Overexpression of lentivirus-mediated glial cell line-derived neurotrophic factor in bone marrow stromal cells and its neuroprotection for the PC12 cells damaged by lactacystin

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Abstract: Objective To construct recombinant lentiviral vectors for gene delivery of the glial cell line-derived neurotrophic factor (GDNF), and evaluate the neuroprotective effect of GDNF on lactacystin-damaged PC12 cells by transfecting it into bone marrow stromal cells (BMSCs). **Methods** pLenti6/V5-GDNF plasmid was set up by double restriction enzyme digestion and ligation, and then the plasmid was transformed into Top10 cells. Purified pLenti6/V5-GDNF plasmids from the positive clones and the packaging mixture were cotransfected to the 293FT packaging cell line by Lipofectamine2000 to produce lentivirus, then the concentrated virus was transduced to BMSCs. Overexpression of GDNF in BMSCs was tested by RT-PCR, ELISA and immunocytochemistry, and its neuroprotection for lactacystin-damaged PC12 cells was evaluated by MTT assay. **Results** Virus stock of GDNF was harvested with the titer of 5.6×10^5 TU/mL. After transduction, GDNF-BMSCs successfully secreted GDNF to supernatant with higher concentration (800 pg/mL) than BMSCs did (less than 100 pg/mL). The supernatant of GDNF-BMSCs could significantly alleviate the damage of PC12 cells induced by lactacystin (10 μ mol/L). **Conclusion** Overexpression of lentivirus-mediated GDNF in the BMSCs cells can effectively protect PC12 cells from the injury by the proteasome inhibitor.

Keywords: Parkinson's disease; proteasome inhibitor; glial cell line-derived neurotrophic factor; lentivirus; gene therapy; bone marrow stromal cells

1 Introduction

Glial cell line-derived neurotrophic factor (GDNF), a member of transforming growth factor- α superfamily, was discovered to promote survival and morphological differentiation of dopaminergic neurons and increase their high-affinity dopamine (DA) uptake in embryonic midbrain cultures in 1993^[1]. Since then, GDNF has been widely studied and proven to be able to protect dopaminergic neurons from 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced damage, which has made it a promising front-runner for therapeutic studies in Parkinson's disease (PD)^[2,3]. How to optimize the delivery of GDNF therefore becomes a critical issue. In recent years, viral vector has proven to be a powerful tool in

gene therapy. Among them, lentivirus is particularly useful and efficient in transferring target genes into nondividing neurons since it is capable to cross an intact nuclear membrane and integrate a gene into the host cell genome, which maximizes the chance of long-term gene expression^[4]. As for cell vehicles in gene therapy, cultured bone marrow stromal cells (BMSCs) are regarded as a mesenchymal progenitor/precursor cell population derived from adult stem cells. BMSCs own advantages of low rate of immunity-assisted rejection and capability of expanding profoundly *ex vivo*, and are the abundant and accessible reservoir for many central nervous system (CNS) diseases (especially PD)^[5].

The ubiquitin-proteasome system (UPS) is a proteolytic system in the cytosol responsible for degrading normal and abnormal intracellular proteins, and loss of activity of this protease results in protein accumulation and cell death^[6]. Mutations in the genes for Parkin (encoding ubiquitin-protein ligase E3) and UCH-L1 (encoding ubiquitin carboxy-terminal hydrolase L1), the components of UPS, have been

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described in familial PD^[6,7]. Further studies in nigral post-mortem tissue from sporadic PD patients demonstrated that the proteasomal activity was selective impaired and the expression of proteasomal subunits was reduced^[8], indicating that UPS plays an important role in nigral degeneration in both familial and sporadic PD. The novel cell and animal PD model induced by lactacystin, a proteasomal inhibitor, could mimic the pathogenesis of PD (namely aggregation of synuclein and formation of Lewy body) more closely. However, whether the supernatant of Lenti-GDNF-BMSCs has neuroprotective effect on lactacystin-induced PC12 cell model has not been addressed so far. Thus, we hope to transduce BMSCs with recombinant Lenti-GDNFs, and to evaluate whether the supernatant can protect PC12 cells from lactacystin-induced injury.

2 Materials and methods

2.1 Materials PLenti6/V5-LacZ, ViraPower Packaging Mix, 293FT, Top10, Lipofectamine2000, and Blasticidin were all from Invitrogen. Polybrene were from Sigma. *Bam*HI, *Xho*I were from NEB. T₄ ligase and Pyrobest DNA polymerase were from TAKARA. DMEM, DMEM-F₁₂, fetal bovine serum, TRIzol and horse serum were from Gibco. GDNF ELISA assay kit was from Promega. PC12 cells were from Institute of Biochemistry and Cell Biology, Chinese Academy of Science. Sprague-Dawley rats were from Shanghai SLAC Laboratory Animal Co. Ltd, China.

2.2 Construction of expressing vector pLenti6/V5-GDNF PLenti6/V5-GDNF was generated by replacing a 3.2-kb *Bam*HI-*Xho*I fragment of pLenti6/V5-LacZ with a 633-bp *Bam*HI-*Xho*I fragment generated by PCR using pcDNA-GDNF (a gift from Zhe-Yu CHEN, The Second Military Medical University, and Bin SUN, Fudan University, China) as the template with the forward primer (5'-CAG GAC CAT GAA GTT ATG GGA TGT C-3') and reverse primer (5'-GTC TCG AGT TGA TAC ATC CAC ACC GT-3'). The Orientation and sequence of the insert were verified by DNA sequencing. Expressing vector contained a V5-tag confluent with GDNF and can be detected by V5 antibody.

2.3 Virus production and concentration^[4] A total of 5×10^6 293FT cells were seeded in a 10-cm-diameter dish 24 h prior to transfection in the DMEM supplement with 10% fetal bovine serum, penicillin (100 IU/mL), and streptomycin (100 µg/mL) in a 5% CO₂ incubator. The culture medium was replaced by the non-antibiotic one 2 h prior to transfection. A total of 12 µg of plasmid DNA (3 µg of expressing plasmid

and 9 µg of packaging plasmid) and 36 µL of Lipofectamine 2000 were diluted in 1.5 mL of Opti-MEM I medium without serum. Plasmids and Lipofectamine2000 were combined after 5 min and then incubated for 20 min. The mixture of DNA and Lipofectamine2000 were added to a 10-cm-diameter dish very gently and were incubated overnight at 37 °C in a CO₂ incubator. The medium was changed 14–16 h later. The conditioned medium was collected after another 24–48 h, purified by low-speed centrifugation, and filtered through a 0.45 µm PVDF filter (Millipore). Thirty-fold concentrated stock was obtained by ultracentrifugation (50 000 g, 150 min, 4 °C). The pellets were resuspended in phosphate-buffered saline (PBS) and stored at –80 °C.

2.4 Titer the lentivirus stock^[4] BMSCs cells were seeded in a 6-well plate before transduction at the density of 2×10^5 /well. Virus stock was serially (10^{-2} – 10^{-6}) diluted to 1 mL of complete medium containing polybrene (8 µg/mL) and also added to the 6-well plate before transduction. The media containing virus were removed and replaced with 2 mL of complete culture medium on the next day. After 48 h, the medium was replaced with complete medium containing the appropriate amount of Blasticidin (5 µg/mL) to select stably transduced cells. The medium was replaced with fresh medium containing antibiotic every 3–4 d. After 10–12 d of selection, there was no live cell in the mock well. Then, other wells were stained with crystal violet to count the blue-stained colonies. Titer of the lentiviral stock was determined by: Number of colonies $\times$ Folds of dilution (TU/mL).

2.5 Preparation and identification of BMSCs^[9,10] Bone marrow was isolated under sterile conditions from 8-week-old male Sprague-Dawley rats. Donor rats were killed by cervical dislocation, and the tibias were dissected. After all attached muscles were scraped off and washed in cold D-Hank's, both ends of the bones were cut off and the marrow was flushed out with 5 mL DMEM using a 25-gauge needle. The collected bone marrow tissue was dissociated by titrating with a pipette and then was filtered through a 100-µm nylon cell strainer. The suspension was centrifuged at 300 g for 5 min and the obtained marrow cells were plated in a 25-cm² tissue culture flask in DMEM (high glucose) containing 20% fetal bovine serum and penicillin/streptomycin. After 24 h, nonadherent cells were removed by replacing the medium. The culture medium was replaced every 3–5 d. After growing to confluence, the adherent cells were lifted by incubation in the solution containing

0.25% trypsin and 1 mmol/L EDTA at 37 °C for 5 min, and then passaged at a density of 6 000 / cm². At the third passage, BMSCs cells were seeded on glass slides, and immunostained with CD71 as Lou's procedure^[10].

2.6 Infection of BMSCs with lentiviral virus^[4] BMSCs were plated in a 10-cm dish with complete medium. Appropriate amount of virus [LacZ or GDNF, at multiplicity of infection of 1 (MOI=1)] were diluted into fresh complete media (contain 6 µg/mL of polybrene) on the next day. Then the old medium was changed with the medium containing virus, and cells were incubated overnight. The following day, the medium containing virus was removed and replaced with fresh and complete culture medium. BMSCs were harvested 72 h after transduction and the total RNA was isolated. Meanwhile, the supernatant of different times (before transduction, and 12, 24, 36, 48 and 72 h after transduction) were collected for later ELISA assay. Half of GDNF-BMSCs were passaged in the 25-cm² flask and supernatant were collected on 1–5 d for ELISA assay.

2.7 Preparation of RNA and semiquantitative RT-PCR of GDNF To detect the GDNF mRNA expression in different groups, total cellular RNA were isolated using TRIzol method. For GDNF RT-PCR amplification, the forward and reverse primers were 5'-CGT CAT CAA ACT GGT CAG GA-3' and 5'-CGG ACG GGA CTC TAA GAT GA-3' respectively, with a 205 bp PCR product^[11]. The β-actin was used as an internal standard, and the forward and reverse primers were 5'-TGACCCAGATCA TGT TTG AGA CC-3' and 5'-CCA TAC CCA AGA AGG AAG GC-3' respectively, with a 413 bp PCR product. The RT-PCR reaction system included dNTPs (10 mmol/L), Moloney murine leukemia virus (M-MLV) reaction buffer (5×), Rnasin rionuclease inhibitor, M-MLV reverse transcriptase, mRNA sample, and oligo-dT₁₈ primer. The Reaction mixture was maintained at 42 °C for 60 min and at 92 °C for 5 min sequentially, and then cycled under the condition: 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 60 s; 35 cycles. Finally the reaction system was incubated at 72 °C for an extra 10 min. PCR products were electrophoresed on 1.5% agarose gel and stained with ethidium bromide (2 µg/mL).

2.8 Immunoassay for detection of GDNF Secretion^[12] For GDNF secretion assay, the supernatants of BMSCs infected with Lenti-GDNF (MOI=1), Lenti-LacZ (MOI=1) or noninfected BMSCs (Control) were collected in different times and stored at -70 °C. Measurement of GDNF in the sampled medium was performed using a commercially avail-

able kit for undertaking enzymelinked immunosorbent assays (GDNF ELISA kit; Promega, Madison, WI, USA) according to the manufacturer's instruction.

2.9 Immunocytochemistry The slides were fixed in ice-cold 4% paraformaldehyde for 10 min, air dried, and blocked with 10% normal goat sera in blocking reagent (0.03% Trinton-100 and 1% BSA in PBS). The slides were then incubated with mouse anti-rat GDNF antibody (1:100, Santa Cruz) and rabbit anti-V5 antibody (1:100, Chemicon) diluted in blocking reagent. Primary antibody was detected with a fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit antibody (1:100, Jackson) and Rhodamine-conjugated goat anti-mouse antibody (1:100, Jackson). The slides were counterstained with Hoechst 33258. Cells were viewed under a fluorescent microscope.

2.10 PC12 cells culture and treatment with lactacystin in different kinds of culture medium PC12 cells grew in DMEM-F₁₂ containing 2.5% fetal bovine serum and 12.5% heat-inactivated horse serum supplement with penicillin/streptomycin. Cells grew to 60% confluence before splitting. The media were changed every 2–3 d.

In experiment I, PC12 cells seeded in 96-well plates (at a density of 3×10⁴ cells/well) were treated with different concentrations of lactacystin (0.1 µmol/L, 1 µmol/L, and 10 µmol/L) diluted in complete medium. No lactacystin was added to the control flasks. Cells were allowed to incubate for 24 h or 48 h before 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

After that, a suitable dose of lactacystin was choosed to treat PC12 cells in experiment II. Cells were plated at a density of 3×10⁴ cells/well in 96-well plate. Different mixing media were added into wells and incubated for 6 h. Then, the lactacystin stock was added into the medium (the final concentration of 10 µmol/L) and incubated for 48 h before MTT assay (Tab. 1, Fig. 1).

2.11 MTT cell viability assay^[13] MTT was absorbed into cell and formed formazan which can be dissolved by DMSO.

Tab. 1 Different mixing medium and treatment of lactacystin

Mixing medium	Lactacystin (10 µmol/L)
PC12 complete medium	–
PC12 complete medium	+
Supernatant of BMSC:PC12 complete medium (2:1)	+
Supernatant of LacZ-BMSC:PC12 complete medium (2:1)	+
Supernatant of GDNF-BMSC:PC12 complete medium (2:1)	+

Fig. 1 Schedule of treatment of PC12 cells in different mediums

Accumulation of formazan reflects the activity of mitochondria directly and the cell viability indirectly. Cells were plated at a density of 3×10^4 cells/well in 96-well plate and treated according to the method described above. MTT stock (5 mg/mL) was added to each well with a final concentration of 500 μg/mL and incubated at 37 °C for 3–4 h. The medium was then removed and the formazan crystals were dissolved in DMSO. Absorbance was determined at 570 nm in a microplate reader. All of results were compensated with optimal density (OD) measured in the same condition well without cell culture.

2.12 Statistical analysis Data were expressed as mean $\pm$ SEM. Results were analyzed by ANOVA. $P < 0.05$ was considered to be statistically significant.

3 Results

3.1 Titer of the virus stock Titers of the concentrated GDNF-expressing virus and LacZ-expressing virus in BMSCs cells were determined as 5.6×10^5 TU/mL and 4.3×10^5 TU/mL, respectively.

3.2 GDNF-BMSCs secreted more GDNF to supernatant Culture media collected from each group of cells were assayed for GDNF expression by ELISA. At 72 h after transduction, GDNF-engineered BMSCs secreted higher levels of GDNF (nearly 860 pg/mL) than non-transduced BMSCs and LacZ-BMSCs (lower than 100 pg/mL; $P < 0.001$, Fig. 2) did. After passage, GDNF-BMSCs still had the ability to secrete an amount of GDNF to the supernatant (Fig. 3).

3.3 Histology and immunocytochemistry of BMSCs The primary passage of BMSCs from the bone marrow grew as separate clones in the flask (Fig. 4A). After passage, the cells were well distributed (Fig. 4B) and were uniformity as the fibroblastic morphology with lower refraction (Fig. 4C, D). In the third generation, cells were 95% positive for CD71 (Fig. 4E–H).

3.4 Immunocytochemistry The result of immunocytochemistry showed that about 95% of BMSCs transduced with lenti-GDNF virus expressed V5 and GDNF in their cytoplasm (Fig. 5).

3.5 RT-PCR The result of RT-PCR confirmed that the

Fig. 2 GDNF produced by GDNF-BMSCs, LacZ-BMSCs, and BMSCs in media. After 24 h, the concentration of GDNF-BMSCs was statistically higher than that of LacZ-BMSCs or BMSCs ($n = 3$, two-way ANOVA, $P < 0.001$). Data are expressed as mean $\pm$ SEM.

Fig. 3 GDNF produced by GDNF-BMSCs after passage in the medium. Data are expressed as mean $\pm$ SEM, $n = 3$.

expression of GDNF mRNA in GDNF-BMSCs cells was statistically higher than that in LacZ-BMSCs and BMSCs cells ($P = 0.01$; Fig. 6, 7).

3.6 The effect of lactacystin in different concentrations to PC12 cells In order to evaluate the viability of PC12 cells exposure to proteasome inhibitor, PC12 cells were treated with different concentrations of lactacystin (0, 0.1, 1, and 10 μmol/L) for 24 h and 48 h. Cell viability was measured by MTT assay. There was significantly reduction of MTT in 10 μmol/L group indicating lower cell in higher concentration (Fig. 8). As an optimal lactacystin concentration, 10 μmol/L lactacystin was selected for the subsequent experiment II.

3.7 Protection of the supernatant of GDNF-BMSCs to PC12 cells treated by lactacystin The supernatant of GDNF-BMSCs had a significant neuroprotection effect on the survival of PC12 cells damaged by lactacystin compared with that of LacZ-BMSCs and BMSCs did (Fig. 9).

Fig. 4 Morphology of BMSCs in primary and passage cultivation (A-D) and fluorescent photomicrographs stained with CD71 (E-H). A: Primary BMSCs, grew to separate clone. B, C, D: The second passage of BMSCs, distributed well. E-H, The third passage of BMSCs: immunostained for CD71 (red, E), stained nuclei by Hoechst 33258 (blue, F) and merged graphs (G, H). Scale bar, 200 μm in A, C; 500 μm in B, H; 50 μm in D, E, F, G.

Fig. 5 Immunocytochemistry showed GDNF (A) and V5 (B) co-expression in the GDNF-BMSCs. About 95% of BMSCs were effectively transduced by GDNF-virus. Scale bar, 50 μm.

Fig. 6 RT-PCR detecting GDNF and β-actin expression level in different groups. Lane 1, 2: BMSCs; lane 3, 4: LacZ-BMSCs; lane 5, 6: GDNF-BMSCs.

Fig. 7 Comparison of GDNF expression level among groups. * $P = 0.01$ vs BMSCs and LacZ-BMSCs.

Fig. 8 Comparison of the viability of PC12 cells in different lactacystin concentrations by MTT assay. Data were represented as mean $\pm$ SEM (percentage of control). Viability of PC12 cells decreased with increasing lactacystin concentration and incubation time. * $P = 0.002$, & $P < 0.0001$ vs control.

Fig. 9 Comparison of viability of PC12 cells in different mixing media. These data were presented as mean $\pm$ SEM (percentage of control). The GDNF-BMSCs mixing medium can protect PC12 cells from damage of lactacystin. * $P = 0.0004$ vs lactacystin group.

4 Discussion

PD is a common neurodegenerative disorder characterized by progressive loss of dopaminergic neurons and concomitant loss of dopamine production in the substantia nigra. *L*-dopa, dopamine agonists and other related agents, are the main treatment strategies for PD and provide dramatic improvements at the early stage of the disease^[14]. At late stages of PD, however, they become less effective in treating movement impairment and several marked deleterious side effects emerge. To slow or reverse the degenerative trend of PD, gene therapies delivering neurotrophic factors such as GDNF are holding great prom-

ise to be beneficial and long-lasting. There are two strategies for introducing transgene into the brain, the *ex vivo* and *in vivo* procedures^[15]. Since *ex vivo* gene transfer had the potential to provide cell replacement therapy at the same time, it is more often applied in animal model studies of PD. As for the approach of transfection, there are two therapeutic strategies as well, non-viral vectors and viral vectors. The former is not suitable for CNS disorders due to its low efficiency. Among the viral vectors, adeno-associated virus (AAV) and lentiviral vectors seem to be the best candidates for delivery of transgene in gene therapy of neurodegenerative disorders. AAV was selected as the vector of delivering glutamic acid decarboxylase(GAD) gene in the first approved clinical trial for PD, though it has the drawback of limited cloning capacity (less than 5 kb)^[16,17]. In recent years, lentiviral vectors are of special interest to be a powerful tool for transferring genes to the brain. Besides the advantages of infecting and integrating into non-dividing cells like neurons, lentiviral vectors show high transduction efficiency and long-term expression in the CNS^[4]. Moreover, the third generation of lentivirus only encode three proteins of virus, which decrease the side effect of immune response, and have successfully expressed genes in *ex vivo* gene transfer paradigms^[18]. In our study, the amount of GDNF in the supernatant of BMSCs (3×10^5 cells) for 3 d was lower than 100 pg/mL. After Lenti-GDNF were transduced, BMSCs secreted higher amount of GDNF in the supernatant at about 800 pg/mL, and approximately 95% GDNF-transduced BMSCs expressed GDNF. That indicates lentiviral vectors are efficient in transduction of GDNF and its expression of GDNF can be long-lasting even after passage. Nevertheless, in order to truly be a viable clinical approach, much research is needed in the future to regulate the expression of target gene and improve its safety.

In animal experiments using *ex vivo* gene transfer into the CNS, several different cell types can be served as gene carriers, including fibroblasts^[19], CNS progenitor cells^[20], and BMSCs^[12]. The problematic issue for fibroblasts is low survival rate after transplantation, and that for CNS progenitor cells is the ethical issue and tumorigenicity. On the contrast, BMSCs, belonging to autologous grafts and have the advantages of safty, no ethically problematic issue and that immunosuppression is not necessary. In addition, BMSCs are known to secrete a substantial amount of neurotrophic factors and cytokines into the soluble stroma and provide a supportive microenvironment for both the growth

and differentiation of the hematopoietic stem cells *in vivo* and *in vitro*^[9,10,21,22]. In the past years, Ye *et al.* have proven that BMSCs can secrete GDNF but the amount was very low^[11] (< 100 pg/mL), which is not enough to protect the damaged neurons. This result was similar to that of our research that the isolated and cultured BMSCs *in vitro* can secrete little GDNF. At the same time, our results *in vivo* (data not shown) also confirmed that BMSCs engineered by Lenti-GDNF can protect lactacystin-induced rat model with PD from neurotoxicity of dopaminergic neurons after transplantation, which indicates that it may be ideal gene carriers for Lenti-GDNF.

Previous studies found that abnormalities in the ubiquitin-proteasome pathway may be responsible for α -synuclein accumulation, Lewy body formation and dopaminergic neuronal death in idiopathic PD. The underlying mechanism may include: 1, activate caspase-3 and increasing apoptosis^[23]; 2, increasing the formation of protein aggregates including nitrated proteins^[24]; and 3, hypersensitizing the cells oxidative damage^[25]. Lactacystin, a powerful UPS inhibitor, was reported to cause dose-dependent degeneration of dopaminergic neurons with the cytoplasmic accumulation and aggregation of α -synuclein to form inclusion bodies^[26]. Selective inhibiting UPS with lactacystin (10 μ mol/L) for 24–48 h caused PC12 cells damage in our experiments. How to rescue the damage caused by UPS dysfunction is becoming a key issue for treatment of PD. Elkon *et al.* have shown that pretreatment with antioxidants (GSH) could reduce cells death induced by lactacystin^[13], and Qiu *et al.* has demonstrated that the apoptosis induced by lactacystin was inhibited by pretreatment with acetyl-aspartyl-glutamylvalyl-aspart-1-aldehyde or overexpression of Bcl-X/L^[23]. Our study showed that pretreatment with supernatant of GDNF-BMSCs for 6 h can alleviate the following damage caused by lactacystin. Further confirmations of the neuroprotection *in vivo* and clarification of the associated mechanisms are therefore highly warranted.

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慢病毒介导的胶质细胞源性神经营养因子在骨髓基质细胞中的表达及其对乳胞素干预 PC12 细胞的保护作用

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摘要: 目的 建立慢病毒介导的胶质细胞源性神经营养因子(glial cell line-derived neurotrophic factor, GDNF)表达系统, 体外感染骨髓基质细胞, 检测过表达 GDNF 对蛋白酶抑制剂引起的 PC12 细胞损伤的神经保护作用。方法 经双酶切和 T4 连接酶构建 pLenti6/V5-GDNF 表达质粒, 经 293FT 细胞包装产生高滴度病毒。用 RT-PCR、ELISA 和免疫细胞化学方法检测感染骨髓基质细胞(bone marrow stromal cells, BMSCs)后 GDNF 的表达, 并检测过表达 GDNF 对蛋白酶抑制剂乳胞素(lactacystin)引起的 PC12 细胞损伤的保护作用。结果 成功构建 pLenti6/V5-GDNF 表达质粒, 获得高滴度具有感染能力的病毒储存液(5.6×10^5 TU/mL)。BMSCs 体外被感染后能大量分泌 GDNF(接近 800 pg/mL), 过表达 GDNF 能减轻乳胞素($10 \mu\text{mol/L}$)引起的 PC12 细胞损伤。结论 慢病毒介导的 GDNF 转染骨髓基质细胞后能分泌具有生物学活性的 GDNF, 对蛋白酶抑制剂引起的 PC12 细胞损伤有保护作用。

关键词: 帕金森病; 蛋白酶抑制剂; 胶质细胞源性神经营养因子; 慢病毒; 基因治疗; 骨髓基质细胞