Sirtuin 2 (SIRT2) enhances 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced nigrostriatal damage via deacetylating Forkhead box O3a (Foxo3a) and activating Bim*

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*Running title: Deletion of SIRT2 prevents MPTP-induced nigrostriatal damage in mice

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Keywords: Parkinson’s disease, SIRT2, sirtuin, MPTP, apoptosis

Background: The functional role of SIRT2 in the MPTP model of Parkinson’s disease is not known.

Results: Deletion of SIRT2 rescues MPTP-induced nigrostriatal damage in mice by increasing acetylated Foxo3a levels, decreasing Bim expression, thereby preventing apoptotic pathways.

Conclusion: SIRT2 deacetylates Foxo3a, increases Bim expression and induces nigrostriatal damage in the MPTP-model of Parkinson’s disease.

Significance: SIRT2 deletion is protective in the MPTP-model of Parkinson’s disease.

Sirtuins are NAD-dependent protein deacetylases that were shown to have beneficial effects against age-related diseases. SIRT2 is a strong deacetylase, which is highly expressed in brain. It has been associated with neurodegenerative diseases. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a dopaminergic neurotoxin that replicates most of the clinical features of Parkinson’s Disease (PD) and produces a reliable and reproducible lesion of the nigrostriatal dopaminergic pathway and neurodegeneration after its systemic administration. Chronic administration of MPTP induces lesion via apoptosis. We show here that SIRT2 deacetylates Foxo3a, increases RNA and protein levels of Bim and as a result enhances apoptosis in the MPTP-model of PD. We also show that neurodegeneration induced by chronic MPTP regimen is prevented by genetic deletion of SIRT2 in mouse. Deletion of SIRT2 leads to the reduction of apoptosis due to an increase in acetylation of Foxo3a and a decrease in Bim levels. We demonstrate that SIRT2 deacetylates Foxo3a, activates Bim and induces apoptosis only in MPP⁺-treated cells. Therefore, designing SIRT2 inhibitors might be helpful to develop effective treatments for PD.

Parkinson’s Disease (PD), the most common movement disorder and the second most common neurodegenerative disease after Alzheimer’s disease, is characterized primarily by the loss of dopaminergic neurons. MPTP is the most commonly used dopaminergic neurotoxin that replicates most clinical features of PD producing reliable and reproducible nigrostriatal damage after systemic administration. It is still the only model that displays reproducible neurodegeneration. The chronic MPTP regimen leads to neurodegeneration via apoptosis (1).

Sirtuins are NAD-dependent protein deacetylases that were shown to have beneficial effects against age-related diseases (2,3). SIRT1 was shown to reduce Abeta peptide formation in an Alzheimer’s disease mouse model and suppress alpha-synuclein aggregation in A53T alpha-synuclein mouse model (4,5). SIRT2 is a
strong protein deacetylase, which is highly expressed in brain. It was shown to be an abundant neuronal protein that accumulates in the central nervous system of aging mice (6). SIRT2 was shown to co-localize with microtubules and functions as alpha-tubulin deacetylase. During G2/M phase, SIRT2 proteins enter nuclei and deacetylate histones (3). Recent study showed that SIRT2 inhibitors protected against dopaminergic cell death both in vitro and in a Drosophila model of PD (7). In addition, SIRT2 inhibition was shown to protect against Huntington’s disease by reducing sterol biosynthesis (8). SIRT2 expression was also shown to increase in cells with oxidative stress, such as hydrogen peroxide treatment. SIRT2 was shown to promote cell death when cells are under severe stress by activating Bim, a proapoptotic factor (9). However, the functional role and the effect of SIRT2 in brain and in a mouse model of a neurodegenerative disease are not known.

Here, we report that SIRT2 deacetylates Foxo3a and activates Bim in MPTP-treated (chronic regimen) mouse brains inducing apoptotic neuronal death. We also show that in MPP+ -treated SH-SY5Y cells, SIRT2 induces caspase-3 activated apoptotic cell death. MPTP-induced nigrostriatal damage is greatly reduced in SIRT2 KO mice indicating SIRT2 deletion is protective against this treatment by preventing apoptosis. In addition, silencing SIRT2 reduces and overexpressing SIRT2 increases caspase-3 activity in MPP+ -treated SH-SY5Y cells. We show that SIRT2 deacetylates Foxo3a, activates Bim and induces apoptosis only in MPP+ -treated cells and only in MPTP-injected mouse brains. Therefore, we show here for the first time that inhibiting SIRT2 in a mouse model of PD might be protective against this disease and helpful in designing effective treatments in the future.

EXPERIMENTAL PROCEDURES

Mouse strains- All mice used were in congenic C57Bl/6. SIRT2 KO mice were generated by the targeted insertion of a puromycin resistance gene into exon 11 of the SIRT2 locus. The insertion introduces a stop codon that should result in nonsense-mediated decay of the SIRT2 mRNA (10). All mice were housed at controlled temperature (25°C) and 12:12 h light-dark cycle.

Plasmids- The plasmids expressing mSIRT2 (13813) and Bim (8804) were purchased from Addgene. The SIRT2-shRNA (RMM3981-9579810) and Bim-shRNA (RHS4533-NM006538) plasmids were purchased from Open Biosystems.

Immunohistochemistry-Mice were perfused with 4% paraformaldehyde, cryoprotected, sectioned 40 µm-thick and collected at 150 µm intervals. 12 sections per brain were analyzed. Vectastain kit (Vector laboratories) was used to perform TH-staining according to manufacturer’s directions using TH antibody (Calbiochem). Nissl staining was performed according to the manufacturer’s protocol (IHCWORLD). TH-positive and Nissl-stained neurons in SNpc were counted by stereology. TH-positive striatal fibers were assessed by optical density.

Western blotting and Immunoprecipitation- Mouse brains were homogenized in RIPA buffer (50 mM Tris-HCl pH: 8.0, 1mM EDTA, 0.1 % SDS, 150 mM NaCl, 1 % NP40, 0.1 % Sodium-deoxycholate) including Complete Protease Inhibitor mixture (Roche), centrifuged, 100 µg of the supernatant was loaded onto 4-15 % gradient SDS-PAGE gels and immunoblotted with anti-SIRT2 (Cell signaling-2313, conc. 1:1000), Foxo3a (Abcam-ab47409, conc. 1:1000), Bim (Abcam-ab7888, conc. 1:1000), actin (Millipore-MAB1501, conc. 1:5000), Ac-K (Immunechem-ICP0380, conc.1:500) antibodies. Western blotting experiments were performed with at least six mice from each genotype and age and the representative blots are shown. For western blotting using cell extracts, cells were harvested and extracted in RIPA buffer as explained above. The immunoprecipitations were carried out by using Pierce Direct IP Kit (Thermo Scientific) according to manufacturer’s directions.

RNA isolation and analysis-Total RNA from mice brains or cells was isolated by using Trizol (Qiagen). For real time q-PCR analysis, cDNA was synthesized from total RNA by SuperScript III reverse transcriptase (Invitrogen) with
random primers. The cDNA was then subjected to PCR analysis with gene specific primers in the presence of SYBR green (Bio-Rad). Relative abundance of mRNA was obtained by normalization to 18S levels. The primers used for Bim qPCR are 5′-CACCATGGCA AGCAACCTTCTGTAGT-3′ (fwd) and 5′-TCAATGCATTCTCCACACC-3′ (rev). The software used to analyzed qPCR data is MxPro-Mx3000P v.4.10 Build 389, Schema 85 Stratagene.

**Cells and transfection**-SH-SY5Y cells (ATCC) were transfected using Effectene transfection reagent (Qiagen). MPP⁺ (Sigma) treatment was performed according to (14).

**Caspase-3 activity assay**- The Apo-Alert kit (Clontech) was used to measure caspase-3 activity according to manufacturer’s protocol.

**Statistical Analysis**-The analysis was performed using Two-way ANOVA. The type of statistical analysis used is indicated in each figure legend. Significant differences are demonstrated by single symbols (*, # , €), indicating p < 0.01. Error bars in figures represent s.e.m.

**RESULTS AND DISCUSSION**

We wished to test whether the deletion of SIRT2 could rescue MPTP-induced nigrostriatal damage by using SIRT2 KO mouse model. SIRT2 KO animals show no differences in brain development and gross anatomy. Gross histological examination of the brain reveals normal morphology in SIRT2 KO mice (data not shown, 10). MPTP is the most commonly used dopaminergic neurotoxin that produces reliable and reproducible nigrostriatal damage after systemic administration. It is accepted as a pharmacological model of Parkinson’s disease (1). The chronic MPTP regimen leads to neurodegeneration via apoptosis (1). We thus administered MPTP i.p. (intra-peritoneal injection) to 3 months old SIRT2 KO mouse and its wild type (wt) littermates via chronic regimen (30 mg kg⁻¹ free base MPTP daily for five consecutive days (11,12)). Nigrostriatal damage caused by the chronic regimen of MPTP is the death of dopaminergic neurons via apoptosis. The number of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the striatal fibers were assessed by TH (tyrosine-hydroxylase) immunoreactivity.

We observed a marked reduction in the number of TH-positive neurons in MPTP-treated wt mice compared to saline-treated wt mice (controls) (Fig. 1A). However, the number of TH-positive neurons in MPTP-treated SIRT2 KO mice did not decrease and was no different than saline treated wt or SIRT2 KO mice. This result indicates that deletion of SIRT2 rescued the nigrostriatal damage caused by MPTP (Fig. 1A). We also analyzed the number of neurons in substantia nigra by Nissl staining and observed that it was decreased after MPTP treatment in wt mice (Fig. 1B). However, the number of neurons in MPTP-treated SIRT2 KO mice did not decrease and was similar to saline treated wt or SIRT2 KO mice (Fig. 1B). Similarly, the density of TH-positive striatal fibers was reduced after MPTP-treatment in wt mice (Fig. 1C). However, the density of TH-positive striatal fibers in MPTP-dosed SIRT2 KO mice were similar to saline-dosed wt or SIRT2 KO mice (Fig. 1C). These data demonstrate that the deletion of SIRT2 prevents the loss of TH-positive neurons of SNpc and the striatal fibers after MPTP treatment.

Since chronic administration of MPTP induces apoptotic neuronal death in mouse brains, we wanted to analyze whether silencing or overexpressing SIRT2 affects the MPP⁺-induced apoptosis in SH-SY5Y (neuroblastoma) cells. Therefore, we overexpressed or silenced SIRT2 in SH-SY5Y cells and assayed apoptosis. We used caspase-3 activity as a measure of MPP⁺-induced apoptosis. Caspase-3 is an active cell-death protease involved in the execution phase of apoptosis, where cells undergo morphological changes such as DNA fragmentation, chromatin condensation, and apoptotic body formation (13). Caspase-3 is activated in response to treatment with pharmacological agents such as MPP⁺. SH-SY5Y cells were treated with media alone or MPP⁺ to a final concentration of 500 μM for different time intervals (2h, 4h, 6h, 8h, 12h, 16h) (14). Caspase-3 activity in cells
treated 16 hours with medium alone was comparable to the activity in untreated cells (0h, Fig. 2A). MPP⁺ treatment increased caspase-3 activity only after 8 hours of treatment, reaching the highest levels after 16 hours (Fig. 2A).

To test the effect of SIRT2 on MPP⁺-induced apoptosis, SH-SY5Y cells were transfected with empty vector (wt), SIRT2 plasmid to overexpress SIRT2, or SIRT2-shRNA plasmid to silence SIRT2 (See Experimental Procedures). Fig. 2B shows the SIRT2 levels in cell lysates after transfection. 48 hours after transfection, cells were treated with MPP⁺ for 16 hours, and then caspase-3 activity was analyzed. SIRT2 overexpression or silencing had no effect on caspase-3 activity in the absence of MPP⁺ treatment (Fig. 2C). After 16 h of MPP⁺ treatment, caspase-3 activity was increased in wt cells (vector, Fig. 2C). SIRT2 silencing decreased caspase-3 activity to baseline while overexpressing SIRT2 increased caspase-3 activity. Scrambled shRNA did not have any effect on caspase-3 activity. This result indicates that silencing SIRT2 prevents MPP⁺-induced apoptosis in SH-SY5Y cells.

SIRT2 was shown to promote cell death when cells are under severe stress by activating Bim, a pro-apoptotic factor (9). It was shown that, in cell culture, SIRT2 deacetylates Foxo3a. Since, Bim is a pro-apoptotic factor that is one of Foxo3a’s target genes, we analyzed whether SIRT2 deacetylates Foxo3a in SH-SY5Y cells and elevates Bim expression. In order to measure the acetylation level of Foxo3a in MPP⁺-treated cells where SIRT2 is overexpressed or silenced, we immunoprecipitated Foxo3a from the extracts of cells transfected with control vector, SIRT2 plasmid or SIRT2 shRNA plasmid using Foxo3a antibody. We then blotted the eluates with acetylated lysine (Ac-K) antibody to detect the acetylation of Foxo3a (Fig. 2D). The acetylation levels of Foxo3a was not changed in the absence of MPP⁺ treatment (no MPP⁺) where SIRT2 was silenced or overexpressed (Fig. 2D, left panel). After 16 h MPP⁺ treatment, we observed that the acetylation levels of Foxo3a was decreased in SIRT2 overexpressing cells and increased in SIRT2-silenced cells compared to empty vector.

This result demonstrates that SIRT2 deacetylates Foxo3a in SH-SY5Y cells only after MPP⁺ treatment (Fig. 2D, right panel).

We then tested whether the deacetylation of Foxo3a by SIRT2 elevates the expression level of Bim in cells. We first analyzed the RNA levels of Bim (Fig 2E). After 16 h of MPP⁺ treatment, Bim RNA levels were increased with overexpressing SIRT2 and decreased with silencing SIRT2. There was no difference in Bim RNA levels with SIRT2 overexpression or silencing in the absence of MPP⁺ treatment (Fig. 2E). The protein levels of Bim in cells were consistent with its RNA levels (Fig. 2F).

We then analyzed whether SIRT2 deacetylates Foxo3a and increases Bim levels in saline or MPTP-injected mouse brains. We observed that acetylation levels of Foxo3a increases only in MPTP-treated SIRT2 KO mouse brains compared to wt brains indicating that SIRT2 deacetylates Foxo3a only after MPTP treatment (Fig. 3A). To investigate whether Bim expression increases as a result of Foxo3a deacetylation, we analyzed the RNA levels of Bim in saline or MPTP-treated wt or SIRT2 KO mouse brains (Fig. 3B). We observed that there is no difference in Bim RNA levels in saline treated wt or SIRT2 KO mice. However, Bim RNA levels increase in MPTP-treated wt mice brains but not in MPTP-treated SIRT2 KO mice (Fig. 3B). Bim protein levels were also consistent with RNA levels in vivo, increasing only in MPTP-treated wt mouse brains (Fig. 3C). These data show that SIRT2 deacetylates Foxo3a and increases Bim levels only after MPTP treatment in mouse brains.

In order to confirm the mechanism that the increase in apoptosis after MPTP treatment is through the increase in Foxo3a deacetylation and Bim expression, we assayed caspase-3 activity in MPP⁺-treated SH-SY5Y cells where we manipulated the levels of SIRT2 and Bim (Fig. 3D). Caspase-3 activity was increased when SIRT2 was overexpressed, and decreased when SIRT2 was silenced. Overexpressing Bim increased caspase-3 activity to similar levels as in SIRT2 overexpression. In addition, silencing Bim decreased caspase-3 activity to similar
levels as in SIRT2 silencing. Importantly, when we overexpress SIRT2 in MPP⁺-treated Bim-silenced cells, Caspase-3 activity was in the levels of wt cells (vector) and not as high as the case of SIRT2 overexpression. This indicates that elevated Bim expression is the main cause of apoptosis. In addition, caspase-3 activity in cells expressing both SIRT2-shRNA and Bim-shRNA were not significantly lower than cells expressing SIRT2-shRNA or Bim-shRNA alone. The latter experiment suggests that SIRT2 and Foxo3a function in the same pathway to elevate caspase-3 activity. Scrambled-shRNAs did not have any effect on the caspase-3 activity (Fig. 3D). Fig. 3E indicates the protein levels of SIRT2 (left panel) or Bim (right panel) after silencing or overexpression in cells of SIRT2 or Bim, respectively. We have also analyzed whether the expression level of SIRT2 is upregulated in MPP⁺-treated cells or MPTP treated mice by western blotting. We did not observe any change in SIRT2 protein levels in MPP⁺-treated cells compared to control cells or in MPTP-injected mice compared to control mice indicating that the increase in Bim expression levels is not caused by the increase in SIRT2 expression (Fig. 3F).

These data show that SIRT2 leads to neurodegeneration in MPTP-injected mice and MPP⁺-treated cells by deacetylating Foxo3a, increasing Bim levels and therefore leading to apoptosis. We also show that deletion of SIRT2 prevents neuronal death in MPTP-treated mice. Similarly, silencing SIRT2 in MPP⁺-treated cells also inhibits apoptosis. The results shown here are also consistent with the fact that sirtuins are stress-response genes. In this study, SIRT2 is shown to deacetylate Foxo3a and increase Bim levels only after MPP⁺-treatment in cells or MPTP-injection in mice. Similarly, in a previous study (5), SIRT1 was shown to deacetylate HSF1 and increase Hsp70 levels only after heat shock in cells or only in the brains of Parkinson’s disease mouse model (A53T alpha-synuclein). These studies show that SIRT1 and SIRT2 are activated as a result of stress condition and turn on their target pathways. Therefore, in the future, designing SIRT2 inhibitors that become activated as a result of the disease condition might be useful in developing treatments for Parkinson’s disease.

REFERENCES


FOOTNOTES
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The abbreviations used are: TH, tyrosine hydroxylase; SNpc, substantia nigra pars compacta; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SIRT2, Sirtuin2; KO, knockout.

FIGURE LEGENDS:

FIGURE 1. Deletion of SIRT2 reduces the MPTP-induced nigrostriatal damage in mouse brains. A, the left panel shows immunostaining of TH-positive neurons in the substantia nigra
pars compacta (SNpc) in saline- or MPTP-dosed wt or SIRT2 KO mice. Scale bar represents 50 uM. The quantification on the right shows the number of SNpc neurons counted by stereology. Bars represent s.e.m (n=8). Statistical analyses were carried out using Two-Way ANOVA. *p<0.01, wt-MPTP vs. wt-saline; #p<0.01, SIRT2 KO-MPTP vs. wt-MPTP.

B, the left panel shows the Nissl staining of the neurons in SNpc of saline- or MPTP-dosed mice. Scale bar represents 50 uM. The quantification on the right shows the number of Nissl-stained neurons in SNpc counted by stereology. Bars represent s.e.m (n=8). Statistical analyses were carried out using Two-Way ANOVA. *p<0.01, wt-MPTP vs. wt-saline; #p<0.01, SIRT2 KO-MPTP vs. wt-MPTP.

C, the left panel shows the TH-positive striatal fibers in saline- or MPTP-dosed wt or SIRT2 KO mice. Quantification on the right shows the loss of striatal fibers in MPTP-dosed mice assessed by optical density (n=8). Statistical analyses were carried out using Two-Way ANOVA. *p<0.01, wt-MPTP vs. wt-saline.

FIGURE 2. SIRT2 deacetylates Foxo3a, increases Bim levels and leads to apoptosis in MPP+-treated cells. A, MPP+ treatment increases caspase-3 activity in SH-SY5Y (neuroblastoma) cells. Caspase-3 activity was determined from the supernatants of SH-SY5Y cell lysates after treatment with MPP+ for the indicated times. Bars represent s.e.m of three independent experiments. Statistical analyses were carried out using Two-way ANOVA. *p<0.01, 8 h vs. 0 h; †p<0.01, 12 h vs. 8 h; ‡p<0.01, 16 h vs. 12 h. B, Relative protein levels of SIRT2 are increased as a result of transfection with SIRT2 plasmid and reduced as a result of silencing with SIRT2-shRNA plasmid. The left panel shows a representative western blot of SIRT2 levels from the lysates of SH-SY5Y cells transfected with SIRT2 (+SIRT2), or SIRT2-shRNA (SIRT2-shRNA) plasmids or empty vector (wt) with anti-SIRT2 antibody. Actin serves as a loading control. Quantification is shown on the right. Bars represent s.e.m of three independent experiments. Statistical analyses were carried out using Two-Way ANOVA. *p<0.01, +SIRT2 vs. wt; †p<0.01, SIRT2-shRNA vs. wt. C, overexpression of SIRT2 increases and silencing SIRT2 decreases the caspase-3 activity that is elevated after MPP+ treatment. The graph shows the caspase-3 activity in the supernatants of SH-SY5Y cell lysates where SIRT2 is overexpressed (+SIRT2) or silenced (SIRT2-shRNA) without (no MPP+) or with MPP+ treatment for 16 h. Bars represent s.e.m of three independent experiments. Statistical analyses were carried out using Two-Way ANOVA. *p<0.01, vector with MPP+ vs. vector no MPP+; †p<0.01, SIRT2-shRNA with MPP+ vs. vector with MPP+. D, SIRT2 deacetylates Foxo3a after MPP+ treatment. The panel shows the lysates of SH-SY5Y cells without MPP+ treatment transfected with SIRT2 (+SIRT2) or SIRT2-shRNA (SIRT2-shRNA) or empty vector (vector) immunoprecipitated with Foxo3a antibody or NRS (Normal Rabbit Serum) and blotted with anti-Foxo3a and anti-acetylated lysine (Ac-K) antibodies without (left panel) or with 16 h of MPP+ treatment (right panel). Quantification of the acetylated bands was carried out by densitometry using the NIH ImageJ program and is shown below the gel. Three independent experiments were performed. Representative blots are shown. E, Relative Bim RNA levels quantified by qPCR from SH-SY5Y cell extracts transfected with vector, SIRT2 or SIRT2-shRNA plasmid with or without MPP+ treatment. Bars represent s.e.m of three independent experiments. Statistical analyses were carried out using Two-Way ANOVA. *p<0.01, vector with MPP+ vs. vector no MPP+; †p<0.01, SIRT2-shRNA with MPP+ vs. vector with MPP+; ‡p<0.01, +SIRT2 with MPP+ vs. vector with MPP+. F, Western blotting of Bim protein extracted from the lysates of SH-SY5Y cells transfected with SIRT2 (+SIRT2) or SIRT2-shRNA (SIRT2-shRNA) or empty vector (vector) with or without MPP+ treatment. Quantification was carried out by densitometry using the NIH ImageJ program and is shown on the right. Three independent experiments were performed. Representative blot is shown. Statistical analyses were carried out using Two-Way ANOVA. *p<0.01, vector with MPP+ vs. vector no MPP+; †p<0.01, SIRT2-shRNA with MPP+ vs. vector with MPP+; ‡p<0.01, +SIRT2 with MPP+ vs. vector with MPP+.
FIGURE 3. SIRT2 deacetylates Foxo3a, increases Bim levels and leads to apoptosis in MPTP-treated mouse brains. A, SIRT2 deacetylates Foxo3a in MPTP-injected mouse brains. The lysates from wt or SIRT2 KO mouse brains were immunoprecipitated with Foxo3a antibody or NRS (Normal Rabbit Serum) and blotted with anti-Foxo3a or anti-acetylated lysine (Ac-K) antibodies in saline- (left panel) or MPTP-injected (right panel) mouse brains. Quantification of acetylated bands was carried out by densitometry using the NIH ImageJ program and is shown below the gel. n=6 in each group (wt-saline, wt-MPTP, SIRT2 KO-saline, SIRT2-KO MPTP). Representative blots are shown. B, Bim RNA levels quantified from whole brains of mice by qPCR. n=6 for each indicated group. Statistical analyses were carried out using Two-Way ANOVA. *p<0.01, wt-MPTP vs. wt-saline. C, Western blotting of Bim protein extracted from whole brains of saline or MPTP-treated wt or SIRT2 KO mouse brains. n=6 for each group. Actin serves as loading control. Representative immunoblot is shown. Quantification of the relative Bim protein levels is shown on the right. Statistical analyses were carried out using Two-Way ANOVA. *p<0.01, wt-MPTP vs. wt-saline. D, Caspase-3 activity of SH-SY5Y cells transfected with SIRT2, SIRT2-shRNA, Bim or Bim-shRNA plasmids and treated with 16 h MPP+. E, Western blotting of SIRT2 or Bim levels from the lysates of SH-SY5Y cells that are transfected with SIRT2 or SIRT2-shRNA plasmids (left panel) or Bim or Bim-shRNA plasmids (right panel). Actin serves as a loading control. Quantifications of the relative SIRT2 or Bim protein levels are shown below the gels. Three independent experiments were performed. Representative blots are shown. Statistical analyses were carried out using Two-Way ANOVA. In the left panel, *p<0.01, SIRT2-shRNA vs. vector; #p<0.01, +SIRT2 vs. vector. In the right panel, *p<0.01, Bim-shRNA vs. vector; ′p<0.01, +Bim vs. vector. F, Left panel shows the western blotting of SIRT2 protein extracted from wt or MPP+-treated SH-SY5Y cells. Three independent experiments were performed. Right panel shows the western blotting of SIRT2 protein extracted from whole brains of saline or MPTP-treated wt mice. n=6 for each group. Actin serves as loading control. Representative immunoblots are shown. Quantifications of the relative SIRT2 protein levels are shown below. Statistical analyses were carried out using Two-Way ANOVA. *p<0.01.
Figure 1

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Number of TH-positive neurons

- wt: 10\(^{4}\) ± 3\(^{4}\)
- SIRT2 KO-saline: 6\(^{4}\) ± 3\(^{4}\)
- wt-MPTP: 2\(^{4}\) ± 3\(^{4}\)
- SIRT2 KO-MPTP: 1\(^{4}\) ± 3\(^{4}\)

* p<0.01

Number of Nissl-stained neurons

- wt: 20\(^{4}\) ± 3\(^{4}\)
- SIRT2 KO-saline: 15\(^{4}\) ± 3\(^{4}\)
- wt-MPTP: 5\(^{4}\) ± 3\(^{4}\)
- SIRT2 KO-MPTP: 10\(^{4}\) ± 3\(^{4}\)

* p<0.01

Density of TH-positive fibers

- wt: 0.3 ± 0.05
- SIRT2 KO-saline: 0.25 ± 0.05
- wt-MPTP: 0.1 ± 0.05
- SIRT2 KO-MPTP: * 0.25 ± 0.05
Figure 2

A. Caspase-3 activity (nmole pNA/mg protein) over time (hours) with media (16 h) treatment.

B. SIRT2 levels with +SIRT2, wt, and shRNA treatments compared to +SIRT2 vector.

C. Caspase-3 activity (nmole pNA/mg protein) with +SIRT2, SIRT2 shRNA, and vector treatments with no MPP+ or 16 h MPP+ treatment.

D. Western blot analysis of Foxo3a and H-AcK with no MPP+ and 16 h MPP+ treatment.

E. Relative mRNA abundance of Foxo3a with no MPP+ and 16 h MPP+ treatment.

F. Bim levels with no MPP+ and 16 h MPP+ treatment.
Figure 3

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D

Caspase-3 activity (nmole pNA/mg protein)

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16 h MPP⁺ treatment

F

cells

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**p<0.01**

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Sirtuin 2 (SIRT2) enhances 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced nigrostriatal damage via deacetylation of Forkhead box O3a (Foxo3a) and activating Bim

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