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Study on the Therapeutic Effect of shRNA Injected into Tail Vein on Parkinson's Disease in Mice Based on SNCA Gene Mutation of α-Synuclein

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Abstract. Aim: This research aims to investigate the therapeutic effect of shRNA injected into the tail vein on Parkinson's disease in mice based on SNCA gene mutation of α-synuclein and explore the migration mechanism of α-synuclein. Methods: SCNA +/+ mice are used to induce α-synuclein by tuberculin pff and then establish the model of Parkinson's disease in mice. Besides, AAV-shRNA is used to set the control group and the experimental group at different times. Open-field test, tail suspension test, and immunofluorescence are adopted to explore the therapeutic effect of shRNA and the migration mechanism of α-synuclein. Results: shRNA inhibits P-α-syn in the brain of mice afflicted with Parkinson's disease, which has obvious behavioral therapy. The whole brain invasion of α-synuclein in OB of the olfactory bulb occurs, while the invasion of α-synuclein in CPu of the dorsal striatum only exists in the downstream brain region. Conclusion: Intravenous injection of shRNA into the tail vein can effectively inhibit α-synuclein with symptomatic therapeutic effect on mice with Parkinson's disease, and the earlier injection enables better therapeutic effect.

Keywords: shRNA; α-Synuclein; Parkinson's Disease; SNCA Gene.

1. Introduction

Parkinson's disease (PD) is the second most common neurological disease after Alzheimer's disease (AD), which mainly occurs in the elderly and is characterized by progressive multiple and occult onset. Its main symptoms are bradykinesia, myotonia, resting tremor, and postural instability. The pathogenesis of PD is the lesion of nigra-striatum in the brain, which triggers the decrease of dopamine in dopaminergic neurons and weakens the dopaminergic nerve function and the predominance of cholinergic nerve function. Besides, their balance is broken with increasing muscle tension, paralysis agitans, and cognitive dysfunction. The main factors of nigrostriatal lesions include genes (pathogenic genes), age, and environment. In addition, the prevalence rate of Parkinson's disease in the whole population is about 0.3% (Ascherio et al., 2016), and that of the elderly has doubled. Moreover, the prevalence rate of the elderly over 65 years old is 1% ~ 2% and that of the elderly over 85 years old is 3% ~ 5% (de et al., 2006). In China, the number of patients with Parkinson's disease has risen sharply. According to a study in 2007, the number of Parkinson's disease patients in China accounted for about half of the world at that time and the period after that. In 2005, the number of Parkinson's disease cases in China was about 1.99 × 106, and that in the world was about 4.10 × 106. It is estimated that such a number in China is to be about 4.94 × 106 in 2030 and that in the world is to be about 8.67 × 106 (Dorsey et al., 2007). Compared with other countries and regions with aging problems worldwide, although the prevalence rate of Parkinson's disease in China is similar to that in the world, the Chinese elderly population prone to be attacked by the disease ranks top with many existing patients and potential patients, which brings challenges to society and medical institutions. Meanwhile, Parkinson's disease features low mortality and a high disability rate, which seriously threatens the health of the elderly. Thus, it is urgent to explore its effective treatment.

The α-synuclein protein gene is located on chromosome 4q21-q23 and belongs to autosomal dominant inheritance, which was first discovered by Polymeropoulos in a hereditary PD family (Contrusi family). α-syn protein gene is widely distributed in nerve tissues and highly expressed in the neocortex, hippocampus, olfactory bulb, and striatum. Its main function is to regulate the size of

the presynaptic vesicle pool of neurons and interact with the synaptic membrane system to affect synaptic function (Luk et al., 2012). A single inoculation of pathological α-synuclein, including α-synPFFs assembled by recombinant proteins, is sufficient to induce extensive CNS α-Syn pathology and accelerate diseases in vivo. Experimental data show that α-Syn can spread to many central nervous system regions at a long distance, including cortical, midbrain, and brainstem neurons affected in DLB/PD (Dickson et al., 2009). According to Braak pathological stage, α-Syn deposits are found in different brain regions of PD patients and are related to each other. In addition, the neurodegeneration of PD can be reproduced by injecting recombinant α-Syn preformed fibrils (α-Syn-pff) into the brain of animals. In the experiment by Guiney et al., recombinant monomer α-Syn and α-Syn-pffs were added in parallel to SN4741 cells for 24h, which showed loss of activity in both cell types, while adding monomer α-Syn did not reduce cell activity in both cell types, indicating that the toxicity targeted aggregated α-Syn (Guiney et al., 2020). Both abnormal aggregation of mutant proteins and injection of fibrils in vitro confirmed that α-Syn oligomer has certain neurotoxic effects. The diffusion of α-Syn with prion-like properties depends on its stable existence outside cells. Therefore, immunotherapy targeting extracellular α-Syn is a new strategy to prevent the progression of PD.

α-Syn was first found in the Lewy body of a patient afflicted with Parkinson's disease in 1997 (Spillantini et al., 1997). The point mutation and gene duplication of the gene SNCA encoding α-Syn are intertwined with the development of Parkinson's disease. It has been proven that the pathological diagnosis of PD is based on the appearance of intracellular inclusion bodies, that is, Lewy body (LB), which are mainly formed by α-Synuclein (α-Syn) aggregation in neurons. There is an interaction between aggregated α-Syn and DA reduction, which can trigger selective death of DA neurons. The Lewy body is the histological marker of the PD brain, with α-Syn as the main component of the Lewy body and Lewy neurite. The abnormal ubiquitin-proteasome system (UPS), oxidative stress, mitochondrial dysfunction, neuroinflammation, and other pathogenic mechanisms will lead to conformational changes, misfolding, and aggregation of α-Syn, which promotes premature neuronal death (Cacabelos et al., 2017).

Recombinant aav (rAAVs) is a commonly used vector for gene transfer in vivo, which is also a promising therapeutic vector. However, aav that can efficiently and non-invasively deliver genes to specific cell populations is needed. Current gene delivery methods such as intraparenchymal surgical injection are invasive, while alternative methods such as intravenous injection require high virus doses and relatively inefficient target cell transduction. Until 2019, Viviana Gradinaru et al. developed AAV targeted evolution technology (CREATE) based on Cre recombination, which was used to design and screen AAV capsids. These capsids can transfer genes to specific cell types more effectively through blood vessels. Injecting specific adeno-associated viruses (AAV) into the tail vein can break through the blood-brain barrier and infect brain tissue, which is one of the basic theories and means of our experiment. Because the olfactory bulb as the front end of the brain is prone to be affected at the earliest, the initial symptom of patients afflicted with Parkinson's disease is usually abnormal olfaction, while behaviors such as tremor and myotonia in the middle and late stage are due to the accumulation of lesions in the dorsal striatum. Therefore, pff is injected into the olfactory bulb to simulate the early lesions, and into the dorsal striatum to simulate the middle and late lesions in this experiment. To find more clinical gene therapy methods, this experiment will be based on TG-SNCA mice, which uses purified α-syn-pff to make the model of Parkinson's disease in mice. Besides, packaging transfection of AAV-shRNA will be given for treatment through immunofluorescence staining, open-field experiment, and tail suspension experiment to explore the effects of shRNA on mouse p-α-syn at the animal molecular level and overall level, so as to provide basic data for the treatment and research of Parkinson's disease.

2. Methods and Materials

2.1 Animals and Models

In this experiment, TG-SNCA+/- mice from Kunming Institute of Zoology that are 8 weeks old, male, 18-22g were selected. All experimental mice were placed in the experimental environment for at least 2 weeks for adaptation, allowing mice to obtain water and food freely. Besides, the use of all animals is approved by the Animal Ethics Committee.

Pentobarbital (35mg/Kg) is injected intraperitoneally into anesthetized animals, and then mouse venous blood is added into the EP tube infiltrated with heparin by tail cutting method. The rectal temperature is maintained at 37.5 ℃ via a feedback control heating pad. In addition, mice are placed in a stereotactic framework and drilled in the skull near the right coronal suture about 1mm in diameter. PFF is injected into the right olfactory bulb (coordinates: X= -0.75mm, Y= +5.10mm, Z= -1.00mm) or the right striatum (coordinates: X= -1.70mm, Y= -0.86mm, Z= -2.40mm) using a microinfusion pump at a rate of 1 μL/min. The needle is taken out to fill the hole with bone wax and then suture the skin incision. Two months later, AAV is given after a tail vein injection.

2.2 Behavioral Experiments

(1) Open-Field Experiment

The experimental device is made of opaque plastic, which is made of a 50cm × 50cm × 50cm cube with a white bottom and inner wall of the box. The mouse is placed in a specific corner facing the central area, and then the timing is started, and the activity of the mouse within 15 minutes is automatically recorded. The open field in the video is divided into the central and surrounding areas by specific behavioral software, with the central area accounting for 25%. Meanwhile, the mouse movement is tracked and recognized synchronously. The movement distance, movement speed, resting time, and the time of mice in the central area are observed, so as to evaluate the exercise ability of mice.

(2) Suspended Tail Test

After lifting the tail of the mice, the movement of the hind limbs within 15s is recorded, and the clasp and muscle strength of the hind limbs are observed.

2.3 Immunofluorescence

Frozen sections are taken after brain fixation with 40μ as the thickness of the sections. 10% goat serum is blocked, the primary antibody is Rabbit-α-syn (sigma), and the secondary antibody is Goat-Rabbit (sigma).

2.4 Statistical Analysis

All the data are expressed by Mean ± SEM, and the comparison between the two groups is made by unpaired student t-test test. According to the comparison between multiple groups made by one-way ANOVA after a post hoc test, $p < 0.05$ is statistically significant. With Prism8 as the device to draw the statistical chart, Flow-Jo is used to analyze the flow cytometry.

3. Results

3.1 Behavioral Results of Treatment at Different Treatment Times in Olfactory Bulb and Striatum

Significant curative effects have appeared in different positions and treatment times. Besides, the effect of shRNA treatment 1.5 months after pff injection in the olfactory bulb is far better than that after 2.5 months of the olfactory bulb injection, and the result of pff injection in olfactory bulb is far better than that in the striatum (Figure 1) for the same 1.5 months.

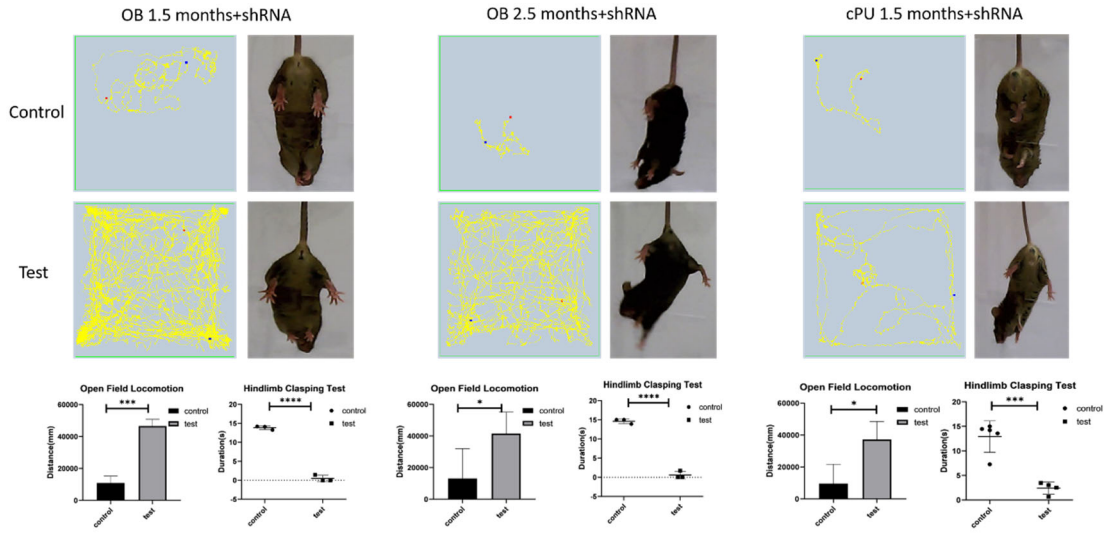

Figure 1. Results of Open-Field Experiment and Tail Suspension Experiment after pff Injection at OB and CPu for Different Treatment Time.

3.2 Immunofluorescence Results of 1.5 Months after pff Injection into Olfactory Bulb and Striatum

According to DAPI staining, p-α-syn was much less than that of cPU at 1.5 months after injecting pff in the olfactory bulb (Figure 2).

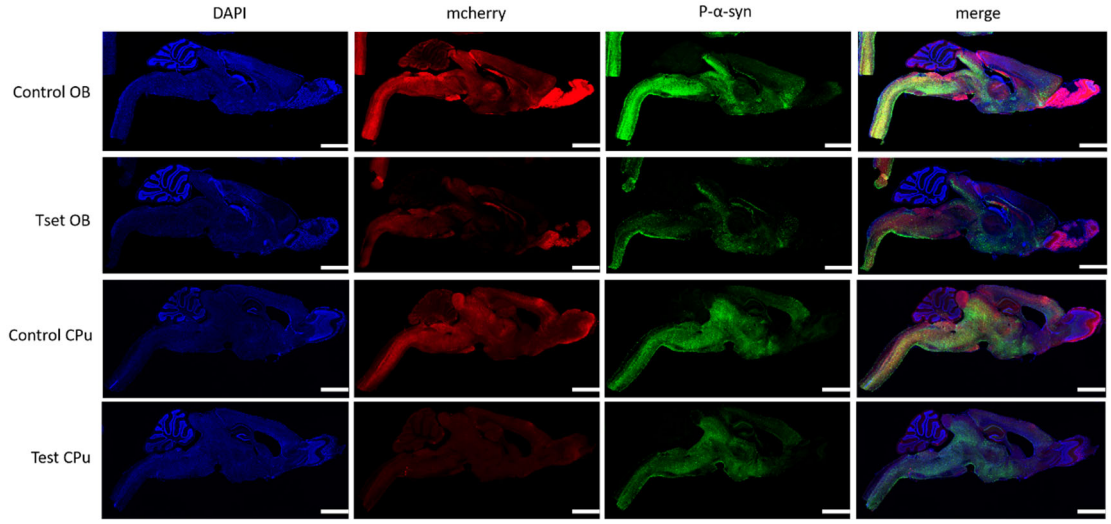

Figure 2. Immunofluorescence Staining after pff Injection at OB and CPu.

The Blue is the DAPI Labeled Nucleus; the Red is the tdT Intensified Signal Labeled by Goat-Chicken-mcherry-546 , which is the shRNA; and the Green is P-α-syn Labeled by Goat-Rabbit-P-α-syn-488 in the Brain.

4. Discussion

Combined with the related literature and results, it is not difficult to find that tuberculin (pff) can induce P-α-syn production in the brain of SNCA +/+ mice, which is a basic condition for the success of the experiment. Another basic condition is that AAV-shRNA can be detected in the brain after being injected into the peripheral vein (tail vein). What is especially gratifying is that the existence of AAV-shRNA has not been detected in the liver, the largest metabolic organ in the body, which also shows that the AAV-shRNA can be directed to the brain to play a role.

After meeting basic conditions, the team set up the experiment and control groups with OB injection for 1.5 months, the experiment and control groups with OB injection for 2.5 months, and the experiment and control groups with CPu injection for 1.5 months. Two neurobehavioral experiments, open field experiment, and tail suspension experiment were carried out to analyze these six groups. According to the results, compared with control groups, the shRNA injection test group shows a more obvious therapeutic effect. In the same OB group, the treatment effect of that for 1.5 months is significantly better than that for 2.5 months, and the treatment effect in OB is significantly better than CPu group, which proves that early treatment and frontal brain areas are also best, because the most common early symptom of Parkinson's patients is olfactory loss.

To further explore the specific relationship, sagittal brain slices are used for immunofluorescence triple staining, and the results are consistent with behavioral experiments. The shRNA-injected test group shows significant therapeutic effects, whether in OB or CPu groups. The gratifying result is that P-α-syn appears in the whole brain after OB injects pff to induce P-α-syn, but P-α-syn appears only in its downstream brain region after CPu injects pff to induce P-α-syn. Thus, the migration of α-syn has a sequence from front to back, which precisely reflects patients afflicted with Parkinson's disease that has experienced small changes such as early olfactory dysfunction to later tremor atrophy (Figure 3).

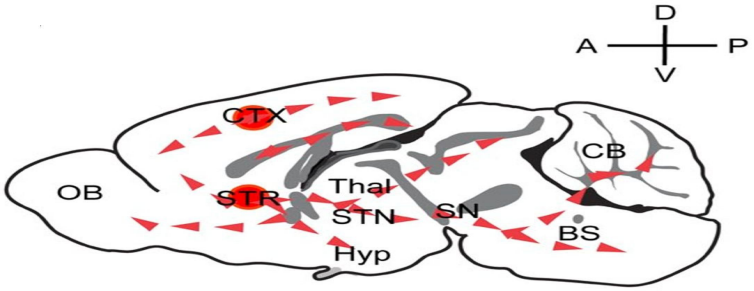

Figure 3. Possible Migration Pathway of α-syn in Brain

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