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Original Research Article

Radiosensitizing effect of polysaccharide in *Schisandrae chinensis* (Turcz.) Baill. Fruit on ovarian cancer SKOV3 cells

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Abstract

Purpose: *To investigate the radiosensitizing effect of polysaccharide from Schisandrae Chinensis fruit (SCFP) on ovarian cancer SKOV3 cell line.*
Methods: *The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate SKOV3 cell viability. Caspase-3, caspase-9, Bax, and Bcl-2 levels in SKOV3 cells were assessed by enzyme-linked immunosorbent assay (ELISA). Furthermore, mRNA expression of caspase-3, caspase-9, Bax and Bcl-2 in SKOV3 cells were determined using quantitative real-time polymerase chain reaction (RT-qPCR) assay.*
Results: *β-Irradiation (10 Gy) did not show obvious inhibitory effects on SKOV3 cell proliferation. However, SCFP at a dose of 1.25, 2.5 and 5 mg/mL significantly enhanced the radiosensitivity of SKOV3 cells to β-irradiation ($p < 0.01$). Furthermore, SCFP at concentrations of 1.25, 2.5 and 5 mg/mL significantly increased the levels of caspase-3, caspase-9 and Bax in SKOV3 cells, but decreased that of Bcl-2 ($p < 0.01$). In addition, SCFP combined with β-irradiation up-regulated mRNA expression of caspase-3, caspase-9 and Bax ($p < 0.01$), but down-regulated that of Bcl-2 in SKOV3 cells ($p < 0.01$).*
Conclusion: *SCFP significantly enhances the sensitivity of nasopharyngeal carcinoma SKOV3 cells to β-irradiation. The possible mechanism of sensitization might be correlated to the induction of mitochondria-mediated apoptosis. This research provides a scientific basis for the development of SCFP for the treatment of ovarian cancer.*

Keywords: *Radiosensitizing, Polysaccharide, Schisandrae chinensis fructus, β-Irradiation, Ovarian cancer, SKOV3 cell line*

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INTRODUCTION

Ovarian cancer has been reported to be the fifth leading cause of oncological-related death in women in the last four decades. In the last 40 years, the mortality rates for ovarian cancer have not significantly declined, with the estimated deaths in 2020 being 5 % of all cancer types [1]. The current treatments for ovarian cancer are mainly chemotherapy and surgery. As an important part of biotherapy, traditional Chinese medicine (TCM) can improve immunity and anti-cancer ability in patients, reduce the side effects of radiotherapy and chemotherapy, and improve

the sensitivity of patients to radiotherapy and chemotherapy [2]. In addition, the possible mechanisms of TCM ovarian cancer treatments include inhibiting tumor cell proliferation by inducing apoptosis, regulating body immunity, and improving the sensitivity of chemotherapy drugs [2].

Schisandrae Chinensis Fructus (SCF), or *Beiweizi* in Chinese, is the dried mature fruit of the *Schisandra chinensis* (Turcz.) Baill. plant, and this fruit is a well-known herbal medicine in China that is commonly used to treat cough, asthma, enuresis, persistent diarrhea, and insomnia [3]. Modern investigations have demonstrated that SCF contains different constituents, including lignans, volatile oils, organic acids, terpenes, flavonoids, and polysaccharides. Modern pharmacology has also shown that SCF possesses antitumor, antioxidant, hepatoprotective, and cardiovascular protective activities [4]. In recent years, several studies have reported that macromolecular compounds, particularly polysaccharides, possess antitumor, antioxidant, and immunoregulatory bioactive effects [5]. It has been reported that polysaccharides in SCF (SCFP) inhibit the proliferation of ovarian cancer SKOV3 cells [6], although the mechanism is unclear. Therefore, the present study aimed to analyze the radiosensitizing effect of SCFP on ovarian cancer SKOV3 cells and determine its possible underlying mechanism

EXPERIMENTAL

Chemicals and reagents

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and DMSO were purchased from Sigma Co. (Shanghai, China). Caspase-3, caspase-9, Bax, and Bcl-2 enzyme-linked immunosorbent assay (ELISA) kits were obtained from Beyotime Institute of Biotechnology (Shanghai, China). RPMI 1640 medium and FBS were purchased from Gibco Biotech. (Shanghai, China). RNA (Ribonucleic Acid) TRIzol Reagent was purchased from Servicebio Company (Wuhan, China). RevertAid First Strand cDNA Synthesis Kit was obtained from Thermo Fisher (MO, USA). All other reagents used in the experiments were of analytical grade.

Polysaccharide extraction from SCF

SCF (Schisandrae Chinensis Fructus) was processed into a powder and exhaustively extracted with 95 % ethanol for 12 h to remove pigments, monosaccharides, and small-molecule materials. The residue was air-dried and extracted in distilled water by refluxing. The extract was left to cool at room temperature, filtered, and concentrated to one-tenth the original volume. Then, ethanol was added slowly to a final concentration of 80 % and this solution was incubated at 4 °C overnight. After incubation, the precipitates were collected by filtering the solution, and were then washed three times with pure ethanol and acetone before being dried at 50 °C to obtain the crude polysaccharides (SCFP). The polysaccharide content in SCFP was 84.12 %.

Cell culture and β-irradiation

Ovarian cancer SKOV3 cell line was obtained from Shanghai SuRan Biotechnology Co., Ltd. SKOV3 cells were cultured in RPMI1640 supplemented with 10 % FBS and grown in an incubator with 5 % CO_2 at 37 °C. The cells were exposed to β-ray irradiation at serial or designed doses using a 2300 C/D linear accelerator (Varian, USA).

MTT assay

Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. SKOV3 cells (1×10^4 cells/well) were seeded in a 96-well plate and cultured for 24h. Then, cells were exposed to different concentrations of SCFP for 24 h. For the radiosensitivity assay, SKOV3 cells were exposed to β-irradiation in addition to the SCFP treatment. Optical density (OD) values were measured at 490 nm using a Multlskan Mk3 microplate reader (Thermo Fisher, Waltham, MA, USA). The experiment was repeated three times. Cell viability was expressed as the OD percentage of normal (untreated) cells.

ELISA assay

After SCFP and β-irradiation treatment, the supernatant of SKOV3 cells was harvested. Then, commercial ELISA kits were used to determine the levels of caspase-3, caspase-9, Bax, and Bcl-2 according to the manufacturer' s instructions.

Quantitative real-time polymerase chain reaction (RT-qPCR)

Total RNA from the SKOV3 cells was extracted using RNA TRIzol Reagent according to the manufacturer's instructions. Sample purity and concentration were determined according to their absorbance at 260 and 280 nm. Then, 2 μg RNA was reverse transcribed into cDNA using the

RevertAid First Strand cDNA Synthesis Kit according to the manufacturer's instructions. RT-qPCR was performed using an ABI StepOnePlus System (Applied Biosystems, CA, USA). The reaction process for the RT-qPCR was as follows: 95 °C for 30 s, cycles of 95 °C for 5 s and 55 °C for 30 s, and a final extension at 72 °C for 30 s. The expression levels of *caspase-3*, *caspase-9*, *Bax*, and *Bcl-2* were normalized to *β-actin* and analyzed using the 2-△△CT method.

Statistical analysis

All the data are expressed as mean ± standard deviation (SD). The student's *t*-test was used to evaluate the significance of differences observed in the results. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Antitumor effects of SCFP and β irradiation on cell proliferation of SKOV3 cells

The MTT assay was conducted to identify relative sub-toxic doses of SCFP and β-irradiation in SKOV3 cells. We found that SCFP treatment at doses of 10 ($p < 0.05$), 20 ($p < 0.01$), and 40 mg/mL ($p < 0.01$) exerted significant cytotoxicity on SKOV3 cells (Figure 1). Additionally, β-ray irradiation also showed notable cytotoxicity on SKOV3 cells at doses greater than 10 Gy (Figure 2). Based on these results, the β-ray irradiation at 10 Gy and 1.25, 2.5, and 5 mg/mL SCFP treatment were selected for subsequent experimentation.

SCFP enhanced radio-sensitivity of SKOV3 cells to β irradiation

The reported cytotoxicity of β-irradiation and SCFP (1.25, 2.5, and 5 mg/mL) combined treatment on SKOV3 cells is presented in Figure 3. We found that 10 Gy β-irradiation did not have significant inhibitory effects on the proliferation of SKOV3 cells ($p > 0.05$). Interestingly, SCFP treatment at concentrations of 1.25 ($p < 0.05$), 2.5 ($p < 0.01$), and 5 mg/mL ($p < 0.01$) significantly enhanced the radiosensitivity of SKOV3 cells to 10 Gy β-irradiation treatment.

Effect of SCFP on the protein expression of caspase-3, caspase-9, Bax, and Bcl-2 in SKOV3 cells

To explore the possible mechanism of the enhanced radiosensitivity of SCFP, proteins related to the apoptosis pathway, including caspase-3, caspase-9, Bax, and Bcl-2 were detected in SKOV3 cells using an ELISA assay.

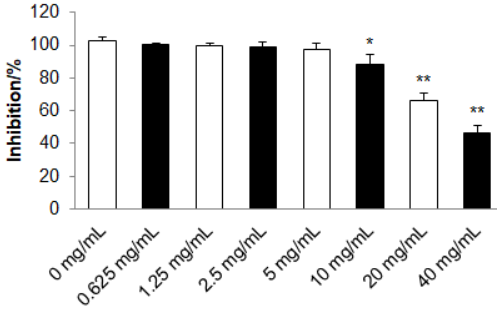

Figure 1: Effect of SCFP on proliferation of SKOV3 cells. Data are expressed as mean ± SD (n = 4); *$p < 0.05$, **$p < 0.01$, compared with control group

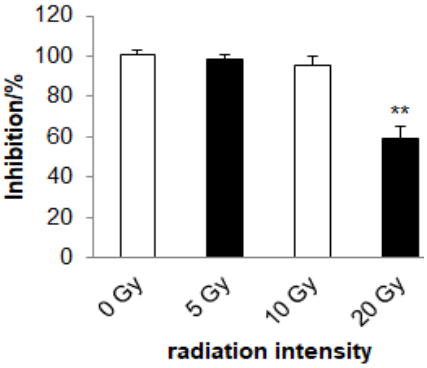

Figure 2: Effect of β irradiation on proliferation of SKOV3 cells. Data are expressed as mean ± SD (n = 4); *$p < 0.05$, **$p < 0.01$, compared with control group

Table 1: The sequence of primers used

Name	Upstream gene	Downstream gene	Gene length
β-actin	GAAGATCAAGATCATTGCTCC	TACTCCTGCTTGCTGATCCA	111bp
Caspase 3	GCACTGGAATGTCAGCTCGCAATG	TTCAGGTCCACAGGTCCGTTCGTT	238bp
Caspase 9	CCTCATCATCAACAACGTGAACTT	TTTTCTTAGCAGTCAGGTCGTTC	146bp
Bax	GGCGAATTGGCGATGAACTGGACAAC	GCTGCCACACGGAAGAAGACCTCTC	100bp
Bcl-2	ACGGTGGTGGAGGAACTCTTCAGG	AGCGTCTTCAGAGACAGCCAGGAG	266bp

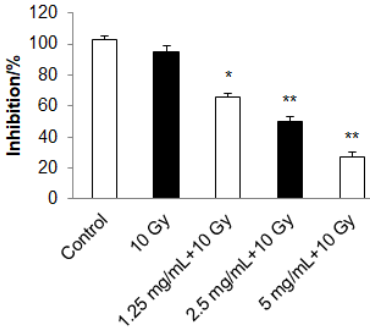

Figure 3: Effects of SCFP combined with β irradiation on cell proliferation of SKOV3 cell. Data are expressed as mean ± SD (n = 4); *p < 0.05, **p < 0.01, compared with control group

As shown in Figure 4, the levels of caspase-3, caspase-9, and Bax significantly increased in SKOV3 cells treated with 1.25, 2.5, and 5 mg/mL SCFP in combination with β-irradiation (p < 0.05), whereas the level of Bcl-2 significantly decreased in SKOV3 cells subjected to the same treatment (p < 0.05).

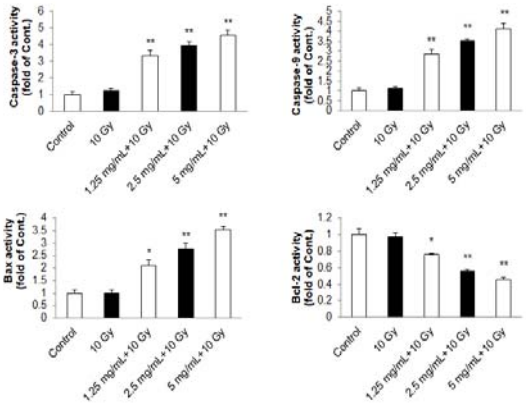

Figure 4: Effect of SCFP combined with β irradiation on protein expression of caspase-3, caspase-9, Bax and Bcl-2 in SKOV3 cells. Data are expressed as mean ± SD (n = 4); *p < 0.05, **p < 0.001, compared with control group

Effect of SCFP on mRNA expression of *caspase-3, caspase-9, Bax, and Bcl-2* in SKOV3 cells

The mRNA expression of *caspase-3*, *caspase-9*, *Bax*, and *Bcl-2* in SKOV3 cells treated with SCFP are shown in Figure 6. We found that mRNA expression of these genes significantly increased after 1.25, 2.5, and 5 mg/mL SCFP treatment when combined with β-irradiation (p < 0.01), whereas the expression of *Bcl-2* decreased in SKOV3 cells subjected to the same treatment (p < 0.01).

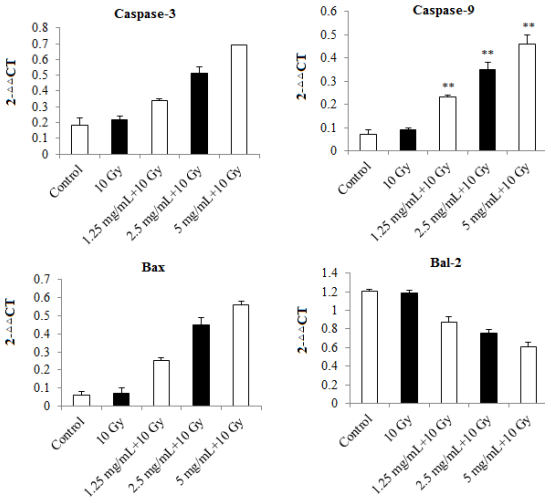

Figure 5: Effect of SCFP combined with β irradiation on mRNA expression of caspase-3, caspase-9, Bax and Bcl-2 in SKOV3 cells. Data are expressed as mean ± SD (n = 4); *p < 0.05, **p < 0.01, compared with Control group; ##p < 0.01, compared with control group (0 mg/mL)

DISCUSSION

Radiotherapy is a common method for treating solid tumors in addition to surgery; however, over 10 % of tumors show resistance to the radiotherapy [7,8] and significantly limit its therapeutic effect. Radiosensitizers are considered a feasible approach to reverse this radio-resistance [9,10]. The results of the present investigation demonstrated that SCFP treatment could significantly sensitize SKOV3 cells to β-ray irradiation and that apoptosis-related mechanism could be involved in its effect [11].

The possible mechanisms of radiosensitizers are complex, and can include apoptosis, DNA damage, and cell cycle arrest [11]. Apoptosis is important for programmed cell death, which is crucial for oncotherapy [12,13]. Caspase family proteins are the executors of cell apoptosis. Caspase-9 initiates the caspase cascade reaction, which can be activated by the release of cytochrome c. Once caspase-9 is activated, caspase-3 is subsequently activated, which acts as a crucial death protease [14,15]. In addition, caspase-3 activates the other caspase proteins, resulting in a cascade reaction [9,15].

Bax directly promotes the release of cytochrome c into the cytoplasm and inhibits anti-apoptotic Bcl-2 proteins [16]. Bcl-2 is a known apoptosis inhibitory factor that suppresses the release of cytochrome c into the cytoplasm [17,18]. In the present study, when SCFP treatment was

combined with β-ray irradiation, the protein and mRNA expression of caspase 3, caspase 9, and Bax increased, while those of Bcl-2 decreased in SKOV3 cells. These results demonstrated that the antitumor mechanism of the SCFP and β-ray irradiation combined treatment is related to the induction of mitochondria-mediated apoptosis.

CONCLUSION

SCFP enhances the sensitivity of ovarian cancer SKOV3 cells to β-irradiation. The mechanism of action is potentially related to the induction of mitochondria-mediated apoptosis. The findings of this research may support the use of SCFP for the clinical management of ovarian cancer; however, further studies are required to confirm these results.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Cai-Xia Wang and Shi-Cong Qia contributed equally to this manuscript. The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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