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Ph.D. Dissertation of Medicine

The inhibition of the polyamine pathway with DFMO as a novel therapeutic strategy for platinum sensitive and resistant epithelial ovarian cancer

백금 민감성 및 저항성 난소암에 대한 새로운 치료 전략으로서 DFMO를 이용한 폴리아민 경로의 억제

August, 2023

Graduate School of Medicine
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Obstetrics and Gynecology Major

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The inhibition of the polyamine pathway with DFMO as a novel therapeutic strategy for platinum sensitive and resistant epithelial ovarian cancer

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Abstract

There is a persistent need for more effective anti-ovarian-cancer drugs with lower toxicity. One anticancer strategy involves the comprehension of molecular functions and the targeting of polyamine metabolism. DFMO, an irreversible ODC inhibitor, has demonstrated anticancer activity by suppressing polyamine synthesis while maintaining a high safety profile. Previously, we discovered that DFMO induced apoptosis in an ovarian cancer cell line, SKOV-3, by increasing AP-1 and JNK phosphorylation expression, either alone or in combination with cisplatin. As a continuation of the previous study, this investigation aims to ascertain the effect of DFMO alone or in combination with conventional chemotherapy, cisplatin, on other ovarian cancer cell lines, including those resistant to cisplatin. In addition, inhibition of tumor growth by DFMO with or without cisplatin will be evaluated in a mouse model. As a result, ovarian cancer cells other than SKOV-3 were found to have a comparable effect in this study. In a mouse xenograft model, DFMO alone or combined with cisplatin suppressed tumorigenesis and altered the mRNA expression of polyamine signaling, angiogenesis, cancer stemness, and apoptosis markers. DFMO also caused apoptosis in cisplatin-resistant ovarian

cancer cells by inhibiting polyamine synthesis signaling. These findings indicate that DFMO treatment, either alone or in combination with cisplatin, could be a promising treatment for ovarian cancer.

Keyword : apoptosis; DFMO; ovarian cancer; polyamines; chemoresistance
Student Number : 2020-37467

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1. Introduction

1.1. Study Background

Ovarian cancer is a serious public health problem that poses a significant risk to public health. Since ovarian cancer is frequently identified at an advanced stage, it is the most lethal gynecological cancer. Patients diagnosed with advanced-stage ovarian cancer have an average 5-year survival rate of only 17% (1). Platinum-based chemotherapy (such as carboplatin or cisplatin) and paclitaxel are usually used as the first line of treatment for ovarian cancer. Although ovarian cancer initially responds favorably to these treatments, more than 80% of patients experience disease recurrence (2). Platinum-resistant recurrence, which is defined as disease recurrence occurring less than 6 months after platinum-based chemotherapy, is often associated with a poor prognosis (3). In order to improve therapeutic efficacy and patient survival, it is vital to comprehend the underlying molecular pathways and biological targets.

Cancer cells have a significantly different metabolism compared to healthy cells. It is becoming increasingly apparent that these distinctions may be the driving force behind cancer cells. This understanding leads to the development of novel strategies for

interfering with the metabolic processes of cancer cells (4). The three main polyamines, putrescine, spermidine, and spermine are required for a variety of biological activities, including cell proliferation and differentiation. The upregulation of polyamines in cancer has rekindled interest in targeting polyamine metabolism as a cancer therapy (5). Ornithine decarboxylase (ODC), a rate-limiting enzyme in polyamine production has emerged as a cancer therapy target (6). DFMO, an irreversible ODC inhibitor, has shown anticancer activity by suppressing the synthesis of polyamines (7). In prior research, we discovered that DFMO induced apoptosis in an ovarian cancer cell line, SKOV-3, by increasing the expression of AP-1 and JNK phosphorylation, either alone or in combination with cisplatin (8)

1.2. Purpose of Research

As a continuation of the previous investigation, the purpose of this study is to determine the effect of DFMO alone or in combination with conventional chemotherapy, cisplatin, on other ovarian cancer cell lines, including those resistant to cisplatin. Furthermore, tumor growth inhibition by DFMO with or without cisplatin will be evaluated in a mouse model. We intend to propose a

new anticancer therapy by revealing the effect of DFMO on ovarian cancer-specific metabolic pathways.

2. Materials and Methods

2.1. Antibodies (Abs) and drugs

Anti-ODC-1 (ab193338; mouse Ab; 1:2000) and anti-BCL-2 (ab692; mouse Ab; 1:1000) Abs were purchased from abCAM (abCAM, MA, USA). Anti-Bax (41162; rabbit Ab; 1:1000), C-C3 (9661; rabbit Ab; 1:1500), C-P (5625; rabbit Ab; 1:1000), β-actin (3700; mouse Ab; 1:4000) Abs were purchased from Cell signaling Technology (CST, MA, USA). Cisplatin was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO) for in vitro study and in 0.9% saline for animal studies.

2.2. Cell culture

OVCAR-3, SKOV3, CAOV-3 and A2780, ovarian cancer cells were obtained from the American Type Culture Collection (VA, USA). OVCAR-3, SKOV3 and CAOV3 cells were maintained in Roswell Park Memorial Institute-1640 culture (RPMI-1640; Gibco; Thermo Fisher Scientific, Inc., Carlsbad, CA, USA) and supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) 100 mg/ml streptomycin and 100

U/ml penicillin (P/S; Gibco; Thermo Fisher Scientific, Inc.). The cells were incubated at 37 ° C in an atmosphere containing 5% CO_2.

2.3. Generation of platinum resistance cell

Cisplatin resistant SKOV3 (Cis-R/SKOV3) cell lines were derived from original parental cell line. The methods about generation of Cis-R/SKOV3 has been described in our previous work. The Cis-R/SKOV3 cells were maintained in cisplatin contained (Cisplatin 20 μM) RPMI 1640 completed media and subcultured upon reaching 70 - 80% confluency. And the cells were incubated at 37 ° C in an atmosphere containing 5% CO_2.

2.4. Cell viability assay

Cell viability was determined by PrestoBlue Cell Viability Reagent (Invitrogen, Carlsbad, CA, USA). The cells were plated at $1x10^4$ cells/well in 96-well plates upon reaching 70 - 80% confluency. The day upon reaching 70 - 80% confluency, cisplatin 0, 1, 5, 10, 50 and100 μM was added to the wells and incubated for 48h. Each treatment was repeated in three independent tests. PrestoBlue Cell Viability Reagent was added to each well for 10% concentration and incubated for 1h. Absorbance was assessed with a Vmax Microplate Reader (Molecular Devices, LLC, Sunnyvale, CA,

USA) at a wavelength of 540nm. The cell viability of each group was calculated by GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). The experiment was repeated 3-5 times, and the data are expressed as the percent of control.

2.5. Cell proliferation assay

The cells were seeded into 96-well plates at a density of 1×10^4 cells/well μL and treated with cisplatin 0, 1, 5, 10, 50 and 100 μM for 48h. The cell counting kit-8 (CCK-8) assay was performed at 48h after cisplatin treatment. For CCK-8 assay, the serum-free medium was replaced at the time and 10 μL of CCK8 were added to each well. After incubation at 37° C and 5% CO2 for 1h, the OD value was measured at 450 nm. Each measurement was performed in quintuplicates.

2.6. Caspase-3 activity

The cells were seeded at $1x10^4$ cells/well in white-walled 96-well plates for 24h and treated with cisplatin 0, 10, 50 and 100 μM for 48h. The treated cells were further incubated with 100 μL of Caspase-Glo 3/7 Reagent (Cat# G8090, Promega, Madison, WI, USA) at room temperature for 30min. The luminescence of each

sample was measured in by the luminometer manufacturer (Molecular Devices, CA, USA). All data are expressed as the percent of control.

2.7. Annexin V and cell death assay

The Annexin V FITC/PI apoptosis detection Assay kit (Invitrogen, Carlsbad, CA, USA) was used to monitor cell death. The ovarian cancer cells were seeded into 6-well culture plates at a density of 2x105 cells/well. After exposure to cisplatin 0, 50 or 100 μM for 48h, the cells were trypsinized and resuspended in RPMI-1640 with 10% FBS at a concentration of $2x10^5$ cells/mL. The cells were incubated with Annexin V FITC and PI solution in the dark at room temperature for 30 min. Finally, the samples were assessed by flow cytometry (FACS Calibur, BD Bioscience, CA, USA).

2.8. Human tumor xenografts

BALB/c nude mice (8 weeks old) were purchased from ORIENT BIO Inc. (Seongnam, Korea). The animals were maintained in specific pathogen free conditions and in a controlled-light and humidity environment. The animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care

and Use of Laboratory Animals. The SKOV3 cells ($1x10^7$) were subcutaneously injected into the right flank of each mouse. Tumor volume (mm^3) was assessed every two days using a Vernier caliper and calculated as follows: [(short length)^2 x long length]/2. Treatment was initiated when the tumors reached a volume of $150mm^3$ (on the 4 week). The mice were fed with 2% DFMO water or 2mg/kg cisplatin (i.p., every 2 day) for 4 weeks. The mice were sacrificed and the experiment was terminated at the end of the 4 weeks. The tumors were isolated, weighed, imaged and performed real-time PCR.

2.9. Determining quantities of polyamines

The concentration of total polyamines determined using Total Polyamine Assay kit (Biovision Inc. CA. USA) according to the manufacturer's instructions. The SKOV3 or Cis-R/SKOV3 cells were harvested and homogenized in ice-cold Buffer for preparation of assay sample. The samples incubated for 30 min at 37° C, protected from light and read the fluorescence (Ex/Em = 535/587 nm) of all reaction, sample background and standard curve wells in endpoint mode.

2.10. Quantitative real-time PCR analysis

Total RNA from isolated tumor samples were extracted using TRIzol reagent (Invitrogen) as per the manufacturer's instructions. Equal quantities of DNA-free RNA were used in reverse transcription reactions for making cDNA using GoScript™ Reverse Transcriptase (Promega). The real-time PCR was carried out in a 25㎕ reaction volume using 3㎕ of a 1:10 cDNA dilution containing SYBR Green master mix (BioRad) and primers for PCR, Ornithine Decarboxylase 1 (ODC-1), spermine synthase (SMS), spermidine synthase (SRM), vascular endothelial growth factor (VEGF), Octamer-binding transcription factor 4 (OCT-4), Nanog homeobox (NANOG), B-cell lymphoma 2 (BCL-2), Bcl-2 associated X (BAX) (Table 1). All PCRs were done in a Qiagen Rotor Gene Q Real Time PCR system and fluorescence threshold values (Ct) were calculated. Relative mRNA levels were assessed by standardization to 18s rRNA. Results are expressed as a fold difference in gene expression.

2.11. Protein preparation and western blot analysis

The cells were treated with the indicated drugs for 48h, and the treated-cells were harvested and lysed in lysis buffer (50 mM

Tris-HCl, 1% NP40, 150 mM NaCl, 1 mM EDTA and 1 mM PMSF) for 30 min at 4 °C. Total cell extracts were separated using 12% SDS/PAGE gels and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% skim milk and incubated with primary antibodies diluted in blocking solution. The signals were visualized using the chemiluminescent substrate method and the Super Signal West Pico kit (Pierce, Thermo Fisher Scientific). β-actin was used as an internal control to normalize the loading materials.

2.12. Statistical analysis

Data are presented as the mean ± standard deviation (SD). The significance of the difference in mean values within and among multiple groups was examined with an ANOVA for repeated measures followed by a Duncan's post hoc test. Student's t-test was used to evaluate the significance of differences between two groups of experiments (SigmaStat; SPSS, Inc., Chicago, IL, USA). A P-value <0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. DFMO decreases cell viability and proliferation in human ovarian cancer cell lines

First, the cytotoxicity of DFMO to human ovarian cancer cell lines SKOV-3, CAOV-3, A2780 and OVCAR-3 was determined using the PrestoBlue assay. The cells were treated with various concentrations of DFMO (0, 1, 5, 10, 50, 100㎛M). Cell viability (Figure 1A-D) and proliferation (Figure 1E-H) were used to examine the cell growth effect of DFMO in human ovarian cancer cell lines for 48h using Presto Blue and CCK-8 assay, respectively. DFMO is decreased dose-dependently the cell viability and proliferation in human ovarian cancer cell lines (DFMO concentration, 0, 1, 5, 10, 50, 100μM). The A2780 cells were decreased in DFMO low concentration (DFMO 10, 50, 100μM) compared to SKOV-3, CAOV-3 and OVCAR-3. The results revealed that DFMO induced the inhibition of the cell viability and proliferation of the various human ovarian cancer cell lines in a dose -dependent manner.

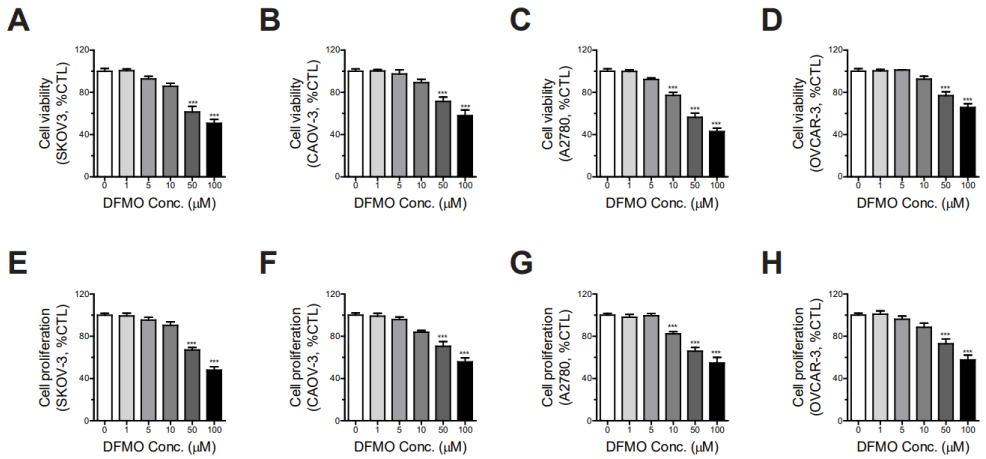

Fig 1. Effect of DFMO on cell toxicity and proliferation in ovarian cancer cell lines. The ovarian cancer cells were treated with DFMO 0 – 100 μM for 48h. (A–D) The cell viability was determined in DFMO–treated ovarian cancer cells by Prestoblue assay. (E–H) Cell proliferation was measured by CCK–8 assay in treated cells. The controls were treated with 0.1% DMSO. Data are expressed as mean ± SD. *P<0.05, **P<0.01, ***P<0.001 compared with the controls.

3.2. DFMO induce apoptotic cell death in human ovarian cancer cell lines

To assess the effect of DFMO on the activities of the caspase-3 activity in human ovarian cancer cells, we treated with different concentrations of DFMO for 48 hours in the cells. The caspase activity was evaluated with Caspase 3/7-Glo assay kit in DMEM completed medium. The results shown that after treatment with DFMO for 48 hours, the cellular caspase 3/7 activities increased in a dose dependent manner (Figure 2A-D). To determine the effects of DFMO on apoptotic cell death in human ovarian cancer cells, the annexin V-FITC/PI staining and flow cytometry were performed (Figure 2E, F). Fluorescence-activated cell sorting (FACS) analysis indicated that the DFMO induced apoptotic cell death. For example, DFMO treatment (100μM) induced a total of early (annexin V FITC+/PI-) and late (annexin V FITC+/PI+) apoptosis of > fold 4.0 in human ovarian cancer cells. And the A2780 and OVCAR-3 cells was increased the apoptotic cell death rate less than the SKOV-3 or CAOV-3 cells. Our results revealed that the DFMO activated apoptosis signaling and induced apoptotic cell death in human ovarian cancer cells.

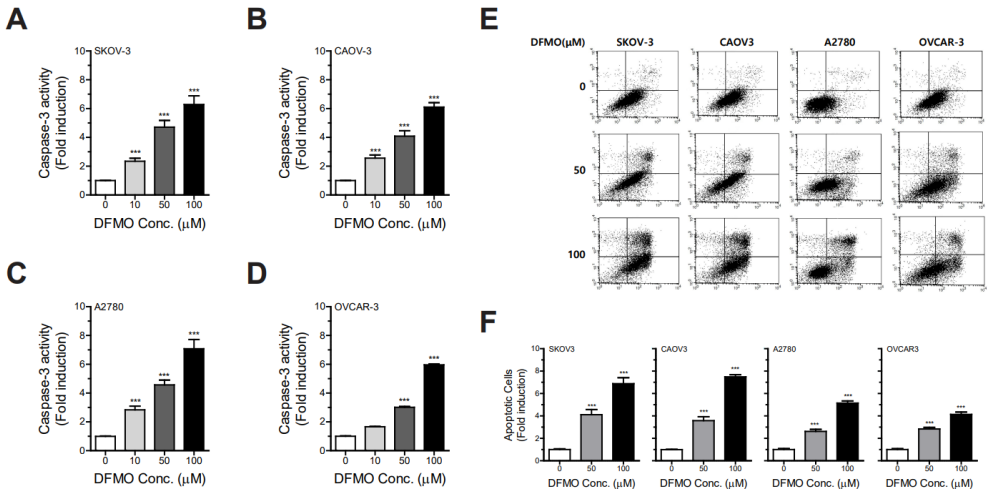

Fig 2. Induction of apoptotic cell death by DFMO in ovarian cancer cell lines. The cells were treated with DFMO 0, 10, 50 or 100 μM for 48h. (A–D) The Caspase–3 activity were determined in DFMO–treated ovarian cancer cells by Caspase–Glo 3/7 assay. (E) Annexin V/propidium iodide (PI) double–staining flow cytometry was performed to determine cell apoptosis, and (F) it was quantified to the levels of apoptotic cell death in the DFMO–treated ovarian cancer cells. Data are expressed as mean ± SD. *P<0.05, **P<0.01, ***P<0.001 compared with the controls

3.3. The combination effect of DFMO and cisplatin in ovarian cancer cells

To evaluate the effects of the combination of DFMO and cisplatin on cell death, we performed the Prestoblue and Caspase-3 activity assay in DFMO/cisplatin-treated cells. Based on the earlier results, we selected the SKOV-3 and A2780 in human ovarian cancer cells. The cell viability indicated that the combination of DFMO and cisplatin reduced cell viability than DFMO or cisplatin single treatment in ovarian cancer cells (Figure 3A, B). Also, in the caspase-3 assay, the similar tendency was observed at the combination of DFMO and cisplatin. When combined with DFMO and cisplatin, the caspase-3 activity was increased to fold induction >6.0, which it is similar to that of SKOV-3 and A2780 cells. So, our result demonstrated that the combined treatment of DFMO and cisplatin was significantly higher than that by either DFMO or cisplatin single treatment in human ovarian cancer cells. Furthermore, to evaluate the underlying mechanisms of the apoptotic effect of DFMO, cisplatin or combination treatment, the protein expression of BCL-2 (anti-apoptotic molecule), BAX (pro-apoptotic molecule), cleaved caspase-3 and cleaved PARP (an effector caspase) were evaluated by immunoblotting analysis. As revealed in Figure 3, following 48h of combined treatment with

DFMO and cisplatin, the protein expression levels of BCL-2, BAX, cleaved caspase-3 and cleaved PARP were revealed to be more marked when compared to those following treatment with DFMO or cisplatin single. The effect of combined treatment with DFMO and cisplatin in ovarian cancer cell may thus be due to promotion of apoptosis through the exogenous apoptotic pathway.

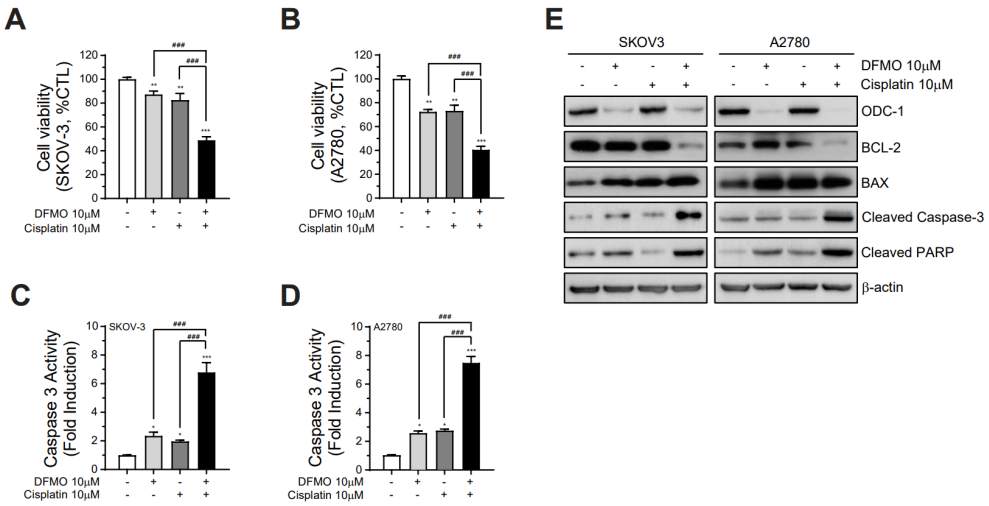

Fig 3. The combination effect of DFMO and cisplatin in ovarian cancer cells.

The combination of cisplatin and DFMO shows enhanced reduction in ovarian cancer. The ovarian cancer cells were treated with cisplatin 10 μM, DFMO 10 μM, or the combination of the two drugs using the same concentrations. The cell viability of cisplatin/DFMO-treated (A) SKOV-3 and (B) A2780 was detected by the Prestoblue assay. Caspase-3 activity of cisplatin/DFMO-treated (C) SKOV-3 and (D) A2780 cells was measured using the Caspase-Glo 3/7 Assay. (E) Western blot for the apoptotic proteins BCL-2, BAX, cleaved caspase-3 and cleaved PARP in cisplatin/DFMO-treated. Data are expressed as mean ± SD. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared with the controls. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ compared to different experimental groups.

3.4. The combination of DFMO with cisplatin reduces tumorigenesis in vivo

To confirm the in vitro results, we performed an in vivo study with human ovarian tumor bearing mice to verify whether significant effects were observed in vivo. We chose to examine the effects of DFMO, cisplatin or combined treatment on the tumor growth of SKOV-3 cell in vivo, and the SKOV-3 cells are highly tumorigenic in nude mice. The female BALB/c nude mice (8 weeks old) were used as an animal model. Each mouse was inoculated subcutaneously with mixture 100 μL of matrigel and human ovarian cancer SKOV-3 cells ($5X10^7$). The mice were divided into 4 groups (n = 10) 30 days after inoculation matched for tumor volume. Mice in groups were treated with vehicle control, cisplatin (2mg/kg), 2% DFMO (in water), or a combination of DFMO and cisplatin. Mice were sacrificed after treatment for 4 week and tumor tissues were collected. We monitored the tumor growth rate and body weight for each mouse group, and the mono-cisplatin and mono-DFMO treatments were found significantly to reduce the tumor growth rate in mice compared to control groups. In addition to, the combined treatment DFMO and cisplatin were decreased dramatically tumor growth rate compared to control groups (Figure 4A). But, all mouse group had no impact on the body weight (Figure 4B). Furthermore,

the combined treatment DFMO and cisplatin reduced the final tumor weight and volume (Figure 4C, D, F) in comparison to single treatment, but there were no significant differences in the final body weight of any mouse group (Figure 4E). These in vivo data strongly suggest that DFMO or combined DFMO and cisplatin treatment suppressed tumorigenesis in mouse tumor xenograft model.

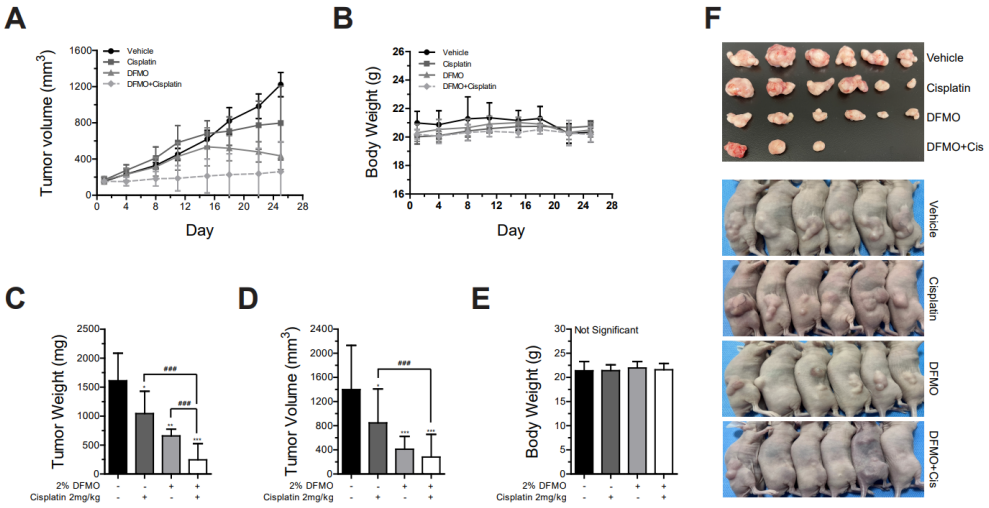

Fig 4. The effect of combination therapy of DFMO and cisplatin in mouse xenograft model. The combination of DFMO and cisplatin therapy leads to improved objective tumor suppression in mouse xenograft model. (A) The growth curve of tumors and (B) body weight was measured every 3 day using digital calipers. (C) Tumor weight, (D) tumor volume and (E) body weight was measured in isolated tumors from mice (F). Data are expressed as mean ± SD. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared with the controls. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ compared to different experimental groups.

3.5. DFMO alter polyamine signaling, angiogenesis, cancer stemness, and apoptosis gene expression levels in tumors

To assess whether DFMO and cisplatin affect the mRNA expression of polyamine signaling, angiogenesis, cancer stemness, and apoptosis markers, the total RNA was isolated from control, DFMO, cisplatin, DFMO/Cisplatin injected mouse tumor, and were analyzed by semi-quantitative real-time-PCR. The mRNA expression level of polyamine signaling genes were markedly decreased (ODC-1, ~70 and ~80%; SRM, ~60 and ~65%; SMS, ~50 and ~40%, respectively) in the DFMO- or DFMO/Cisplatin-injected groups (Figure 5A-C). And, the mRNA expression levels of Vegf, Oct-4 and Nanog were suppressed ~2-fold for DFMO-injected group and ~2.5-fold for DFMO/Cisplatin-injected group (Figure 5D-F). In addition, the mRNA expression levels of Bcl-2 was decreased by 50 and 80%, and the mRNA expression levels of Bax was increased by ~1.5 fold for DFMO-injected group and ~2.0 fold for DFMO/Cisplatin injected group, respectively. These results indicated the inhibition effects of DFMO or DFMO/Cisplatin on polyamine signaling and tumor progression.

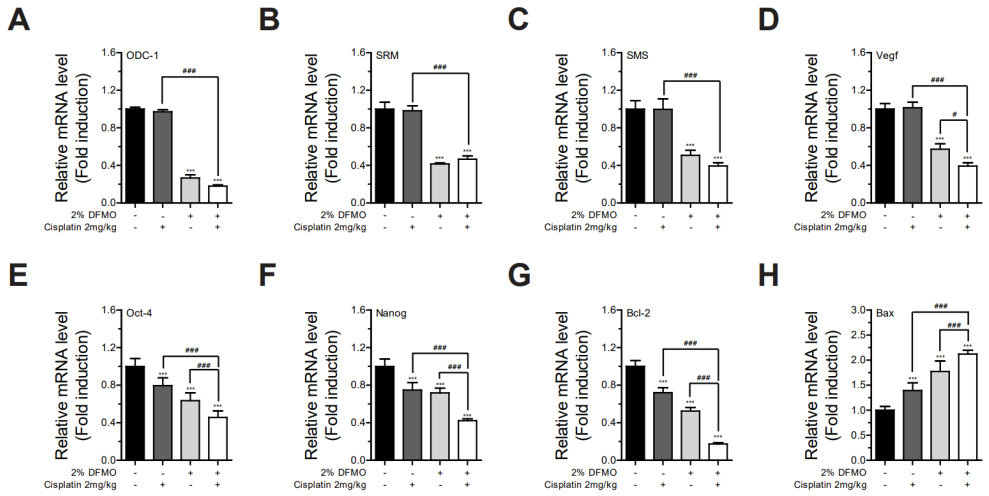

Fig 5. The expression of polyamine signaling and tumorigenesis related genes in mouse tumors. The mRNA expression of ODC-1, SRM, SMS, Vegf, Oct-4, Nanog, Bcl-2 and Bax were determined by semi-quantitative real-time PCR analysis in isolated the tumors. Data are expressed as mean ± SD. $^{*}P<0.05$, $^{*}P<0.01$, $^{*}P<0.001$ compared with the controls. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ compared to different experimental groups.

3.6. DFMO induced apoptosis in cisplatin-resistance ovarian cancer cell through inhibition of polyamine synthesis signaling

We examined whether DFMO could reverse cisplatin resistance in ovarian cancer cells. To explore the effect of DFMO on chemoresistance ovarian cancer in vitro, we established a cisplatin-resistance ovarian cancer cell line (Cis-R/SKOV-3) via extended exposure of cisplatin in normal SKOV-3 cells. First, we performed a cell viability assay and caspase-3 activity assay to confirm whether it have DFMO- or cisplatin-induced cell death in cisplatin resistance ovarian cancer cells. We were treated the cisplatin or DFMO (0, 10, 50 or 100 μM) in normal SKOV or Cis-R/SKOV-3 for 48h. In cisplatin-treated cells, the cell viability decreased and caspase-3 activity increased significantly in normal SKOV-3 cells, but no significant changes were observed in Cis-R/SKOV-3 cells (Figure 6A, B). However, when DFMO treated in normal SKOV-3 and Cis-R/SKOV-3 cells, the cell viability reduced and caspase-3 activity decreased dramatically in both normal SKOV-3 and Cis-R/SKOV-3 cells (Figure 6C, D). The results showed that the DFMO activated induction of cell death and apoptosis signaling in cisplatin resistance ovarian cancer cells. To determine whether the activation of polyamine pathway correlated with chemo-resistance

in ovarian cancer cell, we analyzed level of mRNA in polyamine signaling pathway using reverse-transcriptase PCR (RT-PCR), and determined the polyamine concentration using Total Polyamine Assay. The normal SKOV or Cis-R/SKOV-3 were treated with different concentrations of DFMO 0, 50 or 100 μM for 48h, and isolated total RNA and performed RT-PCR. The mRNA expression level of ODC-1 (Ornithine Decarboxylase 1), SRM (spermidine synthase) and SMS (spermidine synthase), polyamine pathway-related enzymes, increased in Cis-R/SKOV-3 cells compared to normal SKOV-3 cells (Figure 6E). Also, the treated cells were harvested and homogenized the cell lysate, and determine the polyamine concentration in cell lysate of normal SKOV or Cis-R/SKOV-3. Cis-R/SKOV-3 cells had a higher polyamine concentration than normal SKOV-3 cells, and the elevated polyamine concentration dose-dependently decreased in Cis-R/SKOV-3 cells treated with DFMO (Figure 6F).

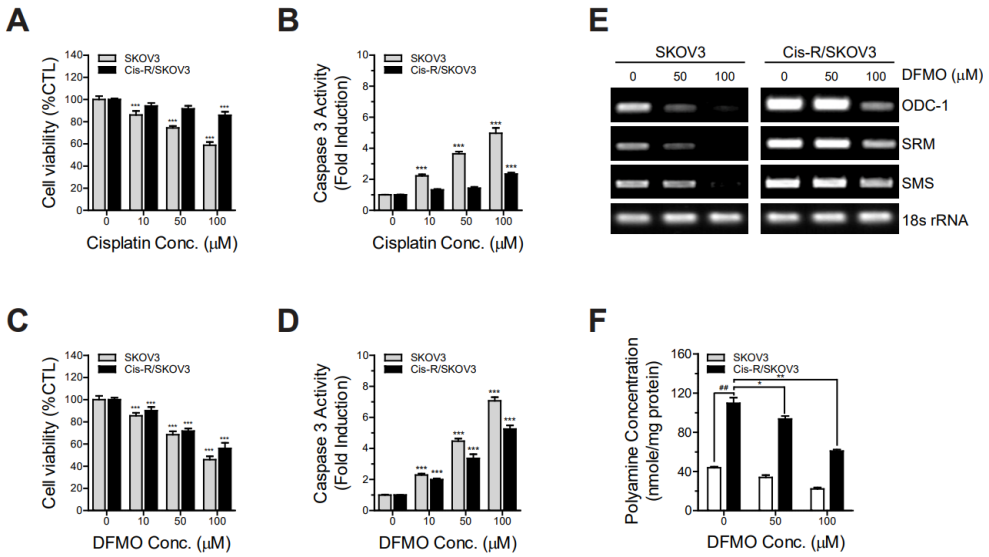

Fig 6. The effect of DFMO in cisplatin-resistance ovarian cancer cell. DFMO is induced the apoptotic cell death in cisplatin-resistant human ovarian cancer cell lines. The SKOV-3 and cisplatin resistance SKOV-3 (Cis-R/SKOV-3) cells were treated cisplatin or DFMO 0, 10, 50, 100 μM for 48h. The cell viability and Caspase 3 activity were determined in cisplatin-treated (A, B) or DFMO-treated (C, D) cells. (E) The polyamine signaling-related genes were measured by RT-PCR in DFMO-treated cells. (F) The level of polyamine was detected using Total Polyamine Assay kit in DFMO-treated cells. Data are expressed as mean ± SD. *P<0.05, **P<0.01, ***P<0.001 compared with the controls. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 compared to different experimental groups.

4. Discussion

In a prior study, ovarian cancer cells appeared to respond to DFMO alone or in combination with cisplatin in a dose-dependent manner by reducing cell viability and proliferation and inducing apoptosis (8). In this study, ovarian cancer cells other than SKOV-3 were found to have a comparable effect. We also evaluated that DFMO inhibited tumor growth in a mouse model with or without cisplatin. These in vivo results indicate that DFMO alone or in combination with cisplatin suppressed tumorigenesis and affected the mRNA expression of polyamine signaling, angiogenesis, cancer stemness, and apoptosis markers in a mouse xenograft model. Furthermore, we discovered that DFMO induced apoptosis in cisplatin resistant ovarian cancer cells by inhibiting polyamine synthesis signaling.

Cancer stem cells are a minor subpopulation of tumor cells, constituting less than 2-5% of the tumor mass (9, 10). Recent research has indicated that the fundamental cause of cancer recurrence and metastasis involves these cancer stem cells, that they may be linked to cancer progression through multiple mechanisms, including the activation of angiogenesis (11, 12, 13). Angiogenesis is a known factor in tumor development and

metastasis, and angiogenesis inhibitors are a treatment option for advanced and recurrent ovarian cancer (14, 15). Our research indicates that DFMO may be a plausible and promising option for inhibiting tumor growth and angiogenesis, as well as a targeted therapy for cancer stem cells.

Drug resistance remains a significant problem in the treatment of recurrent ovarian cancer. Drug resistance is induced after several consecutive lines of chemotherapy treatment, resulting in significant alterations at the cellular level (16, 17). Resistance in cancer cells to treatment are associated with variety of mechanisms for regulating cell signaling pathways (18). This resulted in the recent research employing strategies with a combinational approach that simultaneously target multiple signaling pathways, which may provide the most effective outcome for addressing the issue of drug resistance in cancer therapy (19). We discovered that the polyamine concentration is even higher in platinum resistant ovarian cell lines than platinum sensitive ovarian cell lines, and that DFMO dose-dependently decreased the elevated polyamine concentration in resistant cells. By inhibiting ODC and reducing intracellular polyamine levels, DFMO offers a novel therapeutic strategy for inducing remission in platinum-resistant ovarian cancer patients.

Many of the ovarian cancer treatments that are currently available have issues with cumulative and/or permanent toxicities. The development of more effective and less toxic treatments is required for long-term treatment planning. DFMO is an FDA-approved treatment for African sleeping sickness and female facial hirsutism, that has been used for over three decades at relatively high concentrations with a low profile of systemic toxicity (20, 21). Previous studies with DFMO have demonstrated that the agent is generally well tolerated, with a profile of adverse effects distinct from conventional cytotoxic chemotherapy (22). Numerous studies have identified hearing loss as the dose-limiting effect of DFMO (23, 24). With the exception of one report, DFMO-related hearing loss has been reported to be reversible in all cases (25). Compared to the greater potential for preventing and treating ovarian cancer, the ototoxicity observed with long-term, low-dose administration of DFMO may be of minor concern.

As there is an ongoing need to develop more effective anti-ovarian-cancer drugs with less toxicity, the effect of DFMO on the various subtypes of ovarian cancer cells is significant. To the best of our knowledge, DFMO has not been studied in animal models of ovarian cancer. It is also significant that our study has demonstrated that DFMO is effective in overcoming ovarian cancer

resistance, which is a challenge in the treatment of ovarian cancer. In the immediate future, we intend to expand our research to include in vivo studies of ovarian cancer resistance.

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Abstract

더 낮은 독성을 가진 더 효과적인 난소암 치료 약물에 대한 지속적인 요구가 있다. 한 가지 항암 전략은 분자 기능의 이해와 폴리아민 대사의 표적화를 포함한다. 비가역적인 ODC 억제제인 DFMO는 높은 안전성 프로필을 유지하면서 폴리아민 합성을 억제함으로써 항암 활성을 입증했다. 이전에 우리는 DFMO가 단독으로 또는 시스플라틴과 함께 AP-1 및 JNK 인산화 발현을 증가시킴으로써 난소암 세포주인 SKOV-3에서 세포사멸을 유도한다는 것을 발견했다. 이전 연구의 연속으로, 이 연구는 시스플라틴에 내성이 있는 세포를 포함하여 다른 난소암 세포주에 대한 DFMO 단독 또는 기존 화학 요법인 시스플라틴과의 병합 효과를 확인하는 것을 목표로 하고자 한다. 또한, DFMO 단독 또는 시스플라틴과의 병합에 의한 종양 성장 억제를 마우스 모델에서 평가하였다. 본 연구 결과, SKOV-3 이외의 난소암 세포에서도 비슷한 효과를 나타내는 것으로 나타났다. 마우스 이종이식 모델에서도 DFMO 단독 또는 시스플라틴과의 병합이 종양 형성을 억제하고 폴리아민 신호, 혈관신생, 암 줄기 세포 및 세포자살 마커의 mRNA 발현을 바꾸는 것을 확인했다. 또한 DFMO는 폴리아민 합성 신호를 억제하여 시스플라틴 내성 난소암 세포에서 세포사멸을 일으켰다. 이러한 결과는 DFMO 치료 단독 또는 시스플라틴과의 조합이 난소암에 대한 유망한 치료가 될 수 있음을 시사한다.