

Antiproliferative and pro-apoptotic effects of *Cyclocarya paliurus* polysaccharide and X-ray irradiation combination on SW480 colorectal cancer cells

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Abstract. The anti-hyperglycemic effects of *Cyclocarya paliurus* polysaccharide (CPP) have attracted increasing attention; however, limited research has been conducted on the potential effects of CPP on inhibiting tumor growth. The present study aimed to investigate the functions of CPP in combination with X-ray irradiation on colorectal cancer cells and the underlying mechanisms. SW480 cells were treated with various concentrations of CPP for 24, 48 and 72 h to determine cell viability using a Cell Counting Kit-8 assay. Then, the cells were divided into four groups as follows: Control, CPP (100 μ mol/l), 8 Gy and CPP + 8 Gy. The proliferation and apoptosis, and colony formation of cells were detected using flow cytometry and plate clone formation assays, respectively. Reverse transcription-quantitative PCR and western blot analyses were conducted to determine the expression of proliferation and apoptosis-associated, and PI3K/Akt signaling-associated genes. Treatment with 75 μ mol/l CPP for 48 h significantly decreased cell viability compared with untreated cells. CPP in combination with 8 Gy X-ray treatment significantly promoted the induction of apoptosis, and suppressed cell proliferation and clone formation compared with the control, CPP and 8 Gy groups. The detection of mRNA and protein expression levels by reverse transcription-PCR and western blotting demonstrated that CPP in combination with 8 Gy not only significantly decreased the expression of proliferation marker protein Ki-67, p53 and Bcl-2, but also upregulated the expression of cleaved caspase-3 and Bax, compared with the control. In addition, CPP and 8 Gy combined significantly attenuated the phosphorylation of PI3K and Akt. The present study demonstrated that the combination of CPP with X-ray irradiation suppressed SW480 cell proliferation and promoted cell apoptosis compared with the

control, CPP and 8 Gy groups. The underlying mechanisms may involve inhibition of PI3K/Akt signaling.

Introduction

Colorectal cancer (CRC) is one of the most common cancers threatening human life and health. In recent years, despite social progress, and improvements in living standards, dietary patterns and living habits, the incidence and mortality of CRC are increasing (1). It was reported that ~71,830 men and 65,000 women in the USA were diagnosed with CRC, and that ~26,270 men and 24,040 women succumbed to CRC in 2014 (2). Rectal cancer is the second most common type of colorectal cancer; in the USA, ~136,830 new cases of CRC are diagnosed annually, including 40,000 cases of rectal cancer (3). Rectal cancer can be asymptomatic during early stages, meaning that the majority of patients are diagnosed in advanced stages, and the incidence of local recurrence and distant metastasis following simple surgical treatment are high (4). At present, radiotherapy is one of the most important treatment methods for rectal cancer (5); however, its side effects and individual tolerance prevent increases in radiotherapy doses and limit the curative effects of tumor therapy (6,7).

Cyclocarya paliurus (Batal.) Iljinsk (*C. paliurus*), a plant of the genus *Cyclocarya* (Juglandaceae), is the sole species in its genus and is unique to China (8). It is mainly distributed in Southern China and grows at 420-2,500 m altitude in mountainous humid evergreen forests (8,9). Its branches and leaves taste sweet, have a cooling effect, and may reduce swelling and pain (8). From its leaves, Chinese populations produce health tea, commonly termed 'sweet tea'. Modern pharmacology studies have revealed that *C. paliurus* exhibits various biological activities, including antihyperglycemic, antihyperlipidemic, antihypertensive, anti-oxidative, immune-boosting and anticancer properties (10-13). Various bioactive components have been identified in *C. paliurus*; polysaccharide is one of its main active components (14,15). Polysaccharides and their complexes serve important roles in antitumor, anti-inflammatory, antiviral, antihyperglycemic, anti-aging and anticoagulant activities (16). *C. paliurus* polysaccharide (CPP) exhibits notably high bioactivity (17). At present, research has focused previous studies have focused on the anticancer effects of CPP (18). It can inhibit the growth of gastric cancer MGC803 cells and cervical cancer HeLa

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cells (8,17). Xu and Xu (19) reported that aloe polysaccharide induced pancreatic carcinoma autophagy in combination with radiation. It was hypothesized that CPP in combination with radiation may enhance radiotherapeutic sensitivity. Therefore, in the present study, CPP was combined with radiotherapy to investigate their roles in rectal cancer.

Materials and methods

CPP and cell culture. CPP was purchased from Xiehe Institute of Pharmacology. The content of CPP in *C. paliurus* was ~8.1% and the total sugar content was determined as 75.3%, which was mainly composed of glucose, arabinose, mannose and galactose.

SW480 cells were obtained from BeNa Culture Collection. The cells were cultured in DMEM containing 10% FBS (both Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and streptomycin at 37°C with 5% CO₂ in an incubator. When confluence reached ~75-90%, the cells were digested and subcultured.

Cell Counting Kit-8 (CCK-8) assay. SW480 cells were plated into 96-well plates at a seeding density of 5×10³ cells/well for 24 h. Then, CPP (25, 50, 75 and 100 μmol/l) was added, and the cells were incubated for 24, 48 or 72 h. CCK-8 solution (10 μl; cat. no. HY-K0301; MedChemExpress) was subsequently added to each well prior to incubation for a further 1 h at 37°C. Cell viability was determined by detecting the absorbance at 450 nm using a microplate reader (Thermo Fisher Scientific, Inc.).

Experimental grouping. Subsequently, cells were divided into four treatment groups: Control, CPP (100 μmol/l), Radiotherapy (8 Gy X-ray irradiation treatment) and combined treatment (100 μmol/l CPP + 8 Gy irradiation). Cells were incubated in DMEM containing 10% FBS with or without CPP overnight at 37°C. After 24 h, cells were treated with 8 Gy irradiation in the presence of CPP. Various assays were conducted in the subsequent 48 h following irradiation.

Flow cytometry. SW480 cell proliferation was analyzed using a flow cytometer and PE-Cy5.5 (cat. no. 1341; Tianjin Biolite Biotech Co., Ltd.)-conjugated anti-CXCR4 antibodies (cat. no. ab181020; Abcam). Cells (1×10⁶) were labeled with 50 μg CXCR4 PE-Cy5.5 for 15 min at 37°C. The labeled cells were washed twice with culture medium and then harvested. The resulting fluorescence was measured by flow cytometry (BD FACScanto II; BD Biosciences) and FlowJo 7.6.1 software (FlowJo LLC) (20).

Cells (1×10⁵) were seeded into 6-well plates and treated as aforementioned. Cells were harvested and washed twice with washing buffer. Then, the suspension was incubated with Annexin V-phycoerythrin and propidium iodide (PI; Beijing Solarbio Science & Technology Co., Ltd.) in the dark at 25°C for 15 min. The samples were analyzed by flow cytometry (BD FACScanto II and FlowJo 7.6.1) within 1 h.

Plate clone formation assay. SW480 cells were incubated in four dishes (1.5×10³ cells/dish). The cells were agitated gently and incubated at 37°C with 5% CO₂ for 10-15 days. Cells were constantly monitored and cultures were terminated when clones visibly appeared in the dish. The medium was discarded and

the cells were washed with PBS twice. Then cells were stained with crystal violet (0.1%) for 15 min at room temperature prior to washing with water. The number of colonies containing >50 cells was counted under a light microscope (magnification, ×4; Olympus Corporation) (21).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from culture cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. RNA was reverse transcribed to cDNA using a GoScript™ RT kit (Promega Corporation). RT was conducted at 37°C for 1 h and 90°C for 5 min prior to cooling. qPCR was performed using SYBR Fast qPCR Mix (Invitrogen; Thermo Fisher Scientific, Inc.) to determine proliferation marker protein Ki-67 (Ki67), p53, Bax, Bcl-2 and GAPDH expression levels. The primer sequences are presented in Table I. qPCR was conducted under the following conditions: 95°C for 15 min; 95°C for 10 sec; 40 cycles at 60°C for 30 sec and 60°C for 30 sec. The relative expression levels of the genes were calculated using the 2^{-ΔΔC_q} method (22) and normalized to GAPDH. All primers were synthesized commercially (Sangon Biotech Co., Ltd.).

Western blot analysis. Cellular proteins were extracted using RIPA lysis buffer (Beyotime Institute of Biotechnology). The samples were centrifuged at 12,000 × g for 10 min at 4°C, and the supernatants were collected. The concentration of proteins was determined using a bicinchoninic acid assay (Beyotime Institute of Biotechnology). Aliquots from supernatant with proteins were mixed with loading buffer and mercaptoethanol. Then, samples (50 μg/lane) were subjected to 10% SDS-PAGE and transferred to polyvinylidene fluoride membranes (EMD Millipore), which were blocked in 5% milk with 0.1% for 2 h at room temperature. Triton X-100 and incubated overnight at 4°C with various primary antibodies: Rabbit anti-Ki67 (1:1,000; cat. no. ab16667; Abcam), anti-p53 (1:1,000; cat. no. ab131442; Abcam), anti-cleaved caspase-3 (1:1,000; cat. no. ab2302; Abcam), anti-phosphorylated (p)-PI3K (1:1,000; cat. no. BS4605; Bioworld Technology, Inc.), anti-Akt (1:1,000; cat. no. 9272, Cell Signaling Technology, Inc.) and anti-GAPDH antibody (1:1,000; cat. no. ab22555; Abcam); and mouse anti-Bax (1:1,000; cat. no. sc-20067; Santa Cruz Biotechnology, Inc.), anti-Bcl-2 (1:1,000; cat. no. sc-7382; Santa Cruz Biotechnology, Inc.), anti-PI3K (1:1,000; cat. no. MAB2686; R&D Systems, Inc.) and anti-p-Akt (1:1,000; cat. no. sc-81433; Santa Cruz Biotechnology, Inc.). Following three washes in PBS, blots were incubated with horseradish peroxidase-conjugated goat anti-mouse (cat. no. ab205719) and goat anti-rabbit (cat. no. ab6721) secondary antibodies (both 1:100,000; Abcam) at room temperature for 1 h. Then, the blots washed with TBS four times for 5 min and treated with ECL reagent (Thermo Fisher Scientific, Inc.). The quantification of the relative expression of proteins was performed using Quantity One (version 4.4; Bio-Rad Laboratories, Inc.).

Statistical analysis. Statistical analysis was performed using Prism GraphPad version 6.0 software (GraphPad Software, Inc.). All data are presented as the mean ± standard deviation.

Table I. Primers used for reverse transcription-quantitative PCR.

Gene	Primer	Sequence
Ki67	Forward	5'-ATCATTGACCGCTCCTTTAGGT-3'
	Reverse	5'-TCCTTGGTAGTTCCGCTCG-3'
p53	Forward	5'-TAGTGTGGTGGTGCCCTATGAG-3'
	Reverse	5'-AGTGTGATGATGGTGAGGATGG-3'
Bax	Forward	5'-TGAAGACAGGGGCCTTTTGTG-3'
	Reverse	5'-GCTCACAGAGGCCGCTTAA-3'
Bcl-2	Forward	5'-ATGCCTTTGTGGAAGTATATGGC-3'
	Reverse	5'-CGTAGTGAGACCCACGTATGG-3'
GAPDH	Forward	5'-GAAGGTGAAGGTCGGAGTC-3'
	Reverse	5'-GAAGATGGTGATGGGATTTC-3'

Ki67, proliferation marker protein Ki-67.

Differences were performed using ANOVA following Tukey's multiple comparison test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Various concentrations of CPP affect the viability of SW480 cells. A CCK-8 assay demonstrated that cells treated with 25 and 50 $\mu\text{mol/l}$ CPP exhibited no notable change in viability; however, when cells were treated with 75 or 100 $\mu\text{mol/l}$ CPP, the viability of SW480 cells was significantly suppressed in a time-dependent manner (Fig. 1A). Treatment with 75 $\mu\text{mol/l}$ CPP for 48 h significantly decreased cell viability ($P<0.05$) compared with 0 $\mu\text{mol/l}$ CPP; the effect of treatment with 100 $\mu\text{mol/l}$ CPP for 48 h exhibited a more significant effect compared with 0 $\mu\text{mol/l}$ CPP at 48 h ($P<0.01$). Therefore, treatment for 48 h with 100 $\mu\text{mol/l}$ CPP was selected for subsequent experiments.

Combination of CPP with X-ray irradiation enhances the inhibition of cell proliferation and promotion of cell apoptosis in SW480 cells. Cell proliferation was detected via flow cytometry by CFSE staining. As presented in Fig. 1B and D, CPP ($P<0.05$) and 8 Gy X-ray irradiation ($P<0.01$) treatments significantly attenuated cell proliferation compared with the control. Furthermore, CPP in combination with 8 Gy irradiation exhibited a significant additive effect compared with CPP or 8 Gy ($P<0.01$). An apoptosis assay revealed that CPP single induced a significant increase in apoptosis compared with the control ($P<0.01$; Fig. 1C). Single 8 Gy irradiation promoted cell apoptosis more effectively than CPP ($P<0.01$). Additionally, CPP in combination with 8 Gy X-ray irradiation induced the most significant increase in cell apoptosis (17.55%) compared with the control (3.61%), CPP (7.26%) or 8 Gy (12.07%) treatments ($P<0.01$; Fig. 1C and E).

Combination of CPP with X-ray irradiation suppresses cell clone formation. As presented in Fig. 2, CPP ($P<0.05$; Fig. 2A and B) and 8 Gy ($P<0.01$) significantly inhibited clone

formation compared with the control; the inhibitory effect of 8 Gy was more pronounced than that of CPP ($P<0.01$). CPP in combination with 8 Gy further decreased clone number ($P<0.05$ vs. 8 Gy; $P<0.01$ vs. CPP and control).

Effects of combining CPP treatment with X-ray irradiation on the expression of proliferation and apoptosis-associated genes. To further study the effects of CPP in combination with irradiation on proliferation and apoptosis, the expressions of associated genes were evaluated. mRNA and protein analyses revealed that Ki67, p53 and Bcl-2 exhibited similar expression profiles. CPP and 8 Gy treatment significantly downregulated the mRNA expression levels of Ki67 and Bcl-2 compared with the control ($P<0.01$; Fig. 3A and D). The mRNA expression of p53 was further downregulated by 8 Gy compared with CPP treatment ($P<0.01$; Fig. 3B); the protein expression profiles of p53 and Bcl-2 presented similar results ($P<0.01$; Fig. 4A, C and F). Treatment with CPP did not significantly alter Ki67 protein expression compared with the control ($P>0.05$; Fig. 4A and B). CPP in combination with 8 Gy significantly reduced the expressions of Ki67, p53 and Bcl-2 at the mRNA and protein expression levels compared with the control or CPP ($P<0.05$). CPP in combination with 8 Gy reduced p53 and Bcl-2 mRNA expression (Fig. 3B and D), and Bcl-2 protein expression levels (Fig. 4A and F) to a notably similar degree as 8 Gy X-ray treatment alone ($P>0.05$). The expression profile of Bax in response to the various treatments opposed that of the other genes, as CPP and 8 Gy increased the expression of Bax at the mRNA and protein levels compared with the control (Figs. 3C; 4A and E). Additionally, CPP + 8 Gy significantly upregulated the mRNA and protein expression levels of Bax compared with the control, CPP or 8 Gy groups ($P<0.05$ vs. 8 Gy; $P<0.01$ vs. control or CPP). The protein expression profile of cleaved caspase-3 was similar to Bax; however, CPP in combination with 8 Gy did not significantly affect expression compared with 8 Gy ($P>0.05$; Fig. 4A and D).

CPP and X-ray irradiation combination may inhibit PI3K/Akt signaling. To investigate the underlying molecular mechanisms, PI3K/Akt signaling was evaluated by western blotting. The protein expression of PI3K and Akt was not affected by any of the treatments (Fig. 5A). Treatment with CPP did not significantly affect p-PI3K expression levels compared with the control ($P>0.05$; Fig. 5A and B); however, it significantly downregulated the expression of p-Akt ($P<0.01$; Fig. 5A and C). CPP in combination with X-ray irradiation significantly reduced the phosphorylation levels of PI3K and Akt compared with CPP or 8 Gy ($P<0.01$).

Discussion

Radiation therapy is frequently used for treating malignant tumors (23). It is regularly combined with surgical treatment and/or chemical drug treatment, which are the three major treatment methods for tumors. It is one of the most important treatment methods for treating advanced rectal cancer, including preoperative neo-adjuvant radiotherapy, intra-operative radiotherapy and postoperative adjuvant radiotherapy, which can effectively improve the local control rate and anal protection rate, and reduce the risk of tumor recurrence and

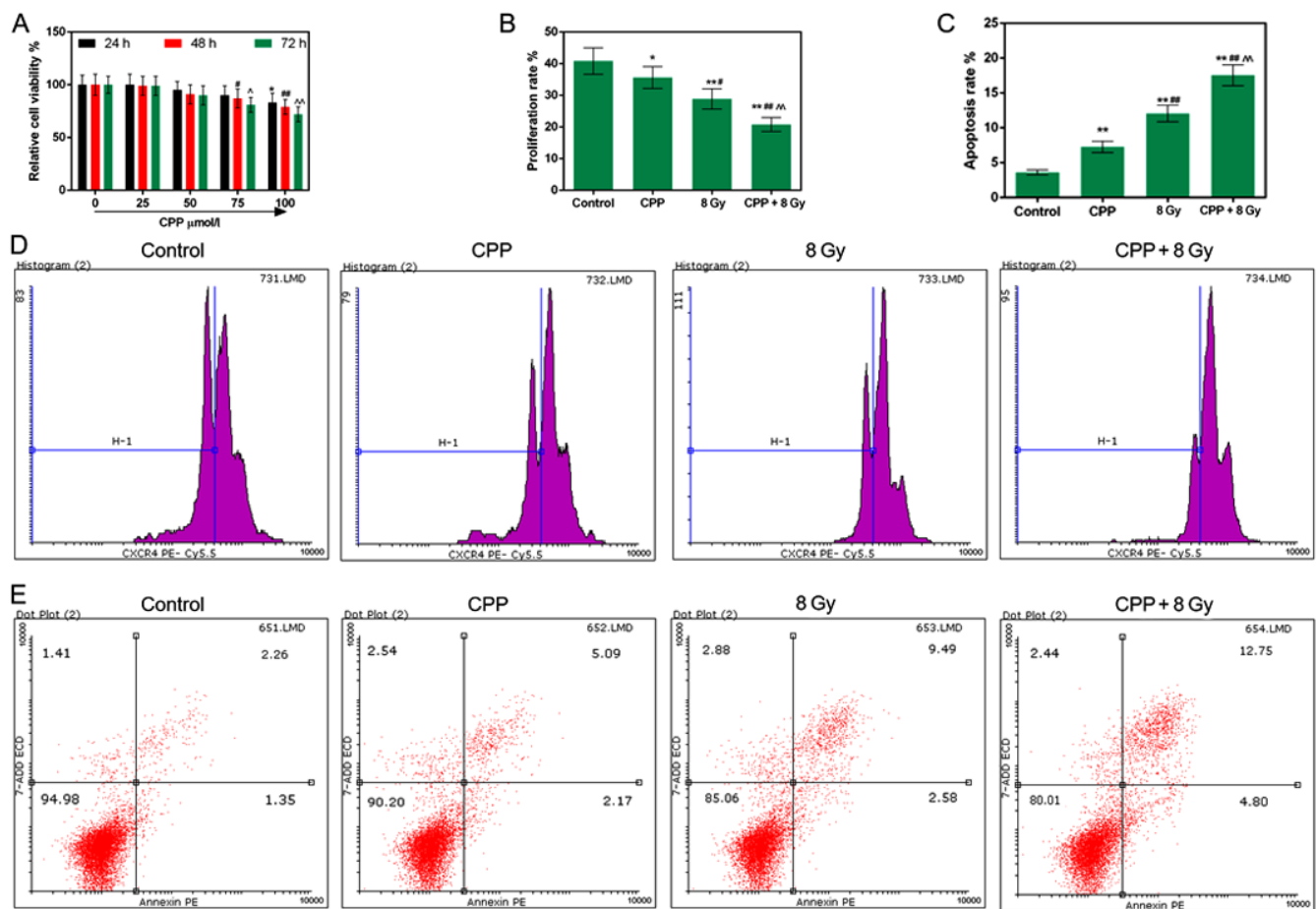


Figure 1. CPP affects cell viability, and combined CPP and X-ray irradiation treatment affects the proliferation and apoptosis of colorectal cancer cells. (A) Effects of treatment with various concentrations of CPP for 24, 48 or 72 h on the viability of SW480 cells. * $P < 0.05$ vs. 0 $\mu\text{mol/l}$ CPP at 24 h; # $P < 0.05$, ## $P < 0.01$ vs. 0 $\mu\text{mol/l}$ CPP at 48 h; ^ $P < 0.05$, ^^ $P < 0.01$ vs. 0 $\mu\text{mol/l}$ CPP at 72 h. (B) Proliferation of SW480 cells following treatment with 100 $\mu\text{mol/l}$ CPP for 48 h and/or 8 Gy irradiation. (C) Apoptosis rate of SW480 cells following the aforementioned treatments. * $P < 0.05$, ** $P < 0.01$ vs. control; # $P < 0.05$, ## $P < 0.01$ vs. CPP; ^^ $P < 0.01$ vs. 8 Gy. (D) Proliferation as determined by the detection of CXCR4 staining by flow cytometry. (E) Apoptosis as assessed by flow cytometry. Data are presented as the mean \pm standard deviation of three independent experiments. CPP, *Cyclocarya paliurus* polysaccharide; CXCR4, chemokine receptor type 4; Cy5.5, cyanine 5.5; PE, phycoerythrin; 7-AAD, 7-aminoactinomycin D.

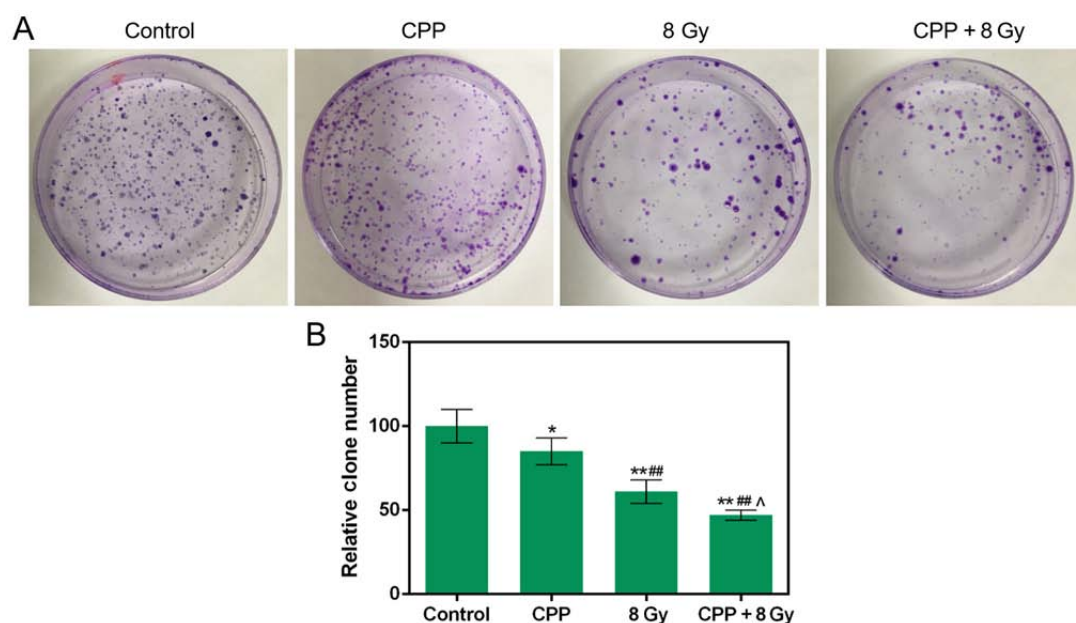


Figure 2. Effects of the combination of CPP and X-ray irradiation on colorectal cancer cell clone formation. (A) SW480 cells were treated with 100 $\mu\text{mol/l}$ CPP for 48 h and/or 8 Gy. (B) Relative clone number following treatment. Data are presented as the mean \pm standard deviation of three independent experiments. * $P < 0.05$, ** $P < 0.01$ vs. control; *** $P < 0.001$ vs. 100 CPP; ^ $P < 0.05$ vs. 8 Gy. CPP, *Cyclocarya paliurus* polysaccharide.

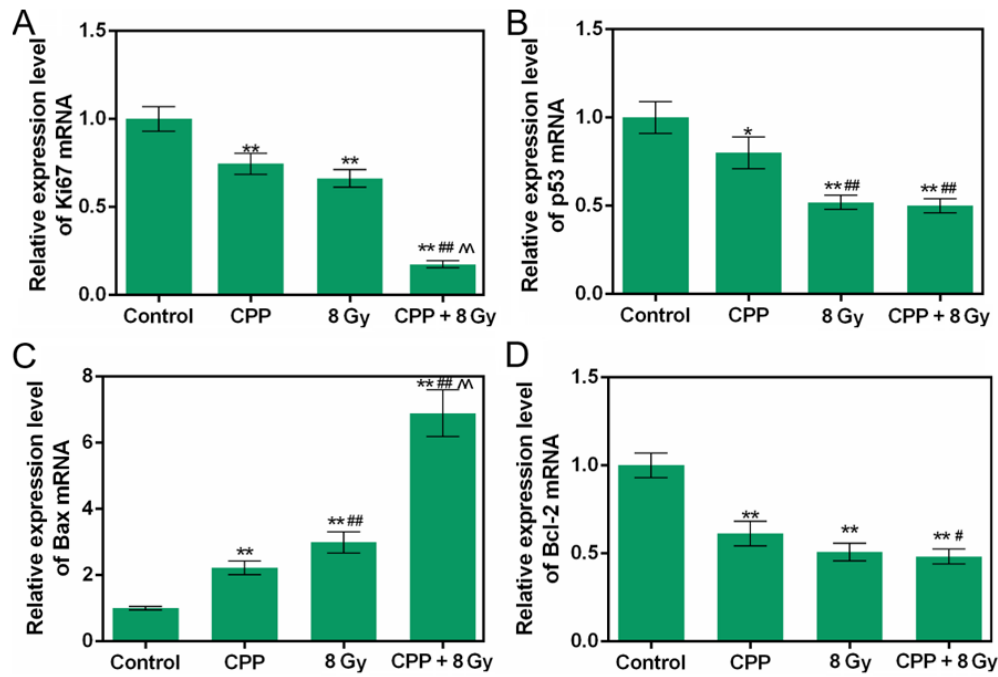


Figure 3. Effects of the combination of CPP and X-ray irradiation on the expression of proliferation and apoptosis-associated mRNAs in colorectal cancer cells. (A) Ki67, (B) p53, (C) Bax and (D) Bcl-2 mRNA expression following treatment with 100 μ mol/l CPP and/or 8 Gy irradiation was detected via reverse transcription-quantitative PCR. GAPDH served as an internal control. Data are presented as the mean \pm standard deviation of three independent experiments. *P<0.05, **P<0.01 vs. control; #P<0.05, ##P<0.01 vs. CPP; ^P<0.01 vs. 8 Gy. CPP, *Cyclocarya paliurus* polysaccharide; Ki67, proliferation marker protein Ki-67.

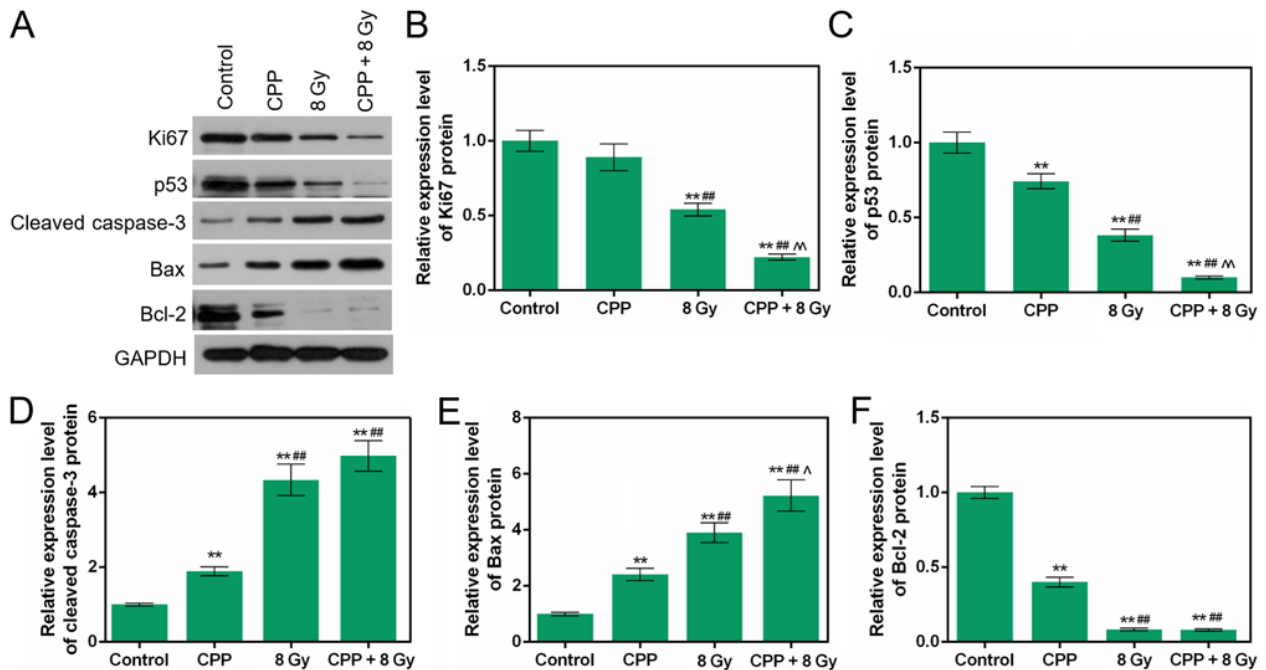


Figure 4. Effects of the combination of 100 μ mol/l CPP for 48 h and X-ray irradiation on the expression of proliferation and apoptosis-associated proteins in colorectal cancer cells. (A) Expression of Ki67, p53, cleaved caspase-3, Bax and Bcl-2 protein as determined by western blotting. Quantification of (B) Ki67, (C) p53, (D) cleaved caspase-3, (E) Bax and (F) Bcl-2 protein expression levels. GAPDH served as an internal control. Data are presented as the mean \pm standard deviation of three independent experiments. **P<0.01 vs. control; ##P<0.01 vs. CPP; ^P<0.05, ^^P<0.01 vs. 8 Gy. CPP, *Cyclocarya paliurus* polysaccharide; Ki67, proliferation marker protein Ki-67.

metastasis (24-30). In the treatment of rectal cancer, radiotherapy and chemotherapy are often used in combination, as chemotherapy often induces systemic toxicity (31). As technology advances, modern radiation therapy technology enables a high degree of conformity, including the use of

computed tomography images to provide high precision and precise treatment, optimizing treatment planning (32,33); however, the limitation of radiation exposure remains an issue with radiotherapy. Therefore, the present study focused on increasing the sensitivity of cells to chemotherapy.

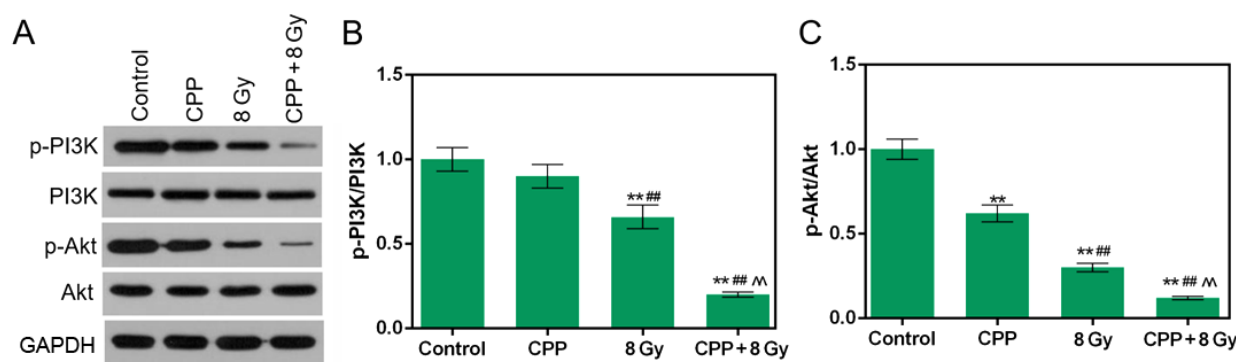


Figure 5. Effects of the combination of 100 μ mol/l CPP for 48 h and X-ray irradiation on PI3K/Akt signaling. (A) Expression of p-PI3K, PI3K, p-Akt and Akt protein as determined by western blotting. Ratios of (B) p-PI3K/PI3K and (C) p-Akt/Akt protein expression levels. GAPDH served as an internal control. Data are presented as the mean \pm standard deviation of three independent experiments. ** P <0.01 vs. control; ## P <0.01 vs. CPP; ^ P <0.01 vs. 8 Gy. CPP, *Cyclocarya paliurus* polysaccharide; p, phosphorylated.

The present study demonstrated that colorectal cancer cells treated with 8 Gy X-ray irradiation exhibited significantly inhibited cell proliferation, promoted apoptosis and suppressed clone formation. In the treatment of tumors, radiation therapy induces various side effects, including impairment of body immunity, local bone marrow suppression, severe gastrointestinal reactions and radioactive dermatitis (34-36). The concept of combined radiotherapy treatment has become one of the focuses of comprehensive tumor treatment in order to increase the sensitivity of tumor cells to radiotherapy, reduce the dose of radiotherapy required during the treatment of tumors and improve the efficacy of radiotherapy.

As an edible and medicinal plant, *C. paliurus* is widely used in the treatment of numerous diseases (37,38). Polysaccharide is the main active component of *C. paliurus* (17). As natural biological macromolecules, polysaccharides exhibit a variety of biological activities and are closely involved in the maintenance of biological processes; for example, various polysaccharides, including those from *Acanthopanax giraldui* Harms Var. *Hispidus* Hoo, deproteinized asparagus, *Rhizoma pleionis*, fungi and *Ganoderma lucidum*, exhibit inhibitory effects on tumor cells (39-43). The potential antitumor properties of CPP have received less attention; however, in the present study, it was observed that treatment for 48 h with 75 μ mol/l CPP significantly reduced the viability of SW480 cells. In addition, treatment with CPP inhibited proliferation, promoted cell apoptosis and suppressed clone formation.

CPP in combination with 8 Gy X-ray irradiation exhibited significantly enhanced antiproliferative and pro-apoptotic effects compared with CPP and radiotherapy alone. The expression profiles of proliferation and apoptosis-associated genes was in accordance with these observations. The Ki67 gene is a cell proliferation-associated gene, and is also considered one of the most reliable indicators of tumor cell proliferation (44). The Ki67 protein is a DNA-binding protein required for tumor cell proliferation and is expressed at all stages of tumor proliferation (45). p53 is one of the earliest discovered tumor suppressor proteins, and its functions include cell cycle arrest, promotion of apoptosis, genome stability maintenance and tumor angiogenesis inhibition (46). The antitumor effects of p53 are achieved by blocking the cell cycle and inducing apoptosis (47). Conversely, in the present study, it was observed that

CPP in combination with 8 Gy X-ray irradiation downregulated the expression of Ki67 and p53 at the mRNA and protein expression levels. Previous studies reported that >50% of tumor cells possess mutations in p53, and that mutant p53 may have the effect of promoting cell proliferation and inhibiting apoptosis (48,49). Additionally, a previous study reported that curcumin promoted apoptosis of colon cancer cells HCT-15 by inhibiting the expression of p53 (50). Further research is required into potential p53 mutations in the SW480 cells used during the present study. Caspase-3, located downstream of the caspase cascade, is considered to be an important mediator of apoptosis induction (51). Cleaved caspase-3 is the active form of caspase-3; therefore, cleaved caspase-3 is frequently used as an indicator of cell apoptosis (52). The present results demonstrated that CPP in combination with 8 Gy increased the protein expression of cleaved caspase-3 and regulated the Bax/Bcl-2 ratio.

The PI3K/Akt signaling pathway is an important signaling pathway in cells, and serves an important role in the proliferation of tumor cells and alterations in the morphology of apoptotic cells (53). Abnormal regulation promotes the expression of tumor-associated factors, and induces cell proliferation and malignant transformation (54,55). PI3K is a kinase located upstream of the Akt/mTOR signaling pathway. Abnormally activated PI3K promotes cell growth, proliferation and metastasis, epithelial-to-mesenchymal cell transformation and angiogenesis (56,57). Akt, also known as protein kinase B, is activated by PI3K (58). It was observed that CPP in combination with 8 Gy significantly inhibited PI3K and Akt phosphorylation. The results suggested that CPP in combination with 8 Gy inhibited PI3K/Akt signaling.

In conclusion, the present study demonstrated that CPP in combination with X-ray irradiation suppressed SW480 cell proliferation and promoted cell apoptosis. The potential underlying mechanisms may involve inhibition of the PI3K/Akt signaling pathway. Future experiments will involve *in vivo* studies to further explore the mechanisms underlying the therapeutic effects of combined CPP and irradiation treatment.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YJJ and ZZJ made substantial contributions to the conception and design of the study. ZZJ and SYJ were involved in data acquisition, analysis and interpretation. YJJ and SYJ drafted the manuscript and critically revised it for intellectual content. All authors gave approval for the final version of the manuscript to be published.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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