농학석사 학위논문

Class III histone deacetylase SIRT2 enhances cisplatin-induced apoptosis of ovarian cancer cells

난소암 세포주에서 **cisplatin**에 의한 세포사멸에 **SIRT2**가 미치는 영향 규명 연구

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Abstract

Resistance to platinum-based chemotherapeutic agents such as cisplatin is one of the most leading causes for progression and recurrence of ovarian cancer. The regulation of intracellular reactive oxygen species (ROS) levels has been associated with cisplatin resistance of ovarian cancer. SIRT2, a member of class III NAD+-dependent histone deacetylases (HDACs), plays a critical role in cellular response to oxidative stress. Recently, several studies have reported that SIRT2 may serve as a tumor suppressor or a tumor promoter depending on the cell type and context. However, its specific function in response to cisplatin in ovarian cancer remains to be defined. In this study, we investigated the role of SIRT2 in response to cisplatin in ovarian cancer cells. For this purpose, genetically matched cisplatin-sensitive and cisplatin-resistant ovarian cancer cell lines were used in our experiments. Cisplatin markedly increased ROS generation in cisplatin-sensitive ovarian cancer cells compared to cisplatin-resistant ovarian cancer cells. Interestingly, we found that cisplatin upregulated the expression level of SIRT2 in cisplatin-sensitive ovarian cancer cells, but not in cisplatin-resistant ovarian cancer cells. Furthermore, the expression levels of SIRT2 and its downstream target in cisplatin-sensitive ovarian cancer cells were associated with cisplatin-induced ROS overproduction. We further

demonstrated that overexpression of SIRT2 improved the response of cisplatin-resistant ovarian cancer cells to cisplatin. These findings suggest that targeting SIRT2 and its downstream pathway could be novel therapeutic strategies to overcome cisplatin resistance in ovarian cancer.

Keywords: Ovarian cancer, Cisplatin, Resistance, Reactive oxygen species, SIRT2

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Contents

List of Figures

List of Abbreviations

CDDP: Cisplatin

ROS: Reactive oxygen species

DHE: Dihydroethidium

DCFH-DA: 2,7-dichloro-dihydro-fluorescein diacetate

NAC: N-acetyl-L-cysteine

SIRT2: NAD-dependent protein deacetylase sirtuin-2

FoxO3: Forkhead box protein O3

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

PARP: Poly ADP-ribose polymerase

Introduction

Ovarian cancer is one of the most common cause of death from gynecological malignancies [1]. Most of ovarian cancer patients are diagnosed at advanced stages due to the lack of symptoms in the early stages of disease, which contributes to the high mortality of ovarian cancer [1]. Currently, the standard of care for advanced ovarian cancer consists of cytoreductive surgery followed by platinum-based chemotherapy. However, the majority of ovarian cancer patients develop resistance to chemotherapy and tumor recurrence, leading to poor prognosis [2-4]. Thus, a better understanding of the molecular mechanisms underlying chemoresistance is required to improve patient's outcome.

A platinum-based chemotherapeutic agent, cisplatin is widely used in various types of cancers including ovarian cancer. It has been reported that the cytotoxic effect of cisplatin is closely associated with an oxidative stress induced by excessive ROS production [5]. Furthermore, several studies have demonstrated that the regulation of intracellular ROS levels is involved in cellular resistance to cisplatin in ovarian cancer, although the mechanisms of resistance to cisplatin appear to be multifactorial [6, 7]. Taken together, these previous studies suggest a close relationship between ROS and cisplatin-induced cytotoxicity in

ovarian cancer.

Sirtuins are class III histone deacetylases (HDACs) that require NAD+ as a cofactor for their enzymatic activities [8]. There are seven sirtuin family proteins (SIRT1 to 7) in mammals. These enzymes differ in their subcellular localizations, and have specificities for various substrates, such as histones, transcription factors, and metabolic enzymes [9]. Many reports have shown that sirtuins are associated with numerous physiological roles including metabolism, cell cycle, and cellular stress responses, and they have complex roles in both tumor promotion and suppression [10, 11]. SIRT2 is a member of the sirtuin family and predominantly resides in the cytoplasm. It can affect gene expression by deacetylating transcription factors that shuttle between the cytoplasm and nucleus [12]. Although SIRT2 has been shown to play a critical role in the response to oxidative stress, its precise function in cancer remains controversial. Some studies have suggested that SIRT2 is a mediator of oxidative stress-induced cell death [13, 14]. In contrast, other studies have indicated that SIRT2 promotes cellular resistance to oxidative stress by modulating various targets via deacetylation [15, 16]. However, the exact role of SIRT2 in the response of ovarian cancer cells to cisplatin is still unknown.

In the present study, we first demonstrated that SIRT2 acts as an important mediator of oxidative stress-induced apoptotic cell death by

cisplatin in cisplatin-sensitive ovarian cancer cells. Furthermore, our data showed that overexpression of SIRT2 enhances cisplatin response in cisplatin-resistant ovarian cancer cells. Therefore, our results suggest that targeting SIRT2 and its downstream pathway could be potential therapeutic strategies to overcome cisplatin resistance in ovarian cancer.

Materials and Methods

1. Reagents and Antibodies

Cisplatin, N-acetyl-L-cysteine (NAC), Dihydroethidium (DHE), and 2',7'-dichloro-dihydro-fluorescein diacetate (DCFH-DA) were purchased from Sigma-Aldrich (St. Louis, MO), and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was from Amresco (Solon, OH). Fluorescein iso-thiocyanate (FITC)-labeled Annexin V (Annexin V-FITC) kit was obtained from BD Biosciences Pharmingen (San Diego, CA). Antibodies for SIRT2, FoxO3, and cleaved caspase-3 were purchased from Cell signaling Technology (Beverly, MA). Antibodies for GAPDH and PARP were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

2. Cell culture

Human ovarian cancer cell line A2780/S and its cisplatin-resistant subline A2780/CP were used in the current study. The cells were maintained in RPMI 1640 (WelGENE, Seoul, South Korea) supplemented with 10% fetal bovine serum (FBS; WelGENE, Seoul, South Korea) and 100 μg/ml penicillin/streptomycin (P/S; Gibco Life Technologies, Gaithersburg, MD) at 37°C in a humidified atmosphere

with 5% CO_2. A2780/CP cells were grown in culture media with 1 μM cisplatin every two passages to maintain drug-resistance.

3. Cell viability assay

For cell viability assay, ovarian cancer cells were seeded into 96-well plates and they were treated with or without cisplatin at various concentrations (1-10 μM) in 100 μl media per well for indicated time. Cell viability was assessed using 2 mg/ml MTT (Amresco, Solon, OH) dissolved in PBS. In brief, 50 μl of MTT solution was added to each well and the cells were incubated in media with the MTT solution for 3 h at 37°C. Then the cells were incubated in 100 μl DMSO at room temperature for 30 min. The absorbance was measured at 540 nm with Multi-scan spectrum (Thermo Scientific, Hudson, NH).

4. Measurement of apoptotic cell death

Ovarian cancer cells were washed with PBS and collected into a FACS tube (BD Falcon, CA) by trypsinization with 0.05% Trypsin-EDTA. For apoptotic cell death analysis by flow cytometer using Cell Quest software (FACS Canto, BD Biosciences, North Ryde, Australia), the cells were stained with Annexin V-FITC Apoptosis Detection Kit I (BD Pharmingen, CA) according to the manufacturer's instructions.

5. Detection of Intracellular ROS

DHE and DCFH-DA were used to measure intracellular ROS. Nonfluorescent DHE is oxidized to 2-hydroxyethidium by superoxide anion, which intercalates into the DNA and exhibits red fluorescence [17, 18]. DCFH-DA is cleaved by intracellular esterases to nonfluorescent DCFH and oxidized to the fluorescent DCF by hydrogen peroxide [19]. After indicated treatment, the cells were harvested and incubated in culture medium with 5 μM DHE for 10 min or 10 μM DCFH-DA for 30 min at 37°C in the dark. Relative fluorescence intensity of 2-hydroxyethidium and DCF was measured using a BD FACS Canto II flow cytometer (BD Bioscience, North Ryde, Australia). 2-hydroxyethidium and DCF fluorescence were measured with excitation at 488 nm and emission at 590 nm, and excitation at 488 nm and emission at 525 nm, respectively [20].

6. Quantitative real time-PCR (qRT-PCR)

Total RNA was isolated from ovarian cancer cells by RNAiso Plus (Takara, Tokyo, Japan). Single-stranded cDNA was synthesized from 1 μg of total RNA using PrimeScript Reverse Transcriptase (Takara, Tokyo, Japan) with oligo$(dT)_{20}$ primers. PCR was performed with specific primers SIRT2 sense 5'-TCC ACC AAG TCC TCC TGT TC-3',

antisense 5'-TGA AGG ACA AGG GGC TAC TC-3'; GAPDH sense 5'-GAG TCA ACG GAT TTG GTC GT-3', antisense 5'-TTG ATT TTG GAG GGA TCT CG-3' using the following amplification conditions: an initial denaturation (95°C, 3 min), followed by 45 cycles of denaturation (94°C, 5 sec), annealing (60°C, 15 sec) and extension (72°C, 10 sec), and a final extension (72°C, 10 min).

7. Western Blotting

Preparation of protein lysates and western blot analysis were performed as described previously [21]. In brief, ovarian cancer cells treated with cisplatin were collected and washed with PBS. The cells were lysed in lysis buffer. Cell extracts were separated by 9-15% SDS-PAGE, and transferred to nitrocellulose membranes. The membranes were incubated with appropriate primary and horseradish peroxidase-conjugated secondary antibodies. Signals were visualized by a chemiluminescence detection kit (AbFrontier, Seoul, South Korea).

8. Transient transfection

Expression vectors for FLAG-tagged human SIRT2 was kindly provided by Prof. Yong-Ho Ahn (Yonsei University, Seoul, South Korea) [22]. Ovarian cancer cells were transfected with the plasmids using

Lipofectamine 3000™ reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol.

9. Statistical Analysis

All experiments were performed in triplicates and the data were expressed as means ± SEM. The statistical significance of the differences between groups was analyzed by Student's *t*-test and One-way ANOVA with Bonferroni's post hoc test using GraphPad Prism 5 and SPSS 20.0 (SPSS Inc., Chicago, IL), and represented at $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

Results

Cisplatin sensitivity correlates with ROS generation in ovarian cancer cells

To examine the role of SIRT2 to the cisplatin response in ovarian cancer, we first used two human ovarian cancer cell lines, cisplatin-sensitive A2780/S cells and its cisplatin-resistant derivative A2780/CP cells [23]. As expected, MTT assay results showed that A2780/CP cells were approximately 4- to 19- fold more resistant to cisplatin than A2780/S cells (Figure 1A). Consistent with the results, cisplatin induced apoptosis in a time-dependent and dose-dependent manner in A2780/S cells, whereas it had little effect on apoptosis in A2780/CP cells (Figure 1B and C).

Previous studies have reported that the cytotoxic effect of cisplatin is closely associated with an increase of intracellular ROS generation [5, 6, 24]. To confirm this, intracellular ROS levels in ovarian cancer cells treated with cisplatin were measured by flow cytometry using DHE and DCFH-DA. As shown in Figure 2A, intracellular ROS levels were markedly increased in A2780/S cells relative to A2780/CP cells. Moreover, cisplatin-induced cell death was reduced when the cells were co-treated with a ROS scavenger, NAC (Figure 2B and C). These data

suggest that the sensitivity of cisplatin correlates with intracellular ROS generation in ovarian cancer cells.

Cisplatin markedly upregulates SIRT2 expression in A2780/S cells compared to A2780/CP cells

To determine the relationship between SIRT2 and cisplatin response in ovarian cancer cells, the relative expression levels of SIRT2 were quantified using qRT-PCR and western blot analysis. As shown in Figure 3A and B, the expression levels of SIRT2 did not differ significantly between A2780/S and A2780/CP cells. Interestingly, SIRT2 expression was markedly upregulated in A2780/S cells treated with cisplatin in a time-dependent and dose-dependent manner, whereas there was little change in A2780/CP cells (Figure 3C and D). In addition, we found that the protein level of FoxO3, which is known as one of the downstream targets of SIRT2, was reduced as opposed to the protein level of SIRT2 in A2780/S cells, but not in A2780/CP cells (Figure 3C and D). FoxO transcription factors are considered to play a central role in the resistance to oxidative stress through regulation of antioxidant defense systems [25-27]. A recent study found that SIRT2-mediated FoxO3 deacetylation leads to its ubiquitination and proteasomal degradation [28]. Thus, the reduced expression of FoxO3 in A2780/S cells was likely to be

associated with the upregulation of SIRT2 caused by cisplatin treatment.

Cisplatin induces the upregulation of SIRT2 through enhancing intracellular ROS levels in A2780/S cells, but not in A2780/CP cells

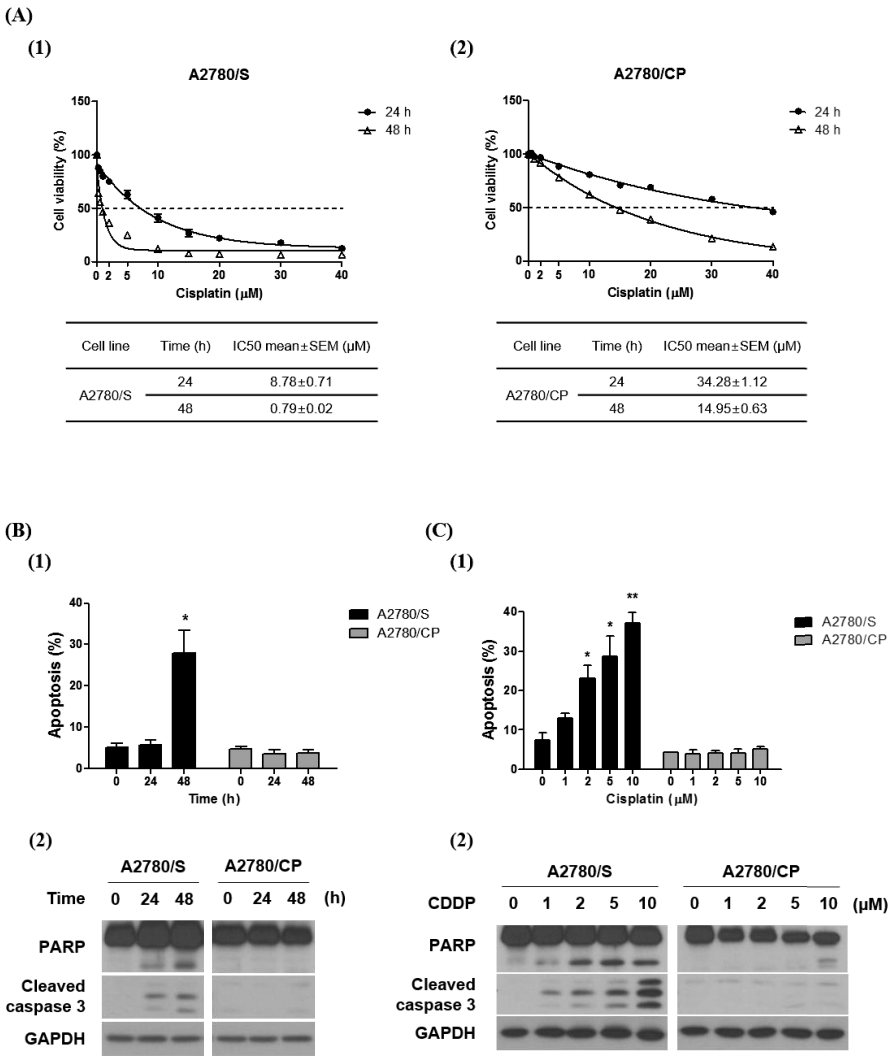
As mentioned in the introduction, SIRT2 is known to play an important role in oxidative stress response [13, 15, 29]. To determine whether SIRT2 upregulation in A2780/S cells is mediated by cisplatin-induced ROS overproduction, ovarian cancer cells were co-treated with cisplatin and NAC. As shown in Figure 4A, NAC treatment significantly reversed the changes in the levels of SIRT2 and FoxO3 caused by cisplatin in A2780/S cells. To further examine whether the level of SIRT2 is mediated by ROS overproduction in ovarian cancer cells, the cells were treated with hydrogen peroxide to mimic cisplatin-induced ROS generation (Figure 4B). Similar levels of ROS production were achieved when the concentrations of hydrogen peroxide were 10 μM and 15 μM in A2780/S and A2780/CP cells, respectively. (Figure 4B). As shown in Figure 4C, apoptosis was induced by each dose of hydrogen peroxide and reversed by NAC, indicating that hydrogen peroxide-induced ROS leads to apoptotic cell death in ovarian cancer cells. In addition, we found that hydrogen peroxide-induced ROS overproduction

increased the expression level of SIRT2, and decreased the expression level of FoxO3 in A2780/S cells (Figure 4C). Surprisingly, however, it had little effect on the expression levels of SIRT2 and FoxO3 in A2780/CP cells (Figure 4C). These data demonstrate that SIRT2 plays a critical role in apoptosis caused by cisplatin-induced intracellular ROS accumulation in cisplatin-sensitive ovarian cancer cells.

Overexpression of SIRT2 enhances cisplatin-induced apoptosis in cisplatin-resistant ovarian cancer cells

To further understand the role of SIRT2 in cisplatin response of cisplatin-resistant ovarian cancer cells, we examined the effect of SIRT2 overexpression in response to cisplatin in A2780/CP cells. Interestingly, overexpression of SIRT2 promoted apoptotic cell death of A2780/CP cells treated with cisplatin (Figure 5A). However, western blot analysis showed that the significant change in the protein level of FoxO3 was not observed (Figure 5A). Consistent with these results, knockdown of FoxO3 did not affect cisplatin-induced apoptotic cell death in A2780/CP cells (Figure 5B). These findings suggest that SIRT2 could contribute to cisplatin sensitivity of cisplatin-resistant ovarian cancer cells through another molecular mechanism independent of FoxO3 transcription factor.

Figures



(A)

Cell line	Time (h)	IC50 mean±SEM (μM)
A2780/S	24	8.78±0.71
	48	0.79±0.02

Cell line	Time (h)	IC50 mean±SEM (μM)
A2780/CP	24	34.28±1.12
	48	14.95±0.63

Figure 1. A2780/S and A2780/CP cells exhibit different sensitivity to cisplatin.

(A) (1) A2780/S and (2) A2780/CP ovarian cancer cells were treated with the indicated concentrations of cisplatin for 24 h or 48 h. Cell viability was evaluated by MTT assay. The IC_{50} values to cisplatin of the two cell lines are displayed below in figure 1A.

(B) Ovarian cancer cells were treated with 2 μM cisplatin for 24h and 48 h. (1) Apoptotic cell death was measured by flow cytometry with Annexin V/PI staining. (2) Expression of apoptosis-related proteins was assessed by western blotting. All experiments were repeated in triplicate, and data are presented as mean ± SEM. **P*<0.05, control versus cisplatin treatment.

(C) Ovarian cancer cells were treated with various concentration (0 to 10 μM) of cisplatin for 48 h. Apoptotic cell death was analyzed by (1) Annexin V/PI staining and (2) western blotting. All experiments were repeated in triplicate, and data are presented as mean ± SEM. **P*<0.05, and ***P*<0.01, control versus cisplatin treatment.

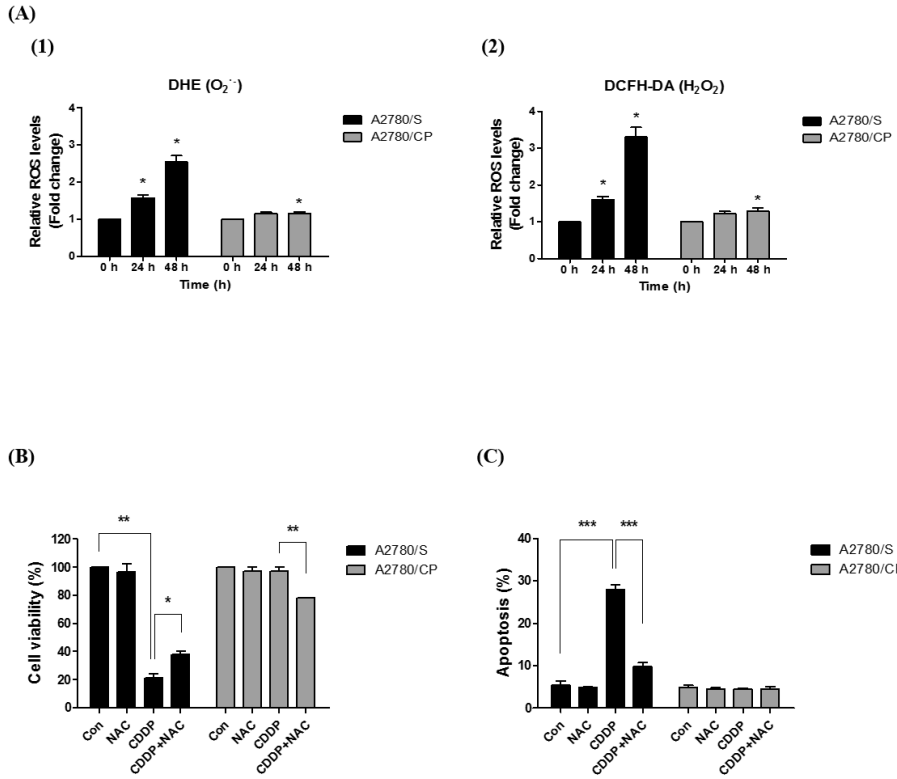

(A)
(1)
DHE ($O_2^{\cdot-}$)
Relative ROS levels (Fold change)
Time (h)
0 h
24 h
48 h
A2780/S
A2780/CP
(2)
DCFH-DA (H_2O_2)
Relative ROS levels (Fold change)
Time (h)
A2780/S
A2780/CP
(B)
Cell viability (%)
Con
NAC
CDDP
CDDP+NAC
A2780/S
A2780/CP
(C)
Apoptosis (%)
Con
NAC
CDDP
CDDP+NAC
A2780/S
A2780/CP

Figure 2. Cisplatin sensitivity correlates with ROS generation in ovarian cancer cells.

(A) Ovarian cancer cells were treated with 2 μM cisplatin for 24 to 48 h and intracellular ROS level was measured using (1) DHE (superoxide) and (2) DCF-DA (hydrogen peroxide) staining by flow cytometric analysis. All experiments were repeated in triplicate, and data are presented as mean ± SEM. **P*<0.05, control versus cisplatin treatment.

(B and C) A2780/S and A2780/CP ovarian cancer cells were treated with 2 μM cisplatin in the presence or absence of 5 mM NAC for 48 h. (B) After treatment, Cell viability was assessed by MTT assay. (C) And apoptotic cell death was analyzed by flow cytometric analysis after Annexin V/PI staining. All experiments were repeated in triplicate, and data are presented as mean ± SEM. **P*<0.05, ***P*<0.01, and ****P*<0.001, control versus cisplatin treatment or cisplatin versus cisplatin + NAC treatment.

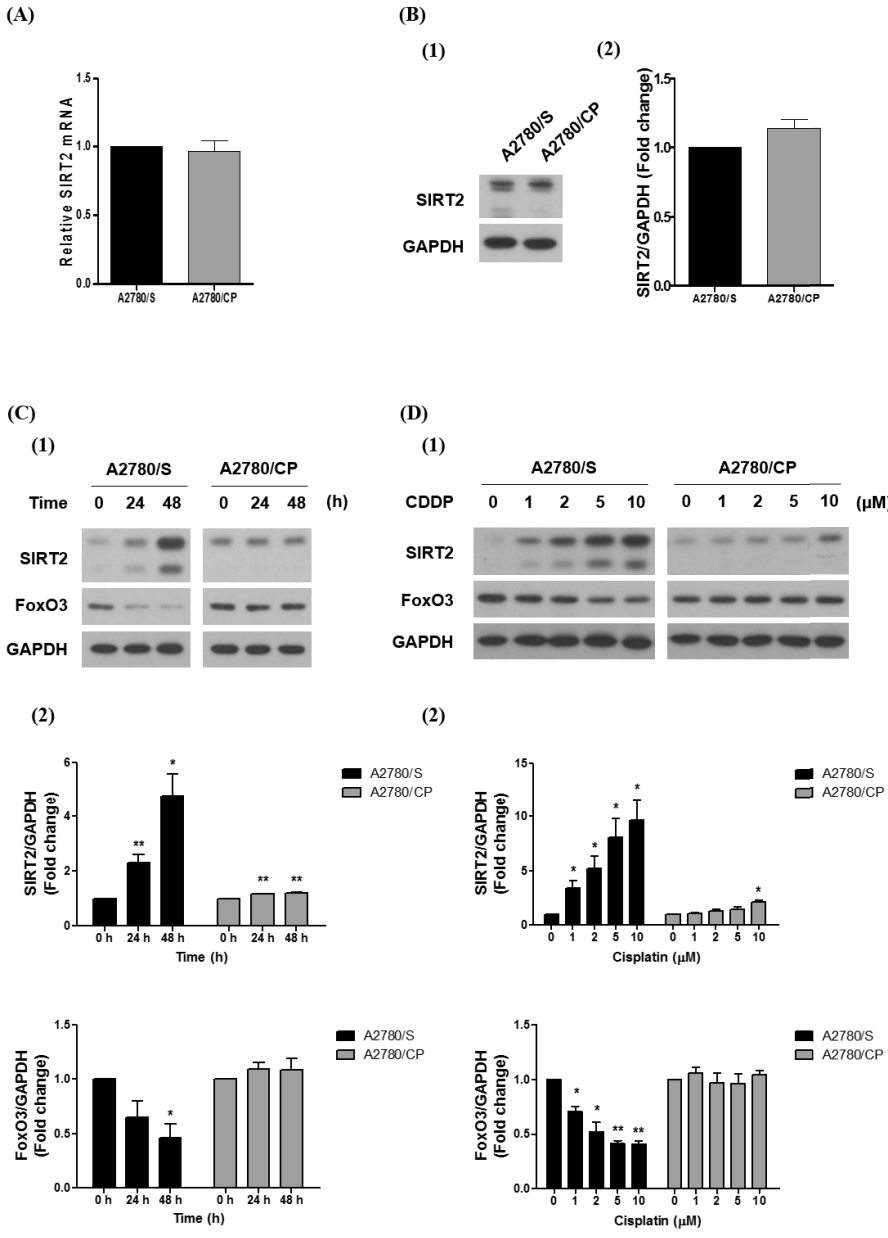

(A)
Relative SIRT2 mRNA
1.5
1.0
0.5
0.0
A2780/S
A2780/CP
(B)
(1)
A2780/S
A2780/CP
SIRT2
GAPDH
(2)
SIRT2/GAPDH (Fold change)
1.5
1.0
0.5
0.0
A2780/S
A2780/CP
(C)
(1)
A2780/S
A2780/CP
Time
0 24 48 0 24 48 (h)
SIRT2
FoxO3
GAPDH
(2)
SIRT2/GAPDH (Fold change)
A2780/S
A2780/CP
0 h 24 h 48 h 0 h 24 h 48 h
Time (h)
FoxO3/GAPDH (Fold change)
A2780/S
A2780/CP
0 h 24 h 48 h 0 h 24 h 48 h
Time (h)
(D)
(1)
A2780/S
A2780/CP
CDDP
0 1 2 5 10 0 1 2 5 10 (μM)
SIRT2
FoxO3
GAPDH
(2)
SIRT2/GAPDH (Fold change)
A2780/S
A2780/CP
0 1 2 5 10 0 1 2 5 10
Cisplatin (μM)
FoxO3/GAPDH (Fold change)
A2780/S
A2780/CP
0 1 2 5 10 0 1 2 5 10
Cisplatin (μM)

Figure 3. Cisplatin markedly increases the expression of SIRT2 in A2780/S cells compared to A2780/CP cells.

(A and B) Basal expression of SIRT2 in A2780/S and A2780/CP ovarian cancer cells was evaluated by (A) qRT-PCR and (B) western blotting. (A) The mRNA level of SIRT2 was normalized to GAPDH. (B) Basal protein expression of SIRT2 was quantified by densitometry and normalized to GAPDH. All experiments were repeated in triplicate, and data are presented as mean ± SEM.

(C) Ovarian cancer cells were treated with 2 μM cisplatin for 24 h or 48 h. Expression of SIRT2 and FoxO3 was analyzed by western blotting (1), and quantified by densitometry and normalized to GAPDH (2). Data are represented as mean ± SEM of three independent experiments. **P*<0.05, and ***P*<0.01, control versus cisplatin treatment.

(D) Ovarian cancer cells were treated with the indicated concentrations of cisplatin (0-10 μM) for 48 h. Expression of SIRT2 and FoxO3 was determined by western blotting (1), and quantified as above (2). Data are represented as mean ± SEM of three independent experiments. **P*<0.05, and ***P*<0.01, control versus cisplatin.

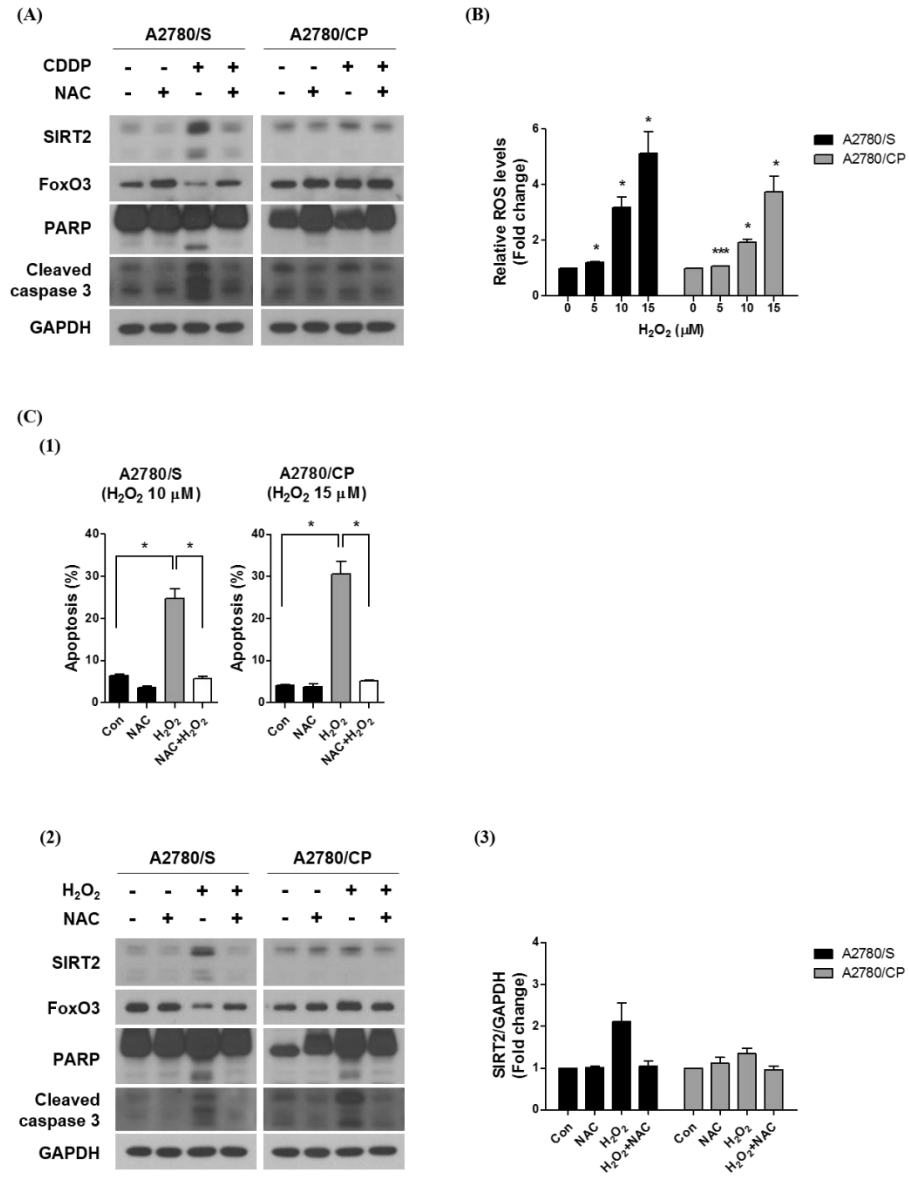

(A)
A2780/S
A2780/CP
CDDP
NAC
SIRT2
FoxO3
PARP
Cleaved caspase 3
GAPDH
(B)
Relative ROS levels (Fold change)
A2780/S
A2780/CP
H_2O_2 (μM)
(C)
(1)
A2780/S (H_2O_2 10 μM)
A2780/CP (H_2O_2 15 μM)
Apoptosis (%)
Con
NAC
H_2O_2
NAC+H_2O_2
(2)
A2780/S
A2780/CP
H_2O_2
NAC
SIRT2
FoxO3
PARP
Cleaved caspase 3
GAPDH
(3)
SIRT2/GAPDH (Fold change)
A2780/S
A2780/CP
Con
NAC
H_2O_2
H_2O_2+NAC

Figure 4. Cisplatin regulates the expression of SIRT2 and its downstream target through ROS overproduction in A2780/S cells, but not in A2780/CP cells.

(A) A2780/S and A2780/CP ovarian cancer cells were treated with 2 μM cisplatin with or without 5 mM NAC for 48 h. Expression of SIRT2 and FoxO3 was assessed by western blotting.

(B) Ovarian cancer cells were treated with the indicated concentrations of Hydrogen peroxide (0-15 μM) for 48 h. Intracellular ROS levels were measured using DCFH-DA (hydrogen peroxide) staining by flow cytometric analysis. Data are represented as mean ± SEM of three independent experiments. **P*<0.05, and ****P*<0.001, control versus H_2O_2 treatment.

(C) Ovarian cancer cells were pretreated with 5 mM NAC for 1 h, followed by hydrogen peroxide treatment (10 or 15 μM) for 48 h. (1) Apoptotic cell death was analyzed by flow cytometry after Annexin V/PI staining. (2) Expression of SIRT2, FoxO3, and apoptosis-related proteins was evaluated by immunoblot analysis. (3) Relative intensities were quantified by densitometry and normalized to GAPDH. All experiments were repeated in triplicate, and data are presented as mean ± SEM. **P*<0.05, control versus H_2O_2 treatment or H_2O_2 versus NAC + H_2O_2 treatment.

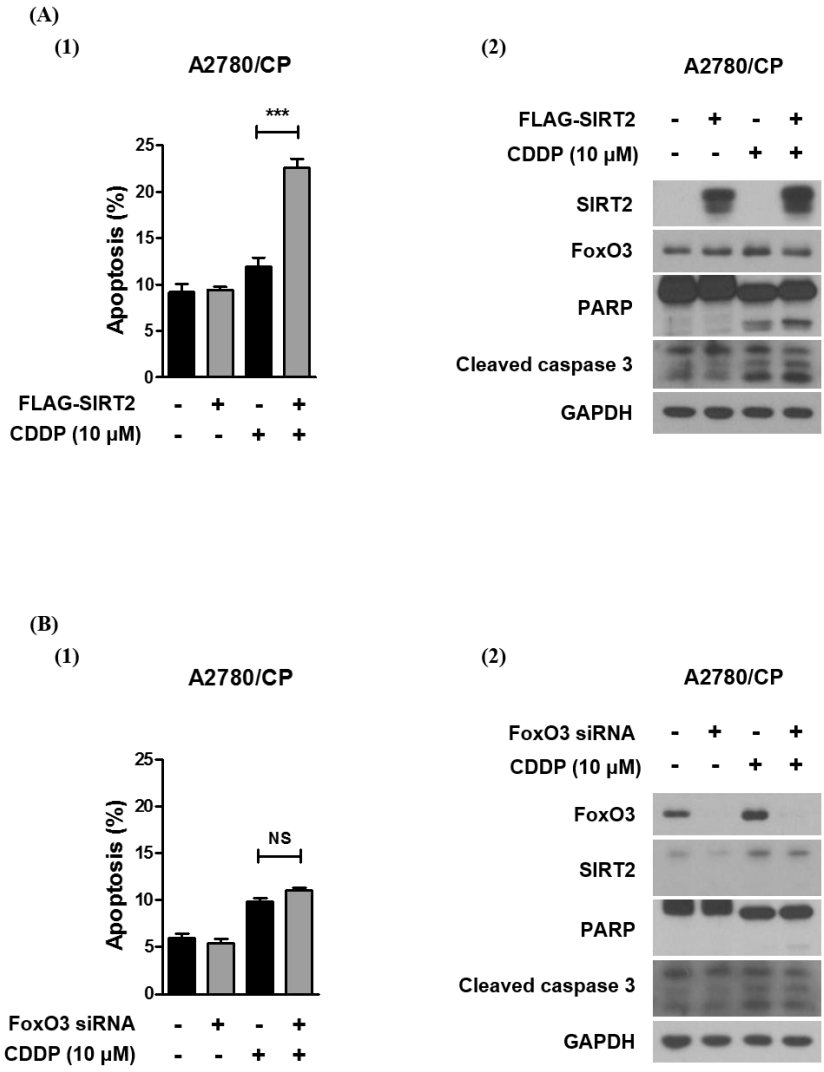

(A)
(1)
A2780/CP
***
Apoptosis (%)
25
20
15
10
5
0
FLAG-SIRT2 - + - +
CDDP (10 μM) - - + +
(2)
A2780/CP
FLAG-SIRT2 - + - +
CDDP (10 μM) - - + +
SIRT2
FoxO3
PARP
Cleaved caspase 3
GAPDH
(B)
(1)
A2780/CP
NS
Apoptosis (%)
25
20
15
10
5
0
FoxO3 siRNA - + - +
CDDP (10 μM) - - + +
(2)
A2780/CP
FoxO3 siRNA - + - +
CDDP (10 μM) - - + +
FoxO3
SIRT2
PARP
Cleaved caspase 3
GAPDH

Figure 5. Overexpression of SIRT2 enhances cisplatin-induced apoptosis in cisplatin-resistant ovarian cancer cells.

(A) A2780/CP ovarian cancer cells were transiently transfected with FLAG-SIRT2 plasmid vector or pcDNA3.1 control vector (5 μg) for 24 h. And the cells were then treated with 10 μM cisplatin for another 48 h. (1) Apoptotic cell death was detected using Annexin V/PI staining by flow cytometry. (2) Expression of SIRT2, FoxO3, and apoptosis-related proteins was assessed by western blotting. All experiments were repeated in triplicate, and data are presented as mean ± SEM. ***P<0.001, pcDNA3.1 control vector transfected cells with cisplatin treatment versus FLAG-SIRT2 vector transfected cells with cisplatin treatment.

(B) A2780/CP ovarian cancer cells were transfected with FoxO3-specific siRNA or control siRNA (100 nM) for 24 h. After the transfection, the cells were treated with 10 μM cisplatin for another 48 h. (1) Annexin V/PI staining-based flow cytometric analysis was used to measure apoptotic cell death. (2) Expression of SIRT2, FoxO3, and apoptosis-related proteins was determined by western blotting. All experiments were repeated in triplicate, and data are presented as mean ± SEM.

Discussion

Conventional therapy using platinum-based chemotherapeutic drugs is currently used in the treatment of ovarian cancer. However, the development of resistance to chemotherapy has become a major obstacle for the success of ovarian cancer therapy. Thus, understanding the molecular mechanisms of resistance to chemotherapy is essential to develop novel therapeutic strategies. In the present study, we showed for the first time that SIRT2 possesses a crucial role in the regulation of cisplatin sensitivity. Our results demonstrated that SIRT2 expression was markedly increased by cisplatin-induced ROS overproduction in cisplatin-sensitive ovarian cancer cells compared to cisplatin-resistant ovarian cancer cells. We also found that overexpression of SIRT2 in cisplatin-resistant ovarian cancer cells enhanced cisplatin-induced apoptosis.

Cisplatin is one of the most effective and commonly used platinum-based chemotherapeutic agents in the treatment of ovarian cancer [30]. The molecular mechanism of its cytotoxic action was established that nuclear DNA adducts generated by interaction of cisplatin with DNA induce excessive DNA damage response in cancer cells [31]. However, recent studies have reported that cisplatin contributes to cell death by inducing ROS overproduction in addition to nuclear DNA damage

response [5, 6, 32]. In our current study, cisplatin increased intracellular ROS levels and induced apoptotic cell death in cisplatin-sensitive ovarian cancer cells, whereas it had no significant effect in cisplatin-resistant ovarian cancer cells. These findings suggest that the oxidative stress response plays a prominent role in the cisplatin sensitivity of ovarian cancer cells.

SIRT2 is one of the seven sirtuin family members (class III HDACs), and its roles remain controversial in many cancers. For instance, some studies indicated that SIRT2 has a tumor suppressive role based on evidence that SIRT2 expression is downregulated in glioma, esophageal cancer, and gastric cancer relative to normal tissues, and overexpression of SIRT2 effectively induced cell death in breast cancer and non-small cell lung cancer [14, 33-35]. On the other hand, other studies observed that SIRT2 is a tumor promoter as it is upregulated and involved in the aberrant proliferation and invasion in acute myeloid leukemia, hepatocellular carcinoma, and pancreatic cancer [36-38]. Most recently, Du *et al* [39] first demonstrated that SIRT2 expression is significantly downregulated in ovarian carcinoma, and it may function as a tumor suppressor. In the present study, we explored the role of SIRT2 in response to cisplatin in ovarian cancer cells. We first observed that cisplatin induced upregulation of SIRT2 in cisplatin-sensitive ovarian cancer cells, but not in cisplatin-resistant ovarian cancer cells. This

response to cisplatin in cisplatin-sensitive ovarian cancer cells was reversed by co-treatment with a ROS scavenger, NAC, indicating that cisplatin-induced ROS generation contributed to SIRT2 upregulation and subsequent apoptotic cell death in cancer cells. We also observed that cisplatin downregulated FoxO3 protein levels only in cisplatin-sensitive ovarian cancer cells. This finding is consistent with the previous report demonstrating a mechanistic link between upregulation of SIRT2 and downregulation of FoxO3 in cancer cells [28]. FoxO3 transcription factor is known to regulate the expression of genes coding for antioxidant enzymes such as superoxide dismutase and catalase [25]. Thus, cisplatin treatment may lead to oxidative stress-mediated apoptotic cell death through inhibition of cellular antioxidant defense system by upregulation of SIRT2 in cisplatin-sensitive ovarian cancer cells.

We further investigated the function of SIRT2 in response to cisplatin in cisplatin-resistant ovarian cancer cells. Our results showed that oxidative stress induced by hydrogen peroxide had little impact on the levels of SIRT2 and its downstream target in cisplatin-resistant ovarian cancer cells. Nevertheless, we first demonstrated that overexpression of SIRT2 improved the response of cisplatin-resistant ovarian cancer cells to cisplatin treatment. The present findings support the previous study describing the role of SIRT2 as a tumor suppressor in ovarian cancer [39]. However, the exact molecular mechanism of apoptotic cell death

triggered by SIRT2 activation in cisplatin-resistant ovarian cancer cells is yet to be elucidated, so further studies are required to clarify this. The results from the current study suggest for the first time that cisplatin resistance in ovarian cancer can be overcome by targeting SIRT2 and its downstream pathway. In addition, we provide significant evidence to support the previous report indicating that the administration of class III HDAC inhibitors should be considered depending on the type of cancer [40].

In conclusion, we determined the role of SIRT2 in the response of ovarian cancer cells to cisplatin treatment. Our findings have demonstrated that SIRT2 has an important role in cisplatin sensitivity of ovarian cancer cells. We therefore suggest that SIRT2 could be a novel therapeutic target to overcome cisplatin resistance in ovarian cancer.

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국문 초록

여성 암 중 가장 예후가 좋지 않은 암으로 알려진 난소암의 재발 및 전이에 주요한 원인으로서 cisplatin과 같은 백금계열 항암제의 저항성에 대한 문제가 지속적으로 인식되고 있다. 따라서 환자의 치료 예후를 개선하기 위해 항암제 저항성의 메커니즘을 이해하는 것이 중요하다. Class III 히스톤 탈아세틸화 효소 중 하나인 SIRT2는 히스톤 외에도 여러 기질과 결합하여 산화 스트레스 반응을 포함한 세포 내 다양한 기능 조절에 관여한다. 그러나 최근 암 세포의 유형과 상황에 따라 SIRT2가 종양 억제자 또는 종양 촉진자의 두 가지 기능을 가진다는 것이 보고되었다. 본 연구에서는 난소암 세포주인 A2780/S와 cisplatin 내성 세포주인 A2780/CP를 이용하여 난소암 세포주에서 cisplatin에 의한 세포 사멸 기전에 SIRT2가 어떠한 역할을 하는 지 규명하고자 하였다. Cisplatin 처리 시, A2780/CP 세포주에 비해 A2780/S 세포주에서 활성산소종 (Reactive oxygen species, ROS)의 생성이 현저히 증가하였고, 이를 매개로 세포사멸이 유도되었다. 이 때, A2780/S 세포주에서 cisplatin에 의해 과잉 유도된 활성산소종이 SIRT2의 발현을 크게 증가시키는 것을 확인하였다. 산화 스

트레스에 의한 SIRT2 활성과 cisplatin 감수성의 관계를 확인하고자 난소암 세포주에 직접 과산화수소를 처리하였다. 그 결과, A2780/S 세포주와 달리 A2780/CP 세포주에서는 SIRT2 발현에 유의한 변화가 관찰되지 않았다. 그러나 SIRT2 유전자를 A2780/CP 세포주에 과발현시켰을 때, cisplatin에 의한 세포사멸이 효과적으로 증진되는 것을 확인하였다. 이러한 연구 결과는 SIRT2와 그의 하위 경로를 표적하는 것이 난소암의 cisplatin 저항성을 극복할 수 있는 새로운 치료 전략이 될 수 있음을 제시한다.