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PRECLINICAL STUDIES

# The poly (ADP-ribose) polymerase inhibitor rucaparib suppresses proliferation and serves as an effective radiosensitizer in cervical cancer

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**Summary**

*Objectives* Our goal was to investigate the effects of rucaparib on the proliferation of cervical cancer cells and sensitivity to radiotherapy. *Methods* We used the human cervical cancer cell lines Hela and Siha and evaluated their viability and activity using various methods. Cellular proliferation was assessed by CCK-8 and clonogenic assays after treatment with rucaparib. Cell cycle analysis was performed using propidium iodide staining. Western immunoblotting analysis was used to detect the expression of cyclin D1 and CDK4. Immunofluorescence staining assay was performed to detect the expression of the DNA injury marker -H2AX after treatment with rucaparib and radiotherapy. Animal experiments were also performed to evaluate tumor size after treatment with rucaparib. Immunohistochemistry was performed to analyze the expression of Ki-67. *Results* Rucaparib suppressed proliferation, induced G2/M phase arrest, and reduced the expression of cyclin D1 and CDK4 in cervical cancer cells. When rucaparib was combined with radiotherapy in cervical cancer cells, clone formation decreased significantly and G2/M phase arrest was accentuated. The expression of the DNA-damage marker -H2AX was increased significantly, and rucaparib suppressed tumor growth *in vivo*. *Conclusions* Rucaparib exerts significant anti-proliferative effects and can serve as an effective radiosensitizer in cervical cancer, suggesting its candidacy in cervical cancer treatment and worthiness for further investigation.

**Key words** Rucaparib · Cervical cancer · Proliferation · Radiosensitivity

## Introduction

Eighty-five percent of cervical cancer cases across the world occur in developing countries, and it has become one of the most common gynecologic malignancies, seriously impairing women's health and lifespan [1, 2]. This situation still exists even though therapeutic methods have gradually improved. However, for advanced and recurrent cervical cancer, treatment efficacy remains unsatisfactory [3]. Ionizing radiation (IR) is a primary treatment for cervical cancer, but there are great differences in the effects of IR on patients [4]. Too much of an IR dose can lead to side effects, and too low a dose will lead to poor efficacy [5]. Therefore, we need to find a safe and effective radiosensitizer that can reduce side effects and achieve the desired therapeutic effect.

Poly (ADP-ribose) polymerase (PARP) is a single-strand DNA repair enzyme that is abundant in eukaryotic cells [6]. There are 18 primary subtypes, but only 6 play a role in DNA repair, and PARP-1 and PARP-2 are the most widely studied [7]. PARP can repair single-strand damage in the DNA replication process, and it plays important roles in maintaining chromosomal integrity, DNA damage repair, and genomic stability [8]. PARP inhibitors impede the activity of PARP, resulting in single-strand DNA breakage that cannot be repaired; and continuous breakage then accumulates in cells, resulting in transformation to double-strand DNA damage during DNA replication [9]. Tumor cells that manifest a functional deficiency in BRCA1/2 gene(s) cannot repair double-stranded DNA damage by homologous recombination (HR), resulting in a halt to DNA replication. This produces cellular toxicity and eventually cell death, with the PARP inhibitor exhibiting an anti-tumor effect [10]. Rucaparib is a PARP

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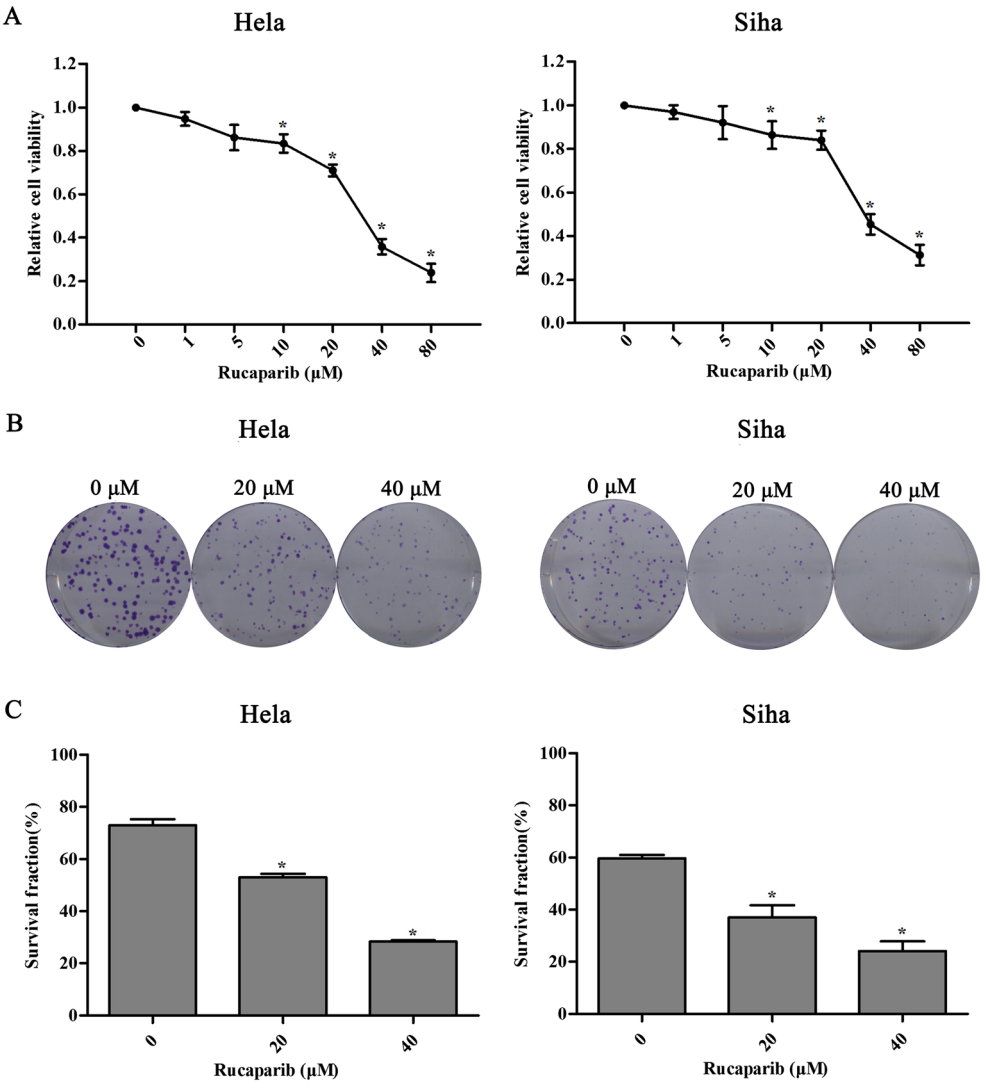



**Fig. 1** Rucaparib inhibits the proliferation of cervical cancer cells. **a** CCK-8 assay was performed on Hela and Siha cells that were treated with different concentrations of rucaparib for 72 h, and their relative viability was decreased in a dose-dependent manner. **b** A clonogenic assay was performed and representative images of Hela and Siha cells showed that clone formation decreased concomitantly with the increase in rucaparib concentration. **c** The survival fraction of Hela and Siha cells was decreased with the increase in rucaparib concentration. Survival fraction (%) = the numbers of cloned cells (more than 50 cells) /the numbers of seeded cells × 100% (*$p$<0.05)

inhibitor that primarily acts on PARP-1, and its molecular formula is C19H18FN3O.

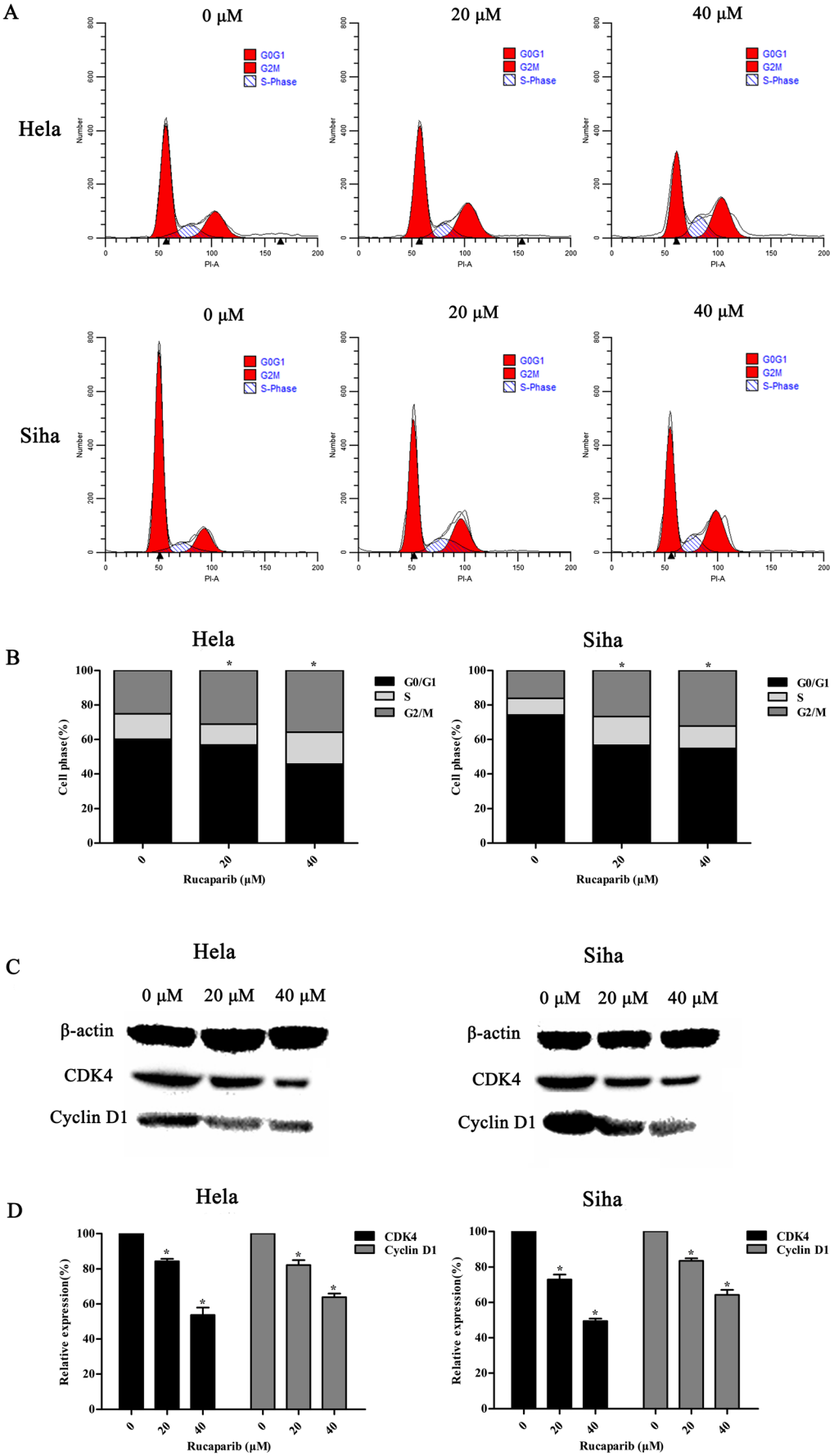
The study of rucaparib on cervical cancer and radiotherapeutic sensitivity has not yet been undertaken. Therefore, the purpose of the present study was to investigate the effects of rucaparib on the proliferation and radiosensitivity of cervical cancer, and to find novel modalities with which to treat cervical cancer and raise radiosensitivity to improve the prognosis for patients.

## Methods

### Cervical cancer cell lines and reagents

The human cervical cancer cell lines Hela and Siha were purchased from ATCC (American Type Culture Collection, USA) and stored in the Cancer Institute of the Affiliated Hospital of Jiangnan University. Hela cells were cultured in DMEM with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 ug/ml streptomycin. Siha cells were cultured in RPMI1640 with 10% fetal bovine serum, 100 units/ml penicillin, and 100 ug/ml streptomycin. These cell lines were cultured under standard conditions at 37°C with 5% $CO_2$ in compressed air and high humidity. Rucaparib was purchased from Abmole, resuspended in dimethylsulfoxide (DMSO), and stored at -80°C. FBS, bull

**Fig. 2** Rucaparib promotes G2/M cell cycle arrest in a dose-dependent manner in cervical cancer cells. **a** Flow cytometry was performed to analyze the cell cycle of Hela and Siha cells, and representative images showed that rucaparib induced G2/M phase arrest in a dose-dependent manner after 48 h of treatment. **b** ModFit 5.0 was used to analyze the results of cell cycle kinetics. The proportion of cells in the G2/M phase increased concomitantly with the increase in rucaparib concentration. **c** Western blot analysis was performed to analyze the expression of CDK4 and cyclin D1, and representative images showed that the expression of CDK4 and cyclin D1 decreased with the increase in rucaparib concentration after 48 h of treatment. **d** Quantification of changes in CDK4 and cyclin D1 of Hela and Siha cells. Untreated samples were denoted as 100% (*$p$<0.05)


A
0 μM
20 μM
40 μM
Hela
Siha
G0G1
G2M
S-Phase
Number
PI-A
B
Hela
Siha
G0/G1
S
G2/M
Cell phase(%)
Rucaparib (μM)
C
Hela
Siha
0 μM 20 μM 40 μM
β-actin
CDK4
Cyclin D1
D
Hela
Siha
CDK4
Cyclin D1
Relative expression(%)
Rucaparib (μM)

serum albumin (BSA), and CCK-8 kits were purchased from Sigma; and all of the primary antibodies to cyclin D1, CDK4, -H2AX, Ki-67, and β-actin were purchased from Cell Signaling Technology.

## Radiation

The X-ray source we used was a Faxitron Cabinet X-ray System (Faxitron, IL, USA). Irradiation methods were as follows: the gantry angle was 180°, dose rate was 200 cGy/min, and field size was 10 cm×10 cm. There was a compensation membrane of about 1.5-cm thickness on the surface of the cell, and SSD was 100 cm. All of the cells were treated at room temperature.

## Cell proliferation assay

During the logarithmic growth phase, Hela and Siha cells were seeded into 96-well plates at a concentration of 1500 cells/well and 2500 cells/well, respectively, for 24 hours. We discarded the original medium and treated cells with different concentrations of rucaparib (0, 1, 5, 10, 20, 40, or 80 umol/L) for a period of 72 hours. Subsequently, 100 ul of 10% CCK-8 medium was added to each well and cells were incubated for 1–4 hours at 37°C in 5% $CO_2$. The results were read by measuring the absorbance at 450 nm with a microplate reader (Tecan, Morrisville, NC, USA). The cellular survival rate (%) = [(the OD value of the experimental well - the OD value of the blank well) / (the OD value of the control well - the OD value of the blank well)] × 100%. Each experiment was repeated at least 3 times.

## Clonogenic assay

During the logarithmic growth phase, Hela and Siha cells were seeded into 6-well plates at a concentration of 300 cells/well for 24 hours. We discarded the original medium and treated cells with different concentrations of rucaparib (0, 20, or 40 umol/L) or different concentrations of rucaparib (0, 1, 5, or 10 μmol/L) combined with different IR doses (0, 1, 2, 4, or 6 Gy). After 10–14 days of culture, the cultures were terminated when the cloned cells could be visualized in the dish. The cells were fixed with 4% paraformaldehyde and stained with crystal violet, and the number of cell colonies was counted under a microscope (more than 50 cells were counted as a clone).

## Cell cycle analysis

During the logarithmic growth phase, Hela and Siha cells were seeded into 6-well plates at a concentration of $10^6$ cells/well for 24 hours. The cells were treated with different concentrations of rucaparib (0, 20, or 40 umol/L); or cells were divided into 4 groups—control, rucaparib (10 μmol/L), radiation (4 Gy), or rucaparib (10 μmol/L) combined with radiation (4 Gy). After 48 hours, the cells were harvested, washed, re-suspended, and fixed in 90% pre-chilled methanol at 4°C. The cells were then washed with phosphate-buffered saline (PBS) and re-suspended with PI (dyeing buffer:25×propidium iodide:RNase A, 400:15:1), followed by incubation for 30 min at 37°C in the dark. The percentage of cells in G0/G1, S, or G2/M phases was counted by flow cytometry. Each experiment was repeated at least 3 times.

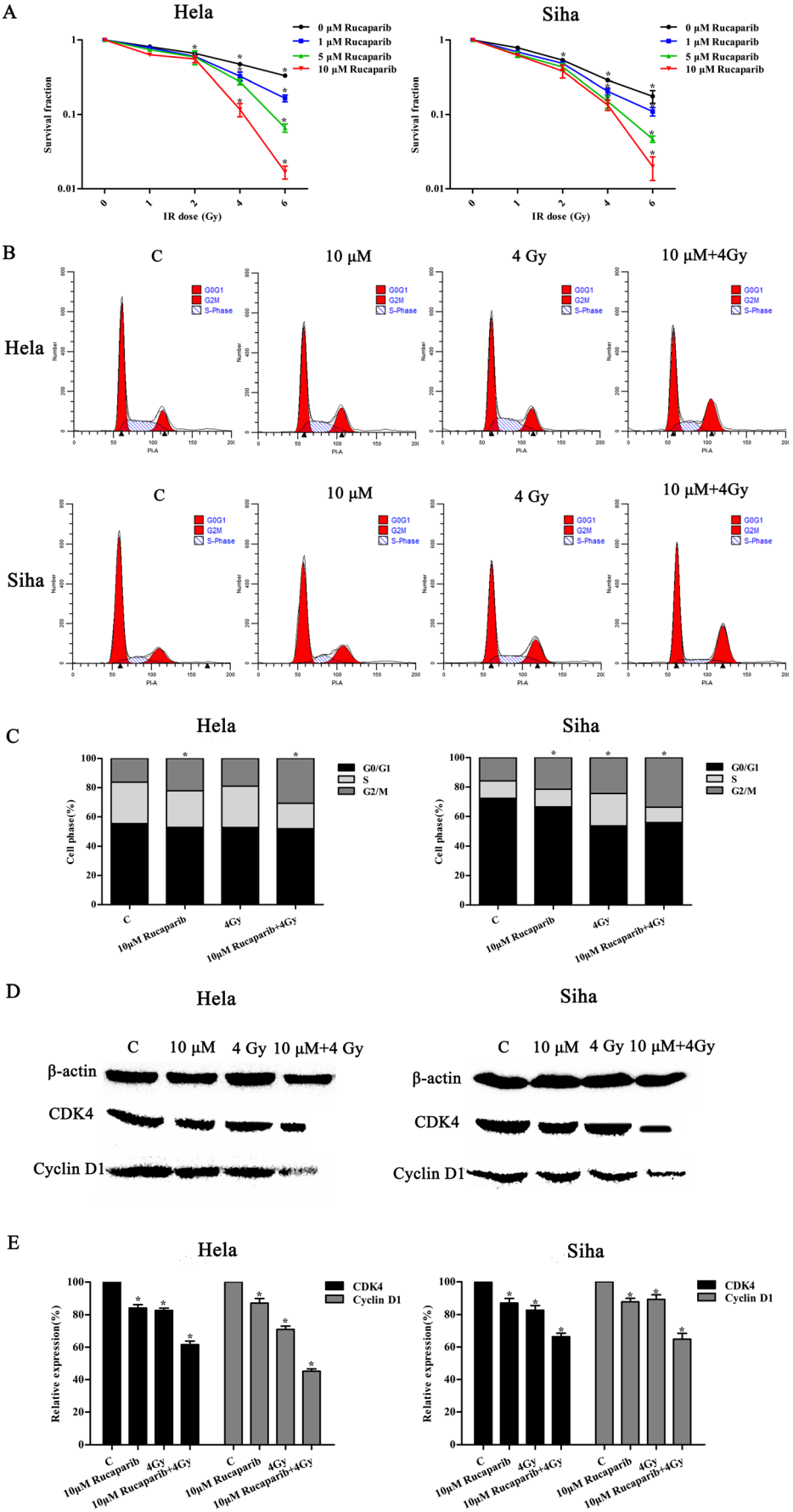
## Western blot analysis

The Hela and Siha cells were plated in 10-cm dishes at a concentration of $2 \times 10^6$ cells for 24 hours. Cells were treated with different concentrations of rucaparib (0, 20, or 40 umol/L); or cells were divided into 4 groups—control, rucaparib (10 μmol/L), radiation (4 Gy), or rucaparib (10 μmol/L) combined with radiation (4 Gy). After 48 h, total protein was extracted from cervical cancer cells using RIPA buffer (Thermo Fisher Scientific, Inc.,Waltham, MA) for 30 min on ice, centrifuged for 30 min at 14000 r/min, 4°C, and then quantified with a BCA assay kit (Thermo Fisher Scientific, Inc.,Waltham, MA). Equal concentrations of total protein were added and separated by 10% SDS-PAGE, transferred to PVDF membranes, and blocked in 5% non-fat milk for 2 hours. PVDF membranes with protein were incubated with primary antibodies overnight at 4°C. The next day, we incubated the membranes with secondary antibodies for 1 hour at room temperature. Finally, the membranes were washed with TBST and visualized with a Tanon-2500 imaging system. The relative expression of protein was calculated using the gray-scale value for each protein compared with the gray-scale value for β-actin.

## Immunofluorescence

Hela and Siha cells were seeded on cover slips in 6-well plates at a concentration of $10^6$ cells/well for 24 hours. Cells were divided into 4 groups: control, rucaparib (10 μmol/L),

**Fig. 3** Rucaparib enhances radiosensitivity in cervical cancer cells after irradiation. **a** A clonogenic assay was performed to show that the survival fraction of Hela and Siha cells was inhibited by rucaparib combined with IR. **b** Flow cytometry was performed to analyze the cell cycle of Hela and Siha cells, and representative images showed that rucaparib combined with IR promoted G2/M cell cycle arrest. **c** ModFit 5.0 was used to analyze the results of cell cycle kinetics. The proportion of cells in the G2/M phase increased when rucaparib was combined with IR compared with other groups. **d** Western blot analysis was performed to assess the expression of CDK4 and cyclin D1. Representative images showed that the expression of CDK4 and cyclin D1 was decreased when rucaparib was combined with IR compared with other groups. **e** Quantification of changes in CDK4 and cyclin D1 of Hela and Siha cells. Untreated samples were denoted as 100% (*$p$<0.05)


A
Hela
Siha
0 μM Rucaparib
1 μM Rucaparib
5 μM Rucaparib
10 μM Rucaparib
Survival fraction
IR dose (Gy)
B
C
10 μM
4 Gy
10 μM+4Gy
Hela
Siha
G0G1
G2M
S-Phase
C
D
Hela
Siha
C 10 μM 4 Gy 10 μM+4 Gy
β-actin
CDK4
Cyclin D1
E
Hela
Siha
CDK4
Cyclin D1
Relative expression(%)
C
10μM Rucaparib
4Gy
10μM Rucaparib+4Gy

radiation (4 Gy), or rucaparib (10 μmol/L) combined with radiation (4 Gy). At different time points (2 h and 24 h), the cells were fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.2% Triton X-100 for 20 min, blocked with 5% BSA at room temperature for 30 min, and incubated with primary antibody ( -H2AX) overnight at 4°C. The next day, we incubated cells with secondary antibody for 1 h in the dark, washed them with PBS 3 times, counterstained with DAPI for 5 min, washed with PBS 3 times, and mounted them on slides. Finally, we observed and photographed the cells under a fluorescence microscope (Olympus Optical, Japan).

### Immunohistochemistry

IHC was performed to analyze paraffin-embedded and formalin-fixed tumor tissue sections from mice. First, tumor tissue sections were deparaffinized, rehydrated, and antigen retrieved. Then, we incubated sections with primary antibody overnight at 4°C. The next day, cells were incubated with secondary antibody for 2 h. We visualized staining using DAB. Lastly, we observed and photographed sections under a light microscope.

### Animal experiments

Four-week-old female BALB/c nude mice (Beijing HFK Bioscience) were used in the experiments, and they were housed under pathogen-free conditions. The animal experiments were approved by the Jiangnan University Ethical Committee and performed strictly following the University Institution Animal Care and Use Committee guidelines. Hela cells ($10^7$) were implanted into the dorsum of 12 nude mice, which were then randomly divided into the control and rucaparib groups, with 6 mice per group. When the tumor volume reached 60 $mm^3$ (at about 10–14 d), we began to administer rucaparib intraperitoneally (8 mg/kg, 5 days per week). The weights of the mice and tumor size were measured every 3 days, and the tumor volume was calculated with the formula tumor volume = (length × $width^2$) /2. After 4 weeks, all of the mice were killed and the tumor tissues were collected for further experiments.

### Statistical analysis

We performed statistical analysis using IBM SPSS Statistics Version 20. The measurement data are presented as means ± standard deviation (SD). $\chi^2$ test and *t* test were applied for different types of data. Graph Pad Prism 5.0 and Image J were used to create photographs. $P<0.05$ was considered to be statistically significant.

## Results

### Rucaparib inhibits the proliferation of cervical cancer cells

We first investigated the effects of rucaparib on the proliferation of the cervical cancer cell lines Hela and Siha, which were treated with different concentrations of rucaparib. CCK-8 results showed that the viability of cells decreased significantly as the concentration of rucaparib gradually increased (Fig. 1a). The IC50 (half-maximal inhibitory concentration) of Hela cells is about 35 μM, and the IC50 for Siha cells is about 40 μM. Therefore, 20 μM and 40 μM were selected for the clonogenic assay. The results from the clonogenic assay showed that the effects of rucaparib on Hela and Siha cells were inversely related in a dose-dependent manner, and the survival fraction also decreased gradually, concomitantly with an increasing concentration (Fig. 1b, c). When the drug concentration was 40 μM, the survival fraction of Hela cells decreased more than 50%, and the survival fraction of Siha cells decreased about 50%, in accordance with the results of the CCK-8 experiments. These results suggested that rucaparib inhibits the proliferation of cervical cancer cells.

### Rucaparib induces cell cycle arrest in cervical cancer cells

Figure 1 shows the results that rucaparib inhibited the proliferation of cervical cancer cells, and that the effect was greater with an increasing concentration. We then explored these phenomena by evaluating cell cycle kinetics and apoptosis of cervical cancer cells by flow cytometry, and we found that rucapaprib exerted no obvious effect on apoptosis but it induced G2/M phase mitotic arrest (Fig. 2a). At rucaparib concentrations of 0 μM, 20 μM, and 40 μM, the proportions of Hela cells in the G2/M phase were 25.11%, 31.11%, and 35.78%, respectively; while the proportions of Siha cells in the G2/M phase were 16.09%, 26.71%, and 32.22%, respectively (Fig. 2b). With an increasing rucaparib concentration, the proportion of the cells that occupied the G2/M phase of the cell cycle increased, indicating that rucaparib induced G2/M phase arrest in cervical cancer cells. To further verify the effect of rucaparib on the cell cycle of cervical cancer cells, we evaluated the expression levels of CDK4 and cyclin D1 in cervical cancer cells treated with different concentrations of rucaparib. The results of Western blotting analysis showed that CDK4 and cyclinD1 protein expression was reduced as the concentration of rucaparib increased (Fig. 2c). Under the 3 different concentrations of rucaparib above, the relative expression of CDK4 in Hela cells was 100.00%, 84.33%, and 53.73%, respectively; and the relative expression of cyclin D1 in Hela cells was 100.00%, 82.24%, and 63.84%, respectively. The relative expression of CDK4 in Siha cells was 100.00%,

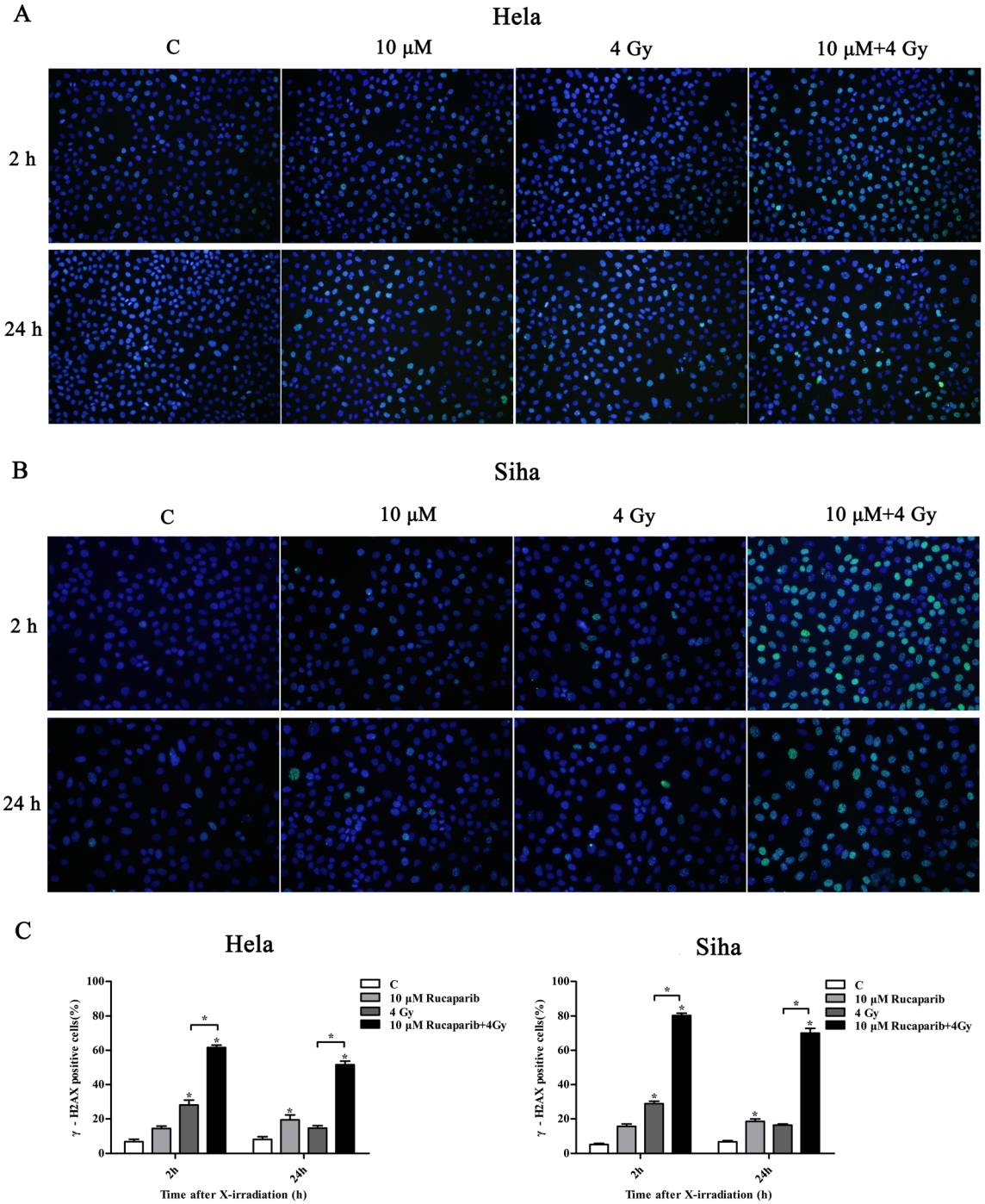



**Fig. 4** Effects of rucaparib and IR on the expression of -H2AX. **a** Immunofluorescence was performed to analyze the expression of -H2AX in Hela cells, and representative images are shown. **b** Representative images of -H2AX expression in Siha cells. (C) The number of -H2AX-positive cells was significantly increased in the combined rucaparib and IR group compared with other groups at 2 h and 24 h. -H2AX-positive cells were defined as cells with >10 -H2AX foci (*$p$<0.05)

72.98%, and 49.42%, respectively; and the relative expression of cyclin D1 was 100.00%, 83.52%, and 64.26%, respectively (Fig. 2d). The expression of cyclin D1 decreased with the increase in rucaparib concentration, further validating our hypothesis that rucaparib inhibits the proliferation of cervical cancer cells by cycle arrest.

## Rucaparib significantly enhances radiosensitivity and promotes G2/M cell cycle arrest in cervical cancer cells after irradiation

Clinically, we have been looking for ways to improve the sensitivity to radiotherapy in cervical cancer. Therefore, our

study not only verified the inhibitory effects of rucaparib on cervical cancer cells, but we also combined rucaparib with radiotherapy to observe whether rucaparib improved the radiosensitivity of cervical cancer. First, we chose different concentrations of rucaparib (0, 1, 5, and 10 μM), and combined these with different doses of radiotherapy (1, 2, 4, and 6 Gy). The clonogenic assay showed that rucaparib combined with radiotherapy inhibited the ability of cervical cancer cells to form colonies, with the effect exacerbated with an increasing drug concentration (Fig. 3a). Then, to further confirm that rucaparib improved the sensitivity to radiotherapy by cervical cancer cells, we chose a dose of 10 μM for rucaparib and 4 Gy to delineate cell cycle changes. The cell cycle results in the Hela cells showed that the G2/M phase of the combined group increased significantly from 16.20% to 30.70% compared with the control group; and that of the combined group increased compared with the radiotherapy group, from 19.00% to 30.70%. Similarly, the cell cycle results in the Siha cells showed that the G2/M phase of the combined group increased significantly from 15.79% to 33.69% compared with that of the control group; and the combined group increased compared with radiotherapy group, from 24.35% to 33.69% (Fig. 3b, c). We therefore showed that small concentrations of rucaparib can greatly improve the sensitivity to radiotherapy. When we further explored the effect of rucaparib combined with radiotherapy, we demonstrated that the expression of CDK4 and cyclin D1 in the rucaparib/radiotherapy group was lower than in the control group, which was also lower than in the radiotherapy-alone group ($P<0.05$) (Fig. 3d, e). We concluded that the combination of rucaparib and radiotherapy inhibited the proliferation of cervical cancer cells, and induced G2/M phase arrest to a greater degree than either alone, implying that rucaparib can enhance the radiosensitivity of cervical cancer cells.

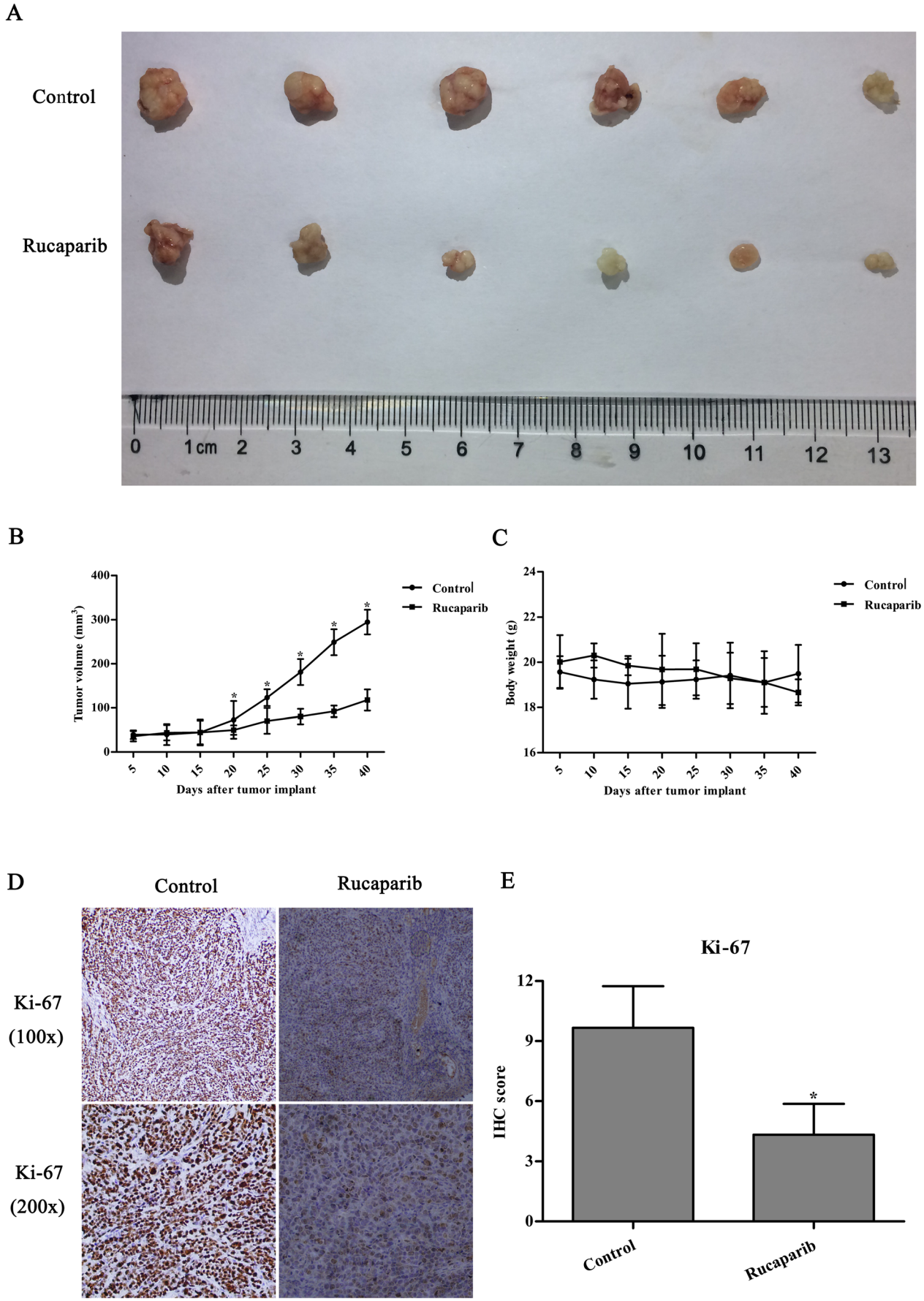
## Rucaparib blocks IR-induced DNA DSB repair in cervical cancer cells

When combined with radiotherapy, rucaparib enhanced the radiosensitivity of cervical cancer cells. IR mainly damages DNA, while rucaparib blocks the repair of DNA by PARP. Therefore, we hypothesized that the combination of rucaparib and radiotherapy would aggravate the degree of DNA damage. When we evaluated the expression of -H2AX by immunofluorescence to reflect the degree of DNA damage, the results showed that the expression of -H2AX in the combination group was significantly higher than in the control or either single group at 2 and 24 h after IR ($P<0.05$) (Fig. 4a, b). Compared with the radiotherapy group, the combined group increased from 28.15% to 61.66% at 2 h after IR, and the combined group increased from 14.62% to 51.62% compared with the radiotherapy group at 24 h after IR in Hela. Compared with the radiotherapy group, DNA damage in the combined group increased from 28.86% to 80.25% at 2 h after IR, and the combined group increased from 16.35% to 70.00% compared with the radiotherapy group at 24 h after IR in the Siha cells ($P<0.05$) (Fig. 4c). The radiotherapy group at 24 h after IR decreased in damage from 28.15% to 14.62% compared with the radiotherapy group at 2 h after IR in the Hela cells, while the combined group decreased from 61.66% to 51.62%. The DNA damage in the radiotherapy group at 24 h after IR decreased from 28.86% to 16.35% compared with the radiotherapy group at 2 h after IR in the Siha cells, while the combined group decreased from 80.25% to 70.00% ($P<0.05$) (Fig. 4c). Compared with the radiotherapy group, the overall expression of DNA damage markers decreased slowly in the combined group, implying that rucaparib can block IR-induced DNA DSB repair, and enhance the sensitivity to radiotherapy by cervical cancer cells.

## Rucaparib delays tumor growth in a mouse cervical cancer model

To support the hypothesis that the inhibitory effect of rucaparib will be observed in cervical cancer *in vivo* (emulating our work *in vitro*), we injected Hela cells into nude mice to elicit the formation of solid tumors. About 2 weeks later, the tumor volume reached 60 $mm^3$ and we then began to administer rucaparib by intraperitoneal injection (8 mg/kg, 5 times per week, for 4 weeks). The control group was given intraperitoneal injections of saline. After 4 weeks of treatment, the mice were killed and the tumors were removed, photographed, and measured. The tumor volume in the rucaparib treatment group was less than that in the control group ($P<0.05$), indicating that rucaparib inhibited tumor growth (Fig. 5a, b). The mice were weighed every 3 days, and they were between 18 and 22 g, with no statistical difference between the 2 groups (Fig. 5c). IHC of Ki-67 mainly reflects the proliferation of tumor cells, and our results showed that the expression of Ki-67 in the rucaparib group was lower than that in the control group ($P<0.05$) (Fig. 5d, e), indicating

**Fig. 5** Rucaparib significantly suppresses cervical tumor growth. **a** ▶ Representative images of tumors from the control and rucaparib groups (n = 6 for each group). **b** Rucaparib significantly suppressed tumor volume. **c** The weight of the mice ranged from 18 to 22 g, and there was no difference between the 2 groups. **d** Immunohistochemistry was performed to analyze the expression of Ki-67, and representative images are shown. **e** The IHC score for Ki-67 and expression of Ki-67 in the rucaparib group were lower than in the control group. The percentage of positive cells was scored as: 0 = 0–10%, 1 = 11–25%, 2 = 26–50%, 3 = 51–75%, or 4 = 76–100%. The staining intensity was scored as: 0 = negative, 1 = weak, 2 = intermediate, or 3 = strong. Scores of IHC = scores of staining intensity × scores of percentages of positive cells (*$p<0.05$)


A
Control
Rucaparib
0 1cm 2 3 4 5 6 7 8 9 10 11 12 13
B
Tumor volume (mm3)
400
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5 10 15 20 25 30 35 40
Days after tumor implant
Control
Rucaparib
C
Body weight (g)
24
22
20
18
16
5 10 15 20 25 30 35 40
Days after tumor implant
Control
Rucaparib
D
Control
Rucaparib
Ki-67 (100x)
Ki-67 (200x)
E
Ki-67
IHC score
12
9
6
3
0
Control
Rucaparib

that tumor cell proliferation was suppressed by rucaparib. To summarize our study, we showed that rucaparib inhibited the growth of cervical cancer *in vivo*, and this was consistent with the results we observed *in vitro*.

## Discussion

Surgery, chemotherapy, and radiotherapy are currently the principal treatments for cervical cancer. Although patients with early cervical cancer may undergo adequate treatment, for advanced and recurrent cervical cancer the prognosis is relatively poor [3]. Radiotherapy is one of the primary treatments for cervical cancer as IR produces ions and free radicals that can damage cancer cells (mainly by inducing DNA double-strand damage) [11]. Although cancer cells can produce a series of reactions to protect themselves and repair injury, when they fail to repair cellular injury induced by radiotherapy, they can become apoptotic or inactive. Even at the same tumor stage and pathologic condition, there is a great difference in the therapeutic effect of radiotherapy for patients with cervical cancer. For example, some patients in the mid- or late stages will exhibit obvious resistance to radiotherapy, which leads to tumor recurrence, metastasis, etc. [12] Therefore, it is necessary to find a safe and effective radiation sensitizer in order to achieve the best treatment effect while reducing adverse radiation-related reactions.

PARP is a type of DNA repair enzyme that was first reported in 1963 by Chambon et al. [13]. PARP is a ribozyme with a relative molecular mass of 116 KD, consists of 1014 amino acid residues, and plays a leading role in DNA single-strand repair [14]. PARP-1 as a receptor for gapped DNA, is activated after DNA injury, and recognized and bound to the DNA fracture site [15]. After binding to the DNA gap, PARP-1's catalytic activity is then increased 10- to 500-fold, and NAD+ is catalyzed to nicotinamide and ADP ribose by glycosylation and homodimer formation [16]. Using ADP ribose as a substrate, the nuclear receptor protein (mainly PARP itself) is glycosylated with poly ADP to form a linear PARP-1-ADP ribose polymer [17]. These poly ADP ribose branches then exhibit a greater negative charge and larger steric resistance, and can prevent the nearby DNA molecules from recombining with the damaged DNA; conversely, this can reduce the affinity between PARP-1 and DNA, dissociate PARP-1 from DNA breaks, and then guide the DNA repair enzyme to bind to the DNA gap and repair the damaged site [18, 19]. The PARP-1-ADP ribose polymer dissociates from DNA and is cleaved by poly ADP-ribose glycohydrolase (PARG), and the cleaved ADP ribose can be reused for the synthesis of NAD + by nicotinamide. After the PARP-1 is separated from the poly ADP ribose polymer, it is reactivated with DNA, and is repeatedly cycled to repair DNA damage [20, 21].

In 2005, investigators discovered that a small-molecule compound inhibited the activity of PARP, and it was subsequently named a PARP inhibitor [22]. The mechanisms underlying PARP inhibition are as follows. IR, alkylation, and oxidants can cause DNA damage, including DNA-single-strand breaks (SSBs) and DNA-double-strand breaks (DSBs). PARP inhibitors mainly then inhibit the repair of these single-strand gaps. SSB repair primarily modifies or replaces missing or incorrect bases through the base excision repair pathway (BER), while DSB repair includes HR and non-homologous terminal connection (NHEJ) [23–25]. HR, as an error-free repair pathway, uses a homologous sister chromosome fragment as a template to repair DNA damage. The NHEJ is forced to adhere to 2 gaps, which leads to excessive or missing nucleotides, and is prone to errors [17, 26]. PARP inhibitors then inhibit the repair of SSBs, and a large number of unrepaired SSBs can lead to the disintegration of the replication fork and further allow the transformation of SSBs to DSBs. Tumor cells with BRCA1 and BRCA2 mutations cannot undergo homologous recombination repair [18, 27, 28], as DNA strand damage depends on NHEJ for such an error-prone repair method; and the accumulation of such damage eventually leads to the death of tumor cells [29]. In2014, the United States Food and Drug Administration (FDA) officially approved the PARP inhibitor olaparib to be listed for advanced ovarian cancer patients with a BRCA mutation [30]. As one of the PARP inhibitors, rucaparib was approved by the FDA for the treatment of ovarian cancer in 2016. These studies show that rucaparib manifests a wide range of prospective actions, but it is now necessary to provide additional *in vivo* and *in vitro* research regarding rucaparib as a novel molecule in the future treatment of cervical cancer.

Our research showed that rucaparib effectively inhibited the proliferation of cervical cancer cells, and that the effect was augmented with an increasing concentration. Simultaneously, rucaparib induces G2/M phase mitotic arrest in cervical cancer cells, and cyclin D1 also decreased concomitantly with an increasing drug concentration. In addition, we found that the combination of rucaparib and radiotherapy significantly inhibited cellular proliferation and induced G2/M phase arrest, and increased DNA damage and delayed repair. Using just a small dose of rucaparib greatly increased the sensitivity of cervical cancer cells to radiotherapy *in vitro*, while, concordantly, our results showed that rucaparib inhibited tumor growth *in vivo*. Our immunohistochemical results showed that the proliferation of tumor tissues in the rucaparib-treatment group was reduced, suggesting that rucaparib was effective in the overall treatment of cervical cancer.

In conclusion, in the present study, we demonstrated that rucaparib inhibited the proliferation of cervical cancer cells *in vitro* and *in vivo*, and induced G2/M phase arrest in the cervical cancer cell cycle. In addition, rucaparib improved the radiosensitivity of cervical cancer and provides a novel choice for the clinical treatment of cervical cancer.

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## Compliance with ethical standards

**Conflict of Interest** Author Mei Tang declares that he has no conflict of interest.

Author Qiuli Liu declares that he has no conflict of interest.
Author Leyuan Zhou declares that he has no conflict of interest.
Author Ling Chen declares that he has no conflict of interest.
Author Xueqing Yang declares that he has no conflict of interest.
Author Jinjin Yu declares that he has no conflict of interest.
Author Yuan Wang declares that he has no conflict of interest.
Author Haifeng Qiu declares that he has no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Informed consent** For this type of study, formal consent is not required.

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