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MTERF4 regulates the mitochondrial dysfunction induced by MPP^+ in SH-SY5Y cells

Xiaofei Ye, Yanyan Han, Linbing Zhang, Wen Liu*, Ji Zuo*

Department of Cellular and Genetic Medicine, School of Basic Medical Sciences, Fudan University, Shanghai 200032, China

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ABSTRACT

Mitochondrial transcription termination factor 4, MTERF4, a member of the MTERF family, has been implicated in the regulation of mitochondrial translation by targeting NSUN4 to the large mitochondrial ribosome. Here, we found a novel role for MTERF4 in regulating mitochondrial dysfunction induced by MPP^+. We observed that knockdown of *MTERF4* in SH-SY5Y cells resulted in increased mitochondrial DNA transcription levels and decreased mitochondrial DNA translation levels. In addition, after treatment with 2 mM MPP^+ for 24 h, the expression levels of MTERF4 were decreased compared to wide-type SH-SY5Y cells. Moreover, after exposure to 2 mM MPP^+ for 24 h, knockdown of MTERF4 in SH-SY5Y cells worsened the mitochondrial dysfunction induced by MPP^+, including increased reactive oxygen species, accumulated cleaved PARP-1, decreased mitochondrial membrane potential and depressed mitochondrial complexes. Furthermore, overexpression of MTERF4 in SH-SY5Y cells partially alleviated the mitochondrial dysfunction induced by MPP^+. Based on these findings, we suggest that the main function of MTERF4 is regulating mtDNA expression, and it is the crucial factor in the mechanism of mitochondrial dysfunction in SH-SY5Y cells induced by MPP^+. MTERF4 probably is the triggering of the pathogenesis of Parkinson's disease induced by environmental toxin.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder and is characterized by the progressive loss of dopaminergic (DA) neurons of the substantia nigra pars compacta (SNpc) and the presence of intraneuronal proteinaceous inclusions known as Lewy bodies [1]. Because of the many potential factors contributing to the occurrence of PD, it is a challenge to study the mechanisms underlying this disease. However, mitochondrial dysfunction induced by various factors, including environmental toxins and deficient expression of mitochondrial DNA (mtDNA), has been shown to play a role in PD [2,3].

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a typical environmental toxin. It crosses the blood–brain barrier and is oxidized into 1-methyl-4-phenylpyridinium (MPP^+), which selectively induces the neurotoxicity of SNpc DA neurons and specifically reduces the activity of mitochondrial complex I [4,5]. In addition, MPP^+ not only induced mitochondrial dysfunctions and ROS generation but also reduced the expression levels of mitochondria-related genes, such as mitochondrial transcription factor A (TFAM) and nuclear respiratory factor (NRF-1), which both contribute to mtDNA expression [6].

Because the key subunits of the respiratory chain and ATP synthase are encoded by mtDNA, mtDNA expression is essential for the control of oxidative phosphorylation. In recent years, the mitochondrial transcription termination factors (MTERFs) have been reported to affect mtDNA expression to varying degrees. MTERF1 specifically binds a 28-bp region between 16S rRNA and $tRNA^{L1}$ and regulates mtDNA transcription termination [7]. MTERF1 also interacts with HSP and activates rRNA transcription by forming an mtDNA loop between the two binding sites [8]. However, recent reports showed that MTERF1 did not regulate heavy strand transcription, but bound mtDNA to prevent transcriptional interference at the LSP [9]. Furthermore, it was demonstrated that MTERF2 binds to the promoter region and affects transcription initiation, but another report suggests that MTERF2 displays a non-sequence-specific DNA-binding activity [10,11]. In addition, mice lacking *Mterf2* are viable, but develop myopathy and memory deficits, which is associated with decreased levels of mitochondrial transcripts [12]. MTERF3 interacts with the mtDNA region and

* Corresponding authors.
E-mail addresses: liuwen@shmu.edu.cn (W. Liu), jzuo@shmu.edu.cn (J. Zuo).

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functions as a negative regulator of mammalian mtDNA transcription [13,14]. Tissue-specific knockout of *Mterf3* in the heart results in the activation of transcription initiation and induces mitochondrial dysfunction [13]. Furthermore, recent studies suggested that MTERF3 is not only involved in mitochondrial transcription but also plays an important role in the regulation of mitochondrial ribosome biogenesis [15]. Moreover, MTERF4 forms a stoichiometric complex with a 5-methylcytosine RNA methyltransferase, NSUN4, and is required for the recruitment of NSUN4 to the large subunit of the mitochondrial ribosome [16,17]. Finally, MTERF4 is necessary for cell proliferation because depletion of *MTERF4* in HeLa cells leads to cell death [18].

Altering the expression of MTERFs affects mtDNA replication, mtDNA transcription and mtDNA translation to induce mitochondrial dysfunction, which is closely related to the pathogenesis of PD. Therefore, we elucidated whether MTERFs influence PD by regulating mitochondrial function. Here, we show that knockdown of MTERF4 in SH-SY5Y cells aggravates the mitochondrial dysfunction induced by MPP^+, which provides new insight into the role of MTERF4 in the regulation of mitochondrial function.

2. Materials and methods

2.1. Plasmid constructions

Full-length human MTERF4 cDNA was cloned into the vector pLVX-IRES-Puro (Clontech) and sequenced. MTERF4-specific knockdown was induced by recombinant lentivirus-mediated shRNA interference. We designed an shRNA targeting the MTERF4 mRNA sequence (5′ to 3′), GTTTCAGCAATGCCCATATTA. It was acquired from the RNAi Consortium shRNA Library of Broad institute (http://www.broadinstitute.org/rnai/public/gene/search). The shRNA oligonucleotides were annealed and cloned into the pLVX-shRNA1 Vector (Clontech, containing the puromycin resistance cassette) as a *Bam*HI/*Eco*RI fragment according to the manufacturer's protocol. A scrambled shRNA sequence was used as a negative control.

2.2. Cell culture and transfection

HEK293T cells and SH-SY5Y cells was cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; BioWest) at 37 °C in 5% CO_2. The sequence verified plasmid (10 μg) together with the lentivirus packaging plasmids were cotransfected into HEK293T packaging cells (~70% confluence in 10 cm^2 flask) using the transfection reagent (Hilymax, Dojindo), according to the manufacturer's protocol. The lentiviral supernatants were harvested at 48 h and 72 h after the start of transfection and filtered through a 0.45-μm filter and were added to the SH-SY5Y cells (~60% confluence in a 6 cm^2 flask). The cells were harvested after selection with puromycin (Sigma).

2.3. Quantitative real-time PCR

Total RNA was extracted from the cells using an RNA simple total RNA kit (TIANGEN), and the cDNA was synthesized using a RevertAid first strand cDNA synthesis Kit (Thermo Scientific). Quantitative real-time PCR reactions were performed on a MiniOpticon real-time PCR detection system (Bio-Rad) using AccuPower 2 × Greenstar qPCR Master Mix (Bioneer). The primers used are listed below (Table 1).

2.4. Quantitative PCR for mtDNA

Total DNA was isolated from the cells using a Wizard genomic DNA purification kit (Promega), and the mtDNA copy number was determined by quantitative PCR, with the *TK2* gene as an internal standard [19].

2.5. Western blots

Immunoblot analysis was performed as previously described [20] using the following primary antibodies: MTERFD2 (Abcam, 1:500), cleaved PARP-1 (Cell Signaling Technology, 1:1000), VDAC (Affinity, 1:1000), MitoProfile total OXPHOS rodent WB antibody cocktail (MitoSciences, 1:500), and β-actin (Sigma, 1:5000).

2.6. Immunofluorescence

The SH-SY5Y cells were grown on coverslips. After 24 h, the cells were fixed with 3.7% paraformaldehyde for 20 min, permeabilized with 0.2% Triton X-100 for 10 min, and blocked with 5% BSA in 37 °C for 30 min. Then, the cells were incubated with mouse monoclonal anti-COX IV (Abcam, 1:200) and rabbit monoclonal anti-MTERF4 antibody (Abcam, 1:200) at 37 °C for 2 h. Next, the cells were incubated with Alexa Fluor 488 conjugated anti-mouse antibody and Alexa Fluor 595 conjugated anti-rabbit antibody as previously described [21]. The images were captured with a fluorescent inverted microscope (Leica DM IL LED).

2.7. Mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) assays

The mitochondrial membrane potential was measured using the mitochondrial membrane potential assay kit with JC-1 (Beyotime Biotech) according to the manufacturer's instructions. Red fluorescence represents aggregates of JC-1, whereas green fluorescence represents monomers of JC-1. The fluorescence ratio of the aggregates/monomers represents the ratio of mitochondrial depolarization. The 2′,7′-dichlorofluorescein diacetate probe (DCFH-DA, Beyotime Biotech) diluted in 1:10000 was used to measure the intracellular ROS. The fluorescence intensity was monitored using a BD Accuri™ C6 flow cytometer (BD Bioscience).

2.8. Cell proliferation assay

A total of 1000 cells per well were plated in sextuplet in 96-well plates and detected using the Cell Counting Kit-8 solution (CCK-8, Dojindo) every 24 h for 6 days. The CCK-8 solution was added to each well for 1 h at 37 °C according to the manufacturer's instructions. Then, the absorbance was measured at 450 nm.

2.9. Cell cycle assay

Determination of the cell cycle was based on a Cell Cycle and Apoptosis Analysis Kit (Beyotime Biotech) according to the manufacturer's protocols, and cell cycle analysis was performed on a BD Accuri™ C6 flow cytometer (BD Bioscience).

2.10. Cell viability assay

To determine the cell viability of the cells treated with MPP^+, ten thousand cells per well were seeded in 96-well plates. After 24 h of culture, the cells were treated with 2 mM MPP^+. After another 24 h, the CCK-8 solution was used to test the cell viability.

Table 1
Primers for quantitative real-time PCR.

Gene	Forward primer	Reverse primer
MTERF4	5′- GGACAGCCCGTACTTCTGTT -3′	5′- CATTGTCCTCCTCATCATCG -3′
GAPDH	5′-TTGCCATCAATGACCCCTTCA -3′	5′- CGCCCCACTTGATTTTGGA -3′
ND4L	5′- CGCTCACACCTCATATCCTC -3′	5′- CGGCAAAGACTAGTATGGCA -3′
ND6	5′- CCCATCATACTCTTTCACCCA -3′	5′- GGGTTGAGGTCTTGGTGAGT -3′
COX1	5′- CGATGCATACACCACATGAA -3′	5′- TCCAGGTTTATGGAGGGTTC -3′
Cytb	5′- GCCTGCCTGATCCTCCAAAT -3′	5′- AAGGTAGCGGATGATTCAGCC -3′
18S rRNA	5′- TAAAGGAATTGACGGAAGGG-3′	5′- CTGTCAATCCTGTCCGTGTC -3′

2.11. Statistical analysis

The data obtained are represented as the mean ± SEM from at least three independent experiments, and statistical significance was ascertained by t test or two-way ANOVA using GraphPad Prism 6.0 software. A p value <0.05 was considered significant.

3. Results

3.1. Knockdown of MTERF4 caused severe mitochondrial dysfunction in SH-SY5Y cells

To confirm whether MTERF4 is a mitochondrial protein in SH-SY5Y cells, we used the MTERF4 antibody to track MTERF4 cellular localization by immunofluorescence. As previously reported [18], the localization of MTERF4 overlapped with mitochondria labeled with COX IV (Fig. 1A).

Because of the important functions of MTERF4 in the regulation of mitochondrial translation [16], we detected mtDNA expression in control and MTERF4 KD SH-SY5Y cells. We assessed MTERF4 mRNA levels by quantitative real-time PCR and showed that the mRNA level of MTERF4 was decreased to 15% in MTERF4 knockdown (KD) SH-SY5Y cells, which was further confirmed by western blotting assays (Fig. 1B, C).

We found that knockdown of *MTERF4* in SH-SY5Y cells increased the mtDNA levels (Fig. 2A). In addition, compared to the control groups, the mtDNA-encoded mRNA levels were significantly increased in MTERF4 KD SH-SY5Y cells as follows: the complex I subunit ND4L, ND6 and Complex III subunit Cytb levels were increased 5-fold or more and the complex IV subunit COX1 was increased 2.2-fold (Fig. 2B). Furthermore, western blot analyses showed that both mtDNA- and nuclear-encoded subunits of the respiratory chain complexes were significantly reduced in MTERF4 KD SH-SY5Y cells (Fig. 2C). Therefore, knockdown of MTERF4 severely impaired the mitochondrial function in SH-SY5Y cells.

Then, we detected the cell proliferation in control and MTERF4 KD SH-SY5Y cells. Unexpectedly, the cell proliferation determined by the cell cycle and cell growth assays displayed no significant difference between the control and MTERF4 KD SH-SY5Y cells (Fig. 2D, E).

3.2. MPP^+ reduced MTERF4 expression in SH-SY5Y cells

MPP^+ is widely utilized in the cellular PD model creation in vitro. MPP^+ induces mitochondrial dysfunction and reduces the expression of mitochondrial-related genes. Interestingly, we found that the expression levels of the MTERF4 protein were significantly decreased (~75% reduction compared to control levels) when cells

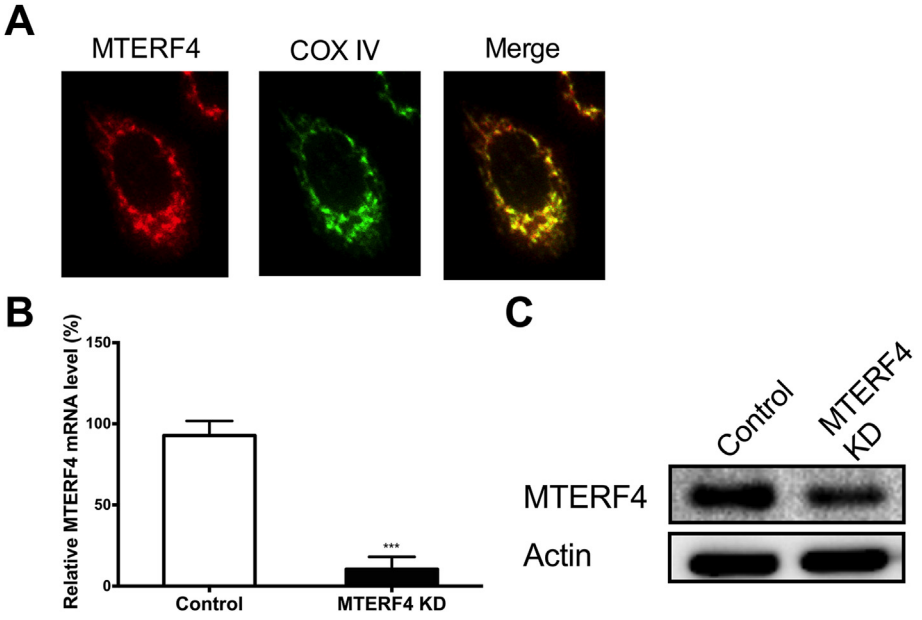

Fig. 1. MTERF4 knockdown SH-SY5Y cells. (A) MTERF4 is a mitochondrial protein. Immunofluorescence of SH-SY5Y cells stained with the anti-MTERF4 antibody plus Alexa Fluor 595 conjugated anti-rabbit antibody (red, left panel) and the anti-COX IV antibody plus Alexa Fluor 488 conjugated anti-mouse antibody (green, middle panel). Co-localization of the green and red signals is displayed in the merged image (yellow, right panel). **(B)** The relative expression of MTERF4 detected by quantitative real-time PCR in MTERF4 knockdown (KD) cells compared to the scramble shRNA vector (Control). Error bars represent the standard error of the mean (SEM); ***P < 0.001, n = 6. **(C)** Western blot analyses of MTERF4 levels in control and MTERF4 KD SH-SY5Y cells. β-actin was used for the loading control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

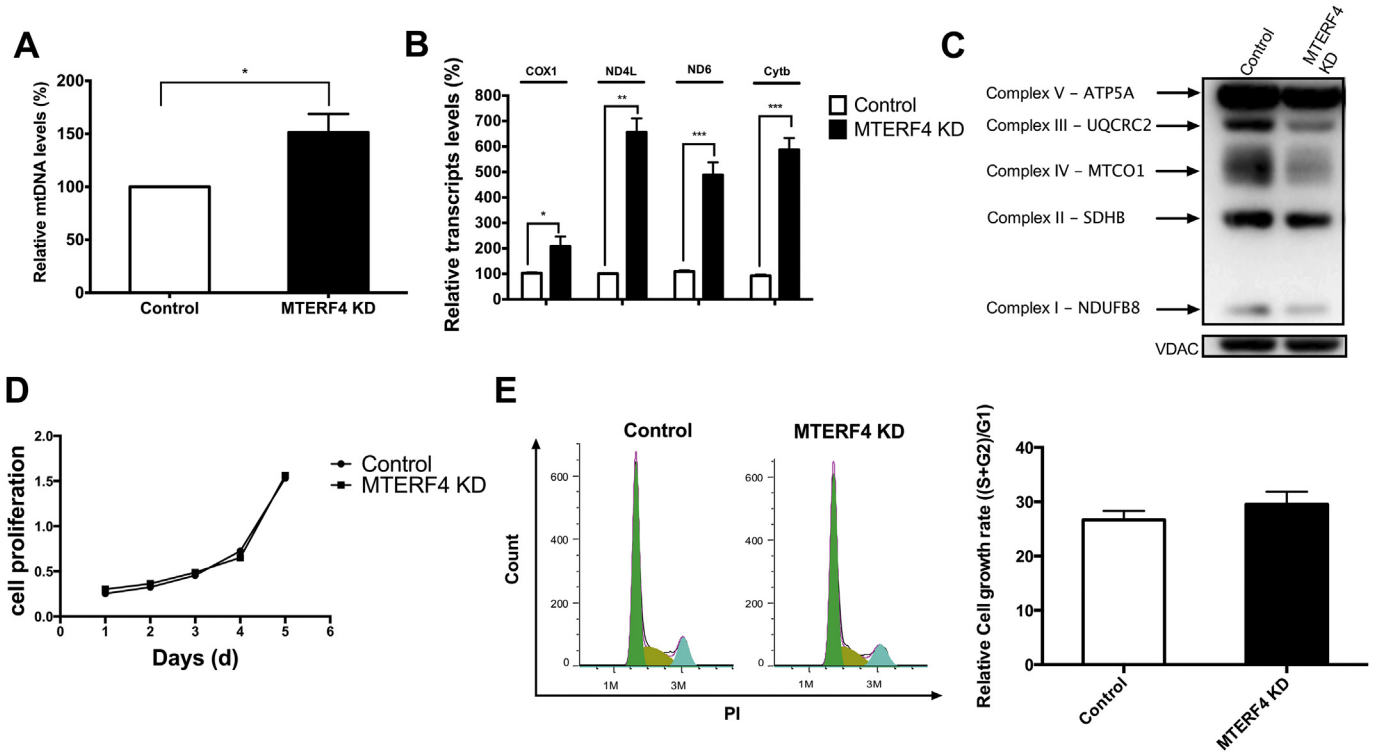

Fig. 2. MTERF4 knockdown causes severe mitochondrial dysfunction. (A) Quantification of the mtDNA copy number determined by quantitative PCR for MTERF4 KD SH-SY5Y cells, normalized to the copy number in control cells. Error bars are the SEM; $^{*}p < 0.05$, n = 6. **(B)** Quantification of the mitochondrial transcript levels of the control and MTERF4 KD SH-SY5Y cells. Cytb: cytochrome b. Error bars are the SEM; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, n = 6. **(C)** Western blot analysis of the subunits of the respiratory chain complexes in the control and MTERF4 KD SH-SY5Y cells. The mtDNA-encoded subunits of complex IV (MTCO1) and nucleus-encoded subunits of complex II (SDHB), complex III (UQCRC2), complex V (ATP5A), and complex I (NDUFB8) were analyzed. VDAC1 was used as a loading control. **(D)** The cell proliferation rates of the control and MTERF4 KD SH-SY5Y cells were determined with CCK-8 solution (n = 3 for each time point). **(E)** The cell cycle for the control and MTERF4 KD SH-SY5Y cells was analyzed by flow cytometry and the quantification of the ratio of G1/(S + G2) phase in the control and MTERF4 KD SH-SY5Y cells. Error bars are the SEM; $p > 0.05$, n = 3.

were treated with 2 mM MPP^+ for 24 h (Fig. 3A). This indicates that a decrease in MTERF4 expression may contribute to the mitochondrial dysfunction induced by MPP^+.

3.3. *MTERF4 knockdown further exacerbated the mitochondrial dysfunction induced by MPP^+*

Based on these findings, we hypothesized that MTERF4 knockdown affects the mitochondrial dysfunction induced by MPP^+. We assessed the ROS and MMP levels in control and MTERF4 KD SH-SY5Y cells. After exposure to 2 mM MPP^+ for 24 h, the levels of DCF fluorescence were significantly increased (approximately 28%) in the MTERF4 KD groups compared to the control cells, consistent with severe mitochondrial depolarization. The ratio of aggregates to monomers was decreased to 68% in the control groups and was decreased to 44% in the MTERF4 KD groups (Fig. 3B, C).

Because the collapse of the mitochondrial membrane potential results in the release of cytochrome c and activation of the caspase cascade, eventually inducing apoptosis [22], we detected cleaved PARP-1 activity in both control and MTERF4 KD SH-SY5Y cells. As shown in Fig. 3D, cleaved PARP-1 activity was elevated in the MTERF4 KD cells exposed to 2 mM MPP^+ for 24 h.

In addition, as shown in Fig. 3E, after exposure to 2 mM MPP^+ for 24 h, the number of normal morphology cells were significantly decreased in both control and MTERF4 KD SH-SY5Y cells. Furthermore, we determined the cell viability in control and MTERF4 KD cells, which revealed that MTERF4 KD groups were more sensitive to MPP^+ because the control groups was decreased to ~60% and the MTERF4 KD groups was decreased to ~43% under MPP^+ stress (Fig. 3F). Taken together, mitochondrial dysfunction induced by MPP^+ was significantly aggravated after MTERF4 knockdown.

3.4. *MTERF4 overexpression restored respiratory chain complex deficiency induced by MPP^+*

To determine whether increasing MTERF4 levels rescue the mitochondrial dysfunction induced by MPP^+, MTERF4 overexpression (MTERF4 overexpression, 3-fold) SH-SY5Y cells and the control cells were used (Fig. 4A). We performed the mtDNA-encoded mRNA levels assays in both control and MTERF4 overexpression cells. After exposure to 2 mM MPP^+ for 24 h, the mtDNA-encoded mRNA levels were reduced, but overexpression MTERF4 restored the decrease (Fig. 4B). Furthermore, the western blotting assays also showed that the respiratory chain complexes' levels were decreased by MPP^+, whereas overexpression of MTERF4 restored complexes I and IV's levels (Fig. 4C).

However, after exposure to 2 mM MPP^+ for 24 h, the levels of ROS and MMP were unchanged in the control and MTERF4 overexpressing SH-SY5Y cells (Fig. 4D, E). Furthermore, the cell viability was not different between the control and MTERF4 overexpressing cells. (Fig. 4F). Therefore, up-regulating MTERF4 conferred partial protection against MPP^+.

4. Discussion

In recent years, it has been reported that mice lacking *MTERF4* are embryonic lethal and that MTERF4 acts as a regulator of

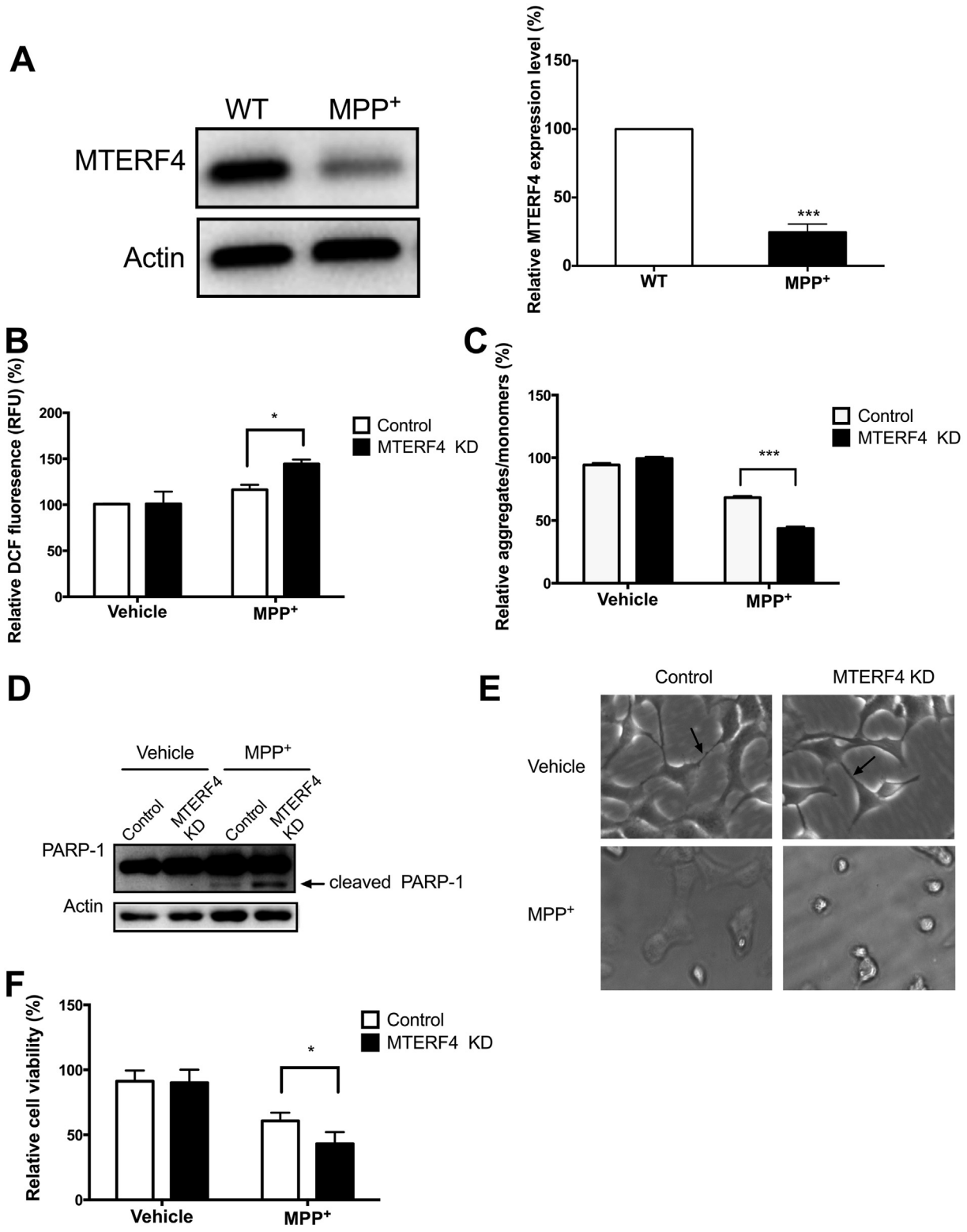

Fig. 3. MTERF4 knockdown further aggravates MPP$^+$-induced mitochondrial dysfunction and cell apoptosis. (A) MTERF4 expression is lower in SH-SY5Y cells treated with MPP$^+$. Western blot analyses for SH-SY5Y cells treated with 2 mM MPP$^+$ for 24 h (MPP$^+$) and the quantification of MTERF4 levels in MPP$^+$ treated SH-SY5Y cells compared to wide-type (WT). Error bars are the SEM; ***$p < 0.001$, $n = 6$. **(B)** Relative ROS accumulation in MTERF4 KD SH-SY5Y cells compared to normal levels under normal conditions (vehicle) or treated with 2 mM MPP$^+$ for 24 h (MPP$^+$). Error bars are the SEM; *$p < 0.05$, $n = 3$. **(C)** The MMP levels detected by JC-1. The aggregates/monomers in MTERF4 KD SH-SY5Y cells compared to normal levels under normal conditions (vehicle) or treated with 2 mM MPP$^+$ for 24 h (MPP$^+$). Error bars are the SEM; ***$p < 0.001$, $n = 6$. **(D)** Representative immunoblot of PARP-1 and cleaved PARP-1 (arrow) protein expression in control and MTERF4 KD SH-SY5Y cells under normal conditions (vehicle) or treated with 2 mM MPP$^+$ for 24 h (MPP$^+$). **(E)** Representative images of cell morphology captured by optical microscopy in control and MTERF4 knockdown (KD) SH-SY5Y cells under normal conditions (vehicle) or treated with 2 mM MPP$^+$ for 24 h (MPP$^+$). The normal SH-SY5Y cells have long synapse, as showed by arrow in the top two pictures. **(F)** Quantification of cell viability determined using CCK8-solution for control and MTERF4 KD SH-SY5Y cells under normal conditions (vehicle) or treated with 2 mM MPP$^+$ for 24 h (MPP$^+$). Error bars are the SEM; *$p < 0.05$, $n = 4$.

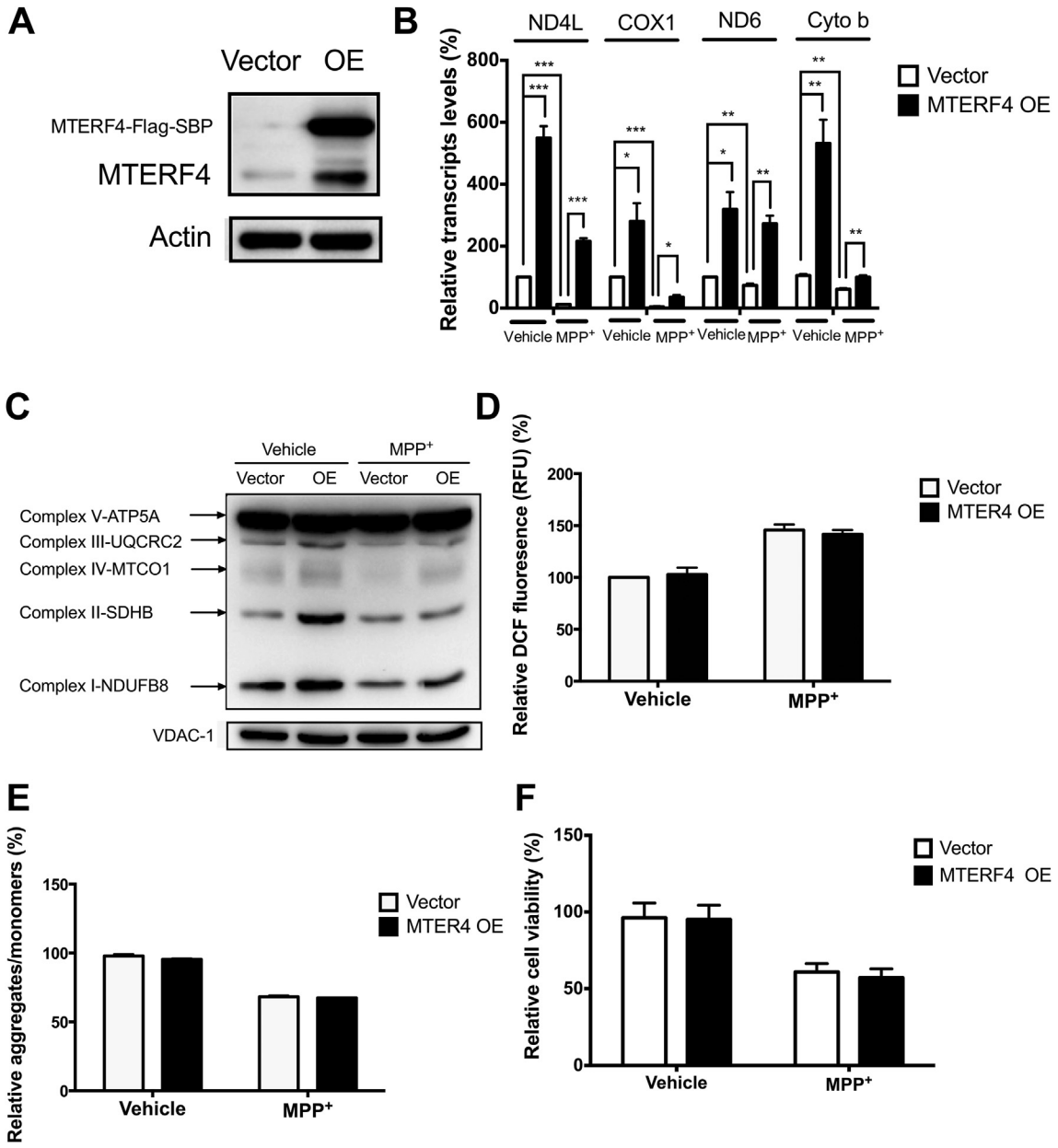

Fig. 4. MTERF4 overexpression restores respiratory chain complex deficiency induced by MPP$^+$. **(A)** Representative immunoblot of the MTERF4 expression levels in empty vector cells (vector) and MTERF4 overexpression (OE) SH-SY5Y cells. The empty vector cells were used as the overexpression control. **(B)** Quantification of the mitochondrial transcript levels of the control and MTERF4 OE SH-SY5Y cells under normal conditions (vehicle) or treated with 2 mM MPP$^+$ for 24 h (MPP$^+$). Error bars are the SEM; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, $n = 3$. **(C)** Representative immunoblot of the subunits of the respiratory chain complexes in control and MTERF4 OE SH-SY5Y cells under normal conditions (vehicle) or treated with 2 mM MPP$^+$ for 24 h (MPP$^+$). **(D)** Relative ROS accumulation in MTERF4 OE SH-SY5Y cells compared to normal levels under normal conditions (vehicle) or treated with 2 mM MPP$^+$ for 24 h (MPP$^+$). Error bars are the SEM; *$p > 0.05$, $n = 3$. **(E)** The MMP levels detected by JC-1. The aggregates/monomers in MTERF4 OE SH-SY5Y cells compared to normal levels under normal conditions (vehicle) or treated with 2 mM MPP$^+$ for 24 h (MPP$^+$). Error bars are the SEM; *$p > 0.05$, $n = 6$. **(F)** Quantification of cell viability in the control and MTERF4 OE SH-SY5Y cells determined using CCK8-solution under normal conditions (vehicle) or treated with 2 mM MPP$^+$ for 24 h (MPP$^+$). Error bars are the SEM; *$p > 0.05$, $n = 4$.

mitochondrial translation by targeting NSUN4 to the large mitochondrial ribosome [16,17].

Here, we showed that knockdown of *MTERF4* impairs mtDNA transcription and mtDNA translation in SH-SY5Y cells, which further indicates that MTERF4 is a crucial regulator of mitochondrial biogenesis. However, we did not find defective cell growth and cell death in the MTERF4 KD SH-SY5Y cells. The decreased expression level of MTERF4 resulted in deficient mtDNA translation and severe respiratory chain deficiency; however, this may be insufficient to impair cell growth or cause cell death in SH-SY5Y cells.

MPP$^+$ is typically used to create the cellular models of PD in vitro and has been strongly demonstrated to induce mitochondrial dysfunction. Interestingly, this was first time that we found that MTERF4 was downregulated when SH-SY5Y cells were treated with 2 mM MPP$^+$ for 24 h. In addition, after exposure to 2 mM MPP$^+$ for 24 h, we demonstrated that the mitochondrial dysfunction induced by MPP$^+$ was further aggravated in MTERF4 KD cells, with the further accumulation of ROS, further decrease of MMP, and further increase of cleaved PARP-1. Therefore, MTERF4 plays an important role in regulating the mitochondrial dysfunction induced by MPP$^+$.

Knockdown of MTERF4 reduced the respiratory chain complex activity, including complex I, which is inhibited under MPP^+ stress. We propose that the decreased expression levels of MTERF4 induced by MPP^+ contribute to the inhibition of complex I in MPP^+ treated SH-SY5Y cells. Therefore, after exposure to 2 mM MPP^+ for 24 h, knockdown of MTERF4 worsened the mitochondrial dysfunction and apoptosis induced by MPP^+.

After exposure to 2 mM MPP^+ for 24 h, overexpression of MTERF4 alleviated the respiratory chain complex deficiency induced by MPP^+; however, it did not rescue the ROS and MMP damage induced by MPP^+ in SH-SY5Y cells. Therefore, MPP^+ may also suppress the expression levels of the remaining MTERFs family members. In addition, MPP^+ may suppress the proteins involved in the regulation of mitochondrial function, such as TFAM [6], and overexpression of MTERF4 only may not be adequate to completely rescue the mitochondrial dysfunction induced by MPP^+.

By contrast, the MTERFs may maintain the dynamic cellular balance. Unpublished data from our lab has found that the expression levels of MTERF3 protein was decreased but the expression levels of MTERF2 protein was increased after exposure to 2 mM MPP^+ for 24 h in SH-SY5Y cells, while both MTERF2 and MTERF3 have been reported to interact with mitochondrial biogenesis [12,15]. Further studies should help clarify whether MTERF2 and MTERF3 play important roles in regulating the mitochondrial dysfunction induced by MPP^+ in SH-SY5Y cells or other cellular models of PD.

In conclusion, our data reveal a novel role for MTERF4 in neurodegenerative disease through the regulation of mitochondrial function. Our study also provides new insight into the mechanism of mitochondrial damage induced by MPP^+. Furthermore, the level of MTERF4 expression may be a good diagnostic indicator for the progression of PD.

Conflict of interest

None.

Acknowledgments

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Transparency document

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