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Original Article

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

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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.

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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