Kaohsiung Journal of Medical Sciences (2017) 33, 535–542

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.kjms-online.com

Original Article

Ginseng polysaccharide serves as a potential radiosensitizer through inducing apoptosis and autophagy in the treatment of osteosarcoma

Xiao-Yu Zhang [a], Ke Sun [b], Qi Zhu [c], Tao Song [d], Yang Liu [d,*]

[a] *Department of Orthopedics, Ningxia People's Hospital, Ningxia Province, China*
[b] *Department of Orthopedics, Shangluo Central Hospital, Shangluo City, Shaanxi Province, China*
[c] *Department of Hand surgery, Ruian Municipal People's Hospital, Ruian City, Zhejiang Province, China*
[d] *Department of Orthopedic Surgery, Hong Hui Hospital Xi'an Jiaotong University, Xi'an City, Shaanxi Province, China*

Received 17 April 2017; accepted 27 June 2017
Available online 18 August 2017

KEYWORDS
Osteosarcomas;
Ginseng polysaccharide;
Ionizing radiation therapy;
Radiosensitizer

Abstract Recent studies have confirmed that the combined use of anti-cancer drugs with ionizing radiation (IR) could improve the sensitivity of osteosarcoma (OS) cells. Therefore, it is necessary to identify potential effective drugs for the enhancement of IR-radiosensitivity. In the current study, we found that 20, 10, 5, and 1 μM of ginseng polysaccharide (GPS) significantly suppressed MG-63 cell viability with or without γ-ray radiation in a dose- and time-dependent manner. Strikingly, 20 μM of GPS combined with 5 Gy treatment suppressed colony formation capacity by nearly 13.75～fold compared with IR treatment alone. Our results showed that GPS could markedly induce early apoptosis and autophagy in MG-63 cells. A higher drug concentration and a greater exposure dose were directly associated with more apoptosis and autophagy in cells. Western blot analysis showed that GPS decreased the phosphorylation of p38 and AKT as well as the protein expression of Bax and cleaved-caspase3. In summary, GPS inhibited proliferation and increased apoptosis and autophagic death in OS cells, indicating that GPS may be a potential effective auxiliary drug for improving the IR sensitivity of OS patients.

Conflicts of interest: All authors declare no conflicts of interest.

* Corresponding author. Department of Orthopedic Surgery, Hong Hui Hospital Xi'an Jiaotong University, No. 76, Nanguo Road, Xi'an City, Shaanxi Province, 710054, China.
E-mail address: yuyonghua126@163.com (Y. Liu).

http://dx.doi.org/10.1016/j.kjms.2017.07.001

Introduction

Osteosarcoma (OS) originates from bone mesenchymal cells and is characterized by the formation of spindle cells and immature bone [1,2]. OS is the most common primary malignant bone tumour and is considered life-threatening because it metastasizes easily and is associated with a poor prognosis [3]. According to the resectability of OS and the sensitivity to chemotherapeutic drugs, radiation therapy can be used as an important adjunct to OS before, during or after surgery [4—6]. High-dose radiation therapy before OS can reduce the size of the tumour, which can decrease the risk associated with surgery and improve the survival rate of patients. The conventional radiotherapy after surgery can further kill the remaining OS tissue. Moreover, if we can find a way to enhance sensitivity to radiotherapy, the treatment of osteosarcoma patients can be improved to some extent. Radiosensitization is a hot topic in the field of oncology, and it is of great significance to enhance the efficacy of radiotherapy [7,8]. Currently, finding a safe and effective drug is the most important issue in the study of radiosensitization for the treatment of OS.

In recent years, more attention has been focused on the anti-tumour effects of Chinese herbal medicines. Multiple basic and clinical studies have been devoted to the study of the corresponding anti-tumour effects and mechanisms [9,10]. Ginseng polysaccharide (GPS) is a type of polymer acidic polysaccharide extracted from ginseng [11,12]. According to the theory of traditional Chinese medicine, GPS can nourish Qi in the spleen and the lungs [13,14]. The most potent pharmacological effect of GPS is to raise the immunity and improve the pathological state of the whole organism [15]. For instance, GPS is found to induce the viability of macrophages and effectively increase the phagocytic function of the macrophages [15]. Additionally, GPS can increase the phagocytic ability of dendritic cells and promote the maturation of dendritic cells [16]. And GPS is also reported to induce cell cycle arrest in the G2/M phase, inhibit the growth of tumour cells and promote cell apoptosis [12]. However, whether GPS can enhance the activity of OS cells after irradiation has never been explored.

In the current study, we first reported that GPS treatment significantly induced OS cell death, which suggests that GPS can enhance the therapeutic effects when combined with radiotherapy.

Materials and methods

Preparation of the GPS solution

Water-soluble ginseng oligosaccharides (purity >90%, Mw: 8 kDa) that were obtained from the water extract of Panax ginseng roots were provided by the Jilin Ginseng Academy at the Changchun University of Chinese Medicine (Changchun, China). The molecular weight was approximately 8 kDa. The GPS was dissolved in phosphate-buffered saline (PBS) at doses of 20, 10, 5 and 1 μM.

Cell lines

The human OS cell line MG-63 and the normal *osteoblast* hFOB1.19 (purchased from ATCC) were used in the study. The cells were cultured in a monolayer in RPMI 1640 medium (Life Technologies Invitrogen, Thermo Scientific, Waltham, MA, USA) that was supplemented with 10% foetal calf serum and 1% Penicillin/Streptomycin at 37 °C in a humid atmosphere with 5% CO_2.

Irradiation

Cells were plated in dishes and incubated at 37 °C and 5% CO_2 under humidified conditions at 70—80% confluence. Cells were irradiated with a ^{137}Cs γ-ray source (Atomic Energy of Canada, Ontario, Canada) at a dose rate of 0, 2, and 5 Gy/min.

Cell proliferation assay

MG-63 cells were seeded in a 96-well plate at a density of 5×10^3 cells per well. After 24 h, the cells were irradiated with a ^{137}Cs γ-ray source (Atomic Energy of Canada, Ontario, Canada) at a dose rate of 5 Gy/min. After radiation, the fresh medium was added and supplemented with GPS at the final concentrations of 20, 10, 5, and 1 μM for 24 h in the presence of 1% FBS. The cell viability was determined using the CCK-8 assay according to the instructions (CCK-8, Beyotime Inst Biotech, China). Each well absorbance was tested at 450 nm using a microplate reader. The proliferation rate was defined in terms of the percentage of surviving cells in each group compared with the untreated group.

Colony-forming assay

MG-63 cells were seeded in a 96-well plate at a density of 5×10^3 cells per well. After 24 h, the cells were irradiated with a ^{137}Cs γ-ray source (Atomic Energy of Canada, Ontario, Canada) at a dose rate of 0, 2, and 5 Gy/min. After radiation, the fresh medium was added and supplemented with GPS at the final concentrations of 10, 5, and 1 μM for 4 consecutive days in the presence of 1% FBS. The colonies were then stained with 0.4% (w/v) crystal violet (Sigma—Aldrich). The plating efficiency (PE) was the percentage of seeded cells that grew into colonies. The survival fraction, which is expressed as a function of the IR dose, was calculated as follows: survival fraction = colonies counted/(cells seeded × PE/100). To evaluate the radiosensitizing effects of GPS, the ratio of the dose (Gy) for IR alone divided by the dose of IR plus ZOL at a survival fraction of 10% was determined.

Detection of apoptotic cells by annexin V staining

For ANNEXIN V-PI staining, cells were washed with ice-cold PBS, trypsinized, and resuspended in 1 × binding buffer [10 mm HEPES/NaOH (pH 7.4), 140 mm NaCl, and 2.5 mm $CaCl_2$] at 1×10^6 cells/mL. After gentle vortex, the cells

were mixed with 5 μL annexin V/fluorescein isothiocyanate (FITC) (BD Biosciences, Franklin Lakes, NJ, USA) and 10 μL propidium iodide (PI) stock solution (50 μg/mL in PBS) followed by a 15 min incubation at room temperature in the dark. Next, 400 μL of 1× binding buffer was added to each sample, and the samples were analysed on a FACScan™ flow cytometer (BD Biosciences). A minimum of 10,000 cells were counted for each sample, and data were analysed using CellQuest™ software (BD Biosciences).

Cell cycle analysis

MG-63 cells were plated onto a 6-well plate after being treated with 20 μM GPS for 48 h. The cells were harvested by trypsin without EDTA, washed 3 times using ice-cold PBS and fixed with 70% ethanol overnight at 4 °C. The cell cycle analysis was performed using RNase A and PI staining (DA0030, Leagene, Beijing, China) and flow cytometry. Experiments were performed in triplicate. The percentage of cells in each cycle phase was assessed using FlowJo software.

GFP-LC3 transient transfection

MG-63 cells were seeded at the density of 5×10^5 cells/well in 6-well plates for 24 h. Subsequently, a GFP-LC3-expressing plasmid was transfected into the cells using Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After 24 h, the cells were treated with 10 μM with or without GPS for 16 h. Then, GFP-LC3-positive dots were counted under a confocal laser microscope, LSM700 (Carl Zeiss, Jena, Germany).

Electron microscopy

The samples were fixed with 2% glutaraldehyde paraformaldehyde in 0.1 M phosphate buffer (PBS, pH 7.4) for 12 h at 4 °C and washed three times for 30 min in 0.1 M PB. Then, the samples were fixed with 1% OsO_4 dissolved in 0.1 M PB for 2 h and dehydrated in an ascending gradual series (50—100%) of ethanol and infiltrated with propylene oxide. Specimens were embedded using a Poly/Bed 812 Kit (Polysciences, Warrington, PA, USA). After pure fresh resin embedment and polymerization at 60 °C in an electron microscope oven (TD-700, DOSAKA, Kyoto, Japan) for 24 h, 350 nm sections were cut and stained with toluidine blue for light microscopy, and 70 nm thin sections were double-stained with 7% uranyl acetate and lead citrate for contrast staining. Sections were cut with a LEICA Ultracut UCT Ultramicrotome (Leica Microsystems, Wetzlar, Germany). All thin sections were observed by TEM (JEM-1011, JEOL, Tokyo, Japan) at an acceleration voltage of 80 kV.

Western blot analysis

Total protein samples were extracted from the culture cells using RIPA buffer (Solarbio, Beijing, China) according to the instructions. The immunoblotting was performed as previously described [17]. The following primary antibodies were purchased from Cell and used as follows, p-AKT (#8200, Cell Signaling Technology, Beverly, MA, USA), AKT (#2920, Cell Signaling Technology, Beverly, MA, USA), p-p38 (#8203, Cell Signaling Technology, Beverly, MA, USA), p38 (#14451, Cell Signaling Technology, Beverly, MA, USA), Bcl-2 (#15071, Cell Signaling Technology, Beverly, MA, USA), Bax (#14796, Cell Signaling Technology, Beverly, MA, USA), c-Caspase3 (#1050, Cell Signaling Technology, Beverly, MA, USA), LC3 (L8918, Sigma—Aldrich, St. Louis, MO, USA) and β-actin (#12413, Cell Signaling Technology, Beverly, MA, USA). The protein of interest was tested with goat anti-mouse or anti-rabbit IgG-HRP secondary antibody.

Statistical analysis

The data were expressed as the means ± standard deviation. Student's t-test was used for two-group comparisons. One-way ANOVA was used for comparison of more than two groups. All statistical analyses were calculated using SPSS 13.0 software (SPSS, Chicago, IL, USA). *$P < 0.05$ was considered to be statistically significant.

Results

GPS suppressed MG-63 cell viability in a dose-dependent manner

First, we explored the effects of GPS on MG-63 cell viability when MG-63 cells were irradiated with a ^{137}Cs γ-ray source at a dose rate of 0, 5 Gy/min for 1, 2, 3, 4 days, respectively. The CCK-8 kit assay demonstrated that 20, 10, 5, and 1 μM of GPS significantly suppressed MG-63 cell viability with or without γ-ray radiation (Fig. 1A—D). Strikingly, 20 μM GPS combined with 5 Gy/min irradiation decreased MG-63 cell viability by approximately 38% compared with that in the absence of the ^{137}Cs γ-ray source at day 4 (Fig. 1D). Meanwhile, at the dose of 5 Gy/min irradiation, 20, 10, 5, and 1 μM GPS reduced MG-63 cell viability by 63%, 33%, 15%, and 7%, respectively, at day 4 (Fig. 1D). Furthermore, we compared the effects of 20, 10, 5, and 1 μM GPS at the dose rate of 5 Gy/min on the normal osteoblast hFOB1.19 for 4 days. As shown in Fig. 1E, GPS decreased hFOB1.19 cell viability by 56%, 37%, 16%, and 6%, respectively, at day 4. Then, we analysed the results of different radiation doses (0, 5, 20, and 40 Gy/min) with or without 20 μM GPS. Compared with the blank control, 20 μM GPS treatment reduced MG-63 cell viability in a synergistic manner (Fig. 1F), which validated the role of GPS as a potential radiosensitizer.

GPS induced MG-63 cell apoptosis and cell cycle arrest

Annexin V staining was used to evaluate the effect of 20 μM GPS and 5 Gy/min irradiation on the apoptosis of MG-63 cells. Compared with the control (4.8%), the percentage of Annexin V-positive cells were increased by 20 μM GPS (14.7%) or 5 Gy/min irradiation (14.2%) alone (Fig. 2A). Strikingly, the combination of 20 μM GPS and 5 Gy/min enhanced the cell apoptosis rate by 57% (Fig. 2A). We also investigated how the cell cycle populations were altered by

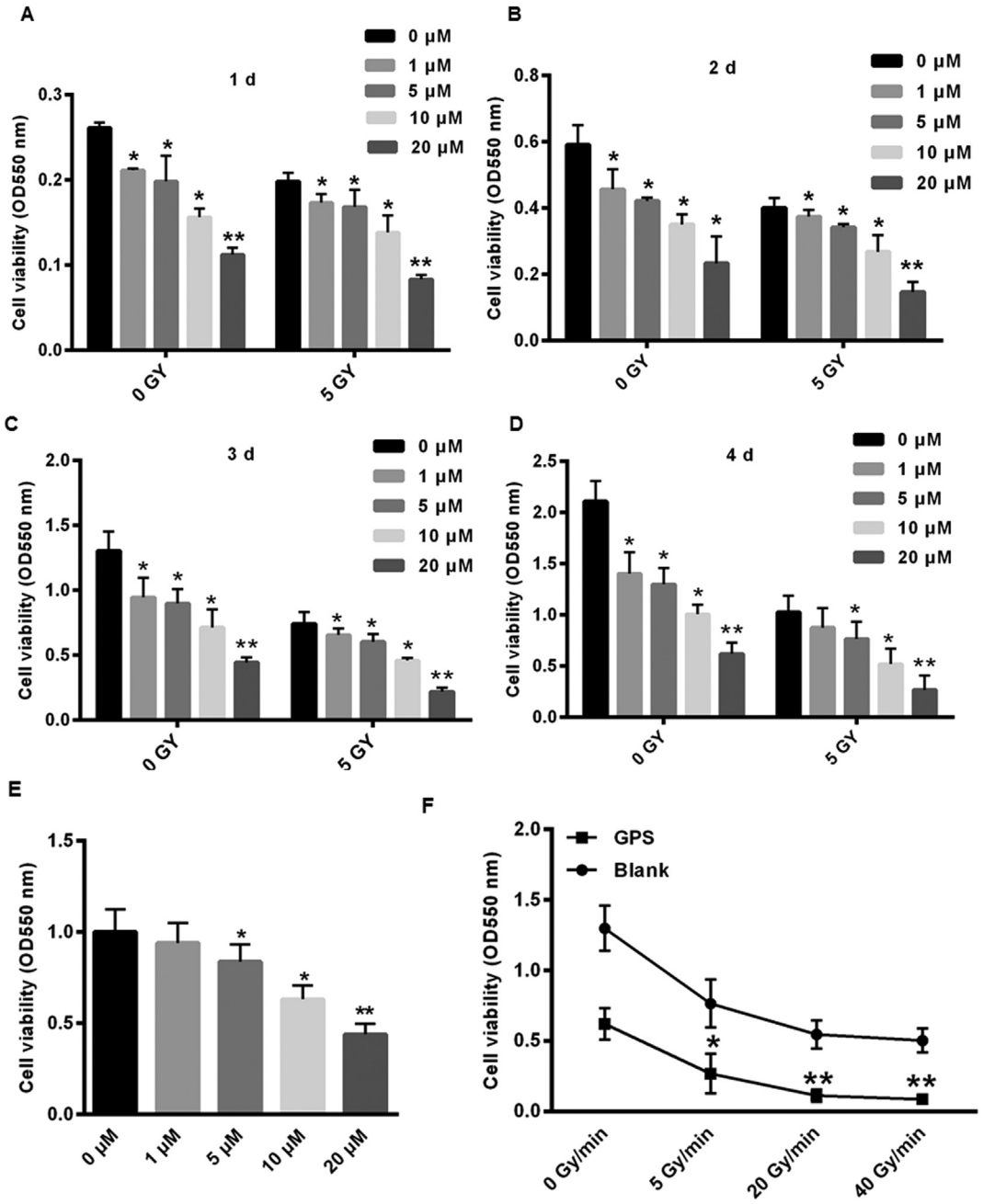

Figure 1. GPS suppressed MG-63 cell viability in a dose-dependent manner. CCK-8 kit assay demonstrated that 20, 10, 5, 1 μM of GPS significantly suppressed MG-63 cell viability at a dose rate of 0, 5 Gy/min for 1 (A), 2 (B), 3 (C), 4 (D) days. (E) GPS decreased hFOB1.19 cell viability by 56%, 37%, 16%, and 6%. (F) Compared with the blank control, 20 μM GPS treatment reduced MG-63 cell viability in a synergistic effect at the dose of different irradiation (0, 5, 20, 40 Gy/min) for 4 days. *$p < 0.05$, **$p < 0.01$ vs. control.

20 μM GPS in the presence or absence of 5 Gy/min irradiation. Cell cycle distribution analysis showed that the number of cells was increased at the G0/G1 phase by 20 μM GPS (52.5%) or 5 Gy/min irradiation (56.1%) alone compared with that of the control (49.1%). More importantly, the combination of 20 μM GPS and 5 Gy/min elicited further increases in the number of cells at the G0/G1 phase (65%), suggesting that GPS served as a radiosensitizer and induced typical cell cycle arrest at the G0/G1 phase in MG-63 cells (Fig. 2B).

GPS inhibited MG-63 cell colony formation capacity in a dose-dependent manner

Furthermore, the rate of cell colony formation capacity was evaluated in MG-63 cells treated with different concentrations of GPS combined with different irradiation doses of γ-ray (5 Gy, 2 Gy, or 0 Gy). As shown in Fig. 3, along with the increase in GPS concentration (5, 10, and 20 μM), the colony formation capacity of MG-63 cells was significantly reduced in a dose-dependent manner in the MG-63 cells

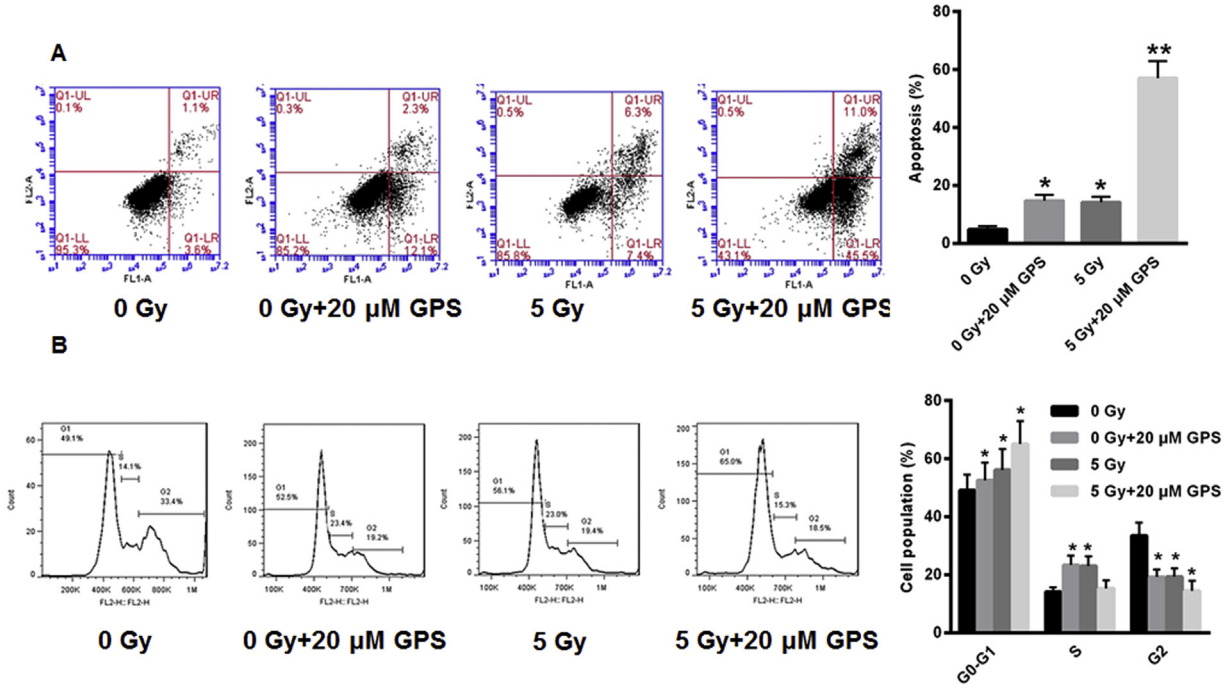

Figure 2. GPS induced MG-63 cell apoptosis and cell cycle arrest. (A) MG-63 cell apoptosis rate was determined by Annexin V — PI staining. (B) Cell cycle distribution analysis. *p < 0.05, **p < 0.01 vs. control.

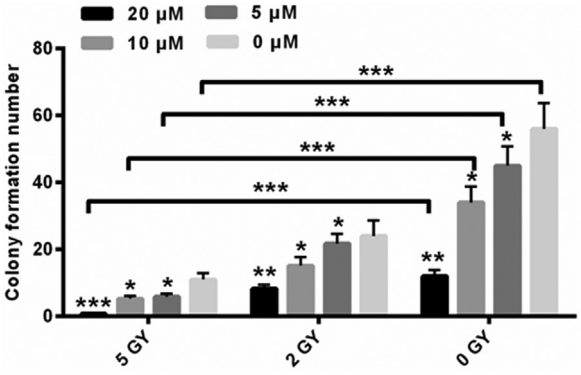

Figure 3. GPS inhibited MG-63 cell colony formation capacity in a dose-dependent manner. *p < 0.05, **p < 0.01 vs. control.

pre-incubated with 5 Gy, 2 Gy, and 0 Gy γ-ray, respectively. More importantly, we found that compared with the 0 Gy γ-ray, the combination use of 5, 10, and 20 μM GPS and 5 Gy γ-ray markedly reduced the colony formation by 10%, 16%, and 44%, respectively. These data indicated that as a radiosensitizer, GPS elicited additional results when combined with the radiation.

GPS induced MG-63 cell autophagy

In addition, the effect of GPS on MG-63 cell autophagy was explored in the presence of 5 Gy irradiation, and rapamycin (Rap) was used as a positive control. Electron microscopy scanning showed that GPS induced MG-63 autophagy in the same pattern as rapamycin (Fig. 4A). Additionally, GFP-LC3 transfection also demonstrated increased autophagy vesicles in the MG-63 cells treated with GPS or Rap (Fig. 4B).

GPS decreased the phosphorylation of p38, AKT and the protein expression of Bax and cleaved-caspase3

To further explore the mechanism by which GPS induces MG-63 apoptosis, we tested the protein expression in MG-63 cells treated with 10 μM of GPS. Previous studies have shown that the activation of MAPK/p38 and AKT plays a key role in the malignancies of OS. Therefore, we tested their expression after GPS treatment. According to our results, the phosphorylation levels of p38 and AKT were significantly reduced by GPS treatment. In addition, the anti-apoptosis protein, Bcl2, was found to be decreased with GPS incubation (Fig. 5). In comparison, the protein levels of pro-apoptotic proteins, including Bax and cleaved-caspase3, were markedly enhanced (Fig. 5). Thus, the anti-cancer effects of GA may be achieved through the suppression of p38, AKT activation and reduction of Bcl-2.

GPS increased the ratio of LC3II/LC3I

GPS treatment also significantly increased the ratio of LC3II/LC3I, which indicated that GPS could activate autophagy in MG-63 cells (Fig. 6).

Discussion

OS is one of the most common malignant bone tumours that seriously endangers the health of adolescents [2,18]. In recent years, the survival rate of OS has improved due to

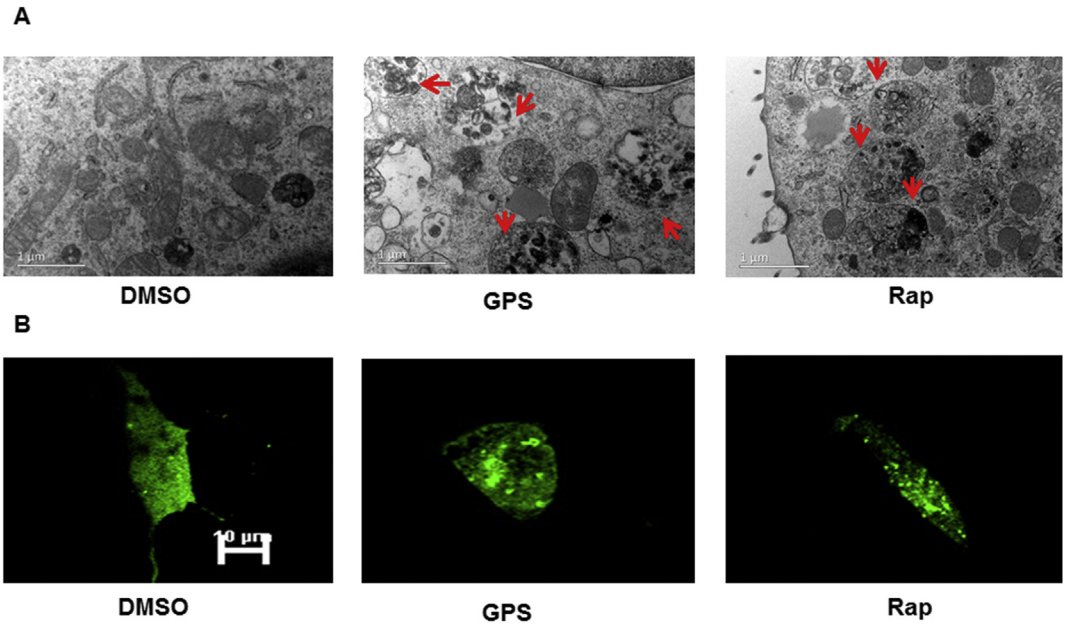

Figure 4. GPS induced MG-63 cell autophagy in the presence of 5 Gy irradiation. (A) Electron microscopy. (B) GFP-LC3 transfection.

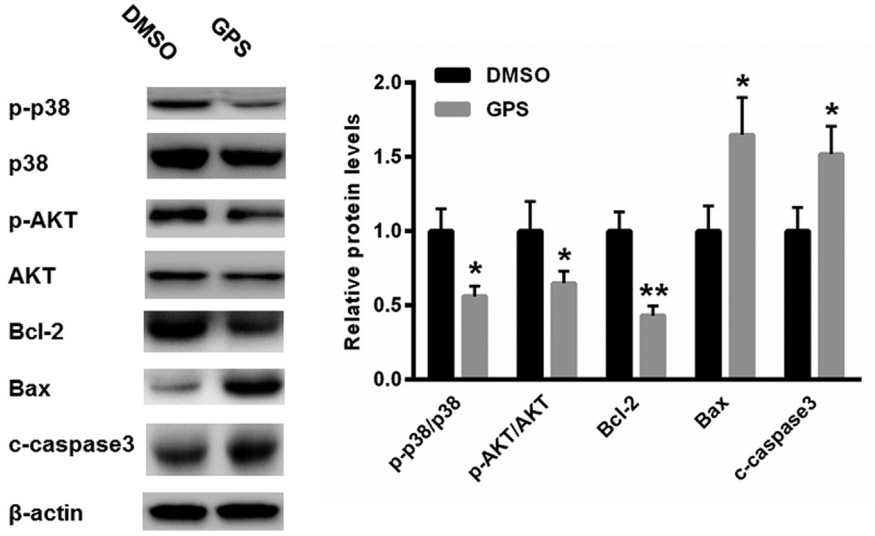

Figure 5. GPS decreased the phosphorylation of p38, AKT and the protein expression of Bax and cleaved-caspase3. *$p < 0.05$, **$p < 0.01$ vs. control.

the development of chemotherapy drugs, but the overall effect is still not ideal [19]. Multi-centre studies show that the residual malignant cells in the tumour bed constitute the root of the recurrence and metastasis, which is a key factor that restricts the improvement in patients with OS [20]. Therefore, radiotherapy combined with surgery can improve the local control rate. However, heterogeneity affects the sensitivity of OS cells to radiotherapy [6]. Therefore, to improve the prognosis for patients with osteosarcoma, an individualized radiotherapy plan must be considered that incorporates improvement in the sensitivity of patients to radiotherapy. As a traditional Chinese medicine, ginseng has a history of thousands of years of clinical application. GPS is the main effective compound [21] and is suggested to enhance the body's immunity [22]. Recent studies found that GPS also plays an important role in antitumour treatment [23,24]. However, the effect of GPS on the biological activity of OS cells is not clear.

Tumour metastasis involves multiple mechanisms, including cell proliferation, invasion, and migration [25–27]. Previous studies have demonstrated that GPS could markedly suppress cancer cell proliferation and invasion [14,28]. Thus, in the current study, we explored the effects of GPS on OS cell proliferation. Here, we demonstrated that GPS + IR had significant inhibitory effects on human OS cell viability in a dose- and time-dependent

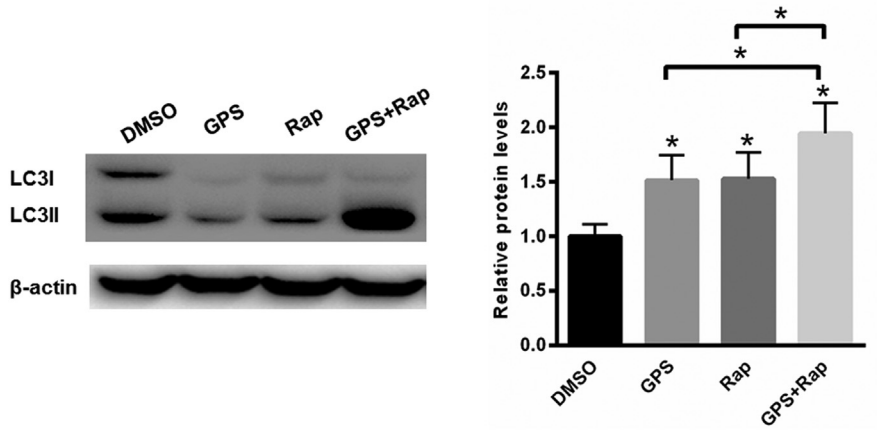

Figure 6. GPS treatment also significantly induced the ratio between LC3II/LC3I. *$p < 0.05$ vs. control.

manner compared with IR treatment alone. Additionally, we found that GPS could significantly induce MG-63 cell apoptosis compared to IR alone. These observations suggested that the combined use of GPS and IR led to an enhancement of in vitro cytotoxicity, which indicates the potential for auxiliary treatment methods after radiotherapy.

Apoptosis is also known as programmed cell death, which is a strictly regulated process in response to cancer therapies [29]. Therefore, we evaluated whether GPS induced MG-63 apoptosis after IR treatment. Compared with IR therapy alone, GPS significantly enhanced OS cell apoptosis in a dose-dependent manner. It was confirmed that the combined use of GPS with γ-ray radiation could induce more MG-63 cell apoptosis compared with that of γ-ray radiation alone and such effects were increased along with the increased GPS concentrations. Three major pathways were suggested to be involved in the underlying mechanisms of cell apoptosis, including the endoplasmic reticulum pathway, mitochondrial pathway and death receptor pathway [29]. Western blot analysis showed that GPS treatment could significantly reduce the protein expression of BcL-2 and enhanced the expression of Bax and cleaved-caspase3. These data indicated that the combined use of GPS with γ-ray radiation prompted MG-63 cell apoptosis by modulating the expression of Bcl-2 members.

Autophagy has a dual role in the regulation of cell death. Mild autophagy protects cells from harmful conditions, and serious or quick autophagy induces programmed cell death, which is called autophagic cell death [30,31]. In the previous studies, the cooperation between autophagy and apoptosis is identified and is suggested to promote cell death [32,33]. Many drugs can activate both apoptosis and autophagy. For example, ceramide induced apoptosis and autophagy in the treatment of breast cancer and colon cancer, respectively [34]. The antibacterial drug, chloroquine chloride, increases autophagy death and apoptosis in leukaemic cells and myeloma cells by disrupting the mTOR signalling pathway [35]. In this study, we first evaluated the effect of GPS on MG-63 cell autophagy in the presence of 5 Gy irradiation. Our data showed that GPS significantly increased cell autophagy, which indicated the synergistic role of autophagy death and apoptosis after GPS treatment.

In conclusion, our study demonstrates that GPS inhibits the proliferation of OS cells and increases apoptosis and autophagy death of OS cells, which suggests that GPS may be a potential effective auxiliary drug for improving IR sensitivity in OS patients.

References

[1] Flores RJ, Kelly AJ, Li Y, Nakka M, Barkauskas DA, Krailo M, et al. A novel prognostic model for osteosarcoma using circulating CXCL10 and FLT3LG. Cancer 2017;123:144—54.
[2] Gandhi D, Naoghare PK, Bafana A, Kannan K, Sivanesan S. Fluoride-induced oxidative and inflammatory stress in osteosarcoma cells: does it affect bone development pathway? Biol Trace Elem Res 2017;175:103—11.
[3] Gill J, Connolly P, Roth M, Chung SH, Zhang W, Piperdi S, et al. The effect of bone morphogenetic protein-2 on osteosarcoma metastasis. PLoS One 2017;12:e0173322.
[4] Gonzalez-Fernandez Y, Imbuluzqueta E, Zalacain M, Mollinedo F, Patino-Garcia A, Blanco-Prieto MJ. Doxorubicin and edelfosine lipid nanoparticles are effective acting synergistically against drug-resistant osteosarcoma cancer cells. Cancer Lett 2017;388:262—8.
[5] Han K, Gan Z, Lin S, Hu H, Shen Z, Min D. Elevated expression of serine/threonine phosphatase type 5 correlates with malignant proliferation in human osteosarcoma. Acta Biochim Pol 2017;64(1):11—6.
[6] Hartemayer R, Kuo C, Kent P. Osteosarcoma metastases with direct cardiac invasion: a case report and review of the pediatric literature. J Pediatr Hematol Oncol 2017 Apr;39(3):188—93.
[7] Bhattarai SR, Derry PJ, Aziz K, Singh PK, Khoo AM, Chadha AS, et al. Gold nanotriangles: scale up and X-ray radiosensitization effects in mice. Nanoscale 2017;9(16):5085—93.
[8] Aoyama N, Ogawa Y, Yasuoka M, Iwasa H, Miyatake K, Yoshimatsu R, et al. Therapeutic response to a novel enzyme-targeting radiosensitization treatment (KORTUC II) for residual lesions in patients with stage IV primary breast cancer, following induction chemotherapy with epirubicin and cyclophosphamide or taxane. Oncol Lett 2017;13:69—76.
[9] Fan JM, Liu ZH, Li J, Wang YP, Yang LY, Huang JJ. Effect of ginseng polysaccharide-induced wnt/beta-catenin signal transduction pathway on apoptosis of human nasopharyngeal cancer cells CNE-2. Zhongguo ZhongYao Za Zhi 2013;38:3332—7.
[10] Kim HJ, Kim MH, Byon YY, Park JW, Jee Y, Joo HG. Radioprotective effects of an acidic polysaccharide of Panax ginseng on bone marrow cells. J Vet Sci 2007;8:39—44.

[11] Bing SJ, Ha D, Hwang I, Park E, Ahn G, Song JY, et al. Protective effects on central nervous system by acidic polysaccharide of panax ginseng in relapse-remitting experimental autoimmune encephalomyelitis-induced SJL/J mice. Am J Chin Med 2016;44:1099—110.
[12] Wang Y, Huang M, Sun R, Pan L. Extraction, characterization of a Ginseng fruits polysaccharide and its immune modulating activities in rats with Lewis lung carcinoma. Carbohydr Polym 2015;127:215—21.
[13] Krylova NV, Besednova NN, Solov'eva TF, Loenko Iu N, Faustov VS, Konstantinova NA, et al. Anti-inflammatory effect of polysaccharide obtained from ginseng cell cultures. Antibiot Khimioter 1990;35:41—2.
[14] Liao K, Bian Z, Xie D, Peng Q. A selenium-modified ginseng polysaccharide promotes the apoptosis in human promyelocytic leukemia (HL-60) cells via a mitochondrial-mediated pathway. Biol Trace Elem Res 2017;177(1):64—71.
[15] Niu J, Pi Z, Yue H, Wang Y, Yu Q, Liu S. Effect of ginseng polysaccharide on the urinary excretion of type 2 diabetic rats studied by liquid chromatography-mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci 2012;907:7—12.
[16] Sun XB, Matsumoto T, Yamada H. Purification of immune complexes clearance enhancing polysaccharide from the leaves of panax ginseng, and its biological activities. Phytomedicine 1994;1:225—31.
[17] Meng X, Guo J, Fang W, Dou L, Li M, Huang X, et al. Liver MicroRNA-291b-3p promotes hepatic lipogenesis through negative regulation of adenosine 5′-monophosphate (AMP)-activated protein kinase alpha1. J Biol Chem 2016;291:10625—34.
[18] Chen P, Wang H, Yang F, Chen H, He W, Wang J. Curcumin promotes osteosarcoma cell death by activating mir-125a/ERRalpha signal pathway. J Cell Biochem 2017;118:74—81.
[19] Dong F, Liu T, Jin H, Wang W. Chimaphilin inhibits human osteosarcoma cells invasion and metastasis through suppressing the TGF-beta1-induced epithelial-to-mesenchymal transition markers via PI-3K/Akt, ERK1/2 and Smad signaling pathways. Can J Physiol Pharmacol 2017. http://dx.doi.org/10.1139/cjpp-2016-0522.
[20] Faisham WI, Mat Saad AZ, Alsaigh LN, Nor Azman MZ, Kamarul Imran M, Biswal BM, et al. Prognostic factors and survival rate of osteosarcoma: a single-institution study. Asia Pac J Clin Oncol 2017;13:e104—10.
[21] Yin SY, Kim HJ, Kim HJ. A comparative study of the effects of whole red ginseng extract and polysaccharide and saponin fractions on influenza A (H1N1) virus infection. Biol Pharm Bull 2013;36:1002—7.
[22] Cai JP, Wu YJ, Li C, Feng MY, Shi QT, Li R, et al. Panax ginseng polysaccharide suppresses metastasis via modulating Twist expression in gastric cancer. Int J Biol Macromol 2013;57:22—5.
[23] Choi HS, Kim KH, Sohn E, Park JD, Kim BO, Moon EY, et al. Red ginseng acidic polysaccharide (RGAP) in combination with IFN-gamma results in enhanced macrophage function through activation of the NF-kappaB pathway. Biosci Biotechnol Biochem 2008;72:1817—25.
[24] Du Xiao F, Jiang CZ, Wu CF, Won EK, Choung SY. Synergistic immunostimulatory effect of pidotimod and red ginseng acidic polysaccharide on humoral immunity of immunosuppressed mice. Pharmazie 2008;63:904—8.
[25] Wang C, Wang W, Wang C, Tang Y, Tian H. Combined therapy with EGFR TKI and gambogic acid for overcoming resistance in EGFR-T790M mutant lung cancer. Oncol Lett 2015;10:2063—6.
[26] Wang S, Yang Y, Wang Y, Chen M. Gambogic acid-loaded pH-sensitive mixed micelles for overcoming breast cancer resistance. Int J Pharm 2015;495:840—8.
[27] Wen C, Huang L, Chen J, Lin M, Li W, Lu B, et al. Gambogic acid inhibits growth, induces apoptosis, and overcomes drug resistance in human colorectal cancer cells. Int J Oncol 2015;47:1663—71.
[28] Hu WW, Yao H, Zhong JJ. Improvement of Panax notoginseng cell culture for production of ginseng saponin and polysaccharide by high density cultivation in pneumatically agitated bioreactors. Biotechnol Prog 2001;17:838—46.
[29] Kashyap D, Mondal R, Tuli HS, Kumar G, Sharma AK. Molecular targets of gambogic acid in cancer: recent trends and advancements. Tumor Biol 2016;37:12915—25.
[30] Keta O, Bulat T, Golic I, Incerti S, Korac A, Petrovic I, et al. The impact of autophagy on cell death modalities in CRL-5876 lung adenocarcinoma cells after their exposure to gamma-rays and/or erlotinib. Cell Biol Toxicol 2016;32:83—101.
[31] Ryter SW, Mizumura K, Choi AM. The impact of autophagy on cell death modalities. Int J Cell Biol 2014;2014, 502676.
[32] Gump JM, Thorburn A. Autophagy and apoptosis: what is the connection? Trends Cell Biol 2011;21:387—92.
[33] Su M, Mei Y, Sinha S. Role of the crosstalk between autophagy and apoptosis in cancer. J Oncol 2013;2013, 102735.
[34] Pattingre S, Bauvy C, Carpentier S, Levade T, Levine B, Codogno P. Role of JNK1-dependent Bcl-2 phosphorylation in ceramide-induced macroautophagy. J Biol Chem 2009;284:2719—28.
[35] Cao B, Li J, Zhou X, Juan J, Han K, Zhang Z, et al. Clioquinol induces pro-death autophagy in leukemia and myeloma cells by disrupting the mTOR signaling pathway. Sci Rep 2014;4:5749.