

The Transmission of Pathological α -Synuclein

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Abstract: Parkinson's disease (PD) is a chronic neurodegenerative disorder. The main pathogenesis is the degeneration and death of dopamine neurons in the substantia nigra caused by a large accumulation of α -synuclein (Syn) and incorrect protein folding. Parkinson's disease is characterized by abundant α -synuclein neuronal inclusions, while the stereotypical progression of α -synuclein pathology through the brain over time suggests that there may be a physical transmission of pathological α -synuclein from one area of the brain to another. In this study, our goal is to prove the transmission of pathological α -synuclein by comparing brain slices of mice in different stages. Genetic type #24 KI-SNCA-tdT (+/-) mice were used as the model, freezing mouse brain sections (CPu+SNC brain region), immunohistochemical staining of mouse brain sections, laser copolymerization microscopy and other experimental techniques were used. We focus on phosphorylated alpha-synuclein and abnormal aggregation of fibrous alpha-synuclein lewy bodies. Finally, we concluded that phosphorylated alpha-synuclein (pSyn) could be transmitted from the Dorsal striatum to the SNC brain region via a neural circuit consisting of dopamine neurons. This leads to the formation of Lewy bodies in dopamine neurons in the SNC brain region, causing neuropathy that leads to Parkinson's disease.

Keywords: Parkinson's disease, α -synuclein, Lewy Body

1. Introduction

Parkinson disease is a neurodegenerative disorder which is able to be characterized and verified by typical clinical symptoms include motor deficits such as rigidity, slowness in movement (bradykinesia), postural instability and a characteristic tremor at rest [1]. The main pathological manifestations are the progressive degeneration and loss of dopamine neurons in the substantia nigra compact area of the midbrain, and the appearance of enormous alpha-synuclein accumulation, which is known as Lewy Body in the neuron cytoplasm. PD now is the second common neurodegenerative disorder that affects 2-3% of the population, especially the old people. The current treatment methods for PD mainly improve relieve and improve symptoms via drugs, but they cannot stop its gradual deterioration. Therefore, it is necessary to attach great importance to PD. The article aims to study that formation of LBs induces Parkinson's disease (PD) in the wild-type mouse model. Accordingly, to elucidate the role of Lewy bodies in the pathogenesis of Parkinson disease once again.[2]

Parkinson's disease represents a progressive neurodegenerative disorder characterized by the degeneration of midbrain neurons due to the accumulation of Lewy bodies primarily composed of the protein α -synuclein, [1] especially the number of nigral dopamine neurons are greatly diminished, then Lewy bodies are present in the remaining neurons. Now PD also be considered as a more complex clinicopathological entity, include the widespread α -syn pathology. Lesions normally occur in several stages, from dorsal motor nucleus of the glossopharyngeal to vagal nerves, anterior olfactory nucleus, then to nuclear grays and cortical areas, finally the cortical involvement: anteromedial temporal mesocortex. [3] The main clinical features of Parkinson's disease are tremor at rest, rigidity, akinesia and postural instability. [4] Bradykinesia (slowness of movement) can also happen, it caused by the basal ganglia's inability to strengthen the cortical processes responsible for initiating and carrying out movement commands. Other features include reduced facial expression (hypomimia), less frequent blinking, monotonous and low-volume speech, and drooling caused by a decrease in spontaneous swallowing. Slower reaction times and premovement cortical excitability increase suggests the abnormal of stored motor commands. [5] Some of the features such as postural instability is non-specific and is usually absent in early disease [6].

α -Synuclein is an intracellular protein localized in the presynaptic terminals, but under pathological conditions, LBs formed in the soma of affected neurons. PD begin in the dorsal IX/X motor nucleus of

the medulla and propagate up through the brainstem, then to cortical regions. [7] Embryonic mesencephalic neuron grafts in PD patients develop LBs many years after grafting indicate the transmissibility of pathologic α -Syn. The experiment shows that the pSyn-positive LB-like accumulations were exclusively ipsilateral to the injection site, with the exception of the amygdala, to which the striatum connects bilaterally shows cell-to-cell transmission followed interneuronal connectivity. [8] These are all the evidence shows the transmission of α -Synuclein. Distinct patterns of α -synuclein pathology within the brain are associated with varying disease symptoms. During the early stages of Parkinson's disease, when motor dysfunction manifests in patients, α -synuclein is localized in the motor-regulating brain regions. As the disease progresses and cognitive function becomes impaired, α -synuclein pathology is observed in brain areas responsible for higher cognitive processing. [7] The spatial distribution of α -synuclein within the brain changes over the course of the disease, correlating with the development of different bodily dysfunctions in patients. These findings suggest the possibility of α -synuclein transmission within the human brain. The emergence of Lewy bodies in patients with Parkinson's disease who received embryonic mesencephalic neuron grafts, several years post-grafting, provides compelling evidence supporting the transmissibility of pathological α -Syn in the disease. [8] Our study aims to establish the propagation of pathological α -synuclein, elucidating the brain sample preparation method, and analyzing the figures through confocal laser scanning.

2. Materials and Methods

2.1 Animal Culture

We used the mouse with the genetic type #24 KI-SNCA-tdT(+/-), and inject alpha-synuclein in the right PFF and Cpu. (α Syn PFF in CPu (R)

2.2 Exposing the heart and Brain isolation

The 20% Urethane was used to anesthetize mice. Perfuse transcardially (left ventricle) with saline water (18g of sodium chloride dissolved in 2L of deionized water) until completely exsanguinated in 5.5rpm, then fix tissue by perfusing with 4% paraformaldehyde in the same rate. Remove brain and place in 4% paraformaldehyde for 24 hr, followed by 30% sucrose solutions at 4°C. [5]

2.3 Brain slicing preparation [9][10]

2.3.1 Dehydration

Transfer the brain sample to a 15 mL tube filled with 30% sucrose solution, place it at 4 °C until it sinks.

2.3.2 Cryosectioning

We section brain sample into 40 μ m thick, prepare a 24-well plate filled with PBS, then collect each five sections of brain sample into one well.

2.4 Immunohistochemistry

The immunohistochemistry technique has been used for the brain slide making. We dissolved one tablet of sigma phosphate buffered saline in 200ml deionized water to make PBS solution, then dissolved 0.7% Tritonx-100 in the PBS solution just made to produce PBST solution. The blocking buffer used is made by goat serum diluted in PBST. Primary antibody is rabbit serum 1:2000 dilute with blocking buffer. Secondary antibody is goat anti-rabbit 1:500 dilute blocking buffer. The DAPI we used is 1mg/ml 1:5000 diluted in PBS solution.

2.5 Brain slice scanning

2.5.1 10 x lens scanning

Using Olympus optical microscope and VS-ASW-S6 software, the holes were selected in fluorescence scanning mode to maintain the loading slide and named. Select the 10x magnification and set the lighting channel and exposure time: FL Blue 10ms, FL Red 50ms, FL Green 50ms. Then check the focus, and finally scan and save.

2.5.2 Confocal laser scanning of brain section

The sample sections were inverted under the microscope, the objective lens was selected using the manual control panel, and the sample was observed under the bright field light source using a halogen lamp to find the focal plane and locate it. Select the fluorescent color filter, switch to fluorescence observation, and set the scanning mode, speed, laser output wavelength, dye, and other parameters in the software. Finally, set the objective lens to 63X Oil, adjust the parameters, and scan.

3. Results

Figure 1 is the mice brain section after a single unilateral injection α -Syn PFFs into the CPu area. There are some very bright parts, according to the image of alpha synuclein, these parts are where more α -Syn gathers. In the figure of tdT scanning the distribution of P- α Syn is most around one point, also some of them locate in the upper left corner. The brighten part of DAPI scanning result also represent the distribution of α -Syn.

We can see from figure 1 that most α -Synuclein are accumulate around one point, which is the injection point, and as the distance increased from the injection point, the accumulation of α -Synuclein is decrease. From this we draw conclusions that the dose of PFF was positively correlated with the accumulation of α -Synuclein.

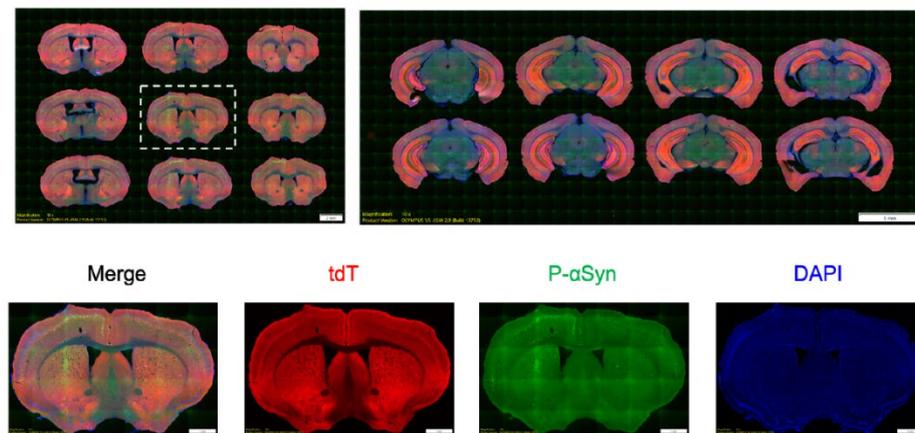


Figure 1. Mice brain section after a single unilateral injection α -Syn PFFs into the CPu area

In figure 2 we can clearly see that the brighten part is α -Synuclein, and the accumulation of α -Synuclein is appear as a fibrous condition.

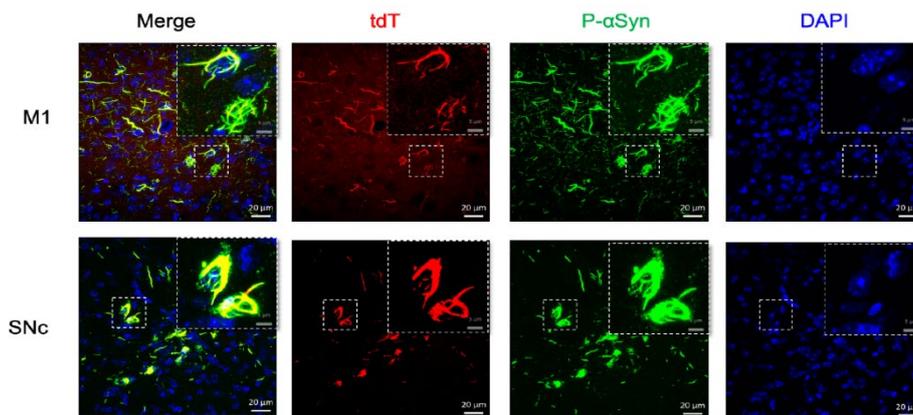


Figure 2. Confocal laser scanning microscope under the magnitude of 63X

Figure a and b shows the structure and different area of the brain, we can see that α -Synuclein distribution change from around CPu area (injection point) to SNc area, therefore we prove the transmissibility of α -Synuclein within the brain. By comparing figure 3 (c) and (d), Figure c and d shows the image we get from Olympus light microscope under the magnitude of 10X.

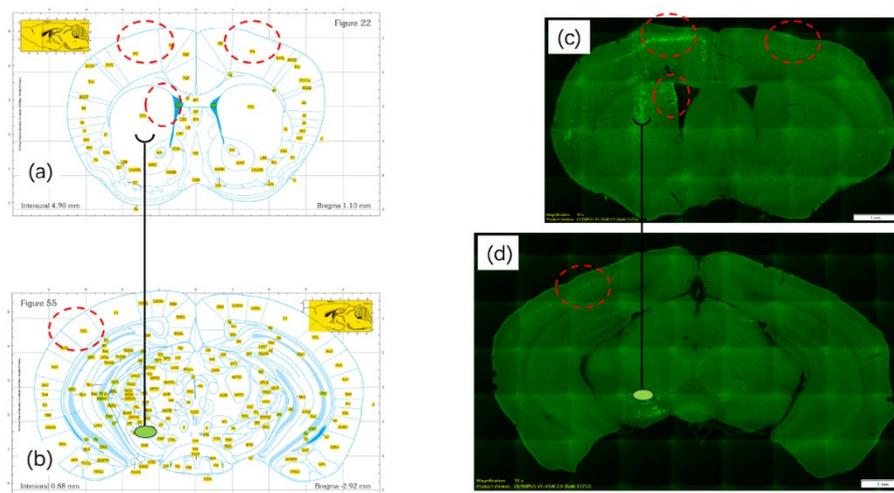


Figure 3. Olympus light microscope under the magnitude of 10X

4. Conclusion

In this article, our study aimed to demonstrate the spread of pathological alpha-synuclein by illustrating the method of brain sample preparation and scanning and analyzing the images. The experiment showed that the dose of PFF was positively correlated with the formation of α -Synuclein aggregates. Through confocal microscopy (63X), the fine structure of α -Synuclein aggregates showed a fibrous aggregation. We demonstrated that injection of exogenously expressed PFF into the brain region of mouse CPU could form α -Synuclein aggregates in the brain of mice.

Through the SNc-CPu loop, we verified the propagation possibility of α -Synuclein aggregates in the brain. KL-SNCA-tdT \pm mouse is an effective animal experimental model for reporting the location of α -Synuclein aggregates, and it can be applied in vivo imaging, cell sequencing, electrophysiology, metabolic detection and other fields in the future.

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