Research Article

Stress increases MHC-I expression in dopaminergic neurons and induces autoimmune activation in Parkinson's disease

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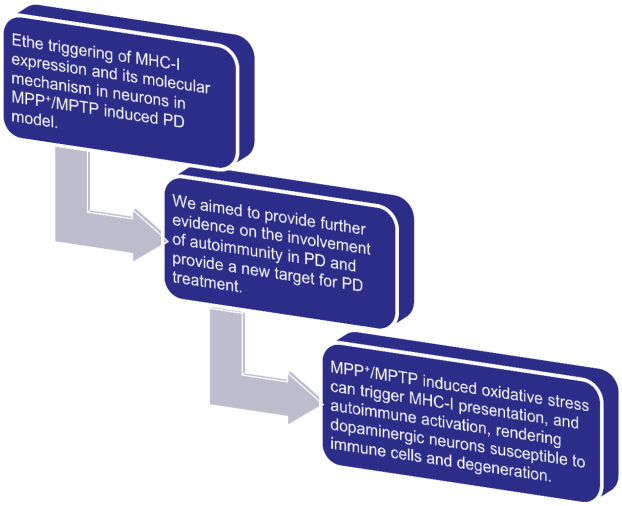
Graphical Abstract *Role of oxidative stress induced major histocompatibility complex class I presentation and the underlying mechanisms in Parkinson's disease (PD)*



Abstract

The expression of major histocompatibility complex class I (MHC-I), a key antigen-presenting protein, can be induced in dopaminergic neurons in the substantia nigra, thus indicating its possible involvement in the occurrence and development of Parkinson's disease. However, it remains unclear whether oxidative stress induces Parkinson's disease through the MHC-I pathway. In the present study, polymerase chain reaction and western blot assays were used to determine the expression of MHC-I in 1-methyl-4-phenylpyridinium (MPP^+)-treated SH-SY5Y cells and a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's disease mouse model. The findings revealed that MHC-I was expressed in both models. To detect whether the expression of MHC-I was able to trigger the infiltration of cytotoxic T cells, immunofluorescence staining was used to detect cytotoxic cluster of differentiation 8 ($CD8)^+$ T cell infiltration in the substantia nigra of MPTP-treated mice. The results indicated that the presentation of MHC-I in dopaminergic neurons was indeed accompanied by an increase in the number of $CD8^+$ T cells. Moreover, in MPTP-induced Parkinson's disease model mice, the genetic knockdown of endogenous MHC-I, which was caused by injecting specific adenovirus into the substantia nigra, led to a significant reduction in $CD8^+$ T cell infiltration and alleviated dopaminergic neuronal death. To further investigate the molecular mechanisms of oxidative stress-induced MHC-I presentation, the expression of PTEN-induced kinase 1 (PINK1) was silenced in MPP^+-treated SH-SY5Y cells using specific small interfering RNA (siRNA), and there was more presentation of MHC-I in these cells compared with control siRNA-treated cells. Taken together, MPP^+-/MPTP-induced oxidative stress can trigger MHC-I presentation and autoimmune activation, thus rendering dopaminergic neurons susceptible to immune cells and degeneration. This may be one of the mechanisms of oxidative stress-induced Parkinson's disease, and implies the potential neuroprotective role of PINK1 in oxidative stress-induced MHC-I presentation. All animal experiments were approved by the Southern Medical University Ethics Committee (No. 81802040, approved on February 25, 2018).

Key Words: antigen presentation; autoimmune; $CD8^+$ T cell; dopaminergic neuron; major histocompatibility complex class I; mitochondria; neuroinflammation; oxidative stress; Parkinson's disease; PINK1

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Introduction

Chronic stress is a critical factor in Parkinson's disease (PD) (Zhang et al., 2020); it can induce the accumulation of alpha-synuclein (α-syn) and lead to the impairment of dopaminergic neurons. The incidence of PD is closely related to age, with a rate of about 1–2% in people over 60 years old, increasing to about 3–4% in people over 80 years old (Corti et al., 2011). Several theories have been proposed regarding the etiology of PD. A number of them are listed here:

(1) Oxidative stress induces mitochondrial dysfunction in dopaminergic neurons. Mitochondrial abnormalities produce reactive oxygen species and disrupt calcium homeostasis. Oxidative stress finally induces neuronal apoptosis (Subramaniam and Chesselet, 2013).

(2) Neuronal inflammation in the substantia nigra. This involves an increase in the number of cluster of differentiation (CD)4^+ T cells (Sulzer et al., 2017) and cytokines (e.g., interleukin-1, interleukin-2, interleukin-6, and interferon-γ), as well as the infiltration of active microglia in the brains of PD patients (Benkler et al., 2012). This may result in the loss of dopaminergic neurons.

(3) Genetic mutations. In hereditary PD patients, common gene mutations have been identified in genes such as PTEN-induced kinase 1 (*PINK1*), DJ-1 (*PARK7*), and leucine-rich repeat kinase 2 (*LRRK2*) (Corti et al., 2011). It has recently been reported that *PINK1* mutations can cause the presentation of mitochondrial proteins on the surface of immune cells, thus triggering attacks from $CD8^+$ T cells (Matheoud et al., 2016).

Aside from the aforementioned etiological factors, it has recently been reported that T cells from PD patients can recognize α-syn (Mosley and Gendelman, 2017; Sulzer et al., 2017), and that γ^+/δ^+ T cells are elevated in the peripheral blood and cerebrospinal fluid of patients with PD (Fiszer et al., 1994); these findings indicate the activation of an immune response toward Lewy bodies in PD patients. Furthermore, autoantibodies against neurons, such as anti-ganglioside GM1 antibodies (Zappia et al., 2002), anti-melanin antibodies (Double et al., 2009), and anti-α-syn antibodies (Papachroni et al., 2007), have also been detected in the peripheral blood and cerebrospinal fluid of PD patients (Benkler et al., 2012). Together, the described evidence suggests that autoimmunity might be involved in the etiology of PD.

With regard to autoimmunity, major histocompatibility complex class I (MHC-I) plays an essential role in the autoimmune activation process. In the human central nervous system, neurons in the lateral geniculate nucleus express MHC-I at 29–31 gestational weeks; this expression decreases with age and completely disappears by adulthood (CebriÃ et al., 2014). Studies spanning long periods have reported an association between the expression of MHC-I in neurons and central nervous system development (Huh et al., 2000; Needleman et al., 2010, Inácio et al., 2016). In recent decades, scientists have uncovered clues that link the expression of MHC-I by central nervous system neurons with immunity. First, MHC-I is expressed in the cortical neurons of patients with Rasmussen's encephalitis, an immune-mediated disease (Bien et al., 2002), as well as in patients with tuberous sclerosis complex, focal cortical dysplasia, and ganglioglioma (Carolina Cebrián et al., 2014). Second, MHC-I expression has been reported in hippocampal endothelial cells in control individuals and patients with Alzheimer's disease (Tooyama et al., 1990), as well as in dopaminergic neurons of the substantia nigra (SN) and norepinephrinergic neurons of the locus ceruleus in both control individuals and patients with PD (Cebrián et al., 2014). Coincidentally, apoptosis of these two neuron types is directly related to the two neurodegenerative diseases with the highest incidences in humans (Alzheimer's disease and PD), suggesting that MHC-I may be involved in aging-induced neuronal apoptosis. However, the relationship between MHC-I expression in dopaminergic neurons and PD remain to be elucidated, as do the underlying mechanisms.

Considering that oxidative stress, *PINK1* mutations, neuronal inflammation, and autoimmune activation all contribute to the onset of PD, they may have a relationship of mutual causality (Quinn et al., 2020). We hypothesized that oxidative stress and PINK1 mutations might trigger the presentation of endogenous proteins via the MHC-I pathway. Misfolded MHC-I protein induces autoimmune inflammation. Thus, this study explored the triggering of MHC-I expression and its molecular mechanism in neurons in 1-methyl-4-phenylpyridinium (MPP^+)-/1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD models to provide further evidence of the involvement of autoimmunity in PD, and to provide support for a new target for PD treatment.

Materials and Methods

Cells

Human neuroblastoma SH-SY5Y cells were acquired from the Central Laboratory of Nanfang Hospital (Guangzhou, China) and were used to examine the molecular mechanisms of antigen presentation in dopaminergic neurons (Fall and Bennett, 1999; Xicoy et al., 2017). The SH-SY5Y cells were cultured in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and incubated at 5% CO_2 and 37°C. To analyze MHC-I expression in a PD model of SH-SY5Y cells, the cells were treated with 0.1 mM MPP^+ (D048: Sigma-Aldrich: St Louis, MO, USA) for 24 hours. Cells in the positive control group were incubated with 100 ng/mL interferon-γ (285-IF-100; R&D Systems, Minneapolis, MN, USA) for 24 hours (Lorenzi et al., 2012, Spel et al., 2015).

Animals

Male specific pathogen-free C57BL/6 mice weighing 23.1 ± 0.8 g and aged 10 weeks were obtained from the Laboratory Animal Centre of Southern Medical University, China. Sixty mice (housed in a specific pathogen-free breeding environment) were randomly assigned to the different groups. All animal experiments were approved by the Southern Medical University Ethics Committee (No. 81802040, approved on February 25, 2018). The experimental procedures followed the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Group assignment and intervention

This study used a completely randomized method for group assignment. The mice were numbered from light to heavy in weight, and were then divided into several (three or four, according to the experiment design) similar-weight groups in sequence. The experimental groups were formed by mice that were randomly picked up from each similar-weight group.

To induce the PD mouse model (Jackson-Lewis and Przedborski, 2007), 36 mice were randomly divided into two groups (n = 18 per group). The first group of 18 mice was randomly divided into two groups of MPTP treatment (1 day or 7 days) and one group of saline treatment (n = 6 per group). Mice in the MPTP treatment group were treated with four intraperitoneal injections of MPTP HCl (18 mg/kg; M103-10MG; Sigma-Aldrich) over 1 day, at 2-hour intervals. In the

control group, mice were injected with an equivalent amount of saline. The other 18 mice were divided into groups and intervened in the same way.

To induce the MHC-I knockdown model (Ye et al., 2018), we used a mouse MHC-I knockdown adenovirus designed by GenePharma Co. (Shanghai, China). Its full length is provided in **Additional Table 1**. Immunofluorescence and western blot assay were used to measure MHC-I expression, to confirm the knockdown efficiency (**Additional Figure 1**). In total, 24 mice were randomly assigned to four groups (n = 6 per group). The mice were then treated with either the adenovirus or the negative control (NC). In the control group, there was no administration of adenovirus to the SN. In the Ad-NC group, both sides of the SN were injected with empty adenoviruses as the NC. In the MPTP + Ad-NC group, both sides of the SN were injected with empty adenoviruses, and after 7 days, the mice were treated with MPTP. In the MPTP + Ad-MHC-I shRNA group, the left side of the SN was stereotaxically injected with MHC-I short hairpin RNA (shRNA) adenoviruses and the right side was stereotaxically injected with empty adenoviruses, and after 7 days, the mice were treated with MPTP.

The stereotaxic injection process was as follows. First, the mice were anesthetized and fixed into a stereotaxic skull-frame (Stoelting Co., Wood Dale, IL, USA). They were then injected with either the adenovirus (the MHC-I knockdown adenovirus, designed by GenePharma Co.; 3 μL; 1×10^{10} infection forming units/μL per construct) or the empty adenovirus (NC) into the SN region (the stereotactic target was anteroposterior: –3.2 mm, mediolateral: 1.0 mm, and dorsoventral: –5.0 mm) (Burguillos et al., 2011). After 1 week, the mice were intraperitoneally injected with either MPTP or saline.

Tissue sampling and handling

The mice were sacrificed on either day 1 or day 7 after the injection of MPTP. After being anesthetized with pentobarbital (50 mg/kg; Sinopharm, Shanghai, China), the mice were infused with 30 mL ice-cold phosphate-buffered saline (PBS) in the left ventricle, and were then perfused with 4% paraformaldehyde for 15 minutes. Next, the brains were collected and fixed in 4% paraformaldehyde for 2 days at 4°C. They were then dehydrated in 30% sucrose for 2 days at 4°C. The midbrain section (12 μm) of the mouse brain was dissected coronally using a microtome (Leica, Heidelberg, Germany) after rapid freezing with liquid nitrogen.

Immunofluorescence and confocal laser microscopy

After being incubated with the reagents (interferon-gamma, MPP^+ and saline) for 24 hours, SH-SY5Y culture slides were fixed with 4% paraformaldehyde (P6148; Sigma-Aldrich) and blocked with 5% bovine serum albumin (A8020; Solarbio; Beijing, China) for 2 hours at room temperature. The slides were then incubated with MHC-I mouse monoclonal antibody against human (1:100; sc-55582; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight, followed by incubation with goat anti-mouse IgG conjugated with Alexa Fluor 488 (1:200; GB2530; Servicebio, Wuhan, China) for 1 hour at room temperature. Images were then captured using a microscope (DM2500; Leica).

Mouse brain sections were incubated in ethylenediaminetetraacetic acid solution for 20 minutes at 95°C for antigen retrieval, and were then blocked in 5% bovine serum albumin (A8020; Solarbio). The following primary antibodies were used for immunofluorescence: for human MHC-I: mouse monoclonal antibody raised against the human leukocyte antigen (HLA) protein (1:100; sc-55582; Santa Cruz Biotechnology); for mouse MHC-I: goat monoclonal antibody against mouse OX-18 conjugated to fluorescein isothiocyanate (FITC; 1:100; ab22367; Abcam, Cambridge, UK); for tyrosine hydroxylase (TH): rabbit polyclonal antibody (1:1000; GB11181; Servicebio); for $CD8^+$: rabbit polyclonal antibody (1:200; GB11068-1; Servicebio). After being incubated with the primary antibodies, the sections were incubated with secondary fluorescent antibodies, as follows: for rabbit polyclonal antibody against mouse TH: Cy3-conjugated goat anti-rabbit IgG (1:300; GB23303; Servicebio); for rabbit polyclonal against mouse $CD8^+$: Cy5-conjugated goat anti-rabbit IgG (1:300; GB23303; Servicebio). In addition, 4′,6-diamidino-2-phenylindole (DAPI; 1:1000; Roche, Shanghai, China) was used to stain cell nuclei. Images were then captured using an inverted laser confocal microscope (LSM 880 with Airyscan, Carl Zeiss Meditec, Jena, Germany) (Yao et al., 2018; Ye et al., 2018). Cells were counted using Stereo Investigator software (Microbrightfield, Williston, VT, USA), and 10 sections covering the entire anteroposterior ventral mesencephalon with a counting frame (100 μm × 100 μm) were selected for each mouse. An 11 μm dissector thickness was used, and the guard zone was set at 2 μm. The coefficient of error values were less than 0.1.

RNA extraction and quantification of gene expression

In the MPP+ time-dependent experiment, total RNA was harvested from the treated cells at 0, 12, 24, and 36 hours using RNAiso Plus (Takara Bio, Kusatsu, Japan). For the other experiment, total RNA was extracted from the cells at 24 hours. To equalize the RNA concentrations, the NanoDrop ND-2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) was used to test and adjust the RNA concentrations to the same levels. To assess mRNA expression, 1 μg of total RNA was obtained to reverse transcript its complementary DNA using the PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara Bio). MHC-I expression was tested using quantitative real-time PCR (Bio-Rad, Hercules, CA, USA) with SYBR® Premix Ex Taq™ II. Beta-actin was used as the control. The sequences of the MHC-I RT-PCR primers are provided in **Additional Table 2**.

siRNA treatment of SH-SY5Y cells

Both the exclusive small interfering RNA (siRNA) for *PINK1* gene and the negative control siRNA were designed by GenePharma Company. The sequences are provided in **Additional Table 3**. The negative control siRNA and the *PINK1* siRNA were transfected into SH-SY5Y cells using Lipofectamine 2000 Reagent (11668019; Invitrogen) in accordance with the manufacturer's instructions. At the end of the 5-hour transfection period, the cells were incubated for 72 hours at 37°C before being harvested and used for western blot assay.

Western blot assay

To confirm the effectiveness of *PINK1* siRNA, a western blot assay was performed to assess the PINK1 protein levels in SH-SY5Y cells after 72 hours of siRNA treatment. Radioimmunoprecipitation assay lysis buffer (P0013B; Beyotime, Jiangsu, China) was used to extract the total protein from SH-SY5Y cells, together with phosphatase and protease inhibitors (B14001 and B15001; BioTools, Olathe, KS, USA). Protein concentrations were determined using the bicinchoninic acid assay (Bio-Rad). Each sample was run on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel (Beyotime Biotechnology, Shanghai, China) and then transferred to polyvinylidene fluoride membranes (IPVH00010; Millipore, Bedford, MA, USA). The membranes were blocked in 5% bovine serum albumin (A8020; Solarbio) for 30 minutes and then incubated with primary antibody

(1:1000) at 4°C overnight. The following primary antibodies were used for western blotting: rabbit polyclonal antibody against human PINK1 protein (BC100-494; Novus Biologicals, Centennial, CO, USA) and mouse monoclonal antibody against human glyceraldehyde 3-phosphate dehydrogenase (GAPDH; ab8245; Abcam). The secondary antibody, goat anti-rabbit IgG-HRP (1:1000; 31460; Life Technologies, Grand Island, NY, USA), was used at room temperature for 2 hours. Finally, the gels were visualized using Image Lab software with HRP Substrate (Millipore), and the band intensities were quantified using ImageJ software and Image Lab 6.0.1 software (Bio-Rad).

Flow cytometry

After being treated with MPP^+ for 24 hours, the SH-SY5Y cells were collected using trypsin/ethylenediaminetetraacetic acid and then resuspended in fluorescence-activated cell sorting (FACS) buffer (1 × PBS with 2% fetal bovine serum). The cells were stained for 20 minutes on ice using allophycocyanin (APC)-conjugated anti-human MHC-I antibody (1:500; 85-17-9958-41; eBioscience, San Diego, CA, USA). MHC-I expression levels in the neurons were detected using an LSR II cytometer (BD Biosciences, San Jose, CA, USA), and the percentage of MHC-I expression was quantified using FlowJo software (version X; TreeStar, Ashland, OR, USA) (Pajtler et al., 2013).

Statistical analysis

Statistical data were analyzed using SPSS 19.0 software (SPSS, Armonk, NY, USA). All data are presented as the mean ± SEM (n = 6) using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA). Statistical analysis was performed using one-way analysis of variance with Bonferroni *post hoc* tests. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

MHC-I is induced in MPP^+-treated SH-SY5Y cells and in dopaminergic neurons of the SN in MPTP-treated mice

There was an increase in MHC-I expression in the SH-SY5Y cells after either MPP^+ or interferon-γ treatment (**Figure 1A**). Moreover, MHC-I transcription levels were both dose-dependent (**Figure 1B**) and time-dependent (**Figure 1C**). Confirmation of MHC-I protein expression levels using immunofluorescence revealed that the cells barely expressed MHC-I under normal conditions, but had a marked increase in MHC-I expression in the PD model (**Figure 1D**). This was consistent with the PCR findings.

To investigate whether MPP also induced MHC-I expression in the SN of mice, we evaluated the expression of MHC-I and the number of dopaminergic (TH^+) neurons in the midbrains of MPTP-treated mice using immunofluorescence (**Figure 1E**). Under normal conditions, dopaminergic neurons in the SN showed no signs of neuronal loss and did not express the MHC-I protein. However, the percentage of MHC-I expression in dopaminergic neurons increased with time after the MPTP injection, and was 75.33 ± 3.71% in the 7-days-after-injection group (**Figure 1G**). Moreover, in the PD model mice, dopaminergic neuronal loss began 1 day after the MPTP injection, and dopaminergic neurons were unable to maintain their morphology. At 7 days after the MPTP injection, there was a significant loss of dopaminergic neurons, and only (6114 ± 529) cells/mm^2 remained (**Figure 1F**).

Expression of MHC-I in dopaminergic neurons causes immune attacks in the SN of PD model mice

In the control mice, dopaminergic neurons did not express MHC-I, and $CD8^+$ T cells were barely detected. In contrast, in the PD model mice, there was MHC-I expression and slight T cell infiltration 1 day after MPTP injection. At 7 days after the MPTP injection, dopaminergic neurons were strongly MHC-I positive, and there was marked T-cell infiltration (**Figure 2A**). In the 7-days-after-injection group, the number of $CD8^+$ T cells increased to 60.01 ± 11.55 cells/mm^2, which was almost 60-fold that of the control group (**Figure 2B**). In some specimens, we even observed contact between the T cells and the MHC-I^+ dopaminergic neurons.

To explore whether the expression of MHC-I can induce a T cell attack and cause the loss of dopaminergic neurons, we silenced the **MHC-I** gene using a stereotaxic injection of MHC-I interference adenovirus (**Figure 2C**). After counting the total number of TH^+ neurons using the optical fractionator method, we revealed that MHC-I knockdown reduced the loss of dopaminergic neurons after MPTP-induced oxidative stress (**Figure 2D**). Observing the sections under higher magnification revealed that, on the MHC-I knockdown side of the brain, the proportion of MHC-I^+ dopaminergic neurons and T cell infiltration was lower than that on the NC side (**Figure 2E**). Statistical analysis demonstrated that MHC-I knockdown reduced MHC-I expression by 31% of the negative control group (**Figure 2G**) and $CD8^+$ T cells by 35% of the negative control group (**Figure 2F**).

PINK1 is related to the expression of MHC-I in SH-SY5Y cells

It is hypothesized that *PINK1* mutations may lead to the presentation of mitochondrial antigens by MHC-I. To confirm this molecular mechanism of the neuronal expression of MHC-I, PINK1 protein expression was knocked down using siRNA in SH-SY5Y cells, and MHC-I expression was analyzed using flow cytometry.

The expression of PINK1 was successfully suppressed by *PINK1*-specific siRNA (**Figure 3C**). The expression of MHC-I was increased in the MPP^+ + si-PINK1 group—more so than in the MPP^+ + NC group (**Figure 3A** and **B**)—suggesting that the PINK1 protein is involved in MHC-I presentation in neurons that are under oxidative stress. Immunofluorescence findings were consistent with the flow cytometry results (**Figure 3D**). Moreover, the elevated MHC-I was located on the membrane of cells transfected with *PINK1*-specific siRNA.

Discussion

As noted in the introduction, MHC-I expression in dopaminergic neurons of the SN can be observed in both control individuals and PD patients. Carolina Cebrián, who discovered this phenomenon, suggested that in the event of the death of a few catecholamine neurons, α-syn and neuromelanin are released to the extracellular space. This release activates microglial cells, which in turn release pro-inflammatory substances, such as interferon-γ (CebriÃ et al., 2014). This induces MHC-I expression on the membranes of catecholamine neurons (Liblau et al., 2013). The main function of MHC-I is to present intercellular peptides on the cell surface for immune surveillance purposes. When $CD8^+$ T cells recognize the peptides as foreign proteins, they attack the presenting cells and induce apoptosis (Neefjes et al., 2011). Thus, to explore whether autoimmune activation occurred in the SN of MPTP-induced PD model mice, immunofluorescence was used to assess the association between MHC-I expression and the infiltration of $CD8^+$ T cells. In the present study, MHC-I expression in dopaminergic neurons was caused not only by interferon-γ secretion, but also by damage to the neurons themselves, and expression levels were related to the severity and time of the damage. In the PD model mice, the antigens of misfolded proteins (such as α-syn) triggered by cellular damage were presented on the cell surface. This phenomenon

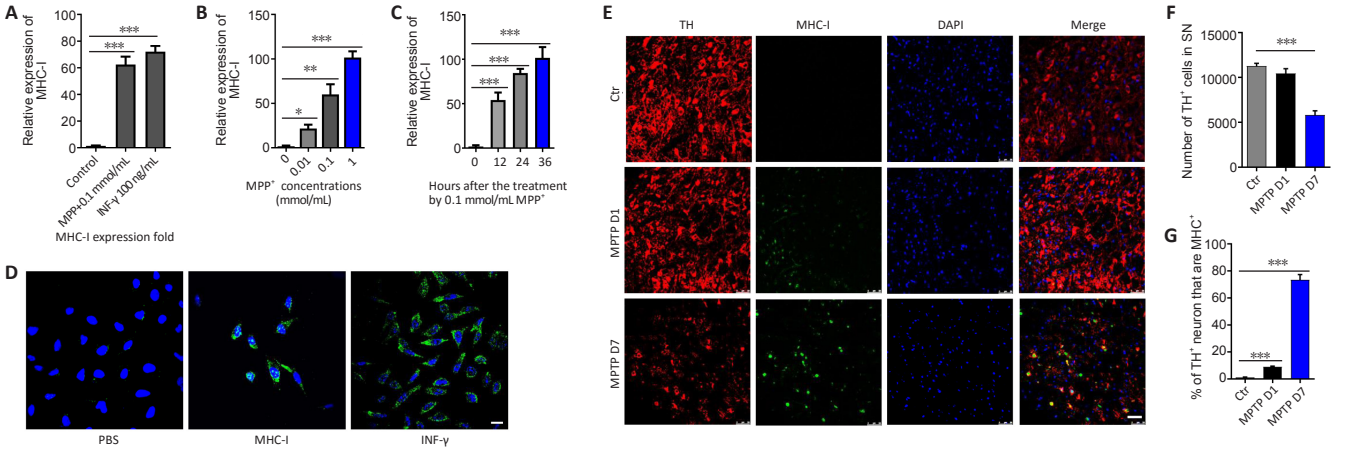

Figure 1 | **Expression of MHC-I in dopaminergic neurons is increased in Parkinson's disease models.**
(A) MHC-I expression in SH-SY5Y cells after being incubated with MPP^+ (0.1 mmol/mL) and INF-γ (100 ng/mL) for 24 hours. (B) MHC-I expression in SH-SY5Y cells after different concentrations of MPP^+ treatment for 24 hours. (C) MHC-I expression in SH-SY5Y cells after different incubation times with MPP^+. (D) Fluorescent images showing MHC-I (green) expression in SH-SY5Y cells. Scale bar: 20 μm. (E) Confocal immunofluorescent labeling of TH (green) and MHC-I (red) in the substantia nigra of mice in the control group, 1-day-after-injection group, and 7-days-after-injection group. Scale bar: 50 μm. (F, G) Numbers of TH^+ neurons in the substantia nigra of mice and the proportion of TH^+ neurons with MHC-I expression. Data are expressed as the mean ± SEM (n = 6; one-way analysis of variance followed by Bonferroni *post hoc* test). *P < 0.05, **P < 0.01, ***P < 0.001. INF-γ: Interferon-γ; MHC-I: major histocompatibility complex class I; MPP^+: 1-methyl-4-phenylpyridinium; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

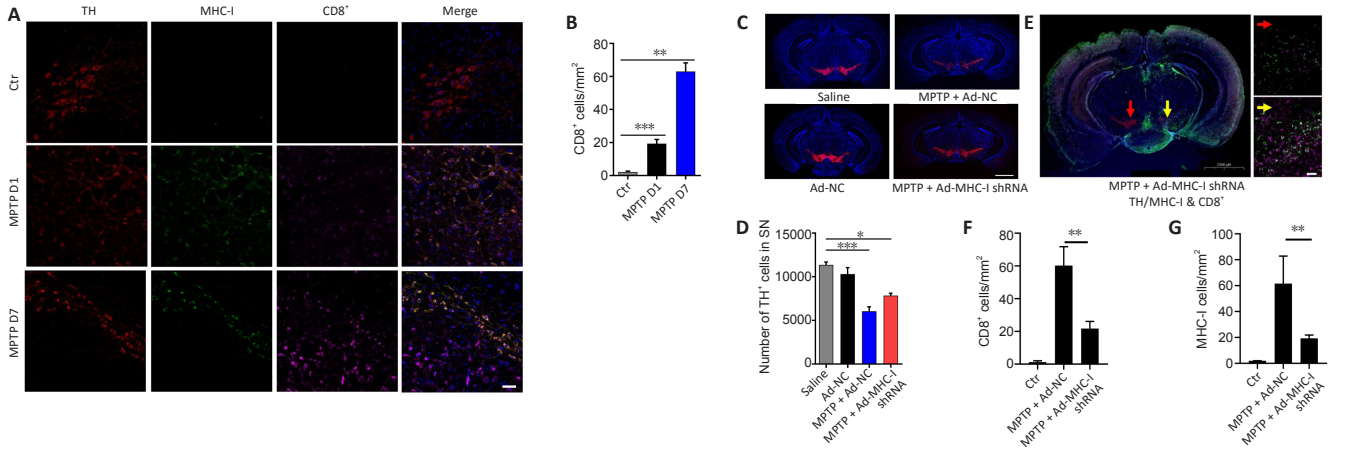

Figure 2 | **Expression of MHC-I causes immune attacks in the substantia nigra of Parkinson's disease model mice.**
(A) Immunofluorescence triple staining for TH (red), MHC-I (green), and $CD8^+$ T cells (pink) in the substantia nigra of the mouse midbrain in the control group, 1-day-after-injection group, and 7-days-after-injection group. Scale bar: 50 μm. (B) Numbers of $CD8^+$ T cells per mm^2. (C) Representative immunofluorescence double staining for the nucleus (DAPI, blue) and TH (red) in the substantia nigra region. Scale bar: 2 mm. (D) Numbers of TH^+ neurons in the substantia nigra of mice. (E) Immunofluorescence triple staining for TH (red), MHC-I (green), and $CD8^+$ T cells (pink) in the MPTP + Ad-MHC-1-shRNA group. The red arrow indicates the side that was injected with Ad-MHC-1-shRNA, while the yellow arrow indicates the side that was injected with Ad-NC. Scale bar: 50 μm. (F, G) Numbers of $CD8^+$ T cells and MHC-1-positive neurons per mm^2. *P < 0.05, **P < 0.01, ***P < 0.001. Data are expressed as the mean ± SEM (n = 6; one-way analysis of variance followed by Bonferroni *post hoc* test). DAPI: 4′,6-Diamidino-2-phenylindole; MHC-I: major histocompatibility complex class I; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NC: negative control; TH: tyrosine hydroxylase.

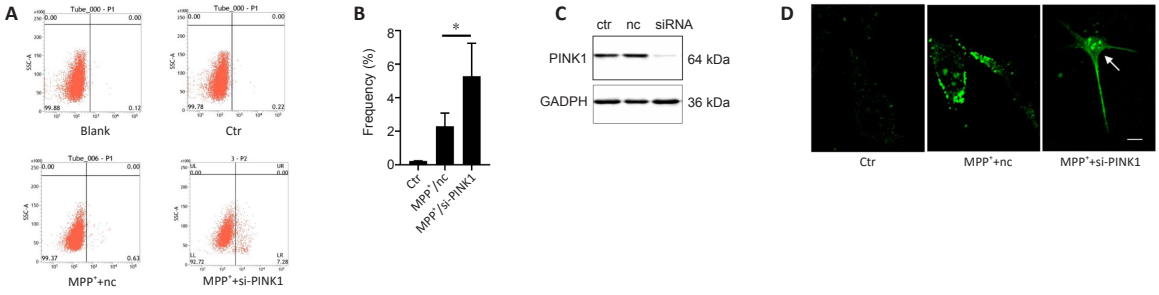

Figure 3 | **PINK1 is related to MHC-I expression in SH-SY5Y cells.**
(A) Flow cytometric analysis of MHC-I expression in neuron membranes. (B) Quantification of the flow cytometric analysis results. Data are expressed as the mean ± SEM (n = 6; one-way analysis of variance followed by Bonferroni *post hoc* test). *P < 0.05. (C) Western blot assay of PINK1 expression in SH-SY5Y cells that had been transfected with control or PINK1 siRNA. (D) Fluorescence images showing the expression of MHC-1 (green) in SH-SY5Y cells. Scale bar: 5 μm. The arrow indicates MHC-I protein presenting on the cell surface. MHC-I: Major histocompatibility complex class I; MPP^+: 1-methyl-4-phenylpyridinium; PINK1: PTEN-induced kinase 1.

activated the autoimmune response and induced T cell attacks, which contributed to the death of dopaminergic neurons. Therefore, MHC-I knockdown in the SN led to a decrease in $CD8^+$ T cell infiltration, and a subsequent decrease in the loss of dopaminergic neurons.

It has been previously reported that *PINK1* mutations can cause familial PD, and PINK1 is also related to mitochondrial antigen presentation by MHC-I in immune cells (Matheoud et al., 2016, Roberts and Fon, 2016). Damaged mitochondria can recruit PINK1 proteins to the outer mitochondrial membrane and activate them. The active PINK1 then phosphorylates parkin, an E3 ubiquitin ligase, resulting in the accumulation of ubiquitin in the damaged mitochondria, which then undergoes mitophagy. Thus, PINK1 plays a significant role in the loss of depolarized mitochondria (Kazlauskaite and Muqit, 2016). Recent evidence has also shown that, when there is a lack of PINK1 and cells are under oxidative stress, mitochondria cannot be eliminated, and the cells instead form mitochondrial-derived vesicles that present at the cell surface and trigger an immune response (Roberts and Fon, 2016).

Oxidative stress is among the consequences of MPP^+ treatment (Islam, 2017), and is caused by impairment of the activity of mitochondrial complexes—mainly complex I and complex III (Zorov et al., 2014). Dysfunctional mitochondria are selectively eliminated by autophagy (a process also known as mitophagy), which is led by the familial PD-related protein PINK1. Recent evidence has indicated a strong association between PINK1 and MHC-I expression in immunocytes; inflammatory conditions can trigger mitochondrial antigen presentation in the absence of PINK1 protein in immune cells (Gu et al., 2003; Matheoud et al., 2016). In the current study, the absence of PINK1 rendered neurons more susceptible to antigen presentation in the PD model. We therefore suggest that *PINK1* mutations make dopaminergic neurons more likely to trigger antigen presentation, to allow the presentation of misfolded proteins to the cell surface and activate an autoimmune response.

The present study provides evidence that autoimmune activation partially contributes to the loss of dopaminergic neurons in PD models. Oxidative stress may induce dopaminergic neurons to present endogenous proteins at the membrane. Some antigens, such as ganglioside GM1, melanin, or α-syn, can activate inflammation to eliminate abnormal neurons, which may explain the detection of these protein-related antibodies in PD patients. Some self-antigen can escape from immune attacks because of immune tolerance, which might be the reason behind the observation of MHC-I expression without the loss of dopaminergic neurons in control individuals. In the future, reducing antigen presentation may be used as a means of repressing PD progression.

However, there are two major limitations in the current study that should be addressed in future research. First, our results do not show a direct interaction between PINK1 and MHC-I, and it remains unknown how PINK1 protein might affect MHC-I presentation. Second, not all antigen presentation is able to activate immune attacks because of immune tolerance; therefore, identifying which antigen is presented on the cell surface and initiating the immune response needs to be addressed in the future.

The results of the present study suggest that dopaminergic neurons present antigens to the cell surface under oxidative stress, resulting in $CD8^+$ T cell attack and causing the loss of dopaminergic neurons. If we are able to identify which antigen activates the immune attack, blocking of the antigen may be a useful treatment to stop PD from progressing.

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Additional Table 1: *The sequence of adenovirus.*
Additional Table 2: *The sequences of MHC-I RT-PCR primers.*
Additional Table 3: *The sequences of PINK1 gene.*
Additional Figure 1: *The MHC-I knockdown efficiency.*

References

Benkler M, Agmon-Levin N, Hassin-Baer S, Cohen OS, Ortega-Hernandez O, Levy A, Moscavitch S, Szyper-Kravitz M, Damianovich M, Blank M, Chapman J, Shoenfeld Y (2012) Immunology, autoimmunity, and autoantibodies in Parkinson's disease. Clin Rev Allerg Immu 42:164-171.

Bien CG, Bauer J, Deckwerth TL, Wiendl H, Deckert M, Wiestler OD, Schramm J, Elger CE, Lassmann H (2002) Destruction of neurons by cytotoxic T cells: a new pathogenic mechanism in Rasmussen's encephalitis. Ann Neurol 51:311-318.

Burguillos MA, Deierborg T, Kavanagh E, Persson A, Hajji N, Garcia-Quintanilla A, Cano J, Brundin P, Englund E, Venero JL, Joseph B (2011) Caspase signalling controls microglia activation and neurotoxicity. Nature 472:319-324.

Cebrián C, Loike JD, Sulzer D (2014) Neuronal MHC-I expression and its implications in synaptic function, axonal regeneration and Parkinson's and other brain diseases. Front Neuroanat 8:114.

Cebrián C, Zucca FA, Mauri P, Steinbeck JA, Studer L, Scherzer CR, Kanter E, Budhu S, Mandelbaum J, Vonsattel JP, Zecca L, Loike JD, Sulzer D (2014) MHC-I expression renders catecholaminergic neurons susceptible to T-cell-mediated degeneration. Nat Commun 5:3633.

Corti O, Lesage S, Brice A (2011) What genetics tells us about the causes and mechanisms of Parkinson's disease. Physiol Rev 91:1161-1218.

De Virgilio A, Greco A, Fabbrini G, Inghilleri M, Rizzo MI, Gallo A, Conte M, Rosato C, Ciniglio Appiani M, de Vincentiis M (2016) Parkinson's disease: autoimmunity and neuroinflammation. Autoimmun Rev 15:1005-1011.

Double KL, Rowe DB, Carew-Jones FM, Hayes M, Chan DKY, Blackie J, Corbett A, Joffe R, Fung VS, Morris J, Riederer P, Gerlach M, Halliday GM (2009) Anti-melanin antibodies are increased in sera in Parkinson's disease. Exp Neurol 217:297-301.

Fall CP, Bennett JJ (1999) Characterization and time course of MPP^+-induced apoptosis in human SH-SY5Y neuroblastoma cells. J Neurosci Res 55:620-628.

Fiszer U, Mix E, Fredrikson S, Kostulas V, Olsson T, Link H (1994) Gamma delta+ T cells are increased in patients with Parkinson's disease. J Neurol Sci 121:39.

Gu Y, Wang C, Roifman CM, Cohen A (2003) Role of MHC Class I in immune surveillance of mitochondrial DNA integrity. J Immunol 170:3603-3607.

Huh GS, Boulanger LM, Du H, Riquelme PA, Brotz TM, Shatz CJ (2000) Functional requirement for class I MHC in CNS development and plasticity. Science 290:2155-2159.

Inácio RF, Zanon RG, Castro MVD, Souza HMD, Bajgelman MC, Verinaud L, Oliveira ALRD (2016) Astroglioma conditioned medium increases synaptic elimination and correlates with major histocompatibility complex of class I (MHC I) upregulation in PC12Cells. Neurosci Lett 634:160-167.

Islam MT (2017) Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. Neurol Res 39:73-82.

Jackson-Lewis V, Przedborski S (2007) Protocol for the MPTP mouse model of Parkinson's disease. Nat Protoc 2:141-151.

Kazlauskaite A, Muqit MM (2015) PINK1 and Parkin-mitochondrial interplay between phosphorylation and ubiquitylation in Parkinson's disease. FEBS J 282:215-223.

Liblau RS, Gonzalez-Dunia D, Wiendl H, Zipp F (2013) Neurons as targets for T cells in the nervous system. Trends Neurosci 36:315-324.

Lorenzi S, Forloni M, Cifaldi L, Antonucci C, Citti A, Boldrini R, Pezzullo M, Castellano A, Russo V, van der Bruggen P, Giacomini P, Locatelli F, Fruci D (2012) IRF1 and NF-kB restore MHC class I-restricted tumor antigen processing and presentation to cytotoxic T cells in aggressive neuroblastoma. PLoS One 7:e46928.

Jackson-Lewis V, Przedborski S (2007) Protocol for the MPTP mouse model of Parkinson's disease. Nat Protoc 2:141-151.

Matheoud D, Sugiura A, Bellemare-Pelletier A, Laplante A, Rondeau C, Chemali M, Fazel A, Bergeron JJ, Trudeau L, Burelle Y, Gagnon E, McBride HM, Desjardins M (2016) Parkinson's disease-related proteins PINK1 and parkin repress mitochondrial antigen presentation. Cell 166:314-327.

Mosley RL, Gendelman HE (2017) T cells and Parkinson's disease. Lancet Neurol 16:769-771.

Needleman LA, Liu XB, El-Sabeawy F, Jones EG, McAllister AK (2010) MHC class I molecules are present both pre- and postsynaptically in the visual cortex during postnatal development and in adulthood. Proc Natl Acad Sci U S A 107:16999-17004.

Neefjes J, Jongsma MLM, Paul P, Bakke O (2011) Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol 11:823-836.

Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, Lees AJ, Schrag A (2012) Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Ann Neurol 72:893-901.

Pajtler KW, Rebmann V, Lindemann M, Schulte JH, Schulte S, Stauder M, Leuschner I, Schmid KW, Köhl U, Schramm A, Eggert A (2013) Expression of NTRK1/TrkA affects immunogenicity of neuroblastoma cells. Int J Cancer 133:908-919.

Papachroni KK, Ninkina N, Papapanagiotou A, Hadjigeorgiou GM, Xiromerisiou G, Papadimitriou A, Kalofoutis A, Buchman VL (2007) Autoantibodies to alpha-synuclein in inherited Parkinson's disease. J Neurochem 101:749-756.

Quinn PMJ, Moreira PI, Ambrósio AF, Alves CH (2020) PINK1/PARKIN signalling in neurodegeneration and neuroinflammation. Acta Neuropathol Commun 9:189.

Roberts RF, Fon EA (2016) Presenting mitochondrial antigens: PINK1, Parkin and MDVs steal the show. Cell Res 26:1180-1181.

Spel L, Boelens JJ, van der Steen DM, Blokland NJ, van Noesel MM, Molenaar JJ, Heemskerk MH, Boes M, Nierkens S (2015) Natural killer cells facilitate PRAME-specific T-cell reactivity against neuroblastoma. Oncotarget 6:35770-35781.

Subramaniam SR, Chesselet M (2013) Mitochondrial dysfunction and oxidative stress in Parkinson's disease. Prog Neurobiol 106-107:17-32.

Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, Liong C, McMurtrey C, Hildebrand WH, Mao X, Dawson VL, Dawson TM, Oseroff C, Pham J, Sidney J, Dillon MB, Carpenter C, Weiskopf D, Phillips E, Mallal S, et al. (2017) T cells from patients with Parkinson's disease recognize α-synuclein peptides. Nature 546:656-661.

Tooyama I, Kimura H, Akiyama H, McGeer PL (1990) Reactive microglia express class I and class II major histocompatibility complex antigens in Alzheimer's disease. Brain Res 523:273.

Xicoy H, Wieringa B, Martens GJ (2017) The SH-SY5Y cell line in Parkinson's disease research: a systematic review. Mol Neurodegener 12:10.

Yao L, Ye Y, Mao H, Lu F, He X, Lu G, Zhang S (2018) MicroRNA-124 regulates the expression of MEKK3 in the inflammatory pathogenesis of Parkinson's disease. J Neuroinflamm 15:13.

Ye Y, He X, Lu F, Mao H, Zhu Z, Yao L, Luo W, Sun X, Wang B, Qian C, Zhang Y, Lu G, Zhang S (2018) A lincRNA-p21/miR-181 family feedback loop regulates microglial activation during systemic LPS- and MPTP- induced neuroinflammation. Cell Death Dis 9:803.

Zappia M, Crescibene L, Bosco D, Arabia G, Nicoletti G, Bagala A, Bastone L, Napoli ID, Caracciolo M, Bonavita S, Di Costanzo A, Gambardella A, Quattrone A (2002) Anti-GM1 ganglioside antibodies in Parkinson's disease. Acta Neurol Scand 106:54-57.

Zhang S, Ma Y, Feng J (2020) Neuroprotective mechanisms of ε-viniferin in a rotenone-induced cell model of Parkinson's disease: significance of SIRT3-mediated FOXO3 deacetylation. Neural Regen Res 15:2143-2153.

Zorov DB, Juhaszova M, Sollott SJ (2014) Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev 94:909-950.

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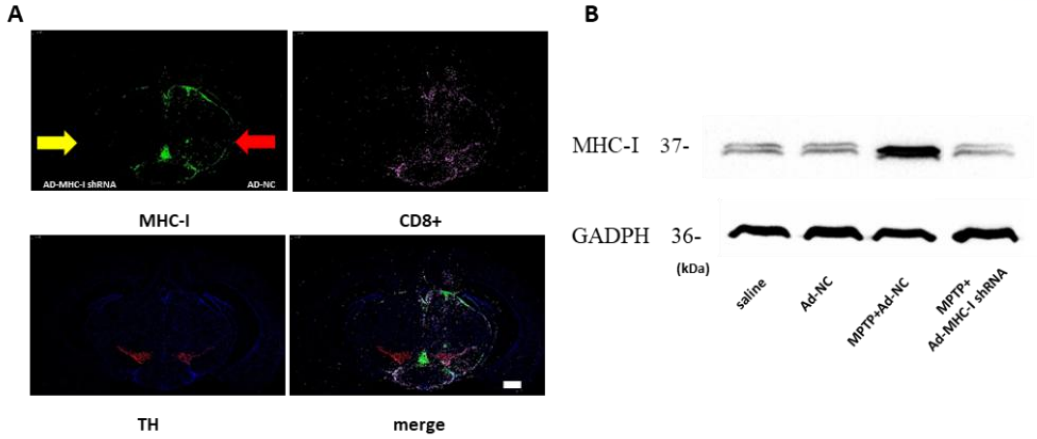

Additional Figure 1 The MHC-I knockdown efficiency.

(A) Immunofluorescence treble staining for MHC-I (green), CD8+ T cell(pink), TH (red), DAPI (blue). Under MPTP-induce PD model, the side (yellow arrow) injected with MHC-I shRNA adenoviruses shows suppression of MHC-I expression, the other side (red arrow) injected with nc adenoviruses indicates the expression of MHC-I. Scale bar: 2000 μm. (B) Western blotting shows the knockdown of MHC-I by MHC-I shRNA adenoviruses in midbrain of the mice. MHC-I: Major histocompatibility complex class I.

Additional Table 1 The sequence of adenovirus

GACAGGTCGGGCAGGAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGG
CTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGA
CGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCAT
ATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAAC
ACCGCAGACCTGAAGATAAAGTCATTCAAGAGATGACTTTATCTTCAGGTCTGCTTTTTTGGATCC
ACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCAGCTTTTGTTCCCTTTAGTGAGGGTTAAT
TGCGCGTTAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTT
TATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAA
CAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCA
AGTAAAACCTCTACAAATGTGGTGAGCTCGTCGACTCGAAGATCCAATAACTTCGTATAGCATAC
ATTATACGAAGTTATAAGTGCCGCCCTATACCTTGTCTGCCTCCCCGCGTTGCGTCGCGGTGCATG
GAGCCGGGCCACCTCGACCTGAATGGAAGCCGGCGGCACCTCGCTAACGGATTCACCACTCCAAG
AATTGGAGCCAATCAATTCTTGCGGAGAACTGTGAATGCGCAAACCAACCCTTGCAGAACATATC
CATCGCGTCCGCCATCTCCAGCAGCCGCACGCGGCGCATCTCGGCTGCATTAATGAATCGGCCAA
CGCGCGGGAGAGGCGGTTTGCGTATTGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCTGCGCTCG
GTCGTTCGGCTGCGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCA
G

Highlight: the Major histocompatibility complex class I (MHC-I) suppression sequence.

Additional Table 2 The sequences of MHC-I RT-PCR primers

Gene	Sequences
MHC-I (β2m)	5’-ATG GCT CGC TCG GTG ACC CTG-3’
	5’-CCG GTG GGT GGC GTG AGT ATA CTT-3’
β-actin	5’-CTA AGG CCA ACC GTG AAA AG-3’
	5’-ACC AGA GGC ATA CAG GGA CA-3’

MHC-I: Major histocompatibility complex class I.

Additional Table 3 The sequences of PINK1 gene

Gene	Primer sequences
PINK1 siRNA	5’-GGA CGC UGU UCC UCG UUA U-3’
PINK1 siRNA-2	5’-GCT GTG TAT GAA GCC ACC ATG CCT ACA TT-3’
Negative Control	5’-UUC UCC GAA CGU GUC ACG U-3’

PINK1: PTEN-induced kinase 1.