Association Between Decreased Srpk3 Expression and Increased Substantia Nigra Alpha‑Synuclein Level in an MPTP-Induced Parkinson’s Disease Mouse Model

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Abstract
Parkinson’s disease (PD) is the second most common neurodegenerative disorder and is caused by the loss of dopaminergic neurons in the substantia nigra (SN). However, the reason for the death of dopaminergic neurons remains unclear. An increase in α-synuclein (α-syn) expression is considered an important factor in the pathogenesis of PD. In the current study, we investigated the association between serine/arginine-rich protein-specific kinase 3 (Srpk3) and PD in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model and in SH-SY5Y cells treated with 1-methyl-4-phenylpyridinium (MPP+). Srpk3 expression was significantly downregulated, while tyrosine hydroxylase (TH) expression decreased and α-syn expression increased after 4 weeks of MPTP treatment. Dopaminergic cell reduction and α-syn expression increase were demonstrated by Srpk3 expression inhibition by siRNA in SH-SY5Y cells. Moreover, a decrease in Srpk3 expression upon siRNA treatment promoted dopaminergic cell reduction and α-syn expression increase in SH-SY5Y cells treated with MPP+. These results suggested that Srpk3 expression decrease due to Srpk3 siRNA caused both TH level decrease and α-syn expression increase. This raises new possibilities for studying how Srpk3 controls dopaminergic cells and α-syn expression, which may be related to PD pathogenesis. Our results provide an avenue for understanding the role of Srpk3 in dopaminergic cell loss and α-syn upregulation in SN. Furthermore, this study supports a therapeutic possibility for PD in that the maintenance of Srpk3 expression inhibits dopaminergic cell reduction.

Keywords Parkinson’s disease · Srpk3 · α-Synuclein · Dopaminergic neuron · Tyrosine hydroxylase

Introduction
Parkinson’s disease (PD) is the second most common neurodegenerative disorder after Alzheimer’s disease. It is characterized by behavioral symptoms such as tremors, bradykinesia, akinesia, and stiffness. The cardinal motor symptoms of PD are caused by the loss of dopaminergic neurons in the substantia nigra pars compacta [1, 2]. Most causes for the death of dopaminergic neurons remain unclear, although some of them are known including risk genes [3, 4], neuroinflammation [5, 6], pesticides [7, 8], and heavy metals [9, 10]. Neurodegeneration in PD is correlated with the occurrence of Lewy bodies, which are intracellular inclusions containing aggregates of the disordered protein α-synuclein (α-syn) [11]. An increase in α-syn expression is considered an important factor in the pathogenesis of PD.

In this study, altered gene expression levels in SN cells were analyzed in a PD mouse model treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce dopaminergic cell reduction [12]. Through the application of a polymerase chain reaction-based expression profiling automated system, which has high sensitivity and reliability [13], several genes with altered expression were detected in the neurodegenerative state caused by MPTP injection. Furthermore, serine/arginine-rich protein-specific kinase 3 (Srpk3) was significantly downregulated after 4 weeks of MPTP treatment.

Srpk3, which phosphorylates serine/arginine repeat-containing proteins, belongs to the serine/arginine...
protein kinase family and is regulated by a muscle-specific enhancer, MEF2 [14]. Srpk3 is expressed in the lungs, skin, spleen, heart, joints, muscles, and brain. This gene encodes a protein kinase specific to the serine/arginine-rich domain (SR domain) family of splicing factors [14]. A similar protein plays a role in muscle development [14].

These intriguing observations led to the hypothesis that Srpk3 downregulation may be related to PD. In PD pathogenesis, dopaminergic cell reduction, which is related to an increase in α-syn expression in mice intoxicated with MPTP, could be affected by changes in Srpk3 expression. To verify this hypothesis, an experiment was performed to show changes in Srpk3 expression in a chronic MPTP-induced PD mouse model, in which tyrosine hydroxylase (TH) expression decreased and α-syn expression increased. Moreover, changes in the expression of dopaminergic cells and α-syn were investigated by siRNA-mediated inhibition of Srpk3 expression in SH-SY5Y cells. Therefore, the principal finding of this study was that changes in Srpk3 expression promoted changes in dopaminergic cell and α-syn expression in the SN regions of mice intoxicated with MPTP, whereas a decrease in Srpk3 expression upon siRNA treatment promoted changes in dopaminergic cell and α-syn expression in SH-SY5Y cells treated with MPP+.

In this regard, our research aimed to investigate the association between Srpk3 and PD and to provide insights into the role of Srpk3 during dopaminergic cell loss and α-syn expression increase in the SN. Furthermore, this study could support a therapeutic possibility for PD in that changes in Srpk3 expression affect dopaminergic cell reduction.

Materials and Methods

MPTP Model of PD

Six-week-old male C57BL/6 mice (20–22 g; DBL, Korea) were divided into two groups: the control group (CTL) and MPTP-intoxicated group (MPTP). Mice in the CTL group (n = 9) were injected with 0.9% (100 μL) saline intraperitoneally once daily for 4 weeks, and mice in the MPTP group (n = 9) were injected with MPTP-HCl (20 mg/kg of free base) in 0.9% (100 μL) saline intraperitoneally once daily for 4 weeks to produce a sustained chronic PD mouse model [15]. On the day after the final MPTP treatment, the mice were anesthetized using Alfaxan and perfused with 0.05 M sodium phosphate buffer (PBS). The Sangji University Animal Experimentation Committee approved all the animal protocols used in this study. The guidelines for animal experiments were followed. All reagents used in the study were purchased from Sigma (USA), unless otherwise stated.

Rotarod Test

To assess the motor ability of mice in CTL and MPTP groups, rotarod tests were conducted before the last MPTP injection. Training was performed in the second week for 15 min at 30 rpm for 2 days, once a day. The diameter of the rotarod treadmill was 28 mm, and the test was conducted in an accelerated mode for 4 min from 10 to 50 rpm in a 5-min running time. A total of 50 rpm was maintained for 1 min until completion after 4 min of accelerated mode. The time until the first fall was measured.

Pole Test

To evaluate the motor ability, the pole test was conducted before the last MPTP injection. A wooden vertical pole with a length of 548 mm and a diameter of 8 mm was used in the pole test. The time the mouse took while moving from the top to the bottom of the pole was measured. Training was performed in the second week for 2 days, once a day.

RNA Extraction and Microarray Analysis

Total RNA was extracted from the bilateral SN using the RNeasy Plus Mini Kit (QIAGEN, USA). Isolated RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA). An aliquot of total RNA was subjected to an Affymetrix GeneChip® Mouse Gene 1.0 ST Array (Affymetrix, USA) according to the GeneChip Whole Transcript Sense Target Labeling Assay manual [16, 17]. T7-(N)6 primers and poly-A RNA control were mixed with 300 ng of total RNA isolated from the SN of the mice in each group. First-strand complementary DNA (cDNA) and second-strand cDNA were synthesized in the first cycle. In the second cycle, single-strand cDNA (ss cDNA) was synthesized and cleaned. Then, the ss cDNA was fragmented and labeled. The labeled ss cDNA was hybridized to the GeneChip. The GeneChip was stained, washed, and scanned using Fluidics 450 station and the GeneChip Operating Software (GCOS, Affymetrix).

Microarray Data Analysis

Quality control of the scanned data was confirmed using the Expression Console software (Affymetrix). Microarray data were analyzed using GenPlex ver. 3.0 (ISTECH, Korea). The mean signal intensities of the genes were obtained from two chips for each group. Following normalization, the genes that satisfied the conditions of
Student’s t-test significance criterion \((p < 0.05)\) and the fold change cutoff \([2]\) were identified using the differentially expressed genes (DEG)-finding module.

**Cell Lines and Cultures**

The neuroblastoma cell line SH-SY5Y was maintained under standard cell culture conditions \((37 \, ^\circ \text{C}, 5\% \, \text{CO}_2)\) in minimum essential medium (MEM; Welgen, Namcheon-myeon) containing 10% fetal bovine serum (BioWhittaker), 0.1 mM nonessential amino acids, 100 U/mL penicillin, and 100 mg/mL streptomycin \((n = 3)\).

**siRNA Knockdown**

Stealth siRNA against \(\text{Srpk3} \) \((5\text{-GAA AUC UGC CUG UUU GUU U-3})\) and negative control duplexes \((\text{i.e., scrambled siRNA against Srpk3}) \ (5\text{-UUC UCC GAA CGU GUC ACG UTT-3})\) were purchased from Bioneer Inc. (Korea).

**siRNA Transfection**

SH-SY5Y cells were incubated in Opti-MEM (Gibco, USA) for at least 1 day before siRNA transfection. The transfection reagent \((\text{Promega, USA})\) and \(\text{Srpk3} \) siRNA \((3.5:1)\) were used for transfection when the density of SH-SY5Y cells was 60%. Transfection was continued for 24 h.

**MPP+ Treatment**

SH-SY5Y cells were treated with 500 \(\mu\text{M MPP+ iodide (Sigma-Aldrich)}\) for 18 h \([18]\).

**Immunohistochemistry**

After 4 weeks, the brains of the mice were resected, fixed in PBS containing 4% paraformaldehyde for 12 h at 4 \(\, ^\circ \text{C}\), rinsed with PBS, immersed in 30% sucrose solution for 12 h at 4 \(\, ^\circ \text{C}\), and then cryo-sectioned. Brain sections were cut using a cryomicrotome \((30\, \mu\text{m thickness})\). Immunohistochemical analyses were performed using an ABC kit and a Mouse on Mouse (M.O.M) immunodetection kit \((\text{Vector Laboratories, CA})\) by a modification of the avidin–biotin–peroxidase method. Briefly, sections encompassing the entire striatum \((\text{ST})\) and SN were incubated in PBS with 3% \(\text{H}_2\text{O}_2\), exposed to 3% bovine serum albumin and 0.3% Triton X-100 for 1 h, and treated with an avidin/biotin blocking kit \((\text{Vector Laboratories})\). Brain sections were treated with an M.O.M. mouse Ig blocking reagent \((\text{Vector Laboratories})\) for 1 h at room temperature, prior to incubation with the primary antibody. The sections were stained overnight with mouse anti-TH \((1:2000; \text{Santa Cruz Biotechnology, USA})\), rabbit anti-\(\alpha\)-syn \((1:500; \text{Santa Cruz Biotechnology, USA})\), rabbit anti-\(\alpha\)-syn \((1:500; \text{Santa Cruz Biotechnology, USA})\), antibodies at 4 \(\, ^\circ \text{C}\). The sections were incubated with biotinylated anti-mouse IgG, followed by an avidin–biotin–peroxidase complex, and then developed using a diaminobenzidine-hydrogen peroxide solution \((0.03\% \, \text{hydrogen peroxide and 0.003}\% \, 3,3\text{-diaminobenzidine in 0.05 M Tris, pH 7.0})\).

**Immunofluorescence**

Cells were mounted on glass and treated using the same procedures as those used for immunohistochemistry. The cells were incubated with primary antibodies: mouse anti-\(\alpha\)-syn \((1:500; \text{BD Biosciences, USA})\), rabbit anti-Srpk3 \((1:1000)\), and mouse anti-TH \((1:2000)\). The sliced brain sections were treated with mouse anti-\(\alpha\)-syn \((1:500)\) and rabbit anti-Srpk3 \((1:1000)\) in a floating state. Cells and brain sections were treated with an avidin/biotin blocking kit and M.O.M. mouse Ig blocking reagent \((\text{Vector Laboratories})\), followed by staining with anti-\(\alpha\)-syn and anti-Srpk3 IgG at 4 \(\, ^\circ \text{C}\) overnight. Each group was treated with biotinylated anti-mouse IgG and anti-rabbit IgG followed by incubation with fluorescein avidin D and rhodamine avidin D \((\text{Vector Laboratories})\). Photographic documentation was performed using a Nikon X-Cite-Series 120 Q microscope \((\text{Nikon, Japan})\). Images were captured using an ECLIPSE Ni-U microscope \((\text{Nikon, Japan})\). Images were acquired using the imaging software NIS-Elements F ver. 4.00.00 \((\text{Nikon})\). Tiff images and quantification data were collected from each image data file using the ImageJ software \((\text{NIH, USA})\).

**Western Blotting**

The ST and SN regions were homogenized in radioimmunoprecipitation assay buffer on ice for 30 min. After centrifugation at 12,000 rpm for 20 min, the soluble supernatant samples were collected, and equal concentrations of protein samples were separated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes \((\text{Bio-Rad, USA})\). The membranes were blocked with 5% skim milk in 0.1% Tris-buffered saline \((\text{TBS})\) for 1 h at room temperature and incubated with mouse anti-TH \((1:2000)\), rabbit anti-\(\alpha\)-syn \((1:500)\), rabbit anti-Srpk3 \((1:2000)\), and mouse anti-\(\beta\)-actin \((1:5000; \text{Santa Cruz Biotechnology})\) antibodies. Thereafter, the membranes were washed with 0.1% TBS containing 0.1% Tween-20 \((\text{TBST})\) and incubated with an anti-mouse IgG-peroxidase antibody \((1:2000; \text{Bio-Rad})\), and the antigen–antibody complexes were visualized using Pierce ECL western blotting substrate \((\text{Thermo Scientific, USA})\). The percentage of densitometric values for the immunoreactive bands was calculated for each experiment. The density was measured using the ImageJ software \((\text{NIH})\).
Statistical Analysis

Statistical analyses were performed using analysis of variance (ANOVA) for three or four groups and Student’s t-test for two groups using SPSS 26 (SPSS Inc., USA). In the ANOVA test, Tukey HSD, Scheffe, or Bonferroni was performed as a post hoc analysis. All values are presented as the mean ± standard error.

Results

Changes in TH and α-syn expression were evaluated to verify the accuracy of the MPTP-induced PD mouse model. The TH levels were noticeably lower in the MPTP group (Fig. 1g and h) than in the CTL group (Fig. 1b and c). Western blot analysis also showed a significant decrease ($p < 0.005$) in the TH levels of the ST and SN of the MPTP group (Fig. 1k and l). α-syn expression in the SN regions was noticeably higher in the MPTP group (Fig. 1i and j) than in the CTL group (Fig. 1d and e). Western blot analysis consistently showed a significant increase ($p < 0.005$) in α-syn expression in the MPTP (Fig. 1k and l). The rotarod and pole tests show reduced values in the MPTP group compared to the control group (Fig. 1m and n).

Srpk3 expression levels in the SN regions were noticeably lower in the MPTP group than in the CTL group (Fig. 2). As seen in the immunoblot assay observations, the results of microarray analysis and western immune blot assay indicated that Srpk3 expression was significantly decreased in the MPTP group compared to the CTL group (Fig. 2h).

To investigate the relationship between Srpk3 and α-syn expressions, we conducted experiments in which Srpk3 was co-localized with α-syn in the SN regions of an MPTP-induced 4-week PD mouse model. In the control, Srpk3 expression was found to be co-localized with α-syn. In the MPTP group, co-localization of Srpk3 and α-syn increased compared with that in the CTL group. It is thought that even though Srpk3 level decreased in the MPTP group, increased α-syn expression caused increased co-localization (Fig. 3).

To investigate the relationship between decreased Srpk3 expression and increased α-syn expression, we conducted

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**Fig. 1** Dopaminergic cell and α-synuclein (α-syn) expression in an MPTP-induced parkinsonism mouse model. The substantia nigra (SN; a, b, d, e, f, g, i, and j) and striatum (ST; c and h) were immunostained with an anti-tyrosine hydroxylase (TH; b and g, 200×; a, c, f and h, 40×) antibody and an anti-α-syn (d and i, 100×; e and j, magnified images of square) antibody. Immunoblot analysis (k) showed that the TH expression of SN and ST decreased, whereas the α-syn expression of SN increased in MPTP; these were statistically significant in comparison with the MPTP normalized to 100% of control (l). The rotarod test shows reduced values in the MPTP group compared to the control group (m, n = 6). The pole test shows reduced values in the MPTP group compared to the control group (n, n = 6). CTL, saline control (n = 3); MPTP, MPTP treated (n = 3).

*p < 0.005 and **p < 0.001

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experiments in which Srpk3 expression was knocked down using Srpk3 siRNA in SH-SY5Y cells. Srpk3 siRNA led to a decrease in Srpk3 expression, but an increase in α-syn expression in SH-SY5Y cells (Fig. 4).

To investigate the effect of decreased Srpk3 expression on the PD state, we performed experiments in which Srpk3 expression was knocked down using Srpk3 siRNA in MPP+-treated SH-SY5Y cells. Western blot analysis showed that decreased Srpk3 expression exacerbated TH reduction and increased α-syn expression in MPP+-treated SH-SY5Y cells (Fig. 5). Immunofluorescence analysis of Srpk3 and α-syn revealed co-localization, and Srpk3 expression was stronger in the CTL group (Fig. 6b) than in the MPP+ group (Fig. 6f). Conversely, α-syn was more strongly expressed in the MPP+ group than in the CTL group (Fig. 6c and g). Even in the merged images (Fig. 6d and h), Srpk3 expression was stronger in the CTL group (Fig. 6d), whereas that of α-syn was stronger in the MPP+ group (Fig. 6h). It was also confirmed that TH expression decreased in the MPP+ group (Supplementary Fig. 1).
Discussion

To verify the accuracy of the MPTP-induced PD mouse model, changes in TH and α-syn expression were evaluated. TH levels in the ST and SN regions were noticeably lower in the MPTP group mice than in the CTL group mice, thereby verifying a statistically significant decrease in TH immunoreactivity due to MPTP treatment. Moreover, α-syn levels in the SN regions were noticeably higher in the MPTP group than in the CTL group. In a previous study, MPTP (20 mg/kg) was intraperitoneally administered once daily for 4 weeks to the MPTP group. The immune responses of TH and dopamine transporter were significantly decreased in the ST and SN regions in the MPTP group compared with those in the control group. Accumulated and oligomeric forms of α-syn appear, and these formations are more strongly deposited in the SN region of MPTP brains [15]. Consistent with this, our results showed accurate establishment of the MPTP-induced PD mouse model. MPTP was used in this study observing the change of α-syn in PD based on the fact that parkinsonism was induced by MPTP in humans [19] and that α-syn was also increased by MPTP in primates [20].

Fig. 3 Serine/arginine-rich protein-specific kinase 3 (Srpk3) co-localized with α-synuclein (α-syn) in the substantia nigra of an MPTP-induced 4-week parkinsonism mouse model. The cells were immunofluorescently labeled with anti-Srpk3 (a, e) and anti-α-syn (b, f) antibodies using rhodamine avidin (a, e; red) and fluorescein avidin (b, f; green). The “merged” panels (c, g) show merged images of the left (a, e) and middle (b, f) panels, respectively. The “merged with dapi” panels (d, h) show merged images of the left (a, e) and middle (b, f) panels with dapi, respectively. Note that the yellow-colored regions in merged panels indicate the co-localization of the Srpk3 and α-syn. CTL, saline control; MPTP, MPTP treated. Scale bar = 10 μm.

Fig. 4 Western blot analysis (a) displaying the administration of a serine/arginine-rich protein-specific kinase 3 (Srpk3) short interfering RNA (siRNA), leading to a decrease in Srpk3 expression but increase in α-synuclein (α-syn) expression in SH-SY5Y cells. (b) Histograms of Srpk3 and α-syn expression were shown in comparison to those of the control group, siRNA 10, and siRNA 100. CTL, control siRNA treatment (100 nM for 2 days; n = 3); siSRPK3 10, Srpk3 siRNA treatment (10 nM for 2 days; n = 3); siSRPK3 100, Srpk3 siRNA treatment (100 nM for 2 days). *p < 0.05 and **p < 0.005 compared to CTL.
and western blot assay indicated that *Srpk3* expression significantly decreased in the MPTP group.

To investigate the relationship between *Srpk3* and α-syn, we conducted experiments in which *Srpk3* was co-localized with α-syn in the SN of an MPTP-induced 4-week PD mouse model. In the control, *Srpk3* expression was found to be co-localized with α-syn. In the MPTP group, co-localization of *Srpk3* and α-syn increased compared with that in the CTL group. It is thought that even though *Srpk3* expression decreased in the MPTP group, increased α-syn level caused increased co-localization. In the CTL group, *Srpk3*, which was not co-localized with α-syn, was observed, but not in the MPTP group. This suggests that *Srpk3* was not sufficient to cover the α-syn in the MPTP group.

We conducted experiments in which *Srpk3* expression was knocked down using *Srpk3* siRNA in SH-SY5Y cells. *Srpk3* siRNA led to a decrease in *Srpk3* expression but increased α-syn expression in SH-SY5Y cells. This suggests that a decrease in *Srpk3* expression was associated with an increase in α-syn expression.

To investigate the effect of decreased *Srpk3* expression on the PD state, we conducted experiments in which *Srpk3* expression was knocked down using *Srpk3* siRNA in MPP⁺-treated SH-SY5Y cells, which was similar to the status of PD. Western immunoblot assay showed that the decrease in *Srpk3* expression exacerbated the increase in α-syn level in MPP⁺-treated SH-SY5Y cells. These results suggested that the decrease in *Srpk3* expression due to *Srpk3* siRNA caused both dopaminergic cell reduction and an increase in α-syn level. This raises a new avenue for studying how *Srpk3* controls dopaminergic cells and α-syn expression, which may be related to PD pathogenesis. SRPKs regulate self-aggregation of proteinaceous cytomatrix at the active zone and this regulation is mediated through modulating...
aggregation of coiled-coil domain [21, 22]. Interestingly, it has been reported that α-syn has a structure similar to that of a right-handed coiled-coil [21, 22]. SRPKs are also closely related to E3 ubiquitin ligase pathway [23]. Therefore, it can be inferred that Srpk3 affects the intraneuronal accumulation of α-syn, but it is elusive whether Srpk3 acts on α-syn directly or indirectly. Thus, more research is needed on how Srpk3 regulates α-syn.

Srpk3 phosphorylates its substrates at serine residues located in regions rich in arginine/serine dipeptides [14]. Several types of α-syn phosphorylated at serine have been reported to be involved in the pathogenesis of PD [24]. Based on these previous studies and our results, Srpk3 might be involved in α-syn phosphorylation-related neurodegeneration of dopaminergic neurons. In previous research, striated muscles have been reported to be highly sensitive to Srpk3 expression [25]. Srpk3-null mice displayed a new type 2 fiber-specific myopathy with a significant increase in centrally placed nuclei [25]. Myopathy in Srpk3 mutant mice is characterized by no signs of muscle degeneration or regeneration, such as myofiber disarray, muscle cell death, or neutrophil infiltration [25]. Combining these studies with our results suggests that motor dysfunction in PD may be associated with decreased Srpk3 levels. In a previous study, we reported decreased Srpk3 expression in the muscles of MPTP-induced PD mice, which might be related to the increased expression of α-syn [26]. In this study, we confirmed a decrease in Srpk3 expression at both gene and protein levels in the SN, a major lesion site in PD. The decreased expression of Srpk3 in the brain might be one of the causes of dopaminergic cell degeneration, as siRNA-induced decrease in Srpk3 expression led to an increase in α-syn expression in SH-SY5Y cells. However, it has been reported that Srpk3 is expressed in GABAergic neuron [23]. Therefore, it is necessary to investigate the expression of Srpk3 more in different types of neurons, glial and endothelial cells in that there are few studies on Srpk3.

There has been a report that SRPKs are mutated in neurodevelopmental disorder including intellectual disability disorders [23]. SRPKs may also have potential roles in neurodegeneration and Srpk2, in particular, participates in the disorders [23]. SRPKs may also have potential roles in neurodevelopmental disorder including intellectual disability [14]. Several types of α-syn phosphorylated at serine have been reported to be involved in the pathogenesis of PD [24]. Based on these previous studies and our results, Srpk3 might be involved in α-syn phosphorylation-related neurodegeneration of dopaminergic neurons. In previous research, striated muscles have been reported to be highly sensitive to Srpk3 expression [25]. Srpk3-null mice displayed a new type 2 fiber-specific myopathy with a significant increase in centrally placed nuclei [25]. Myopathy in Srpk3 mutant mice is characterized by no signs of muscle degeneration or regeneration, such as myofiber disarray, muscle cell death, or neutrophil infiltration [25]. Combining these studies with our results suggests that motor dysfunction in PD may be associated with decreased Srpk3 levels. In a previous study, we reported decreased Srpk3 expression in the muscles of MPTP-induced PD mice, which might be related to the increased expression of α-syn [26]. In this study, we confirmed a decrease in Srpk3 expression at both gene and protein levels in the SN, a major lesion site in PD. The decreased expression of Srpk3 in the brain might be one of the causes of dopaminergic cell degeneration, as siRNA-induced decrease in Srpk3 expression led to an increase in α-syn expression in SH-SY5Y cells. However, it has been reported that Srpk3 is expressed in GABAergic neuron [23]. Therefore, it is necessary to investigate the expression of Srpk3 more in different types of neurons, glial and endothelial cells in that there are few studies on Srpk3.

In conclusion, our results demonstrated that Srpk3 expression was decreased in the MPTP-induced PD mouse model, and decreased Srpk3 expression increased α-syn levels in SH-SY5Y cells induced by Srpk3 siRNA. These results suggest an association between Srpk3 and PD and provide an avenue to comprehend the role of Srpk3 during dopaminergic cell loss and α-syn level increase in the brains of PD mice. Furthermore, this study sheds light on the maintenance of Srpk3 as a therapeutic target for controlling α-syn expression in the pathogenesis of PD.

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Author Contribution SY designed the study, interpreted the data, performed the protein assay, prepared several samples for immunoblot and immunohistochemistry, and wrote the manuscript. MS performed immunoblotting and immunohistochemistry, performed the protein assay, prepared several samples for immunoblotting and immunohistochemistry, and interpreted the data. All the authors have read and approved the manuscript.

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Data availability Not applicable

Code Availability Not applicable

Declarations

Ethics Approval Not applicable

Consent to Participate Not applicable

Consent for Publication Not applicable

Competing Interests The authors declare no competing interests.

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