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Metformin Inhibits Cisplatin-Induced Epithelial-to-Mesenchymal Transition in Chemoresistant Ovarian Cancer by Repressing Akt Signaling

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ABSTRACT

Objective: The aim of this study was to investigate the role of metformin in reversing the chemoresistance of ovarian cancer and the underlying mechanism.

Material and Methods: The expression of E-cadherin、vimentin and p-Akt in A2780 and A2780/DDP cells was determined by Western blot. The regulatory effects of metformin on proliferative and apoptosis in A2780/DDP cells were evaluated by the Cell Counting Kit 8 (CCK-8) and the Annexin V-FITC Kit. The expression of E-cadherin、vimentin and p-Akt in different groups of A2780/DDP cells was determined by Western blot.

Results: The expression levels of E-cadherin were significantly lower in A2780/DDP cells than in A2780 cells ($p<0.05$); in contrast, the expression levels of vimentin and p-Akt were significantly higher ($p<0.05$). The combination of metformin and cisplatin reduced cell viability compared with cisplatin treatment only. However, the combination of cisplatin and metformin had no effect on apoptosis in A2780/DDP cells. In addition, cisplatin was shown to induce EMT in A2780/DDP cells ($p<0.05$), while the combination of metformin and cisplatin reversed EMT. Cisplatin was also shown to increase the expression levels of p-Akt in A2780/DDP cells ($p<0.05$), while the combination of metformin and cisplatin reversed the expression of p-Akt.

Conclusion: Metformin can sensitize A2780/DDP cells to cisplatin by inhibiting EMT. We hypothesize that the mechanism responsible for this effect involves the inhibition of the Akt signaling pathway.

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Introduction

Ovarian cancer is a common malignancy in women and represents the leading cause of cancer-related mortality in the field of gynecology [1]. The most common form of ovarian malignancy is epithelial ovarian cancer (EOC); this accounts for more than 90% of all ovarian malignancies. Because of the absence of, or vague, symptoms and the lack of screening methods, more than 70% of EOC cases are diagnosed at an advanced stage (usually stage III/IV). The current gold standard for treating ovarian cancer is optimized cytoreductive surgery followed by platinum-based combination chemotherapies; this is now considered as the standard treatment for advanced EOC [2, 3]. Recent advances in radical surgery and chemotherapy have improved the treatment outcomes for patients with ovarian cancer. Of the platinum-based compounds, cisplatin has been proven to be the most effective drug against cancer. However, the 5-year survival rate for ovarian cancer is usually lower than 40%; this is because of the development of resistance to platinum-based therapies and recurrence. The advent of chemoresistance has emerged as a major obstacle for the treatment of ovarian cancer. The mechanisms underlying platinum resistance in ovarian cancer, which have been studied extensively, include reduced platinum accumulation in cells, impaired DNA damage repair, and enhanced platinum detoxification. However, the specific mechanisms that confer resistance to platinum-based drugs have yet to be fully elucidated [4, 5]. Consequently, there is an urgent need to investigate the molecular mechanisms underlying cisplatin resistance. Such work will facilitate the development of effective and targeted therapeutic strategies for cisplatin resistance in EOC and the development of more efficacious combined regimens with the ability to reverse drug resistance.

Metformin is well-tolerated, belongs to the biguanide class of anti-diabetes drugs and is the most widely prescribed oral hypoglycemic agent. This drug appears to have a number of pleiotropic effects, including the potential for being repurposed as an anticarcinogenic drug. Metformin has been the focus of a number of recent reviews by virtue of the fact that it exerts

potential anti-tumorigenic effects and because its safety profile has been well characterized [6-9]. Several epidemiological studies have demonstrated a correlation between metformin treatment and a reduction in both the incidence of cancer and cancer-related mortality; however, the molecular mechanisms underlying these effects remain largely unknown. The predominant hypothesis for the molecular mechanism underlying the anti-cancer effects of metformin is that AMPK activation and mTORC1 inhibition are involved; collectively, these processes inhibit the growth of tumor cells [10]. One unique aspect of the anti-cancer effects of metformin is its capacity to act as a chemosensitizer. For example, in colon cancer cells, metformin is known to enhance the effects of 5-fluorouracil and oxaliplatin [11-13]. Metformin has also been shown to enhance the tumor-suppressing effect of chemotherapy in breast cancer cells, both *in vitro* and *in vivo* [14-16]. With regards to gynecological cancers, metformin has been shown to inhibit the proliferation of ovarian cancer cell lines *in vitro* [17]. In addition, metformin has also been shown to improve the efficacy of chemotherapy, both *in vitro* and *in vivo*. However, the molecular mechanisms underlying such effects have yet to be elucidated. Acquiring a better understanding of these mechanisms will help pave the way for the use of metformin in routine clinical treatment of cancer [18-20].

In the present study, we investigated the effects of metformin in a chemoresistant ovarian cancer cell line and demonstrated that EMT plays a role in chemoresistance. Furthermore, the combination of metformin and cisplatin resulted in a clear reduction in cell viability when compared with cisplatin treatment alone. However, the combination of cisplatin and metformin treatment had no effect on apoptosis in A2780/DDP cells. We also found that cisplatin could induce EMT in A2780/DDP cells and that the combination of metformin and cisplatin reversed EMT. Cisplatin was also shown to increase the expression levels of p-Akt in A2780/DDP cells. Collectively, these results suggest that metformin can sensitize A2780/DDP cells to cisplatin by inhibiting EMT. We hypothesize that the mechanism responsible for this effect involves the inhibition of the Akt signaling pathway. We hope that our findings will pave the way to the successful translation of metformin for the clinical treatment of ovarian cancer.

Materials and Methods

Cell Culture

Two ovarian cancer cell lines (A2780 and A2780/DDP) were purchased from the Cellular Institute in Shanghai, China. A2780 cells were cultured in DMEM supplemented with 10% FBS, 100 U/ml of penicillin, and 1% streptomycin. A2780/DDP cells were cultured in RPMI 1640 supplemented with 10% FBS, 100 U/ml of penicillin, 1% streptomycin, and 1000ng/ml of DDP. Both cell types were maintained in a humidified atmosphere at 37°C and 5% CO_2. Media were refreshed completely every 3 days. Cell passage was conducted at 70% of confluence. Three generations of well grown cells were collected for testing.

Cytotoxicity Assays

Cell viability was assessed using the Cell Counting Kit 8 (CCK-8) (Tongren, China) assay. In brief, 5×10^5 A2780/DDP cells (100µl) were seeded into 96-well plates. After 24 h of incubation, cisplatin (Sigma, Merck KGaA, Germany) was added at concentrations of 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml. Metformin (Abcam, Cambridge, England) was also added as well at a concentration of 100 mmol/l (each concentration was repeated over three experiments). Cells were then incubated for 24 h. After 24 h, 10µl of CCK-8 solution was added to each well, followed by 1 to 2 h of incubation at 37°C. Optical density (OD) values were then read at dual wave lengths of 450 nm to determine cell viability using a microplate reader (Thermo Fisher Lab systems).

Analysis of Apoptosis

The percentage of apoptotic cells was quantitated using the Annexin V-FITC Kit (Beyotime, Shanghai, China) in accordance with the manufacturer's instructions. In brief, 1×10^6 A2780/DDP cells were seeded into 6-well plates. After 24 h of incubation, the cells were divided into three groups: placebo control, DDP (25µg/ml) and MET (100 mmol/l), and DDP (50 µg/ml) and MET (100 mmol/l). The cells were then incubated for 24 h and 48 h. During the first 15 min of staining, 1×10^6 cells were analyzed by flow cytometry using a FAC Scan instrument (Beyotime, Shanghai, China). Flow cytometry data were subsequently analyzed by Flow Jo software (Tree Star Inc.).

Western Blotting

For western blotting, A2780/DDP cells were washed twice with ice-cold PBS. Cell lysates were then prepared by incubation in RIPA buffer (Beyotime, Shanghai, China) for 30 min on ice followed by centrifugation at approximately 15 000 g for 20 min. The concentration of total protein in each extract was then determined using a Bradford protein assay kit (Millipore, Billeride, MA, USA). Equal amounts of protein (30 mg) were then separated by electrophoresis and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billeride, MA, USA). The membrane was then blocked with 5% non-fat dried milk for 2h and incubated overnight with a range of diluted primary antibodies at 4°C, including Phospho-akt (thr308) (D25E6) XP, rabbit mAb (1:1000), vimentin rabbit polyclonal antibody (10366-1-AP 1:5000); AKT rabbit polyclonal antibody (10176-2-AP 1:5000); and E-cadherin rabbit polyclonal antibody (20874-1-AP 1:10000). The Phospho-akt (thr308) antibody was purchased from Cell Signaling Technology (Cell Signaling Technology, Inc, MA, USA). All the other antibodies were purchased from Proteintech Group (Proteintech Group, Inc, Wuhan, China). The following morning, membranes were washed and incubated for 1 h with a peroxidase-conjugated secondary antibody (diluted 1:50 000). Antibody binding was then detected using an ECL detection system (Solarbio). Densitometric analysis was hen carried out on each western blot using an Alpha Imager 2200 gel documentation system with image analysis software. Using this system, we quantified the band intensities for E-cadherin, vimentin, Akt and p-Akt, and normalized these values against those for β-actin. Each experiment was repeated in triplicate.

Statistical Analysis

All experiments were repeated at least three times. Data are expressed as the mean ± standard error (SE). An unpaired Student's t-test was used to compare differences in the expression levels of different proteins between A2780 cisplatin-sensitive and A2780/DPP cisplatin-resistant cell lines. Differences were considered to be statistically significant when $p < 0.05$.

Results

A2780/DDP Cells Exhibited Resistance to Cisplatin

In order to investigate the chemoresistance of A2780/DDP cells, we quantified cell viability in the presence of an increasing concentration of cisplatin. Compared with the A2780 cell line, A2780/DDP cells demonstrated enhanced resistance to cisplatin (Figure 1).

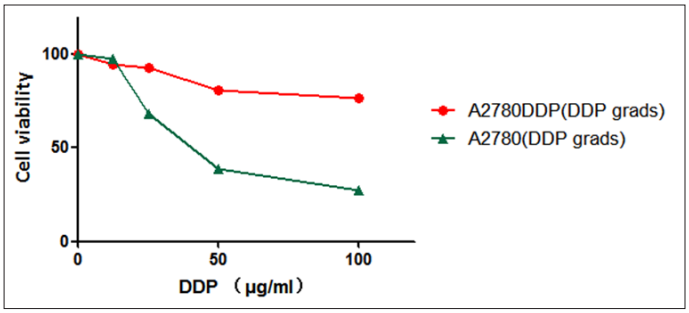

Figure 1: Cell viability of A2780 and A2780/DDP cells in the Presence of an Increasing Concentration of Cisplatin

A2780/DDP Cells Exhibited Increased Expression Levels of EMT Markers

Compared with that in A2780 cells, western blotting showed a significant increase in the expression levels of mesenchymal markers vimentin in A2780/DDP cells ($p<0.05$). In contrast, the expression levels of the epithelial marker E-cadherin were significantly reduced in A2780/DDP cells when compared with A2780 cells ($p<0.05$). We also used western blotting to investigate the potential involvement of the AKT pathway and found that the levels of p-Akt were significantly higher in A2780/DDP cells ($p<0.05$). There was no significant difference between A2780/DDP cells and A2780 cells with regards to the expression of Akt ($p<0.05$) (Figure 2a, b).

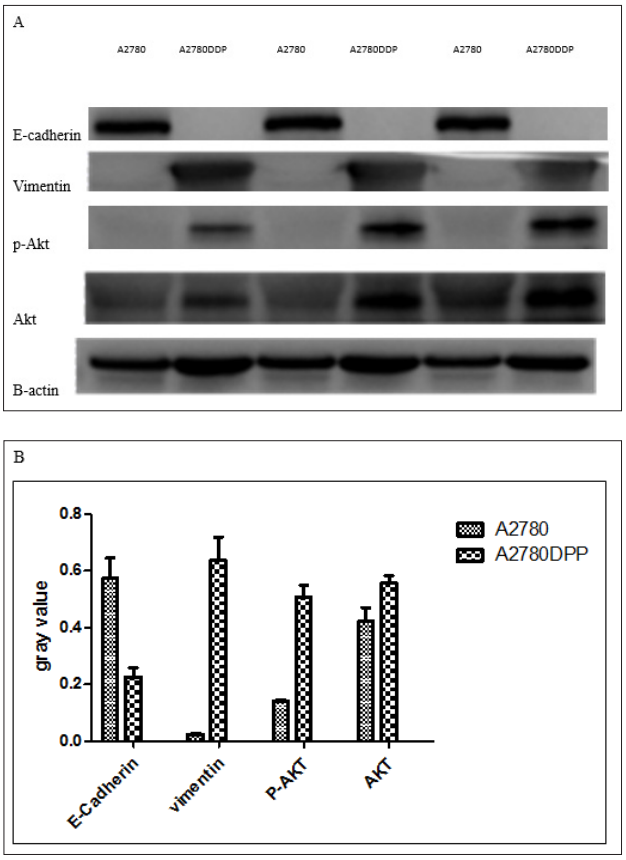

Figure 2: The levels of E-cadherin, vimentin, p-Akt, Akt, and β-actin, in A2780 and A2780/DDP cells. (A) Western blot images showing E-cadherin, vimentin, p-Akt, Akt, and β-actin. (B) Quantified western blotting data (mean ± SE) from three independent experiments. The expression levels of E-cadherin in A2780/DDP cells were significantly lower than those in A2780 cells ($p<0.05$). The expression levels of vimentin and p-Akt were significantly higher in A2780/DDP cells than in A2780 cells ($p<0.05$). There was no significant different between the two cell lines with regards to the expression levels of Akt.

Metformin Sensitized A2780/DDP Cells to Cisplatin

The first part of our analysis showed that the combination of metformin and cisplatin led to a reduction in cell viability compared with that in cells treated with only cisplatin. This suggested that metformin increased the chemosensitivity of A2780/DDP cells. However, further analysis showed that combination of cisplatin and metformin led to an increased proportion of cells undergoing apoptosis compared with cells treated with cisplatin alone (Figure 3a, b).

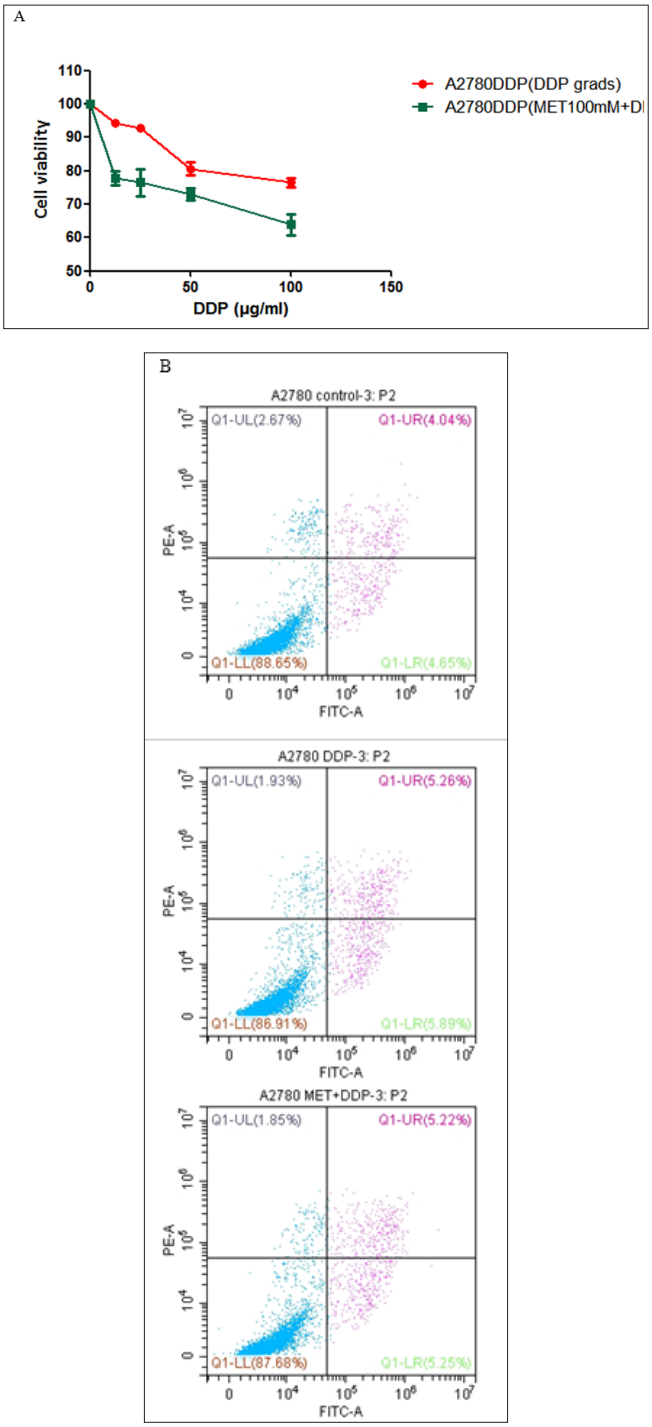

Figure 3: Cell viability and apoptosis in A2780/DDP cells treated with cisplatin and with a combination of cisplatin and metformin.

(A) Cell viability of A2780/DDP cells treated with cisplatin and with a combination of cisplatin and metformin. (B) The proportion of apoptosis in A2780/DDP cells treated with cisplatin and those treated with a combination of cisplatin and metformin.

Cisplatin Induced EMT in A2780/DDP Cells while the Combination of Metformin and Cisplatin Inhibited EMT

Data showed that cisplatin induced EMT in A2780/DDP cells. Cisplatin decreased the expression of E-cadherin and increased that of vimentin. In contrast, the combination of metformin and cisplatin reversed EMT. Furthermore, cisplatin treatment increased the expression levels of p-Akt in A2780/DDP cells. In contrast, the combination of metformin and cisplatin reversed the higher expression levels of p-Akt. Collectively, these results suggest that metformin sensitizes A2780/DDP cells to cisplatin by inhibiting EMT via suppression of the Akt signaling pathway (Figure 4).

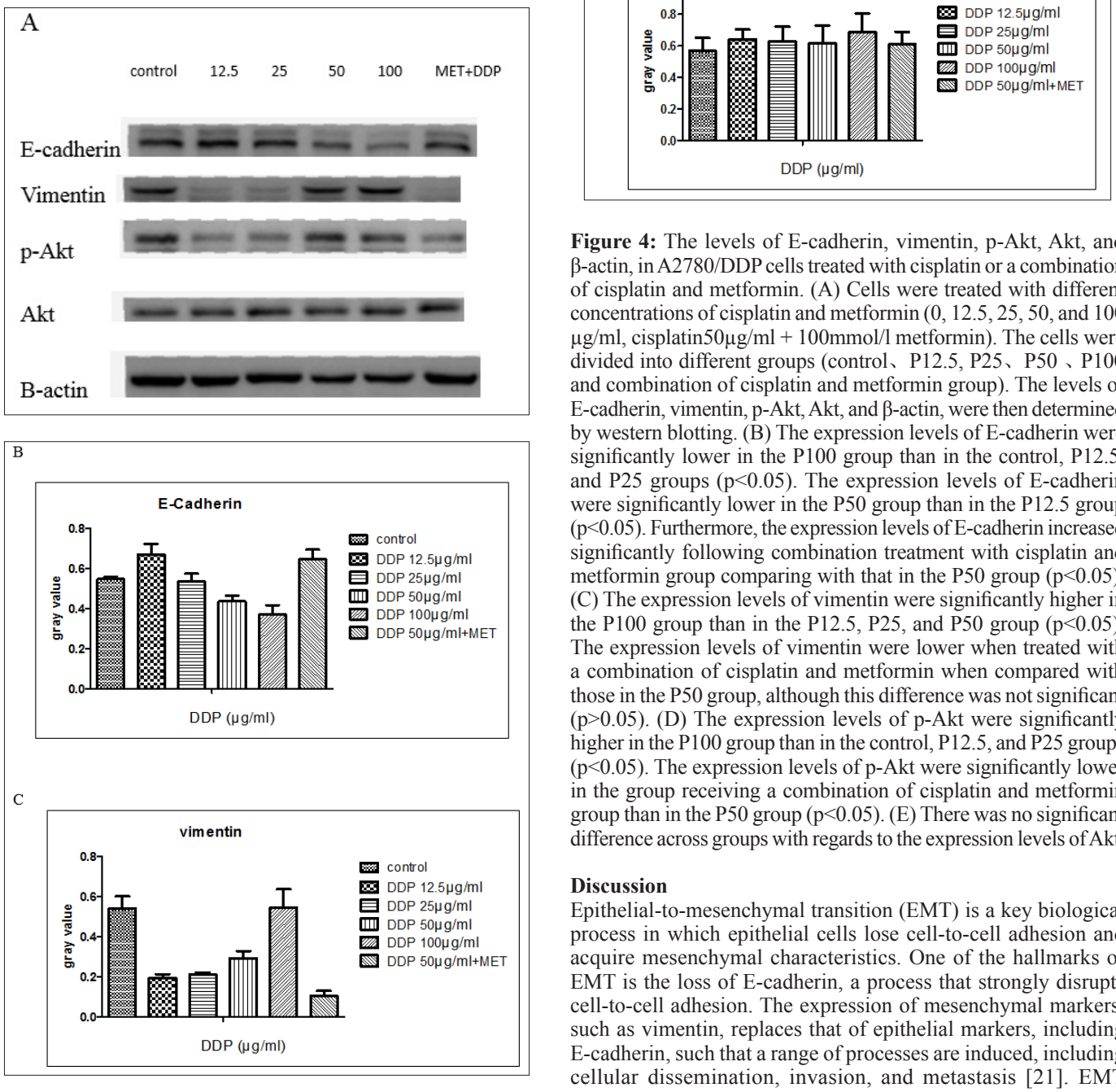

Figure 4: The levels of E-cadherin, vimentin, p-Akt, Akt, and β-actin, in A2780/DDP cells treated with cisplatin or a combination of cisplatin and metformin. (A) Cells were treated with different concentrations of cisplatin and metformin (0, 12.5, 25, 50, and 100 μg/ml, cisplatin50μg/ml + 100mmol/l metformin). The cells were divided into different groups (control、P12.5, P25、P50 、P100 and combination of cisplatin and metformin group). The levels of E-cadherin, vimentin, p-Akt, Akt, and β-actin, were then determined by western blotting. (B) The expression levels of E-cadherin were significantly lower in the P100 group than in the control, P12.5, and P25 groups ($p<0.05$). The expression levels of E-cadherin were significantly lower in the P50 group than in the P12.5 group ($p<0.05$). Furthermore, the expression levels of E-cadherin increased significantly following combination treatment with cisplatin and metformin group comparing with that in the P50 group ($p<0.05$). (C) The expression levels of vimentin were significantly higher in the P100 group than in the P12.5, P25, and P50 group ($p<0.05$). The expression levels of vimentin were lower when treated with a combination of cisplatin and metformin when compared with those in the P50 group, although this difference was not significant ($p>0.05$). (D) The expression levels of p-Akt were significantly higher in the P100 group than in the control, P12.5, and P25 groups ($p<0.05$). The expression levels of p-Akt were significantly lower in the group receiving a combination of cisplatin and metformin group than in the P50 group ($p<0.05$). (E) There was no significant difference across groups with regards to the expression levels of Akt.

Discussion

Epithelial-to-mesenchymal transition (EMT) is a key biological process in which epithelial cells lose cell-to-cell adhesion and acquire mesenchymal characteristics. One of the hallmarks of EMT is the loss of E-cadherin, a process that strongly disrupts cell-to-cell adhesion. The expression of mesenchymal markers, such as vimentin, replaces that of epithelial markers, including E-cadherin, such that a range of processes are induced, including cellular dissemination, invasion, and metastasis [21]. EMT is known to be governed by a variety of signaling pathways, including those involving TGF-β, fibroblast growth factor, and hepatocyte growth factor. EMT also increases the capacity of cells

to migrate and invade [22, 23]. This complex process occurs during embryonic development, tumor progression, and tissue fibrosis. Many studies have indicated that the activation of EMT represents an important step in the acquisition of malignant phenotypes in EOC. In ovarian cancer, EMT increases the ability of cells to migrate and invade [24, 25]. Experimental evidence also indicates the EMT is associated with chemoresistance in ovarian cancer [26, 27]. Consequently, EMT may represent a key therapeutic target for EOC.

The clinical outcomes of patients with ovarian cancer are determined by timely diagnosis and access to appropriate surgery and systemic therapies. Therefore, the prognosis of such patients can be considered as an important indicator of the effectiveness of the health care system in any given country. Despite advances in both surgery and chemotherapy, the survival rates for ovarian cancer have only shown a modest improvement over recent decade. The major obstacles facing patients with ovarian cancer are chemoresistance and relapse. Cisplatin is a commonly used first-line anti-tumor agent for patients with ovarian cancer. This drug binds to and cross-links DNA in cancerous cells, thus leading to apoptosis [28, 29]. However, the overall 5-year survival rate of EOC patients remains low at present, largely because of resistance to cisplatin. Current evidence indicates that processes involved in EMT play an important role in the development of chemoresistance [30]. For example, Marchini et al. reported that several genes that are involved in EMT are also associated with chemoresistance in EOC [31]. Another study reported that the upregulation of genes that induce EMT was associated with resistance to chemotherapeutic drugs in patients with ovarian cancer [26]. In the present study, we found that the expression levels of E-cadherin were significantly lower in A2780/DDP cells than in A2780 cells, while the expression levels of vimentin were significantly higher. These findings are consistent with those reported previously.

Platinum-based drugs such as cisplatin and carboplatin exhibit a broad range of activity in malignant diseases and are used to treat many different types of cancer, including ovarian cancer [32]. Since the introduction of cisplatin into clinical trials, this drug has had a major impact on the therapeutic management of several different tumor types [33]. The cytotoxic action of cisplatin is mediated by its interaction with DNA to form adducts. These intra-strands crosslinked adducts then activate several signal transduction pathways and trigger apoptosis. However, the emergence of primary or acquired resistance eventually limits their efficacy in treatment. Chemotherapy appears to be a double-edged sword. Recent studies have reported the biological deterioration of cancer cells following chemotherapy. In one study, cyclophosphamide pretreatment induced the metastasis of fibrosarcoma cells in a nude mouse model [34]. Exposure to chemotherapeutic agents has also been shown to enhance metastatic potential in colorectal, pancreatic, breast, and ovarian carcinoma cells [35-38]. Therefore, chemotherapy is considered to induce EMT and enhance the metastatic potential of tumor cells. In our study, we found that cisplatin could induce EMT in A2780/DDP cells. However, the combination of metformin and cisplatin reversed EMT.

The phosphoinositide-3-kinase (PI3K)/AKT signaling cascade controls a wide range of cellular functions, including proliferation, differentiation, tumorigenesis, angiogenesis, and apoptosis. A high proportion of ovarian carcinomas are known to exhibit molecular aberrations within the PI3K/AKT signaling pathway, including in PI3K itself as well as in AKT and mTOR [39, 40]. A mounting body of evidence demonstrates that the PI3K/Akt signaling pathway is involved in the development of chemoresistance, because these pathways regulate the expression of genes that are critical for cell survival and the evasion of apoptosis [41]. The signal of apoptosis is known to be primarily activated through AKT, and the activation of AKT has been observed in patients with both high-grade and late-stage serous ovarian cancer. The activation of AKT exerts anti-apoptotic effects and antagonizes cell cycle arrest [42]. A previous study reported that inhibition of the PI3K/AKT pathways led to an increase in cisplatin sensitivity in cisplatin-resistant breast cancer cells [43]. The AKT pathway has also been confirmed to be activated in cisplatin-resistant ovarian cancer [44]. The findings of the present study are consistent with previous studies, in that we observed a significant increase in the expression levels of p-Akt in A2780/DDP cells compared with A2780. Furthermore, we found that cisplatin increased the expression levels of p-Akt in A2780/DDP cells. Previous research has shown that the PI3K/AKT/mTOR signaling pathway plays a significant role in the process of EMT [45]. In our study, the combination of metformin and cisplatin reversed the higher expression levels of p-Akt and reduced cell viability by inhibiting EMT.

There were some limitations to the present study. First, although we found that metformin could reverse EMT *via* the Akt signaling pathway, the specific mechanism underlying this effect remains to be identified. Second, our study was only carried out *in vitro*. It is now necessary to confirm our findings by carrying out research *in vivo* with nude mice.

Conclusion

In the present study, we demonstrated that chemoresistance in EOC is associated with EMT and that metformin can reverse such chemoresistance by reversing cisplatin-induced EMT. Our findings indicate that metformin is a promising therapeutic option for patients with EOC.

Conflict of Interests

None of the authors have any conflicts of interest to declare.

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References

1. Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020. CA Cancer J Clin 70: 7-30.
2. Maringe C, Walters S, Butler J, Coleman MP, Hacker N, et al. (2012) Stage at diagnosis and ovarian cancer survival: evidence from the International Cancer Benchmarking Partnership. Gynecol Oncol 127: 75-82.
3. Vaughan S, Coward JI, Bast RC Jr, Berchuck A, Berek JS, et al. (2011) Rethinking ovarian cancer: recommendations for improving outcomes. Nat Rev Cancer 11: 719-725.
4. Siddik ZH (2003) Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 22: 7265-7279.
5. Martin LP, Hamilton TC, Schilder RJ (2008) Platinum resistance: the role of DNA repair pathways. Clin Cancer Res 14: 1291-1295.
6. Pollak M (2010) Metformin and other biguanides in oncology: advancing the research agenda. Cancer Prev Res (Phila) 3: 1060-1065.
7. Heckman-Stoddard BM, De Censi A, Sahasrabuddhe VV, Ford LG (2017) Repurposing metformin for the prevention of cancer and cancer recurrence. Diabetologia 60: 1639-1647.
8. Kobayashi Y, Banno K, Kunitomi H, Tominaga E, Aoki D (2019) Current state and outlook for drug repositioning

anticipated in the field of ovarian cancer. J Gynecol Oncol 3: e10.
9. Quinn BJ, Kitagawa H, Memmott RM, Gills JJ, Dennis PA (2013) Repositioning metformin for cancer prevention and treatment. Trends Endocrinol Metab 24: 469-480.
10. Meric-Bernstam F, Gonzalez-Angulo AM (2009) Targeting the mTOR signaling network for cancer therapy. J Clin Oncol 27: 2278-2287.
11. Huang WS, Lin CT, Chen CN, Chang SF, Chang HI, et al. (2018) Metformin increases the cytotoxicity of oxaliplatin in human DLD-1 colorectal cancer cells through down-regulating HMGB1 expression. J Cell Biochem 119: 6943-6952.
12. Zhang Y, Guan M, Zheng Z, Zhang Q, Gao F, et al. (2013) Effects of metformin on CD133+ colorectal cancer cells in diabetic patients. PLoS One 8: e81264.
13. Park JH, Kim YH, Park EH, Sun-Joo Lee, Hyewon Kim, et al. (2019) Effects of metformin and phenformin on apoptosis and epithelial-mesenchymal transition in chemoresistant rectal cancer. Cancer Sci 110: 2834-2845.
14. Hirsch HA, Iliopoulos D, Tsichlis PN, Struhl K (2009) Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. Cancer Res 69: 7507-7511.
15. Iliopoulos D, Hirsch HA, Struhl K (2011) Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. Cancer Res 71: 3196-3201.
16. Hirsch HA, Iliopoulos D, Struhl K (2013) Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. Proc Natl Acad Sci USA 110: 972-977.
17. Zou G, Bai J, Li D, Chen Y (2019) Effect of metformin on the proliferation, apoptosis, invasion and autophagy of ovarian cancer cells. Exp Ther Med 18: 2086-2094.
18. Dos Santos Guimarães I, Ladislau-Magescky T, Tessarollo NG, Zipinotti Dos Santos D, Rodrigues Pereira Gimba E, et al. (2018) Chemosensitizing effects of metformin on cisplatin- and paclitaxel-resistant ovarian cancer cell lines. Pharmacol Rep 70: 409-417.
19. Zheng Y, Zhu J, Zhang H, Liu Y, Sun H (2018) Metformin inhibits ovarian cancer growth and migration in vitro and in vivo by enhancing cisplatin cytotoxicity. Am J Transl Res 10: 3086-3098.
20. Yang C, Zhao N, Li D, Zou G, Chen Y (2019) Metformin improves the sensitivity of ovarian cancer cells to chemotherapeutic agents. Oncol Lett 18: 2404-2411.
21. Kalluri R, Weinberg RA (2009) The basics of epithelial-mesenchymal transition. J Clin Invest 119: 14201428.
22. Hong B, Zhang J, Yang W (2018) Activation of the LKB1 SIK1 signaling pathway inhibits the TGF β mediated epithelial mesenchymal transition and apoptosis resistance of ovarian carcinoma cells. Mol Med Rep 17: 2837-2844.
23. Lo HW, Hsu SC, Xia W, Xinyu Cao, Jin-Yuan Shih, et al. (2007) Epidermal growth factor receptor cooperates with signal transducer and activator of transcription 3 to induce epithelial-mesenchymal transition in cancer cells via up-regulation of TWIST gene expression. Cancer Res 67: 9066-9076.
24. Solheim O, Førsund M, Tropé CG, Kraggerud SM, Nesland JM, et al. (2017) Epithelial-mesenchymal transition markers in malignant ovarian germ cell tumors. APMIS 125: 781-786.
25. Lili LN, Matyunina LV, Walker LD, Wells SL, Benigno BB, et al. (2013) Molecular profiling supports the role of epithelial-to-mesenchymal transition (EMT) in ovarian cancer metastasis. J Ovarian Res 6: 49.
26. Haslehurst AM, Koti M, Dharsee M, Nuin P, Evans K, et al. (2012) EMT transcription factors snail and slug directly contribute to cisplatin resistance in ovarian cancer. BMC Cancer 12: 91.
27. Kajiyama H, Shibata K, Terauchi M, Yamashia M, Ino K, et al. (2007) Chemoresistance to paclitaxel induces epithelial-mesenchymal transition and enhances metastatic potential for epithelial ovarian carcinoma cells. Int J Oncol 31: 277-283.
28. Siddik ZH (2003) Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 22: 7265-7279.
29. Zamble DB, Lippard SJ (1995) Cisplatin and DNA repair in cancer chemotherapy. Trends Biochem Sci 20: 435-439.
30. Polyak K, Weinberg RA (209) Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 9: 265-273.
31. Marchini S, Fruscio R, Clivio L, Beltrame L, Porcu L, et al. (2013) Resistance to platinum-based chemotherapy is associated with epithelial to mesenchymal transition in epithelial ovarian cancer. Eur J Cancer 49: 520-530.
32. Martin LP, Hamilton TC, Schilder RJ (2007) Platinum resistance: role of DNA repair pathways. Clin Cancer Res 14: 1291-1295.
33. Prestayko AW, D'Aoust JC, Issell BF, Crooke ST (1979) Cisplatin (cis-diamminedichloroplatinum II). Cancer Treat Rev 6: 17-39.
34. Yamauchi K, Yang M, Hayashi K, Jiang P, Yamamoto N, et al. (2008) Induction of cancer metastasis by cyclophosphamide pretreatment of host mice: an opposite effect of chemotherapy. Cancer Res 68: 516-520.
35. Yang AD, Fan F, Camp ER, Van Buren G, Liu W, et al. (2006) Chronic oxaliplatin resistance induces epithelial-to-mesenchymal transition in colorectal cancer cell lines. Clin Cancer Res 12: 4147-4153.
36. Shah AN, Summy JM, Zhang J, Park SI, Parikh NU, et al. (2007) Development and characterization of gemcitabine-resistant pancreatic tumor cells. Ann Surg Oncol 14: 3629-3637.
37. De Larco JE, Wuertz BR, Manivel JC, Furcht LT (2001) Progression and enhancement of metastatic potential after exposure of tumor cells to chemotherapeutic agents. Cancer Res 61: 2857-2861.
38. Kajiyama H, Shibata K, Terauchi M, Yamashita M, Ino K, et al. (2007) Chemoresistance to paclitaxel induces epithelial-mesenchymal transition and enhances metastatic potential for epithelial ovarian carcinoma cells. Int J Oncol 31: 277-283.
39. Gao N, Flynn DC, Zhang Z, Zhong X, Walker V, et al. (2004) G1 cell cycle progression and the expression of G1 cyclins are regulated by PI3K/AKT/mTOR/p70S6K1 signaling in human ovarian cancer cells. Am J Physiol Cell Physiol 287: C281-91.
40. Easton JB, Houghton PJ (2006) mTOR and cancer therapy. Oncogene Rev 25: 6436-6446.
41. Kim SH, Juhnn YS, Song YS (2007) Akt involvement in paclitaxel chemoresistance of human ovarian cancer cells. Ann Ny Acad Sci 1095: 82-89.
42. Olvera N, Stevens EV, She QB, Aghajanian C, King TA, et al. (2012) Genomic complexity and AKT dependence in serous ovarian cancer. Cancer Discovery 2: 56-67.
43. Gohr K, Hamacher A, Engelke LH, Kassack,MU (2017) Inhibition of PI3K/Akt/mTOR overcomes cisplatin resistance in the triple negative breast cancer cell line HCC38. BMC Cancer 17: 711.
44. Zhao Z, Wang J, Tang J, Liu X, Zhong Q, et al. (2012) JNK- and Akt-mediated Puma expression in the apoptosis of cisplatin-resistant ovarian cancer cells. Biochem J 444:

291-301.

45. Wei R, Xiao Y, Song Y, Yuan H, Luo J, et al. (2019) FAT4 regulates the EMT and autophagy in colorectal cancer cells in part via the PI3K-AKT signaling axis. J Exp Clin Cancer Res 38: 112.