

# Leukemia inhibitory factor promotes nestin-positive cells, and increases gp130 levels in the Parkinson disease mouse model of 6-hydroxydopamine

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## ABSTRACT

**الأهداف:** التحقيق من دور عامل مثبط اللوكيميا (LIF) في نموذج الفأر من 6-OHDA، وعرض كيفية تحسين أعراض عامل مثبط اللوكيميا لمرض باركنسون.

**الطريقة:** أجريت هذه الدراسة في مختبر علم المناعة والالتهابات، مركز بحوث العلوم الطبية الأساسية، جامعة تيانجين الطبية، وتيانجين، الصين، خلال الفترة أبريل 2008م ويناير 2010م. تم تقسيم 72 فأر إلى مجموعة الشاهد (CON)، ومجموعة خادعة (SHA) مجموعة المحلول الملحي (NS)، ومجموعة مثبط اللوكيميا (LIF) عدد=18 في كل مجموعة. تم حقن 6-OHDA في الفأر منتصف المخطط الأيسر لبناء 6-OHDA في نموذج الفأر مرض باركنسون PD. وتلقى كل من الفأر 10 ملغرام من 6-OHDA، وبعد ساعتين تم إطلاق LIF أو كلوريد الصوديوم في CSF عن طريق قسطرة مضخة الأزميوم على التوالي في مجموعة عامل مثبط اللوكيميا ومجموعة مرض باركنسون. واستمر العلاج لمدة 3 أسابيع، وجرى تقييم وظائف المحرك من الفئران في اليوم الأخير من كل أسبوع (7، 14، 21 يوماً)، وتحسب عدد الخلايا الجذعية العصبية الذاتية في كل مجموعة من الفئران باستخدام فحوصات المناعي، والكشف عن مستوى gp130 باستخدام تحليل لطخة.

**النتائج:** بعد تسريب السائل المخي الشوكي من عامل مثبط اللوكيميا، لاحظنا زيادة في عدد الخلايا الجذعية العصبية الذاتية في مخ الفأر، كما تمت زيادة التعبير عن المكونات الرئيسية لمجمع مستقبلات عامل مثبط اللوكيميا، gp130، وفي 14، 21 يوم. كما حسن عامل مثبط اللوكيميا LIF من وظيفة محرك نموذج الفأر في مرض باركنسون.

**خاتمة:** تشير هذه النتائج إلى إمكانية إدارة عامل مثبط اللوكيميا كوسيلة علاجية لمرض باركنسون.

**Objective:** To investigate the roles of leukemia inhibitory factor (LIF) in a 6-hydroxydopamine (6-OHDA) mouse model of Parkinson disease (PD), and explore how the LIF improves PD symptoms.

**Methods:** This study was performed in Tianjin Medical University, Tianjin, China, between April

2008 and January 2010. Seventy-two mice were allocated into a control group (CON), sham operation group (SHA), physiological saline (NS) treatment group (PD), and LIF treatment group (LIF), n=18 for each group. The 6-OHDA was injected into the mice's left mid-striatum to build a 6-OHDA model of PD. The LIF or NS was slowly released into the CSF through the ALZET osmotic pump catheters duct, in the LIF or NS treated groups. The whole treatment lasted 3 weeks, and the motor functions of the mice were assessed on the seventh, fourteenth, and twenty-first day during the treatment. The nestin-positive cells in the mice were counted by immunofluorescence assays, and the level of gp130 was detected with western blot analysis.

**Results:** After CSF infusion in the LIF-treated group, we observed an increased number of nestin-positive cells in the mice's brains. The expression of a major component of the LIF receptor complex, gp130, was also increased. In the fourteenth and twenty-first day time periods; LIF treatment continuously improved the motor functions of the mouse model of PD.

**Conclusion:** These results suggest the potential of LIF administration as a therapeutic method for PD.

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Parkinson disease (PD) is a neurodegenerative disorder affecting millions of people in the aging population. Patients with PD show many movement problems, which have negative impacts on their life quality.<sup>1</sup> The development of PD in patients involves both genetic and environmental factors, and the exact etiology of this disease remains unclear.<sup>2</sup> Currently, therapies for PD patients focus on symptomatic management, but with no curative and preventive treatments. Levodopa (L-dopa) is currently the most commonly used medication for PD, but this treatment has some side effects and motor defect complications.<sup>3</sup> As a neurodegenerative disorder, PD is characterized by the progressive loss of dopaminergic neurons in the substantia nigra. Cell-based neurogenic therapeutic methods are considered to be potential treatments for PD.<sup>4</sup> One approach involving cell-based therapy is the transplantation of exogenous stem cells. Although clinical trials yielded positive results in some patients, this treatment was not always beneficial.<sup>5,6</sup> Graft-induced dyskinesias developed in some patients, most likely due to the impure composition of the graft and the incomplete reinnervation of the striatum. Since this approach proved to be less efficient than deep brain stimulation, transplantations have not been carried out in patients with PD in recent years.<sup>7</sup>

Another approach using cell-based therapy depends on the endogenous neural stem cells (ENSCs). The ENSCs have been identified in the adult brain in the subventricular zone (SVZ) of the lateral ventricles, the subgranular layer (SGL) of the dentate gyrus as well as other regions.<sup>8</sup> Self-renewal and the migration of ENSCs may regenerate damaged neurons in the CNS. The successful repair of damaged neurons depends on the correct regulation of ENSCs proliferation and differentiation. In the partial progressive 6-hydroxydopamine (6-OHDA) lesion model of PD, increased SVZ- and midbrain-derived progenitor cell proliferation and astrogenesis were observed in the mouse brain.<sup>9</sup>

Previous research found that the administration of LIF caused a statistically significant improvement in the number and density of nestin-positive NSCs in the LIF-treated group compared with the untreated PD group, and nestin-positive NSCs were rarely observed in the control, and control + LIF groups in the brain

of the PD mouse model.<sup>10</sup> However, it has not been concluded whether the treatment of LIF can improve PD symptoms by increasing the number of nestin-positive cells in the brain in the mouse model of PD. Therefore, we aimed to investigate the roles of LIF in a 6-OHDA mouse model of PD, and explore how the LIF improves PD symptoms.

**Methods.** This study was performed from April 2008 to January 2010. The C57BL mice, at the age of 8 weeks, were purchased from Better Biotechnology Co., Ltd., (Nanjing, China). The animals were housed and fed in a specific pathogen-free animal facility at the Experimental Animal Center of Tianjin Medical University, Tianjin, China and kept on a 12-hour light-dark cycle. The experiments were conducted in accordance with the guidelines for animal care and were approved by the Animal Ethics Committee of Tianjin Medical University.

**Mice, 6-OHDA PD model development, and LIF treatment.** Seventy-two C57BL mice aged 8 weeks were allocated to a control group (CON, n=18), a sham operation group (SHA, n=18), a PD mouse model with physiological saline (NS) treatment group (PD, n=18), and a PD mouse model with LIF treatment group (LIF, n=18). The mice in the SHA, LIF, and PD groups were anesthetized by intraperitoneal injection of pentobarbital 60 mg/Kg (Sigma-Aldrich, St. Louis, MO, USA). After shaving the hair and disinfecting the skin with 10% povidone-iodine and 75% ethanol, these mice were placed in a stereotactic frame. Then, 6-hydroxydopamine (6-OHDA) (Sigma-Aldrich, St. Louis, MO, USA) was injected into the left mid-striatum (anterior 0.4 mm, lateral 1.8 mm, ventral 3.5 mm), being measured from the bregma and the surface of the skull. The dopamine-innervated striatum was wounded unilaterally. Each mouse received 10  $\mu$ g of 6-OHDA dissolved in 2  $\mu$ l of physiological saline containing 0.02% ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA). A 22-gauge 10  $\mu$ l micro syringe was used to infuse the solution with a velocity of 0.1  $\mu$ l per minute. The syringe stayed in the injection position for 5 minutes after the infusion. Two hours later, ALZET Osmotic pump catheters (model 2002; Alzet, Cupertino, CA) were implanted in the mice of the LIF and PD groups. Mice in the SHA group were subjected to the implant operation without receiving the implanted osmotic pump. The pump was implanted into the dorsal subcutaneous space between the shoulder blades of each mouse. A caudally cannula was threaded subcutaneously and inserted into the subarachnoid space at the L5 level. The LIF (25  $\mu$ g/kg/d)<sup>10</sup> or physiological

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saline was slowly, continuously intrathecally delivered with chronically implanted spinal catheters and Alzet osmotic pumps in the LIF and PD groups. The treatment lasted for 3 weeks, and the motor functions of the mice were assessed on the last day of every week, namely, the seventh, fourteen, and twenty-first day of the treatment period. To confirm the 6-OHDA lesion mouse model of PD, a week after the surgery, the mice were subcutaneously injected with morphine (apomorphine, Sigma-Aldrich, St. Louis, MO, USA, 0.5 mg/Kg). Most of the 6-OHDA mice turned to the right (7 turns/minutes), which meant the model was successful. We assessed the mice once a week and found that the symptoms were stable in all mice.

**Motor function tests.** The motor functions of the experimental mice were detected by 3 different tests. In order to obtain objective results, 3 motor function tests were completed in this study. Three investigators who were blinded to each study group performed these tests. The scores resulted from averaging the scores assessed by those investigators. (1) Rotarod test: Performance was defined as the duration each mouse was held in the rotary shaft (3.6-cm diameter; speed of rotating, 16 rpm) without falling down, up to 180 seconds. Then, data were averaged from 3 experiments. (2) Bar grabbing test: To test the capacity of reflexive grabbing of a wooden bar (5-mm-diameter) in response to the touching of a mouse's right hind foot. Bar grabbing test was monitored in different groups. The following scales were used in this test: 0 points, no response; one point, curl foot without grabbing; 2 points, weak grabbing without pushing response; 3 points, moderate grabbing and pushing response; 4 points, strong grabbing and pushing response as a normal mouse.<sup>11</sup> (3) Tremor analysis: The degrees of tremor on the mouse limbs were assessed as: 1, no tremor; 2, light tremor without restricted action; 3, interrupted tremor with action gently restricted; 4, frequent tremor with rigidity and restricted action; 5, continual tremor and action inability.

**Western Blot analysis.** After the motor function tests, the mice were terminated by decapitation (Sigma-Aldrich, St. Louis, MO, USA). The left striatum was mechanically minced in one ml buffer, containing the phosphatase and protease inhibitors of phenylmethanesulfonyl fluoride (PMSF) and aprotinin (Sigma-Aldrich, St. Louis, MO, USA). Soluble protein was quantified by the Bradford protein quantitative method (Qingdao MDBio Biotech Co. Ltd., Qingdao, China). Fifty  $\mu$ g of protein was separated on SDS-PAGE gels and then transferred onto nitrocellulose membranes (Roche, Basel, Switzerland). After blocking in 5% milk

diluted in 0.05% Tween-20 in phosphate buffered saline (PBST), the membranes were incubated overnight at 4°C with gp130 (1:500) and  $\beta$ -actin (1:1000) antibodies (Cell Signaling Technology, Boston, USA) diluted in 1% PBST. After washing with PBST, the blot was incubated for one hour at room temperature with HRP-conjugated goat anti-mouse IgG (Cell Signaling Technology, Boston, USA) (1:1000) diluted in 1% PBST. The membrane was then washed and incubated for 3 minutes at room temperature with an ECL Chemiluminescence kit (Millipore, Billerica, MA, USA). Images were obtained and quantitatively analyzed using the BandsScan software.

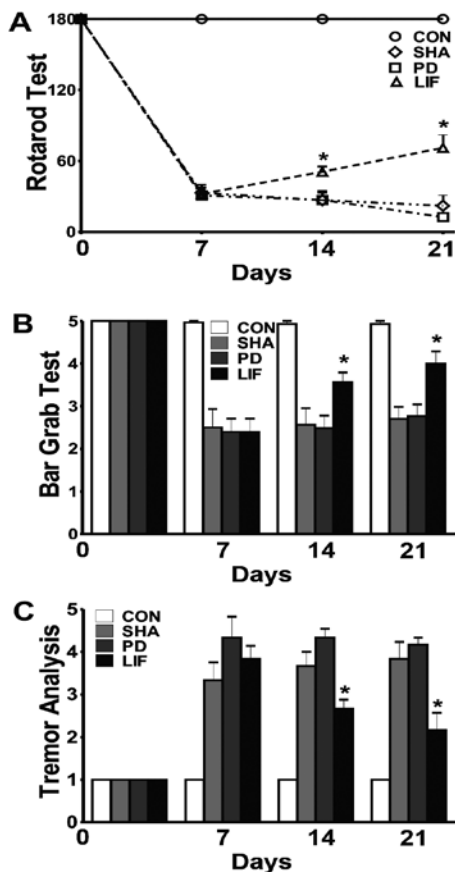
**Immunofluorescence.** After 3 weeks of treatment, 6 mice from each group were terminated by lethal intraperitoneal injection with 200 mg/kg of sodium pentobarbitone (Sigma-Aldrich, St. Louis, MO, USA) and transcardially perfused with 0.1 M phosphate buffered saline (PBS), followed by 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA). The brains were removed with aseptic techniques. The left striatum from each mouse was isolated and immersed in 30% sucrose (Sigma-Aldrich, St. Louis, MO, USA) overnight at 4°C. The tissues were sectioned coronally at 10  $\mu$ m and mounted on super frost coated slides. After blocking with 5% mouse serum, these sections were incubated with an anti-nestin monoclonal antibody (Millipore, Billerica, Massachusetts, USA) overnight at 4°C and then labeled with a monoclonal antibody labeling kit (Alexa Fluor<sup>®</sup> 488, Molecular Probes, Invitrogen, Oregon, USA). All antibodies were diluted in PBS containing 5% mouse serum in the presence of 0.3% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA). The number of nestin-positive cells was counted under fluorescence microscopy using green color illumination with a confocal counting system. The images of nestin-positive cells in the left striatum were merged by the Spot software (Diagnostic Instrument, Inc., Sterling Heights, MI, USA). All photos were reviewed and verified by 2 radiologists.

**Statistical analysis.** A 2-way ANOVA with Tukey's posthoc-test was performed using the Prism software, version 5.0 (GraphPad Software Inc., San Diego, CA, USA). Differences were considered statistically significant at  $p < 0.05$ .

**Results. Leukemia inhibitory factor administration improves motor function in the 6-OHDA PD mouse.** 6-hydroxydopamine has the same catecholaminergic transportation system as dopamine and norepinephrine. Treatment with 6-OHDA causes specific degeneration of catecholaminergic neurons.<sup>12</sup> The degeneration

induced by 6-OHDA has been proven to be correlated with oxidative stress-related mechanisms. 6-hydroxydopamine can be stereotactically targeted into the substantia nigra, the nigrostriatal tract or the striatum to mimic most of the clinical and pathological features of PD.<sup>13</sup> The 6-OHDA mouse model had been widely used to evaluate the management efficacy of PD. To determine the effects of LIF infusion on the 6-OHDA PD mouse model, we detected motor function in different groups of experimental mice using the rotarod test, the bar grabbing test, and tremor analysis.

As shown in Figure 1A, prior to lesion (0 day), all mice could balance on the rotarod for 180 seconds.



**Figure 1** - Leukemia inhibitory factor (LIF) improves motor function in the 6-hydroxydopamine (6-OHDA) Parkinson disease (PD) mouse model. Compared with SHA and PD groups: A) The results of Rotarod test, the mice in the LIF treatment group showed progress on the fourteenth ( $p=0.004$ ), and twenty-first day ( $p=0.001$ ). B) The results of the bar grab test, the mice in the LIF treatment group showed progress on the fourteenth ( $p=0.001$ ), and twenty-first day ( $p=0.001$ ). C) The results of tremor analysis, the mice in the LIF treatment group show progress on the fourteenth ( $p=0.008$ ), and twenty-first day ( $p=0.012$ ).

After operation, the balancing ability of the mice in the 3 operated groups continued to degrade in the rotarod test. Compared with the CON group, the balance time in the SHA group ( $33.00 \pm 16.67$ s on the seventh day,  $26.83 \pm 18.78$ s on the fourteenth day, and  $22.17 \pm 21.96$ s on the twenty-first day), in PD group ( $30.83 \pm 15.94$ s on the seventh day,  $27.00 \pm 14.97$ s on the fourteenth day,  $12.67 \pm 10.01$ s on the twenty-first day), and in the LIF group ( $32.17 \pm 13.53$ s on the seventh day,  $50.83 \pm 10.68$ s on the fourteenth day,  $71.00 \pm 26.85$ s on the twenty-first day) significantly decreased ( $p=0.012$ ). In contrast, although the LIF group presented with a balancing defect that was similar to that of the SHA and PD group on the seventh day ( $p=0.833$ ), significant improvement was observed on the fourteenth day ( $p=0.004$ ) and the twenty-first day ( $p=0.001$ ).

In the bar grabbing test (Figure 1B), full scores were observed for each group prior to lesion. After operation, the bar grabbing scores of mice in the 3 operated groups significantly decreased. Compared with CON group, the scores of SHA group ( $2.50 \pm 1.06$  on the seventh day,  $2.57 \pm 0.95$  on the fourteenth day,  $2.70 \pm 0.70$  on the twenty-first day), in PD group ( $2.40 \pm 0.76$  on the seventh day,  $2.48 \pm 0.73$  on the fourteenth day,  $2.77 \pm 0.67$  on the twenty-first day), and in LIF group ( $2.45 \pm 1.02$  on the seventh day,  $3.57 \pm 0.55$  on the fourteenth day,  $4.00 \pm 0.70$  on the twenty-first day) significantly decreased ( $p=0.001$ ). A similar defect in the bar grabbing test was detected in the LIF group on the seventh day ( $p=0.630$ ). However, an improvement in the bar grabbing test score was observed in this group on the fourteenth day ( $p=0.001$ ), and further improvement was shown on the twenty-first day ( $p=0.001$ ) in the LIF group.

For the tremor analysis (Figure 1C), no tremor was detected in any of the mice prior to lesion. After operation, tremor phenotype started to emerge in the 3 operated groups. Compared with CON group, the tremor scores in SHA group ( $3.33 \pm 1.03$  on the seventh day,  $3.67 \pm 0.82$  on the fourteenth day,  $3.83 \pm 0.98$  on the twenty-first day), in the PD group ( $4.33 \pm 1.21$  on the seventh day,  $4.33 \pm 0.56$  on the fourteenth day,  $4.16 \pm 0.41$  on the twenty-first day), and in LIF group ( $3.83 \pm 0.75$  on the seventh day,  $3.83 \pm 0.75$  on the fourteenth day,  $2.17 \pm 0.98$  on the twenty-first day) significantly increased ( $p=0.001$ ). Compared with SHA and PD groups, the tremor scores in LIF group did not show significant difference on the seventh day ( $p=0.430$ ), but they significantly improved on the fourteenth day ( $p=0.008$ ), and the twenty-first day ( $p=0.012$ ).

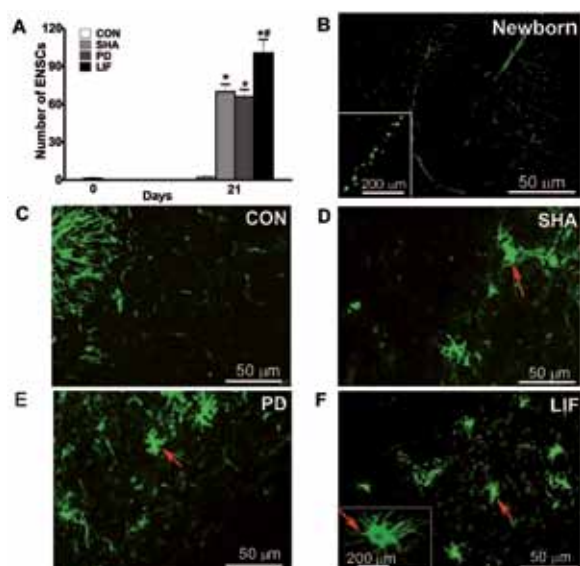
*The LIF treatment increases nestin-positive cells in the brain of the PD mouse.* Nestin, type VI intermediate

filament protein, is a marker of undifferentiated endogenous neural precursor cells (NPCs). To determine whether the improvement in the motor function of the LIF-treated mice was associated with an increase in the number of NPCs, we counted nestin-positive cells in each group of mice using immunofluorescence assays. As shown in Figure 2A, nestin-positive cells were rarely observed prior to the lesion in all groups. Additionally, few nestin-positive cells were detected in the CON ( $2.00 \pm 0.63$ ) group on the twenty-first day (Figures 2A, & 2C). An increased number of nestin-positive cells were observed in mice of the SHA group ( $69.83 \pm 14.54$ ), PD group ( $66.00 \pm 13.65$ ) and LIF group ( $100.50 \pm 26.23$ ) on the twenty-first day ( $p=0.001$ ). However, compared with the SHA and PD groups, a more significant increase in the number of nestin-positive cells was observed in the LIF group ( $p=0.001$ ).

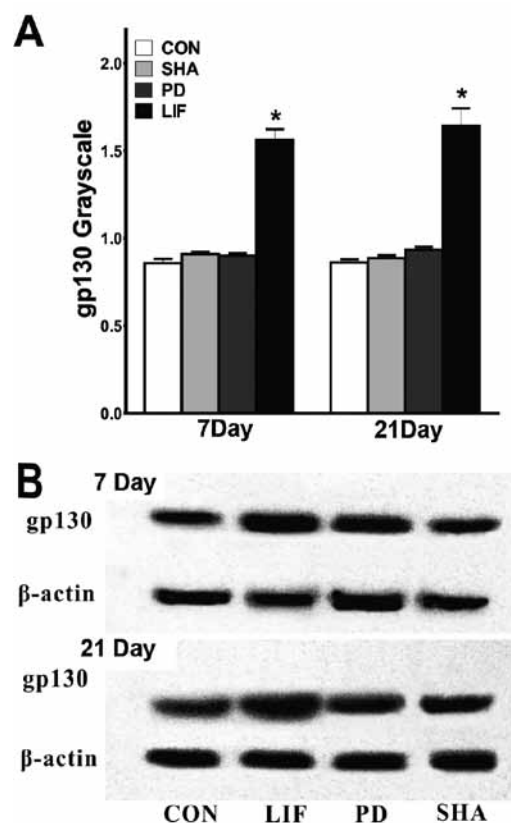
We also tested nestin-positive cells in the brain of newborn mice. The nestin-positive cells were detected

in the hippocampus of a mouse that was 3 days post-natal as shown in Figure 2B. Figures 2C-2F show the distribution of nestin-positive cells in the section of the mouse brain from each group. The nestin-positive cells were rarely observed in the mouse brain from the CON group (Figure 2C). The number of nestin-positive cells was largest in the mice from the LIF group (Figure 2F).

*The LIF increases the expression of gp130 in PD mouse brain.* The LIF signal is transferred through a heterodimeric complex on the cell surface containing LIF receptor (LIFR) and glycoprotein 130 (gp130). We detected the level of gp130 in the LIF-treated mice using western blot analysis (Figure 3). Quantitative analysis (Figure 3A) showed that the gp130 protein level in the CON group was  $0.858 \pm 0.058$  on the seventh day and  $0.861 \pm 0.043$  on the twenty-first day. A similar level of gp130 was detected in both the SHA ( $0.91 \pm 0.034$  on the seventh day, and  $0.89 \pm 0.038$  on the twenty-first day) and the PD group ( $0.90 \pm 0.03$  on the



**Figure 2** - Leukemia inhibitory factor (LIF) treatment increases nestin-positive cells in the brain of Parkinson disease (PD) mouse model. Histogram A) The numbers of nestin-positive cells in the brain of the control group, sham (SHA) group, PD group, and LIF treatment group at the first, and twenty-first day. The nestin-positive cells were markedly increased in the SHA, and PD group, and the increase was more significant in the LIF treatment group on the twenty-first day ( $p=0.001$ ). There is a 152.3% increase in the number of nestin-positive cells in the LIF treatment group compared with the PD group ( $p=0.001$ ). Photomicrograph B) shows nestin-positive cells in hippocampus of a mouse at post-natal day 3, a higher magnification view of nestin-positive cells is shown in the insert of the panel. Photomicrograph C, D, E, & F) shows the distribution of nestin-positive cells in the section of the mouse brain from each group. A higher magnification view of nestin-positive cells is shown in the insert of the panel F).\*,# -  $p < 0.05$



**Figure 3** - The expression of gp130 was significantly higher in the leukemia inhibitory factor (LIF) treatment group. In the LIF treatment group, increased expression of gp130 was detected on the seventh day ( $1.56 \pm 0.14$ ,  $p=0.001$ ) and the twenty-first day ( $1.64 \pm 0.247$ ,  $p=0.001$ ).

seventh day and  $0.934 \pm 0.04$  on the twenty-first day),  $p=0.146$ . However, compared with other groups, the increased expression of gp130 was detected in the LIF group ( $1.56 \pm 0.14$  on the seventh day,  $p=0.001$ , and  $1.64 \pm 0.247$  on the twenty-first day,  $p=0.001$ ).

**Discussion.** A number of neurotrophic factors play important roles in CNS development;<sup>14</sup> these factors include LIF, which is a member of the IL-6 subfamily of cytokines. The LIF has been reported to promote the self-renewal of mouse embryonic stem (ES) cells and the long-term growth of embryonic human neural stem cells (NSCs) *in vitro*.<sup>15</sup> Leukemia inhibitory factor is also involved in the induction of NSCs into definitive NSCs.<sup>16</sup> The over-expression of LIF *in vivo* promotes NSC self-renewal and inhibits differentiation, resulting in the expansion of the NSC pool.<sup>17</sup> *In vitro* treatment with LIF stimulates the differentiation of NSCs into tyrosine hydroxylase-positive neural precursor cells to be used in transplantation treatment of PD.<sup>18</sup> The levels of LIF and LIF receptor have also been reported to be increased in the brains of PD patients.<sup>19</sup>

Leukemia inhibitory factor can act as a neuroprotective and neurotrophic factor in nervous system injury. In a low-dose pilocarpine injury model, LIF knockout mice showed reduced astrocyte and microglial activation in response to injury, indicating that LIF was required for normal glial responses to brain damage.<sup>20</sup> In the case of optic nerve injury, LIF functions as a cofactor with ciliary neurotrophic factor in lens injury-mediated regeneration and neuroprotection of retinal ganglion cells.<sup>21</sup> The potential of LIF as a therapeutic factor in several neuron system diseases has also been studied. For example, LIF expression was found to be increased in CSF in multiple sclerosis patients.<sup>22</sup> In experimental autoimmune encephalomyelitis mouse model, LIF inhibits cuprizone-induced demyelination and oligodendrocyte loss.<sup>23</sup> Treatment with LIF could delay development of motor impairment in the SOD1 G93A murine model of amyotrophic lateral sclerosis.<sup>24</sup>

In this study, we investigated the therapeutic effects of LIF for PD in the 6-OHDA mouse model. We first investigated the effects of LIF to improve motor functions in 6-OHDA mice. Motor functions were detected using 3 different tests. The rotarod test is a passive movement test for cooperative activity and stamina, but its results are objective. The bar grabbing test is used to quantify a mouse's response to stimulation, but its results can be affected by the cooperation of the tested mouse in the experiments. Tremor analysis is used to observe a status change when a mouse is at rest; thus, this test is not affected by the mouse's cooperation, fatigue, or stamina.

The results of both the bar grabbing test and the tremor analysis were scaled at relatively objective levels. We combined the 3 tests results to more accurately quantify any changes in motor functions. The LIF was injected into the CSF through an ALZET osmotic pump. The implantation of the ALZET osmotic pump itself had no influence on motor functions. As shown by Figure 1, all results from the 3 tests showed that after LIF administration, the motor functions in the PD mouse model improved continuously in both the second and the third week when compared with that of the SHA and PD groups (Figure 1). Our results suggested that LIF administration may be an effective therapeutic method for PD. In our study, after 3 weeks, the motor functions of the LIF-treated PD mice were still lower compared with those of the control group. Further study over a longer time period may help to determine the eventual motor function recovery.

Secondly, we investigated the number of nestin-positive cells in mouse brain from each group by immunofluorescence. Nestin, as a marker of undifferentiated NPCs, is expressed mostly in dividing nerve cells where they are implicated in the radial growth of the axon. After differentiation, nestin is down regulated and replaced by tissue-specific intermediate filament proteins. During neurogenesis and gliogenesis, nestin is replaced by cell type-specific intermediate filaments; for example, neurofilaments and glial fibrillary acidic protein. Nestin is also expressed by reactive astrocytes after injury, which is transient, and decreasing to near baseline level on the seventh day after injury.<sup>25</sup> Our results showed that nestin-positive cells were barely found in the brain of the PD and LIF-treated mice on the seventh day (data not shown), but an increased number (152.3%) of nestin-positive cells were found in the brain of LIF-treated mice on the twenty-first day (Figure 2). This result is consistent with our previous results.<sup>10</sup> So these new cells might be neural precursor cells, which are associated with the role of LIF. We presume that LIF could improve the motor functions of PD mice model by increasing the numbers of nestin-positive neural precursor cells. Moreover, LIF could also act as an activator of astrocytes,<sup>20</sup> exert an immune-modulatory action, and increase oligodendrocyte or neuron survival,<sup>26</sup> these may contribute to the improvement of the motor functions in LIF-treated mice in this study.

Glycoprotein 130, which is a major component of the LIF receptor complex, plays an important role in LIF signal transduction. The binding of LIF to the gp130/LIFR complex activates several signaling pathways that regulate the differentiation and proliferation of NPCs in

vitro, including the Janus kinase/signal transducer and activator of transcription pathway, the mitogen-activated protein kinase pathway and the phosphoinositide 3-kinase/Akt signaling pathway.<sup>17</sup> Glycoprotein 130 has been shown to be the major factor in the activated gp130/LIFR complex.<sup>14</sup> Glycoprotein 130 has been shown to stimulate and maintain stem cells; this function is related to the Notch-1 signaling pathway and the phosphatidylinositol 3 kinase-Akt pathway.<sup>14</sup> Glycoprotein 130 signaling has been suggested to upregulate Notch-1 expression and to activate the self-renewal of neural stem cells in mice.<sup>27</sup> Glycoprotein 130 signaling has also been shown to support ENSC survival via activation of the phosphatidylinositol 3 kinase-Akt pathway.<sup>28</sup>

In our study, we also detected the expression of gp130 in the mouse brain from each group. After LIF administration, the gp130 protein and mRNA expression levels in the 6-OHDA mouse model were higher than those in other groups on the seventh and twenty-first day. The increased expression of gp130 was observed after the administration of LIF in PD mice, which was consistent with the observed improvement in the motor function tests. These results demonstrated that gp130 was involved in the promotion of nestin-positive cells proliferation by LIF in the brain of the 6-OHDA mouse.

In summary, our studies provide evidence that LIF could improve motor functions in the PD mouse model by enhancing the numbers of nestin-positive cells and the level of the gp130 protein. This finding suggests that LIF may be used as a therapeutic method to treat PD patients.

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