ZKSCAN3 affects the autophagy-lysosome pathway through TFEB in Parkinson's disease

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Abstract. The present study aimed to explore the effects of zinc finger with KRAB and SCAN domains 3 (ZKSCAN3)/transcription factor EB (TFEB) on autophagy-lysosome pathway in Parkinson's disease (PD). SH-SY5Y cells were treated with 6-hydroxydopamine to establish a PD cell model. A ZKSCAN3 overexpression vector and short interfering (si)RNAs were also constructed. The TFEB overexpression vector was transfected into the cells with ZKSCAN3 siRNA and the TFEB siRNA was transfected into the cells with the ZKSCAN3 overexpression vector. Reverse transcription-quantitative and western blotting were performed to detect the expression levels of Beclin-1, LC3II/I, α-synuclein and lysosomal-associated membrane protein 1 (Lamp-1). Lysosomes were labelled with LysoTracker Red and morphological changes in the lysosomes were detected by using laser confocal scanning microscopy. Transmission electron microscopy was used to observe changes in autophagosomes and lysosomes. Compared with those in the normal group, the model group presented decreases in the LC3B, ZKSCAN3, TFEB, Beclin-1 and Lamp-1 mRNA levels and increases in the LC3A, LC3II/I and α-synuclein protein levels. ZKSCAN3 overexpression resulted in a decrease in Beclin-1, LC3I mRNA, LC3 II/I protein and α-synuclein levels, as well as an increase in LC3II mRNA levels. ZKSCAN3 interference resulted in an increase in LC3A mRNA, LC3 II/I protein, Beclin-1, α-synuclein mRNA and Lamp-1 and a decrease in LC3B mRNA and α-synuclein. TFEB reversed the effects of ZKSCAN3. The results of lysosome detection revealed that, compared with that of the normal group, the fluorescence intensity of the model group was lower. The fluorescence intensity of the ZKSCAN3 interference group and TFEB interference group was greater than that of the interference empty group. Compared with those in the overexpression empty group, the fluorescence intensity and number of lysosomes in the ZKSCAN3 overexpression group and the TFEB overexpression group were lower. In conclusion, ZKSCAN3 affected the occurrence and development of PD through the TFEB-mediated autophagy-lysosome pathway.

Introduction

Parkinson's disease (PD) is a neurodegenerative disease that affects the dopaminergic (DA) neurons in the nigrostriatal circuit of the basal ganglia. The etiology of PD is complex and involves gene-environment interactions that interfere with cellular function. The main risk factors for PD are ageing, mitochondrial dysfunction, aggregation of α-synuclein, proteotoxic stress and oxidative stress (1-3). Current treatments for PD, such as medicine, brain surgery and supportive treatment, primarily focus on symptom management (4,5). Although gene therapy is a promising therapeutic approach under evaluation, many challenges remain (6,7). At present, PD is still difficult to treat. Therefore, it is important to explore specific pathways that can target disease progression.

The DNA binding protein zinc finger with KRAB and SCAN domains 3 (ZKSCAN3) is a transcriptional repressor of many autophagy-related genes, including LC3 and WIPI2 (8). ZKSCAN3 plays an important role in the progression of PD. It has been reported that in the PD model, the A30P mutant α-synuclein is more prone to oligomerization than the wild-type α-synuclein. It activates ZKSCAN3 in a JNK-dependent manner to promote its nuclear translocation,

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thus inhibiting the autophagy of dopaminergic neurons (9). ZKSCAN3 knockout induces autophagy and promotes lysosomal biogenesis and ZKSCAN3 overexpression reduces sirolimus-induced autophagy.

ZKSCAN3, an antagonist of transcription factor EB (TFEB), can regulate the autophagy-lysosome pathway (10). Lysosomes play crucial roles in PD (11). More than two-thirds of lysosomal storage diseases involve dysfunction of the central nervous system (12). Lysosomal dysfunction and the accumulation of undegraded autophagosomes in neurons were observed in PD patients and an animal model. The lysosomal Ca^{2+} channel mucolipin 1 (MCOLN1) and lysosomal potassium ion channel TMEM175 affect the autophagy-mediated degradation of α-synuclein oligomers (13,14). It was hypothesized that ZKSCAN3 affects the occurrence and development of PD through TFEB-mediated autophagy-lysosome pathway. Studies on the function of the ZKSCAN3-mediated autophagy-lysosome pathway are helpful for identifying the potential pathological mechanism of PD.

Materials and methods

Reagents. 6-hydroxydopamine (6-OHDA; cat. no. HY-B1081; MedChemExpress), overexpression vector (Jiangxi Zvast-Biotechnology Co.). Primary antibodies: Rabbit anti-ZKSCAN3 (1:1,000; cat. no. DF7752; Affinity Biosciences), rabbit anti-transcription factor EB (1:1,000; TFEB; cat. no. AF7015; Affinity Biosciences), rabbit anti-Beclin-1 (1:500; cat. no. AF5128; Affinity Biosciences), rabbit anti-LC3 I/II (1:1,000; cat. no. 12741T; Cell Signaling Technology), rabbit anti-α-synuclein (1:500; cat. no. AF0402; Affinity Biosciences), rabbit anti-lysosomal-associated membrane protein 1 (1:1,000; Lamp-1; cat. no. 21997-1-AP, Proteintech), mouse Anti-β-Actin (1:2,000; cat. no. HC201; TransGen Biotech). Secondary antibodies: HRP conjugated Goat Anti-Mouse IgG (H+L; 1:2,000; cat. no. GB23301; Wuhan Servicebio Technology Co., Ltd.), HRP conjugated Goat Anti-Rabbit IgG (H+L; 1:2,000; cat. no. GB23303; Wuhan Servicebio Technology Co., Ltd.). LysoTracker Red (cat. no. C1046; Beyotime Institute of Biotechnology) and Hoechst 33342 (cat. no. C1026; Beyotime Institute of Biotechnology) were used. ZKSCAN3 overexpression vector was constructed by cloning the CDS of ZKSCAN3 into VP001-CMV-MCS-EF1-ZsGreen-T2A-PURO lentivirus vector (General Biol (Anhui) Co., Ltd.). TFEB overexpression vector was constructed by cloning the CDS of TFEB into pcDNA3.1(+).

Cell transfection. The SH-SY5Y cell line was obtained from Procell Life Science & Technology Co., Ltd. (cat. no. CL-0208). The short tandem repeat (STR) profiling method was used for the authentication of the SH-SY5Y cell line. The cells were prepared for transfection when the cell density reached 70%. The cell culture medium was replaced with 1 ml of serum-free medium. A total of 125 μl of Opti-MEM was added to two sterilized EP tubes. Lipofectamine 3000® (Invitrogen; Thermo Fisher Scientific, Inc.; 5 μl) was added to one EP tube and 12.5 μl of siRNA (125 μl/1OD) was added to the other EP tube. The sequences of siRNAs used in the present study are shown in Table I. After incubation at room temperature for 5 min, the reagents in the two EP tubes were mixed and incubated at room temperature for 15 min. The mixture was added to a 6-well plate, after which the cells were placed in the incubator. At 6 h later, 1 ml of complete culture medium supplemented with 20% serum was added to each well of the 6-well plate. At 48 h later, the cells were collected for testing.

CCK8 assay. The cells were digested, resuspended, counted and seeded at a density of 500 cells per well. The cells were treated for 24 h when they had adhered to the wall of the plate. The media in the 96-well plate was replaced with fresh culture media (100 μl per well). CCK8 reagent (10 μl) was added to each well and incubated at 37˚C for 2 h. The absorbance value of each well was detected at a wavelength of 450 nm using a microplate reader.

Reverse transcription-quantitative (RT-q) PCR. Cells ($5x10^5$) were collected and lysed with TRIzol® reagent. Total RNA was extracted for concentration and purity determination. cDNA was synthesized via the use of a reverse transcription HiFiScript cDNA first strand synthesis kit. RT-qPCR was performed using the ChamQ Universal SYBR qPCR Master Mix (cat. no. Q711-02; Vazyme Biotech Co., Ltd.) and conducted on a fluorescence quantitative PCR instrument. RNA extraction, cDNA synthesis, and qPCR were performed according to the manufacturers' protocols. The thermocycling conditions were: Initial denaturation at 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 sec, 58˚C for 30 sec and 72˚C for 30 sec, and final extension at 72˚C for 10 min. β-actin was used as the internal reference. The relative expression levels of genes were calculated. The primers used are shown in Table II. These experiments were replicated for 3 times. The relative gene expression was calculated using the $2^{-\Delta\Delta Cq}$ method (15).

Western blotting. RIPA cell lysis buffer (cat. no. C1053; Applygen Technologies Inc.) was added to the cells or tissues, which were placed on ice for 15 min and then centrifuged at 13,523 x g at 4˚C for 10 min. The supernatant was collected and the protein concentration was determined via a BCA protein quantification kit. The samples (50 μg) were loaded and subjected to 5% SDS-PAGE at 60V and 80V for 120 min. The sponge, filter paper, gel, membrane, filter paper and sponge were placed in sequence, followed by membrane transfer at 300 mA. Proteins were blocked at 37˚C with 3% skimmed milk in 1X TBST (0.1% Tween-20) for 1 h. The PVDF membrane was incubated with the primary antibodies at 4˚C overnight. After being washed with 1X TBST 3 times, the PVDF membrane was incubated with the secondary antibodies at 37˚C for 2 h. The membrane was subsequently washed with 1X TBST 3 times. SuperSignal west pico chemiluminescent substrate (Thermo Fisher Scientific Inc.) was then added and the membranes were placed in an ultrahigh-sensitivity chemiluminescence imaging system for image development. Band density was analyzed using the Image-Pro Plus software (version 6.0; Media Cybernetics, Inc.).

Transmission electron microscopy. The cells were fixed with 2.5% glutaraldehyde, washed with PBS three times and fixed with 1% osmium acid at 4˚C for 2 h. After dehydration with graduated alcohol and acetone, the cells were embedded

Table I. Sequences of siRNAs used in the present study.

Name	Sequences
siRNA-NC	UUCUCCGAACGUGUCACGUTT
	ACGUGACACGUUCGGAGAATT
ZKSCAN3 siRNA-1	GAGAAGCCAUGUAGGGAAATT
	UUUCCCUACAUGGCUUCUCTT
ZKSCAN3 siRNA-2	CAGAGAAGAUAAAGUGGUATT
	UACCACUUUAUCUUCUCUGTT
ZKSCAN3 siRNA-3	AUGGAAAGCCAGUUGGAAATT
	UUUCCAACUGGCUUUCCAUTT
TFEB siRNA	CUGAAAUGCAGAUGCCCAATT
	UUGGGCAUCUGCAUUUCAGTT

siRNA, short interfering RNA; NC, negative control; ZKSCAN3, zinc finger with KRAB and SCAN domains 3; TFEB, transcription factor be.

Table II. Primers used in the present study.

Primer name	Sequences (5'-3')
β-actin forward	TGGCACCCAGCACAATGAA
β-actin reverse	CTAAGTCATAGTCCGCCTAGA AGCA
Beclin-1 forward	TCCCGTGGAATGGAATGAGA
Beclin-1 reverse	GTAAGGAACAAGTCGGTATCT CTG
LC3A forward	CTCAGACCGGCCTTTCAAGC
LC3A reverse	GCTCGATGATCACCGGGATTT
LC3B forward	CATCCAACCAAAATCCCGGT
LC3B reverse	GAGCTGTAAGCGCCTTCTAA
α-synuclein forward	TGTTGGAGGAGCAGTGGTGA
α-synuclein reverse	GGCATTTCATAAGCCTCATTG TC
Lamp-1 forward	GAAGGACAACACGACGGTGA
Lamp-1 reverse	CGCGTTGCACTTGTAGGAAT

with Spurr812 epoxy resin by immersion in 1:3, 1:1, and 3:1 solutions of resin in acetone at room temperature for 2 h, 3 h and overnight respectively. The cell clumps were placed into the embedding mold, the embedding agent was added and the samples were then subjected to solidification by heating. The samples were sectioned at 70-90 nm with an ultrathin slicer (Leica UC 7; Leica Microsystems GmbH). Then, double staining was performed with 2% uranium acetate and lead citrate at room temperature for 30 min. The cells were observed under a transmission electron microscope (JEM-1230; 80 KV; JEOL Ltd.).

Lysosome staining. The cell culture solution was discarded and diluted LysoTracker Red working solution (1:2,000) was added to the Petri dish and incubated at 37˚C for 30 min. The medium was then discarded and Hoechst 33342 staining solution was added. The stained sample was fully covered and incubated at 37˚C in darkness for 20-30 min. Then, the cells were observed and images were collected under a fluorescence microscope (BX53; Olympus Corporation). The nucleus stained with Hoechst 33342 was blue and the nucleus labelled with LysoTracker Red was red.

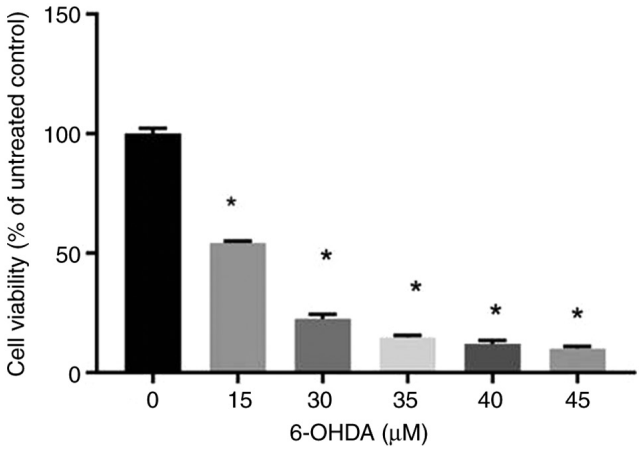

Figure 1. The proliferation of SH-SY5Y cells was inhibited when cells were treated with 6-OHDA at a concentration of 15 μM and above. *P<0.05 vs. the control group. 6-OHDA, 6-hydroxydopamine.

Statistical analysis. SPSS 20.0 software (IBM Corp.) was used for statistical analysis. All the experiments were repeated three times and the quantitative results are expressed as the means ± standard deviations. Comparisons of data between two groups were conducted via independent unpaired sample t-tests, whereas comparisons between multiple groups were conducted via one-way ANOVA. The subsequent pairwise comparison was conducted via Tukey's test. P<0.05 was considered to indicate a statistically significant difference.

Results

Selection of the 6-OHDA concentration. The CCK-8 results revealed that cell proliferation decreased significantly when the cells were treated with 6-OHDA at concentrations of ≥15 μM (Fig. 1). The cell viability was decreased to <50% when cells were treated with 6-OHDA >15 μM, making it difficult to conduct normal experiments. Therefore, 15 μM was selected as the modelling concentration.

Verification of ZKSCAN3 interference and overexpression. The RT-qPCR results revealed that, following transfection of the three interference vectors, the mRNA expression of ZKSCAN3 was significantly lower than that in the negative control (NC) group. The siRNA-2 vector had the most significant effect (Fig. 2A). Compared with the NC group, the ZKSCAN3-overexpressing group showed a significant increase in ZKSCAN3 mRNA expression (Fig. 2C). The western blotting results revealed that, after transfection with the three interference vectors, the protein expression of ZKSCAN3 was reduced compared with that in the NC group. The siRNA-2 vector had the most significant effect (Fig. 2B). Compared with the NC group, the overexpression group showed a significant increase in ZKSCAN3 protein expression (Fig. 2D).

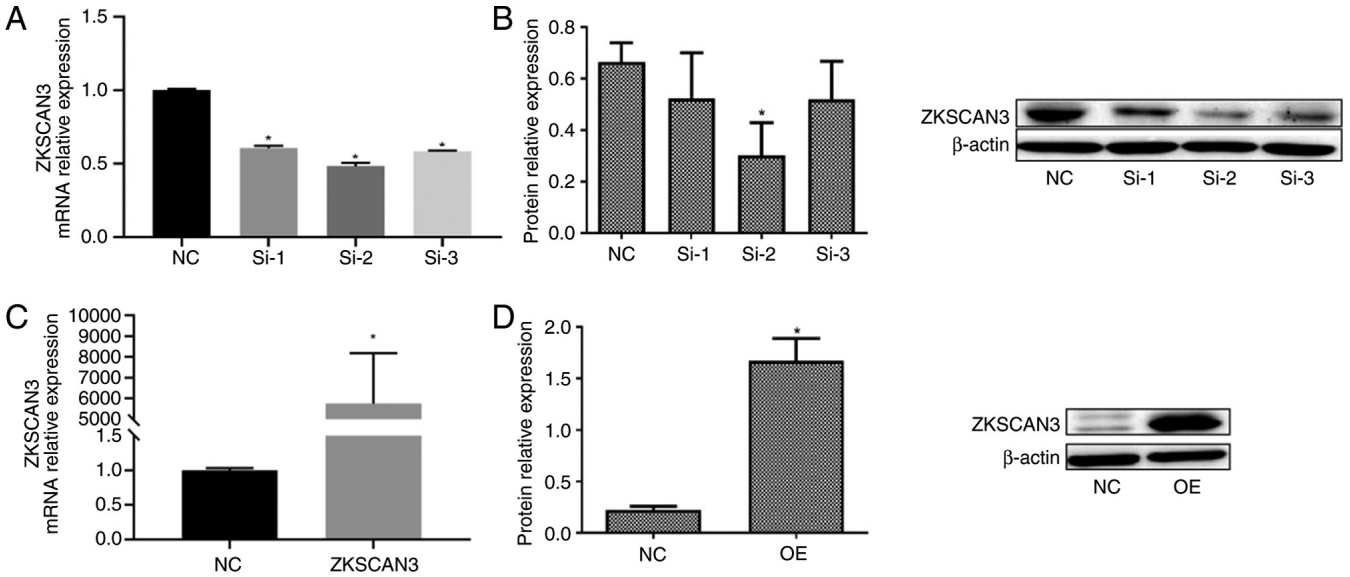

Figure 2. Verification of interference and overexpression of ZKSCAN3. (A) ZKSCAN3 mRNA expression was significantly reduced when cells were transfected with ZKSCAN3 siRNAs. (B) ZKSCAN3 protein expression was significantly reduced when cells were transfected with ZKSCAN3 siRNA-2. (C) ZKSCAN3 mRNA expression was significantly increased when cells were transfected with ZKSCAN3 overexpression vector. (D) ZKSCAN3 protein expression was significantly increased when cells were transfected with ZKSCAN3 overexpression vector. *P<0.05 vs. the NC group. siRNA, short interfering RNA; NC, negative control; OE, overexpression; ZKSCAN3, zinc finger with KRAB and SCAN domains 3.

Effect of ZKSCAN3 on the expression of LC3, α-synuclein, Lamp-1 and Beclin-1 in a PD cell model. The RT-qPCR results revealed that, compared with the NC group, the model group showed increased LC3A expression, whereas the expression of Beclin-1 and LC3B was decreased. Compared with the model group, the ZKSCAN3 overexpression group showed significant decreases in Beclin-1 and LC3A and an increase in LC3B. Compared with the interference control group, the ZKSCAN3 interference group showed an increased expression of LC3A, α-synuclein and Lamp-1 and decreased expression of LC3B (Fig. 3A).

The western blotting results revealed that, compared with the normal control group, the model group showed increased ZKSCAN3 expression and decreased TFEB expression (Fig. 3B). Compared with the normal control group, the model group showed decreases in Beclin-1 and Lamp-1 expression and increases in LC3II/I and α-synuclein expression. Compared with the empty vector group, the ZKSCAN3 overexpression group showed increases in Beclin-1 and Lamp-1 and decreases in LC3 II/I and α-synuclein expression. Compared with the interference control group, the ZKSCAN3 interference group showed increases in Beclin-1, Lamp-1 and LC3 II/I expression, whereas the expression of α-synuclein was decreased (Fig. 3C).

Effect of TFEB on the expression of Beclin-1, LC3, α-synuclein and Lamp-1 in a PD cell model. The RT-qPCR results revealed that, compared with that in the normal control group, the expression of Beclin-1 in the model group was increased. Compared with the Model + ZKSCAN3 + TFEB SiNC group, the Model + ZKSCAN3 + TFEB siRNA group showed decreased expression of Beclin-1, LC3A and LC3B, whereas the expression of Lamp-1 was increased. Compared with the Model + ZKSCAN3 siRNA + TFEB-NC group, the Model + ZKSCAN3 siRNA + TFEB group showed an increased expression of Beclin-1 and LC3B, whereas the expression of α-synuclein, LC3A and Lamp-1 was decreased (Fig. 4A).

The western blotting results revealed that, compared with those in the normal control group, Beclin-1 levels were lower, LC3 and α-synuclein levels were higher and Lamp-1 levels were not significantly different in the model group. Compared with the Model + ZKSCAN3 + TFEB siNC group, the Model + ZKSCAN3 + TFEB SiRNA group showed decreased expression of Beclin-1, α-synuclein and Lamp-1, whereas the expression of LC3 was increased. Compared with the Model + ZKSCAN3 siRNA + TFEB-NC group, the Model + ZKSCAN3 siRNA + TFEB group showed decreased expression of Beclin-1, Lamp-1 and α-synuclein and increased expression of LC3 (Fig. 4B).

Detection of lysosomes. Lysosomal staining revealed that, compared with that of the normal control group, the fluorescence intensity of the model group was lower. Compared with those in the ZKSCAN3 NC group, the fluorescence intensity and number of lysosomes in the ZKSCAN3-overexpressing group were lower. Compared with the ZKSCAN3 siRNA NC group, the ZKSCAN3 interference group showed an increase in fluorescence intensity (Fig. 5A). Compared with that of the interference empty group, the fluorescence intensity of the TFEB interference group was greater. Compared with that of the empty group, the fluorescence intensity of the TFEB-overexpressing group was lower and the number of lysosomes was lower (Fig. 5B).

Transmission electron microscopy. The results of transmission electron microscopy revealed that the autophagy level in the model group was increased. The autophagy level was decreased in the ZKSCAN3 overexpression group and increased in the ZKSCAN3 interference group (Fig. 6A). The level of autophagy was decreased in the TFEB-overexpressing group (Fig. 6B).

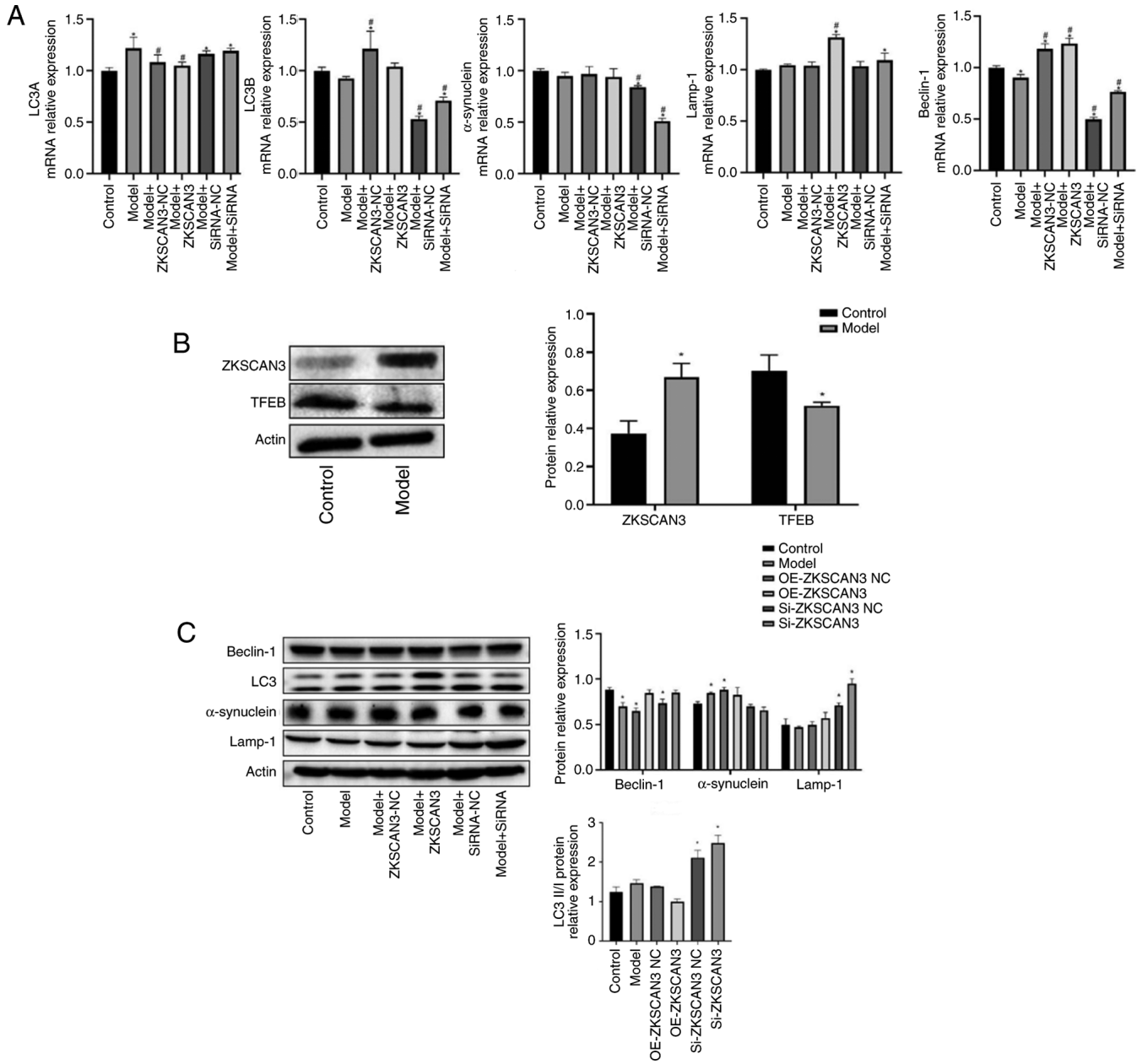

Figure 3. Effect of ZKSCAN3 on the expression of TFEB, LC3, α-synuclein, Lamp-1 and Beclin-1 in PD cell model. (A) The mRNA expression of LC3A, LC3B, α-synuclein, Lamp-1 and Beclin-1 when cells were transfected with ZKSCAN3 overexpression vector or ZKSCAN3 siRNA. (B) The protein expression of ZKSCAN3 and TFEB in PD cell model. (C) The protein expression of LC3, α-synuclein, Lamp-1 and Beclin-1 when cells were transfected with ZKSCAN3 overexpression vector or ZKSCAN3 siRNA. *P<0.05 vs. the control group; #P<0.05 vs. the model group. PD, Parkinson's disease; NC, negative control; ZKSCAN3, zinc finger with KRAB and SCAN domains 3; TFEB, transcription factor EB; Lamp-1, lysosomal-associated membrane protein 1.

Discussion

Autophagy occurs widely in eukaryotic cells. It is a highly evolutionary and conserved process in which cells maintain homeostasis via phagocytosis and degradation of damaged organelles, proteins and biological macromolecules through the autophagy-lysosome system (16). The basal level of autophagy removes aggregate-prone proteins and allows cells to recycle cytoplasmic components, whereas unrestrained autophagic activity triggers apoptotic cell death and is considered a contributing factor in the pathogenesis of several disorders. Autophagy plays important roles in tumors, brain diseases and neurodegenerative diseases. Autophagy affects the progression of diseases by regulating cell death and survival (17,18). In recent years, increasing evidence has suggested that autophagy is involved in PD (18,19).

The ratio of LC3II/LC3I is an important indicator of autophagy (20). Beclin-1 is another key protein involved in the formation of autophagosomes (21). Zhuang *et al* reported that Beclin-1 is released when the interaction between Beclin-1 and Bcl-2 is disrupted, thus increasing the basal level of autophagy (21).

PD is a common neurodegenerative disease. α-Synuclein is closely related to PD (22). Additionally, Beclin-1 and LC3 are key autophagy-related proteins (23). The present study found that the expression of ZKSCAN3 was increased and

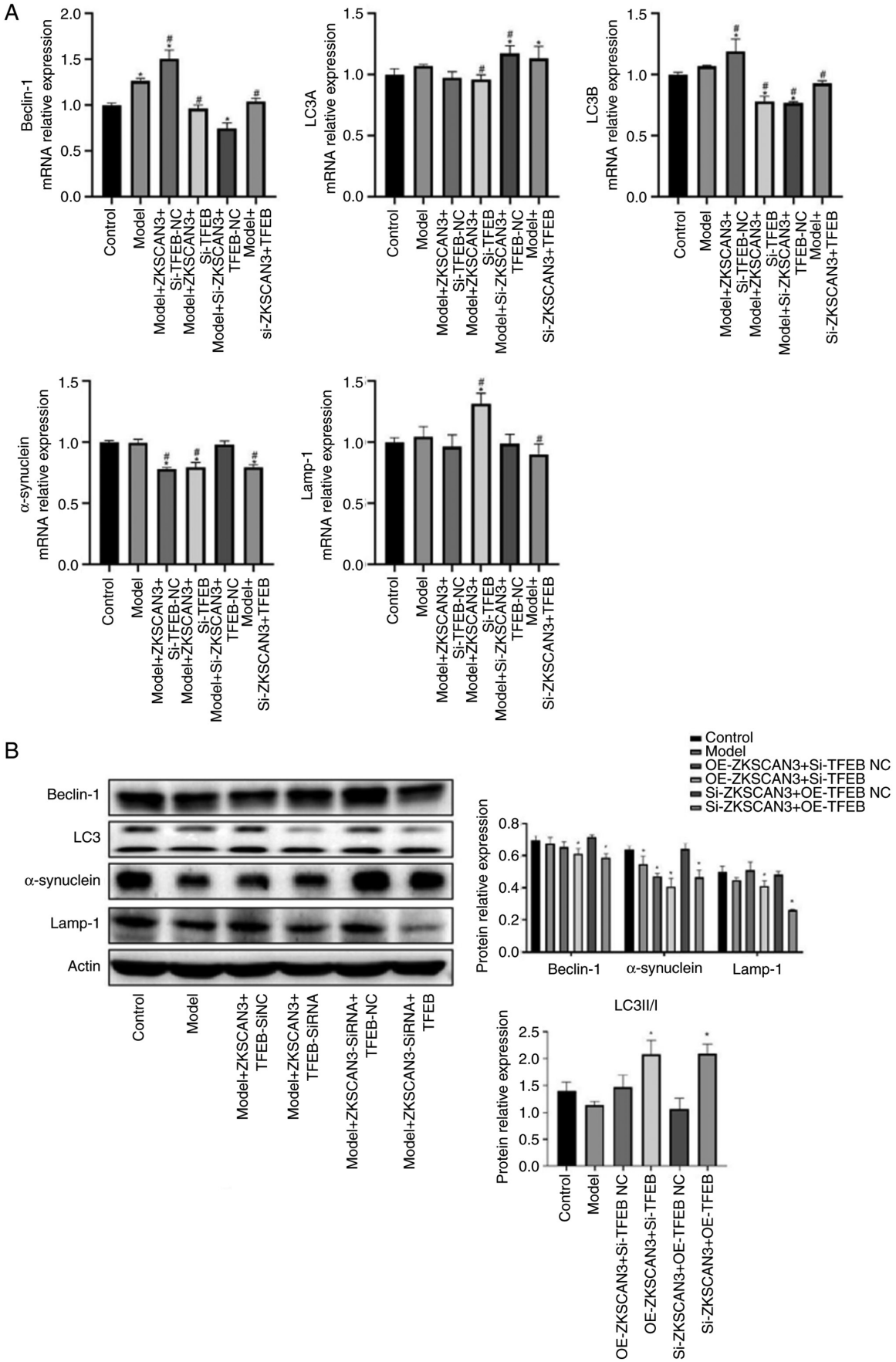

Figure 4. Effect of TFEB on the expression of Beclin-1, LC3, α-synuclein and Lamp-1 in PD cell model. (A) The mRNA expression of LC3A, LC3B, α-synuclein, Lamp-1 and Beclin-1 when cells were transfected with ZKSCAN3/TFEB overexpression vector or siRNA. (B) The protein expression of LC3, α-synuclein, Lamp-1 and Beclin-1 when cells were transfected with ZKSCAN3/TFEB overexpression vector or siRNA. *P<0.05 vs. the control group; #P<0.05 vs. the model group. PD, Parkinson's disease; NC, negative control; ZKSCAN3, zinc finger with KRAB and SCAN domains 3; TFEB, transcription factor EB; Lamp-1, lysosomal-associated membrane protein 1.

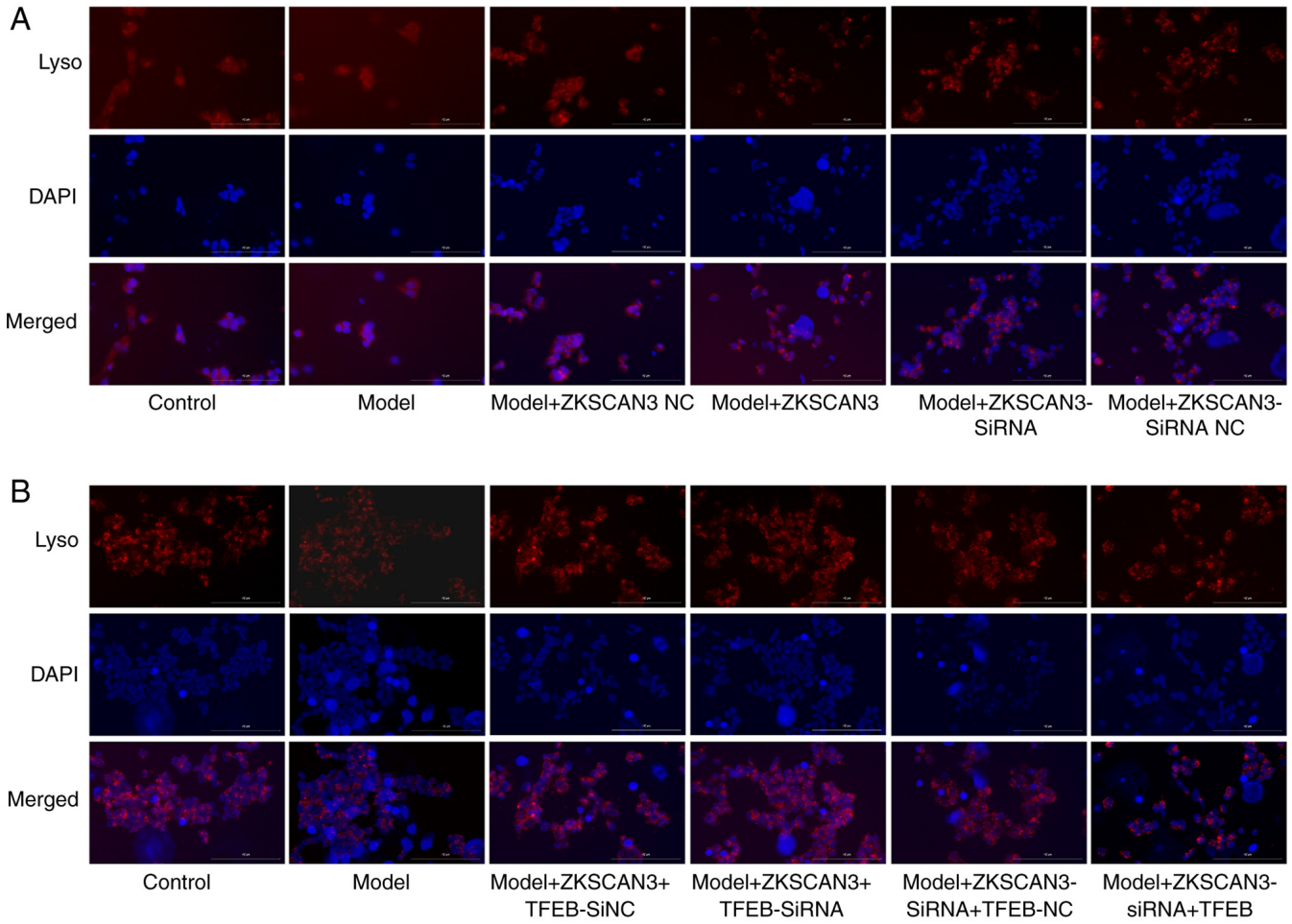

Figure 5. Effect of ZKSCAN3/TFEB on lysosomes determined by lysosomal staining. (A) Lysosomal staining when cells were transfected with ZKSCAN3 overexpression vector or siRNA. (B) Lysosomal staining when cells were transfected with ZKSCAN3/TFEB overexpression vector or siRNA. Magnification, x200. NC, negative control; ZKSCAN3, zinc finger with KRAB and SCAN domains 3; TFEB, transcription factor be; siRNA, short interfering RNA.

that the expression of TFEB was decreased in the model group compared with the normal group. Compared with those in the normal group, the Beclin-1 and Lamp-1 levels in the model group were lower, whereas the LC3II/I and α-synuclein expression levels were greater. Compared with the empty group, the ZKSCAN3-overexpressing group showed increases in Beclin-1 and Lamp-1 as well as decreases in LC3 II/I and α-synuclein expression levels. Compared with the interference control group, the ZKSCAN3 interference group showed increased levels of Beclin-1, Lamp-1 and LC3 II/I, whereas the level of α-synuclein was decreased. ZKSCAN3 promotes autophagy in PD.

The present study explored the interaction between TFEB and ZKSCAN3. Compared with the normal group, the model group showed a decrease in Beclin-1 levels as well as increases in LC3 and α-synuclein levels, but the expression of Lamp-1 did not change. Compared with those in the Model + ZKSCAN3 + TFEB SiNC group, the levels of Beclin-1, α-synuclein and Lamp-1 were lower and the level of LC3 was greater in the Model + ZKSCAN3 + TFEB SiRNA group. Compared with those in the Model + ZKSCAN3 siRNA + TFEB-NC group, the expression of Beclin-1, Lamp-1 and α-synuclein was decreased and the expression of LC3 was increased in the Model + ZKSCAN3 siRNA + TFEB group. In PD, TFEB can reverse the regulatory effect of ZKSCAN3.

LysoTracker Red was used for lysosome-specific fluorescence staining (24). The results of lysosomal staining revealed that, compared with that of the normal group, the fluorescence intensity of the model group was lower. Compared with those in the overexpression empty group, the fluorescence intensity and number of lysosomes in the ZKSCAN3 overexpression group were lower. Compared with the interference empty group, the ZKSCAN3 interference group showed an increase in fluorescence intensity. Compared with that of the interference empty group, the fluorescence intensity of the TFEB interference group was greater. Compared with those in the empty control group, the fluorescence intensity and number of lysosomes in the TFEB overexpression group were lower. Functional assays to confirm the role of ZKSCAN3 in autophagy and lysosomal function could provide more robust evidence for the proposed mechanisms. Co-Immunoprecipitation assay for protein interactions, detection of autophagic flow and related experiments on downstream signaling pathways could be performed as further directions. Further research is planned to be conducted in animal experiments.

A limitation of the present study is that all the experiments were performed *in vitro*. *In vivo* studies will need to be

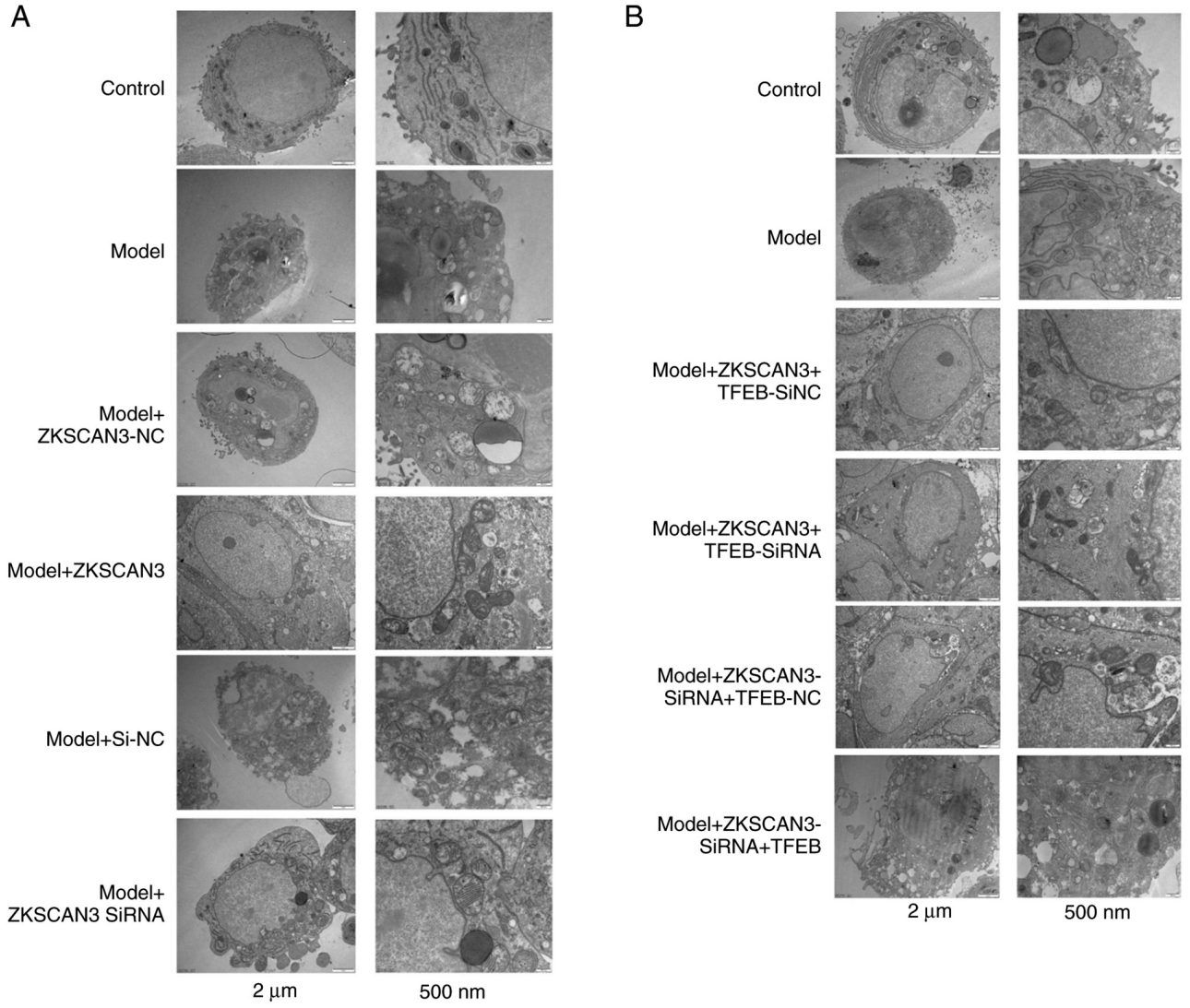

Figure 6. Transmission electron microscopy detection for autophagy. (A) Transmission electron microscopy detection when cells were transfected with ZKSCAN3 overexpression vector or siRNA. (B) Transmission electron microscopy detection when cells were transfected with ZKSCAN3/TFEB overexpression vector or siRNA. Magnification, x5,000 (for 2 μm group); x20,000 (for 500 nm group). si, short interfering RNA; NC, negative control; ZKSCAN3, zinc finger with KRAB and SCAN domains 3; siRNA, short interfering RNA; TFEB, transcription factor EB.

conducted in the future to confirm the *in vitro* results. Treating SH-SY5Y cells with 6-OHDA is a common method for constructing a cell model of PD. The results of the CCK8 assay represented the fundamental phenotype indicator for model validation. Measuring more indicators will make the model more convincing. In the present study, 15 μM was selected as the modelling concentration of 6-OHDA and the limitation is that the concentration within 15 μM was not tested.

In the autophagy-lysosome pathway, TFEB regulated the biogenesis of lysosomes and participates in the regulation of lysosomal hydrolases and autophagy-related proteins. Under normal conditions, TFEB is phosphorylated and located on the surface of lysosomes in a deactivated state. When TFEB is dephosphorylated, it is activated to enter the nucleus, where it promotes the expression of autophagy- and lysosome-related proteins and enhances the autophagy-lysosome pathway (25). The typical pathological change in PD is the progressive loss of dopaminergic neurons. Misfolded α-synuclein is related to this process. α-synuclein changes the transcriptional activity of TFEB and impairs the degradation function of lysosomes. An increase in exogenous TFEB activity can promote the clearance of α-synuclein and protect dopamine neurons (26).

In normal brain tissue, ZKSCAN3 is located in the cytoplasm and cannot inhibit autophagy. In a model of PD, ZKSCAN3 accumulates in the nucleus, inhibits the expression of autophagy-related genes and reduces lysosome production, thus leading to the accumulation of α-synuclein and the progression of PD (27). Protein accumulation may be caused by increased synthesis or decreased clearance. Unnecessary proteins are usually removed from cells via the ubiquitin-proteasome system or autophagy-lysosome system. Functional defects in the ubiquitin-proteasome system and lysosomes have been detected in patients with PD. The elimination of unwanted proteins is one method for developing candidate drugs for PD (28). Therapeutic strategies to enhance the function of lysosomes and autophagy may also be appropriate for

drug development (29). In conclusion, the present study demonstrated that ZKSCAN3 affects the occurrence and development of PD through the TFEB-mediated autophagy-lysosome pathway.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MY, SL, MC and JL contributed to the study conception and design. Material preparation, data collection and analysis were performed by SL, BS and MC, BS and WC conducted the experiments. MY, SL, MC and JL confirmed the authenticity of all the raw data. The first draft of the manuscript was written by MC and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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