International Journal of Sciences

Research Article Volume 8 – April 2019 (04)

MPP+ Attenuates the Inhibition of p53 by Parkin in SH-SY5Y Cells

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Abstract: Parkin acts as a p53 transcriptional repressor, binds to p53 gene promoter in the nucleus, thereby inhibiting the transcription of p53 gene. However, whether this process take part in Parkinson's disease (PD) is unclear. In order to detect the inhibitory effect of parkin on p53 in the PD model, parkin-transfected SH-SY5Y cells were treated with 1-methyl-4-phenylpyridinium (MPP^+). The results showed that parkin could inhibit the expression of p53 protein under physiological conditions, but in the PD cell model, the function of parkin impaired, which reduced the inhibitory function of p53.

Keywords: Parkin, p53, Parkinson's Disease

Introduction

Parkinson's disease (PD) is a common neurodegenerative disease, in which the main pathological change is the selective loss of dopamine (DA) neurons in the substantia nigra[1, 2], accompanied by the specific accumulation of iron in the substantia nigra[3]. At present, there are 18 pathogenic genes found in PD. The parkin gene is the second disease-causing gene cloned in 1998, and its mutation leads to autosomal recessive adolescent PD (AR-JP)[4, 5]. The parkin protein is a ubiquitin ligase that catalyzes the binding of specific substrates to ubiquitin and participates in the degradation of abnormal protein in cells[5, 6].

In addition to E3 ubiquitin ligase activity, parkin protein also functions as a p53 transcriptional repressor and binds to the p53 gene promoter in the nucleus, thereby inhibiting the transcription of p53 gene[7, 8]. How the interaction between parkin and p53 played in PD were investigated in this study. Using SH-SY5Y cells, the effect of overexpression of parkin on p53 was observed. We also explored whether the regulation of p53 was affected by parkin was observed under 1-methyl-4-phenylpyridinium (MPP^+) treatment.

Materials and Methods

Cell culture and cell treatment

The SH-SY5Y cells was bought from the Chinese Academy of Sciences Cell Bank. The SH-SY5Y cells were cultured in RPMI 1640 Medium (Gibco, USA) containing 15% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin in a culture environment containing 5% CO_2 at 37 ℃. The parkin plasmid was transfected into SH-SY5Y cells by Lipofectamine 2000 (Thermo Fisher Scientific, USA) to prepare cells transiently overexpressing parkin. 24 hrs after cell transfection, cells were treated with 200 μM MPP^+ (Sigma, Ronkonkoma, NY, USA) for 24 hrs.

Cell viability measurement

Cell viability was detected using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. SH-SY5Y cells were seeded in a 96-well plate and cultured overnight for cell attachment. At the end of the treatment, MTT was added to the medium medium of each well at a final concentration of 5 mg / mL, and cultured at 37 ℃ for 4 hrs. The insoluble formazan was then dissolved in dimethyl sulphoxide (DMSO). After treatment, The plates were shook until the crystal was dissolved. Cell viability was assessed at the wavelength of 494 nm and 630 nm using a microplate reader (Molecular Device, M5, Sunnyvale, CA, USA).

Western blotting analysis

Cells were lysed with protease inhibitors (Roche Diagnostics, Germany) and RIPA lysis buffer (ComWin Biotech Co, China) for 30 min on ice and the insoluble material was removed after centrifugation (12000 r/min, 20 min, 4 ℃). Protein concentrations were determined by the BCA Protein Assay Kit (Thermo Fisher Scientific, USA). The supernatant (20 μg/channel) was mixed with 5 × SDS sample buffer, and boiled for 5 min, and then separated by 10% SDS-PAGE gel. After electrophoresis, the proteins were transferred to the PVDF membrane by electrophoretic transfer, which

Published at: http://www.ijsciences.com/pub/issue/2019-04/
DOI: 10.18483/ijSci.1967; Online ISSN: 2305-3925; Print ISSN: 2410-4477

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was blocked subsequently with 10% skim milk powder for 2 hrs. The membranes were incubated with anti-parkin antibody (1:1000, Millipore, USA), anti-p53 antibody (1:1000, Millipore, USA), anti-β-actin antibody (1:10000, Bioss, China) overnight at 4°C. Membranes were incubated with a suitable source of secondary antibody (1:10000, Bioss, China) for 1 hr at room temperature. Finally, blots and data statistics were performed using the BioSpectrum Imaging System (UVP, USA).

Statistical analysis

Data were presented as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests. Values of $P < 0.05$ were considered signifcantly.

Result

Parkin overexpression reduces the level of p53 in SH-SY5Y cells

First, we transfected the parkin plasmid into SH-SY5Y cells and observed the changes of p53 levels in parkin-transfected cells. We found p53 levels were decreased by 26.5% with overexpression of parkin, as shown in Fig. 1A and 1B. The results showed that overexpression of parkin inhibited the expression of p53.

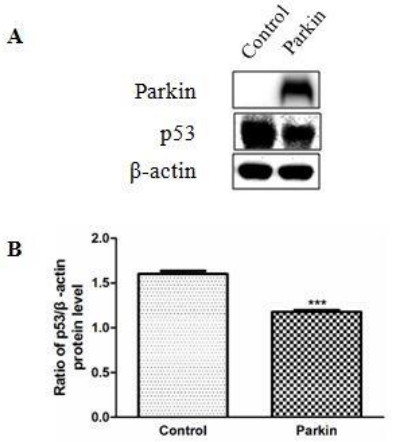

Fig. 1. Parkin overexpression down-regulates p53 expression in SH-SY5Y cells. Data were presented as the ratio of p53 to β-actin. Each bar represented as the mean ± SEM of 3 independent experiments. ***$P <$ 0.001 compared to the control.

Effects of MPP^+ on the SH-SY5Y cell viability

To detect the effect of MPP^+ on SH-SY5Y cell viability, we performed the MTT assay to determine the cell survival in response to different concentrations of MPP^+ treatment (0, 50, 100, 200, 300, 500, 1000 μmol/L) for 24 hrs. The results showed that treatment with the low dose MPP^+ (50, 100 μmol/L) had no effect on SH-SY5Y cell viability, while the high dose MPP^+ (200, 300, 500, 1000 μmol/L) signifcantly decreased the cell viability (Fig. 2), suggesting that MPP^+ induced cell death in a dose dependent manner. Based on the minimum effective dose of neurotoxicity, treatment with MPP^+ at 200 μmol/L for 24 hrs was used to prepare a PD cell model.

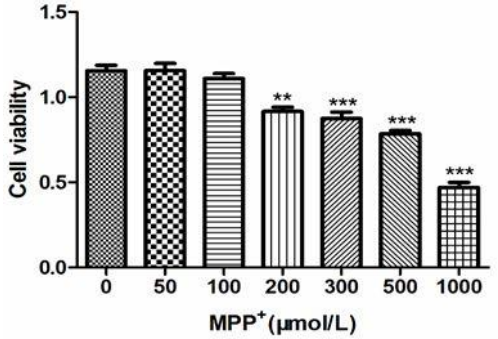

Fig. 2. The changes of cell viability under MPP^+ treatment. SH-SY5Y cells' viability following MPP^+ treatment was determined by MTT assay. All data were presented as mean ± SEM of 6 independent experiments. **$P < 0.01$, ***$P < 0.001$ compared with the control (0).

MPP^+ affects the inhibitory effect of parkin on p53 expression in SH-SY5Y cells

When cells were treated with 200 μM MPP^+ for 24 hrs, the levels of p53 showed a 0.76-fold increase in MPP^+ treated overexpressed parkin SH-SY5Y cells. There was no significant change in p53 level compared with the MPP+ group without parkin. These results indicate that MPP^+ affects the inhibitory effect of parkin on p53 expression.

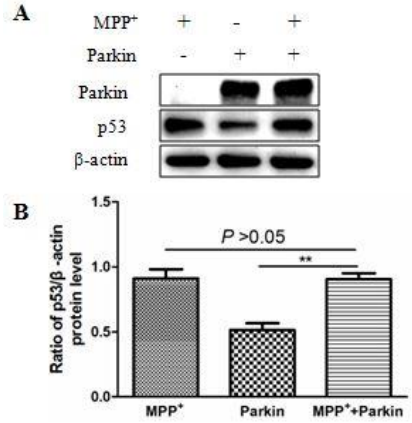

Fig. 3. MPP^+ up-regulates p53 expression in SH-SY5Y cells overexpressing parkin. Data were presented as the ratio of p53 to β-actin. Each bar represented as the mean ± SEM of 3 independent experiments. **$P < 0.01$ compared to the parkin group.

Discussion

In the present study, we demonstrated that parkin

reduced its activity as a p53 gene expression inhibitor in the PD cell model prepared by MPP^+, resulting in up-regulation of p53 protein. Our data suggest that inhibition of parkin function contributes to the expression of p53 and partly to the pathophysiology of sporadic PD.

Parkin has been reported to mediate neuronal protection against neurotoxicity induced by 6-OHDA[9]. Overexpression of parkin in Parkinson's animal model improves exercise capacity and reduces dopaminergic neuron degeneration[9]. But its molecular mechanism is not well explained. p53 is an important transcription factor involved in the regulation of cell cycle, cellular metabolism and autophagy, apoptosis, etc. through a variety of signaling pathways[10]. Many studies have demonstrated that abnormalities in p53 protein promote the development of PD by targeting the regulation of apoptosis-related genes[11, 12]. Induction of apoptosis is an important mechanism by which p53 exerts tumor suppressor function in cancer[13, 14]. Interestingly, p53 activation was observed in brain and PD mouse models of PD patients, suggesting that p53 activation and p53-mediated apoptosis promote PD[15-18]. Therefore, inhibition of p53-mediated apoptosis may be an important mechanism of parkin-related neuroprotection.

In this study, we observed the effect of parkin on p53 protein in the MPP^+-induced PD cell model. We found that after MPP^+ treatment of cells, the level of p53 protein was significantly increased with the increase of parkin content, and the inhibitory function of parkin on p53 was impaired.

In conclusion, parkin is a neuroprotective molecule that inhibits the activity of p53 under physiological conditions. Current studies indicate that in the PD cell model prepared by MPP^+, parkin function is impaired and its ability to bind to the p53 promoter sequence is lost. This leads to an increase in the level of p53 protein, which may result in the death of apoptotic cells in the affected neurons. Our research indicates that parkin impaired is critical for the presence of intracellular p53, which probably provides a new target for the prevention and treatment of PD.

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