increased significantly when EBs were plated and cultured in selective medium plus fibronectin. This is in agreement with previous reports of a proliferative effect of fibronectin on EGF-responsive NSCs and adherent mouse neurospheres [40–42].

We found in our research that most of the engrafted NPCs express the dopamine-synthesizing enzymes TH. And these results were repetitive in our experiments and were also reported by Yang et al. [43] in neural stem cell research. Yang found that when an undifferentiated NSC is transplanted into the intact or 6-hydroxydopamine lesioned striatum, cells express markers associated with neuronal but not glial differentiation. And 2–5 weeks post-grafting in the majority of these transplants, nearly all engrafted cells express the dopamine-synthesizing enzymes tyrosine hydroxylase and aromatic L-amino decarboxylase. Our results confirmed that the adult brain contains intrinsic cues sufficient to direct the specific expression of dopaminergic traits in immature multipotential NPCs. In our research, only $2.10 \pm 0.41\%$ grafted NPCs survived. However, in rat models of Parkinson disease, survival of as few as 200–500 dopaminergic neurons can reverse Parkinson symptoms [44]. Considering the large amount of NPCs we transplanted and high differentiation ratio of the survival cells, $2.10 \pm 0.41\%$ of the survival cells could have a substantial impact on animal behavior. Of course, more cells the better.

NPCs can replace damaged neurons and glia, and produce therapeutic effect, however, functional improvements of stem cell transplantation were often a result of stem cell-induced self-repair and neuroprotection, rather than cell replacement [45]. Our results demonstrated that the grafted NPCs differentiated into DA-like neurons which may integrat into the host CNS. Further research is necessary to test the electrophysiological characters of the grafted DA-like neurons.

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