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Corilagin reduces resistance to PARP inhibitors by inhibiting the ERK signaling pathway in ovarian cancers

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Running title: LUAN et al: Corilagin helps to overcome PARP inhibitor resistance

Abstract

Background: Corilagin is a compound with hepatoprotective and antiviral activity extracted from *Phyllanthus niruri L.* Our previous work demonstrated that corilagin inhibits the growth of ovarian cancer cells by regulating the TGF- β /AKT/ERK signaling. Corilagin was also found to sensitize ovarian cancer cells to paclitaxel and carboplatin by inhibiting the Snail-glycolysis pathway. We have now studied whether corilagin could overcome resistance of ovarian cancer cells to poly ADP ribose polymerase inhibitors (PARPi). PARPi block DNA base excision repair and have been approved for treatment of ovarian cancers. Drug resistance has limited efficacy of PARPi.

Methods: We have assessed the effect of corilagin alone and in combination with PARPi in two pairs of ovarian cancer cell lines - A2780CP/A2780CP_R and UWB1.289/UWB1.289_R - that are sensitive or resistant to PARPi. CulcuSyn software (BIOSOFT - Software for Science, Cambridge, U.K.) was used to calculate synergy between two drug combinations.

Results: Corilagin was active against all four cell lines and enhanced BMN673 activity synergistically in both PARPi resistant cell lines. PARPi -BMN673 down-regulated the expression levels of PARP and up-regulated pH2AX, it decreased pERK activity in sensitive cell lines, but not in resistant cell lines. While corilagin affected DNA repair function to some extent, it inhibited pERK activity in both PARPi sensitive and resistant cells in a dose dependent manner. Corilagin, but not the BMN673, inhibited ZEB1 in resistant cells.

Conclusions: Corilagin deserves further evaluation as a drug that could enhance the activity of PARPi in PARPi-resistant ovarian cancer cells.

Keywords: corilagin, drug resistance, ERK signaling pathways, ovarian cancer, PARP inhibitors,

Background

Poly ADP ribosylation is required for base excision repair following damage to DNA by alkylating agents.¹ This process is catalyzed primarily by a 113-kDa enzyme poly(ADP-ribose)polymerase-1 (PARP1), the major isoform of PARP.² PARP inhibitors (PARPi) block PARP activity in cells and have been used extensively in cancer therapy, particularly for cancers with defects in homologous recombination (HR) repair that mends DNA double strand breaks and limits genetic instability.

In ovarian cancer, up to 50% of all high grade serous ovarian cancers (HGSOC) have inactivating germline mutations, somatic mutations or epigenetic silencing of genes involved in HR DNA repair (e.g., BRCA1/2).³ PARPi have been used to treat HR deficient, BRCA1/2-mutated, ovarian cancers. Mutations of other genes can predispose cancer cells to apoptosis induced by PARPi, including PALB2, ATM, BRIP1, CHEK2, and RAD51.⁴

Three PARPi - olaparib, rucaparib and niraparib – have been approved by the U.S. Food and Drug Administration for maintenance therapy of remissions of recurrent ovarian cancers in women with germline BRCA mutations.⁵ While long-lasting remissions have been observed, inevitably ovarian cancer cells become resistant to PARPi and there is an urgent need to find drugs that will prevent or reverse the emergence of PARPi resistance.

Corilagin is a compound with hepatoprotective and antiviral activity extracted from the herb *Phyllanthus niruri L.*⁶ Our previous work demonstrated that corilagin inhibits the growth of ovarian cancer cells by regulating the TGF- β /AKT/ERK signaling pathways.⁷ Corilagin was also found to sensitize ovarian cancer cells to paclitaxel and carboplatin by inhibiting the Snail-glycolysis pathway.⁸ We have now studied whether corilagin can help to overcome resistance of ovarian cancer cells to PARPi.

Materials and Methods

Cancer cell line cultures. Two pairs of PARPi sensitive/resistant ovarian cancer cell lines - A2780CP/A2780CP_R and UWB1.289/UWB1.289_R - were obtained from Dr. Gordon Mills,^{9, 10} M. D. Anderson Cancer Center, Houston, TX, and were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. Different concentration of BMN673 and corilagin was added after 24 hours and cell growth was measured after 72 hours of treatment.

Reagents. Antibodies against pERK, TERK, ZEB1, PARP, and pH2AX were purchased from Cell Signaling Technology (Danvers, MA), and an anti-GAPDH antibody was purchased from Kang Chen Bio Co. (Shanghai, China). The PARPi BMN673 was purchased from Selleckchem (Houston, TX).

Extraction and purification of corilagin. Corilagin was extracted and purified by the Xiamen Overseas Chinese Subtropical Plant Introduction Garden as previously described.⁶

Cell proliferation assay. Sulforhodamine B (SRB) was used to detect the effect of drugs on growth of ovarian cancer cell lines, as previously described⁷. For each cell line, we have tested different concentrations of each drug by SRB, defining the **inhibitory concentrations** (IC) for 10, 25 and 50 percent of ovarian cancer cells.

Western blot analysis. Cells were seeded in 60-mm plates ($1-2 \times 10^5$ /plate) and incubated with corilagin, BMN673 or DMSO (as a control) for 24, 48 or 72 hours, using the concentration defined for IC10, IC25, IC50 for each pair. Cell lysates were harvested and analyzed as previously described.⁸

Statistical analysis. Triplicate or quadruplicate experiments were performed in each study. CalcuSyn software (BIOSOFT - Software for Science, Cambridge, U.K.) was used to calculate synergy between two drug combinations. The combination index (CI)-isobologram equation allows quantitative determination of drug interaction, where $CI < 1$, $= 1$, and > 1 indicate synergism, an additive effect, or antagonism, respectively.

Results

Corilagin inhibits growth of PARPi-sensitive and PARPi-resistant ovarian cancer cell lines. Pairs of putatively PARPi sensitive and PARPi-resistant cancer cell lines - 2780CP/ A2780CP_R and UWB1.289/ UWB1.289_R - were sub-cloned and tested for their sensitivity to the PARPi BMN673. The IC50 of BMN673 was 0.02mM for A2780CP, >10mM for A2780CP_R, 0.12mM for UWB1.289 and 60mM for UWB1.289_R (**Figure 1**), confirming that the putatively resistant cell lines were, in fact, resistant to the PARPi. By contrast, when the same cell lines were treated with different concentrations of corilagin, identical sensitivity was observed between A2780CP and A2780CP_R, but remarkably UWB1.289_R was more sensitive than UWB1.289 to corilagin (**Figure 2**).

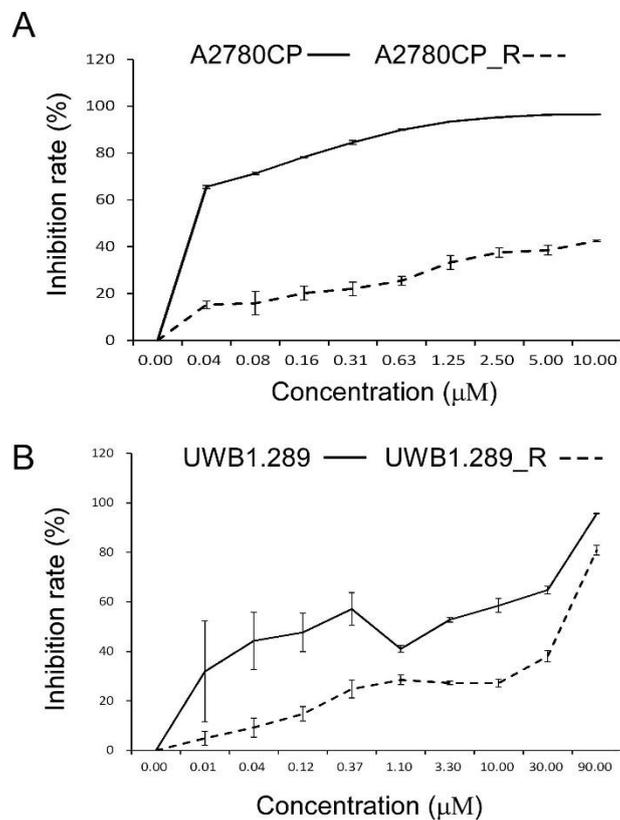


Figure 1. Effect of BMN673 on two pairs of putatively PARPi-sensitive and PARPi-resistant ovarian cancer cell lines. (A) A2780CP and A2780CP_R; (B) UWB1.289 and UWB1.289_R. Different concentration of BMN673 was added after 24 hours. SRB assays were performed at least in triplicate after 72 hours of treatment.

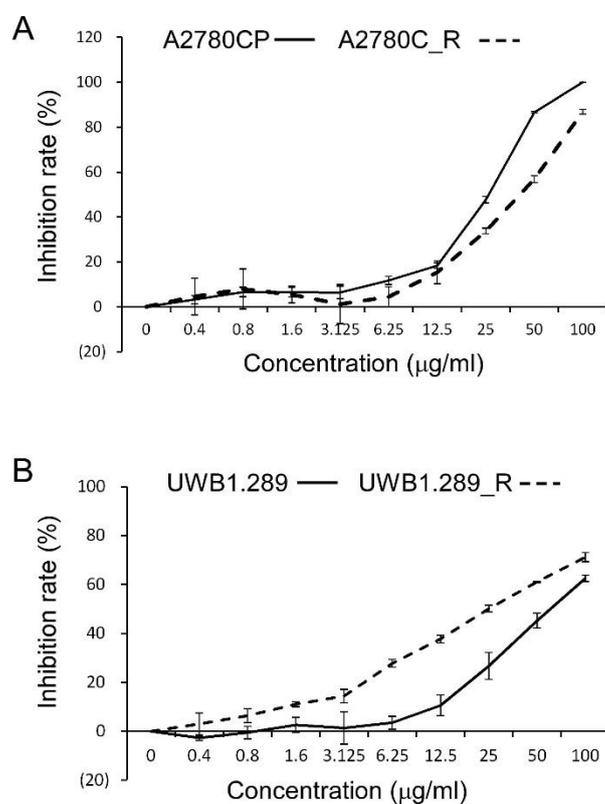
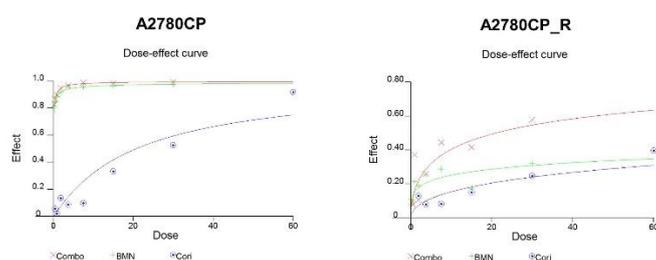


Figure 2. Effect of corilagin on two pairs of putatively PARPi-sensitive and PARPi-resistant ovarian cancer cell lines. (A) A2780CP and A2780CP_R; (B) UWB1.289 and UWB1.289_R. Different concentration of corilagin was added after 24 hours. SRB assays were performed at least in triplicate after 72 hours of treatment.

A combination of corilagin and the PARPi BMN673 exert synergistic growth inhibition in PARPi-sensitive and PARPi-resistant ovarian cancer cell lines. Four ovarian cancer cell lines were incubated with different concentrations of corilagin, BMN673 or both drugs. Combination indices (CI) were calculated for each cell line at ED50, ED75 and ED90. The PARPi-sensitive A2780CP exhibited synergistic activity (CI<1) only at ED90, with sub-additive interactions (CI>1) at ED50 and ED75 (**Figure 3**). By contrast, the PARPi-resistant cell line A2780CP_R exhibited strong synergy (CI<1) at all three doses. Both the PARPi-sensitive UWB1.289 and the PARPi-resistant UWB1.289_R exhibited strong synergy (CI<1) at all doses (**Figure 4**).



Combination of BMN673 and Corilagin on A2780CP

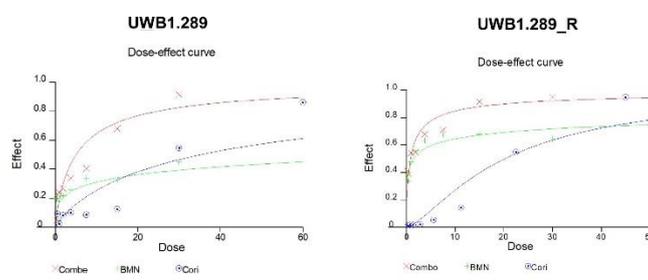
Summary table						
Drug	Combination Index Values at			Dm	m	r
	ED50	ED75	ED90			
BMN (Not a combination)	N/A	N/A	N/A	0.01055	0.46631	0.98109
Corilagin (Not a combination)	N/A	N/A	N/A	20.76893	1.02704	0.88915
Combo (1:2)	3.03914	1.36211	0.61471	0.03205	0.70843	0.97394

Combination of BMN673 and Corilagin on A2780CP_R

Summary table						
Drug	Combination Index Values at			Dm	m	r
	ED50	ED75	ED90			
BMN (Not a combination)	N/A	N/A	N/A	665.432	0.25984	0.79691
Corilagin (Not a combination)	N/A	N/A	N/A	294.8206	0.50346	0.8269
Combo (1:2)	0.17365	0.13379	0.11944	20.95509	0.52483	0.83637

Figure 3. Synergistic effect of a combination of corilagin and BMN673 in PARPi-

sensitive A2780CP and PARPi-resistant A2780CP_R ovarian cancer cells. Cell proliferation and drug inhibition were performed by SRB. Each study was performed at least in triplicate. CalcuSyn software was used to calculate the ED50, ED75 and ED90, the doses that produce 50%, 75% and 90% growth inhibition, respectively. Dm: Median-effect dose. m: The shape parameter for the dose-effect curve. r: The conformity parameter for goodness of fit to the median-effect principle of the mass-action law.



Combination of BMN673 and Corilagin on UWB1.289

Summary table						
Drug	Combination Index Values at			Dm	m	r
	ED50	ED75	ED90			
BMN	N/A	N/A	N/A	110.6656	0.33134	0.64925
(Not a combination)						
Corilagin	N/A	N/A	N/A	35.29742	0.85243	0.82414
(Not a combination)						
Combo (1:2)	0.32785	0.28462	0.27559	4.99023	0.86193	0.92924

Combination of BMN673 and Corilagin on UWB1.289_R

Summary table						
Drug	Combination Index Values at			Dm	m	r
	ED50	ED75	ED90			
BMN	N/A	N/A	N/A	1.42766	0.30428	0.91343
(Not a combination)						
Corilagin	N/A	N/A	N/A	20.25793	1.40415	0.91299
(Not a combination)						
Combo (1:1.5)	0.62787	0.2154	0.3284	0.81069	0.6799	0.93965

Figure 4. Synergistic effect of a combination of corilagin and BMN673 in PARPi-sensitive UWB1.289 and PARPi-resistant UWB1.289_R ovarian cancer cells. Cell proliferation and drug inhibition were performed by SRB. Each point was performed at least in triplicate. CalcuSyn software was used to calculate the ED50, ED75 and ED90,

the doses that produce 50%, 75% and 90% growth inhibition, respectively. Dm: Median-effect dose. m: The shape parameter for the dose-effect curve. r: The conformity parameter for goodness of fit to the median-effect principle of the mass-action law.

BMN673 down-regulates the expression of PARP and up-regulates pH2AX.

Treatment with BMN673 decreased PARP protein expression and increased pH2AX, a marker of double strand DNA breaks, in these cell lines in a dose dependent manner, aside from A2780CP_R that did not express PARP and pH2AX (**Figure 5**). Corilagin affected PARP to some extent, but increased pH2AX at high concentrations (**Figure 5**).

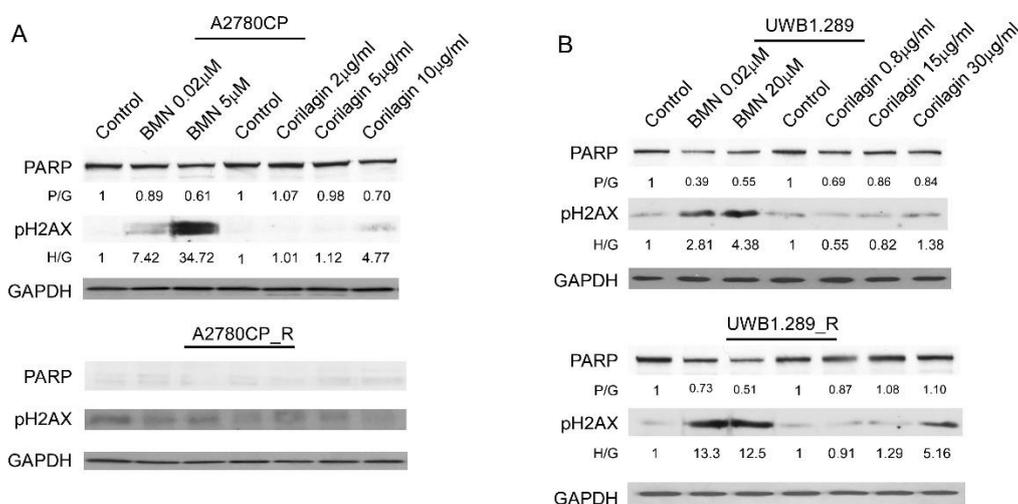


Figure 5. BMN673 down-regulated the expression of PARP and up-regulated pH2AX.

Two pairs of PARPi sensitive/resistant ovarian cancer cells: (A) A2780CP/A2780CP_R; (B) UWB1.289/UWB1.289_R. were treated with corilagin (mg/ml), or BMN673 (mM) at IC10, IC25, IC50 concentrations for each pair, and analyzed with Western Blots.

GAPDH was used as a loading control. P/G means the ratio of PARP/GAPDH by bands scan, H/G means the ratio of pH2AX/GAPDH by bands scan.

Corilagin inhibits the pERK pathway in resistant cells, whereas BMN673 does not.

High concentrations of BMN673 inhibited pERK activity in sensitive A2780CP cells, but not in resistant A2780CP_R cells and UWB1.289/UWB1.289_R cells (**Figure 6**). By contrast, corilagin inhibited pERK activity in both PARPi-sensitive and resistant cells in a dose dependent manner (**Figure 6**).

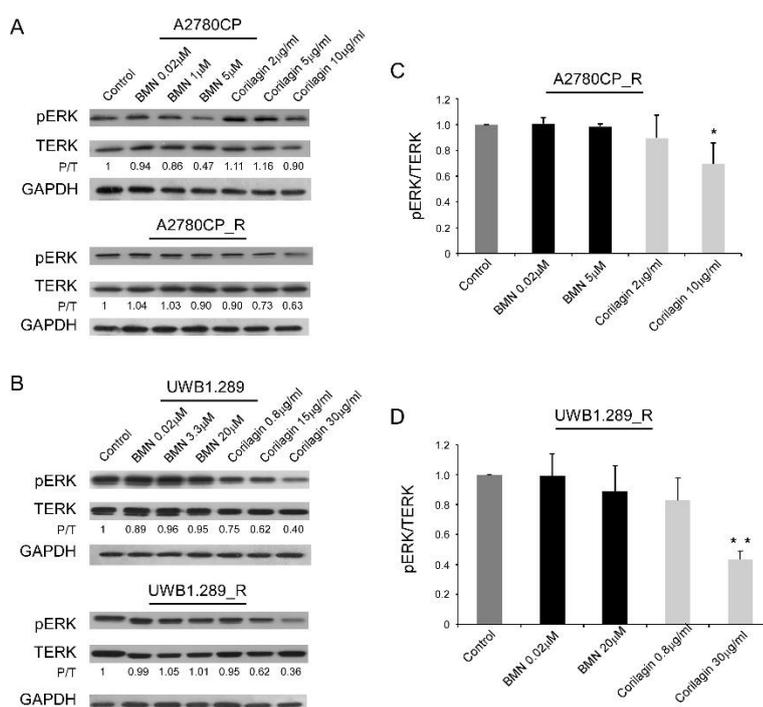


Figure 6. Corilagin inhibits the pERK pathway in resistant cells, whereas BMN673 does not. (A) A2780CP/A2780CP_R; (B) UWB1.289/UWB1.289_R were treated by corilagin (μg/ml), or BMN673 (μM) at the concentration of IC10, IC25, IC50 for each pair and used for Western Blots analysis. Total ERK (TERK) was used as a loading

control; P/T means the ratio of pERK/TERK by bands scan. (C) A2780CP_R: (D) UWB1.289_R were treated by corilagin (mg/ml), or BMN673 (mM) at the concentration of IC10, IC50 and Western Blots analysis was repeated three times (*p<0.05, **p<0.01).

Corilagin inhibits expression of ZEB1. There are several reports regarding the relation between epithelial-mesenchymal transition (EMT) and drug resistance.¹¹ Expression of several molecules involved in EMT are inhibited by corilagin such as Snail and CD44.⁸ In this study, the expression of CD44 and Snail could not be detected in either of the two pairs on Western blot analysis. ZEB1, which is an important regulator of EMT, could not be detected in UWB1.289 and UWB1.289_R cells, but is expressed in A2780CP and A2780CP_R cells. Corilagin inhibited ZEB1 in A2780CP and A2780CP_R. BMN673 inhibited ZEB1 expression in the PARPi-sensitive cells, but not in the PARPi-resistant cells (**Figure 7**).

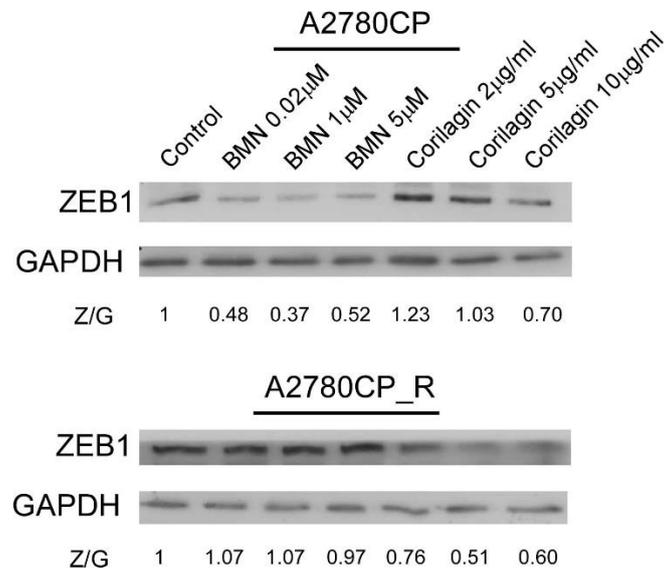


Figure 7. Corilagin down-regulates the expression of ZEB1 in A2780CP and A2780CP_R cells. A2780CP/A2780CP_R were treated by corilagin (mg/ml), or BMN673 (mM) at the concentration of IC10, IC25, IC50 for each cell line and used for Western Blots analysis. GAPDH was used as a loading control. Z/G means the ratio of ZEB1/GAPDH by bands scan.

Discussion

While PARPi benefit both breast and ovarian cancer patients whose tumors have defects in DNA repair, the response is limited by the development of resistance.¹² Our study documents that corilagin, a novel natural product, enhances the sensitivity of the PARPi BMN673 in PARPi-sensitive and PARPi-resistant ovarian cancer cell lines. Corilagin inhibited pERK signaling in PARPi-resistant cells. Sun *et al.* previously reported that RAS/MAPK pathway activity was upregulated in PARPi resistant clones. MEKi have the potential to re-sensitize PARPi resistant human tumors to PARPi.⁹ This group also reported that BRD4i resensitizes cancer cells to overcome multiple mechanisms of acquired PARPi resistance.¹⁰ There are also reports that Pglycoprotein and c-Met may be involved in PARPi resistance.^{13,14}

In our own study, the PARPi BMN673 down-regulated the expression of PARP and up-regulated pH2AX, a biomarker for double strand DNA breaks, in both sensitive and resistant cancer cells. BMN673 down-regulated the expression levels of pERK only in

PARPi-sensitive cells, but not in PARPi-resistant cells, suggesting a role for pERK in PARPi resistance. By contrast, corilagin inhibited pERK activity in both PARP-sensitive and PARP-resistant cells (Figure 6). Corilagin inhibited ZEB1 in A2780CP and A2780CP_R. BMN673 inhibited ZEB1 expression in the PARPi-sensitive cells, but not in the PARPi-resistant cell line (Figure 7). These observations may explain why corilagin has synergistic effects with PARPi when treating PARPi resistant cells.

Our previous study revealed corilagin inhibited multiple signaling pathways in ovarian cancer. Cyclin B1, Myt1, phospho-cdc2 and phospho-Weel were all down-regulated by treatment with corilagin.⁷ Corilagin also inhibited TGF- β secretion and the TGF- β -induced stabilization of Snail;⁷ Corilagin blocked the activation of both the canonical Smad and non-canonical ERK/AKT pathways.⁷ Furthermore, corilagin sensitized ovarian cancer cells to paclitaxel and carboplatin treatment by primarily inhibiting the Snail-glycolysis pathways.⁸

Several recent studies have focused on the role of EMT in chemo-resistance. EMT is a complex molecular program that regulates changes in cell morphology and function during embryogenesis and tissue development. EMT is associated with therapy resistance and tumor recurrence¹¹. Several transcription factors, including the Snail/Slug family, Twist, δ EF1/ZEB1, SIP1/ZEB2 and E12/E47, function as molecular switches for the EMT program.¹¹ Among these factors, the zinc finger E-box binding transcription factor ZEB1 is considered to play a crucial role in ovarian cancer

EMT. ZEB1 promotes EMT by repressing genes contributing to the epithelial phenotype while activating those associated with the mesenchymal phenotype.¹⁵ Suppression of ZEB1 inhibits cell invasion and metastasis, restoration of paclitaxel sensitivity of chronic chemo-resistant ovarian carcinoma cells.¹⁶ In our study, corilagin downregulated ZEB1, suggesting that PARPi-resistance might function through the ZEB1/EMT pathway in ovarian cancers.

In this paper, we document that corilagin interacts with PARPi to exert synergistic anti-tumor activity by inhibiting pERK and ZEB1 in PARPi-resistant cancer cells. This herbal medicine deserves further evaluation and may provide a new way to overcome PARPi-resistance.

List of abbreviations

EMT: Epithelial-Mesenchymal Transition; SRB: Sulforhodamine B; IC: inhibiting concentration; CI: combination indexes; PARP: poly ADP ribose polymerase; PARPi: poly ADP ribose polymerase inhibitors.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests: These authors report no financial or intellectual conflicts of interest regarding this study.

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Authors' contributions: BL, HZ and YY: data generation; YY, ZL, RCB: data analysis; YY, ZL and RCB: manuscript writing; YY, ZL and RCB: overall supervision and study guidance.

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Figure legends

Figure 1. Effect of BMN673 on two pairs of putatively PARPi-sensitive and PARPi-resistant ovarian cancer cell lines. (A) A2780CP and A2780CP_R; (B) UWB1.289 and UWB1.289_R. Different concentration of BMN673 was added after 24 hours. SRB assays were performed at least in triplicate after 72 hours of treatment.

Figure 2. Effect of corilagin on two pairs of putatively PARPi-sensitive and PARPi-resistant ovarian cancer cell lines. (A) A2780CP and A2780CP_R; (B) UWB1.289 and UWB1.289_R. Different concentration of corilagin was added after 24 hours. SRB assays were performed at least in triplicate after 72 hours of treatment.

Figure 3. Synergistic effect of a combination of corilagin and BMN673 in PARPi-sensitive A2780CP and PARPi-resistant A2780CP_R ovarian cancer cells. Cell proliferation and drug inhibition were performed by SRB. Each study was performed at least in triplicate. CulcuSyn software was used to calculate the ED50, ED75 and ED90, the doses that produce 50%, 75% and 90% growth inhibition, respectively. Dm: Median-effect dose. m: The shape parameter for the dose-effect curve. r: The conformity parameter for goodness of fit to the median-effect principle of the mass-action law.

Figure 4. Synergistic effect of a combination of corilagin and BMN673 in PARPi-sensitive UWB1.289 and PARPi-resistant UWB1.289_R ovarian cancer cells. Cell proliferation and drug inhibition were performed by SRB. Each point was performed at least in triplicate. CulcuSyn software was used to calculate the ED50, ED75 and ED90, the doses that produce 50%, 75% and 90% growth inhibition, respectively. Dm: Median-effect dose. m: The shape parameter for the dose-effect curve. r: The conformity parameter for goodness of fit to the median-effect principle of the mass-action law.

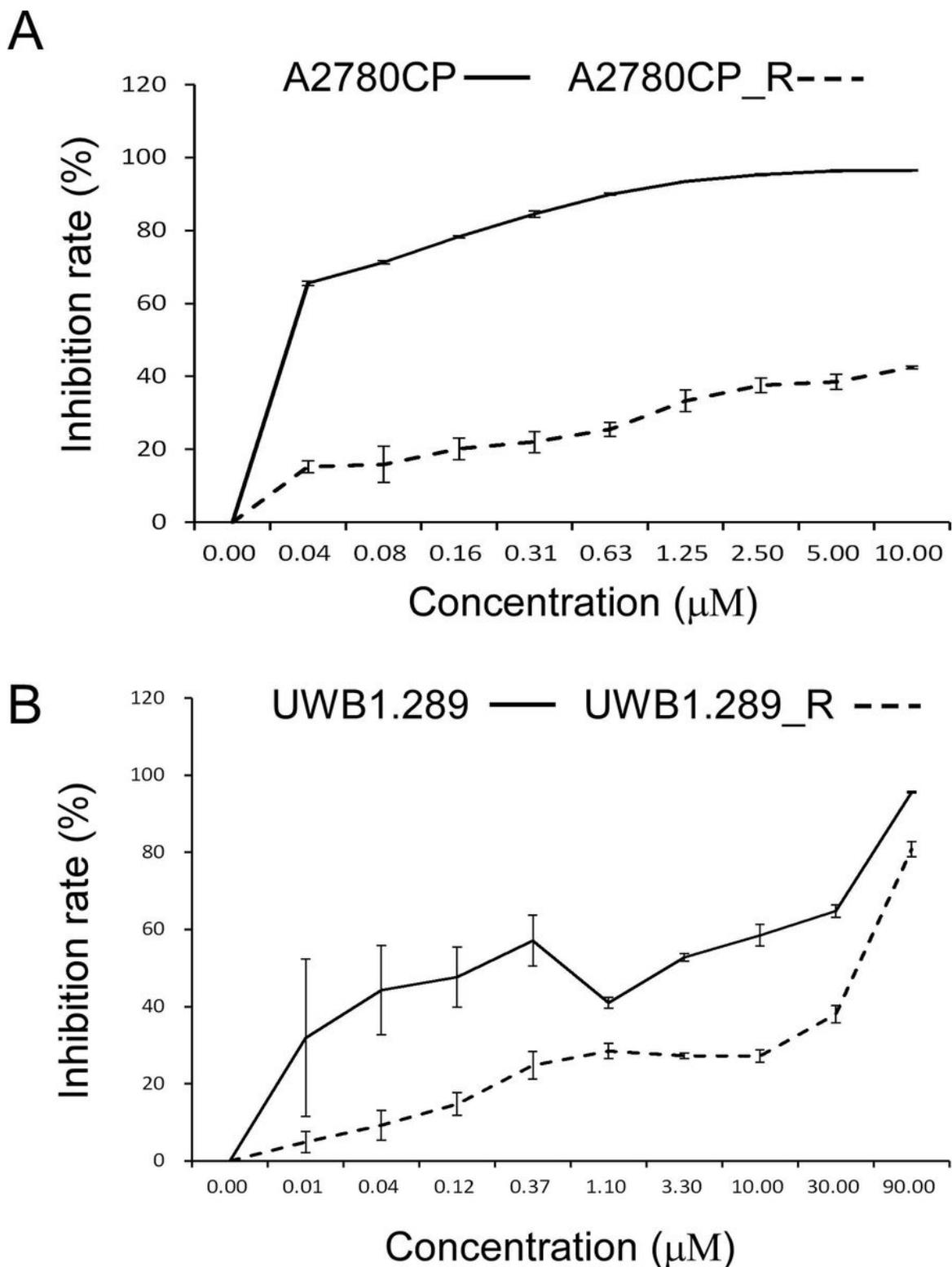
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Figure 6. Corilagin inhibits the pERK pathway in resistant cells, whereas BMN673 does not. (A) A2780CP/A2780CP_R; (B) UWB1.289/UWB1.289_R were treated by corilagin (mg/ml), or BMN673 (mM) at the concentration of IC10, IC25, IC50 for each pair and used for Western Blots analysis. Total ERK (TERK) was used as a loading control; P/T means the ratio of pERK/TERK by bands scan. (C) A2780CP_R: (D) UWB1.289_R were treated by corilagin (mg/ml), or BMN673 (mM) at the concentration of IC10, IC50 and Western Blots analysis was repeated three times (*p<0.05, **p<0.01).

Figure 7. Corilagin down-regulates the expression of ZEB1 in A2780CP and A2780CP_R cells. A2780CP/A2780CP_R were treated by corilagin (mg/ml), or BMN673 (mM) at the concentration of IC10, IC25, IC50 for each cell line and used for Western Blots analysis. GAPDH was used as a loading control. Z/G means the ratio of ZEB1/GAPDH by bands scan.

Figures



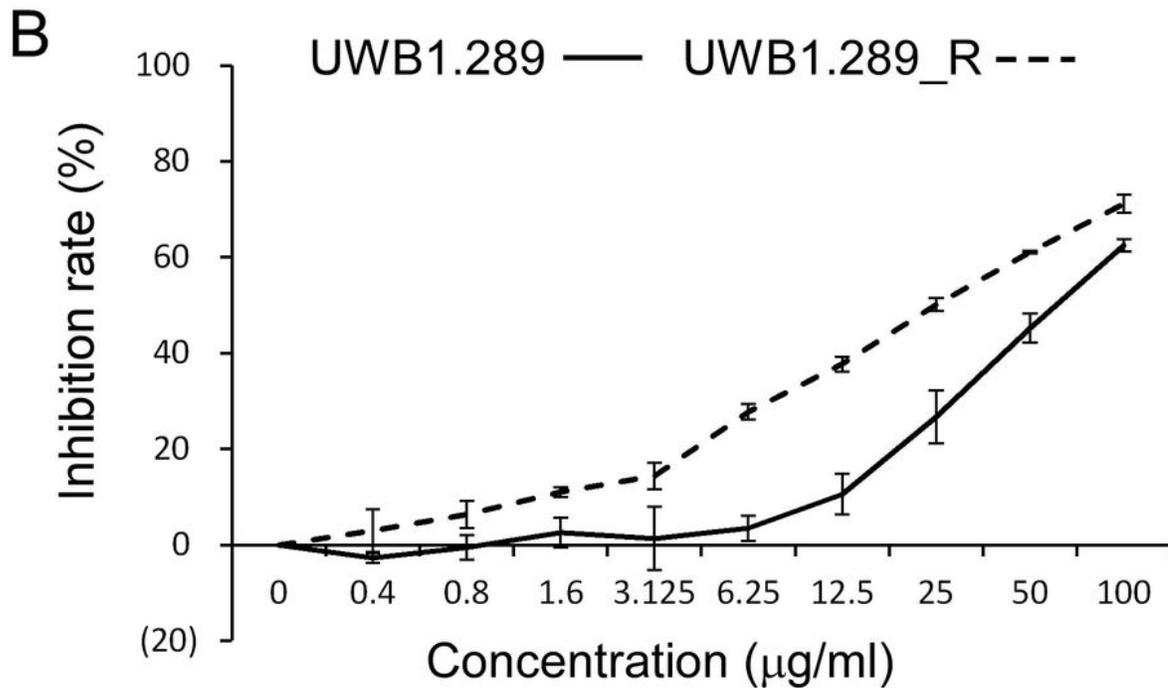
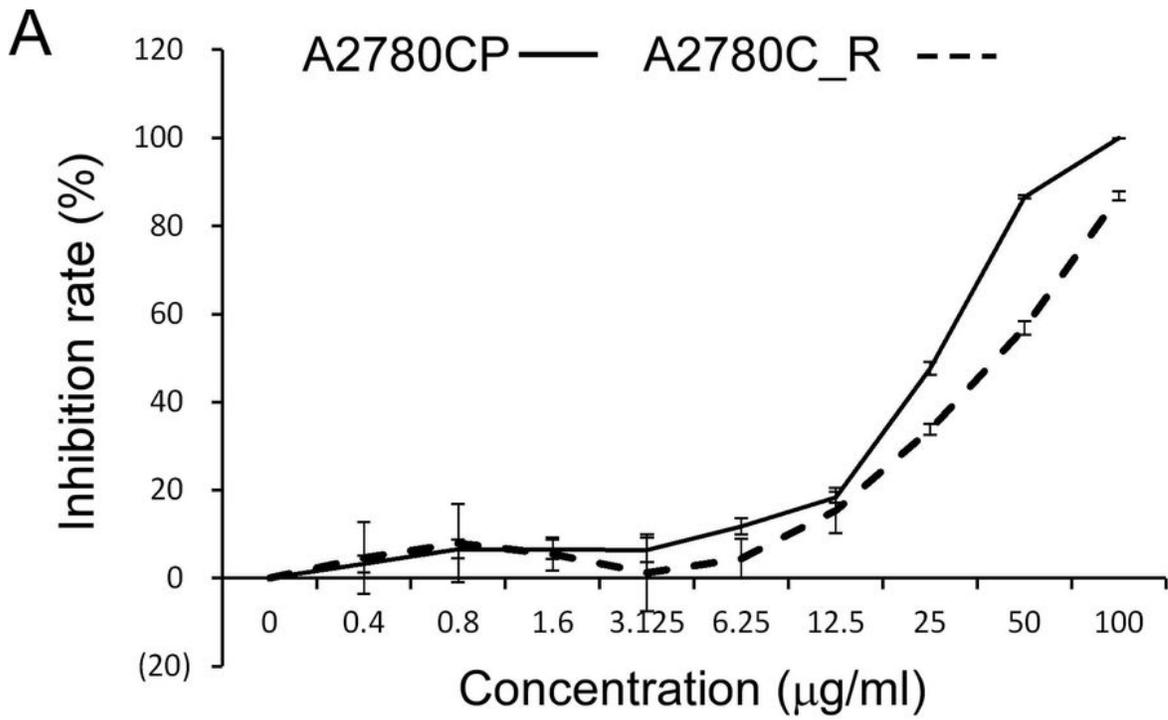
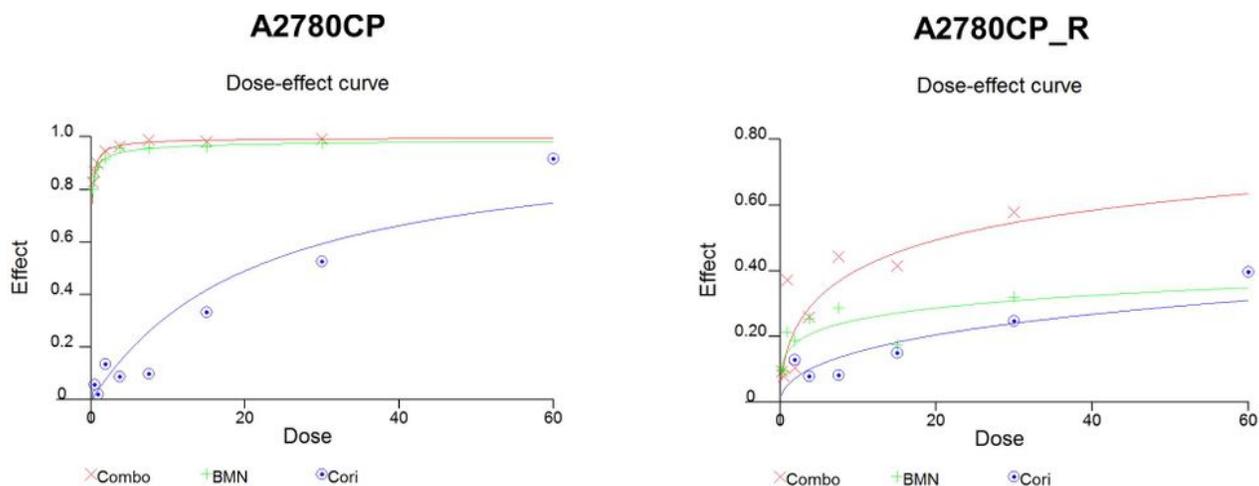


Figure 2

Effect of corilagin on two pairs of putatively PARPi-sensitive and PARPi-resistant ovarian cancer cell lines. (A) A2780CP and A2780CP_R; (B) UWB1.289 and UWB1.289_R. Different concentration of corilagin was added after 24 hours. SRB assays were performed at least in triplicate after 72 hours of treatment.



Combination of BMN673 and Corilagin on A2780CP

Summary table						
Drug	Combination Index Values at					
	ED50	ED75	ED90	Dm	m	r
BMN	N/A	N/A	N/A	0.01055	0.46631	0.98109
(Not a combination)						
Corilagin	N/A	N/A	N/A	20.76893	1.02704	0.88915
(Not a combination)						
Combo (1:2)	3.03914	1.36211	0.61471	0.03205	0.70843	0.97394

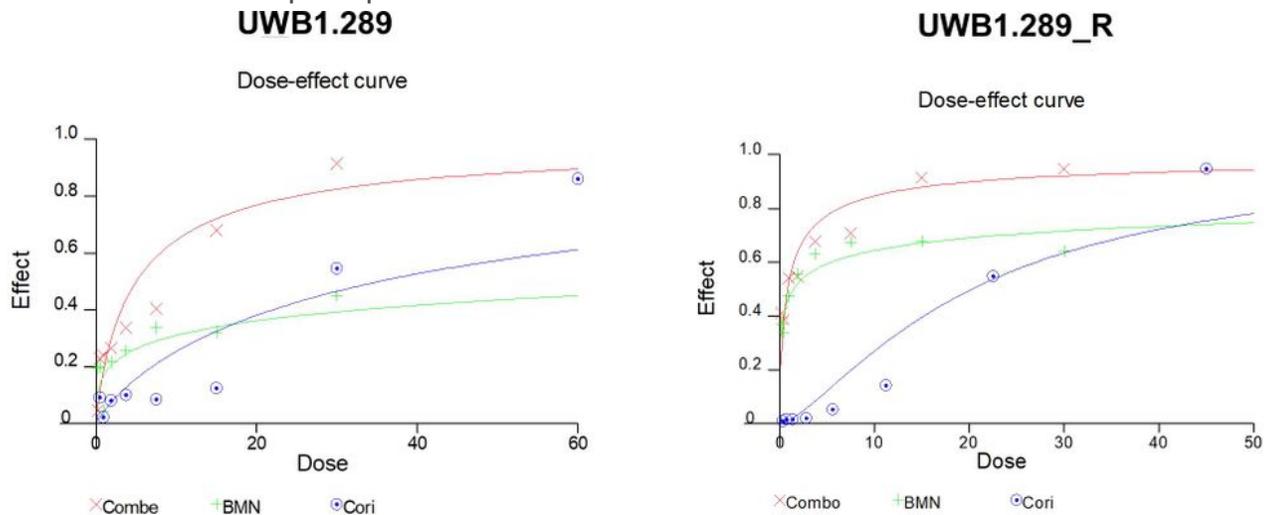
Combination of BMN673 and Corilagin on A2780CP_R

Summary table						
Drug	Combination Index Values at					
	ED50	ED75	ED90	Dm	m	r
BMN	N/A	N/A	N/A	665.432	0.25984	0.79691
(Not a combination)						
Corilagin	N/A	N/A	N/A	294.8206	0.50346	0.8269
(Not a combination)						
Combo (1:2)	0.17365	0.13379	0.11944	20.95509	0.52483	0.83637

Figure 3

Synergistic effect of a combination of corilagin and BMN673 in PARPi-10 sensitive A2780CP and PARPi-resistant A2780CP_R ovarian cancer cells. Cell proliferation and drug inhibition were performed by SRB. Each study was performed at least in triplicate. CalcuSyn software was used to calculate the ED50, ED75 and ED90, the doses that produce 50%, 75% and 90% growth inhibition, respectively. Dm: Median-effect

dose. m: The shape parameter for the dose-effect curve. r: The conformity parameter for goodness of fit to the median-effect principle of the mass0-action law.



Combination of BMN673 and Corilagin on UWB1.289

Summary table						
Drug	Combination Index Values at			Dm	m	r
	ED50	ED75	ED90			
BMN	N/A	N/A	N/A	110.6656	0.33134	0.64925
(Not a combination)						
Corilagin	N/A	N/A	N/A	35.29742	0.85243	0.82414
(Not a combination)						
Combe (1:2)	0.32785	0.28462	0.27559	4.99023	0.86193	0.92924

Combination of BMN673 and Corilagin on UWB1.289_R

Summary table						
Drug	Combination Index Values at			Dm	m	r
	ED50	ED75	ED90			
BMN	N/A	N/A	N/A	1.42766	0.30428	0.91343
(Not a combination)						
Corilagin	N/A	N/A	N/A	20.25793	1.40415	0.91299
(Not a combination)						
Combo (1:1.5)	0.62787	0.2154	0.3284	0.81069	0.6799	0.93965

Figure 4

Synergistic effect of a combination of corilagin and BMN673 in PARPi-sensitive UWB1.289 and PARPi-resistant UWB1.289_R ovarian cancer cells. Cell proliferation and drug inhibition were performed by SRB. Each point was performed at least in triplicate. CulcuSyn software was used to calculate the ED50, ED75

and ED90, the doses that produce 50%, 75% and 90% growth inhibition, respectively. Dm: Median-effect dose. m: The shape parameter for the dose-effect curve. r: The conformity parameter for goodness of fit to the median-effect principle of the mass-action law.

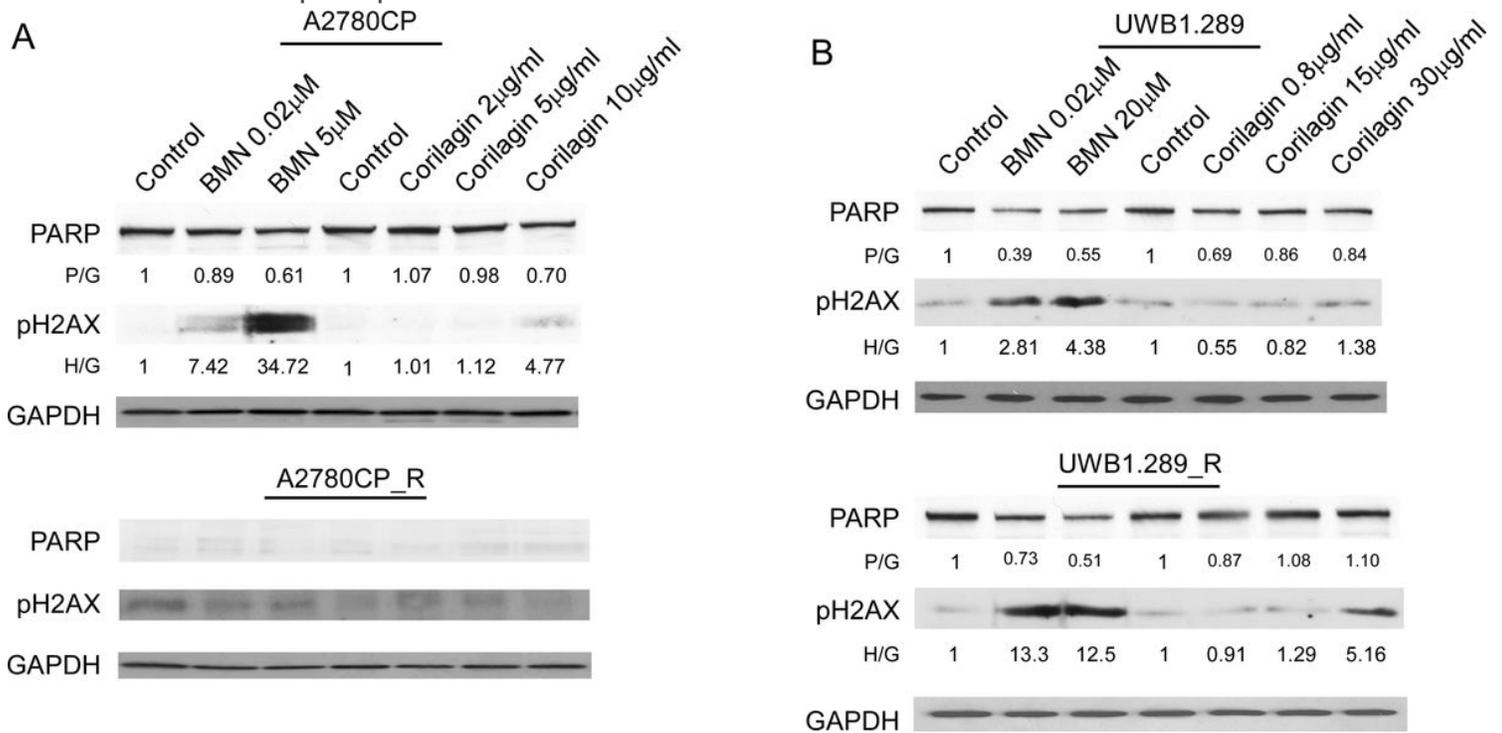


Figure 5

BMN673 down-regulated the expression of PARP and up-regulated pH2AX. Two pairs of PARPi sensitive/resistant ovarian cancer cells: (A) A2780CP/A2780CP_R; (B) UWB1.289/UWB1.289_R. were treated with corilagin (mg/ml), or BMN673 (mM) at IC10, IC25, IC50 concentrations for each pair, and analyzed with Western Blots. GAPDH was used as a loading control. P/G means the ratio of PARP/GAPDH by bands scan, H/G means the ratio of pH2AX/GAPDH by bands scan.

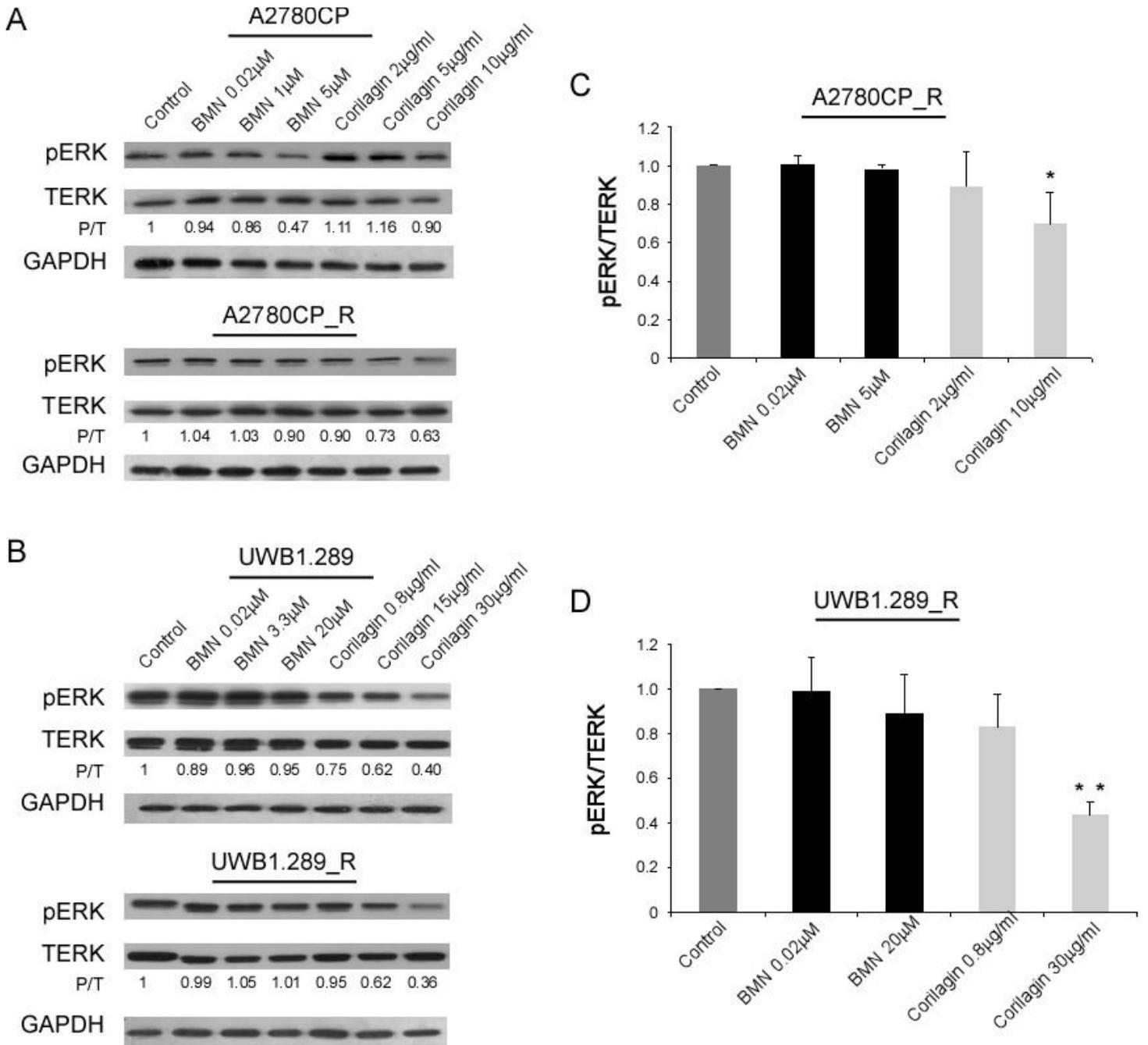
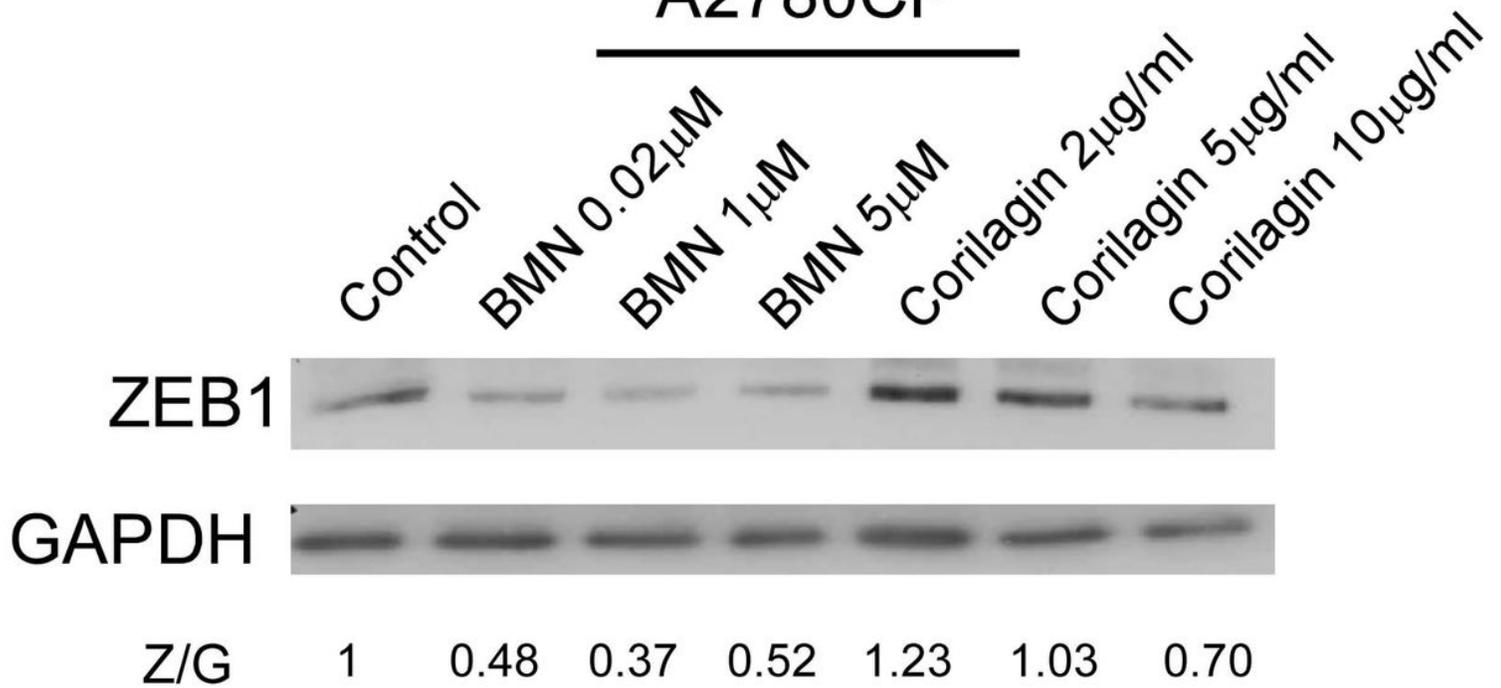


Figure 6

Corilagin inhibits the pERK pathway in resistant cells, whereas BMN673 does not. (A) A2780CP/A2780CP_R; (B) UWB1.289/UWB1.289_R were treated by corilagin (mg/ml), or BMN673 (mM) at the concentration of IC10, IC25, IC50 for each pair and used for Western Blots analysis. Total ERK (TERK) was used as a loading control; P/T means the ratio of pERK/TERK by bands scan. (C) A2780CP_R; (D) UWB1.289_R were treated by corilagin (mg/ml), or BMN673 (mM) at the concentration of IC10, IC50 and Western Blots analysis was repeated three times (* $p < 0.05$, ** $p < 0.01$)

A2780CP



A2780CP_R

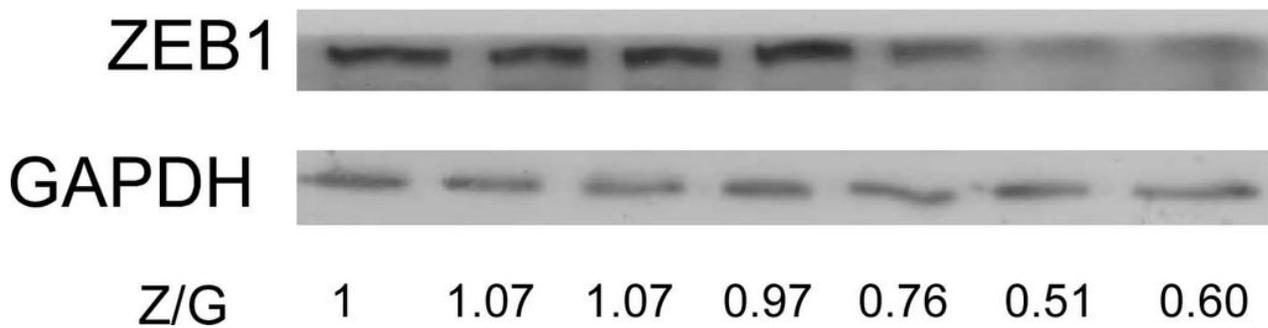


Figure 7

Corilagin down-regulates the expression of ZEB1 in A2780CP and A2780CP_R cells.

A2780CP/A2780CP_R were treated by corilagin (mg/ml), or BMN673 (mM) at the concentration of IC10, IC25, IC50 for each cell line and used for Western Blots analysis. GAPDH was used as a loading control.

Z/G means the ratio of ZEB1/GAPDH by bands scan