AG1031 induces apoptosis through suppressing SIRT1/p53 pathway in human neuroblastoma cells

Jingxuan Fu1 · Hui Zhang1 · Yuling Zhang1 · Tao Zhang1

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Abstract
Neuroblastoma is the most common extra-cranial tumor in childhood. As an antineoplastic medicine, the effect of AG-1031 on the neuroblastoma is still unclear. Silent information regulator 1 (SIRT1) is a conserved NAD+-dependent deacetylase, which plays a key role in carcinogenesis through the deacetylation of important regulatory proteins, including p53. The purpose of the present study was to determine whether there was a significant anti-tumor effect of AG-1031 on the human neuroblastoma cells through suppressing SIRT1/p53 pathway. Our study showed that AG1031 treatment resulted in a dose-dependent decrease in human neuroblastoma SH-SY5Y cell viability. The data, obtained from both Western blot assay and Hoechst 33258 staining, further showed that AG1031 exhibited strong anti-tumor activity closely associated with significantly increasing apoptotic indices and enhancing oxidative stress levels. Moreover, AG1031 treatment could down-regulate SIRT1 in a dose-dependent manner and up-regulate p53 acetylation, while overexpression of SIRT1 significantly attenuated the anti-tumor effect of AG1031 in SH-SY5Y cells. AG1031 potently induced SH-SY5Y cells apoptosis through suppressing SIRT1/p53 signaling. These data suggest that AG1031 may be used for therapeutic intervention in neuroblastoma treatment.

Keywords AG1031 · Neuroblastoma · SIRT1 · Apoptosis · p53 · Overexpression

Introduction
Neuroblastoma, derived from primitive cells of the sympathetic nervous system, is a most common extra-cranial solid tumor in childhood [1]. It exhibits very complex biological, clinical heterogeneities and susceptibility [2–4]. Several factors including age at diagnosis, extent of disease, and tumorous biology are associated with the possibility of the cure; consequently, the likelihood of cure is quite different [5]. In spite of the fact that the benign form of the disease can reach spontaneous and complete recession, the morbidity and mortality rates of the malignant form is very high [6]. Even if providing dramatic increases in the intensity of therapy, the cure rates among children with high-risk neuroblastoma displayed only modest improvement [6, 7]. Therefore, a great deal of efforts has been direct toward developing new drugs by probing molecular mechanism to treat high-risk neuroblastoma.

Sirtuins, which are conserved from bacteria to eukaryotes, are NAD+-dependent class III histone deacetylases. Silent information regulator 1 (SIRT1) is the most extensive studied family member because it is said to prolong the lifespan of some species [8–10]. It is reported that SIRT1 plays a wide variety of roles in many biologic systems related to aging [9], metabolic diseases, cellular senescence, stress, and inflammation [8, 9, 11]. In the past decades, there have been extensive studies of SIRT1 performing functions in cancers. Several previous studies showed that SIRT1 deacetylated histone proteins and other key transcriptional regulators such as p53 [12], the forkhead box O (FoxO) transcription factors [13], NF-κB [14], PPARγ [15], and the hypoxia-inducible transcription factors [16]. Nevertheless, there is a debate about SIRT1 in cancer, i.e., if it acts as either a tumor suppressor or tumor promoter. On the one hand, overexpression of SIRT1 was observed in many cancers such as lung cancers, prostate, colon and acute myeloid leukemia [17, 18]. In view of the increasing levels of SIRT1 in cancers, it hypothesizes that SIRT1 serves as a tumor promoter [19, 20]. On the other hands, SIRT1 may inhibit
tumor cell growth by suppressing the transcription factor NF-κB in some kinds of cancer [21].

AG-1031 is a small-molecule drug (MW = 780 Da) for human approved by Food and Drug Administration (FDA). Using an established high throughput assay system, AG-1031 was identified as the first small-molecule inhibitor of human transcriptional positive cofactor 4 (PC4). Preliminary investigations have implied that AG-1031 may inhibit the viability of several cancer cell lines. A previous study showed that AG-1031 induced autophagic cell death and apoptosis in C6 glioma cells associated with Notch-1 signaling pathway [22]. However, in the present study, we aimed to investigate if there were any other pathways involved in its effect on neuroblastoma cells. Therefore, a hypothesis was raised that AG1031 could induce apoptosis through inhibiting SIRT1/p53 pathway in human neuroblastoma cells. Accordingly, human neuroblastoma cells, SH-SY5Y, was used to investigate the effect of AG-1031 on SH-SY5Y cell lines proliferation. And we further assessed the anti-tumor activity of AG1031 on SH-SY5Y cells and explored the role of SIRT1/p53 pathway in human neuroblastoma cells.

Materials and methods

Reagents

Dulbecco’s modified eagle media (DMEM) cell culture medium was purchased from Invitrogen GIBCO. The fetal bovine serum (FBS) was purchased from Omega Scientific (United States). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) was obtained from Beijing Ding guo Changshen Biotechnology co LTD (China). Antibodies against acetylated-p53 (Lys382), cytochrome c, Bcl-xl, Bax, caspase 9, caspase 3, SIRT1 were purchased from Abcam (United States). Antibody against p53 was purchased from Cell Signaling Technology. Antibody against β-actin was purchased from Santa Cruz Biotechnology, Inc. CA (United States). Antibody against Flag was purchased from sigma (United States). Superoxide dismutase (SOD) assay kit, Malondialdehyde (MDA) assay kit and Hochest 33258 kit were purchased from the Beyotime Biotechnology (China). The chemiluminescent HRP substrate was purchased from Millipore Corporation (United States).

Cell culture and treatment

Human neuroblastoma SH-SY5Y cell line was obtained from FDCC, which was cultured with the DMEM medium contain 10% FBS. The early passage (P11–P17) human neuroblastoma SH-SY5Y cell line was used in the experiment. Differentiation was induced by adding 10 μM all-trans retinoic acid (RA) with the DMEM medium containing 3% FBS on day 1, 24 h after plating, and continued for 5 days. Every 72 h the old medium was replaced by new one. All treatments began on day 6. The cells were maintained in 95% O₂ and 5% CO₂ in a sterile atmosphere.

Establishing stable SIRT1 overexpression in human neuroblastoma cells

The lentiviral vector (packaging byG358, Ubi-MCS-3FLAG-SV40-EGFP-IRES-puromycin) was obtained from Shanghai Genechem Co., Ltd. (Shanghai, China). SH-SY5Y cells were plated at a concentration of 5×10³ cells/well in 96-well plates for transfection. The cells were transfected either with the GFP lentiviral vectors (lvGFP, negative GFP control group) or with SIRT1-GFP lentiviral vectors (lvSIRT1, SIRT1 overexpression group) in serum-free medium for 12 h. According to the manufacturer’s protocol, the cells were selected with puromycin (0.5 μg/ml) for 72 h. After infection, the reporter gene expression was examined by using fluorescence microscopy to make sure that the efficiency of the infection was approximately 100%. Finally, the cells were stained with Hoechst 33258 and harvested for Western blot analysis.

Cell viability assay

SH-SY5Y cell viability was assessed with MTT assay. First of all, as untreated normal cells, lvGFP cells and lvSIRT1 cells were plated in 96-well microtiter plates 1×10⁵ per well and the cells were grown overnight prior to studies. And then they were incubated with final concentrations of AG-1031. After incubating for 24 h, MTT (0.5 mg/ml) was used to react with living cells for 4 h at 37 °C. Afterwards, culture medium was removed, and 100 μl DMSO was added to each well. After shaking for 15 min, the mixture was dissolved completely and the absorbance of each well was measured at 492 nm on an ELISA reader (Elx800, Bio-TEK, USA).

Western blot analysis

The cells, incubated with final concentrations of AG-1031, were homogenized in lysis buffer containing a 1% PMSF similarly like a previous report [23]. Insoluble debris was precipitated by centrifugation at 12,000 rpm for 15 min at 4 °C, and the supernatants were collected as the total cellular proteins and assayed for protein concentration using the BCA reagent kit. Subsequently, 5× loading buffer was added to the supernatant (ratio is 5:1) and boiled at 100 °C metal bath for 15 min. Samples (40 μg protein) were extracted, separated by electrophoresis, and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked for 1 h. Subsequently, the membranes with the protein of normal
cells and DIF cells were incubated with primary antibodies against SIRT1, MAP2 (1:1000 dilution), and β-actin (1:2000 dilution). The membranes with the protein of untreated normal cells were incubated with primary antibodies against caspase 3, Bax, cytochrome c, and β-actin (1:2000 dilution). The membranes with the protein of the lvGFP cells and lvSIRT1 cells were incubated with primary antibodies against caspase 3, Bax, cytochrome c, Bcl-xl, Bax, caspase 3, caspase 9, Flag (1:1000 dilution), and β-actin (1:2000 dilution) during the night at 4 °C following by several TBST washes. The membranes were then probed with the immunoreactivity by adding secondary antibody diluted to 1:5000 detecting it with chemiluminescent HRP detection kit.

**Nuclear staining analysis by Hoechst 33258**

Since Hoechst 33258 dye can bind to DNA in living cells, it is used to assess the changes in the nuclear morphology. lvGFP and lvSIRT1 SH-SY5Y cells were seeded a 12-well plate and treated with AG1031 at 1 μM for 24 h. They were washed with phosphate buffer saline (PBS) three times and then cultured with Hoechst 33258 dye (5 μg/ml) for 20 min at a temperature of 37 °C in the dark. After washing three times with PBS, we employed the fluorescence microscope to visualize the Hoechst-stained nuclei.

**Measurement of MDA and T-SOD**

The assay kits were used to evaluate the level of total SOD (T-SOD) activity and malondialdehyde (MDA) level in lvGFP and lvSIRT1 SH-SY5Y cells. The cells were plated in 6-well plates. As soon as reaching 60–70% confluent, the cells were treated with AG1031 (1 μM) for 24 h. After incubation, the cells were harvested and tested by following the manufacturer’s instructions resemble as our previous study [24].

**Statistical analysis**

All data were obtained from at least three independent experiments and presented as mean ± SEM. The statistical significance was assessed by one-way analysis of variance (ANOVA) with supporting by LSD post hoc test when more than two groups. Student’s t test was used for two-group comparisons. All analyses were performed using SPSS 22.0 software and the significant difference was taken when \( p < 0.05 \).

**Results**

**The effect of AG1031 on SH-SYSY cell viability and apoptosis**

The MTT assay was performed to measure the viability of AG1031-treated SH-SYSY cells, and the data are presented in Fig. 1a. The cell viability in the CON group was defined as 100%, while the cell viabilities of other five AG1031 groups were expressed as the percentage of that in the CON group. The SH-SY5Y cells were treated by 0.1 μM, 0.3 μM, 1 μM, 3 μM, and 10 μM AG1031 for 24 h, respectively. The data showed that cell growth was significantly inhibited in a dose-dependent manner (Fig. 1a, \( p < 0.001 \)). Since it was nearly the 50 percentage of the cell viability in the CON group, the AG1031 treatment with 1 μM was employed during following experiments.

After treatment with 1 μM AG1031 for 24 h, it could be seen that the apoptotic protein was significantly increased (Fig. 1b–e). Western blot analysis showed that the pro caspase 3 to cleaved-caspase 3, levels of Bax and cytochrome c were much higher in the AG1013 group than that in the CON group (Fig. 1c, \( p < 0.001 \), Fig. 1d, \( p < 0.01 \), Fig. 1e, \( p < 0.01 \)).

**The effect of AG1031 on SIRT1**

In order to investigate an underlying mechanism of SH-SYSY cell apoptosis induced by AG1031, we measured the expression of SIRT1. It was found that the level of SIRT1 expression of the differentiated SH-SYSY cells, named as DIF, was lower than that in the CON group (Fig. 2c, d, \( p < 0.01 \)). In addition, the level of MAP2 was significantly enhanced in the DIF group compared to that in the CON group (Fig. 2a, b, \( p < 0.05 \)). Moreover, after the treatment of AG1031 for 24 h, the levels of SIRT1 expression were significantly reduced in a dose-dependent manner in the AG1013 group compared to that in the CON group (Fig. 2e, f, \( p < 0.01–0.001 \)).

**The effect of AG1031 on cell viability in overexpressed SIRT1 SH-SYSY cells**

In order to further investigate the role of SIRT1 in SH-SYSY cells treated by AG1031, the overexpressed SIRT1 SH-SYSY cell line was established. Figure 3a showed that the green fluorescence was evidently detected in the lvGFP group and the lvSIRT1 group. In addition, it can be seen that there is no evidence of Flag protein in both the lvGFP
Fig. 1 AG1031 induces apoptosis in SH-SY5Y cells. a The effects of AG1031 on cell viability measured by MTT assay. SH-SY5Y cells were treated with AG1031 at various concentrations (0, 0.1, 0.3, 1, 3, 10 μM) for 24 h. b Antibody responded to SH-SY5Y cells treated with 1 μM AG1031 for 24 h. The representative images of Western blot for apoptosis related proteins. c Quantitative expression of cleaved-caspase 3 to pro caspase 3. d Quantitative expression of Bax. e Quantitative expression of cytochrome c. **p < 0.01; ***p < 0.001 as compared with the CON group.

Fig. 2 AG1031 decreased the expression of SIRT1 in SH-SY5Y cells. a Representative images of Western blot for MAP2 protein in differentiated SH-SY5Y cells. b Quantitative expression of MAP2. c Representative images of Western blot for SIRT1 protein in differentiated SH-SY5Y cells. d Quantitative expression of SIRT1. e Representative images of Western blot for SIRT1 protein in SH-SY5Y cells treated at various concentrations (0, 0.3, 1, 3 μM) for 24 h. f Quantitative expression of SIRT1 in SH-SY5Y cells treated at various concentrations for 24 h. **p < 0.01; ***p < 0.001 as compared with the CON group.
and lvGFP+1031 groups, however, there is a clear evidence of Flag protein in both the lvSIRT1 and lvSIRT1+AG1031 groups (Fig. 3b), suggesting that SIRT1 has been successfully overexpressed in the lvSIRT1 and lvSIRT1+AG1031 groups.

Figure 3c showed that there was an evident effect of AG1031 on the cell viability in lvGFP SH-SY5Y cells but not overexpressed SIRT1 SH-SY5Y cells. The cell viability of both lvGFP and lvSIRT1 groups was defined as 100% and it was expressed as the percentage of them in other two groups. It could be seen that the cell viability was significantly decreased in the lvGFP+AG1μM group compared to that in the lvGFP group (50.95 ± 0.023, p < 0.001). Moreover, it was much higher in the lvSIRT1+AG1μM group than that in the lvGFP+AG1μM group (p < 0.001).

### The effect of AG1031 on apoptosis in overexpressed SIRT1 SH-SY5Y cells

The Hoechst 33258 staining was used to measure the change of nuclear morphology (Fig. 4a). The rate of apoptosis was evidently increased in the lvGFP+1031 group compared to that in the lvGFP group (Fig. 4b, p < 0.001). Interestingly, the rate of apoptosis was much lower in the lvSIRT1+AG1031 group than that in the lvGFP+AG1031 group (Fig. 4b, p < 0.01).

In order to determine the effect of AG1031 on apoptosis in overexpressed SIRT1 SH-SY5Y cells, both caspase 3 and caspase 9 were measured by using Western blot assay (Fig. 5a). As shown in Fig. 5b, c, the level of either cleaved/pro caspase 3 or cleaved/pro caspase 9 was higher in the lvGFP+AG1031 group than that in the lvGFP group (p < 0.001). However, the level of them was lower in the lvSIRT1+AG1031 group than that in the lvGFP+AG1031 group (p < 0.05–0.01).

To determine whether the effect of AG1031 on anti-apoptosis in overexpressed SIRT1 SH-SY5Y cells was associated with the p53 pathway, the level of p53, acetylated-p53 (Lys382), and their downstream proteins such as Bcl-xl, Bax and cytochrome c expressions were measured (Fig. 5d). It was found that the expression levels of both p53 and acetylated-p53 (Lys382) were higher in the lvGFP+AG1031 group.
than that in the lvGFP group, while they were lower in the lvSIRT1+AG1031 group than that in the lvGFP+AG1031 group (p < 0.01–0.001, Fig. 5e, f). The level of Bcl-xl was much lower in the lvGFP+AG1031 group than that in the lvGFP group, while it was higher in the lvSIRT1+AG1031 group than that in the lvGFP+AG1031 group (p < 0.01, Fig. 5g). Moreover, the levels of Bax (p < 0.001, Fig. 5h) and cytochrome c (p < 0.001, Fig. 5i) were higher in the lvGFP+AG1031 group than that in the lvGFP group, meanwhile they were much lower in the lvSIRT1+AG1031 group than that in the lvGFP+AG1031 group (p < 0.001, Fig. 5h, i).

The effect of AG1031 on oxidative stress in overexpressed SIRT1 SH-SY5Y cells

In order to examine if AG1031 effectively changed the level of ROS in overexpressed SIRT1 SH-SY5Y cells, the levels of MDA and SOD were measured individually by using commercial test kits (Fig. 6). The level of T-SOD was lower in the lvGFP+AG1031 group compared to that in the lvGFP group (Fig. 6a, p < 0.01), however, it was significantly enhanced by overexpressed SIRT1 in the lvSIRT1+AG1031 group compared to that in the lvGFP+AG1031 group (p < 0.01). Moreover, the level of MDA was much higher in the lvGFP+AG1031 group compared to that in the lvGFP group (Fig. 6b, p < 0.01), but it was significantly reduced by overexpressed SIRT1 in the lvSIRT1+AG1031 group compared to that in the lvGFP+AG1031 group (Fig. 6b, p < 0.01).

Discussion

In patients younger than 15 years, neuroblastoma accounts for more than 7% of malignancies and around 15% of all pediatric oncology deaths [5]. The objective of the study was to reveal the effect of AG-1031 on human neuroblastoma SH-SY5Y cells. One of our previous study showed that AG1031 could inhibit C6 glioma cells proliferation via AKT-mTOR-Notch1 signaling [25]. The role of SIRT1 in cancers has been extensively studied in the past decade [25]. Therefore, in the study, we aimed to explore if there was another anti-tumor pathway of AG1031 associated with SIRT1. The data showed that AG1031 was a potent inhibitor of SH-SY5Y cell viability. The AG1031 treatment significantly induced apoptosis in SH-SY5Y cells. In addition, the overexpression of SIRT1 considerably weakens the apoptosis protein expression and oxidative status in AG1031 treated SH-SY5Y cells. It suggests that AG1031 induces neuroblastoma apoptosis by regulating the expression of SIRT1.

SIRT1 is a highly conserved NAD+-dependent deacetylase that has been implicated in the modulation of transcriptional silencing and cell survival. It also plays a crucial role in carcinogenesis through the deacetylation of important regulatory proteins [26]. In some human cancers, such as breast, lung and colon cancers, SIRT1 is highly expressed [27–29]. In the present study, it was found that the level of SIRT1 expression was higher in undifferentiated SH-SY5Y cells than that of differentiated cells, implying that there was an important role of SIRT1 in the survival of cancer cells. In addition, the inhibition of SIRT1 expression and activity by siRNA or small-molecule inhibitors impeded the growth, invasion and metastasis of many tumors, and induced apoptosis in cancer cells [30, 31]. Interestingly, our data showed that AG1031 down-regulated SIRT1/p53 pathway with significantly improving intracellular caspase 3 activity and up-regulated the levels of Bax and cytosolic cytochrome c protein. It suggests that AG1031 may restrain the growth of tumors via inhibiting SIRT1 signaling. Similarly, a previous study showed that the downregulation of SIRT1...
Fig. 5 The effect of AG1031 induces SH-SY5Y cells apoptosis via SIRT1/p53 pathway. a Representative images of Western blot for caspase 3 and caspase 9. b Quantitative expression of leaved caspase 3 to pro caspase 3. c Quantitative expression of cleaved-caspase 9 to pro caspase 9. d Representative images of Western blots for p53, acetylated-p53, Bcl-xl, Bax and cytochrome c (Cyto-c). e Quantitative expression of p53. f Quantitative expression of acetylated-p53-K382. g Quantitative expression of Bcl-xl. h Quantitative expression of Bax. i Quantitative expression of cytochrome c. ***p < 0.001 as compared with that in the lvGFP group; ##p < 0.01, ###p < 0.001 as compared with that in the lvGFP+AG1031.
expression and the mitochondrial death pathway could be actually enhanced by AG1024 in MCF-7 breast cancer cells [32].

P53 mutations have been detected in 50% of all human cancers. It is also emphasized by accumulating evidences that p53 protein function and stability are often abrogated via posttranslational mechanisms in the remaining human cancers [33]. No doubt about it, tumor suppressor gene p53 plays an important role in the prevention of human cancer development and progression. Since cancers need to normally deactivate p53, it will trigger apoptosis and cell growth arrest as soon as p53 is activated, which are detrimental to cancer cells [34]. Alteration of p53 acetylation other than phosphorylation status is essential for its role as both a transactivator and transcription-independent cell death inducer [35, 36]. It was found that the attenuated p53 response in SH-SY5Y cells was able to result from the high expression of a negative regulator of p53, such as SIRT1 [37]. In the p53 protein, SIRT1 can deacetylate K382, the deacetylation site of SIRT1.

In addition, there are many chemotherapeutic drugs which inhibit the growth of cancer cells and induce apoptosis via the intrinsic mitochondrial apoptotic pathway [38]. Bcl-xl, Bax, caspase 3, caspase 9 and cytochrome c are important members of this pathway [39]. Cytochrome c releasing from mitochondria is a critical step in the apoptotic process [40]. Furthermore, Bcl-xl, as an important regulator of apoptosis and overexpressed in human cancer, is a close homolog of Bcl-2 [41]. And Bax is the p53-dependent pro-apoptotic gene. By preventing the release of cytochrome c from mitochondria, Bcl-xl blocks apoptosis [34]. Whereas, as a member of the Bcl-2 protein family, Bax induces the release of cytochrome c, activation of caspase 3, and cell death [42]. It reports that p53β binds the p53-responsive promoter and enhances p53 transcriptional activity on the BAX promoter [43]. Moreover, p53-dependent apoptosis could be obstructed by Bcl-xl [44, 45]. And p53 prompts apoptosis through the releasing from mitochondrial cytochrome c via caspase activation [46]. In the study, the data showed that AG1031 treatment could significantly inhibit SIRT1 and motivated acetylated p53 in SH-SY5Y cells. In addition, the antitumorous effect of AG1031 was efficiently attenuated in the overexpressed SIRT1 SH-SY5Y cells, such as increasing the viability of SH-SY5Y cells and reducing apoptosis and oxidative status. These results suggest that AG1031 inhibits the growth of neuroblastoma by down-regulating SIRT1 in part through increasing p53 acetylation.

Oxidative stress, involving numerous metabolic pathways, is a multi-factorial process in the cells [3, 47]. And oxidative damage is that the reactive species (RS) generation exceeds the capacity of the antioxidant defense system [48, 49], resulting in damage to lipids, proteins and DNA, and also inhibiting the normal cellular function [50]. Superoxide dismutase (SOD) is an enzyme that catalyzes the removal of superoxide free radicals. The activity of SOD in cancer tissues increases with the progression of stage, and changes with the depth of invasion [23, 51]. Malondialdehyde (MDA), which is a by-product of lipid peroxidation, is one of the reference values of principal parameters of oxidative stress [52, 53]. The elevated level of MDA and the reduced activities of SOD can cause oxidative stress [54]. Besides, many anticancer drugs are capable of generating superoxide, typically by redox cycling with oxygen [55]. SIRT1 may participate in the intrinsic mitochondrial apoptotic pathway [56]. Mitochondria has been considered as a source of reactive oxygen species (ROS) during apoptosis and plays a key role in the maintenance of cellular redox potential [57]. The enhanced ROS production, induced by anti-tumor medicine, was connected to the apoptotic responses [58]. We measured the expression level of p53 and acetyl-p53 since the protein p53 was required for cell death induced by oxidative damage [59]. Exposure to oxidative stress is able to

**Fig. 6** The effect of AG1031 on T-SOD and MDA in overexpressed SIRT1 SH-SY5Y cells. a T-SOD activity measurement. b MDA activity measurement. **p < 0.01; as compared with that in the lvGFP group. *p < 0.01 as compared with that in the lvGFP+AG1031
induce a loss of proliferative capacity and neoplastic transformation that resembles cellular senescence, suggesting that the accumulation of oxidative damage with age lead to senescence through a p53-mediated activation of p21 [60]. Furthermore, the upregulation of SIRT1/p53 signaling pathway could alleviate oxidative stress and induce apoptosis in murine mesangial cells [61]. Our results show that AG1031 causes oxidative stress in SH-SY5Y cells, while the effect of AG1031 on oxidative stress was significantly attenuated in the lvSIRT1+AG1031 group. These findings suggest that AG1301 elevates oxidative status in SH-SY5Y cells through SIRT1/p53 pathway.

Based on the results obtained from both the present study and the previous studies, it suggests that AG1031 promotes the acetylation of p53 by reducing the expression of SIRT1. Furthermore, the activation of p53 could induce cytochrome c, initiate caspase cascade and oxidative stress, and eventually lead to SH-SH5Y human neuroblastoma cell apoptosis (Fig. 7). Although a possible mechanism of AG-1031 in SH-SH5Y cells has been validated, further understandings of how AG-1031 regulates SIRT1 pathway is still remain unclear. Moreover, several signaling pathways, such as Notch and NF-κB, have also been an implicated in the process of apoptosis associated with AG1031. Consequently, it will be interesting to investigate whether these signaling pathways are also involved in AG1031-induced apoptosis.

Conclusions

The results suggested that the neuroblastoma cell growth was significantly inhibited by AG1031 treatment through down-regulating SIRT1/p53 pathway. Accordingly, numerous benefits of AG1031 make it a robust candidate in a potential therapeutics for neuroblastoma.

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Author contributions Conceived and designed the experiments: JF, TZ. Performed the experiments and analyzed the data: JF, HZ, YZ. Wrote the manuscript: JF, TZ. Authors approved final version for publication.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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