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The Role of PRMT5 in Chemoresistance of Ovarian Cancer Cells

Do Thi Yen

2022년 2월

석 사 학 위 논 문

The Role of PRMT5 in Chemoresistance of Ovarian Cancer Cells

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The Role of PRMT5 in Chemoresistance of Ovarian Cancer Cells

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Acknowledgment (감사의 말씀)

First of all, I would like to give my warmest thank to my Professor So-Jin Shin, Professor Ji Hae Seo, Professor Eunyoung Ha who patiently have guided and given me advice through all the stages of studying my thesis. I would like to thank professors in the Department of Obstetrics & Gynaecology, and the Department of Biochemistry, who taught and encouraged me during the master degree program. Furthermore, I would like to thank the university committees for supporting me in my defense and giving meaningful suggestions in my thesis.

In the master degree program, despite many difficulties, I learned a lot of important lessons and gained confidence over 2 years. With my strong background knowledge, I would continue working smarter and harder to become a better person in the future.

Finally, I would like to thank my parents who always encouraged and supported me through all the problems. Without you, none of these would have been indeed be possible. Thank you all for your helps.

2022년 02월

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Table of Contents

1. Introduction ······ 1

2. Materials and Methods ······ 4

3. Results ······ 10

4. Discussion ······ 24

5. Summary ······ 27

References ······ 28

Abstract ······ 35

국문초록 ······ 37

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List of Table

Tables 1. Details of PCR Primer Pair Sequences 9

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List of Figures

Figure 1. Establishment of chemoresistant ovarian cancer cell line ··· 16

Figure 2. The essential role of PRMT5 for chemoresistance in ovarian cancer cells ··· 17

Figure 3. The stimulation of cell migration by PRMT5 in chemoresistant ovarian cancer cells ··· 18

Figure 4. The activation of EMT by PRMT5 in chemoresistant ovarian cancer cells ··· 19

Figure 5. The increase of stem cell-like properties by PRMT5 in chemoresistant ovarian cancer cells ··· 20

Figure 6. The regulation KLF4 protein stability by PRMT5 in chemoresistant ovarian cancer cells ··· 21

Figure 7. The effect of KLF4 on EMT and stemness of chemoresistant ovarian cancer cells ··· 23

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1. Introduction

Epithelial ovarian cancer (EOC) is one of the most common cancers in women worldwide and ranks the first leading cause of gynaecological cancer-associated mortality (1,2). The standard of EOC treatment includes the tumor debulking surgery and platinum/taxane-based chemotherapy (2). Regimes containing single-agent or combination of platinum and/or taxane have been the first-line chemotherapeutic methods in both early or advanced stage of ovarian cancer (3). Although these chemotherapy treatments could be highly successful and remain efficacious over four decades, unfortunately, approximately 50-70% of chemotherapy-received patients experience chemoresistance-associated recurrence (4). Paclitaxel resistance also occurs in numerous patients, leading to poor progression (5). The molecular mechanisms of paclitaxel resistance in ovarian cancer cells still remain unknown (6,7). Overcoming chemoresistance of ovarian cancer is one of the main challenges that helps to increase overall and disease-free survival of patients. Hence, the development of alternative or combination therapies to overcome chemoresistance is needed.

Protein arginine methyltransferase 5 (PRMT5) is classified as the major type II arginine methyltransferase in PRMT family. PRMT5 catalyzes symmetric methylation of the arginine residues of target proteins in various types of cancer (8). Recent studies have found that PRMT5 functions as an oncogene that promotes proliferation, differentiation, migration and stem-cell like properties of cancer cells (9). For example, PRMT5 which serves as the binding protein partner of sterol regulatory element binding protein 1a (SREBP1a) symmetrically dimethylates SREBP1a on R321, thereby accelerating the growth of lung,

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liver and breast cancer cells in vivo and in vitro (10). In breast cancer cells, PRMT5 accelerates tumor growth by suppressing programed cell death 4 (PDCD4). Elevated level of PRMT5 positively correlates with worse clinical outcome of breast cancer patients (11). In myeloproliferative neoplasm (MPN) pathogenesis, PRMT5 is overexpressed in primary MPN cells, and inhibition of PRMT5 potentially decreases MPN cell proliferation ex vivo. Inhibition of PRMT5 also increases apoptotic cells through downregulating the expression of E2F transcription factor 1 (E2F1) (12). In EOC, elevated expression of PRMT5 promotes tumor cell growth and is associated with poor disease prognosis (13). PRMT5 regulates cancer stem cell markers such as octamer-binding transcription factor 4 (Oct4), Krüppel-like factors 4 (KLF4) and c-Myc, rendering cancer cells to be sensitive to doxorubicin in breast cancer (14).

KLF4 is a key transcriptional regulator that regulates diverse cellular processes such as cell growth, migration or stemness (15). In osteosarcoma cancer cells, KLF4 expression is upregulated and closely associated with cancer stemness through activation of the p38 mitogen-activated protein kinase (MAPK) signaling pathway. Conversely, KLF4 knockdown reduces cancer stemness in vivo (16). In breast cancer cells, accumulated KLF4 leads to increase cell migration, cell invasion and cancer stem cell population (17). In similar, KLF4 plays an integral function as an oncogene promoting growth and migration of melanoma, bladder, pancreatic and esophageal cancer cells (18-21).

The relation between PRMT5 and KLF4 was indicated in several studies (22-24). In breast cancer cells, PRMT5 stabilizes the protein levels of the KLF4 (25). PRMT5 methylates triple arginine sites on KLF4 and prevents KLF4 from ubiquitin-mediated proteasomal

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degradation (26). PRMT5-mediated KLF4 methylation is required to license DNA resection and homologous recombination in cellular processes (27).

Recently, amongst PRMT5 inhibitors, GSK3326596 was identified as a potential anti-cancer drug (28). GSK3326595 inhibits tumor growth and activates p53 pathway by regulating murine douple minute 4 protein (MDM4) (29,30). In breast cancer, GSK3326595 inhibits AKT signaling pathway and its oncogenic function (31). In addition, the combination of GSK3326595 with immune checkpoint therapy limits growth of murine melanoma tumor (32,33). A clinical phase I/II study of GSK3326595 identified the safety and effects of PRMT5 in advanced solid tumors and non-Hodgkin lymphoma (34,35). In this study, I investigated the anti-tumor activity of GSK3326595 in chemoresistant ovarian cancer cells. GSK3326595 suppresses the proliferation, migration, epithelial-mesenchymal transition (EMT) and stem-cell like properties of chemoresistant cancer cells. These results suggest that GSK3326595 is a potent anti-tumor candidate for overcoming the chemoresistance of ovarian cancer.

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2. Materials and Methods

2.1. Cell culture and reagents:

Human ovarian cancer cell line OVCAR-3 (Ovcar3) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Paclitaxel-resistant ovarian cancer cells, Ovcar3ptx, were generated by exposing Ovcar3 cells to gradually increasing doses of paclitaxel, then were subcultured until resistance to 10 nM paclitaxel. Cells were cultured in culture media Roswell Park Memmorial Institue (RPMI 1640) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycine at 37 °C in an incubator with 5% CO_2.

Paclitaxel and GSK3326595 were purchased from Medchemexpress (Monmouth Junction, NJ, USA).

2.2. Cell proliferation assay:

Ovarian cancer cells were plated in 96-well plates (3 × 10^3 cells/well) and then treated with paclitaxel or GSK3326595 for 72 hr. Cell viability was evaluated using cell counting kit 8 (CCK-8, Dojindo Molecular Technologies, Kumamoto, Japan). Cells were incubated with CCK-8 reagent for 1 hr at 37 °C. The plates were read at 450 nm with TECAN microplate reader (TECAN, Mannedolf, Switzerland).

2.3. Fluorescence-activated cell sorting (FACS):

Cells were seeded in 6-well plates (1 × 10^5 cells/well) and then

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treated with paclitaxel or GSK3326595 for 72 hr. Cells were harvested and then stained with FITC annexin V apoptosis detection kit with 7-AAD (BioLegend, San Diego, CA, USA). The population of apoptotic cells was detected by flow cytometry BD FACSCanto Ⅱ (BD, Franklin, NJ, USA).

2.4. Western blotting:

Harvested cell pellets were lysed in radioimmunoprecipitation assay (RIPA) buffer (Thermo Fisher Scientific, Waltham, MA, USA) including protease inhibitor cocktail (PI) (Thermo Fisher Scientific) and phenylmethylsulfonyl fluoride (PMSF) (Thermo Fisher Scientific) and then centrifuged at 12000 rpm for 30 min at 4 °C.

Supernatants were quantified using bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific). Thirty μg protein in each sample was separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to nitrocellulose membranes (GE Healthcare Life Sciences, Piscataway, NJ, USA). After being blocked in 5% skim milk in TBS-T (10 mmol/L Tris-HCl, 50 mmol/L NaCl, and 0.25% Tween-20) for 1 hr at room temperature, membranes were incubated with primary antibodies overnight at 4 ℃. In the next day, membranes were incubated with the second antibodies for 2 hr. The protein signals were detected using LAS-3000 (Fujifilm, Tokyo, Japan).

2.5. Wound healing assay:

Cells were seeded in 6-well plates (5 x 10^5 cells/well). Cells were

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wounded with 1 mL pipette tips and treated with 500 µM of thymidine and GSK3326595 for 24 hr. The migrated cells were captured by a microscope.

2.6. Immunofluorescencent staining:

Cells were seeded in 24-well plate (1 x 10^4 cells/well). After that, cells were treated with GSK3326595 for 72 hr. Cells were fixed in 4% paraformaldehyd (PFA) for 30 min at room temperature. Permeabilization was carried out by 0.1% Triton X-100 and non-specific binding was blocked with 1% bovine serum albumin (BSA). Cells were stained using the primary antibody overnight at 4 °C. Then, samples were incubated with the secondary antibody for 1 hr. Nucleus of cells were stained by 1 µg/ml 4′,6′-diamidino-2-phenylindole (DAPI). Mounted samples were dried for 24 hr. Protein expressions were imaged by fluorescence microscope (Carl Zeiss, Oberkochen, Germany).

2.7. Tumorsphere formation:

Cells were resuspended in 24-well ultra-low attachment plates (1 × 10^4 cells/well) and treated with GSK3326595 in serum free B-27/neurobasal media (Thermo Fisher Scientific) including 20 ng/ml EGF, 10 ng/ml bFGF (R&D system, Minneapolis, MN, USA), 2 mM L-glutamine, 20 mM HEPES (Sigma Aldrich, Saint Louis, MO, USA), 2.5 µg/ml amphotericin B (Thermo Fisher Scientific) and 1% penicillin/streptomycin for 7 days. The number of spheres was calculated using a microscope.

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2.8. Cell migration assay:

Cells were resuspended with serum-free medium and seeded (1×10^4 cells/well) in the upper chamber of transwell with 8 μm chamber (Corning, Tewksbury, MA, USA). Complete medium was added into the lower portion. After 24 hr incubation, the upper chambers were fixed in 4% PFA for 30 min and then stained with 0.1% crystal violet for 30 min. Migrated cells were captured and calculated using a microscope.

2.9. Reverse transcription polymerase chain reaction (RT-PCR) & real-time PCR:

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized with M-MLV reverse transcriptase (Promega, Madison, WI, USA) using 2 μg of RNA. PCR amplification was performed using specific primer for targeted gene and Go Taq Flexi DNA Polymerase (Promega). Amplified PCR product was assessed by electrophoresis on 1.2% agarose gel and visualized by ethidium bromide (Sigma Aldrich).

For real-time PCR, cDNA was amplified with SYRBR green PCR master mix using a LightCycler 96 instrument (Roche, Basel, Switzerland).

2.10. Statistical analysis:

Data were generated with at least three independent experiments and were represented as means ± S.D. Data were analyzed by Student's

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t-test with $p < 0.05$ being considered as statistical significance.

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Table 1. Details of PCR Primer Pair Sequences

Name	Sequences of primers	℃	bp
KLF4	Forward : 5 '- GAAACCTTACCACTGTGACTG - 3' Reverse : 5' - CAGTCACAGACCCCATCTGTT - 3'	60	175
GAPDH	Forward : 5' - GGCCTCCAAGGAGTAAGACC - 3' Reverse : 5' - AGGGGTCTACATGGCAACTG - 3'	60	147

KLF4: Krüppel-like factor 4.

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

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3. Results

3.1. Establishment of chemoresistant ovarian cell line:

Paclitaxel-resistant ovcar3 cells (Ovcar3ptx) were generated by exposing paclitaxel with gradually increasing doses. The cytotoxic effect of paclitaxel on parental Ovcar3 and Ovcar3ptx was examined by cell proliferation assay. After 72 hr paclitaxel treatment at the indicated doses, paclitaxel showed significant inhibitory effects on non-resistant cells, not Ovcar3ptx cells (Figure 1A). The morphology of 2D cultured cells displayed cellular shrinkage, a further trait of apoptosis. Paclitaxel strongly induced cell shrinkage in Ovcar3, but not in Ovcar3ptx as shown in Figure 1B.

To further investigate whether paclitaxel could induce cell apoptosis, the population of apoptotic cells was determined by flow cytometry with annexin V/7-AAD staining 72 hr after paclitaxel treatment. As shown in Figure 1C, paclitaxel clearly increased the number of apoptotic cells in Ovcar3 compared with Ovcar3ptx. I also checked apoptotic marker proteins containing cleaved PARP and cleaved caspase-3. Paclitaxel induced an increase in apoptotic marker expressions in Ovcar3 compared with Ovcar3ptx 3 days after paclitaxel treatment as shown in Figure 1D. These results demonstrate that paclitaxel induces apoptosis in chemosensitive ovarian cancer cells compared with its chemoresistant cells.

3.2. The essential role of PRMT5 for chemoresistance in ovarian cancer cells:

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Previous study showed that the elevated expression of PRMT5 protein was correlated with poor survival of ovarian cancer patients (13). PRMT5 is well-known to enhance cell proliferation and tumorigenesis (22-24). However, the role of PRMT5 in chemoresistance is still unclear. As shown in Figure 2A, I observed that PRMT5 expression was upregulated in Ovcar3ptx compared with Ovcar3. Notably, either knockdown of PRMT5 or inhibition of PRMT5 activity using GSK3326595 restored the chemosensitivity of Ovcar3ptx to paclitaxel as shown in Figure 2B&C. These results suggest that PRMT5 is required for the survival of chemoresistant cancer cells against paclitaxel treatment.

To determine whether GSK3326595 or knockdown of PRMT5 could trigger apoptosis signalling pathway, western blot assay was performed to analyze apoptotic marker proteins. As shown in Figure 2D&E, both GSK3326595 and PRMT5 depletion elevated the cleavage of caspase-3 and PARP following exposure to paclitaxel. These results suggest that PRMT5 is essential for the acquirement of chemoresistance in ovarian cancer cells.

3.3. The stimulation of cell migration by PRMT5 in chemoresistant ovarian cancer cells:

Cancer cell migration and invasion are the initial steps of the tumor cells metastasis (36). As shown in Figure 3A, compared to sensitive Ovcar3 cells, resistant Ovcar3ptx cells increased cell migration. The percentage of wound closure rose to 40% in Ovcar3ptx compared to Ovcar3 cells. Previous studies showed that PRMT5 regulates cell migration and invasion in colorectal, breast and pancreatic cancer cells

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(37-39). To investigate whether PRMT5 stimulated cell migration and invasion of chemoresistant ovarian cancer cells, cell migration assay was performed. Knockdown of PRMT5 and GSK3326595 treatment decreased cellular migration of Ovcar3ptx (Figure 3B&C). The proportion of wound closure exhibited approximately 30-40% and 50-60% reduction, respectively, compared to control group. These results suggest that PRMT5 enhances migration and invasion of chemoresistant ovarian cancer cells.

3.4. The activation of EMT by PRMT5 in chemoresistant ovarian cancer cells:

EMT is the essential process for tumor dissemination and metastasis. Cancer cells become more motile and display invasive abilities through EMT (40,41). To further determine the role of PRMT5 in cancer cell metastasis and EMT, protein expressions of E-cadherin (E-cad), an epithelial marker, N-cadherin (N-cad) and vimentin, mesenchymal markers, were examined. As shown in Figure 4A, the expression of E-cad was downregulated in Ovcar3ptx compared with Ovcar3. However, N-cad and vimentin were upregulated in Ovcar3ptx. These results indicate that EMT phenotype is strongly associated with the chemoresistance in ovarian cancer cells.

Intriguingly, depletion of PRMT5 or PRMT5 inhibitor reversed EMT-related protein expressions in chemoresistant Ovcar3ptx (Figure 4B&C). For further confirmation, immunofluorescence staining was performed with E-cad, N-cad and vimentin antibodies. As shown in Figure 4D, elevated mesenchymal marker expression and reduced epithelial marker expression were observed in Ovcar3ptx compared to

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Ovcar3. In addition, mesenchymal marker expression were reduced in Ovcar3ptx after depleting PRMT5 and treating with PRMT5 inhibitor (Figure 4E&F). These results demonstrate PRMT5 plays a major role in promoting EMT in chemoresistant ovarian cancer cells.

3.5. The increase of stem cell-like properties by PRMT5 in chemoresistant ovarian cancer cells:

Cancer stem cells (CSCs) present a small subpopulation of cancer cells with an increase in tumor-initiating potential, self-renewal, differentiation and tumorigenicity (42). In addition, stem cell-like characteristics are known to contribute to chemoresistance of ovarian cancer cells (44). As shown in Figure 5A, the number of sphere increased in Ovcar3ptx compared with Ovcar3. Consistent with this result, the expression of stemness markers, including sox2, nanog and oct4, increased in Ovcar3ptx compared to Ovcar3 (Figure 5C). To investigate the function of PRMT5 on cancer stemness, sphere formation assay was carried out with PRMT5 inhibitor. As shown in Figure 5B, GSK3326595 treatment decreased the number of sphere formation. Consistently, PRMT5-knockdown and the inhibition of PRMT5 activity reduced expressions of stemness markers (Figure 5D&E). These results suggest that PRMT5 attributes to stemness in chemoresistant ovarian cancer cells.

3.6. The regulation KLF4 protein stability by PRMT5 in chemoresistant ovarian cancer cells:

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Previous studies revealed the key interaction of PRMT5 with other binding proteins such as p53, FOXP3, BCL6, NF-κB, E2F1 or KLF4 (9,22). Among of these proteins, KLF4 was significantly increased in Ovcar3ptx compared with Ovcar3 (Figure 6A). Meanwhile, the methylation of PRMT5 on triple arginine sites of KLF4 led to KLF4 protein stabilization from ubiquitin-mediated proteasomal degradation pathway (25,26). As shown in Figure 6B&C, knockdown of PRMT5 and PRMT5 inhibitor reduced KLF4 protein level in Ovcar3ptx. However, there were no significant changes in the mRNA level of KLF4 following the indicated doses of GSK3326595 exposure (Figure 6D).

To further verify whether PRMT5 regulates the protein stability of KLF4 in chemoresistant ovarian cancer cells, I treated cells with a proteasome inhibitor MG132. The results showed that MG132 reversed the decrease of KLF4 protein that was induced by GSK3326595 as shown in Figure 6E. For further confirmation, cycloheximide (CHX), an inhibitor of protein biosynthesis, was administrated into chemoresistant ovarian cancer cells, with or without GSK3326595. KLF4 protein level was strongly reduced by GSK3326595 treatment. However, the protein level of KLF4 was slightly decreased in the absence of GSK3326595 after CHX treatment (Figure 6F). These results suggest that PRMT5 enhances the protein stability of KLF4 by inhibiting its degradation.

3.7. The effect of KLF4 on EMT and stemness of chemoresistant ovarian cancer cells:

To confirm the regulation of PRMT5 on chemoresistance of ovarian cancer cells through stabilization of KLF4 protein level, I investigated the role of KLF4 in stimulation of EMT and cancer stemness. As

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shown in Figure 7A, the number of migrated cells was reduced approximately 50% in KLF4-depleted Ovcar3ptx cells. In addition, the number of spheres also decreased after knockdown of KLF4 in Ovcar3ptx cells (Fiugre 7B). Next, the role of KLF4 in drug sensitivity of Ovcar3ptx was examined. As shown in Figure 7C, depletion of KLF4 reversed drug sensitivity of chemoresistant ovarian cancer cells. Furthermore, depletion of KLF4 also activated the apoptosis pathway after paclitaxel exposure in chemoresistant ovarian cancer cells (Figure 7D). These results suggest that KLF4 modulates EMT signalling and stem cell-like properties in Ovcar3ptx. Likewise, PRMT5 contributes to chemoresistance of ovarian cancer cells via the accumulation of KLF4 protein in chemoresistant ovarian cancer cells.

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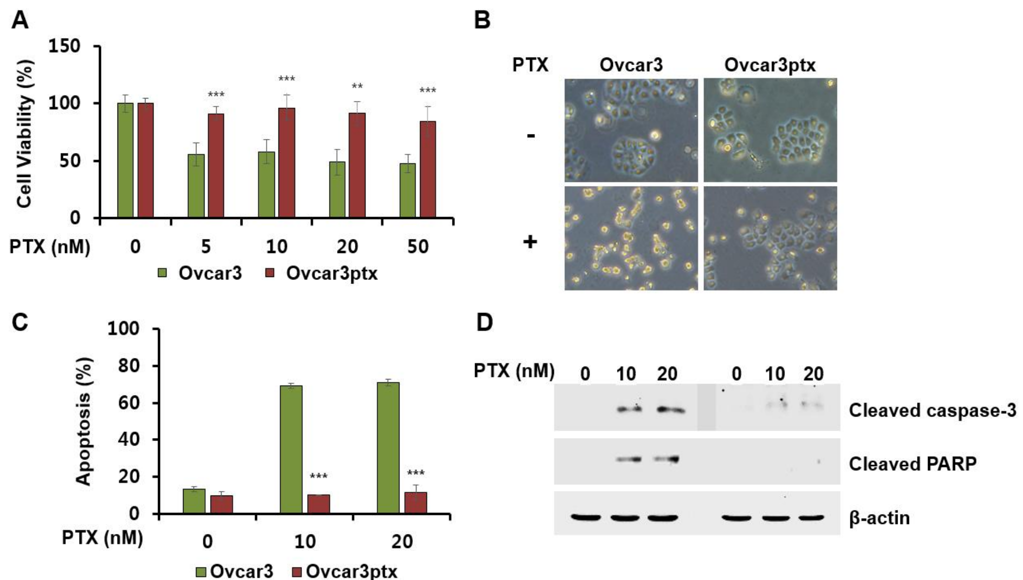

Figure 1. Establishment of chemoresistant ovarian cancer cell line. (A) PTX showed significant inhibitory effects on Ovcar3 compared with Ovcar3ptx. Cells were treated with PTX at the indicated doses for 3 days. Cell viability was measured by CCK-8 assay. (B) PTX strongly induced cell shrinkage in Ovcar3, but not in Ovcar3ptx. The morphology of 2D cultured cells were captured under a microscope 3 days after PTX exposure. (C) PTX clearly increased apoptotic cells in Ovcar3 as opposed to Ovcar3ptx. Apoptosis analysis was performed by flow cytometry with annexin V/7-AAD staining. (D) PTX increased protein levels of apoptosis markers in Ovcar3. Cells were treated with different concentrations of PTX for 3 days. β-actin was detected as a control. All values were presented as the mean ± S.D (n = 3). PTX: paclitaxel; Significant differences were indicated; ***, $p < 0.001$.

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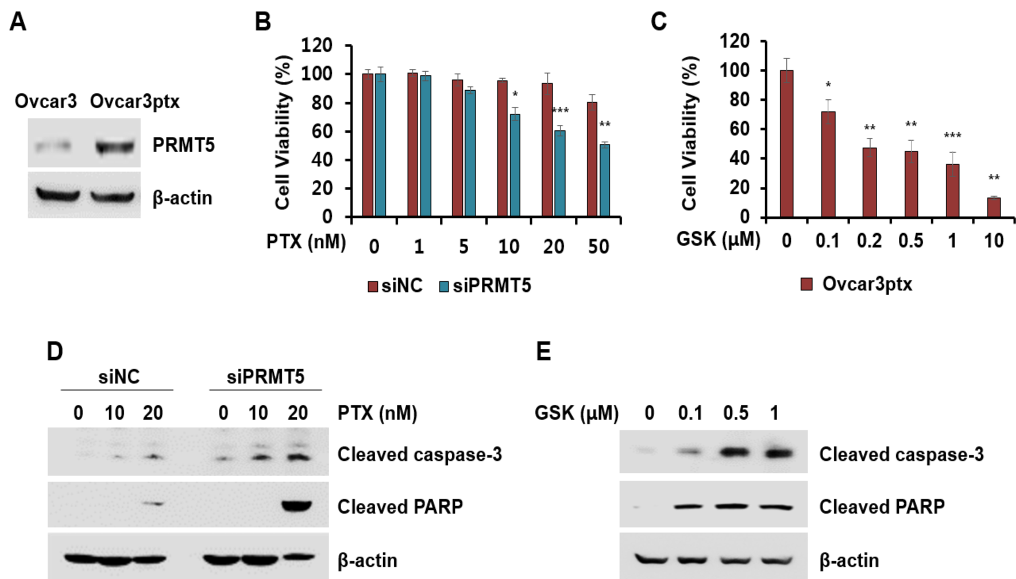

Figure 2. The essential role of PRMT5 for chemoresistance in ovarian cancer cells. (A) PRMT5 protein level was upregulated in Ovcar3ptx compared with Ovcar3. Cell lysates were subjected to western blotting analysis. (B) Knockdown of PRMT5 decreased cell viability of Ovcar3ptx. Cells were treated with PTX at the indicated doses for 3 days. Cell viability was determined by CCK-8 assay. (C) PRMT5 inhibitor, GSK reduced Ovcar3ptx viability. Ovcar3ptx cells were treated with GSK at the indicated concentrations for 3 days. (D) Depletion of PRMT5 increased the level of apoptosis markers in Ovcar3ptx following PTX treatment. (E) GSK elevated apoptosis marker proteins in Ovcar3ptx. Cells were treated with GSK at indicated doses for 3 days. β-actin was detected as a control. All values were presented as the mean ± S.D (n = 3). PTX: paclitaxel; GSK: GSK3326595; Significant differences were indicated; ***, $p<0.001$, **$p<0.001$, *$p<0.05$.

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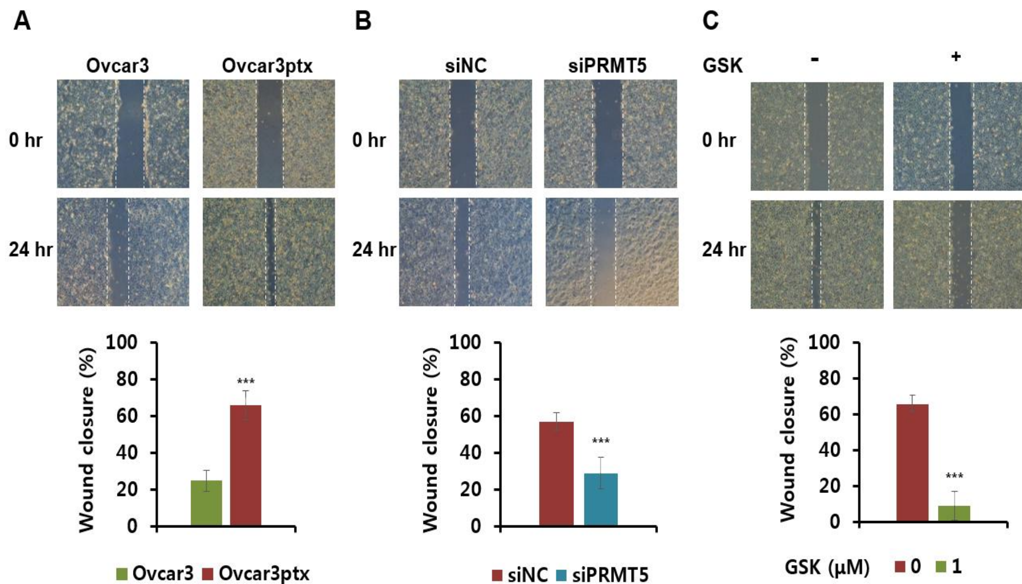

Figure 3. The stimulation of cell migration by PRMT5 in chemoresistant ovarian cancer cells. (A) Ovcar3ptx exhibited the increase in cell migration compared with Ovcar3 (upper panel). The percentage of wound closure was determined (lower panel). (B) Depletion of PRMT5 decreased migration of Ovcar3ptx cells (upper panel). The proportion of wound closure was measured (lower panel). (C) GSK decreased migration of Ovcar3ptx. Cells were treated with 1μM GSK (upper panel). The percentage of wound closure was determined (lower panel). Cells were scratched for wound healing assay. Images were captured 24 hr after wounding. All values were presented as the mean ± S.D (n = 3). GSK: GSK3326595; Significant differences were indicated; ***, $p<0.001$.

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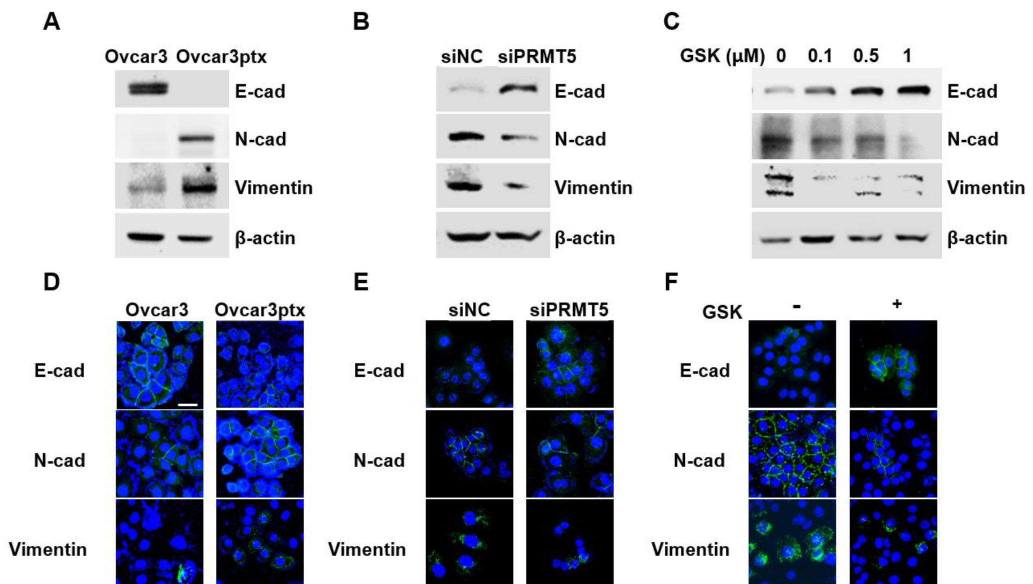

Figure 4. The activation of EMT by PRMT5 in chemoresistant ovarian cancer cells. (A) The expression of E-cad was down-regulated and N-cad, vimentin were upregulated in Ovcar3ptx compared with Ovcar3. (B) PRMT5 depletion in Ovcar3ptx reversed EMT-related proteins expression (E-cad, N-cad and vimentin). (C) GSK converted EMT-related proteins expression in Ovcar3ptx. β-actin was detected as a control. (D) Elevated mesenchymal markers and reduced epithelial marker expression were observed in Ovcar3ptx compared to Ovcar3. (E) Expressions of mesenchymal markers reduced in Ovcar3ptx after depleting PRMT5. (F) Expressions of mesenchymal markers decreased in Ovcar3ptx after treating with PRMT5 inhibitor. Images were obtained by immunocytochemistry. DAPI was used to stain the nuclei (Blue, x 200). GSK: GSK3326595.

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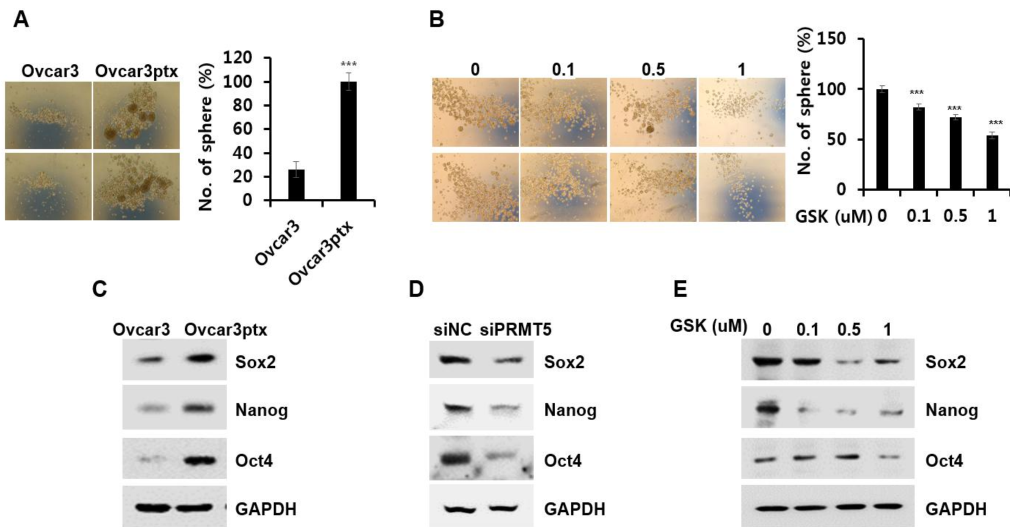

Figure 5. The increase of stem cell-like properties by PRMT5 in chemoresistant ovarian cancer cells. (A) Ovcar3ptx increased the number of spheres compared with Ovcar3. (B) PRMT5 inhibitor treatment in Ovcar3ptx reduced the number of spheres. (C) Stemness marker expression including sox2, nanog, oct4 increased in Ovcar3ptx compared to Ovcar3. (D) Knockdown of PRMT5 reduced the expression of stemness markers. (E) PRMT5 inhibitor decreased protein level of stemness markers. GAPDH was detected as a control. All values were presented as the mean ± S.D (n = 3). GSK: GSK3326595; Significant differences were indicated; ***, $p<0.001$.

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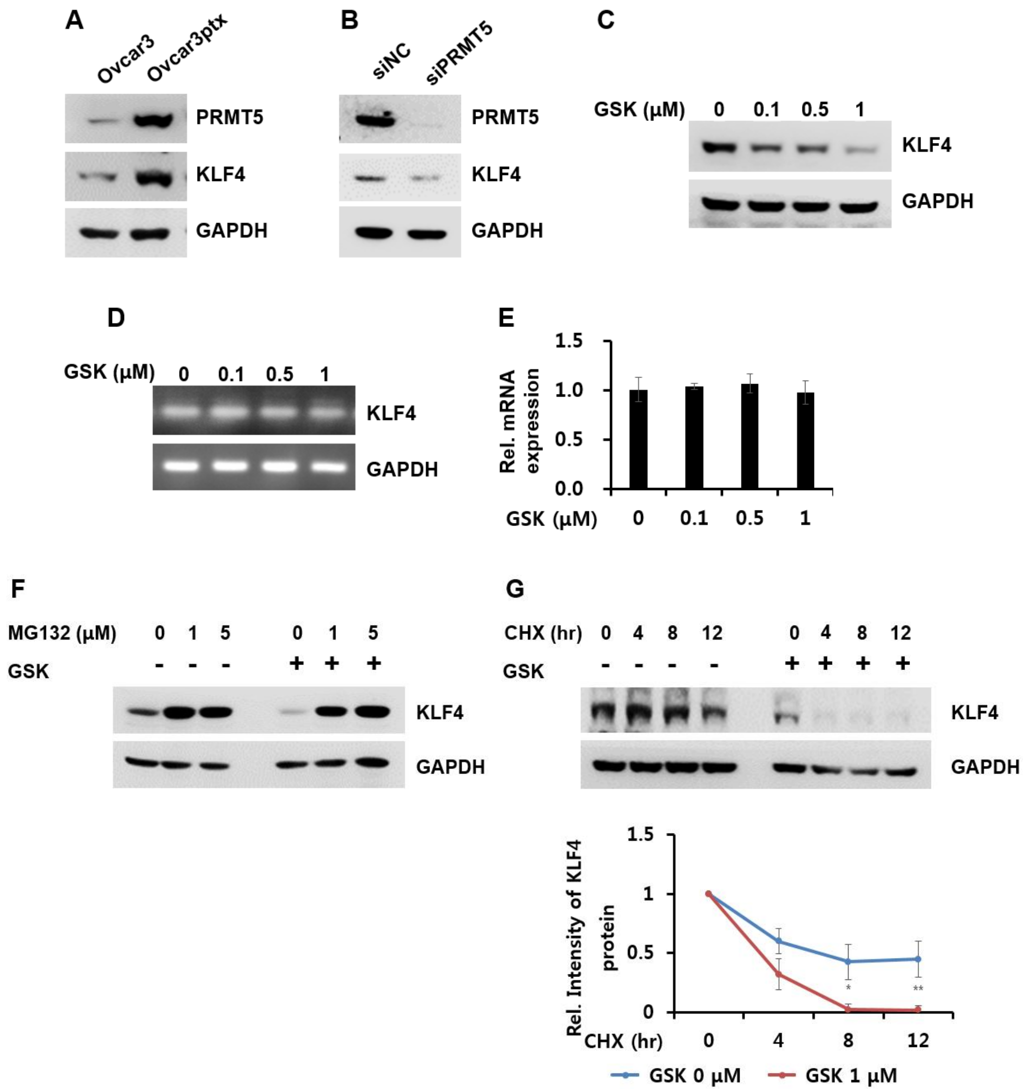

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Figure 6. The regulation KLF4 protein stability by PRMT5 in chemoresistant ovarian cancer cells. (A) KLF4 protein level was upregulated in Ovcar3ptx compared with Ovcar3 cells. (B) Knockdown of PRMT5 and (C) inhibition of PRMT5 by GSK reduced KLF4 protein level in Ovcar3ptx. (D,E) Relative mRNA levels of KLF4 were not changed by treatment of PRMT5 inhibitor. (F) MG132 treatment abolished the effect of PRMT5 inhibitor on KLF4 protein level. (G) PRMT5 inhibitor enhanced the degradation of KLF4 protein. Graph in the lower panel represented the quatification of avarage protein expression of three independent experimental groups. All values were presented as the mean ± S.D (n = 3). GSK: GSK3326595; Significant differences were indicated; ***, p<0.001, **p<0.001, *p<0.05.

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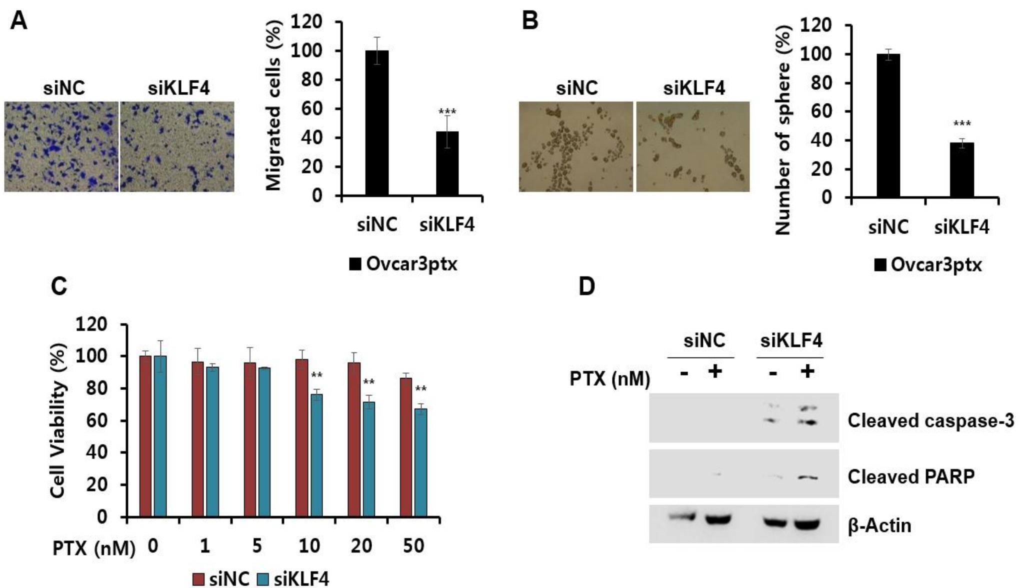

Figure 7. The effect of KLF4 on EMT and stemness in chemoresistant ovarian cancer cells. (A) Depletion of KLF4 decreased the number of migrated cells of Ovcar3ptx. (B) Depletion of KLF4 inhibited the sphere formation of Ovcar3ptx cells. (C) Knockdown of KLF4 rendered Ovcar3ptx to become more sensitive to PTX. (D) KLF4 depletion induced apoptosis in Ovcar3ptx following 3 days paclitaxel exposure. β-actin was detected as a control. All values were presented as the mean ± S.D (n = 3). PTX: paclitaxel; GSK: GSK3326595; Significant differences were indicated; ***, $p<0.001$, **$p<0.001$, *$p<0.05$.

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4. Discussion

PRMT5 has been recently characterized as an oncogene in various types of cancer such as lung, prostate, bladder and ovarian cancers (8). Accumulated PRMT5 in cancer tissues is positively related with poor overall survival, high-grade tumor and metastasis. Previous study reported that PRMT5 expression was highly upregulated in ovarian cancer (13). In this study, the protein level of PRMT5 was highly increased in chemoresistant ovarian cancer cells compared to non-resistant cancer cells. PRMT5 inhibition restored chemosensitivity of resistant cancer cells. These results support the oncogenic function of PRMT5 in ovarian cancers.

Targeting PRMT5 activity may represent multiple therapeutic approaches to strengthen anti-tumor treatment. There are plenty of selective PRMT5 inhibitors such as GSK3235025. LLY-283, JNJ-64619178 or GSK3326595 (28). Amongst numerous selective PRMT5 inhibitors, GSK3326595 showed its potential anti-tumor effect in multiple types of cancer. GSK3326595 displayed specificity and high potency in vitro with IC_{50} value of 3-9.9 nM. Previous study investigated that GSK3326595 decreased tumorigenesis through inhibiting AKT signal pathway or p53-MDM4 regulatory axis (31). GSK3326595 was also demonstrated as a candidate in anti-tumor immunity response (32). In addition, GSK3326595 was designed in PhaseI/II clinical trials from 2016 and showed its safety, mechanisms and efficacy in multiple tumor models (34,35). Therefore, further studies regarding GSK3326595 would be of great merit.

Chemoresistance in ovarian cancer is closely related to tumor metastasis and stemness (43,44). Tumor recurrence accounted for

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approximately 70% of chemotherapy-treated patients (45). In spite of the promising chemotherapy response, frequent relapses highly raise a major therapeutic challenge in the field cancer research. EMT and stem cell-like properties contribute to chemoresistance. EMT governs the biochemical alterations by which cancer cells become more motile and migrate nearby tissues. A study showed that chemoresistant ovarian cancer cells exhibits mesenchymal morphology and higher migratory ability (46). Cancer stem cell markers, such as CD44, CD133 and sox2, also enhance the development of drug resistance in ovarian cancer (47). Therefore, in this study, I investigated whether the progressive development of chemoresistance in ovarian cancer is related with EMT and stemness. The results confirmed the critical relation between chemoresistance and cell migration and stem cell-like properties. Indeed, GSK3326595 decreased cell migration and invasion of resistant cancer cells. Moreover, PRMT5 knockdown strongly reduced the expressions of stemness markers and sphere formation. These results are supported by previous studies, demonstrating that the changes in EMT and stemness show a reversible response in chemoresistance. Therefore, PRMT5 plays an integral role in chemoresistance of ovarian cancer cells through promoting cell migration and stimulating stemness properties.

I investigated the molecular mechanism of PRMT5 in regulating metastasis, stemness and chemoresistance. Previous studies showed that PRMT5 methylates several targeted proteins such as E2F1, FAM47E, KLF4 and others. In this study, I observed that KLF4 protein level was highly upregulated in chemoresistant ovarian cancer cells. These results show that KLF4 is a crucial candidate of PRMT5 regulation in chemoresistance of ovarian cancer cells.

In conclusion, I propose that PRMT5-KLF4 axis is a decisive pathway in chemoresistance of ovarian cancer. Inhibition of PRMT5

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activity by GSK3326595 could reverse the chemosensitivity of resistant cancer cells to paclitaxel. Furthermore, EMT and cancer stemness are downregulated by inhibition of PRMT5. Therefore, targeting PRMT5-KLF4 axis could be a potential therapeutic strategy of overcoming chemoresistance of ovarian cancer.

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5. Summary

This study aimed to investigate the role of PRMT5 in chemoresistance of ovarian cancer cells. PRMT5 is overexpressed in paclitaxel-resistant ovarian cancer cells compared to non-resistant cancer cells. Otherwise, PRMT5 inhibitor could overcome paclitaxel-resistance in ovarian cancer cells. Furthermore, PRMT5 enhances stem cell-like properties and motility of resistant cancer cells. Regarding to molecular mechanism, PRMT5 regulates the protein stability of KLF4. Taken together, this study suggests that PRMT5-KLF4 axis is a potential target for treatment of chemoresistant ovarian cancer.

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The regulation of PRMT5 in chemoresistant ovarian cancer cells.

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(Abstract)

Protein arginine methyltransferase 5 (PRMT5) is a methyltransferase enzyme which symmetrically dimethylates arginine residues of target proteins. Recently, many studies reported that PRMT5 is elevated in various types of cancer such as lung, gastric, colorectal, and ovarian cancer and that high expression of PRMT5 in cancer tissues is related to poor outcomes of cancer patients.

In this study, I found that PRMT5 was upregulated in paclitaxel-resistant ovarian cancer cells compared to non-resistant cancer cells. PRMT5 inhibitor or PRMT5 depletion diminished paclitaxel-resistance of ovarian cancer cells. In addition, both knockdown of PRMT5 and inhibition of PRMT5 activity using GSK3326595

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decreased cell migration, EMT and cancer stemness. Regarding molecular mechanism, I found that PRMT5 regulated KLF4 protein stability. Similar to PRMT5 depletion, knockdown KLF4 inhibited cell proliferation, reduced EMT signalling and stem cell-like properties in chemoresistant ovarian cancer cells. Therefore, PRMT5-KLF4 axis enhances the chemoresistance of ovarian cancer cells by increasing stem cell-like characteristics and promoting cell migration and invasion.

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난소암 세포에서 PRMT5의 항암제 내성 조절 역할 규명

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(지도교수 신 소 진)

(초록)

단백질 아르기닌 메틸트랜스퍼라제5 (PRMT5)는 표적 단백질의 아르기닌 잔기를 대칭적으로 메틸화시키는 메틸트랜스퍼라제 효소이다. 최근 많은 연구에서 PRMT5는 폐암, 위암, 대장암, 난소암 등 다양한 암에서 발현이 증가되어 있으며 암 조직에서 PRMT5의 발현 수준은 환자의 나쁜 예후와 관련이 있다고 보고하고 있다.

본 연구에서는 PRMT5가 일반 암세포에 비해 파클리탁셀 내성 난소암 세포에서 과발현되는 것을 관찰했다. 내성 암 세포에서 PRMT5의 발현을 저해하면 파클리탁셀에 대한 내성이 감소되었다. 또한, PRMT5의 활성 억제제인 GSK3326595를 처리한 내성 암 세포에서는 세포 이동력, EMT 및 암 줄기능이 모두 감소되었다. 이를 매개하는 분자 기전으로 PRMT5가 KLF4 단백질 안정성을 조절한다는 것을 발견했다. PRMT5의 발현을 저해하였을 때와 유사하게, KLF4의 발현을 억제하면 내성 암 세포의 세포 증식이 억제되고 EMT 신호 전달 및 줄기 세포능이 감소되었다. 이러한 결과

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는 PRMT5-KLF4 축이 암줄기능을 증가시키고 세포 이동 및 침윤 촉진하

여 난소암 세포의 내성을 향상시킴을 나타낸다.