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MTERF2 contributes to MPP$^+$-induced mitochondrial dysfunction and cell damage

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ABSTRACT

Parkinson's disease (PD) is a common neurodegenerative disorder whose pathogenesis is under intense investigation. Substantial evidence indicates that mitochondrial dysfunction plays a central role in the pathophysiology of PD. Several mitochondrial internal regulating factors act to maintain the mitochondrial function. However, how these internal regulating factors contribute to mitochondrial dysfunction in PD remains elusive. One of these factors, mitochondrial transcription termination factor 2 (MTERF2), has been implicated in the regulation of oxidative phosphorylation by modulating mitochondrial DNA transcription. Here, we discovered a new role of MTERF2 in regulating mitochondrial dysfunction and cell damage induced by MPP$^+$ in SH-SY5Y cells. We found that MPP$^+$ treatment elevated MTERF2 expression, induced mitochondrial dysfunction and cell damage, which was alleviated by MTERF2 knockdown. These findings demonstrate that MTERF2 contributes to MPP$^+$-induced mitochondrial disruption and cell damage. This study indicates that MTERF2 is a potential therapeutic target for environmentally induced Parkinson's disease.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by preferential loss of dopaminergic neurons in the substantia nigra pars compacta and the presence of intraneuronal inclusions known as Lewy Bodies [1]. Because of many potential factors contributing to the occurrence of PD, the mechanisms underlying PD development remain elusive. However, the pivotal role of mitochondrial dysfunction in the pathophysiology of PD has been substantiated by evidence from cellular models, animal models and postmortem human brain tissue [2,3].

Although the majority of proteins present in the mitochondria are encoded by the nuclear genome, mitochondria have their own DNA in the mitochondrial matrix, which encodes a small set of mitochondrial proteins (13 subunits of the respiratory chain complexes), rRNAs and tRNAs. Accordingly, mitochondria contain several internal regulating factors that are involved in mitochondrial DNA (mtDNA) maintenance, replication, transcription and translation. Mitochondria have quality-control systems to maintain mitochondrial functions, and selectively respond to stress through mechanisms acting at the molecular, organellar and even cellular levels [4]. Moreover, the importance of mitochondrial functions is highlighted by the fact that mitochondrial dysfunction is involved in the occurrence of PD [2]. Therefore, the role of mitochondrial internal regulating factors in the pathogenesis of PD is a meaningful and ongoing research topic.

In recent years, a family of mitochondrial transcription termination factors (MTERF1, MTERF2, MTERF3, MTERF4) have been found to regulate mitochondrial functions in mammalian cells. MTERF1 can regulate mtDNA transcription termination [5] and activate rRNA transcription [6]. There is also a report showing that MTERF1 bound mtDNA to prevent transcriptional interference at the light-strand promoter (LSP), but did not regulate heavy strand transcription [7]. MTERF3 not only functions as a negative regulator of mammalian mtDNA transcription [8] but also is involved in the biogenesis of the mitochondrial ribosomes [9]. Moreover, MTERF4 regulates translation by targeting the 5-methylcytosine RNA methyltransferase NSUN4 to the large subunit of the mitochondrial

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ribosome [10,11].

MTERF2 (also known as MTERFL, MTERFD3) was identified as one of the mitochondrial genes that respond to serum starvation in 2005 and the expression of MTERF2 was elevated after serum starvation [12]. MTERF2 is localized in the matrix and is ubiquitously expressed [13]. But relatively higher levels of MTERF2 mRNA expression were detected in tissues with higher energy demand, such as heart, brain, kidney and testis [13]. Some recent research [13,14] indicates that MTERF2 is a modulator of mitochondrial transcription. When *Mterf2* knock-out mice fed on a ketogenic diet (high fat, low carbohydrates), loss of MTERF2 caused a respiratory defect in certain tissues, due to decreased levels of the oxidative phosphorylation (OXPHOS) complexes [13]. When MTERF2 was overexpressed in HeLa cells, the mtDNA copy numbers and expression levels of mitochondrial genes were significantly reduced [14]. However, opposing models for the regulation molecular mechanism were reported: (1) MTERF2 has the capacity to bind DNA in a non-sequence specific way [14,15]; (2) MTERF2 binds to the heavy-strand promoter (HSP) region together with MTERF1 and MTERF3 [13]. Thus, the molecular mechanisms of MTERF2 function remain unclear.

Here, we examined the potential role of MTERF2 in PD using SH-SY5Y cells treated by 1-methyl-4-phenylpyridinium (MPP^+), which induces mitochondrial dysfunction and is widely used to construct the cellular model of PD [16,17]. We showed that MTERF2 knockdown in SH-SY5Y cells alleviated the mitochondrial dysfunction and cell damage induced by MPP^+. Our work provides new insights into understanding the pathogenesis and developing effective therapeutics for Parkinson's disease.

2. Materials and methods

2.1. Cell culture and cell viability assay

SH-SY5Y cells and HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco). To determine the cell viability of 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 different concentration gradient or certain concentration of MPP^+. After another 24 h, the Cell Counting Kit-8 solution (CCK-8, Dojindo) was used to test the cell viability.

2.2. Quantitative real-time PCR

Total RNA was isolated from the cells using a total RNA kit (TIANGEN), and the cDNA was synthesized using a HiScript II 1st Strand cDNA Synthesis Kit (Vazyme Biotech). Total DNA was extracted from the cells using a Wizard genomic DNA purification kit (Promega). The mRNA expression of MTERF2 and the mtDNA copy number (primers referenced [18]) was determined on a MiniOpticon real-time PCR detection system (Bio-Rad) with AccuPower 2 × Greenstar qPCR Master Mix (Bioneer). The following primers were also used: MTERF2 (sense 5′-CCGGAAGCAATTGTCTGTAG-3′ and antisense 5′-GCAGACCAACTGCCAGAGT-3′), actin (sense 5′-CACTCTTCCAGCCTTCCTTC-3′ and antisense 5′-GGATGTCCACGTCACACTTC-3′).

2.3. Western blots

Immunoblot analyses were performed according to standard methods with the following primary antibodies: MTERF2 (Abcam, 1:250), β-actin (Sigma, 1:5000), GAPDH (Sigma, 1:5000), VDAC1 (Affinity, 1:1000), cytochrome c (Cyto C, Abcam, 1:1000), and MitoProfile total OXPHOS rodent WB antibody cocktail (MitoSciences, 1:500). Immunoblot of β-actin or GAPDH was performed to demonstrate equal whole-cell proteins and cytosolic proteins loading, while VDAC1 for mitochondrial protein.

2.4. Plasmid constructions and transfection

Full-length human cDNA of MTERF2 was cloned into the vector pLVX-IRES-Puro (Clontech) and sequenced. Short hair-pin RNA (shRNA) targeting MTERF2 (5′-TTAACCCTGTGGCACCATTAA-3′) was inserted into the pLVX-shRNA1 Vector (Clontech) and sequenced. The sequence verified plasmid and the lentivirus packaging plasmids were cotransfected into HEK293T cells, using HilyMax transfection reagent (Dojindo). Then the culture supernatants were collected at 48 h and 72 h after transfection and filtrated (0.45 μm filter) to get lentivirus to transduce SH-SY5Y cells. Stable transfectants were harvested after selection with puromycin (Sigma).

2.5. Mitochondrial membrane potential ($\Delta\psi m$) and reactive oxygen species (ROS) assays

The mitochondrial membrane potential was measured with JC-1 (Beyotime Biotech). JC-1 exhibits potential-dependent accumulation in mitochondria. Mitochondrial depolarization is indicated by a fluorescence emission shift from red (aggregates of JC-1) to green (monomers of JC-1). The images were captured by a fluorescent inverted microscope (Leica DM IL LED). The 2′,7′-dichlorofluorescein diacetate probe (DCFH-DA, Beyotime Biotech) was used to measure the intracellular ROS. The fluorescence intensity was monitored by a BD Accuri™ C6 flow cytometer (BD Bioscience).

2.6. Cell cycle assay

Determination of the cell cycle used a Cell Cycle Kit (Beyotime Biotech) according to the manufacturer's protocols. Briefly, the harvested cells were pelleted by centrifuge and fixed in 70% ethanol for at least 2 h at 4 °C. Cells were then washed twice with cold PBS and treated with the mixture of Rnase A and propidium iodide for 30 min at 37 °C. After that, the cells were detected and analyzed on a BD Accuri™ C6 flow cytometer (BD Bioscience).

2.7. Mitochondria isolation

Mitochondria were isolated using a mitochondria isolation kit (Thermo) according to the manufacturer's instructions. Briefly, about 2×10^7 cells were harvested by centrifuging cell suspension and then centrifuged after cells were broken at 700 g for 10 min at 4 °C. The supernatant was further centrifuged at 12000 g for 15 min at 4 °C. Then the pellet (the mitochondrial fraction) was washed and lysed with RIPA lysis (Beyotime Biotech); the supernatant was concentrated by the 3K filtrate tube (Millipore), centrifuged at 4500 g for 45 min at 4 °C and designated as the cytosolic fraction [19].

2.8. Statistical analysis

Data obtained were expressed as the mean ± standard deviation (SD) from at least three independent experiments, and statistical significance was analyzed by *t*-test or two-way ANOVA using GraphPad Prism 6.0 software. A p value < 0.05 was considered statistically significant.