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Psoralen inhibits the proliferation and promotes apoptosis through endoplasmic reticulum stress in human osteosarcoma cells

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Abstract

Introduction. Psoralen is a main active component of *Psoralea corylifolia* Linn. (Leguminosae). Psoralen has been reported to show antitumor effects and activity to accelerate osteoblastic proliferation. Nevertheless, the antitumor mechanism of psoralen in osteosarcoma has never been elucidated. The current study is aimed to investigate the therapeutic function of psoralen in human osteosarcoma cells and its potential regulatory mechanism.

Material and methods. Effects of psoralen (0–70 μg/mL) on the viability of two osteosarcoma cell lines cultured for 48 h was evaluated by MTT assays. The concentration of IC_{10} (8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells) was regarded to be a non-cytotoxic dose selected as the working concentration in the subsequent experiments. Effects of psoralen on cell proliferation for 48 h was assessed by colony formation assays. Flow cytometry analyses were performed to measure cell cycle and apoptosis. RT-qPCR and Western blotting were carried out to assess RNA expression and protein levels of endoplasmic reticulum (ER) stress associated factors.

Results. Psoralen inhibited osteosarcoma cell viability (IC_{50} 25 μg/mL for MG-63 cells and IC_{50} 40 μg/mL for U2OS cells) in a dose-dependent manner and growth inhibition rate reached the highest level when cells were treated with 70 μg/mL psoralen. Psoralen induced cell cycle arrest in the G0/G1 phase and promoted apoptosis of both MG-63 and U2OS cells. The treatment of psoralen resulted in an increase in ATF-6 and CHOP protein levels as well as a decrease in Bcl-2 protein level, indicating that cell apoptosis induced by psoralen was associated with ER stress. Treatment with 4-PBA, the ER stress inhibitor, attenuated the ability of psoralen to promote apoptosis of MG-63 and U2OS cells.

Conclusions. Psoralen showed growth-inhibitory effects in osteosarcoma cells, and induced apoptosis *via* the ER stress pathway, which might be a potential drug to suppress the development of osteosarcoma. *(Folia Histochemica et Cytobiologica 2022, Vol. 60, No. 1, 101–109)*

Key words: Psoralen; osteosarcoma cells; cell death; ER stress; unfolded protein response; apoptosis

Introduction

Osteosarcoma is the most common primary sarcoma of bone, and it is a leading cause of cancer death among children and adolescents [1]. It accounts for 3–6% of all childhood cancers, ranking only after lymphomas and brain cancers in the most common cancers among children and adolescents [2]. The rapid bone growth at puberty is predicted to be correlated with osteosarcoma development, which has not been confirmed by any study until now [3]. Standard treatments of osteosarcoma include targeted radiation therapy, systemic chemotherapy, and aggressive surgical resection [4]. Nearly 70% of osteosarcoma

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patients have been effectively cured after surgical resection with adjuvant chemotherapy using methotrexate, ifosfamide, cisplatin or doxorubicin [5]. Furthermore, the survival rates of osteosarcoma patients are demonstrated to substantially increase by 60–70% after combination chemotherapy [6]. Nevertheless, the overall 5-year survival in osteosarcoma patients with metastasis or relapse has remained unchanged at about 20% in the past three decades due to chemotherapy resistance [7]. Therefore, novel treatments are urgent to be developed for osteosarcoma surgery.

Previously, multiple studies demonstrated that bioactive natural compounds present in medicinal plants can act as substitutes for chemically designed anticancer drugs [8]. Currently, natural products deriving from plants are sources of numerous anticancer agents including combretastatins, epipodophyllotoxin, camptothecin derivatives, paclitaxel, vinblastine, vincristine, and others [9], playing significant roles in the field of cancer treatment research. For example, phellamurin, a flavonone glycoside extracted from *Phellodendron amurense* Rupr. (Rutaceae), is demonstrated to repress cell growth and induce cell apoptosis by suppressing the PI3K/AKT/mTOR pathway in osteosarcoma U2OS and Saos-2 cells [10]. Pristimerin, a triterpene compound derived from plant extracts, is considered a new therapeutic agent for osteosarcoma treatment since it suppresses osteosarcoma cell proliferation *in vitro* and *in vivo* [11]. *Psoralea corylifolia* Linn. (Suppl. Fig. 1A) is an important plant species belonging to Leguminosae [12]. It is a significant medicinal plant used in traditional Chinese medicine to cure several diseases such as hypertension [13], nephritis [14], and pollakiuria [15]. It has been reported that its plant extracts possess antioxidant, anti-inflammatory, antibacterial, antitumor, and antifungal effects [16]. Psoralen, a tricyclic furocoumarin, is the most active furocoumarin in *Psoralea corylifolia* [17]. The chemical structure of psoralen was shown in Suppl. Fig. 1B. Psoralen was reported many times to play an antitumor effect in numerous human cancers. For instance, psoralen inhibits cell proliferation, induces cell apoptosis, and suppresses bone metastasis in animal models of breast cancer [18–20]. Psoralen activates endoplasmic reticulum stress to repress cell growth and promote cell apoptosis in human hepatocellular carcinoma [21]. Psoralen represses the HOXB7-HER2 signaling pathway to increase chemotherapeutic sensitivity of gastric cancer cells [22]. In addition, psoralen has been confirmed to suppress the growth of transplanted osteosarcoma tumors in nude rats and induce tumor cell necrosis or apoptosis [23]. Nevertheless, the influence of psoralen on human osteosarcoma cells is not clear.

Endoplasmic reticulum stress (ER stress) is the basic cell response against a variety of extracellular factors involving drug agonists, toxic substances, anoxia, UV and nutrient deficiency, which leads to the unfolded proteins accumulating in the endoplasmic reticulum and breaks Ca^{2+} homeostasis balance [24]. In the early stage of ER stress, the unfolded protein response (UPR) helps cells against the stress and protects cell functions by improving the capacity of ER to fold proteins, clearing unfolded/misfolded proteins, and attenuating protein synthesis [21]. Nevertheless, prolonged activation of UPR induces apoptosis pathway after ER stress, including JNK activation pathway, the GADD153/DITT3/CHOP gene activation pathway, and ER-specific cysteine caspase-12 activation pathway [25, 26].

In the present study, the influence of psoralen on the morphology and growth of human osteosarcoma cells were evaluated. Furthermore, we investigated how psoralen influenced cell cycle and cell apoptosis through regulating ER stress.

Materials and methods

Materials. Psoralen was purchased from the National Institute of Control of Pharmaceutical and Biological Products. Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Roswell Park Memorial Institute (RPMI) 1640 medium and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). TRIzol, streptomycin and penicillin were purchased from Invitrogen Life Technologies (Grand Island). Radio Immunoprecipitation Assay (RIPA) lysis buffer was bought from Beyotime Biotechnology (Shanghai, China). PrimeScript® RT reagent Kit was purchased from TaKaRa Bio Inc (Otsu, Japan). The AnnexinV-FITC/propidium iodide (PI) apoptosis detection kit was purchased from Pharmingen (Becton-Dickinson Company, San Jose, CA, USA). Antibodies against cyclin A1 (ab133183), cyclin B1 (ab215436), cyclin D1 (ab40754), CDK2 (ab32147), GAPDH (ab181602), Bcl-2 (ab32124), Bax (ab32503) were bought from Abcam (Cambridge, MA, USA). Total caspase 3 (#14220), cleaved caspase 3 (#9661), GRP78 (#3177), CHOP (#5554), IRE1α (#3294), XBP-1s (#40435), ATF-6 (#65880), PERK (#5683) antibodies and HRP-linked antibody were purchased from Cell signaling technology (Beverly, MA, USA).

Cell culture and treatment. Human osteosarcoma cell lines (MG-63 and U2OS) and normal hFOB 1.19 cells were purchased from Procell Life Science & Technology (Co. Ltd, Wuhan, China). Cells were incubated in RPMI-1640 medium containing 1% penicillin-streptomycin and 10%

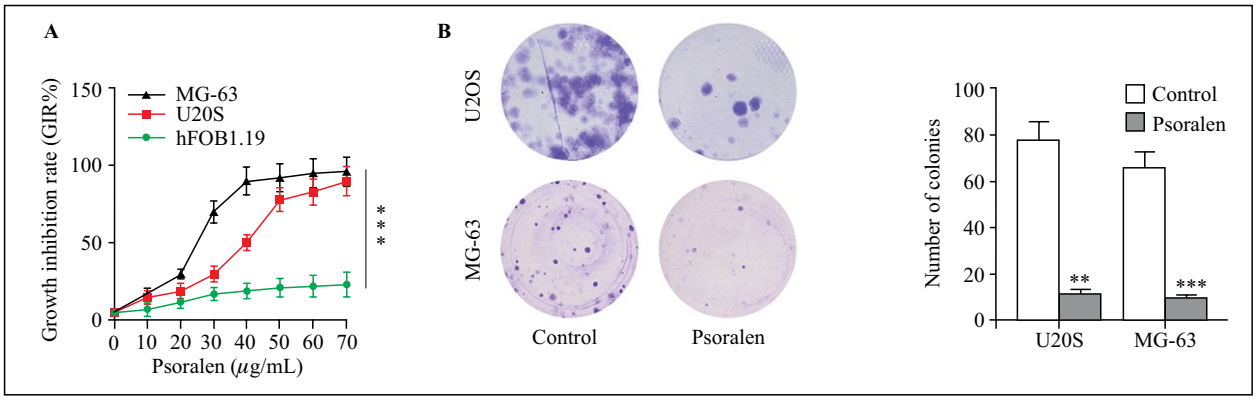

Figure 1. Influence of psoralen on the growth of osteosarcoma cells. **A.** Effects of psoralen (0–70 μg/mL) on the viability of U2OS and MG-63 osteosarcoma cells compared with normal hFOB 1.19 cells for 48 h were detected by MTT. **B.** Colony formation assay evaluated the proliferation of U2OS and MG-63 cells after psoralen treatment for 48 h. $^{**}p < 0.01$, $^{***}p < 0.001$.

fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 at 37°C.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). TRIzol (Invitrogen) was applied to extract total RNA from MG-63 and U2OS cells (1×10^5) according to protocols of the manufacturers. Then, for each sample, 2 μg of RNA was reverse transcribed to obtain the cDNA template by using a PrimeScript® RT reagent Kit (TaKaRa). The real-time PCR reaction was performed using a SYBR Green PCR Kit (Kapa Biosystems, Wilmington, MA, USA) on an ABI PRISM 7500 System (Applied Biosystems, Foster City, CA, USA). The primers used in the present study were: GRP78 (forward: 5'-CTGTCCAGGCTGGTGTGCTCT-3', reverse: 5'-CTTGGTAGGCACCACTGTGTTC-3'), DDIT3 (forward: 5'-ATTGCCTTTCTCCTCGGAC-3', reverse: 5'-TTCATCTGAAGACAGGACCTC-3'), GADD34 (forward: 5'-CCTCTGGCAATCCCCCATAC-3', reverse: 5'-TCTCGCTCACCATACATGCC-3'), EDEM1 (forward: 5'-CTGAACATCAATGATGTACTAGGG-3', reverse: 5'-CGGATGAATTTCCCATTATTGC-3'), S1P (forward: 5'-TGCGGGAAGGGAGTATGTTT-3', reverse: 5'-CGATGGCGAGGAGACTGAAC-3'), GDF15 (forward: 5'-TAGACAAGGAGAGCCAGGA-3', reverse: 5'-ATGAGGACACCATTCCAGC-3') and GAPDH (forward: 5'-TCAAGATCATCAGCAATGCC-3', reverse: 5'-CGATACCAAAGTTGTCATGGA-3'). Relative mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

MTT assay. Osteosarcoma cell viability influenced by psoralen treatment was detected by MTT assay. MG-63 and U2OS cells were incubated in 96-well plates (5×10^3 cells/well) in 100 μL of RPMI 1640 for 24 h. Then the medium was replaced by a fresh medium supplemented with different concentrations of psoralen (0, 10, 20, 30, 40, 50, 60, and 70 μg/mL) and the cells were cultured for 48 h. Then, to each well 20 μL of MTT was added (Sigma) and cultured at 37°C for 4 h. Absorbance was measured by an automatic microplate reader (Bio-Rad, Hercules, CA, USA) at 490 nm.

Colony formation assay. U2OS and MG-63 cells were treated with psoralen (8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells) and cultured in six-well plates (2×10^3 cells/well). Culture medium was replaced every two days. After 2 weeks, the cells were stained with 0.1% crystal violet (Beyotime) for 5 min after being washed twice with phosphate-buffered saline (PBS). The colonies were observed and counted under a microscope.

Cell cycle assay by flow cytometry. U2OS and MG-63 cells were seeded into 6-well plates (1×10^5 cells/well). After being incubated for 24 h at 37°C, cells were treated with psoralen (8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells) for 48 h. After the treatment, cells were harvested, fixed with pre-cooled 70% ethanol for 24 h and washed twice with PBS. Then, cells were stained with propidium iodide (PI) working solution containing RNase at room temperature for 30 min. The samples were analyzed using FACScan flow cytometer (Becton-Dickinson, Franklin Lakes, NJ, USA) and cellular DNA content was measured using the ModFit LT 3.2 software. The proportion of apoptotic peaks as well as the cell cycle at the G0/G1, S, and G2/M phases were determined.

Apoptosis assessment by flow cytometry. U2OS and MG-63 cells were seeded into 6-well plates (1×10^5 cells/well). After being incubated for 24 h at 37°C, cells were treated with psoralen (8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells) for 48 h. After the treatment, cells were harvested and washed twice with PBS. An AnnexinV-FITC/propidium io-

dide (PI) apoptosis detection kit (Pharmingen) was applied to determine cell apoptosis. The ImageJ2 software was used to analyze the apoptosis rate.

Western blotting. Total proteins were extracted from U2OS and MG-63 cells using RIPA lysis buffer (Beyotime) containing protease inhibitors. Then, equivalent proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA) after separation with the 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The membrane was subsequently incubated at 4°C overnight with primary antibodies against cyclin A1, cyclin B1, cyclin D1, CDK2, Total caspase 3, Bcl-2, Bax, cleaved caspase 3, CHOP, IRE1α, XBP-1s, PERK, and ATF-6 diluted at 1:1000, GAPDH diluted at 1:10000, followed by incubation with HRP-conjugated secondary antibody. GAPDH was considered as an internal reference. The enhanced chemiluminescence (ECL) plus kit (Millipore) was applied to visualize protein expression.

Statistical analysis. All data are expressed as the mean ± standard deviation. One-way analysis of variance followed by Dunnett's multiple comparison tests were utilized for difference evaluation among multiple groups and Student's *t* test was for comparison between two groups using SPSS 13.0 software (SPSS, Chicago, IL, USA). The value of $p < 0.05$ was deemed as statistically significant.

Results

Psoralen inhibited human osteosarcoma cell proliferation

Initially, the effects of psoralen on the cytotoxicity of human osteosarcoma cell lines were investigated. MTT assay revealed that psoralen suppressed osteosarcoma cell viability (IC_{50} 25 μg/mL for MG-63 cell and IC_{50} 40 μg/mL for U2OS cell) in a dose-dependent way but exerted no such significant effect on normal hFOB 1.19 cells (Fig. 1A). The IC_{10} of psoralen was 8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells. Therefore, the concentration of IC_{10} was a non-cytotoxic dose selected as the working concentration in the subsequent experiments. In addition, we also evaluated cell morphological changes induced by psoralen in human osteosarcoma cell lines. U2OS cells were treated with psoralen (40 μg/mL) for 12, 24, and 48 h to investigate how different incubation times with psoralen influenced cell morphology. The results revealed increased intercellular spaces and mild shrinkage of osteosarcoma cells. Osteosarcoma cells shrunk significantly with the prolonged treatment, followed by the observation of dead and floated cells. These results demonstrated that psoralen induced cell shrinkage and attenuated the adhesion of osteosarcoma cells in a time-dependent manner (Suppl. Fig. 2A). Then, the morphology of U2OS cells in the negative control group, the low-dose psoralen (10 μg/mL) group, the high-dose psoralen (50 μg/mL) group, and the positive control group (treated with 250 μg/mL of 5-FU) was observed under an inverted microscope after 48 h. Compared to untreated cells, the number of osteosarcoma cells was significantly reduced after treatment with elevated concentration of psoralen (data not shown), and psoralen induced progressive morphological changes in U2OS cells in a dose-dependent manner (Suppl. Fig. 2B). Next, we detected whether psoralen affects human osteosarcoma cell proliferation. Colony formation assay showed that the colony numbers of psoralen-treated MG-63 and U2OS cells were significantly reduced compared to the control group, which showed that psoralen (8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells) inhibited osteosarcoma cell colony formation (Fig. 1B). In conclusion, psoralen suppressed the proliferation of osteosarcoma cells.

Psoralen induced cell cycle arrest in human osteosarcoma cells

Subsequently, we evaluated cell cycle progression by analyzing cell cycle distribution with flow cytometry. The analyses showed that psoralen (8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells) caused a significant increase in the percentage of U2OS and MG-63 cells at the G0/G1 phase (Fig. 2A). We additionally investigated the influence of psoralen on the level of cell cycle proteins. Relative to the control group, the levels of cyclin A1, cyclin B1, cyclin D1, and CDK2 proteins were significantly suppressed by psoralen (Fig. 2B). Taken together, psoralen induced cell cycle arrest in cultured human osteosarcoma cells.

Psoralen promoted human osteosarcoma cell apoptosis

Next, the influence of psoralen on osteosarcoma cell apoptosis was determined. Flow cytometric analyses revealed that psoralen treatment (8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells) promoted apoptosis of MG-63 and U2OS osteosarcoma cells, significantly increasing cell apoptosis rate compared with the control groups (Fig. 3A). Then, we measured the effects of psoralen on apoptosis-related proteins by western blotting. The results demonstrated that the levels of cleaved caspase 3 and Bax proteins were increased while the level of Bcl-2 protein was reduced after treatment with psoralen (8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells) (Fig. 3B).

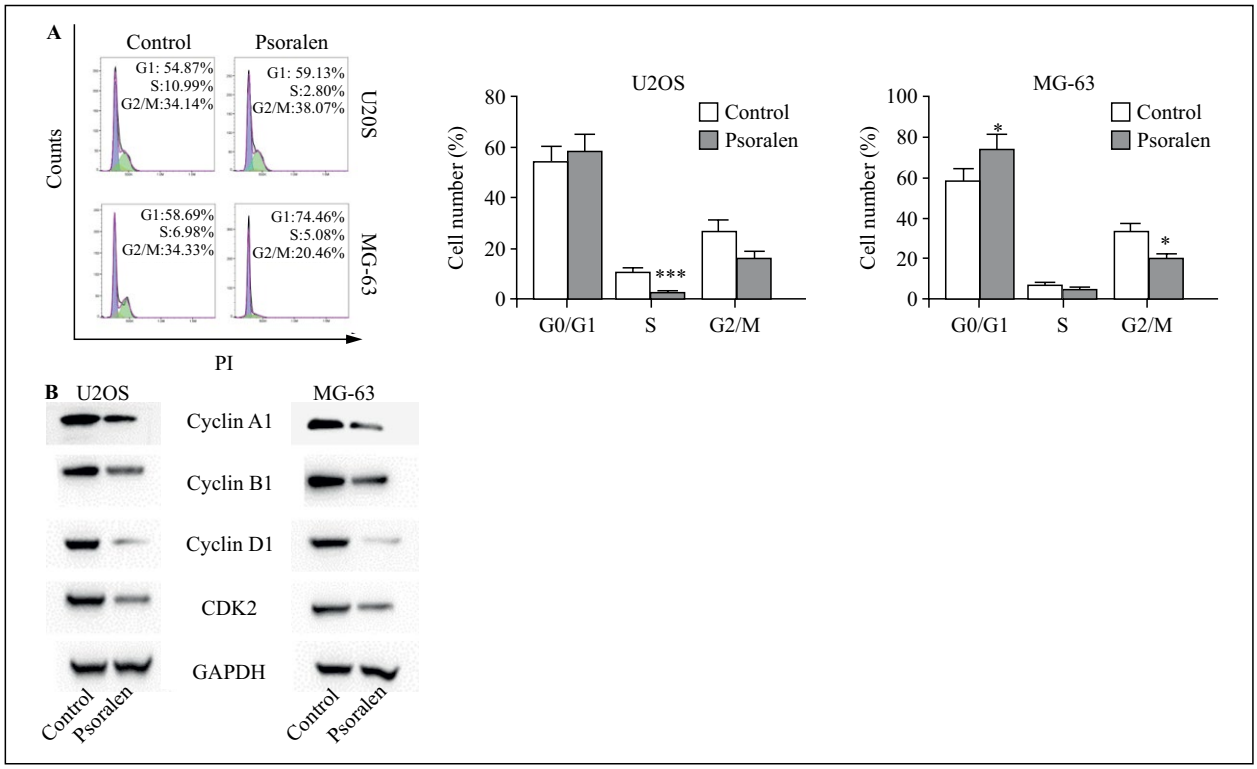

Figure 2. Influence of psoralen on the cell cycle in osteosarcoma. **A.** The cell cycle of U2OS and MG-63 cells treated with psoralen for 48 h compared to the control group was measured by flow cytometric analysis. **B.** The influence of psoralen on the level of cell cycle proteins was determined by western blotting. $^{*}p < 0.05$, $^{***}p < 0.001$.

The level of total caspase 3 was not influenced. Taken together, psoralen induced cell apoptosis in human osteosarcoma.

Psoralen upregulated the expression of ER stress-related genes and proteins in human osteosarcoma cells

U2OS and MG-63 cells were treated with psoralen (8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells) to Fig out the influence of psoralen on the expression of ER stress-associated key genes and proteins. In comparison with the control groups, GRP78, DDIT3, GADD34, EDEM1, GDF15, and S1P mRNA expression were upregulated after psoralen treatment in both osteosarcoma cell lines (Fig. 4A–4F). Furthermore, Western blotting demonstrated that levels of CHOP, IREα, XBP-1s, GRP78, PERK, and ATF-6 proteins were also upregulated after psoralen treatment (Fig. 4G). Thus, psoralen activated ER stress in osteosarcoma cells.

Psoralen induced osteosarcoma cell apoptosis through ER stress

Finally, we further explored whether psoralen-induced cell apoptosis is associated with ER stress. U2OS and MG-63 cells were pretreated with the ER stress inhibitor 4-PBA for 2 h and then treated with psoralen (8 μg/mL for MG-63 cells and 9 μg/mL for U2OS cells) for 24 h. The results of flow cytometry demonstrated that the combination of 4-PBA and psoralen reduced the apoptosis rate of U2OS and MG-63 cells compared with psoralen alone (Fig. 5). These data suggested that psoralen induced ER stress, which participated in the process of psoralen-induced cell apoptosis.

Discussion

Osteosarcoma remains an important cause of cancer-related deaths around the world [27]. Currently, even though the synthetic inhibitors applied in targeted therapies have improved patient prognosis, toxicity and resistance to these agents is still a challenge [28]. Plant-derived natural products and their derivatives have already been used to produce chemotherapeutic agents [29]. Furthermore, many crude herbal or botanical preparations have also been used for cancer treatment [29]. Psoralen is a tricyclic furocoumarin extracted from *Psoralea corylifolia*, which has been confirmed to have antitumor effects in some types of

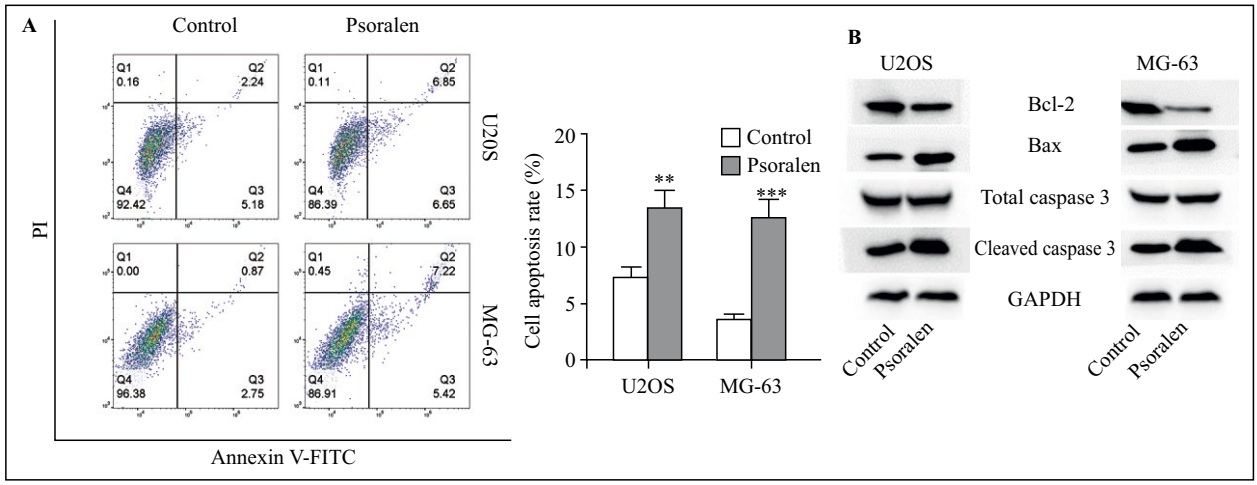

Figure 3. Influence of psoralen on human osteosarcoma cell apoptosis. **A.** Flow cytometric analyses detected the influence of psoralen on osteosarcoma cell apoptosis. **B.** Western blotting showed apoptosis-associated protein levels in osteosarcoma cells treated with psoralen for 48 h. $^{**}p < 0.01$, $^{***}p < 0.001$.

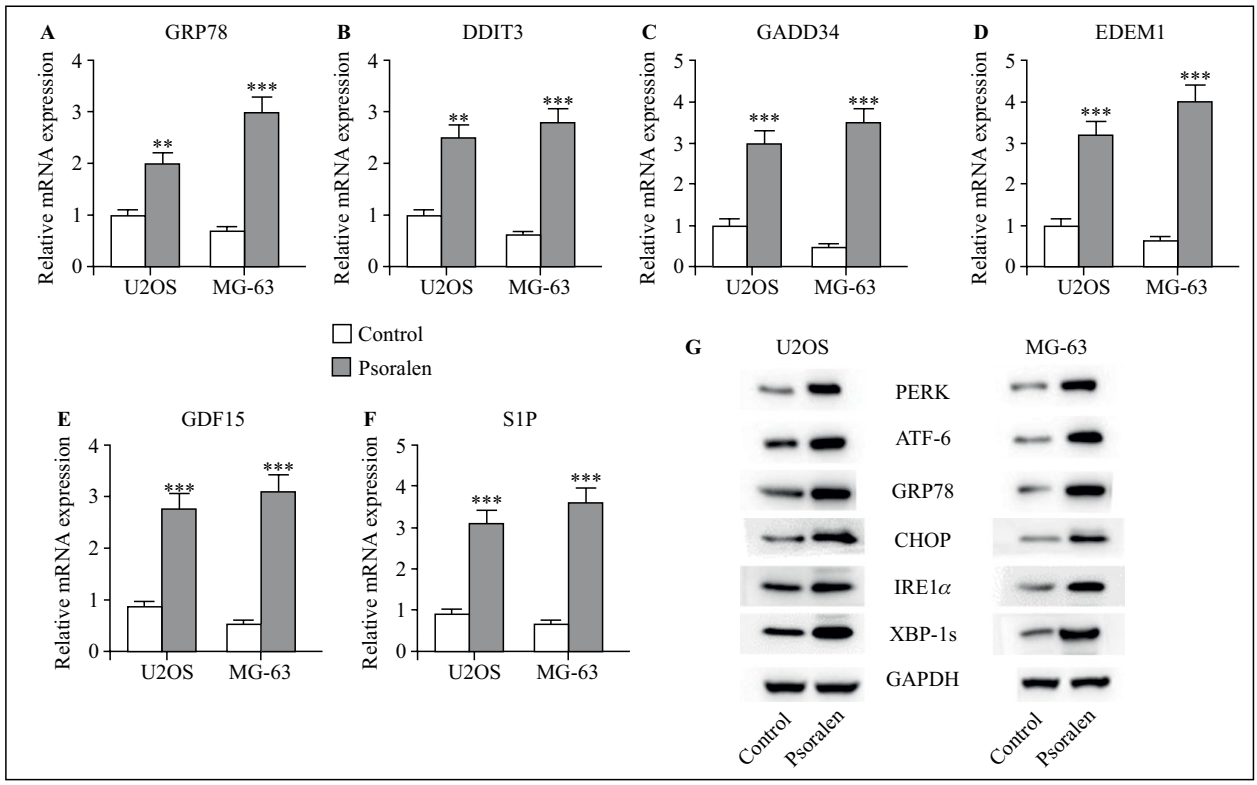

Figure 4. Influence of psoralen on ER stress in human osteosarcoma cells. **A–F.** The mRNA expression of key genes related to ER stress under psoralen treatment for 48 h compared to the control groups. **G.** Western blotting showed the influence of psoralen on ER stress-associated protein levels. $^{**}p < 0.01$, $^{***}p < 0.001$.

cancers [30]. However, whether psoralen influences human osteosarcoma cells and in turn regulates the tumorigenesis of osteosarcoma is not clear. Therefore, our study investigated for the first time the effects of psoralen in human osteosarcoma cell lines. We found that psoralen inhibits cell proliferation in a dose-dependent manner. Furthermore, psoralen induced cell apoptosis via the activation of ER stress. Collectively, psoralen exhibited anti-proliferative effects in osteosarcoma cells.

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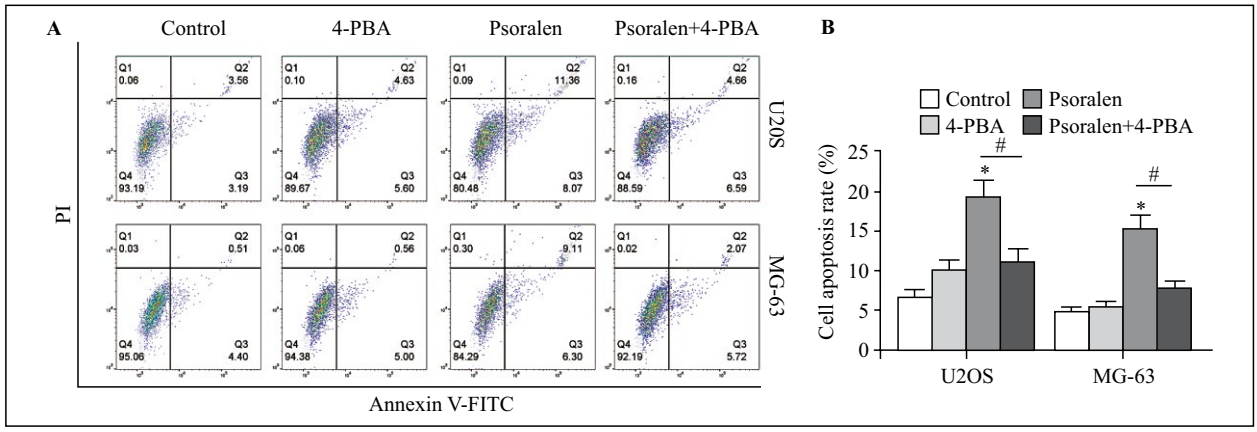

Figure 5. ER stress was associated with psoralen-induced apoptosis of human osteosarcoma cells. The apoptosis of U2OS and MG-63 cells treated with psoralen, 4-PBA or both for 48 h were measured by flow cytometry. $^{*}p < 0.05$ *versus* control, $^{\#}p < 0.05$ *versus* psoralen.

Previously, the antitumor effects of psoralen have been reported in breast cancer and hepatocellular carcinoma cell lines. For example, psoralen inhibits proliferation of human SMMC7721 hepatoma cells in a dose- and time-dependent manner and promotes cell apoptosis in a dose-dependent manner [21]. Psoralen inhibited cell proliferation in a dose-dependent manner and induced cell cycle arrest and apoptosis of breast cancer cell lines MCF-7 and MDA-MB-231 [18, 31]. Based on these results, we investigated the influence of psoralen on MG-63 and U2OS cells. In our study, we initially discovered that psoralen changed osteosarcoma cell morphology in a dose- and time-dependent manner. Osteosarcoma cells demonstrated progressive morphological changes to psoralen with increased duration or concentration. Furthermore, we investigated the influence of psoralen on osteosarcoma cell behaviors. We found that psoralen treatment reduced the proliferation of MG-63 and U2OS cells in a dose-dependent manner, as revealed by MTT and colony formation assays. Additionally, since the malignant transformation of tumor cells is also characterized by cell cycle disorder, we then performed flow cytometry analysis to explore cell cycle progression after psoralen treatment. Cyclin B binds to cyclin-dependent protein kinase 1 (CDK1), and the active complex formed is mitosis-promoting factor (MPF), also known as M-phase-promoting factor [32]. MPF can translocate into the nucleus and phosphorylate its nuclear substrates, which promote the G2/M phase transition [33]. The key proteins associated with cell cycle and apoptosis have been investigated in multiple types of human cancers. For example, in human gastric cancer SGC-7901 cells, cannabidiol suppresses the levels of CDK2 and cyclin E proteins, thereby resulting in cell cycle arrest at the G0/G1 phase [34]. Cannabidiol also significantly increases the level of Bax protein and decreases the levels of Bcl-2 and cleaved caspase-3 proteins, thereby inducing apoptosis in SGC-7901 cells [34]. In cholangiocarcinoma HCCC-9810 and RBE cells, knockdown of targeting protein for Xenopus kinesin-like protein 2 (TPX2) expression elevates the levels of cyclin A1 and cyclin B1 proteins, but reduces the levels of cyclin D1 and CDK2 proteins, blocking the cell cycle at G2/M phase [35]. TPX2 downregulation also enhances the expression of Bax protein while inhibits the expression of Bcl-2 protein, suggesting that cholangiocarcinoma cell apoptosis was facilitated by TPX2 knockdown [35]. In lung cancer A549 cells, coptisine derivative 8-cetylcoptisine (CCOP) induces apoptosis and G0/G1 cell cycle arrest, which can be explained by the increased levels of Bax and cleaved caspase 3 proteins as well as the decreased levels of Bcl-2, cyclin D, cyclin E, CDK2, CDK4 and CDK6 proteins [36]. Similarly, in our study, psoralen treatment also reduced the levels of cyclin A1, cyclin B1, cyclin D1, CDK2 and Bcl-2 proteins, and elevated the levels of cleaved caspase-3 and Bax proteins. Thus, we have demonstrated that in the studied human osteosarcoma cells psoralen induced the G0/G1 cell cycle arrest and apoptosis.

As well known, apoptosis is modulated by various pathways, including the ER stress-mediated apoptosis signaling pathway [24]. ER is a dynamic organelle that is involved in multiple cellular functions by controlling proteostasis, lipid metabolism and calcium stores [37–39]. Under stressful situations, ER environment and protein maturation are impaired, which causes the accumulation of misfolded proteins and a typical stress response known as unfolded protein response

(UPR) [40]. UPR protects cells from stress and helps cellular homeostasis re-establishment, while UPR activation induces cell apoptosis during prolonged ER stress [41]. ER stressors regulate autophagy and in turn induce cell survival or death according to the situation. The UPR in cells mainly involves three pathways: activating transcription factor 6 (ATF-6) pathway, inositol-requiring enzyme 1 (IRE1) pathway, and double-strand RNA-activated protein kinase-like ER kinase (PERK) pathway [42]. Under normal conditions, ATF6, IRE1 and PERK are all combined with glucose regulatory protein 78 (GRP78/ /Bip) in an inactive state [43]. When ER stress occurs, GRP78 expression is increased, and many unfolded or misfolded proteins snatch GRP78, making three kinds of responsive proteins exposed and activated [44]. ATF-6 is a type II ER transmembrane protein, and activated ATF-6 facilitates transcription and expression of CHOP in the nucleus, thereby repressing Bcl-2 and leading to apoptosis [45]. PERK is a type I ER transmembrane protein, which can promote the translation of activating transcription factor-4 (ATF-4) and in turn activate the CHOP apoptotic pathway [41]. Therefore, we studied the mRNA and the protein levels of ER-associated functional molecules in MG-63 and U2OS cells after psoralen treatment. Our findings of the psoralen-induced upregulation of ER stress-related genes and proteins levels including PERK, ATF-6, CRP78, CHOP, IRE1α, and XBP-1s have not yet been reported in osteosarcoma MG-63 and U2OS cells. Previously, in human SMMC7721 hepatoma cells, Wang *et al*. also found that different incubation times of psoralen led to different changes in ER stress-related genes and proteins levels [21]. The level of IRE1α was upregulated time-dependently. The levels of GRP78 and XBP-1s proteins were also markedly increased after psoralen treatment for 24 h and 48 h. However, psoralen had no significant effect on the level of CHOP protein in SMMC7721 cells. Thus, the effects of psoralen on the expression of ER stress-related molecules in different types of cells are different.

In summary, we demonstrated that psoralen suppressed the proliferation and induced the apoptosis of human osteosarcoma cells through activating ER stress. These results provided a molecular basis for the further development of psoralen as novel anticancer drugs for human osteosarcoma.

Disclosure statement

The authors declare that they have no conflicts of interest.

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