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ORIGINAL ARTICLE

Rotenone affects p53 transcriptional activity and apoptosis via targeting SIRT1 and H3K9 acetylation in SH-SY5Y cells

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Abstract

The protein deacetylase SIRT1 has been recognized to exert its protective effect by directly deacetylasing histone and many other transcriptional factors including p53. However, the effect of SIRT1 on p53 expression at the transcriptional level still remains to be elucidated. In this study, we found that rotenone treatment decreased cell viability, induced apoptosis, reduced SIRT1 level, and promoted p53 expression. Pre-treatment with resveratrol, a SIRT1 activator, could attenuate rotenone-induced cell injury and p53 expression, whereas down-regulation of SIRT1 directly increased p53 expression. Moreover, chromatin immunoprecipitation experiments showed that SIRT1 bound to H3K9 within the p53 promoter region, and this binding resulted in decreased H3K9 acetylation and increased H3K9 tri-methylation, thereby inhibiting p53 gene transcription. In conclusion, our data indicate that rotenone promotes p53 transcription and apoptosis through targeting SIRT1 and H3K9. This leads to nigrostriatal degeneration, the main pathogenic mechanism of motor features of Parkinson's disease.

Keywords: H3K9-ac, H3K9-me3, p53, resveratrol, SIRT1.

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Progressive dopaminergic neuron degeneration in the substantia nigra pars compacta has been considered as one of the most important pathological features of Parkinson's disease (PD) (Dragicevic *et al.* 2014). Although several important advances have been made in elucidating the pathogenesis of PD, the underlying mechanisms implicated in dopaminergic neuronal degeneration and the relevant neuroprotective therapeutic strategies still require further studies (Sunico *et al.* 2013; Lu *et al.* 2014; Neuhaus *et al.* 2014; Schapira *et al.* 2014).

Resveratrol (trans-3,4′,5-trihydroxystilbene) is a natural non-flavonoid polyphenolic phytoalexin with antioxidant and anti-inflammatory properties (Soleas *et al.* 2001). A growing body of evidence has provided support for resveratrol's cardioprotective effects (Bernhaus *et al.* 2009) and as a potential neuroprotective agent in neurodegenerative diseases such as PD (de la Lastra and Villegas 2005; Lin *et al.* 2014). One of the mechanisms postulated to explain the wide

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Abbreviations used: AMPK, AMP-activated protein kinase; BrdU, bromodeoxyuridine; CCK8, cell counting kit-8; ChIP, chromatin immunoprecipitation; DMEM, Dulbecco's modified eagle's medium; DMSO, dissolved in dimethyl sulfoxide; IP, immunoprecipitation; PD, Parkinson's disease; PGC-1α, proliferator-activated receptor-gamma coactivator-1α; PI, propidium iodide; PVDF, polyvinylidene fluoride; ROS, reactive oxygen species; siRNA, small interfering RNA; SIRT1, silent information regulator1; SNpc, substantia nigra pars compacta.

biological and pharmacological effects of resveratrol is through activation of SIRT1 (Clasen *et al.* 2014; Tamaki *et al.* 2014). It was previously demonstrated that the resveratrol-AMPK-SIRT1 pathway exerts neuroprotective effect in Parkin-associated familial PD through induction of proliferator-activated receptor-gamma coactivator-1α, resulting in enhanced mitochondrial oxidative function (Ferretta *et al*. 2014). Our previous study also implicated the protective effect of the resveratrol-AMPK-SIRT1 pathway in cellular models of PD, which, at least in part, results from the induction of autophagic function (Wu *et al.* 2011). Impaired autophagy has been postulated to play an important role in many neurodegenerative diseases, including PD (Pan *et al.* 2008; Ghavami *et al.* 2014).

Since its discovery, the role of p53 in various cellular mechanisms has expanded from an oncogene to a tumor suppressive gene, and as a transcriptional factor modulating an increasing number of genes involved in cell cycle arrest, autophagy, and apoptotic pathway (Hao and Cho 2014). While under normal conditions, both p53 protein level and activity are tightly regulated, p53 is activated in response to various cellular stress such as ribosomal stress, DNA damage, and hypoxia, resulting in up-regulation of pro-apoptotic proteins such as Bax and PUMA, and leading to apoptosis (Vousden 2000). One of the important functions of p53 gene is the contribution of its mutations to hereditary cancer syndromes, such as Li-Fraumeni syndrome and familial adenomatous polyposis (Agarwal *et al.* 2014). In addition, p53 has also been implicated in neuronal death associated with neurodegenerative diseases including PD (Checler *et al.* 2014). Previous studies have focused on the regulation of p53 at post-transcriptional level, such as phosphorylation and ubiquitination, but especially acetylation (Marouco *et al.* 2013). Studies have demonstrated that p53 can be deacetylated and inactivated by SIRT1 at lys 382 in mouse embryonic fibroblasts (Luo *et al.* 2001; Vaziri *et al.* 2001). Moreover, it has been reported that SIRT1 is sufficient to deacetylate and inactivate p53 to inhibit neuron apoptosis (Langley *et al.* 2002; Okawara *et al.* 2007; Hernandez-Jimenez *et al.* 2013).

In this study, we sought to investigate whether resveratrol-activated SIRT1 can target p53 not only at the protein level but also at the transcriptional level to regulate p53 expression, thus inhibiting apoptosis and providing protection from rotenone-induced cell death.

Materials and methods

Cell culture and treatments

SH-SY5Y cells were routinely grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, Gaithersburg, MD, USA) and cultured at 37°C under humidified 5% CO_2 atmosphere. Rotenone (Sigma, St. Louis, Mo., USA) stocks were dissolved in dimethyl sulfoxide from a stock concentration of 1 mM which was stored at −20°C. Resveratrol (Sigma) was prepared in dissolved in dimethyl sulfoxide at a stock of 25 mM. Other agents were also purchased from Sigma.

CCK8 cell viability assay

Cell viability was measured with CCK8 assay (cell counting kit-8, Dojindo, Kumamoto, Japan). All groups of SH-SY5Y cells were seeded in 96-well plates at a cellular density of 5000 cells per well with 100 μL DMEM. After cells were subjected to various conditions, 10 μL CCK8 was added to each well for another 1 h at 37°C. The wells containing only culture medium was regarded as blanks. Absorbance was recorded at 450 nm by a microplate reader and the results were expressed as percentages of control values. All the experiments were independently repeated at least three times.

Immunoblotting and immunoprecipitation

After each specific treatment, total protein was isolated with a mammalian cell lysis/extraction reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacture's protocol. Equal amount of protein was separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. After incubation in blocking solution for 1 h at 37°C, the membranes were then incubated with specific primary antibodies: anti-p53, anti-ac-p53, anti-Bcl-2, anti-Bax, anti-sirt1 (Cell Signaling Technology, Inc., Danvers, MA, USA), respectively. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and β-actin were used to confirm equal protein loading (Table 2). Then membranes were washed with TBST (1 × Tris-buffered saline, 0.1% Tween-20) for three times and 15 min for each. After incubation with secondary antibodies for 45 min at 37°C and washing with TBST, immunoreactivity was visualized using an NIR scanning device Odyssey scanner, LI-COR Biosciences (4647 Superior Street P.O. Box 4000 Lincoln, NE 68504, USA). Specific proteins were quantified using Image J software (National Institutes of Health).

Table 1 The sequences of qRT-PCR primers

Gene product	Forward (F) and reverse (R) primers (5′→3′)
SIRT1	F: AGAGATGGCTGGAATTGTCC
	R: CCAGATCCTCAAGCGATGTT
p53	F: ATGACTGAGGTCGTGAGACGCTGCCC
	R: GGAGCCAGGCCGTCACCATCAGAGC
Bax	F: AGCTTCTTGGTGGACGCAT
	R: CAGAGGCGGGGTTTCATC
Bcl-2	F: GAGAAATCAAACAGAGGCCG
	R: CTGAGTACCTGAACCGGCA
18S rRNA	F: TGCGGAAGGATCATTAACGGA
	R: AGTAGGAGAGGAGCGAGCGACC
SIRT1 siRNA1	F: UACAAAUCAGGCAAGAUGCUGUUGC
	R: GCAACAGCAUCUUGCCUGAUUUGUA
SIRT1 siRNA2	F: GCAAUAGGCCUCUUAAUUA
	R: UAAUUAAGAGGCCUAUUGC
SIRT1 siRNA3	F: UUCAACAUUCCUAGAAGUUUGUACUUC
	R: GAAGUACAAACUUCUAGGAAUGUUGAA
SIRT1 siRNA4	F: ACAGUUUCAUAGAGCCAUGAAGUAU
	R: AUACUUCAUGGCUCUAUGAAACUGU

For immunoprecipitation (IP) experiments, extracted proteins were incubated with anti-SIRT1 at 4°C overnight with rotation, followed by precipitation with protein A agarose breads. Immunoprecipitates were then washed three times with saline and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis separation. Subsequent immunoblotting procedures were the same as described above.

RNA extraction and analysis by quantitative real-time PCR (RT-PCR)

Total RNA from each group of cells were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. All RNA samples were treated with DNase I (Sigma-Aldrich), quantified, and reverse-transcribed into cDNA using a ReverTra Ace-α first strand cDNA synthesis kit (TOYOBO, Osaka island city shibei hall two block, Japan). qRT-PCR was conducted using a RealPlex4 real-time PCR detection system from Eppendorf Co., Ltd. (HIGASHIKANDA 1-CHOME, CHIYODA-KU, TOKYO), with SYBR-Green real-time PCR Master Mix (Toyobo) as the detection dye. A comparative threshold cycle (Ct) was used to determine relative gene expression normalized to 18s rRNA. For each sample, the Ct values of the genes were normalized using the formula ΔCt = Ct_genes − Ct_18s rRNA. To determine relative expression levels, the following formula was used ΔΔCt = ΔCt_all_groups − ΔCt_blank control group. The values used to plot relative expression of markers were calculated using the formula $2^{-\Delta\Delta Ct}$. The cDNA of each gene was amplified with primers as previously described (Table 1).

Annexin V-FITC/PI assay

Apoptosis was evaluated using an Annexin V-FITC/PI kit (Beyotime Institute of Biotechnology, 602 Jiefang Road, Haimen, Jiangsu, China) according to the manufacturer's instructions. Cells (5×10^4) were plated on coverslips. After incubation under various conditions, each group of cells was washed twice with phosphate-buffered saline. Then, 195 μL Annexin V-FITC/PI binding buffer was added to each group, followed by 5 μL Annexin V-FITC/PI. After gently vortexing, all the cells were incubated for 30 min at 20–25°C without light. After removing the supernatant, 190 μL binding buffer and 10 μL propidium iodide (PI) was added to the cells. Immunofluorescence microscopy was used to examine the morphology of cells that underwent early apoptosis (stained green) or late apoptosis or necrosis (stained green and red).

Table 2 Primary antibodies, their source and dilutions

Antibodies	Companies	Applications
Rabbit anti-human Bax	Cell Signaling Technology	WB (1 : 1000)
Rabbit anti-human Bcl-2	Cell Signaling Technology	WB (1 : 1000)
Rabbit anti-human p53	Cell Signaling Technology	WB (1 : 1000)
Rabbit anti-human ac-p53	Cell Signaling Technology	WB (1 : 1000)
Rabbit anti-human SIRT1	Cell Signaling Technology	WB/IP (1 : 1000)
Mouse anti-human H3K9-me3	Cell Signaling Technology	ChIP (1 : 1000)
Mouse anti-human H3K9-ac	Cell Signaling Technology	ChIP (1 : 1000)
Mouse anti-human β-actin	Cell Signaling Technology	WB (1 : 1000)
Rabbit anti-human AMPK	Cell Signaling Technology	WB (1 : 1000)
Rabbit anti-human p-AMPK	Cell Signaling Technology	WB (1 : 1000)

Bromodeoxyuridine (BrdU) labeling and flow cytometry assay

Cells were plated in 6 cm dishes, and incubated with DMEM containing BrdU. After cells were treated with various drugs, anti-BrdU antibody was added according to the manufacturer's instruction. Cells were harvested with ethanol at −20° for no less than 48 h. Then all groups of cells were resuspended in PI. Fluorescence intensity was determined by flow cytometry for cell cycle detection.

Chromatin immunoprecipitation (ChIP)

The ChIP assay was performed using an EZ ChIP chromatin immunoprecipitation kit (Millipore, Bedford, MA, USA). SH-SY5Y cells (1×10^7) were fixed in 10 mL of DMEM with 1% formaldehyde at 4°C for 10 min. Cells were resuspended in 1 mL of sodium dodecyl sulfate Lysis Buffer containing 1× Protease Inhibitor Cocktail II. All ChIP procedures was performed as described previously (Lim *et al.* 2010). The chromatin solution was incubated with primary antibodies against H3K9-me3, H3K9-ac, SIRT1 (Cell Signaling Technology) or control immunoglobulin G and protein A/G plus-agarose immunoprecipitation reagent overnight at 4°C with rotation. Human Ig G was used as the negative control and DNA (Input) as positive control. Immunoprecipitates were eluted from the protein A/G plus-agarose reagent and precipitated. Then the purified DNA was subjected to PCR amplification with the specific primers to human P53 promoter: 5′-AAAAGTTTTGAGTTTTTTAAAAGTTTAGAG-3′ and 5′-AAAAACAATCTAACTACCAATCCAAAA-3′.

RNA interference (RNAi) and transfection

siRNA oligomers targeting SIRT1 (si-SIRT1) and a scrambled oligomer (si-control) were purchased from Genepharma Co., Ltd. (Shanghai, China) and the transfection method was performed according to the manufacturer's instructions. Briefly, SH-SY5Y cells were transfected with 0.3 μg siRNA-SIRT1 or a siRNA-control, respectively, by using a commercial transfection kit (Shanghai GenePharma).

Statistical analysis

Each experiment was performed at least three times and all data were expressed as mean values ± SD. Statistical significance was determined by Student's *t*-test. All data analyses were performed using SPSS version 11.5 (SPSS Inc, Chicago, IL, USA).

Results

Rotenone and resveratrol affects apoptosis in SH-SY5Y cells

To evaluate the neurotoxicity of rotenone in SH-SY5Y cells, we first treated cells with rotenone at various concentrations

(0, 0.1, 1, 10, 100 μM) for 24 h, or at 1 μM for different time periods (0, 3, 6, 12, 24, 36 h). As shown in Fig. 1a and b, cell viability was significantly reduced both dose- and time-dependently. Compared to the control group, cells treated with rotenone at a concentration of 1 μM achieved an approximate 40–50% reduction in cell viability. A significant decrease in cell viability was also observed after cells were treated with rotenone at 1 μM for 24 h. To determine the effect of resveratrol on rotenone-induced neurotoxicity, we first checked the effect of resveratrol on cell viability and found that concentrations at or lower than 25 μM of resveratrol caused no significant cell death (data not shown). We therefore used 25 μM resveratrol for all subsequent experiments. When cells were pre-treated with resveratrol for 12 h, rotenone-induced cell death was dramatically attenuated (Fig. 1c).

We further studied the effect of rotenone and resveratrol on apoptosis. Our results showed that rotenone treatment could increase pro-apoptotic Bax protein levels while decreasing anti-apoptotic Bcl-2 protein levels (Fig. 2a). Pre-treatment with resveratrol reversed the neurotoxicity of rotenone by inhibiting Bax and promoting Bcl-2 protein levels (Fig. 2a). qRT-PCR analysis also confirmed that rotenone-induced Bax gene expression and inhibited Bcl-2 gene expression, which could be alleviated by resveratrol pre-treatment (Fig. 2b). Furthermore, Annexin V-FITC/PI assay revealed that the number of apoptotic cells was significantly increased after rotenone-exposure, whereby increases in apoptotic induction was reversed by resveratrol pre-treatment for 12 h (Fig. 2c). These results suggest that rotenone is toxic to SH-SY5Y cells, whereas resveratrol could attenuate rotenone-induced cellular injury by inhibiting apoptosis.

The role of AMPK–SIRT1–p53 signal pathway in neuroprotection by resveratrol

Our previous results (Wu *et al.* 2011) and studies from others have demonstrated that resveratrol is an activator of SIRT1 through AMPK induction (Kulkarni & Canto 2015, Tamaki *et al.* 2014). Consistent with these findings, this study also confirmed that resveratrol could induce SIRT1 expression, along with AMPK phosphorylation and consequent activation. In addition, we found that rotenone treatment resulted in reduction of SIRT1 protein levels and AMPK phosphorylation; and resveratrol pre-treatment could attenuate rotenone-induced reduction of SIRT1 protein levels and AMPK phosphorylation (Fig. 3a).

Next we sought to validate whether p53 was involved in the neuroprotection by resveratrol. Western blot showed that both total and acetylated p53 protein levels were significantly increased upon rotenone treatment; and these increases were attenuated with resveratrol pre-treatment. Similarly, qRT-PCR results also showed that rotenone treatment led to an increase in p53 mRNA levels and a decrease in SIRT1 mRNA levels; and such changes were attenuated with pre-treatment of resveratrol (Fig. 3b and c).

Cell cycle regulation by p53

To further explore a possible mechanism for p53-dependent resveratrol function, we assayed for alterations in cell cycle progression by flow cytometry. We found that rotenone in large part arrested SH-SY5Y cells in G0/G1, and the percentage of cells in S phase was significantly decreased comparing to control group. Resveratrol treatment alone, however, induced cell progression to S phase, indicating that resveratrol may promote cell proliferation. With resveratrol pre-treatment, the number of cells in G0/G1 decreased comparing to rotenone treatment alone, and cells in G2/M phase increased, suggesting that resveratrol may function as a mitogenic agent (Fig. 4).

Resveratrol-mediated neuroprotection is SIRT1-dependent

To determine whether SIRT1 is involved in rotenone-induced cellular injury, we transfected SH-SY5Y cells with SIRT1 small interfering RNA (siRNA) that dramatically

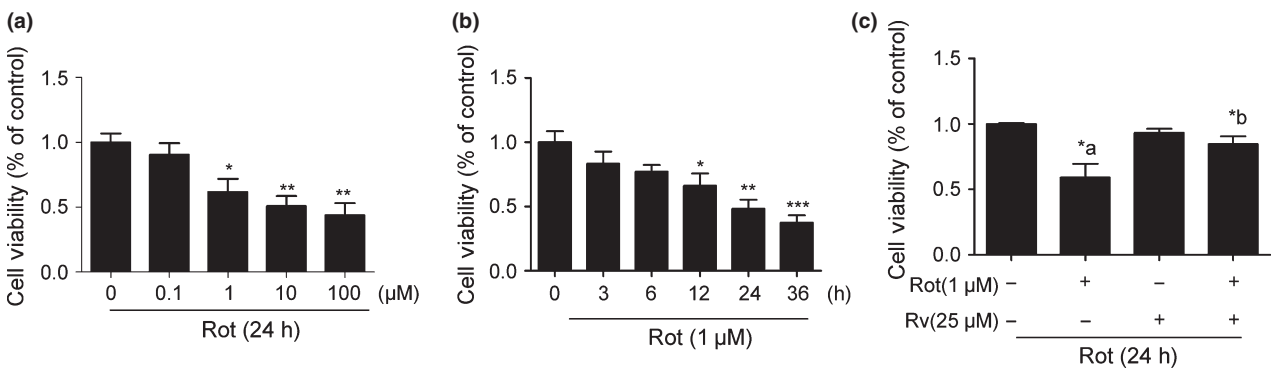

Fig. 1 Effects of rotenone and resveratrol on cell viability. SH-SY5Y cells were treated with rotenone for 24 h at various concentrations (a) or at 1 μM for different time periods (b). Neurons were pre-treated with resveratrol at 25 μM for 12 h, followed by rotenone treatment at 1 μM for 24 h (c). Data were expressed as mean ± SD. *$p < 0.05$; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared to control (a) or rotenone (b).

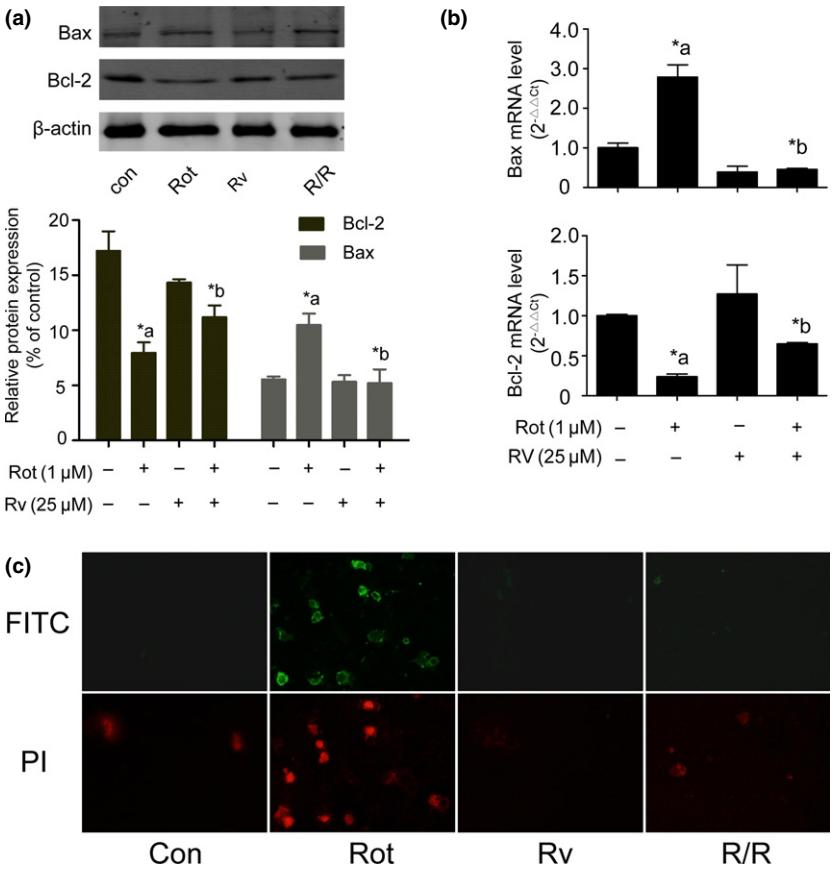

Fig. 2 Rotenone-induced apoptosis is attenuated by resveratrol pre-treatment. Western blot (a) and qRT-PCR (b) assay to detect apoptotic related genes Bax and Bcl-2 expression. (c) Immunofluorescence assay via Annexin V-FITC/PI method to test apoptosis. Data were expressed as mean ± SD. $^*p < 0.05$ as compared to control (a) or rotenone (b).

decreased SIRT1 protein levels (Fig. 5a). We found that the acetylated p53 levels were significantly increased upon SIRT1 down-regulation, indicating that SIRT1 could directly deacetylate p53. In addition, both p53 mRNA and protein levels were also increased upon SIRT1 down-regulation, suggesting that SIRT1 could inhibit p53 gene expression (Fig. 5).

Taken together, our results suggest that SIRT1 activation with resveratrol leads to suppression of p53, which may at least in part, explain why resveratrol's attenuation of rotenone-induced cell toxicity (Fig. 5).

SIRT1 target on H3K9

To further investigate the mechanism underlying the regulation of p53 by resveratrol, we performed ChIP assays to analyze changes in histone H3-K9 levels within the p53 promoter region. Following exposure to the drugs indicated, cross-linked DNA fragments were immunoprecipitated using specific anti-H3K9-me3 and anti-H3K9-ac antibodies and subjected to semi-quantitative PCR. The resulting PCR products were quantified to determine levels of H3K9 bound to the p53 promoter. We found that acetylated H3K9 levels were relatively high but the level of tri-methylated H3K9 was relatively low after cells were treated with rotenone, indicating that rotenone promotes p53 transcription and p53-mediated apoptosis. When cells were pre-treated with resveratrol for 12 h, we observed a reduced acetylation level and an increased methylation level in H3K9, compared to cells treated with rotenone alone, indicating that resveratrol pre-treatment targets the p53 promoter region to restrain p53 transcription, and thus inhibiting p53-mediated apoptosis (Fig. 6a). Moreover, we transfected SH-SY5Y cells with SIRT1 siRNA. Compared to the mock group, cells transfected with SIRT1 siRNA exhibited a significant increase in the H3K9-ac, accompanied with reduced H3K9-me3, indicating a means for enhancing p53 expression (Fig. 6c).

SIRT1 modulation of post-translational p53 modifications

To test protein complex interactions between SIRT1 and p53, co-immunoprecipitation assays were performed. After incubation under varying conditions, cells were lysed and total cell lysates were subjected to immunoprecipitation with an anti-SIRT1 antibody. Western blot analysis revealed that ac-p53 and H3K9-ac levels were markedly increased following exposure to rotenone for 24 h. This increase was reversed by resveratrol pre-treatment for 12 h. Thus resveratrol may prevent cell apoptosis by activating SIRT1 and targeting not only H3K9 at transcriptional level but also p53 at protein level (Fig. 6b).

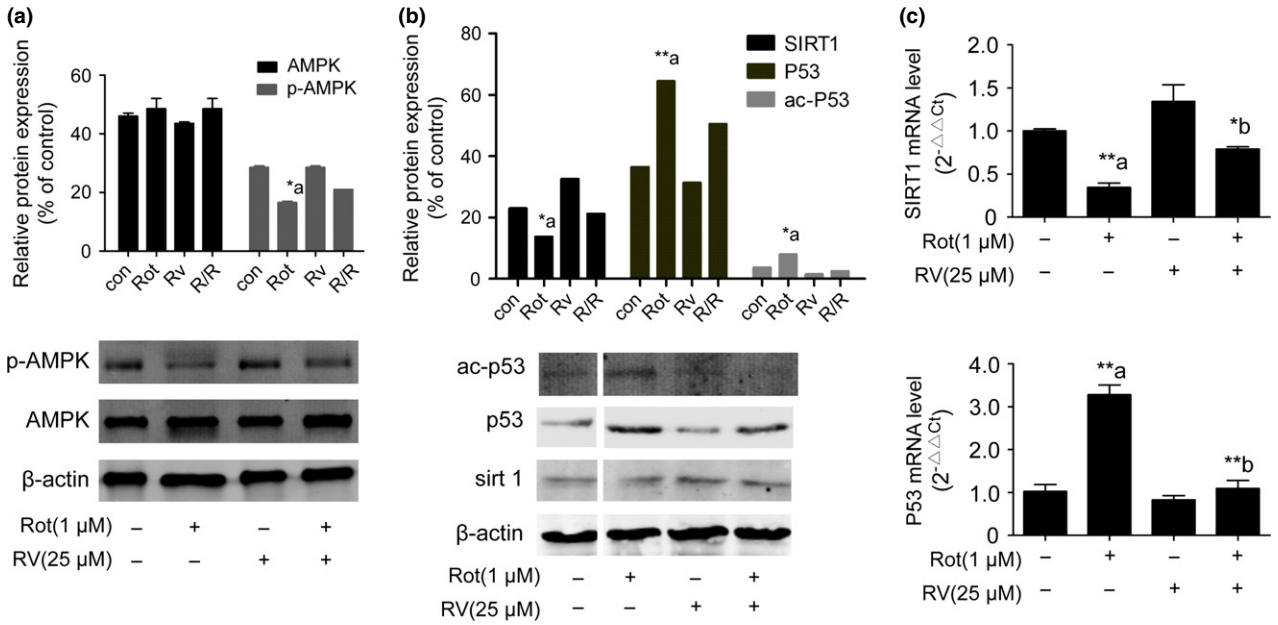

Fig. 3 Involvement of the AMPK-SIRT1-p53 signaling pathway in resveratrol dependent neuroprotection. Western blot assay and quantification of related proteins after different treatments (a and b). qRT-PCR analysis of SIRT1 and p53 expression in SH-SY5Y cells under various conditions (c). Data were expressed as mean ± SD. $^*p < 0.05$, $^{**}p < 0.01$ as compared to control (a) or rotenone (b). *Note*: The image of the first lane was from the same exposure of the image of the remaining three lanes.

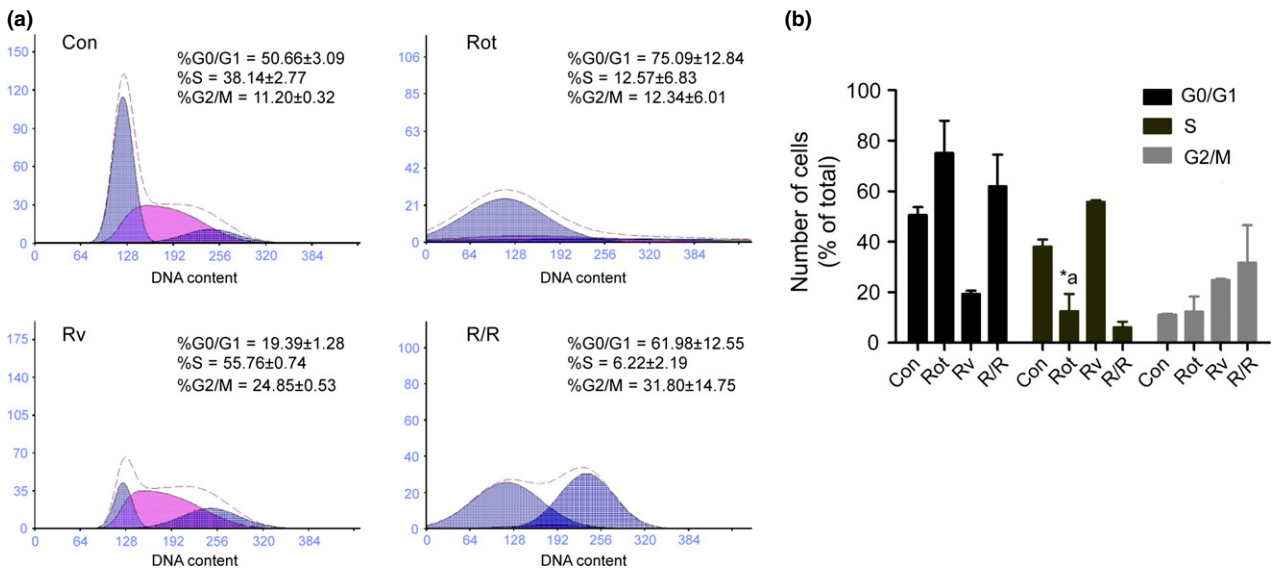

Fig. 4 Effects of rotenone or resveratrol on cell cycle progression. Data are expressed as mean ± SD. $^*p < 0.05$ as compared to control (a). Fig. 4b was the quantification based on Fig. 4a.

Discussion

Our study and those of others have indicated that SIRT1 plays a neuroprotective role in PD, mainly through autophagy induction and enhanced mitochondrial oxidative function (Wu *et al.* 2011; Mudo *et al.* 2012; Ferretta *et al.* 2014). Our findings provide evidence that resveratrol represses p53 by activating SIRT1, especially through epigenetic modulation at the transcriptional level, thus inhibiting apoptosis and enhancing neuron survival.

In the study, we applied rotenone to SH-SY5Y cells to recreate an *in vitro* cellular model of PD. A commonly used

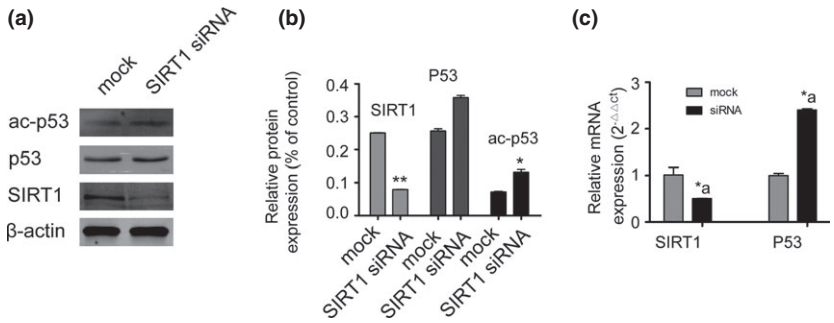

Fig. 5 Neuroprotection of resveratrol in a cellular model of Parkinson's disease (PD) is SIRT1-dependent. Western blot (a) and quantification (b) of SIRT1 and p53 protein levels after siRNA transfection. (c) qRT-PCR assay to test SIRT1 and p53 changes on mRNA levels. Data are presented as mean ± SD. $*p < 0.05$ as compared to mock group (a); $**p < 0.01$ as compared to mock (a).

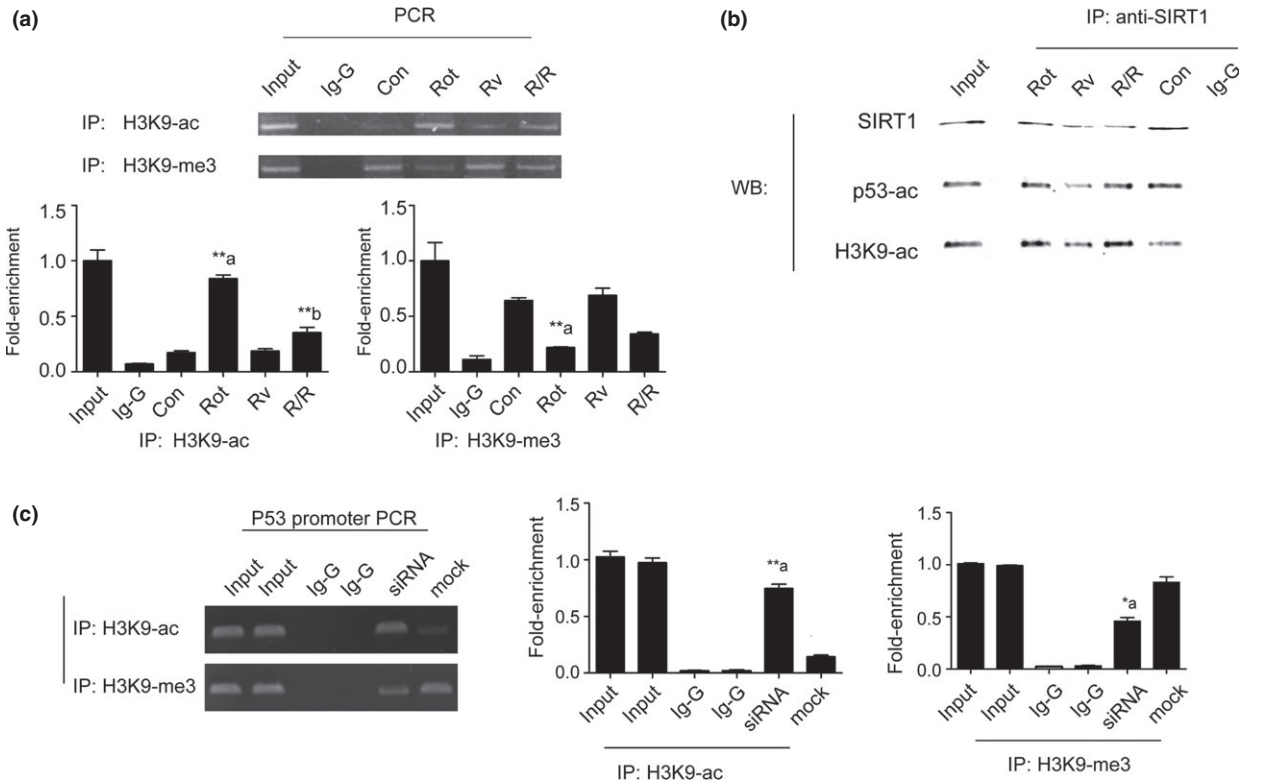

Fig. 6 Characterizing regulation of the p53 promoter region through SIRT1 by ChIP and CO-IP analysis. (a) ChIP assay with specific H3K9-me3 and H3K9-ac antibodies was used to detect H3K9-me3 and H3K9-ac levels after resveratrol or rotenone treatment (b) CO-IP assay to test the interaction of SIRT1 with p53 and H3K9. (c) The role of SIRT1 in regulating H3K9. Data were expressed as means ± SD. $*p < 0.05$, $**p < 0.01$ as compared to control or mock (a) or rotenone (b).

toxin for modeling PD, rotenone selectively inhibits mitochondrial electron transport chain complex I activity, which contributes to the release of reactive oxygen species, ultimately leading to the induction of neuronal apoptosis (Mao *et al.* 2007; Pal *et al.* 2014). Our results showed that rotenone-induced apoptosis could be alleviated by resveratrol pre-treatment and altered p53 and H3K9 expression. Several studies have identified the role of AMPK-SIRT1 in mediating neuroprotective effects of resveratrol (Lin *et al.* 2010; Wang *et al.* 2013; Ferretta *et al.* 2014). In agreement with previous reports, our current study also showed that resveratrol-induced expression of SIRT1 is accompanied by AMPK phosphorylation and activation. We found that resveratrol, by activating SIRT1, repress the function of p53, a vital cell cycle regulator (Hao and Cho 2014). While rotenone blocks cell proliferation by arresting cells at the G0/G1 phase, SIRT1 promotes cell cycle progression to S and G2/M, thus contributing to cell proliferation. These results suggest that p53-regulated cell cycle progression may be a possible mechanism through which its neuroprotective effect is mediated.

SIRT1, silent information regulator 1, also represses gene expression by acting as an NAD^+-dependent histone deacet-

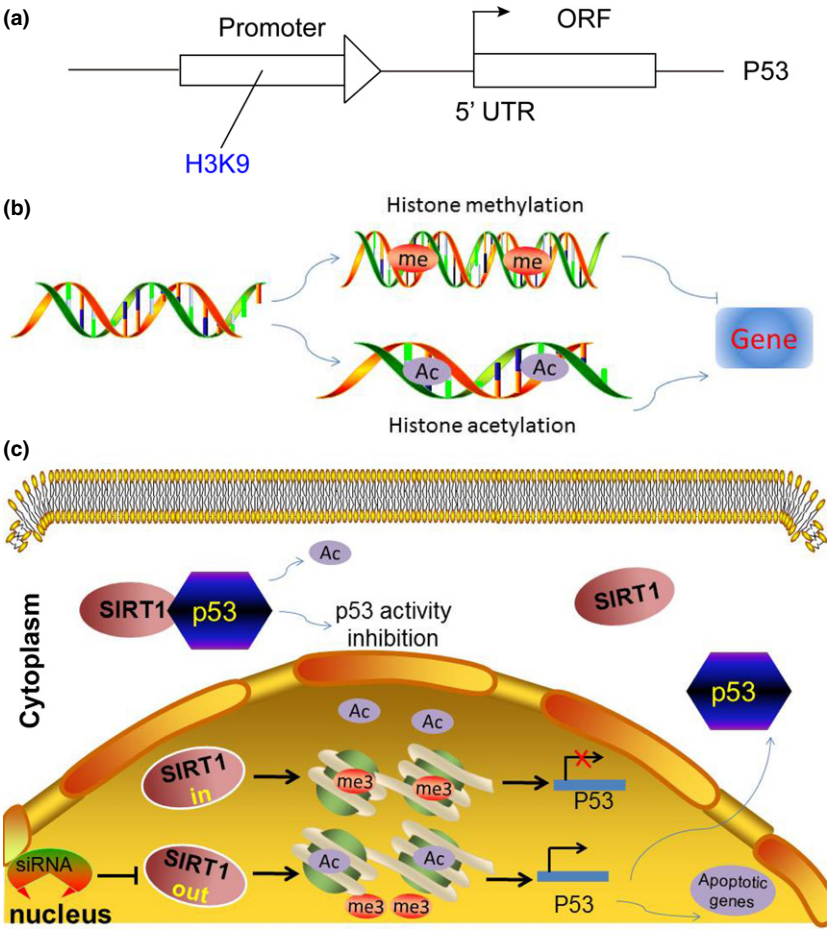

Fig. 7 Model depicting epigenetic regulation of p53 gene expression by SIRT1 in SH-SY5Y cells. (a) The brief structure of P53 gene and the location of H3K9. (b) The relationship of histone methylation and acetylation with gene transcription. (c) The role of SIRT1 in regulating p53 gene expression in SH-SY5Y cells.

ylase (Petegnief and Planas 2013). Furthermore, SIRT1 can directly act on p53 protein at lysine 382 (K379 in mouse p53), inhibiting p53 activity in response to DNA damage (Luo *et al.* 2001; Saunders and Verdin 2007). Using ChIP assay, we demonstrated for the first time that SIRT1 regulates p53 promoter at the transcriptional level. Furthermore, we found that activated SIRT1 can target H3K9, leading to decreased acetylation and increased tri-methylation of H3K9, thereby resulting in chromatin compaction. Consequently, wound DNA under these circumstances impedes the action of other transcriptional factors on the p53 promoter, thus ultimately repressing p53 gene expression. In contrast, rotenone treatment results in a loosened chromosome which facilitates binding of transcriptional factors to promoting p53 expression (Fig. 7).

In addition to the effects described above, recent studies have suggested that inflammatory stimuli may lead to SIRT1 inhibition and transcription activation of p53 and p65, resulting in apoptosis and neurodegeneration (Shinozaki *et al.* 2014).

In conclusion, our results show that resveratrol protects against neurotoxicity in a SIRT1-dependent manner. We demonstrate that resveratrol activates SIRT1, which targets H3K9 and regulates p53 gene expression at the transcriptional level, thus inhibiting p53 gene expression to enhance neuroprotection. Further studies of epigenetic regulation of p53 gene by SIRT1 may lead to the development of disease-modifying therapies in PD (Feng *et al.* 2015).

Acknowledgments and conflict of interest disclosure

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All experiments were conducted in compliance with the ARRIVE guidelines.

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