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Transmissibility of phosphorylated α-synuclein in the brain of Parkinson's Disease model mice

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Abstract. Parkinson's disease (PD) is a chronic neurodegenerative disease. The pathogenesis of PD is the misfolding and accumulation of the alpha-synuclein protein leading to degeneration and death of dopaminergic neurons in the Substantia nigra. This study focuses on the transmissibility of phosphorylated α-synuclein in the brain of PD model mice. In this study, DAT-IRES-Cre+/-/LSL-SNCA-GFP+/- mice was used as a model, and experimental techniques including frozen section of mouse brain, immunohistochemical staining, and confocal microscopy were used to investigate the transmission of α-synuclein as well as Lewy bodies, which are abnormal aggregates of α-synuclein, in the mouse brain. We conclude that phosphorylated α-synuclein (pSyn) can propagate from the dorsal striatum to the Substantia Nigra Pars Compacta (SNc) through the dopaminergic pathway, resulting in the formation of Lewy bodies in dopaminergic neurons in the SNc, causing neuronal death and thus leading to the pathogenesis of Parkinson's disease.

Keywords: Parkinson's disease, dopaminergic neurons, phosphorylated α-synuclein, Lewy bodies.

1. Introduction

Parkinson's Disease is a globally prevalent neurodegenerative disease. The main manifestations of PD are motion disorders including tremor, abnormal gait, bradykinesia, balancing disorders, and autonomic dysfunction [1], as well as neuropsychiatric disorders including olfactory deficits, visual hallucinations, and dementia [3]. In recent years, the incidence of PD has manifested growth globally, increasing by 118% between 1990 and 2015 [4]. In 2040, nearly 17.5 million people are expected to be living with the disease globally [5].The prevalence of PD poses a serious threat to the health of aging populations around the globe; however, the mechanisms of pathogenesis of Parkinson's disease have not yet been fully elucidated and effective treatment not yet made widely available.

Lewy bodies, which are abnormal aggregates of α-synuclein found in neurons, are key pathological hallmarks of PD. In healthy neurons, α-synuclein exists in discrete monomeric structures that can bind to phospholipids and are believed to function for the transport of lipids [6]. However, α-synuclein monomers can aggregate abnormally to form oligomers. Oligomers aggregate to form amyloid fibrils and eventually form Lewy bodies [6]. Phosphorylation is considered as an essential factor contributing

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to the abnormal aggregation of α-synuclein: it has been discovered that phosphorylation of serine 129 promotes α-synuclein fibrillation and is commonly found in synucleinopathies [7].

Dense dopaminergic neurons (DA) are present in the Substantia Nigra Pars Compacta (SNc), which play an important role in regulating motor functions in the body. The formation of Lewy bodies in DA can lead to neuronal lesions and death, consequently impairing bodily motor functions and leading to the movement disorders commonly seen in PD [8]. It has been found that phosphorylated α-synuclein (pSyn) can propagate from the dorsal striatum to the SNc brain region through the dopaminergic pathway, resulting in the formation of Lewy bodies in dopaminergic neurons in the SNc brain region, causing neuronal lesions [9]. In this study, we verify the transmissibility of pSyn in the brain using a Parkinson's disease model mouse, and develop a method for fluorescence labeling of pSyn via genetic modification which can potential allow for live-cell study of Lewy bodies.

2. Method and Materials

2.1. Experimental design

In this study, we designed DAT-IRES-Cre+/-/LSL-SNCA-GFP+/- model mice based on the Cre-LoxP recombination system. The DAT promoter is specifically expressed in dopaminergic neurons, allowing the Cre-LoxP recombinant system to be expressed only in these cells. Cre recombinase knocks out the terminator in the LSL (LoxP-stop-LoxP) sequence, allowing human-derived α-synuclein (SNCA) and green fluorescent protein (GFP) to be expressed in the dopaminergic neurons of model mice, achieving green fluorescent labeling of α-synuclein. The F1 generation mice were knocked in with DAT-IRES-Cre gene sequence and LSL-SNCA-GFP gene sequence with adenovirus vectors, respectively. First-generation mice were bred to produce second-generation DAT-IRES-Cre+/-/LSL-SNCA-GFP+/- model mice, in which the dopaminergic neurons specifically express human pSyn with GFP label.

Model mice were injected with in vitro induced fibrous pSyn (PFF) in the dorsal striatum (CPu) via brain stereotactic injection and the brain tissues were observed six months post-injection. It is hypothesized that the injected PFF would propagate from the CPu to the SNc via synaptic junctions, producing Lewy body-like structures in both brain regions.

2.2. Experiments

2.2.1. Mouse whole brain sampling. Prepare cardiac perfused mice, scalpel, scissors, blunt tweezers, aluminum foil, centrifuge tube, PFA solution, and 30% sucrose solution.

Remove the head of the mouse intact from behind the ears and remove all tissues around the skull. Holding the scalpel diagonally, make scratches along the midline of the bone suture and the posterior lambdoidal suture. Open a small slit in the skull at the lambda by holding the tweezer horizontally. Pry open the skull behind the posterior lambdoidal suture and to the left and right of the midline of the bone suture to expose the brain. Separate brain tissue from skull. Immerse brain tissue in PFA solution and freeze overnight. Replace PFA with 30% sucrose solution and keep frozen away from light for 1 to 2 days.

2.2.2. Mouse brain frozen section (CPu+SNc). Prepare mice brain tissue, 24-well plate, slides, sample tray, blades, frozen sectioning machine, glass needle, OCT solution, and PBS buffer.

Prepare an OCT plate on the sample tray. Remove brain tissue from sucrose solution and trim off some tissue at the back of the brain until the anterior-posterior midline and dorso-ventral axis are perpendicular to the horizontal surface of a slide. Place the trimmed mouse brain upright on the sample tray so that the dorso-ventral axis is at a 90˚ angle to the groove on the sample tray. Apply OCT solution on the brain sample and place it in a frozen slicer for 10 minutes to allow the solution to solidify. Repeat until the sample is completely covered in OCT. Fix sample tray on the operation platform of the frozen sectioning machine and begin sectioning brain tissue. After cutting through the olfactory bulb, lower the

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glass stopper to start collecting brain slices. Immerse brain slices in PFA solution. Repeat until only the cerebellum remains. Store brain slices on ice and protected from light.

2.2.3. Immunohistochemical staining and DAPI staining of mouse brain sections. Prepare glass needle, 24-well plate, slide, forceps, pipette, shaker, aluminum foil, collected brain slices (CPu+SNc), sigma PBS flakes, ddH2O, triton-X-100, goat serum, PSYN primary antibody (rabbit source) master mix, secondary antibody (goat source) master mix, and 1mg/ml DAPI master mix.

Dissolve sigma flake PBS into ddH2O (1 flake:200ml) to obtain PBS solution. Dilute triton-X-100 into PBS solution (7:1000) to obtain PBST solution. Mix goat serum into PBST solution (1:10) to obtain containment solution. Inject containment solution into 24-well plates. Immerse brain slices in containment solution, protected from light overnight on a shaker at 120rpm, 4˚C. Dilute the primary antibody master mix into containment solution (1:2000) to obtain primary antibody dilution. Transfer brain slices into the primary antibody dilution using a glass rod, protect from light and shake at 120rpm at room temperature for 6 hours. Wash three times with PBST. Dilute the secondary antibody master mix into containment solution (1:300) to obtain secondary antibody dilution. Transfer brain slices into secondary antibody dilution and shake at 120rpm at room temperature for 2 hours. Wash three times with PBST. Dilute DAPI master mix into PBS (1:5000) to obtain DAPI solution. Transfer brain slices into the DAPI solution and shake at 120rpm at room temperature for 15 minutes. Wash with PBST for 15 minutes.

3. Results

Olympus VS 120 fluorescence tenfold microscope and LSM 180 laser scanning confocal microscope were used to observe the brain sections of PD model mice. Fluorescence microscope was used to observe the distribution of pSyn in brain tissue of model mice. Laser scanning confocal microscopy with an imaging magnification of 63× was used to observe the morphological features of pSyn.

Fluorescence tenfold scanning results of immunohistochemically stained pSyn signals in the CPu and SNc in the brain tissue of model mice show the presence of pSyn in these brain regions. Green florescence also show that pSyn is present in dopaminergic neurons in the SNc brain region, suggesting that Lewy body-like structures are able to form in dopaminergic neurons.

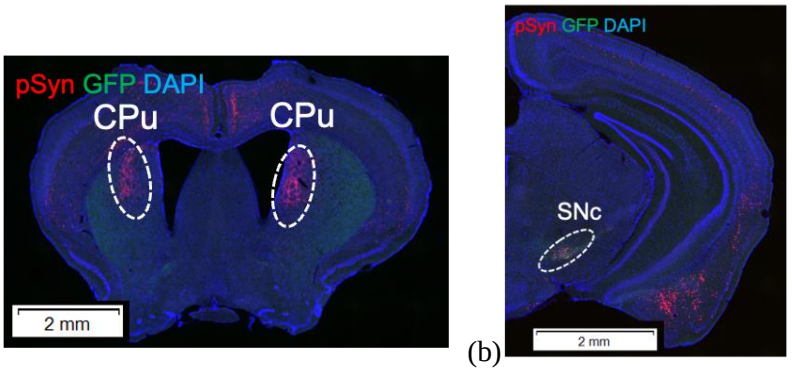

Figure 1. Three-channel scanning results with Olympus VS 120 fluorescence tenfold microscope. Red fluorescent signal is immunohistochemical staining of pSyn, green fluorescent signal is GFP-labeled pSyn, and blue fluorescent signal is DAPI stained nuclei. (a) Strong red fluorescent signal is present near the PFF injection site in the CPu and some red fluorescent signals are present in the cerebral cortex. (b) Intense red and green fluorescent signals are present in the SNc. Red fluorescent signals indicate presence of pSyn in the SNc and green fluorescent signals indicate the presence of pSyn in the dopaminergic neurons in the SNc.

On the basis of fluorescence tenfold scanning imaging demonstrating the presence of pSyn in the CPu and SNc, the CPu and SNc were observed with a 63× laser scanning confocal microscope. Red

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channel imaging results demonstrate that Lewy body-like structures are present in large numbers in neurons in both brain regions and pSyn has a denser distribution in the CPu compared to the SNc. Red channel imaging reveals that pSyn aggregates show a distinct fiber-like, irregular shape at the edges. Green channel imaging demonstrates great number of green fluorescent signals in the SNc, indicating that pSyn is widely present in dopaminergic neurons. Co-localization of green and red fluorescent signals are highly present in the SNc but limited in the CPu, demonstrating that GFP can specifically label pSyn in dopaminergic neurons of the model mice and indicating the presence of Lewy body-like structures in dopaminergic neurons.

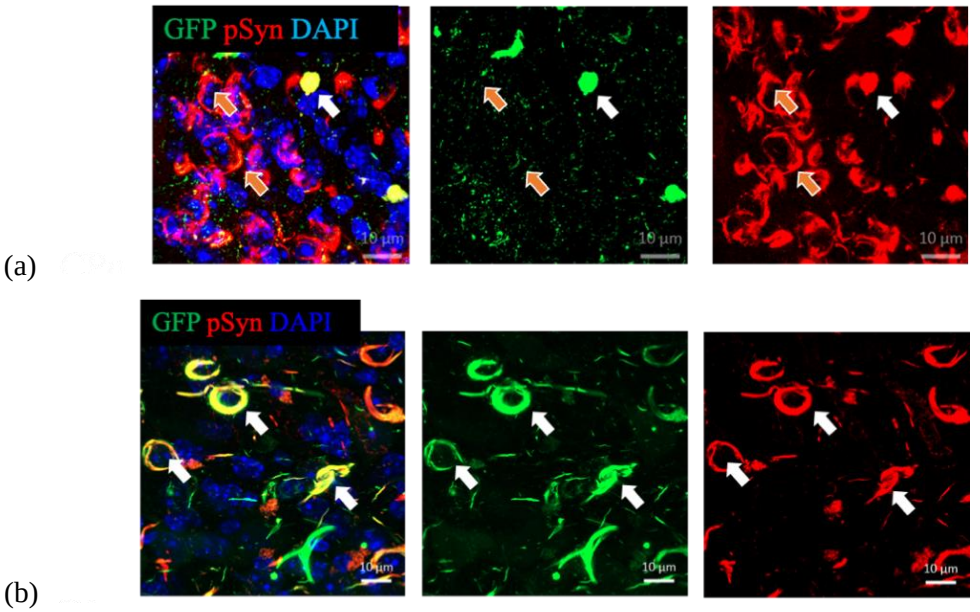

Figure 2. Scanning results with LSM 180 laser scanning confocal microscope. Red fluorescent signal is immunohistochemical staining of pSyn, green fluorescent signal is GFP labeled pSyn, blue fluorescent signal is DAPI cell nucleus staining, and yellow signal is co-localization of immunohistochemical staining and GFP labeling. (a) Laser confocal scanning of CPu of model mice. Red florescent signals are abundant. In the left panel, there are few yellow co-localized signals, and in most cases, there are only red fluorescent signals without overlapping green fluorescent signals. (b) Laser confocal scanning of SNc brain region of model mice. pSyn signals are abundant in the SNc. Green fluorescence indicates presence of pSyn in dopaminergic neurons. Co-localization signals indicate precise labeling of pSyn in dopaminergic neurons.

4. Discussion

After six months of in vitro induced synthesis of PFF via brain stereotactic injection into the CPu brain region of PD model mice, pSyn aggregates were present in the CPu, SNc and cerebral cortex of the mice brain. Thus, in vitro synthesis and localized injection of fibrillar α-synuclein into brain tissues induced the aggregation of α-synuclein oligomers in healthy neurons. Moreover, pSyn signals in the SNc brain region and the cerebral cortex indicate that pSyn can propagate from the CPu brain region to the SNc and the cortex through synaptic junctions, leading to the formation of Lewy bodies in these brain regions. Green fluorescent labeling of pSyn specifically expressed in dopaminergic neurons further elucidate that intracerebral propagation of pSyn could lead to the formation of Lewy bodies in dopaminergic neurons in the SNc, resulting in neuronal pathology and death. Distribution of pSyn in brain suggests that Lewy bodies form in immediate vicinity of injection site of PFF and Lewy body-like structures will gradually form in other brain regions via neural pathways.

In this study, we confirm that the GFP tag can label pSyn in dopaminergic neurons in the brain tissues of DAT-IRES-Cre+/-/LSL-SNCA-GFP+/- model mice. This method can be used for in vivo imaging

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analysis of Lewy bodies and thus for study of the formation of Lewy bodies and the molecular mechanisms leading to pathogenesis of dopaminergic neurons. A potential limitation of this method is that fluorescent signal of GFP and immunohistochemical staining of pSyn are not completely overlapped in the SNc, indicating certain range of error in GFP fluorescence labeling in dopaminergic neurons.

5. Conclusion

In summary, we conclude that phosphorylated α-synuclein (pSyn) can propagate from the dorsal striatum to the SNc brain region through the dopaminergic pathway, resulting in the formation of Lewy bodies in dopaminergic neurons in the SNc brain region, leading to the development of Parkinson's disease. The present study reveals the transmissibility of phosphorylated α-synuclein between the brain regions of PD model mice, further elucidating the mechanism of pathogenesis of Parkinson's disease.

6. Ethical statement

I guarantee that the information provided is true, and that the ethical norms for scientific research on experimental animals, national or municipal regulations on experimental animals, and the rules and regulations of the institution and its operational practices are complied. I guarantee the scientific rationality of the use of experimental animals in this program, the principle of minimizing and optimizing the use of animals, the correct use of anesthetics, the protection of the welfare of experimental animals and scientific research ethics, and the realization of the scientific research value of animals. I assure that this study properly uses and handles laboratory animal hazardous factors and biological materials.

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