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·Original Article·

Ex vivo non-viral vector-mediated neurotrophin-3 gene transfer to olfactory ensheathing glia: effects on axonal regeneration and functional recovery after implantation in rats with spinal cord injury

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Abstract: **Objective** Combine olfactory ensheathing glia (OEG) implantation with *ex vivo* non-viral vector-based neurotrophin-3 (NT-3) gene therapy in attempting to enhance regeneration after thoracic spinal cord injury (SCI). **Methods** Primary OEG were transfected with cationic liposome-mediated recombinant plasmid pcDNA3.1(+)-NT3 and subsequently implanted into adult Wistar rats directly after the thoracic spinal cord (T9) contusion by the New York University impactor. The animals in 3 different groups received 4×10^5 OEG transfected with pcDNA3.1(+)-NT3 or pcDNA3.1(+) plasmids, or the OEGs without any plasmid transfection, respectively; the fourth group was untreated group, in which no OEG was implanted. **Results** NT-3 production was seen increased both *ex vivo* and *in vivo* in pcDNA3.1(+)-NT3 transfected OEGs. Three months after implantation of NT-3-transfected OEGs, behavioral analysis revealed that the hindlimb function of SCI rats was improved. All spinal cords were filled with regenerated neurofilament-positive axons. Retrograde tracing revealed enhanced regenerative axonal sprouting. **Conclusion** Non-viral vector-mediated genetic engineering of OEG was safe and more effective in producing NT-3 and promoting axonal outgrowth followed by enhancing SCI recovery in rats.

Keywords: functional recovery; gene therapy; neurotrophin-3; olfactory ensheathing glia; regeneration; spinal cord injury; non-viral vectors

1 Introduction

Impairment in motor and sensory function following spinal cord injury (SCI) has been attributed to disruption of descending motor pathways and ascending sensory ones. After SCI, very little regenerative response occurs in the adult mammalian central nervous system (CNS), leading to permanent loss of function and paralysis. There is no available therapy for restoration of function at present. Many investigators have researched strategies for the restoration of function resulting from SCI, and cellular transplantation has emerged as a promising strategy[1-3].

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Both normal and transected olfactory axons have the unusual property of being able to grow into adult CNS, find their appropriate targets, and form synaptic contacts with them[4]. A critical element in the ability of olfactory axons to regenerate and form specific connections is the presence of a unique supporting cell, the olfactory ensheathing glia (OEG). These cells uniquely present both Schwann cell-like and astrocyte-like characteristics[5] and seem to have great potential to repair damaged spinal cord[2,5-9]. However, not all axonal populations grow into OEG implants[10]. Additional vector-mediated neurotrophin expressed could enhance their regeneration-supporting properties, because endogenous neurotrophic factor expressed by OEG is low[11,12].

Neurotrophins are well known for their beneficial effects on neurite outgrowth and neuroprotection. Neurotrophin-3 (NT-3) is one of important factors in regenerative milieu and exerts variety of physiological effects on nervous system development[13]. Many studies are focused on the usage of

NT-3 stimulating nerve regeneration, but the safe and efficient delivery of NT-3 to the site of injury is still problematic because of its short half-life and the barrier between brain and vessel. Gene transfer technique provides a new strategy for application of NT-3 and allows insertion of NT-3 gene into OEG, which would then produce NT-3 continuously and exert the physiological action.

The present experiment represents a new approach to repair SCI by applying *ex vivo* plasmid-transfected OEG implants in attempting to create more conducive conditions for axonal regeneration. We examined the effects of plasmid vector-mediated hypersecretion of NT-3 on functional recovery and axonal regeneration after thoracic injury. Anatomical and functional analyses were used to assess regeneration in animals over a 12-week observation period.

2 Materials and methods

2.1 Recombinant plasmid pcDNA3.1(+)-NT3 production Coding sequence of NT-3 was amplified by PCR from gene got from rat liver cells and cloned into pcDNA3.1(+) eukaryotic expression vector. Analysis by restricting enzyme digestion and DNA sequencing were carried out to demonstrate the sequence of plasmid as described previously[14].

2.2 Culturing of OEG The isolation of OEG from the olfactory bulb nerve layer has been described previously [15]. Briefly, newborn Wistar rats were killed by intraperitoneal injection of a lethal dose of pentobarbital and decapitated. A craniotomy was quickly performed under sterile conditions to expose the olfactory bulb. The olfactory bulb was dissected and rapidly placed to in a Petri dish with ice cold HBSS (Hanks balanced salt solution). The bulbs were rinsed twice with ice cold HBSS and then minced with a sterile scalpel blade. The minced bulb tissue was then suspended in 5 mL of HBSS with 1 mg/mL trypsin and incubated at 37 °C for 15 min with continual shaking. DMEM (Sigma) and Ham's F-12 (DMEM/F-12; 1:1 mixture; Sigma) supplemented with 10% fetal bovine serum (DF-10S; Invitrogen) was added to stop the trypsinization and the specimen was centrifuged briefly. The supernatant was aspirated and the cells were multiply rinsed with DF-10S. DF-10S culture medium supplemented with L-glutamine (20 mmol/L), and penicillin/streptomycin (10 000 μg/mL) was added and the cells passed through a 70-μm mesh filter. An aliquot of cells was checked for viability with 0.1% trypan blue, and cells were counted with a hemocytometer. The cell suspension was diluted to 1×10^6 cells/mL and plated onto 25 cm^2 flask pretreated with poly-D-lysin (0.2 mg/mL). Cells were incubated for 7 d at 37 °C with 5% CO_2, with culture medium changed every 2 d. Six days following the plating, OEGs were purified from contaminated cells by using the serum-free medium (SFM) instead of DF-10S. The SFM consisted of DMEM, biotin (10 ng/mL), insulin (5 μg/mL), transferrin (50 μg/mL), sodium selenate (5.2 ng/mL), hydrocortisone (10 ng/mL) and glutamine (292 mg/L). Consequently, cells were incubated at 37 °C with 5% CO_2 with culture medium changed every 3 d.

2.3 Transfection of OEG cultures with cationic liposome We used Lipofectamine2000 (Invitrogen) as the media to transfer the plasmids into OEG. Transfection procedure was performed according to the instructions of manufacturer. Briefly, one day before transfection, cells were plated in growth medium (without antibiotics) in a 24-well plate so that they would be 90%-95% confluent at the time of transfection. Dilute plasmid in 50 μL of Opti-MEM I Reduced Serum Medium without serum and mix gently. Mix Lipofectamine2000 gently before use, then dilute the appropriate amount in 50 μL of Opti-MEM I Medium. Mix gently and incubate for 5 min at room temperature. After the 5-min incubation, combine the diluted DNA with the diluted Lipofectamine2000. Mix gently and incubate for 20 min at room temperature to allow the formation of plasmid- Lipofectamine2000 complexes. Add the complexes to each well. Mix gently by rocking the plate back and forth. Incubate the cells at 37 °C in a CO_2 incubator for 24-48 h until they were ready to assay for transgene expression.

2.4 NT-3 production from transfected OEG The NT-3 levels of secreted plasmid in conditioned medium from transfected OEG cultures were determined by ELISA, as described by Woodhall *et al*.[12]. The medium was refreshed 3 d post-transfection and the cultures were left for 24 h for determination of the production per day.

The Emax immunoassay system (Promega) was used to assess the NT-3 levels in OEG conditioned medium. The amount of NT-3 secreted from 1×10^5 transfected cells per day was recorded.

2.5 Biological activity of plasmid vector-derived NT-3 To determine whether recombinant NT-3 protein was biologically active, we studied their effects on the neurite outgrowth in human neuroblastoma cells. Primary human neuroblastoma cells were collected and cultured routinely. The attached cells

were trypsinized by 0.05% trypsin (Invitrogen) for 10 min, and then the reaction was stopped by adding DMEM (Sigma) and Ham's F-12 (1:1; Sigma) supplemented with 10% fetal bovine serum (DF-10S; Invitrogen). The suspension was washed twice in serum-free medium and seeded onto 4 plates at a density of 1×10^4. Then 500 μL medium from the cultures of OEGs transfected with pcDNA3.1(+)-NT3 or pcDNA3.1(+), or OEGs without plasmid transfection, was added to 3 different plates. The rest plate was added with serum-free medium. Three days later, neurite outgrowth was photographed.

2.6 Preparation of OEG for spinal implantation OEG suspensions were prepared as Ruitenberg *et al*. described[16]. Purified OEGs were seeded onto poly-L-lysine-coated dishes at a density of 1×10^6 cells in DF-10S. The next day, the medium was replaced with fresh medium containing pcDNA3.1(+)-NT3 or pcDNA3.1(+), and cells were left for 72 h. After that, the OEG cultures were washed with L-15 medium, detached by trypsinization and washed twice in serum-free DMEM/F-12 medium. Next, cells were pelleted by low-speed centrifugation, carefully resuspended, and diluted in appropriate volume of DMEM/F-12 to obtain the OEG suspension at 50 000/μL. The viability of OEG suspensions, which was assessed by counting the percentage of dead cells using Trypan blue staining, was >95%.

2.7 Experimental design Wistar rats (200-240 g) were raised under standard conditions with a 12/12 h light/dark cycle, and accessed to water and food ad libitum. All experimental procedures were conducted in accordance with the guidelines of the local Animal Welfare Committee for use and care of laboratory animals. Forty four adult female rats were subjected to the thoracic spinal cord contusion and then divided into four groups: group 1 (n =10) was untreated and did not receive implantation; groups 2 and 3 received implantation of untransfected OEG (n = 10) or pcDNA3.1(+)-transfected OEG (n = 12), respectively; and group 4 (n = 12) received implantation of pcDNA3.1(+)-NT3-transfected OEG. The recovery of rats' hindlimb performance was evaluated weekly for 12 weeks. Seven days after implantation, two animals each group were taken for evaluation of transgene expression. Fourteen days before being killed, another two rats from each group were retrogradely labeled with fluorochrome (FG; Vector Laboratories).

2.8 Surgical procedures Implantation of OEG into complete thoracic transection injury model was described previously in detail[16]. In brief, animals were anesthetized and underwent laminectomy at T9-T10, and then they were subject to a 25 g/cm spinal cord contusion using the New York University impactor[17]. Once a technically complete injury was confirmed, the OEG were carefully injected into the middle of spinal cord at 1 mm distance, both proximal and distal, from the edges of the lesion area. From ventral to dorsal, cells were injected into the following four sites of each cord stump: (1) ventral funiculus, (2) gray commissure, (3) dorsal corticospinal tract, and (4) gracile fasciculus. Coordinates of the four injection sites were 1.75 mm, 1.25 mm, 1 mm, and 0.5 mm, respectively. Each site received 1 μL of a suspension containing 5×10^4 cells, and thus we injected total 2×10^5 cells into each cord stump. Injections were performed using a sterile glass micropipette, Hamilton syringe, and a micromanipulator. All animals received a postoperative subcutaneous injection of 2 mL physiological salt solution to compensate for blood loss, and their bladders were expressed twice daily until reflexes returned. The rats were maintained in pairs in separate cages. At the end of surgical procedures, the viability of OEG suspensions used for implantation still ranged between 90% and 95% as determined by Trypan blue staining.

2.9 *In vivo* transgene expression RT-PCR was performed 7 d after implantation to evaluate transgene expression. Briefly, total cellular mRNA was isolated from the injury area by using Test Kit following the manufacturer's instructions. The resulting cDNA was amplified by PCR. PCR reaction was performed in 25 μL volume containing: 2 μL of cDNA, 2 U of Taq polymerase, 2 μL primers (forward and reverse), and 3 μL β-actin (inner reference). Reaction conditions were at 94 °C for 10 min, then at 94 °C for 1 min, at 55 °C for 1 min, and at 72 °C for 1 min, for 35 cycles; followed by a final extension at 72 °C for 10 min.

2.10 Locomotor analysis After surgery, animals were videotaped in an open field environment on 1, 14, 28, 42, 56, and 84 d post-operation. Videotapes were reviewed by a blinded scorer, and the Basso Beattie Bresnahan (BBB) scores[18] were assigned for each animal at each testing session. Scores from the left and right side were averaged and a single score was submitted for each animal at each testing session.

2.11 Retrograde axon tracing The regenerated axons was retrogradely traced with FG, as a neuronal label, 14 d before perfusion. In brief, the animals were held in a stereotaxic frame for laminectomy under deep ketamine–xylazine anesthesia.

FG tracer in glass pipettes was subsequently delivered to the spinal cord caudad to the lesion region. The injector was left in place for 5 min, followed by careful withdrawal of the glass capillary and suturing of the skin.

2.12 Histological analysis Rats were sacrificed 12 weeks post-operation, and the spinal cords were harvested for immunohistochemical staining of neurofilament (NF). In brief, the anesthetized rats were perfused with cold PBS (0.1 mol/L, pH 7.2) containing heparin, followed by 4% buffered paraformaldehyde. Spinal cord from the contused site was cut and fixed in a 10% formalin solution for 2 h, embedded in paraffin, then cut in 4 μm sections. The sections were treated with 0.3% H_2O_2 in methanol to inactivate the endogenous peroxidase, and nonspecific staining was blocked with 3% normal serum. Primary antibodies raised against NF (mouse anti-Neurofilament SMI31, 1:1 000; Sternberger Monoclonals Inc., Baltimore, MD, USA) was diluted in 1% normal serum, applied to the tissue sections at 37 °C for 1 h and then at 4 °C overnight. Sections were incubated with an appropriate biotinylated secondary antibody (1:200; Vector Laboratories, Burlingame, CA, USA) followed by ABC complex for 45 min at room temperature. Diaminobenzidine (DAB-brown color; Sigma Chemical Co., St. Louis, MO, USA) was used as the chromatogen. The sections were counterstained with hematoxylin, dehydrated in graded alcohols, and mounted. The sections stained with normal serum were used for control. Omission of primary antibodies resulted in only weak, non-specific labeling of the blood vessels. Quantification of the proportional area at the center of the lesion at 40× was performed by using a computer-assisted digital image analysis system (MPIAS-500), and by manually setting the relative optical density threshold to identify positive stained properties within a defined target area. FG-labeled neurons were visualized as mentioned above and then observed under fluorescent microscope.

2.13 Statistical analysis Data obtained from histological analysis and the BBB scores were analyzed for statistical differences between animal groups using one-way ANOVA and Student-Newman-Keuls tests. $P < 0.05$ was considered statistically significant. All data incorporated in the analysis were obtained from the animals survived the entire study.

3 Results

3.1 *In vitro* analysis of transgenic neurotrophin expression Transfection of purified OEG cultures with plasmid-liposome complex resulted in comparatively high levels of transgene expression in many cells as determined by in situ hybridization at 3 d after infection (data not shown). Transfection efficiency came up to 30% as determined by fluorescence assay. Numerous cells expressed high levels of NT-3 mRNA without signs of toxicity or cytopathological effects. No staining for any of the transgenes was observed in untransfected OEGs that served as control cultures or after hybridization with sense probe.

Conditioned medium from transfeced OEG cultures was analyzed for the presence of NT-3 using an ELISA assay 4 d later after transfection. NT-3 was not detectable in the medium samples ($n = 4$) taken from control or pcDNA3.1(+)-transfected OEG cultures. After transfection with pcDNA3.1(+)-NT3, ELISA analysis of medium samples revealed that at least (21.7±4.8) ng of recombinant NT-3 was secreted from 1×10^5 cells per day. These results indicate that, at the time-point of implantation, high amounts of recombinant neurotrophin were released from NT-3-transfected OEG as compared with controls.

Biological activity of plasmid-derived NT-3 was demonstrated in a coculture experiment. For this, human neuroblastoma cells ($n = 12$) were cultured with medium from OEG cultures to determine the effect of transgenic neurotrophic factor production and were subsequently analyzed for neurite outgrowth at 3 d after the initial plating. Moderate extensions of neurites were observed when human neuroblastoma cells were cocultured with medium from untransfected or pcDNA3.1(+)-transfected OEG (Fig. 1A, B). No visible differences in radial neurite outgrowth were observed between these groups, indicating that plasmid vector transfection did not interfere with the growth supporting properties of OEG. A robust outgrowth of neurites from neuroblastoma cells was found when medium from OEG transfected with a plasmid vector-encoding NT-3 was added (Fig. 1C). These results demonstrate that plasmid vector-derived NT-3 was biologically active and confirm that recombinant NT-3 was secreted by transfected OEG.

3.2 *In vivo* analysis of transgene expression At 7 d after implantation, transgene expression in OEG implants was examined by RT-PCR. The tissue from rats in pcDNA3.1(+)-NT3-tansfected OEG group expressed high levels of NT-3 mRNA (Fig. 2).

3.3 Axonal survival and regeneration To evaluate axonal

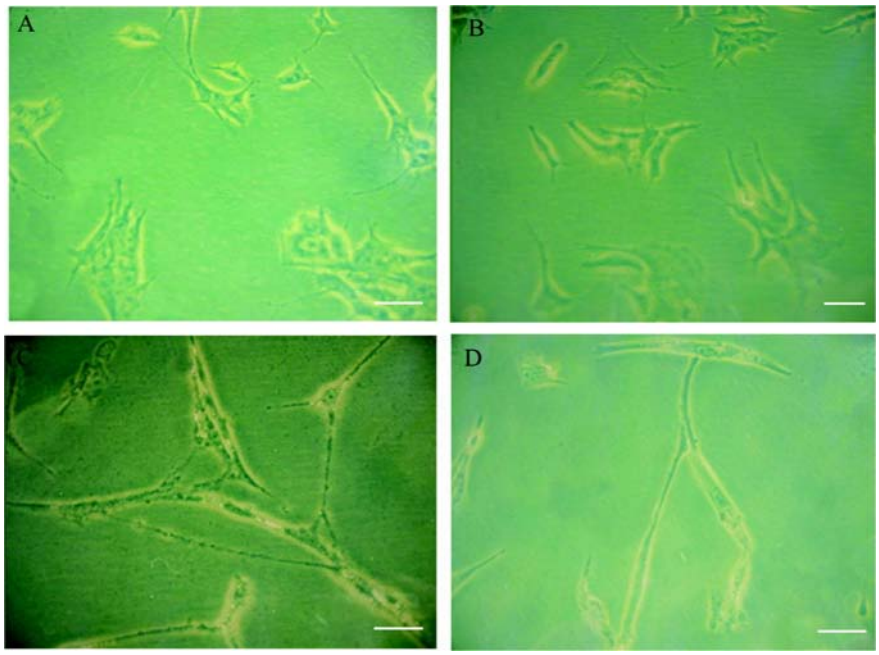

Fig. 1 Human neuroblastoma cells cultured with medium from olfactory ensheathing glia (OEG) cultures. Moderate extensions of neurites were observed when human neuroblastoma cells were cocultured with medium from untransfected or pcDNA3.1(+)-transfected OEG (A, B). Robust outgrowth of neurites from neuroblastoma cells (C) and suspected synapse connection (D) were found when medium from the OEG transfected with NT-3-encoding plasmid vector was added. Scale bar, 8 μm.

survival and regeneration, NF immunoreactivity was assessed at 12 weeks post-injury. The significantly more NF-positive axons survived in the pcDNA3.1(+)-NT3-tansfected OEG group than in pcDNA3.1(+)-transfected and untransfected OEG groups, or untreated group (Fig. 3; $P < 0.05$).

The FG labeling showed that retrograde transportation of regenerated fibers overcame the glial scar and regrew into the distal part of spinal cord through the lesion regions. Some labeled neurons could be seen in the cervical spinal cord and some parts of the cerebrum except for cortex (Fig. 4).

3.4 Functional recovery Unfortunately, some of the rats died from cystitis or other infections, so the numbers of rats survived until the 12th week after SCI were 10 in the pcDNA3.1(+)-NT3-transfected OEG group, 9 in the untransfected OEG group, 9 in the pcDNA3.1(+)-transfected OEG group and 7 in untreated group. Blind functional assessments and analyses were performed by using the BBB open field locomotion scale. The rats uniformly exhibited either paralysis or durative uncoordinated hindlimb spasms. Three weeks after SCI, initial paralysis was attenuated in the animals implanted with pcDNA3.1(+)-NT3-transfected OEG (Fig. 5). Six weeks after SCI, they demonstrated extensive joint movement in their hindlimbs, and their BBB score increased significantly compared

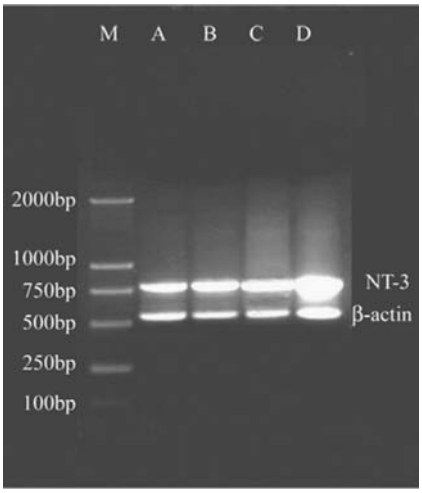

Fig. 2 *In vivo* transgene expression. Lane A: untreated group; Lane B: OEG group; Lane C: pcDNA3.1(+)+OEG group; Lane D: pcDNA3.1(+)-NT3+OEG group. The pcDNA3.1(+)-NT3-tansfected OEG group expressed high level of NT-3 mRNA.

with the other three groups ($P < 0.05$). The mean BBB scores at 12-week were 12.50, 10.39, 10.22 and 3.78, respectively, in pcDNA3.1(+)-NT3+OEG,OEG, pcDNA3.1(+)+OEG, and untreated groups. Rats in the pcDNA3.1(+)-NT3-transfected OEG group had better performances compared with those in other groups ($P < 0.05$). The pcDNA3.1(+)-transfected

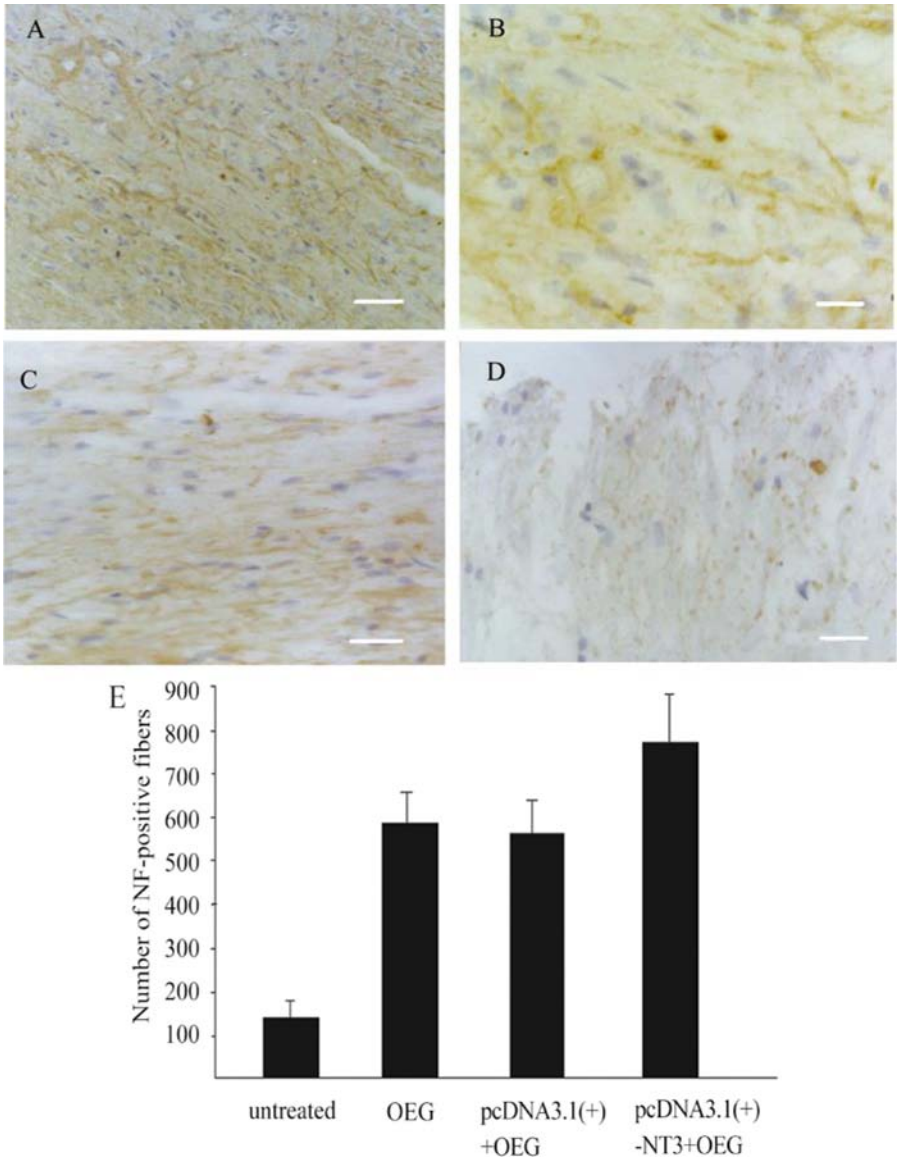

Fig. 3 Immunohistochemical staining for neurofilament (NF) at the contusion injury site. Twelve weeks after injury, fewest NF-positive neuronal fibers were observed at the lesion site in the untreated group (A), more fibers were found in the OEG group (B) and pcDNA3.1(+) transfected OEG group (C). Most NF-positive neuronal axons survived in the spinal cords of rats in the pcDNA3.1(+)-NT3 transfected OEG group (D). E: This difference of stained NF among every two groups (represented as mean±SD) was significant ($P < 0.05$). Scale bar, 40 μm.

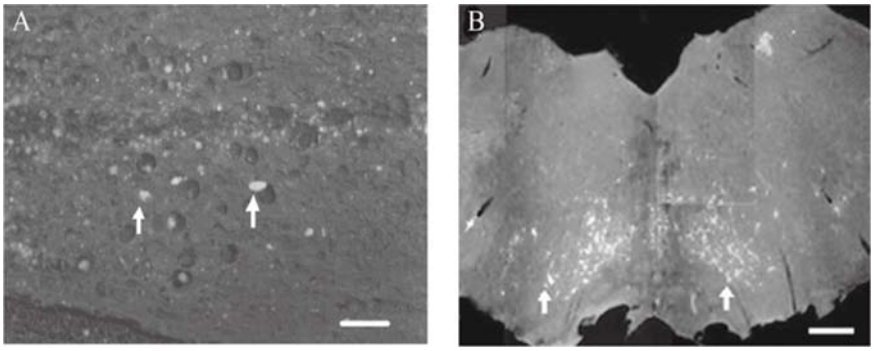

Fig. 4 Labeled neurons (arrows) in the proximal cervical spinal cord (A) and the reticular nucleus of pons (B) were visible in rats of pcDNA3.1(+)-NT3 group. Scale bar, 50 μm.

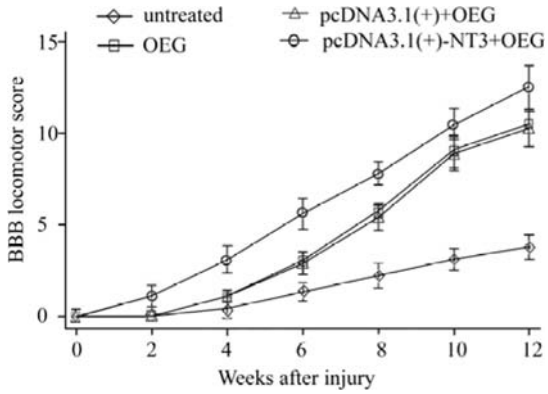

Fig. 5 Basso Beattie Bresnahan (BBB) locomotor score (mean±SD) of rats in different groups. Rats that received pcDNA3.1(+)-NT3 transfected OEG revealed active coordinated hindlimb movement six weeks later after injury. The only OEG implantation and untreated rats exhibited either paralysis or occasional uncoordinated hindlimb spasms after injury. Differences between every two weeks were determined by *post hoc* means-corrected *t*-test ($P < 0.05$).

OEG group and the untransfected OEG group had no significantly difference .

4 Discussion

By combining OEG implantation with *ex vivo* plasmid vector-mediated NT-3 gene transfer, we demonstrated the following results in this study: (1) OEG can be transfected efficiently with plasmid, a non-viral vector, mediated with cationic liposome. Furthermore, OEG can survive both *ex vivo* and *in vivo*, expressing the recombinant NT-3 protein with biological activity. (2) The NT3-transfected OEG implants improved recovery of hindlimb function as determined by behavioral testing. (3) Quantitative histological showed that the NT3-transfected OEG implants did promote the axonal regeneration to repair injured spinal cord, indicating genetic modified OEG can repair the injured spinal cord better than simple OEG. Although many problems remain unsolved, we consider that OEG combined with gene therapy is a promising strategy.

Gene therapy may be described as delivery of nucleic acids to patients via a vector for therapeutic purposes. This is a promising therapeutic method though currently enormous failure of clinical trials. The primary reason for current failure and frustration is that no adequate vectors could be used to deliver therapeutic nucleic acids to their desired site of action in the chosen cells. There are two kinds of vectors, viral and non-viral vector system, used for gene delivery. Synthetic non-viral vector systems have many advantages compared with viral systems, including significantly lower toxicity/immunogenicity and potential for oncogenicity, size independent delivery of nucleic acids (from oligonucleotides to artificial chromosomes), simpler quality control, and substantially easier pharmaceutical and regulatory requirements[19]. Increasing public alarm particularly with the toxic side effects of virus is also strengthening these significant advantages. Basic clinical confidence in non-viral vectors is growing and the various advantages listed above inherent in synthetic non-viral vector systems should ensure substantial clinical uptake once the science and technology of these vector systems can be appropriately matured for routine clinical use. In our original proof of present study, we prove that cationic liposome could mediate delivery of recombinant NT-3 cDNA to OEG, thereby resulting in production of therapeutic levels of functional NT-3 protein in injured spinal cord.

Neurotrophins exert biological effects dependent on their specific combination with the receptor. A kind of neurotrophin can only affect some specific neurites. As to SCI of mammalian, the recovery of corticospinal tract (CST) and main sensory pathway, where NT-3 receptors exist widely, will be significative. So NT-3 is the appropriate candidate[20]. Many researches have shown that NT-3 can maintain the survival of sensory, motor and sympathetic neurons, prevent neuronal apoptosis after SCI and induce the axonal regeneration. In the ability of promoting axonal regeneration, NT-3 excels nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF)[21]. To date, a number of axon populations were reported to regenerate through OEG implants in different injury models, including sensory axons of the dorsal root axons[21,22] as well as serotonergic axons[8,9,24,25] and CST axons[7,8]. Some controversy has arisen on the regenerative response of the CST because others found that OEG induced sprouting was limited to the proximal injury site[26]. In the present study, a significant axonal regeneration and better recovery of locomotion function was found after implantation of OEG transfected with NT-3-encoding plasmid. But no fully recovery of locomotion was observed. Maybe it is due to no sufficient NT-3 was produced by the OEG transfected with non-viral vector. But we do not think so because no fully recovery of locomotion was observed either in previous study using viral vector[16,27]. As we know, the repair of injured spinal cord depends on the

balance between promoter and inhibitor. So the single use of promoter such as OEG or neurotrophin without dealing with inhibitor such as Nogo would lead to unbalance which is not optimum for nerve regeneration. It is therefore important to obtain more insight in overall requirements of injured spinal cord. Extended knowledge of both promotion factors and inhibition ones involved in regeneration of nerve tracts will allow the development of more optimal microenvironment.

The combination of neural transplantation and neurotrophin delivery has emerged as a promising strategy to augment regeneration and functional recovery after spinal cord injury[28,29]. We demonstrate that *ex vivo* transfection of OEG with NT-3-encoding plasmid vectors did: (1) significantly promote axonal regeneration, (2) enhance the growth-promoting properties of these cells, and (3) improve functional recovery after implantation. A limitation of only neurotrophin-transfected OEG implants is that they can not counteract inhibitors directly, which is another important factor affecting repair of injured spinal cord. Combination of promoter and inhibitor antagonist could significantly improve the results obtained, giving hope for the use of these modified cells in both acute and chronic SCI models.

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神经营养素 -3 基因非病毒载体转染的嗅鞘细胞移植促进脊髓损伤大鼠轴突再生及功能恢复

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摘要：目的 将神经营养素 -3（neurotrophin-3，NT-3）基因转染的嗅鞘细胞（olfactory ensheathing glia，OEG）移植到脊髓损伤大鼠体内，以期促进大鼠胸脊髓损伤的恢复。**方法** 将自行构建的质粒 pcDNA3.1(+)-NT3，应用脂质体介导的方法导入体外培养的 OEG，将其移植入急性脊髓损伤大鼠体内，连续观察 12 周，与接受单纯 OEG 移植和空白质粒转染OEG移植及无OEG移植的脊髓损伤大鼠进行比较。**结果** pcDNA3.1(+)-NT3转染的OEG移植后能在体内长期存活，表达 NT-3 基因，并较对照组更能促进脊髓损伤区轴突的再生和后肢功能的恢复。**结论** OEG 是脊髓损伤基因治疗较好的受体细胞。转染OEG 移植后可以在体内较长时间存活。能明显促进急性脊髓挫伤神经纤维再生和功能恢复的作用，为基因修饰嗅鞘细胞在脊髓损伤治疗的应用提供了实验和理论依据。

关键词：功能恢复；基因治疗；神经营养素 -3；嗅鞘细胞；再生；脊髓损伤；非病毒载体