

RESEARCH ARTICLE

Blocking PARP activity with the inhibitor veliparib enhances radiotherapy sensitivity in endometrial carcinoma

Jing Wang^{1,2}  | Weizhen Xing³ | Yanling Lin³ | Nuray Uskenbayeva⁴ |
Hongchao Yan¹ | Yang Xu¹ | Lisha Fang¹

¹Department of Obstetrics and Gynecology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, China

²Department of Reproductive Medicine, The Second Affiliated Hospital of Soochow University, Suzhou, China

³Department of Gynecology, Sanya Women and Children's Hospital (Sanya Maternal and Child Health Care Hospital), Sanya, China

⁴Graduate School, Xuzhou Medical University, Xuzhou, China

Correspondence

Yang Xu and Lisha Fang, Department of Gynaecology and Obstetrics, The affiliated Hospital of Xuzhou Medical university, 99 Huaihai West Road, Xuzhou221002, Jiangsu Province, China.
Emails: zhizi20150823@126.com(Y.X.); 120335540@qq.com(L.F.)

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Abstract

Objective: Our study aimed to investigate the potential clinical utility of a poly(ADP-ribose) polymerase (PARP) inhibitor, veliparib (ABT-888), as a radiosensitizer in the medication of endometrial carcinoma (EC).

Methods: Human Ishikawa endometrial adenocarcinoma cells were treated with veliparib, radiotherapy (RT), or combination treatment. The viabilities, radiosensitivity enhancement ratio (sensitizer enhancement ratio (SER), and apoptosis of Ishikawa cells were, respectively, evaluated by Cell Counting Kit-8 (CCK-8), colony formation experiment, and flow cytometry. The tumor growth was assessed by xenograft mice models. Western blot assay investigated the expression of DNA damage and apoptosis-related proteins in vivo and in vitro.

Results: Cell Counting Kit-8 revealed that the 10% inhibition concentration (IC_{10}) and 50% inhibition concentration (IC_{50}) values of veliparib-treated Ishikawa cells were 1.7 and 133.5 μ M, respectively. The SER of veliparib combined with RT was 1.229 in vitro. Flow cytometry analysis results indicated that the apoptosis rate of the veliparib + RT group was markedly higher than that of the RT group in vitro ($p < 0.05$). Furthermore, in vivo data revealed that veliparib + RT treatment significantly decreased tumor growth compared with single treatments of veliparib or RT and with the control group ($p < 0.05$). Then western blot confirmed the levels of anti-phospho-histone (γ H2AX), caspase-3, and B-cell lymphoma 2 (Bcl-2) associated protein X (Bax) were significantly higher in the veliparib + RT group, while the level of Bcl-2 was lower compared with that of the RT group ($p < 0.05$), both in vivo and in vitro.

Conclusion: Our results indicate that veliparib in combination with RT markedly improved the therapeutic efficiency in human endometrial carcinoma.

KEYWORDS

apoptosis, endometrial carcinoma, poly(ADP-ribose) polymerase, radiotherapy, veliparib

Jing Wang and Weizhen Xing contributed equally to this work and they should be considered as co-first authors.

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1 | INTRODUCTION

Endometrial carcinoma (EC) is the most frequent type of reproductive system cancer in women aged 55–65 years around the world. In the USA, there were 66,570 new cases of EC diagnosed in 2021, with 12,940 fatalities, and incidence rates have risen by around 1% per year in recent years.¹ The number of women diagnosed with EC will double to 122,000 cases per year by 2030 if current trends continue in the USA.² Although the preferred treatment for EC is staging surgery,³ high-risk factors of early EC mean that additional treatment, such as external pelvic radiotherapy (RT) or vaginal brachytherapy, is necessary. Clinical trials have confirmed the significant improvement of local control obtained by RT but an absence of survival benefit, and the rationale for the abandonment of RT for intermediate-risk EC has been confirmed.⁴ As RT is often limited by radiation resistance, it is important to elucidate targeted therapies to improve radiosensitivity.

Treatments targeting the DNA repair pathway have been promising for enhancing the effectiveness of RT in tumor cells.⁵ Poly(ADP-ribose) polymerases (PARPs) are a group of enzymes that serve a significant role in oncology therapy. Poly(ADP-ribose) polymerase-1 (PARP-1) was the first to be discovered and is the most abundant and the best characterized member of the PARP family.⁶ The main mechanisms of DNA injury repair are as follows: Base excision repair (BER), nucleotide excision repair, homologous recombination (HR), mismatch repair, nonhomologous end-joining, and single strand annealing.⁷ PARP is involved in BER DNA damage repair.⁸ DNA fracture can enhance the catalytic activity of PARP, leading to DNA damage binding to PARP via its notch site. PARP catalyzes nicotinamide adenine dinucleotide (NAD), reducing it to (ADP)-ribose and nicotinic acid.⁹ ADP-ribose then combines with PARP protein, further polymerizing histones and other DNA repair related proteins, forming the ADP-ribose polymer (PAR) which transfers the DNA repair protein to the site of damage.^{10,11}

It has been manifested that the inhibition of PARP expression induces lethality in HR pathway-deficient cancer cells, resulting in apoptosis.¹² A previous study revealed that the PARP inhibitor, veliparib (ABT-888), may be used as a chemosensitizer in combination with cytotoxic chemotherapy.¹³ Furthermore, the study indicated that veliparib was well tolerated when combined with cytotoxic agents at full dosages, unlike most other PARP inhibitors.¹⁴ Additionally, it has been revealed that veliparib elicited a clinical response in 37.5% (3 of 8) patients with breast cancer and breast cancer 1 (BRCA1) gene mutations in a phase II clinical trial.¹⁵ Veliparib has also been tested in stage I and II clinical trials in combination with RT for breast, ovarian, pancreatic, non-small cell lung, rectal cancers, and glioma.^{16–22}

The aim of our current study was to evaluate the effect of veliparib treatment administered at different concentrations and to assess Ishikawa cell sensitivity to veliparib when applied in combination with RT.

2 | MATERIALS AND METHODS

2.1 | Antibodies and reagents

Dojindo Molecular Technologies, Inc. provided the Cell Counting Kit-8 (CCK-8) assay. System Biosciences offered the Annexin V-FITC/PI (fluorescein isothiocyanate/propidium iodide) apoptosis detection kit. Anti-phospho-histone (H2AX; Ser139) was purchased from ABclonal Biotechnology Co., Ltd. Antibodies against caspase-3, B-cell lymphoma 2 (Bcl-2), Bcl-2 associated protein X (Bax), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were purchased from Proteintech Group, Inc.

2.2 | Cell culture and drug treatments

Human Ishikawa endometrial adenocarcinoma cells were obtained from the Institute of Obstetrics and Gynecology, Xuzhou Medical University Affiliated Hospital (Xuzhou, China) and cultured at 37°C in a humidified atmosphere with 5% CO₂ in Dulbecco's Modified Eagle Medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and also 1% penicillin/streptomycin (Gibco; Thermo Fisher Scientific, Inc.). Veliparib (ABT-888; Target Molecule Corp.) was dissolved in dimethyl sulfoxide (DMSO; Cayman Chemical Company) to 10 mmol/L stock solution aliquots and stored at –20°C. All experiments were operated according to the regulations and guidelines that pertain to biological studies.

2.3 | Cell counting Kit-8 assay

Ishikawa cells were seeded in 96-well plates at a density of 5000 cells per well and cultivated for 24 h until cell attachment was seen. In each group, six wells were set up, and different concentrations of veliparib (0, 0.5, 1, 2.5, 10, 25, 50, 100, 125, 150, and 200 μmol/L) were delivered for 24 h at 37°C in a humidified incubator with 5% CO₂. Following the addition of 10 μl CCK-8 solution, cells were cultured for another 2 h at 37°C in a humidified incubator with 5% CO₂. At a wavelength of 450 nm, the absorbance of each well was measured in optical density (OD). The following formula was used to compute the survival rate: Survival rate was calculated as follows: (Experimental group OD value of each well/blank control group OD) × 100%. A subcytotoxic 10% inhibition concentration (IC₁₀) was used as a follow-up concentration of veliparib.

2.4 | Colony formation assay

Radiotherapy and veliparib + RT groups were assessed in this experiment. Logarithmic phase cells were inoculated into three wells of a 6-well plate at a density of 800–3000 cells/well. After cells adhered to the wall of the well plate, veliparib was added to the drug group (veliparib + RT) 2 h prior to irradiation. Using a 6 MV Precise linear

accelerator (Elekta), the two groups were irradiated at doses of 0, 2, 4, 6, and 8 Gy as follows: Isocenter irradiation with an X-ray (dose rate of 1 Gy/min); the center of the irradiation field was 10×15 cm. A 1.5-cm thick equivalent organic glass was placed on the bottle surface. Irradiated cells were cultivated for 10–14 days at 37°C in 5% CO₂. After fixation, staining, and dry processing, the number of colonies was counted (>50 cells per colony) at each dose. The following formula was used to calculate the survival score (survival fraction; SF): Average colony number of the dose/(number of inoculated cells \times inoculation efficiency). The radiation sensitization ratio (sensitivity enhancement ratio; SER) was calculated as follows: D₀ (RT group)/D₀ (veliparib + RT group).

2.5 | Flow cytometry

Control, veliparib, RT, and veliparib + RT groups were assessed. Cells were inoculated at 3.5×10^5 cells per well into 6-well plates and grown for 24 h before receiving 2 Gy irradiation. Veliparib was added to the drug groups (veliparib and veliparib + RT) 2 h prior to irradiation. Cells were removed, digested, washed, and centrifuged at 400 \times g for 5 min after being cultured for 24 h. Annexin V buffer (500 μ l) was added, cells were resuspended, Annexin V/FITC (fluorescein isothiocyanate) (5 μ l), and propidium iodide (PI; 5 μ l) were added. Flow cytometry was performed to count cells after a 15 min incubation. The right upper and lower quadrants represent late and early apoptotic cells, respectively.

2.6 | Western blotting of Ishikawa cells

Cells were collected (veliparib was added to drug groups when cells adhered to the wall of the plates), and radioimmunoprecipitation assay (RIPA) buffer was used to lyse cells that had been grown for 24 h. The protein concentration in the sample was then adjusted. Protein samples (45 μ g) were isolated using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a constant current of 250 mA. After that, samples were transferred to polyvinylidene difluoride (PVDF) membranes, which were then closed for 2 h before being placed in the first antibody sealing fluid and incubated overnight at 4°C. Membranes were then incubated for 2 h at 4°C with horseradish peroxidase-conjugated immunoglobulin G (IgG) secondary antibodies (Secondary Antibody Dilution Buffer, 1:1000). The Enhanced Chemiluminescence (ECL) Plus reagent was used to develop color. With use of GAPDH as an internal control, ImageJ software version 1.52a (National Institutes of Health) was used to assess the film gray value. Relative target protein expression was calculated as follows: Gray value/gray reference expression value.

2.7 | Tumor xenografts in nude mice

Female BALB/c mice, aged 4 weeks, were purchased from Xuzhou Medical University's Experimental Animal Center (Xuzhou, China). The Animal Care and Use Committee of Xuzhou Medical University

gave its approval to all of the procedures. After 1 week of acclimation in a pathogen-free facility, Ishikawa cells in the logarithmic phase were processed into single-cell suspensions and implanted subcutaneously into the right thigh of each mouse (6×10^6 cells/mouse).²³ After 2 weeks, the average tumor size was 220–350 mm³. Mice were randomly assigned to one of the four groups ($n = 6$): (i) vehicle control (10% DMSO in PBS/10% daily for a total of 5 days; ingested through oral gavage), (ii) veliparib (50 mg/kg daily for a total of 5 days; ingested through oral gavage), (iii) 10 Gy fractionated RT (2 Gy daily for a total of 5 days), and (iv) veliparib and 10 Gy fractionated RT (5 \times 2 Gy fractionated RT). Tumor volume (mm³) was determined using the formula: Tumor volume = $L \times W^2 \times 0.5$, where L denotes the longest axis and W denotes the shortest. Mice were CO₂-sacrificed at the end of the experiment, and tumors were excised, weighed, and extracted for western blot analysis.

2.8 | Western blot analysis of tumor tissue

Tumor tissue (20 mg) was extracted from mice, after which 100 μ l protein lysis buffer was added. After homogenization, the samples were centrifuged at 16,000 \times g for 30 min at 4°C. The supernatant was obtained to measure protein concentrations. Proteins were separated by electrophoresis on a 12% SDS-PAGE gel and then transferred to PVDF membranes (EMD Millipore). After 2 h of blocking, the samples were put in the primary antibody sealing solution and incubated overnight at 4°C. After washing, the PVDF membranes were incubated at 4°C with horseradish peroxidase-conjugated secondary antibodies (Secondary Antibody Dilution Buffer, 1:1000; Wuhan Boster Biological Technology, Ltd.) for 2 h. Subsequently, enhanced chemiluminescence (ECL) fluid was added and ImageJ software version 1.52a was used to analyze the gray value of images (National Institutes of Health). The target protein's relative expression was calculated as follows: Gray value/gray reference expression value.

2.9 | Statistical analysis

For statistical analysis, SPSS 19.0 software (SPSS, Inc.) was used, and data were expressed as the mean \pm standard deviation. When comparing two groups, a *t*-test was used, and when comparing multiple groups, a one-way analysis of variance (ANOVA) was used. A statistically significant difference was defined as $p < 0.05$.

3 | RESULTS

3.1 | Inhibitory effect of veliparib on Ishikawa cell proliferation

To investigate the inhibitory effects of veliparib as a single agent on Ishikawa cellular toxicity, a CCK-8 assay was performed to evaluate cell proliferation and to calculate cell survival rate with

a concentration gradient (0, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 125, 150, and 200 $\mu\text{mol/L}$). The experiment was repeated three times. The results revealed that the OD value of cells in the respective concentration gradients ranged from 0.79 ± 0.01 to 0.27 ± 0.01 at a wavelength of 450 nm ($p < 0.05$; Figure 1A). The survival rate of cells in the concentration gradient ranged from $100.00 \pm 0.00\%$ to $34.31 \pm 0.02\%$. The IC_{10} and 50% inhibition concentration (IC_{50}) values were 1.7 $\mu\text{mol/L}$ and 133.5 $\mu\text{mol/L}$, respectively. The results revealed that the inhibitory effect of veliparib as a single drug treatment increased at higher concentrations ($p < 0.05$; Figure 1B). However, as veliparib exhibited dose toxicity to Ishikawa cells in vitro, the low dose IC_{10} value was selected for subsequent experimentation.

3.2 | Cell survival in cell colony-forming experiment

The current study aimed to determine if veliparib treatment enhanced the radiation effect of Ishikawa cells via colony formation assays. Cells in the veliparib + RT group were treated with veliparib 2 h prior to receiving radiation. The results of the cell colony-forming experiment revealed that veliparib treatment enhanced Ishikawa cell sensitivity to radiation. Additionally, veliparib treatment reduced the survival of Ishikawa cells treated with radiation, the effect of which was more prominent as the radiation dose increased. To determine whether veliparib promoted cell death, the average colony number and survival fraction of the RT and veliparib + RT groups were calculated ($p < 0.05$ vs. RT) at Gy dosages of 0, 2, 4, 6, and 8 Gy (Figure 2A,B). Furthermore, cell survival curves were plotted according to the single-hit multitarget model ($\text{SF} = 1 - [1 - \exp(-D/D_0)]^N$; $p < 0.05$ vs. RT; Figure 2C). The model's conclusions determined that the D_0 values of biological indicators (Gy) in the RT and veliparib + RT groups were 1.654 and 1.346, respectively. The results also indicated that veliparib treatment in combination with RT had a SER of 1.229 (1.654/1.346). SER was calculated as follows: D_0 (RT group)/ D_0 (veliparib + RT group).

3.3 | Veliparib enhances RT-induced cell apoptosis

Ishikawa cell apoptosis was analyzed following exposure to veliparib (1.7 $\mu\text{mol/L}$) or irradiation (2 Gy) via flow cytometry. The results showed that cells in the veliparib + RT group cultured for 24 h after 2 Gy irradiation exhibited a significantly higher apoptosis rate compared with the other three groups (all $p < 0.05$). The apoptotic rates of the Ishikawa cells in the veliparib + RT, RT, veliparib, and control groups were $35.53 \pm 1.63\%$, $16.27 \pm 1.10\%$, $9.97 \pm 1.56\%$, and $2.13 \pm 0.42\%$, respectively (Figure 3). These data indicated that the cytotoxic and cytostatic effects of veliparib in combination with irradiation were enhanced when compared with veliparib or irradiation administered alone.

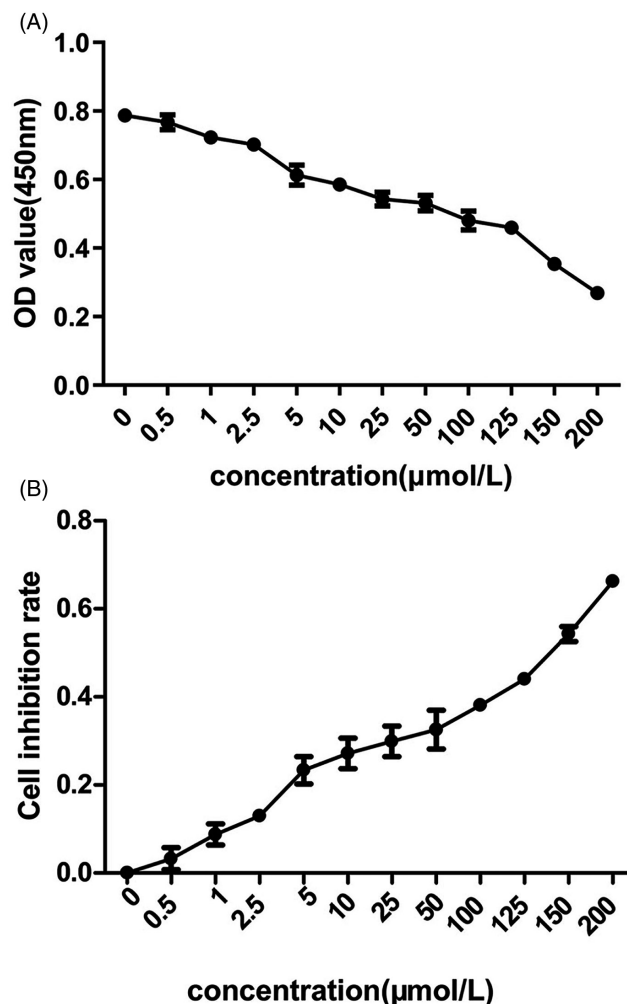


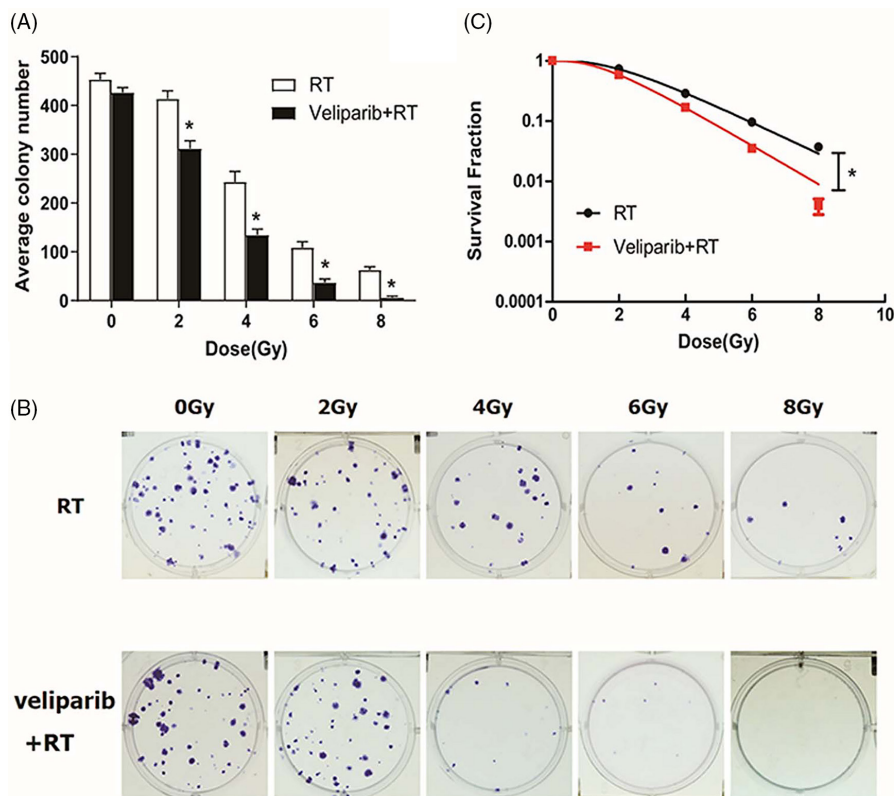
FIGURE 1 Inhibitory effect of veliparib on the proliferation of Ishikawa cells, as determined via the CCK-8 assay. (A) The OD values of Ishikawa cells treated at different concentrations were determined at a wavelength of 450 nm. (B) Ishikawa cell inhibition rate was calculated following veliparib treatment at different concentrations. CCK-8, Cell Counting Kit-8; OD, optical density

3.4 | Veliparib with RT significantly increases apoptotic protein expression

Veliparib is a PARP-1 and Poly(ADP-ribose) polymerase-2 (PARP-2) inhibitor,²⁴ and PARP-1 is responsible for nearly 90% of the universal PAR synthesis following DNA strand breakage.²⁵ Therefore, PARP-1 protein was selected for western blotting to verify our inhibition efficiency.

To elucidate veliparib's potential role in regulating double strand break (DSB) repair, γH2AX (a DNA double strand break-related protein) expression was detected using western blot analysis. Based on radiation survival assays, veliparib (1.7 $\mu\text{mol/L}$) was introduced 2 h prior to irradiation. Irradiation was performed using a dose of 2 Gy at room temperature. The results demonstrated that γH2AX expression increased by $6.14 \pm 0.63\%$ in the veliparib group compared with the control group ($p < 0.05$) and increased by $13.16 \pm 1.69\%$

FIGURE 2 Cell survival, as determined by a cell colony-forming assay. (A) Average Ishikawa cell colony number at each Gy dose. (B) Suppressive effects of RT, veliparib + RT on colony formation of Ishikawa cells ($n = 3$). The insets are representative images of a colony-forming assay. (C) Ishikawa cell survival curves plotted using the single-hit multitarget model. * $p < 0.05$ vs. RT. RT, radiotherapy



in the veliparib + RT group compared with the RT group ($p < 0.05$). The pro-apoptotic proteins Bax and caspase-3, as well as the anti-apoptotic protein Bcl-2, were analyzed using western blotting to further clarify the role of veliparib in apoptosis. The results revealed that the expression of Bax and caspase-3 increased by 7.79 ± 1.65 and $6.84 \pm 1.29\%$, respectively, and that the expression of Bcl-2 decreased by $23.99 \pm 1.40\%$ in the veliparib + RT group compared with the RT group in vitro ($p < 0.05$; Figure 4A,B). Collectively, the results revealed that veliparib shortage may induce a delay in DNA damage repair and cause cells to maintain greater γ H2AX levels to induce cell apoptosis.

3.5 | Transplanted tumor volumes and tumor weights of nude mice

Before treatment, there was no statistically significant difference in tumor size between the four groups of nude mice ($p > 0.05$). During treatment, the growth rate of transplantation tumors in control and veliparib-treated groups markedly increased compared with the RT and veliparib + RT groups. After treatment, the volume and weight of transplanted tumors in the veliparib + RT treatment group ($22.5 \pm 1.76 \text{ mm}^3$; $0.19 \pm 0.049 \text{ g}$) were smaller compared with the RT group ($27 \pm 0.18 \text{ mm}^3$; $0.27 \pm 0.033 \text{ g}$), veliparib treatment ($445.5 \pm 46.47 \text{ mm}^3$; $0.56 \pm 0.042 \text{ g}$) and control groups ($850 \pm 72.46 \text{ mm}^3$; $0.67 \pm 0.051 \text{ g}$). There were statistically significant differences between the groups ($p < 0.05$; Figure 5A-D).

3.6 | Veliparib with RT significantly increases apoptotic protein expression in vivo.

In each treatment group, Western blotting was used to detect the expression of γ H2AX. The results revealed that γ H2AX expression increased by $7.74 \pm 0.85\%$ in the veliparib group compared with the control group ($p < 0.05$) and increased by $20.15 \pm 0.29\%$ in the veliparib + RT group compared with the RT group ($p < 0.05$). To further define the role of veliparib in apoptosis, the pro-apoptotic proteins, Bax and caspase-3, and the anti-apoptotic protein, Bcl-2, were examined via western blotting. The results demonstrated that Bax and caspase-3 expression increased by $7.55 \pm 0.27\%$ and $4.93 \pm 0.85\%$, respectively, and the expression of Bcl-2 decreased by $31.03 \pm 1.90\%$ in the veliparib + RT group compared with the RT group ($p < 0.05$; Figure 6A,B). The current study assessed whether suppressing PARP-1 makes cancer cells more sensitive to ionizing radiation by causing apoptosis and/or enhancing DNA damage.

4 | DISCUSSION

The majority of studies hypothesized that the occurrence and development of EC involves complex biological processes such as oncogene activation and tumor suppressor gene loss. Theoretically, cell proliferation, invasion, and apoptosis can be regulated via vascular endothelial growth factor (VEGF), matrix metalloproteinases, Bcl-2 and certain caspases, etc.²⁶ Preliminary clinical studies have revealed that epidermal growth factor receptor (EGFR) inhibitors, mammalian

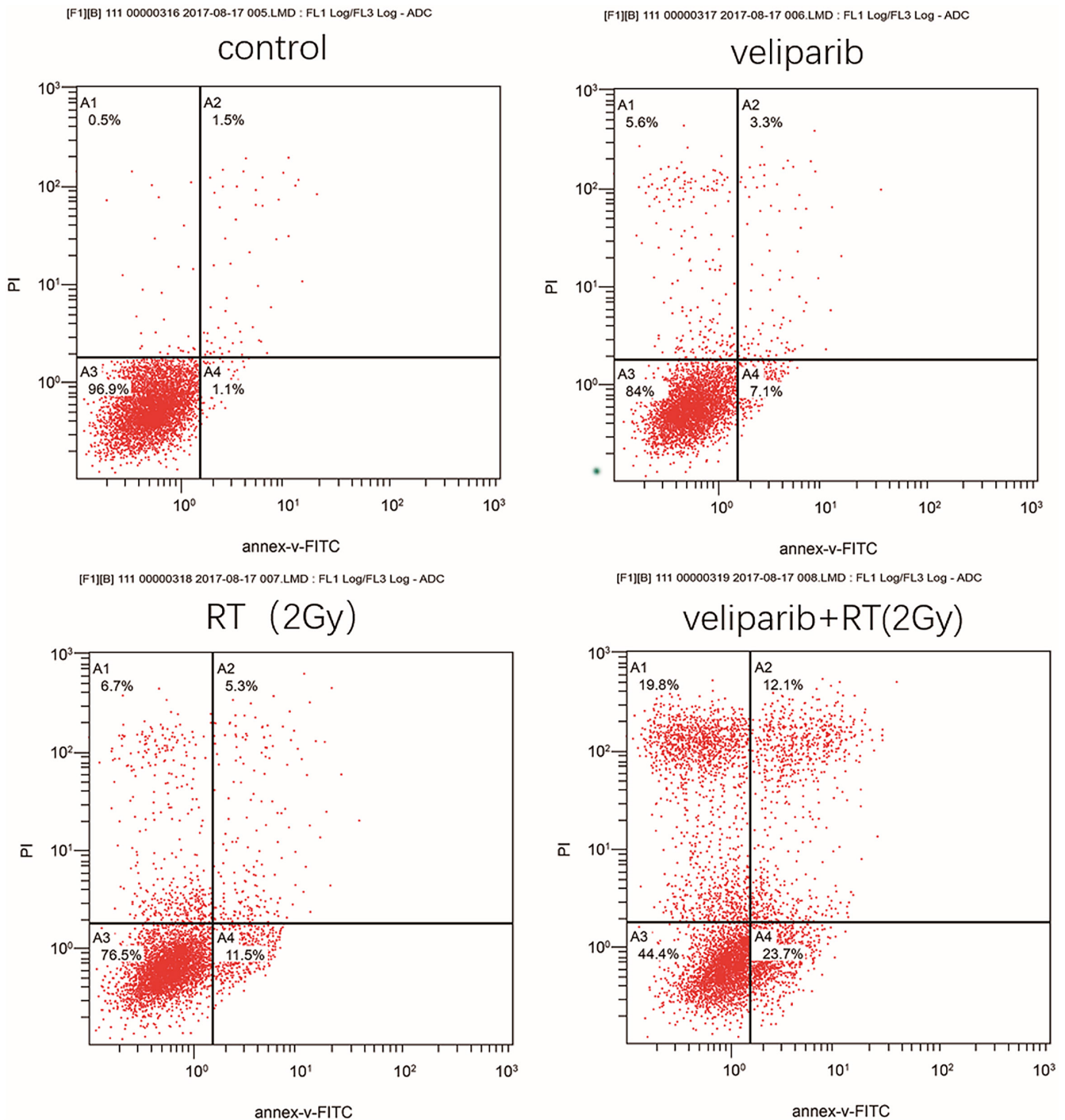


FIGURE 3 In vitro differential cell apoptosis rate. Apoptotic rate was measured via flow cytometry analysis. Cells in the veliparib + RT group cultured for 24 h after 2 Gy irradiation exhibited a significantly higher apoptosis rate compared with the other three groups ($p < 0.05$ vs. all groups)

target of rapamycin (mTOR) inhibitors, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mTOR pathway related PI3K inhibitors, and Akt inhibitors were effective for the treatment of advanced and recurrent EC, and further studies are required to confirm these data.²²⁻²⁷ However, no successful targeted therapy for EC-related genes has been demonstrated, making it important to elucidate an effective target therapy gene. Research has revealed that PARP, a DNA repair protein-activating factor, has a higher expression in

EC compared with normal endometrial tissue.²⁸ Our earlier experiments also determined that lentivirus-transfected Ishikawa EC cells with PARP gene silencing are associated with increased radiosensitivity in human EC Ishikawa cells.²⁹ In this study, veliparib, a PARP inhibitor, was selected to evaluate the radiosensitivity of Ishikawa EC cells treated in vitro and in vivo. Our present study proved a higher proportion of apoptotic cells in the veliparib + RT group, exhibiting higher expressions of γ H2AX, caspase-3, and Bax, and

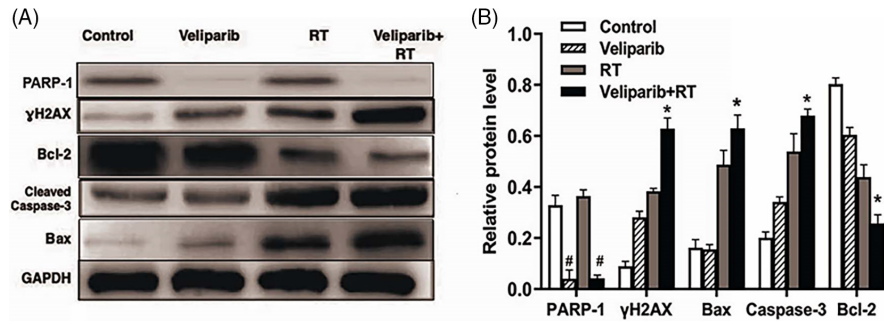


FIGURE 4 In vitro DNA damage and apoptosis-related protein expression in Ishikawa cells. (A) The relative protein levels of DNA damage and apoptosis-related proteins were measured via western blotting after Ishikawa cells were irradiated in vitro (2 Gy). (B) Quantification of protein levels. $p < 0.05$ vs. all groups. # $p < 0.05$ vs. control group. γ H2AX, Anti-phospho-histone; Bax, Bcl-2 associated protein X; Bcl-2, B-cell lymphoma 2; PARP-1, poly (ADP-ribose) polymerase-1

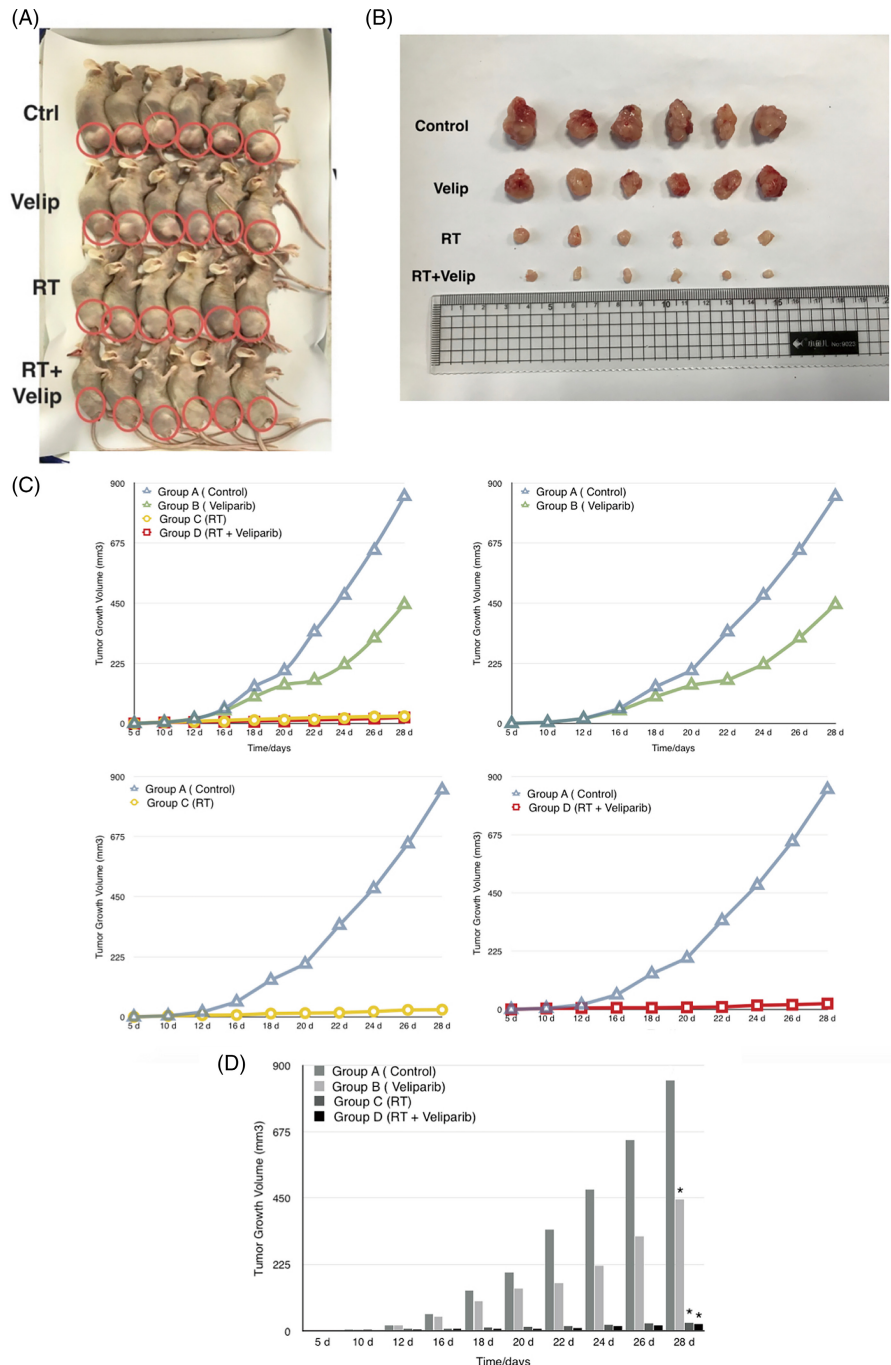


FIGURE 5 Tumor-bearing mice and xenograft tumor growth curves. (A) Tumor-bearing mice without xenograft rupture. (B) Xenografts were isolated for next-day detection after irradiation. (C) Tumor growth curves of Ishikawa xenografts. (D) Statistical analysis of the tumor volume changes $p < 0.05$ vs. control group

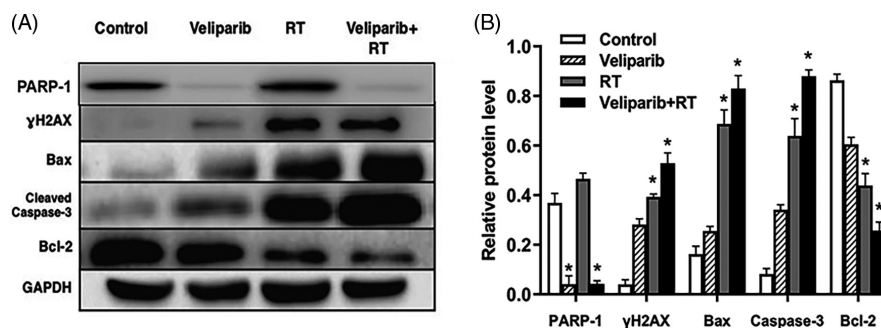


FIGURE 6 DNA damage and apoptosis-related protein expression of Ishikawa tumor xenografts. (A) Relative protein levels of γ H2AX, Bax/Bcl-2, and caspase-3 were measured via western blotting after Ishikawa cells were transplanted and treated (2 Gy \times 5 d). (B) Quantification of protein levels. $p < 0.05$ vs. control group. γ H2AX, Anti-phospho-histone; Bax, Bcl-2 associated protein X; Bcl-2, B-cell lymphoma 2; PARP-1, poly (ADP-ribose) polymerase-1

lower expressions of Bcl-2 protein. The radiation sensitization ratio of the veliparib + RT group was 1.229. It was determined that veliparib exerted radiosensitizing effects on Ishikawa cells. Although PARP inhibitors have been used in recent years as monotherapy or in combination therapy for EC,³⁰ veliparib has not been reported to enhance EC radiosensitivity.

Miyasaka et al.³¹ utilized the PARP inhibitor, Olaparib, for the evaluation of EC cell lines. The results indicated that Olaparib inhibited the proliferation of 16 EC cell lines and enhanced their radiosensitivity. Recently, Romana et al.²³ reported that a defect in the HR-related protein, MRE11, may be a cause of EC susceptibility to PARP inhibitors, indicating that the synthetic death caused by HR defects may result in PARP inhibitor sensitivity. EC has been proved to be a homologous recombination-deficient tumor. However, there is limited knowledge about the clinical significance of HR deficiency in EC.³² To obtain more convincing experimental results, our research team will further study the correlation between PARP inhibitors and HR defects in EC.

Ionizing radiation-induced DNA damage in tumor cells primarily manifests as single strand breaks (SSBs) and DSBs. Compared with normal tissue, in patients receiving RT and chemotherapy, the DNA damage repair pathway is overactivated in tumor cells and serves a negative regulatory role.³³ PARP serves a key role in BER after SSBs.³⁴ When PARP is inhibited, SSBs accumulate in cells and are converted to DSBs by fork folding.^{11,35} Research has demonstrated that γ H2AX phosphorylation is the most effective biological marker for the early detection of DSBs,³⁶ and it plays an important role in the regulation of apoptosis, as well as being strongly associated to the development, progression, and drug susceptibility of malignancies, serving key roles in the regulation of apoptosis.³⁷ The current study assessed the extent of DNA damage via western blotting, and the results revealed that Ishikawa cells significantly increased the formation of DSBs when treated with a low dose of veliparib in combination with RT in vitro. Olaparib has been revealed to exert a radiosensitization effect on ovarian cancer H460 and H1299 cell lines, and olaparib combined with RT significantly upregulates the expression of γ H2AX.³⁸ This result is consistent with those of the current study.

Apoptosis is one of the main biological effects of ionizing radiation exerted by tumor cells. Additionally, apoptosis regulates

mitochondrial membrane permeability, releasing active substances into the cytosol and inducing the caspase cascade and apoptosis via Bax and Bcl-2.³⁹ The current study indicated veliparib in combination with RT significantly induced cell apoptosis compared with veliparib administered alone, maybe via the Bax/Bcl-2 pathway and caspase-3 protein, which deserves further study.

A 2-part study to evaluate the incorporation of veliparib into chemoradiotherapy (CRT) for stage III non-small-cell lung cancer showed that veliparib with CRT was feasible and well tolerated.⁴⁰ A randomized phase II trial of veliparib, radiotherapy, and temozolomide in patients with unmethylated O⁶methylguanine-DNA methyltransferase glioblastoma illuminated that the veliparib-containing regimen was effective, safe, and well tolerated.²¹ While veliparib was investigated for its recent clinical progress at the time, many other PARP inhibitors have since been studied. Multiple clinical trials have examined or are examining the safety of PARP inhibitors such as olaparib (NCT03462342), rucaparib (NCT03521037), and niraparib (NCT04734665). In brief, PARP inhibitors have great potential application prospects, and there have been preliminary studies on endometrial cancer combined with radiotherapy, but veliparib combined with radiotherapy in the treatment of endometrial cancer has hardly been reported. Our current study provides support for the use of PARP inhibitors in endometrial cancer radiotherapy research.

In conclusion, the current study confirmed the radiosensitization effect of veliparib on Ishikawa EC cells and transplanted tumor xenografts. Veliparib enhanced the biological effects of irradiation by increasing the formation of DSBs and the expression of apoptosis-related proteins in irradiated Ishikawa cells. However, the further and precise mechanisms need to be elucidated and validated in more endometrial cancer cell lines. The findings could pave the way for the use of PARP inhibitors in combination with RT in the future and may also provide an enhanced radiosensitization effect for the clinical treatment of EC, inducing apoptosis by inhibiting DNA repair and increasing DNA DSBs.

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CONFLICT OF INTEREST

The authors state that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Available upon request.

ORCID

Jing Wang  <https://orcid.org/0000-0002-6533-4082>

REFERENCES

- Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2021. *CA Cancer J Clin*. 2021;71(1):7-33.
- Makker V, MacKay H, Ray-Coquard I, et al. Endometrial cancer. *Nat Rev Dis Primers*. 2021;7(1):1-22.
- Bogani G, Papadia A, Buda A, et al. Sentinel node mapping vs. sentinel node mapping plus back-up lymphadenectomy in high-risk endometrial cancer patients: results from a multi-institutional study. *Gynecol Oncol*. 2021;161(1):122-129.
- Creutzberg CL, Nout RA, Lybeert ML, et al. Fifteen-year radiotherapy outcomes of the randomized PORTEC-1 trial for endometrial carcinoma. *Int J Radiat Oncol Biol Phys*. 2011;81(4):e631-e638.
- Huang R, Zhou PK. DNA damage repair: historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. *Signal Transduct Target Ther*. 2021;6(1):1-35.
- Eleazer R, Fondufe-Mittendorf YN. The multifaceted role of PARP1 in RNA biogenesis. *Wiley Interdiscip Rev RNA*. 2021;12(2):e1617.
- Sharova NP. How does a cell repair damaged DNA. *Biochemistry (Mosc)*. 2005;70:275-291.
- Ronson GE, Piberger AL, Higgs MR, et al. PARP1 and PARP2 stabilise replication forks at base excision repair intermediates through Fbh1-dependent Rad51 regulation. *Nat Commun*. 2018;9(1):1-12.
- Langelier MF, Zandarashvili L, Aguiar PM, et al. NAD(+) analog reveals PARP-1 substrate-blocking mechanism and allosteric communication from catalytic center to DNA-binding domains. *Nat Commun*. 2018;9(1):1-13.
- Cerrato A, Morra F, Celetti A. Use of poly ADP-ribose polymerase [PARP] inhibitors in cancer cells bearing DDR defects: the rationale for their inclusion in the clinic. *J Exp Clin Cancer Res*. 2016;35(1):1-13.
- Harrison D, Gravells P, Thompson R, et al. Poly(ADP-Ribose) glycohydrolase (PARG) vs. Poly(ADP-Ribose) polymerase (PARP) – function in genome maintenance and relevance of inhibitors for anti-cancer therapy. *Front Mol Biosci*. 2020;7:191.
- Lee JM, Ledermann JA, Kohn EC. PARP Inhibitors for BRCA1/2 mutation-associated and BRCA-like malignancies. *Ann Oncol*. 2014;25(1):32-40.
- Rodler ET, Kurland BF, Griffin M, et al. Phase I study of veliparib (ABT-888) combined with cisplatin and vinorelbine in advanced triple-negative breast cancer and/or BRCA mutation-associated breast cancer. *Clin Cancer Res*. 2016;22(12):2855-2864.
- Audeh MW. Novel treatment strategies in triple-negative breast cancer: specific role of poly(adenosine diphosphate-ribose) polymerase inhibition. *Pharmacogenomics Pers Med*. 2014;7:307-316.
- Lehmann BDPJ, Tan AR. Triple-negative breast cancer: molecular subtypes and new targets for therapy. *Am Soc Clin Oncol Educ Book*. 2015:e31-e39.
- Jagsi RGK, Bellon JR, Woodward WA, et al. Concurrent veliparib with chest wall and nodal radiotherapy in patients with inflammatory or locoregionally recurrent breast cancer: the TBCRC 024 phase I multicenter study. *J Clin Oncol*. 2018;36(13):1317-1322.
- Argiris A, Miao J, Cristea MC, et al. A dose-finding study followed by a phase II randomized, placebo-controlled trial of chemoradiotherapy with or without veliparib in stage III non-small-cell lung cancer: SWOG 1206 (8811). *Clin Lung Cancer*. 2021;22(4):313-323. e311.
- Reiss KA, Herman JM, Armstrong D, et al. A final report of a phase I study of veliparib (ABT-888) in combination with low-dose fractionated whole abdominal radiation therapy (LDFWAR) in patients with advanced solid malignancies and peritoneal carcinomatosis with a dose escalation in ovarian and fallopian tube cancers. *Gynecol Oncol*. 2017;144(3):486-490.
- Tuli R, Shiao SL, Nissen N, et al. A phase 1 study of veliparib, a PARP-1/2 inhibitor, with gemcitabine and radiotherapy in locally advanced pancreatic cancer. *EBioMedicine*. 2019;40:375-381.
- Baxter PA, Su JM, Onar-Thomas A, et al. A phase I/II study of veliparib (ABT-888) with radiation and temozolomide in newly diagnosed diffuse pontine glioma: a Pediatric Brain Tumor Consortium study. *Neuro Oncol*. 2020;22(6):875-885.
- Sim HWM, Lwin KL, Barnes Z, et al. A randomized phase II trial of veliparib, radiotherapy, and temozolomide in patients with unmethylated MGMT glioblastoma: the VERTU study. *Neuro Oncol*. 2021;23(10):1736-1749.
- Czito BG, Deming DA, Jameson GS, et al. Safety and tolerability of veliparib combined with capecitabine plus radiotherapy in patients with locally advanced rectal cancer: a phase 1b study. *Lancet Gastroenterol Hepatol*. 2017;2(6):418-426.
- Koppensteiner R, Samartzis EP, Noske A, et al. Effect of MRE11 loss on PARP-inhibitor sensitivity in endometrial cancer in vitro. *PLoS One*. 2014;9(6):e100041.
- Berlin J, Ramanathan RK, Strickler JH, et al. A phase 1 dose-escalation study of veliparib with bimonthly FOLFIRI in patients with advanced solid tumours. *Br J Cancer*. 2018;118(7):938-946.
- Talhaoui I, Lebedeva NA, Zarkovic G, et al. Poly(ADP-ribose) polymerases covalently modify strand break termini in DNA fragments in vitro. *Nucleic Acids Res*. 2016;44(19):9279-9295.
- Werner HM, Berg A, Wik E, et al. ARID1A loss is prevalent in endometrial hyperplasia with atypia and low-grade endometrioid carcinomas. *Mod Pathol*. 2013;26(3):428-434.
- Ejskjaer K, Sorensen BS, Poulsen SS, et al. Expression of the epidermal growth factor system in endometrioid endometrial cancer. *Gynecol Oncol*. 2007;104(1):158-167.
- Ghabreau L, Roux JP, Frappart PO, et al. Poly(ADP-ribose) polymerase-1, a novel partner of progesterone receptors in endometrial cancer and its precursors. *Int J Cancer*. 2004;109(3):317-321.
- Wang J, Lu XY, Zhu XF, et al. Depletion of PARP-1 enhances the radiosensitivity of Ishikawa endometrial cancer cell. *Shandong Med J*. 2016;56:33-35. (in Chinese).
- Shen K, Yang L, Li FY, et al. Research progress of PARP inhibitor monotherapy and combination therapy for endometrial cancer. *Curr Drug Targets*. 2022;23(2):145-155.
- Aki Miyasaka KO, Ikeda Y, Wada-Hiraike O, et al. Anti-tumor activity of olaparib, a poly (ADP-ribose) polymerase (PARP) inhibitor, in cultured endometrial carcinoma cells. *BMC Cancer*. 2014;14(1):1-10.
- Siedel JH, Ring KL, Hu W, et al. Clinical significance of homologous recombination deficiency score testing in endometrial Cancer. *Gynecol Oncol*. 2021;160(3):777-785.
- Genois MM, Paquet ER, Laffitte MC, et al. DNA repair pathways in trypanosomatids: from DNA repair to drug resistance. *Microbiol Mol Biol Rev*. 2014;78(1):40-73.
- Masahiko S, Satoh TL. Role of poly(ADP-ribose) formation in DNA repair. *Nature*. 1992;356(6367):356-358.
- Chan CY, Tan KV, Cornelissen B. PARP inhibitors in cancer diagnosis and therapy. *Clin Cancer Res*. 2021;27(6):1585-1594.
- Tu WZ, Li B, Huang B, et al. γ H2AX foci formation in the absence of DNA damage: mitotic H2AX phosphorylation is mediated by the DNA-PKcs/CHK2 pathway. *FEBS Lett*. 2013;587(21):3437-3443.

37. Zhang YJ, Lu CR, Cao Y, et al. Imatinib induces H2AX phosphorylation and apoptosis in chronic myelogenous leukemia cells in vitro via caspase-3/Mst1 pathway. *Acta Pharmacol Sin.* 2012;33(4):551-557.
38. Kong Y, Xu C, Sun X, et al. BLM helicase inhibition synergizes with PARP inhibition to improve the radiosensitivity of olaparib resistant non-small cell lung cancer cells by inhibiting homologous recombination repair. *Cancer Biol Med.* 2021;18:1-32. 1:j.issn.2095-3941.2021.0178.
39. Li K, van Delft MF, Dewson G. Too much death can kill you: inhibiting intrinsic apoptosis to treat disease. *EMBO J.* 2021;40(14):e107341.
40. Argiris A, Miao J, Cristea MC, et al. A dose-finding study followed by a phase II randomized, placebo-controlled trial of

chemoradiotherapy with or without veliparib in stage III non-small-cell lung cancer: SWOG 1206 (8811). *Clin Lung Cancer.* 2021;22(4):313-323. e311.

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