이학석사 학위논문

ROS-mediated SIRT2 activation by genistein induces pro-survival autophagy in cisplatin resistant ovarian cancer cells

항암제 내성 난소암 세포주에서 활성산소에 의해 유도된 자가포식의 세포사멸억제와 기전 연구

2015 년 8 월

서울대학교 대학원

의과대학 협동과정 종양생물학 전공

음 영 훈

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지도교수 송 용 상

이 논문을 이학석사 학위논문으로 제출함
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음 영 훈

음영훈의 이학석사 학위논문을 인준함
2015 년 5 월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

ROS-mediated SIRT2 activation by genistein induces pro-survival autophagy in cisplatin resistant ovarian cancer cells

by
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A Thesis submitted to the Interdisciplinary Graduate Program in Partial Fulfillment of the Requirements for the Degree of Master of Science in Cancer Biology at the Seoul National University

May 2015

Approved by Thesis Committee:

Professor _____Chairman

Professor _____Vice chairman

Professor _____

ABSTRACT

The most serious problem of ovarian cancer is chemoresistance. Autophagy is one of the phenotype contributing to chemoresistance and also has the bidirectional effect during chemotherapy. In this study, we demonstrated that ROS-induced autophagy by genistein has opposing functions regarding chemoresistance in ovarian cancer cell line A2780/CP (cisplatin resistant) and A2780/S (cisplatin sensitive). Genistein induced cell death through ROS generation. Interestingly, with ROS scavenging, the cell death rate was increased in A2780/CP in contrast to decrease in A2780/S. Genistein increased autophagy in both cell lines and the addition of autophagy inhibitor, 3MA, showed the same pattern of cell death rate as that of ROS scavenging. It means that the function of autophagy by genistein was different, pro-survival in A2780/CP while pro-death in A2780/S. SIRT2 promotes autophagy as a defense against the oxidative stress. AGK2, SIRT2 inhibitor, similarly inhibited cell viabilities in both cell lines. Moreover, AGK2 inhibited pro-survival autophagy in A2780/CP and overcame the cisplatin resistance with genistein. Taken together, understanding the autophagy could help to develop the therapeutic design to

overcome the chemoresistance in ovarian cancer.

Keywords: ROS, Autophagy, SIRT2, Chemoresistance

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LIST OF ABBREVIATIONS

ROS: reactive oxygen species

NAC: N-acetyl-L-cystein

LC3B: microtubule-associated proteins 1A/1B light chain 3B

3MA: 3-methyladenine

SIRT2: sirtuin-2

AO: acridine orange

AVO: acidic vesicular organelle

INTRODUCTION

Ovarian cancer is the most lethal in gynecologic malignancies. According to statistics of the United States, approximately 22,000 women are diagnosed and 14,000 will die each year. Ovarian cancer is the leading cause of death among women.[1, 2] Currently, the standard for treatment is aggressively cytoreductive surgery and chemotherapy. Although diagnosed women has good response to initial therapy, roughly 75% of patients will recur due to chemoresistance and 56% among relapsed women die.[3] Therefore, new therapeutic approach is needed to overcome chemoresistance.

ROS are secondary signaling molecules in diverse pathways. At high level of ROS, ROS induces cell death or growth arrest for oxidative damage to DNA, proteins and lipids. Cancer cells are vulnerable to ROS than normal cells because cancer cells have rapid proliferative activity and vigorous metabolism resulting in excessive ROS levels. Thus, additory ROS insults by ROS-generating drugs is therapeutic approach to enhance tumor cell death.[4] However, partial population of cancer cells which are intrinsic or acquired

resistance to ROS has enhanced antioxidant system. In addition, another resistant pathway is autophagy, which is a cellular defense against oxidative stress. When ROS harm proteins and organelles, if not removed damaged components, these components are toxic to cellular conditions and cause apoptosis, necrosis.[5] However, thereby degradation of harmful constituents during autophagic process, cancer cells escape ROS-induced toxic condition.

Autophagy is a stress-mediated catabolic process, which has diverse functions of maintaining house in starvation self-adjustment, degradation of dysfunctional proteins such as damaged and misfolded proteins.[6] During cancer chemotherapy as stress condition, autophagic process has four functional states such as cytotoxic, cytoprotective, cytostatic, nonprotective.[7] The cytotoxic and cytoprotective states are commonly main, can be opposing functions in stress conditions by treatment of chemoagents. The cytotoxic functions are not generally showed with conventional chemotherapeutics, which foster cancer cell deaths alone and/or interplay with apoptotic cell death as a precursor. The cytoprotective functions that protect cancer cells death from chemotherapy, which may be a chemoresistance mechanism. In previous studies, autophagy is activated by clinically

used anticancer drugs (e.g., cisplatin, crizotinib) in chemoresistant cell lines (ovarian cancer cell, lung cancer cell lines) and inhibition of autophagy increased sensitivity to kill tumor cells to anticancer drugs. [8, 9] As above, autophagy has bidirectional effects during chemotherapy, hence these phenomena reflect hardship for cancer treatment using chemoagents.

SIRT2 is a class Ⅲ histone deacetylase (HDAC) and required for regulation of cellular process by deacetylation of non-histone proteins.[10] SIRT2 is upregulated by oxidative stress and interfere with autophagy as stress defense molecule.[11, 12] NCBI Geo data has shown that SIRT2 expression is elevated in cisplatin resistant ovarian cancer cells (Profile GDS3754). It may SIRT2 encourage chemoresistance as stress defense by oxidative stress.

Our lab demonstrated that genistein-generated ROS induces autophagic cell death in ovarian cancer cells. Many standard chemotherapeutic drugs induces ROS generation and autophagy.[13] However, ROS and autophagy underlying mechanism is not fully understood. Especially, regulation of autophagy regarding chemoresistance are unclear. In this study, we investigated that the role of autophagy and regulatory molecules in

ovarian cancer cells.

MATERIALS AND METHODS

1. Reagents and Antibodies

Cisplatin, genistein, 3-methyladenine (3-MA), acridine orange (AO), 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT), N-Acetyl-L-cystein (NAC), 2', 7'-Dichlorofluorescin diacetate (DCF-DA) were all obtained from Sigma-Aldrich (St. Louis, MO). AGK2 was from Santa Cruz Biotechnology (Santa Cruz, CA). Hydrogen peroxide was purchased from Junsei Chemical (JUNSEI, Japan). Anti-SIRT2 (1:1000), anti-Beclin1 (1:1000), anti-ATG5 (1:1000), anti-LC3B (1:1000) were purchased from Cell Signaling Technology (Danvers, MA).

2. Cell lines and Cell culture

The human ovarian cancer cell line A2780/S (cisplatin sensitive) and A2780/CP (cisplatin resistant) were cultured in RPMI 1640 medium (WelGENE, Korea) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL, Gaithersberg, MD) and 1% penicilin-streptomycin (Invitrogen, Carlsbard, CA). These cells were incubated at 37℃ in an atmosphere of 5% CO_2.

3. MTT assay

The cells were seeded in 96-well plates in 100 ㎕ of medium per well and incubated for 24h. The medium was removed and the cells were treated with various concentration of genistein for 48h. MTT solution (2mg/ml) was added to each well for 50 ㎕ and incubated for 3h. The MTT solution was discarded, and 100 ㎕ dimethyl sulfoxide (DMSO) was added to solubilize formazan crystals formed. The absorbance of each well was measured by Multiskan spectrophotometer (Thermo Scientific, Hudson, NH) at 540nm.

4. Measuring intracellular ROS levels

Intracellular ROS levels were measured using ROS-sensitive dye 2', 7-dichlorofluorescein diacetate (DCF-DA). The control and treated-cells were harvested, and then incubated with 20 ㎕M DCF-DA for 30min in the dark. Cells were suspended in PBS and quantified intensity of fluorescence by FACS Calibur flow cytometry (BD Bioscience, CA).

5. Detection of acidic vesicular organelles

The cells were seeded in 6-well plates and incubated for 24h. The medium was discarded and treated with 50 ㎕M genistein for 48h in

the presence or absence of N-Acetyl-L-cystein (NAC). Cell were stained by 1 μg/ml acridine orange (AO) at 37℃ for 15min in the dark. The stained-cells were harvested and washed with PBS, then analyzed by flow cytometry. The cells incubated in same way in a coverglass bottom dish were analyzed by confocal microscopy.

6. Apoptosis assay

The cells were incubated in 6-well plates for 24h and treated with genistein on various conditions. Cells were washed with PBS and trypsinized, then collected in a FACS tube (BD Falcon, CA). These cells were centrifugated 1500 rpm for 5 min and re-suspended with 100 μl 1X Annexin V-binding buffer, then stained by using Annexin V-FITC Apoptosis Detection Kit I (BD Pharmigen). After staining, the intensity of fluorescence was measured by flow cytometry.

7. Western blotting

Cells were lysed in lysis buffer (1 M NaCl, 1 M Tris-HCl, 0.1 M EDTA, 0.1 M EGTA) containing sodium deoxycholate, 10% TritonX-100, 0.1 M phenyl methyl sulfonyl fluoride (PMSF), 0.1M Na_3VO_4, and EDTA free. The protein concentrations were

determined using BCA protein assay kit (Thermo Scientific, Hudson, NH). The equal amounts of protein were loaded in SDS-PAGE (polyacrylamide gel electrophoresis) and transferred to nitrocellulose membrane (Bio-rad, Hercules, CA) for 1h at 4℃. After blocking with 5% skim milk in TBS-T, the membranes were incubated with primary antibodies (1:1000) overnight at 4℃ and washed three times in 0.1% TBS-T. After incubation with secondary antibodies conjugated horseradish peroxidase (1:5000) for 2h at room temperature, protein bands were visualized using chemilminescence detection system.

8. Isolation of Human peripheral blood mononuclear cells from buffy coat

Buffy coats were diluted by PBS. Peripheral blood mononuclear cells (PBMC) were separated with Ficoll-Paque™ (GE Healthcare, NJ). The plasma was discarded and separated PBMC were washed by PBS twice. PBMC were cultured with RPMI 1640 at 37℃ in an atmosphere of 5% CO_2.

9. Statistical analysis

Data were represented as the mean ± SEM of three independent experiments. The values were analyzed by one-way ANOVA test. A p values of <0.05 were considered significance.

RESULTS

Genistein enhances cell death in ovarian cancer cell lines

Anticancer effects of genistein in human ovarian cancer cell lines A2780/S (cisplatin sensitive) and A2780/CP (cisplatin resistant) were determined. First, to verify resistance of chemoagent, increasing concentration of cisplatin was applied to both ovarian cancer cells. As expected, A2780/S was significantly decreased in cell viability than A2780/CP was decreased (Figure 1A). Cells were treated with genistein in dose-dependent manner (Figure 1B). Consistent with response to cisplatin, genistein treated cells had similar appearance as well. In treatment of genistein for 48h, the cell viability of A2780/CP is 63.41 % while A2780/S is 29.51 %. Confirmed apoptosis in genistein treated cells, genistein induced apoptosis in dose- and time-dependent manner. Slightly increased apoptosis of A2780/CP in contrast to steeply increased apoptosis of A2780/S were showed (Figure 1C). Genistein has no effect of cell viability in PBMC that genistein induces cytotoxicity in only cancer cells (Figure 1D). Therefore, A2780/CP was resistant to genistein for inhibition of proliferation and apoptosis than A2780/S.

ROS have different cell death in chemoresistance

Our previous studies showed that genistein as a pro-oxidant induced intracellular ROS accumulation in parallel with apoptosis, autophagic cell death in ovarian cancer cell lines. To verify ROS generation by genistein, 2', 7'-dichlorofluorescin diacetate (DCF-DA) was used to measure intracellular ROS levels. Genistein generated intracellular ROS in both cell lines, however A2780/CP was significantly increased on intensity of value of relative ROS accumulation than A2780/S in dose- and time-dependent manner. However, ROS were not increased in PBMC (Figure 2A, B and C). In accordance with cell death rate and ROS generation, we conducted scavenging of ROS in genistein treated cells to understand response to ROS. With pretreatment of N-acetyl-L-cysteine (NAC), A2780/CP was increased in cell death rate, which of decreased the cell viability and increased apoptosis. On the other hand, A2780/S was decreased in cell death rate (Figure 2D and E). Thus, scavenging of genistein-induced ROS affected cell death differentially in relation to the chemoresistance.

Genistein induces autophagy in both cell lines

We conducted whether generation of ROS by genistein induces autophagy to understand why the respective cell line has different response to ROS. To verify induction of autophagy by genistein, autophagy marker (Beclin1, ATG5, LC3B) and detection of acidic vesicular organelles (AVOs) stained by acridine orange were used. Expression level of Beclin1 and ATG5, conversion LC3B Ⅰ to Ⅱ was increased in time-dependent manner in both cell lines (Figure 3A). Genistein or hydrogen peroxide (exogenous ROS) treated cells were represented red colored vesicles in both cell lines (Figure 3B). And we performed quantification of AVOs by FACS analysis. When was treated genistein, AVOs in A2780/CP were significantly increased approximately two-fold than A2780/S (Figure 3C). As a result, ROS production by genistein induced autophagy in both cell lines and we subsequently determine the role of autophagy by ROS.

Inhibition of autophagy increases cell death in cisplatin resistant cells

To confirm ROS by genistein induces autophagy immediately, detection of AVOs was used to measure induction of autophagy in presence or absence with NAC. Scavenging of ROS inhibited

autophagy in both ovarian cancer cell lines (Figure 4A). Moreover, the addition of autophagy inhibitor, 3MA, inhibits autophagy (Figure 4B) and has the similar pattern as ROS scavenging, which of increased cell death in A2780/CP and decreased in A2780/S (Figure 4C and 4D). Therefore, ROS-induced autophagy has different appearance regarding chemoresistance and cisplatin resistant cells were sensitized by autophagy inhibition. In other words, autophagy was bidirectional for anticancer effects on chemosensitive in return for chemoresistance in ovarian cancer cell lines.

ROS-induced autophagy is regulated by SIRT2

We investigated whether SIRT2 is regulated by ROS and induces autophagy. First, we determined SIRT2 expression after treatment of genistein as ROS inducer. Genistein increased SIRT2 expression as well as hydrogen peroxide and ROS scavenging inhibited forced SIRT2 expression by genistein that ROS by genistein upregulated SIRT2 expression (Figure 5A). Depending on elevation of SIRT2 expression, we verified autophagy induction by SIRT2 using AGK2, SIRT2 inhibitor, impeding SIRT2 enzymatic activity. Inhibition of SIRT2 suppressed autophagic flux, Beclin1, ATG5 were decreased as well as AVOs (Figure 5B and 5C). After treatment of 3MA,

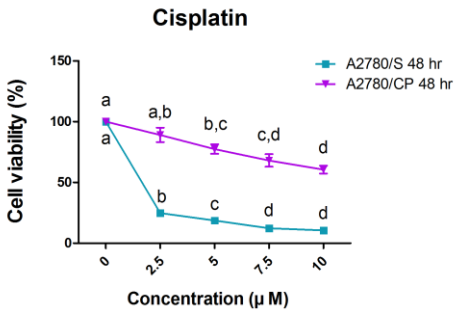
SIRT2 expression was not changed (Figure 5D). These data suggested that genistein induced autophagy by generation of ROS via SIRT2 activation.

Inhibition of SIRT2 potentiates genistein-induced cytotoxicity through autophagy inhibition in cisplatin resistant cells

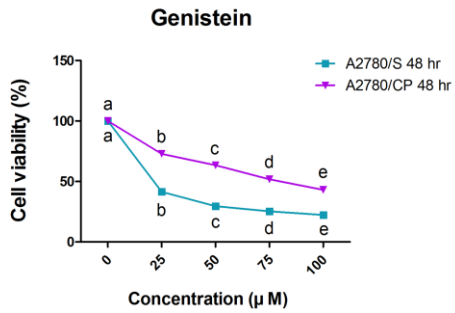
AGK2, anticancer-small molecule, inhibited cervical cancer cell proliferation.[14] As expected, AGK2 suppressed cell viabilities in both cell lines, highly interestingly, AGK2 very significantly inhibited cisplatin resistant cells like A2780/S. The effect of AGK2 has showed a similar falling curve in both cell lines (Figure 6A). Pretreatment of AGK2 and then added genistein inhibited cell viability than genistein alone (Figure 6B). Combination of genistein and AGK2 has synergistic effects for anticancer therapy. Cells were exposed to non-constant ratio (1. 50:1, 2. 20:1, 3. 10:1, 4. 5:1). Combination index (CI) value is approximately 0.78 and 0.82, which means two combination (1, 3) have synergism for the cytotoxicity effect. While the other combination (2, 4) have additive effect (Figure 6C). Taken together, inhibition of SIRT2 potentiated genistein-induced growth inhibition through autophagy inhibition.

FIGURES

A



B



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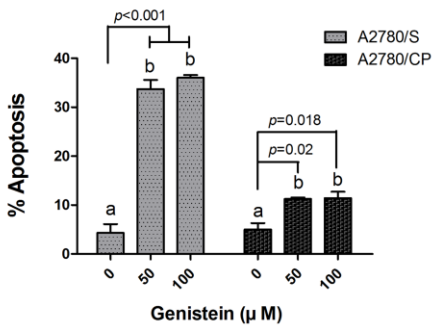
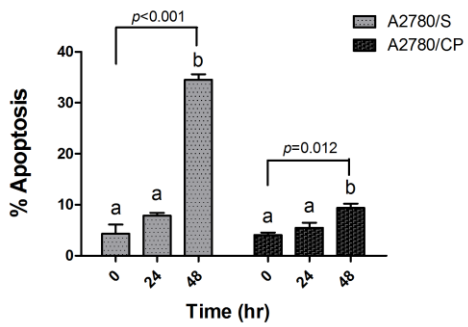




D

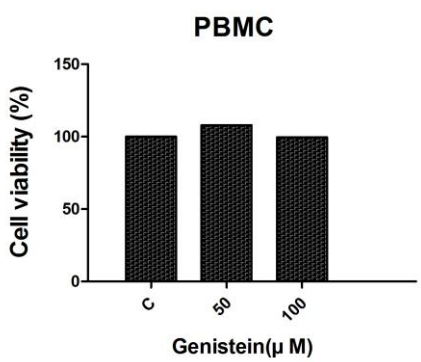


Figure 1. Anticancer effects of genistein in ovarian cancer cell lines

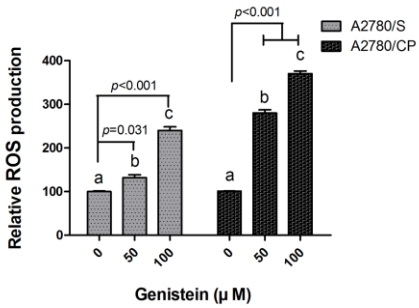
A. B. The human ovarian cancer cell lines A2780/S (cisplatin sensitive) and A2780/CP (cisplatin resistant) were treated with cisplatin (2.5, 5, 7.5 and 10 μM) and genistein (25, 50, 75 and 100 μM) for 48h. The cell viability was measured by the MTT assay. The values were divided significantly (p<0.05) into groups (a, b, c, d, e at each cell line).

C. Cells were treated with increasing doses of genistein for 48 hours. And cells were treated with genistein (50 μM) in the indicated times. Apoptosis was determined using FITC annexin V and propidium iodide (PI) by flow cytometry.

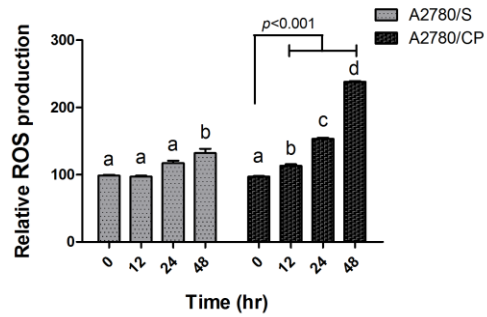
D. PBMC were treated with genistein (50, 100 μM) for 48h. The MTT assay was conducted for measurement of cell viability.

All results were shown as mean ± SEM of the three independent experiments.

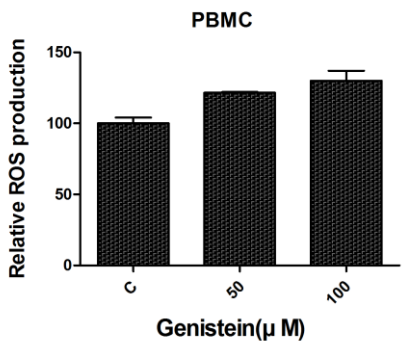
A


Relative ROS production
A2780/S
A2780/CP
p<0.001
p=0.031
p<0.001
a
b
c
a
b
c
0
100
200
300
400
0
50
100
0
50
100
Genistein (μ M)

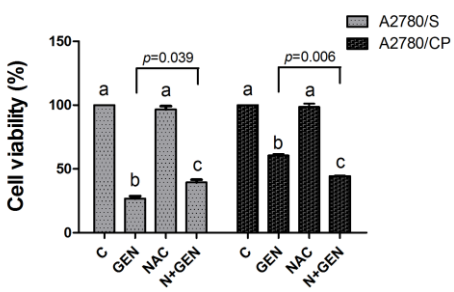
B


Relative ROS production
A2780/S
A2780/CP
p<0.001
a
a
a
b
a
b
c
d
0
100
200
300
0
12
24
48
0
12
24
48
Time (hr)

C


PBMC
Relative ROS production
0
50
100
150
C
50
100
Genistein(μ M)

D


Cell viability (%)
A2780/S
A2780/CP
p=0.039
p=0.006
a
b
a
c
a
b
a
c
0
50
100
150
C
GEN
NAC
N+GEN
C
GEN
NAC
N+GEN

E

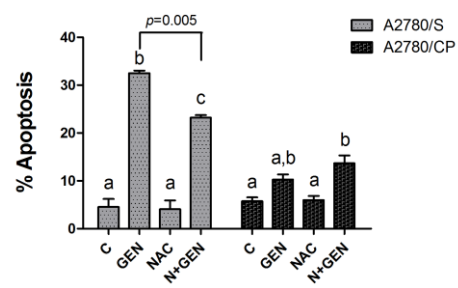

% Apoptosis
A2780/S
A2780/CP
p=0.005
a
b
a
c
a
a,b
a
b
0
10
20
30
40
C
GEN
NAC
N+GEN
C
GEN
NAC
N+GEN

Figure 2. Generation of ROS by genistein in ovarian cancer cell lines

A. Two ovarian cancer cells were treated with 50, 100 μM of genistein for 48h.

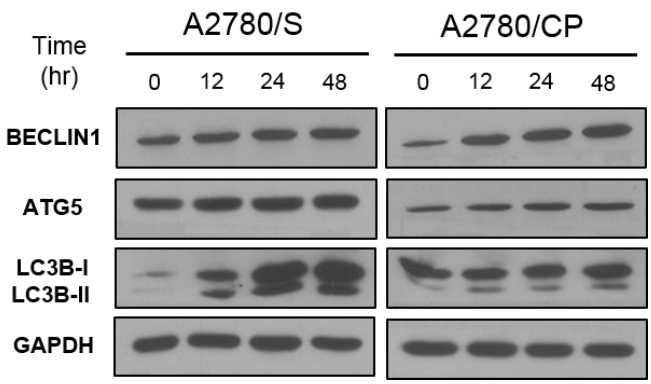
B. Cells were treated with genistein (50 μM) in time-dependent manner. ROS accumulation was assessed by 2', 7'-dichloro-fluorescin diacetate (DCF-DA) staining by flow cytometry.

C. PBMC were treated with genistein (50, 100 μM) and measured on ROS accumulation.

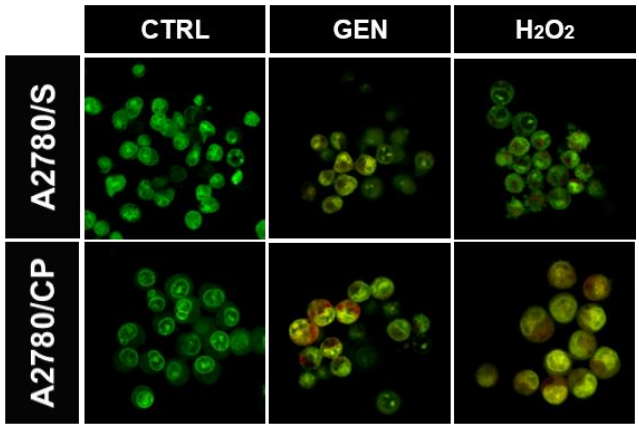
D. E. Cells were pretreated N-acetyl-L-cysteine (NAC, 10 mM) before treatment of genistein (50 μM), then cell viabilities were determined by MTT assay and apoptosis was detected by staining cells with FITC annexin V and PI by flow cytometry.

All results were shown as mean ± SEM of the three independent experiments.

A


Time (hr)
A2780/S
A2780/CP
0
12
24
48
0
12
24
48
BECLIN1
ATG5
LC3B-I
LC3B-II
GAPDH

B


CTRL
GEN
H2O2
A2780/S
A2780/CP

C

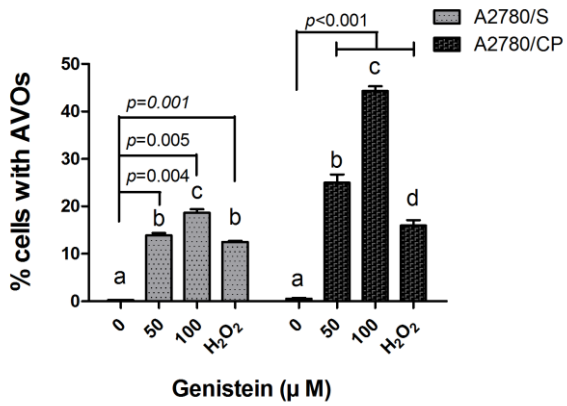

A2780/S
A2780/CP
p<0.001
p=0.001
p=0.005
p=0.004
% cells with AVOs
Genistein (μ M)
0
50
100
H2O2

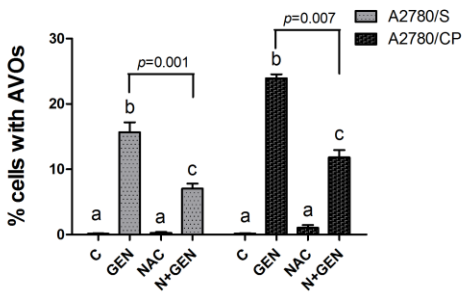
Figure 3. Induction of autophagy by genistein-mediated ROS

A. Cells were treated with genistein (50 μM) for the indicated times and induction of autophagy was measured using autophagy markers (Beclin1, Atg5, LC3B) by Western blotting.

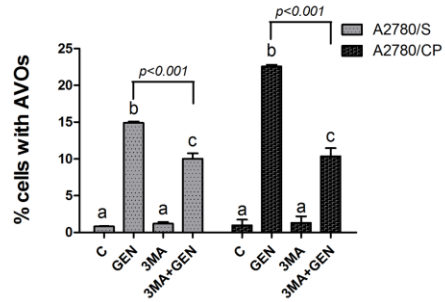
B.C. Cells were treated with genistein (50, 100 μM) and hydrogen peroxide (10 μM) as exogenous ROS, then autophagy was assessed by 1 μg/ml acridine orange (AO) staining for detection of AVOs by confocal microscopy and flow cytometry.

All results were shown as mean ± SEM of the three independent experiments.

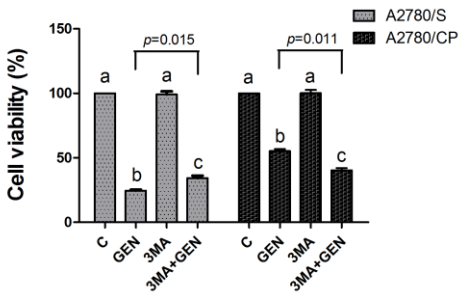
A


% cells with AVOs
A2780/S
A2780/CP
p=0.001
p=0.007
C
GEN
NAC
N+GEN

B


% cells with AVOs
A2780/S
A2780/CP
p<0.001
p<0.001
C
GEN
3MA
3MA+GEN

C


Cell viability (%)
A2780/S
A2780/CP
p=0.015
p=0.011
C
GEN
3MA
3MA+GEN

D

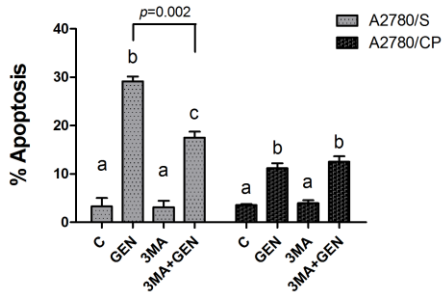

% Apoptosis
A2780/S
A2780/CP
p=0.002
C
GEN
3MA
3MA+GEN

Figure 4. Enhancement of cell death by inhibition of autophagy in cisplatin resistant cells

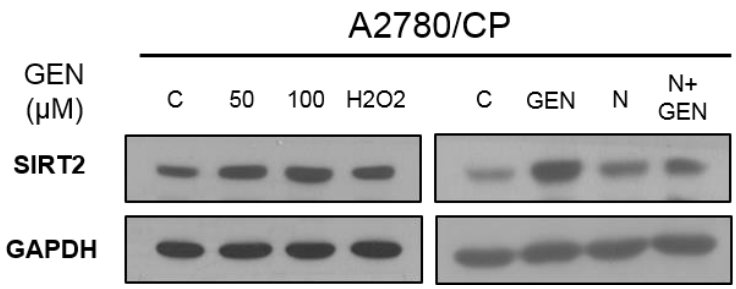
A. After treatment of genistein (50 μM) in the presence of absence of NAC (10 mM), autophagy was analyzed using AO staining by flow cytometry.

B. Cells were treated with 3MA or not before treatment of genistein and measured on induction of autophagy.

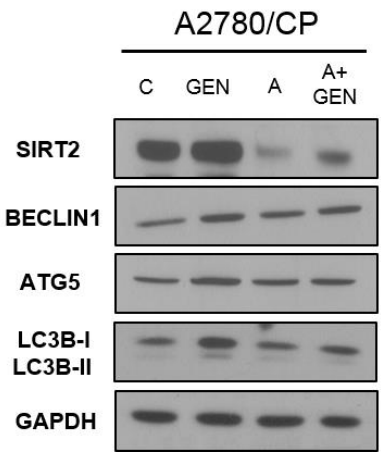
C. D. With pretreatment of 3MA, cell viabilities and apoptosis were measured to confirm cell death rate.

All results were shown as mean ± SEM of the three independent experiments.

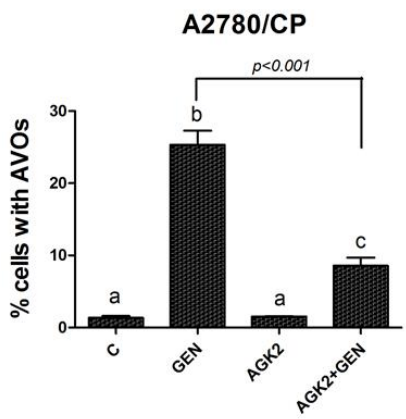
A


A2780/CP
GEN (μM)
C
50
100
H2O2
C
GEN
N
N+ GEN
SIRT2
GAPDH

B


A2780/CP
C
GEN
A
A+ GEN
SIRT2
BECLIN1
ATG5
LC3B-I
LC3B-II
GAPDH

C


A2780/CP
p<0.001
% cells with AVOs
30
20
10
0
a
b
a
c
C
GEN
AGK2
AGK2+GEN

D

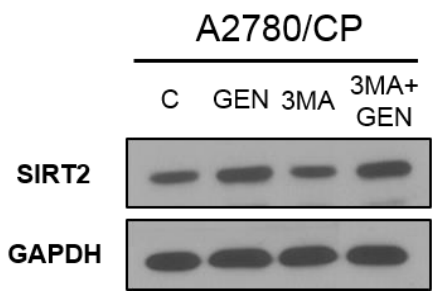

A2780/CP
C
GEN
3MA
3MA+ GEN
SIRT2
GAPDH

Figure 5. Modulation of SIRT2 by genistein-induced ROS

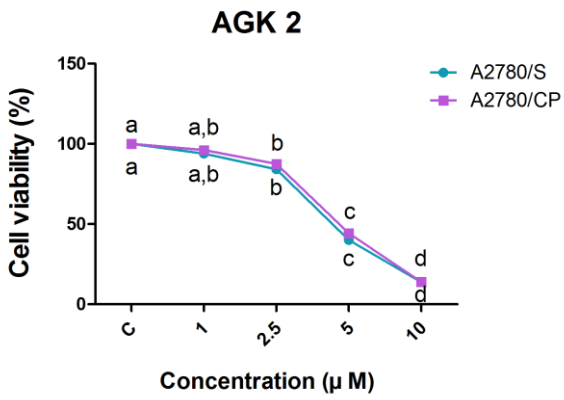
A. Genistein treated cells with NAC or not and hydrogen peroxide (10 μM) treated cells as positive control were lysed and loaded equivalent amount of lysates. SIRT2 (48 kDa) protein was evaluated by Western blot analysis with GAPDH as a loading control.

B.C. After pretreatment of AGK2 (1 μM), SIRT2 inhibitor, for 1 hour, genistein is treated for 48h. Expression level of SIRT2, autophagy marker were visualized by Western blotting and AVOs were determined by flow cytometry.

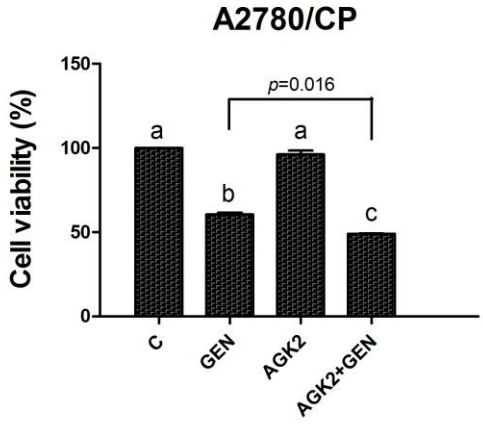
D. After treatment of 3MA, expression of SIRT2 was determined by Western blotting.

All results were shown as mean ± SEM of the three independent experiments.

A


AGK 2
A2780/S
A2780/CP
Cell viability (%)
150
100
50
0
a
a
a,b
a,b
b
b
c
c
d
d
C
1
2.5
5
10
Concentration (μ M)

B


A2780/CP
Cell viability (%)
p=0.016
150
100
50
0
a
b
a
c
C
GEN
AGK2
AGK2+GEN

C

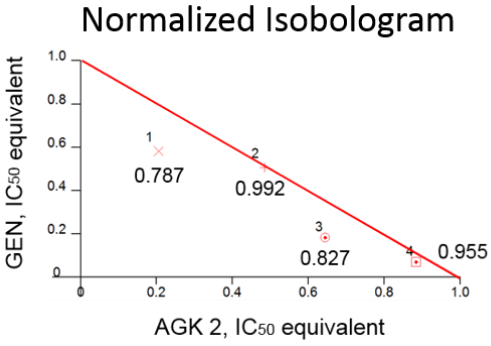

Normalized Isobologram
GEN, IC50 equivalent
1.0
0.8
0.6
0.4
0.2
0
1
0.787
2
0.992
3
0.827
4
0.955
0
0.2
0.4
0.6
0.8
1.0
AGK 2, IC50 equivalent

Figure 6. Potentiating genistein-induced cell death with AGK 2

A. Cells were treated with AGK 2 (1, 2.5, 5 and 10 μM) for 48h and cell viabilities were assessed by MTT assay. The values were divided significantly (p<0.05) into groups (a, b, c, d, e at each cell line).

B. Genistein treated cells in presence or absence with AGK2 (1 μM) were used to measure cell viability.

C. Synergism of genistein and AGK2 were analyzed using CalcuSyn Software. Genistein to AGK2 of combination ratios were 50:1, 20:1, 10:1 and 5:1 respectively. Combination Index (CI) was indicated <1 synergism, =1 additive, >1 antagonism.

All results were shown as mean ± SEM of the three independent experiments.

DISCUSSION

Our goal of study is to determine the role of autophagy as oxidative stress defense mechanisms and crucial autophagy regulatory molecule in relation to chemoresistance. The findings demonstrated that oxidative stress-mediated autophagy has protective function in cisplatin resistant cells in contrast to cytotoxic function in sensitive cells, consequently, inhibition of autophagy enhances cell death in cisplatin resistant cells. Moreover, inhibition of SIRT2, ROS-mediated autophagy regulator, potentiates genistein-induced growth inhibition and restore sensitivity of chemoresistance.

Cancer cells with excessive ROS levels are vulnerable to additional ROS insults by exogenous agents. However, a number of cancer cells can be adjusted under persistent intrinsic oxidative conditions, suggesting that various signaling pathway by ROS are associated with stress response and survival mechanisms.[15] He Hao *et al.*, reported that Physalin A, ROS-generating agents, induces autophagy as a cell survival mechanism against apoptosis in melanoma.[16] They have shown that autophagy has protective role to deal with oxidative stress in cancer treatment. Autophagy is a

potential chemoresistance mechanism associated with handling to ROS by anticancer drugs. Cisplatin, well-known ROS-generating drug, induces autophagy to contribute cisplatin resistance via ERK pathway.[17, 18] Consistent with our results, genistein as pro-oxidant isoflavones induces autophagy to protect cell death in cisplatin resistant cells, indicating that oxidative stress-mediated autophagy contributes to drug resistance for cell survival.

The role of autophagy during chemotherapy is controversial according to cell type and chemoagents. A number of recent studies reported that many chemoagents induces ROS generation and autophagy.[13] Many strategies to treat cancer take advantage of ROS and autophagy to induce cytotoxicity. According to previous studies, 2-ME induced both ROS production and autophagic cell death in glioblastoma and cervical cancer cells.[19] Compound K induces autophagic cell death via generation of ROS and activation of JNK in colon cancer cells.[20] In contrast, resveratrol induces autophagy and inhibition of autophagy potentiates the resveratrol-induced cytotoxicity.[21] Inhibition of autophagy by sulforaphane enhances sulforaphane-induced apoptosis.[22] Above all, autophagy represents dual role for cancer treatment depending on cancer type and chemotherapy drugs. Therefore, in doing chemotherapy, understanding the role of autophagy in ovarian

cancer is required. We found that genistein has showed different autophagy role in A2780/S and A2780/CP. Genistein-induced autophagy has dual role, cytotoxic in cisplatin sensitive cells and cytoprotective in cisplatin resistant cells acquired drug resistance, and then blockade of autophagy enhances cell death. Accumulated evidences associated with oxidative stress, it is important to how manage chemotherapy for enhancement of efficacy.

SIRT2 has double role as both tumor suppressive and tumorigenesis. Protein and RNA expression levels of SIRT2 is decreased in gliomas, melanomas and gastric cancer, and inhibits proliferation in glioma cells.[23] SIRT2 knock-out mice develop cancer by instability of chromosome and aneuploidy, thus indicating tumor suppressive role.[24] Although many studies have been shown that SIRT2 is cancer regression molecule, however, SIRT2 has also tumorigenic role. SIRT2 expression is increased in AML cells and inhibition of SIRT2 activity induces apoptosis.[25] AK1, a specific SIRT2 inhibitor, induces cell cycle arrest and inhibits migration in colon cancer cells.[26] Acetylated FoxO1 interacts ATG7 and induces tumor suppressive autophagy through SIRT2 inhibition.[27] In our findings, SIRT2 induces cytoprotective autophagy in response to oxidative stress. Inhibition of SIRT2 activity by AGK2

inhibits autophagic flux and increased cell death in cisplatin resistant cells. Also, SIRT2 inhibition dramatically decreased cell viability, implying that SIRT2 encourage chemoresistance except for induction of autophagy.

Although determining the regulation of autophagy is controversial, our findings provided evidence that autophagy contributes to chemoresistance in response to oxidative stress in cisplatin resistant ovarian cancer cells. These suggested that it could be a key to overcome chemoresistance by understanding autophagy regulatory mechanism.

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국문 초록

난소암은 높은 재발율과 항암제 내성으로 인해 부인종양 중 가장 치명적인 암종이다. 자가포식작용은 항암제 내성을 나타내는 표현형 중 하나로 항암화학요법에 있어 세포의 유형과 항암제의 종류에 따라 세포의 생존과 사멸의 양방향성 특성을 갖기도 한다. 본 연구에서는, cisplatin 내성 세포주인 A2780/CP와 감수성 세포주 A2780/S에서 genistein에 의해 발생한 활성산소가 일으키는 자가포식작용이 항암제 내성에 따라 상반된 기능을 갖고 있음을 연구하였다. Genistein에 의해 발생한 활성산소가 항암제 내성에 따라 반대되는 양상으로 세포사를 초래하였다. 활성산소 저해제를 처리하였을 때, A2780/CP 세포주는 세포 사멸이 증가한 반면 A2780/S 세포주에서는 세포 사멸이 감소하였다. Genistein은 두 세포주 모두에서 자가포식작용을 일으켰고 자가포식작용 저해제인 3MA를 처리하였을 때, 활성산소를 저해하였을 때와 같은 양상으로 세포사를 유도하였다. 이는 genistein에 의해 유도된 자가포식작용이 A2780/CP 세포주에서는 세포 생존을 유도한 반면 A2780/S 세포주에서는 세포사멸을 유도함을 의미한다. SIRT2는 산화 스트레스에 대한 방어 기작으로 자가포식작용을 유도한다. SIRT2의 저해제인 AGK2를 처리하였을 때 두 세포주에서 비슷한 양상으로 세포 생존능을 감소시켰다. 또한 AGK2 처리시 A2780/CP 세포주에서 genistein에 의해 유도되는 세포 생존의 자가포식작용을 저해하였고 이때 cisplatin 내성의 감수성을 민감하게

하였다. 따라서, 항암화학요법에 있어 자가포식작용의 조절을 이해하는 것이 난소암의 항암제 내성을 극복하는 데 도움이 될 수 있다고 제시한다.

주요어: 활성산소, 자가포식작용, SIRT2, 항암제 내성

학 번: 2013-23518