Psoralidin inhibits osteosarcoma function by down-regulating ITGB1 expression through FAK and PI3K/Akt signaling pathways

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Abstract

Background

Psoralea is a medicinal plant of legume, which has been used in many diseases for a long time. Psoralidin (PSO) is the main extract of psoralea, which has antibacterial, anti-tumor, anti-inflammatory, antioxidant and other pharmacological activities. The inhibitory effect of PSO on tumor has been found, but its inhibitory effect on osteosarcoma has not been reported. Therefore, this study aimed to evaluate the inhibitory effect of PSO on osteosarcoma and its underlying molecular mechanism.

Materials and Methods

Crystal violet assay, CCK8 assay, and EdU stain assay were used to confirm the inhibitory effect of PSO on the proliferation of 143B and MG63 osteosarcoma cells. Wound healing and Transwell assays were conducted to evaluate the effects of PSO on osteosarcoma cell migration and invasion. The cell cycle and apoptosis were observed by flow cytometry. RNA sequencing was performed to determine the possible relevant molecular mechanisms, and protein expression levels were analyzed using Western blot. The inhibitory effect of PSO on osteosarcoma in vivo was analyzed by an orthotopic OS tumor animal model and immunohistochemistry.

Results

According to crystal violet assay, cck8 assay, and EdU stain assay, PSO inhibited cell proliferation in a concentration-dependent manner. Moreover, PSO inhibited the migration and invasion of the osteosarcoma cells. Flow cytometry analysis showed that PSO induces cell cycle arrest and apoptosis in OS cells. To elucidate the molecular mechanism of PSO, we performed RNA-seq analysis and found that PSO treatment significantly inhibited FAK and PI3K/Akt signaling pathways by down-regulating the expression of ITGB1 in MG63 and 143B cells. Furthermore, we confirmed that PSO restrained osteosarcoma growth in vivo mouse models.

Conclusion

PSO may play an anti-osteosarcoma role via FAK and PI3K/Akt signaling pathways by down-regulating ITGB1 expression

1 | Background

Psoralea corylifolia, a traditional Chinese medicine, is a member of the Leguminosae plant family. Psoralidin (PSO), a coumestan derivative, is an extract of psoralen and has an array of pharmacological activities, such as anti-inflammatory, antioxidant, and antibacterial properties. (Fig. 1A) According
to a recent study, PSO effectively inhibits cancer cell proliferation and promotes apoptosis. It can induce apoptosis of prostate cancer cells through autophagy. [5] At the same time, psoralidin significantly downregulated Notch1 Signaling and induced growth inhibition in breast cancer cells. [6] It can also induce cell cycle arrest and apoptosis in colon cancer. [7] In addition, PSO has been reported to have a protective effect on the heart by activating the SIRT1/PPARγ signaling pathway. [8] PSO can also have a therapeutic effect on lung tissue injury after radiotherapy. [9] Therefore, the multi-target anticancer activity and potential organ protective function of PSO are beneficial as anti-osteosarcoma drug candidates. However, the role and mechanism of PSO in osteosarcoma are still unclear and need further research.

Osteosarcoma (OS) is the most common primary bone malignancy in children, adolescents, and young adults with a high tendency for local invasion and distant metastases. [10] Current therapy for osteosarcoma includes neoadjuvant chemotherapy, surgery, and adjuvant (post-surgery) chemotherapy. [11, 12] A great deal of progress has been made in OS treatment in recent years, and the five-year survival rate for patients with only local diseases has increased to about 65%. [13, 14] However, in cases of metastatic lesions, the five-year survival rate remains dismal at less than 20%. [15] Tumor migration and invasion are the key factors involved in tumor metastasis which are closely related to the treatment and prognosis of patients. [16] Therefore, it is of great significance to develop adjuvant drugs that can effectively inhibit osteosarcoma metastasis.

In this study, we investigated PSO's antitumor effect and molecular mechanism in OS cell lines. Our results suggest that PSO may inhibit the initiation and progression of OS via the FAK and PI3K/Akt signaling pathways by down-regulating ITGB1 expression.

2 | Material And Methods

2.1 | Cell Culture and Drug Preparations

Human osteosarcoma cell lines 143B and MG63 were purchased from the American Type Culture Center (ATCC, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum, and Penicillin-streptomycin liquid were purchased from Gibco, Carlsbad, CA. PSO of 20mg, Cell count kit-8, and dimethyl sulfoxide (DMSO) were purchased from China MCE. Crystal violet staining solution was purchased from Beijing Solarbio Science & Technology Co., Ltd. EdU 488 kit and BCA protein concentration kit was purchased from Beyotime Biotechnology. Transwell was purchased from Guangzhou Jet Bio-Filtration Co., Ltd. Matrigel was purchased from BD Biosciences, USA.

PSO of 20mg was purchased from China MCE, with a purity of over 99%. Take 20 mg of PSO, dissolve it in 595µl DMSO until the final concentration is 100 mM, and store it at −20°C. Dilute with the fresh medium before use.

2.2 | CCK8 Assay
A logarithmic growth phase of MG63 and 143B cells was digested, counted, and mixed with 10% FBS to prepare 5 x 10^3 cells/well, seeded into 96-well culture plates for 24h. After cell adhesion, different concentrations of PSO were added for 24 hours and 48 hours. The cells were also incubated for 2 h at 37°C in 10% cell count kit-8 liquid diluted in a normal medium. Each well's spectroscopic absorbance was measured using an automated plate reader at 450nm.

### 2.3 Crystal Violet Assay

OS cell lines at a density of 5 x 10^3 per well were seeded in 96-well plates overnight and were treated with different concentrations of the drugs. The cells were cultured for 24 and 48 hours and stained with crystal violet. Finally, the crystal violet in 96 well plates was fully dissolved with 20% acetic acid solution, and the OD value of each well was measured at 590 nm wavelength of the enzyme marker to reflect the cell proliferation activity.

### 2.4 EdU cell staining assay

MG63, 143B cells were cultured in 6-well plates (2 x 10^5 cells/well). Cells were incubated at 37°C and 5% CO_2 for 24h. The EdU solution was then incubated at 37°C for 4h and fixed with 4% paraformaldehyde for 20min according to the manufacturer's protocol, permeate with 0.3% triton-100 for 15min. Incubate with 500ul of click reaction solution for 30min. Nuclei were stained with DAPI, and EdU/DAPI positive rate was calculated. Images were taken with a fluorescence microscope. (Nikon Clipstie, Japan)

### 2.5 Wound Healing Assays

MG63 and 143B cells were seeded into 6-well plates. After cellular fusion, scratched with a 200µl pipette tip and then washed three times with 1 x PBS to remove cell debris and suspended cells. Then, a new medium with different concentrations of PSO was added. The scratched area was photographed at different time points (6 hours, 12 hours, and 24 hours) and the recovery area was calculated with image-J software.

### 2.6 Cell Migration and Invasion Assays

Thaw the Matrigel at 4°C and dilute it with serum-free DMEM at a ratio of 1:8; Then, the mixed solution was added to the transwell chamber with 80µl medium/well and incubated at 37°C and 5% CO_2 for 4 hours. Subsequently, 2.5 x 10^4 cells were added to the upper cavity of the transwell chamber, and different concentrations of PSO were added to the lower cavity of the transwell. After incubation for 24 hours, the cells that penetrated the matrix gel coating and migrated to the lower chamber were stained with crystal violet (0.1%) and photographed. The steps described above are to reflect the intrusion capability. For cell migration measurement, the experiment was conducted under the same conditions, except for the removal of Matrigel in the upper cavity.

### 2.7 Flow cytometry analysis

Flow cytometry was used to determine the effect of PSO on osteosarcoma apoptosis and cell cycle. After treatment with PSO, the cells were then harvested, washed, and resuspended three times in PBS, and
500µl of PBS was added to resuspend the cells. The apoptosis rate was determined by flow cytometry according to the method provided by Annexin V-FITC/PI double labeling staining kit. For the cell cycle, osteosarcoma cells were harvested and washed and then fixed with 70% ethanol overnight at 4°C. Cell cycle analysis was performed on a flow cytometer (CytoFLEX, Beckman Coulter, Fullerton, CA, USA).

2.8 | Western Blot Assay

OS cells were treated with different concentrations of PSO for 24 hours. RIPA then lyses the cells and extracts the protein. The samples were separated by SDS-PAGE gel electrophoresis and transferred to a PVDF membrane (0.45µm). After blocking with 5% skim milk for 1 h and washing three times with TBS, the membranes were left with primary antibodies overnight at 4°C. After washing 3 times with 1×TBST, the membranes were incubated with a secondary antibody (goat anti-rabbit/mouse IgG, 1:5000) for 2 hours at room temperature. Pictured and analyzed the protein bands by ChemiDoc MP Imaging System and Image Lab Software (Bio-Rad, California, USA). The anti-bodies used were Anti-PCNA antibody (ab92552, Abcam), Anti-MMP2 antibody (ab92536, Abcam), Anti-MMP9 antibody (ab76003, Abcam), Snail Rabbit mAb (#3879, CST), bcl-2 Rabbit mAb (#4223, CST), Bax Rabbit mAb (#41162, CST), cleaved caspase-3 Rabbit mAb (#9654, CST), β-actin Rabbit mAb (#4970, CST), FAK (ab40794, Abcam), p-FAK (phospho Y397) (ab81298, Abcam), PI3 Kinase p85 alpha (ab191606, Abcam), p-PI3 Kinase p85 alpha (phospho Y607) (ab182651, Abcam), Akt (ab179463, Abcam), p-Akt (phospho S472 + S473 + S474) (ab192623, Abcam).

2.9 | RNA Sequencing

143B cells and MG63 cells were treated with 30 µM PSO for 24 hours. RNA Extraction Kit (Thermo Fisher Scientific, Waltham, MA) was used to extract total RNA from cells. Subjected to RNA sequencing to determine the changes in mRNA expression profiles. The obtained RNA samples were sent to Beijing Qingke Biotechnology Co., LTD., China, and RNA sequence manipulation was performed at DNBSEQ-T7.

2.10 | Kaplan-Meier analysis

The GSE21257 OS microarray dataset was downloaded from GEO database (https://www.ncbi.nlm.nih.gov/geo/), including 53 OS biopsy samples before chemotherapy, including metastatic status and time information, including 34 metastatic samples and 19 non-metastatic samples. And contains transition status and time information. Kaplan-Meier (K-M curve) graph was constructed by R package survminer (version 0.4.6) to visualize the survival curve.

2.11 | Molecular docking

The core protein structure of the PPI network was downloaded from the PDB database (http://www.rcsb.org/) and its ligands and water molecules were removed with pymol. Using the Pubchem database (https://pubchem.ncbi.nlm.nih.gov) to download the core ingredient of the SDF file, its use OpenBabel software into mol2 format, Finally, the obtained protein structure (receptor) and active
component (ligand) were docked by autodock software, and the docking results were visualized by Pymol software.

### 2.12 | Orthotopic OS Tumor Animal Model

Balb/c-nude female mice (3–4 weeks old) weighing from 15 to 20 g were purchased from HUNAN SJA LABORATORY ANIMAL Co., Ltd. After adaptive breeding (1 week), 50ul of 143B suspension (2×10⁷ cells /ml) was injected into the proximal tibia of mice. Then, the rats were given different doses of PSO (5, 10, 20 mg/kg) or dimethyl sulfoxide (DMSO) by gavage every two days, and the animals were sacrificed after 3 weeks of treatment. The formula for calculating the tumor volume was 0.5×L×W² (L is the length of the tumor, and W is the width of the tumor). All animal experiments were approved by the Animal Care and Use Organization Committee IACUC of Chongqing Medical University.

### 2.13 | Hematoxylin and Eosin Staining and Immunohistochemistry

Tumor tissues were collected, fixed in 4% paraformaldehyde for 24 hours, and washed in PBS. The fixed tissue samples were embedded in paraffin. Dewaxing and dyeing with hematoxylin and eosin (H&E).

Immunohistochemical staining of tumor sections with PCNA (1:100), Bax (1:100), and MMP2 (1:100) antibodies. All sections were imaged using a light microscope (magnification, x40 and x100).

### 2.14 | Statistical Analysis

SPSS 22.0 software package was used for statistical processing. The in vitro experiment was repeated three times, and the in vivo experiment was repeated five times. The differences among multiple groups were analyzed by one-way analysis of variance, and the Tukey test was used for comparison between groups. The experimental data were expressed as mean ± SD (mean ± SEM), and P < 0.05 was statistically significant.

### 3 | Results

#### 3.1 | PSO Inhibits the Growth of 143B and MG63 Cells

To investigate the anti-proliferation effect of PSO on osteosarcoma cells, 143B and MG63 cells were treated with 0–50 µM PSO, and measured the cell viability at 24 and 48 hours, respectively. The experimental control group was treated with DMSO and set as the Control (Con) group. After 24 hours of treatment, the half inhibitory concentration (IC50) of the drug on osteosarcoma cells was calculated, (IC50, MG63 = 31.44 µM, 143B = 29.52 µM). Based on these data, we selected 10, 20, 30, and 40µM for
143B cells and 20, 30, 40, and 50µM for MG63 cells as working concentrations for subsequent experiments. CCK-8 assay further confirmed the inhibitory effect of PSO on the OS cell line (143B, MG63). (Fig. 2A-B, P < 0.05) we found that PSO inhibited the proliferation of OS cells in a dose and time-dependent manner via crystal violet staining and EdU staining assay. (Fig. 2C-J, P < 0.05) In addition, we found that PSO reduced the protein expression level of proliferating cell nuclear antigen (PCNA), a well-recognized marker for evaluating osteosarcoma growth. (Fig. 2K-N, P < 0.05) Based on these results, PSO inhibited the proliferation of human osteosarcoma cells effectively.

3.2 | PSO Inhibited OS Cells Migration and Invasion

The effect of PSO on the metastasis ability of OS cells was investigated by wound healing and transwell assay. Wound-healing assays showed that PSO inhibited the migration ability of OS cells, resulting in a reduced wound healing rate. (Fig. 3A-D, P < 0.05) Transwell migration assay further demonstrated that PSO inhibited the migration of OS cells compared with the Control group. (Fig. 3E-H, P < 0.05) According to the Matrigel transwell test, PSO inhibited OS cells' invasive potential compared with the control group. (Fig. 3I-L, P < 0.05) Epithelial-mesenchymal transition (EMT) and matrix metalloproteinase (MMP) play key roles in tumor metastasis. [17, 18] In addition, PSO significantly reduced the expression of EMT transcription factors Snail, matrix metalloproteinase 2 (MMP2), and matrix metalloproteinase 9 (MMP9) by Western blotting. (Fig. 2M-P, P < 0.05) As a result, these in vitro experiments demonstrated that PSO inhibited OS cell migration and invasion.

3.3 | PSO induces OS cell cycle arrest at G0/G1 phase and promotes apoptosis

Two main reasons for cell proliferation inhibition are cell cycle arrest and apoptosis induction. Next, we used flow cytometry to investigate whether PSO had any effect on the cell cycle in OS cells. We performed flow cytometry analysis and found that PSO treatment increased the G0/G1 phase of OS cells. (Fig. 4A-D, P < 0.05) In addition, we quantified the apoptotic rate of 143B and MG63 cells by flow cytometry and found that PSO treatment significantly increased the percentage of apoptotic rate in 143B and MG63 cells. (Fig. 5A-D, P < 0.05) Following PSO treatment, we detected apoptosis-related proteins with Western blot, and the results showed that the protein level of anti-apoptotic factor Bcl-2 decreased, while the protein level of pro-apoptotic factor Bax increased. In addition, levels of cleaved caspase-3, a classical marker of apoptotic activation, were increased by PSO stimulation. (Fig. 5E-H, P < 0.05) These results indicated that Cell cycle arrest and apoptosis were induced in OS cells by PSO treatment.

3.4 | PSO down-regulated ITGB1 expression and attenuated FAK and PI3K/Akt signaling pathways

To explore the possible molecular mechanism behind the antitumor effect of PSO, RNA was extracted from 143B, MG63 cells after 24 hours of PSO treatment, and RNA sequence analysis was performed. Compared with the control group, RNA sequence analysis showed that 1686 up-regulated genes and 1993 down-regulated genes were found in 143B cells treated with PSO, and 1585 up-regulated genes and
1523 down-regulated genes were found in MG63 cells. Among them, there are 797 common up-regulated genes and 792 down-regulated genes. (Fig. 6A-F) Then, all genes identified were then categorized based on gene ontology (GO) into three groups: biological processes (BP), cellular components (CC), and molecular functions (MF). (Fig. 6G)

The KEGG pathway enrichment analysis was used to analyze differentially expressed genes and rank the results of pathway enrichment analysis. (Fig. 7A) We took the intersection of genes with the same trend of gene expression change in the two cell lines and screened only the enrichment with NES absolute value ≥ 1 and Qvalue ≤ 0.05 by GSEA enrichment analysis. (Fig. 7B) After GESA, Venn interaction of the abovementioned top five pathways, ITGB1, PIK3CD, MAPK3, and PRKCA were identified as highly enriched DEGs. Among them, the expression trend of ITGB1, MAPK3 and PRKCA was decreasing, and the expression trend of PIK3CD was increasing. (Fig. 7C) Download the GSE21257 OS microarray dataset from the GEO database, including 53 OS biopsy samples. For the above four genes, Kaplan-Meier analysis was performed on the data set, and the samples were divided into high risk and low risk groups. All patients in the high-risk group developed OS metastasis within 5 years compared with the low-risk group. Kaplan-Meier analysis showed that of the four genes, the higher PIK3CD and MAPK expression indicated patients had better overall survival, the higher the expression of ITGB1 and PRKCA, the lower the overall survival rate and ITGB1 had the highest hazard ratio. (Fig. 7D, P < 0.05) In addition, the above proteins were further docked with PSO. The molecular docking of ITGB1 with PSO showed that the compound had 2.6 A hydrogen bond with amino acid residue LEU275, 2.2 A hydrogen bond with amino acid residue VAL274, and 2.2 a hydrogen bond with amino acid residue GLY439. These interacting binding proteins form a stable complex with compound PSO. (Fig. 7E, Figure S1)

Finally, the expression levels of related proteins were detected by Western Blot. Our results showed that 24 h after PSO treatment, ITGB1 expression was significantly decreased in both cell lines, PRKCA expression was down-regulated only in 143B cell line, while PIK3CD and MAPK3 expression were not significantly changed in both cell lines. (Fig. 7F-H, P < 0.05) We then further analyzed the total and phosphorylated levels of FAK, PI3K/ Akt. We found that the levels of FAK, and the ratios of p-FAK /FAK, p-PI3K /PI3K, and p-Akt /Akt in 143B, MG63 cells were significantly decreased after 24h PSO treatment, which was consistent with the results of our RNA sequence analysis. (Fig. 8A-D, P < 0.05) These results suggest that PSO may inhibit the growth of OS cells through FAK and PI3K/Akt signaling pathway by down-regulating ITGB1 expression.

**3.5 | PSO inhibits Tumor Development in vivo**

Then, we established an OS model using 143B cells and tested the in vivo anti-OS therapeutic effect of PSO. The results indicated that PSO significantly inhibited tumor growth in a dosage-dependent manner. (Fig. 8A-B) Besides, the weight of mice was not significantly reduced (Fig. 8C). The HE staining results revealed that the tumor's structure in the control group was clear and compact, but in the PSO treatment group, we could observe irregular morphology, elevated karyokinesis, and nuclear heterogeneity. Immunohistochemical showed that the expression of PCNA and MMP2 was decreased, and the
expression of Bax was increased in the treatment group. (Fig. 8D) These results further confirmed that PSO could inhibit tumor growth and metastasis in vivo.

4 | Discussion

Osteosarcoma is a highly malignant primary bone tumor. Despite the level of clinical treatment constantly improving, the survival rate of OS patients is still unsatisfactory, especially in patients with lung metastases [19, 20]. Currently used anti-OS drugs such as ifosfamide, methotrexate, and cisplatin are prone to resistance. What's worse, although these drugs have an anti-osteosarcoma effect, most of them are highly cytotoxic to normal cells and can cause liver toxicity and kidney dysfunction. [21–23] Therefore, it is necessary to explore safe and effective drugs for the treatment of OS with relatively low side effects.

Psoralidin (PSO) is the main extract of psoralen. [8] In addition to its antioxidant, antibacterial, and anti-inflammatory effects, PSO also has significant inhibitory effects on various cancers. [9–14, 24, 25] In this study, we first determined that PSO can inhibit the proliferation, migration, and invasion of OS cells and promote the apoptosis of OS cells in vitro and inhibit the tumor growth of OS cells in vivo.

The malignant proliferation and excessive migration and invasion of osteosarcoma cells are the main reasons for the poor prognosis of the tumor. [26] We found that PSO significantly inhibited the proliferation of OS cells in a dose-dependent manner by crystal violet assay, CCK-8 assay, and EdU staining assay. PCNA is closely related to DNA synthesis and plays an important role in the initiation of cell proliferation.[27] In this study, we found that PSO significantly downregulated PCNA in a dose-dependent manner. In addition, we found that the arrest of OS cells by PSO mainly occurred in G0/G1 phase from flow cytometry analysis. This further confirmed that PSO played an anti-proliferative role in osteosarcoma cells.

Tumor metastasis constitutes one of the most significant biological characteristics of malignant tumors and an important cause of death in tumor patients. Therefore, the key to the treatment of tumors is limiting metastasis. [28, 29] We demonstrated that PSO inhibited the migration and invasion of OS cells by scratch wound healing assays and transwell assays. Epithelial-mesenchymal transition (EMT) is an important process in tumorigenesis, which is closely related to tumor invasion, metastasis, and prognosis. Snail is a key transcription factor in EMT, and regulates the connections between cells.[18, 30] Matrix metalloproteinases (MMP) can destroy the matrix-degrading balance, and promote cancer invasion through the histological barrier (constituted by the basement membrane and ECM) into the surrounding tissues and metastasis to distant tissues.MMP-2 and MMP9 are the most essential for tumor metastasis and invasion.[31, 32] We found that MMP-2, MMP-9, and Snail were down-regulated after PSO treatment. These findings suggest that PSO may inhibit OS cell migration and invasion by blocking the EMT process and inhibiting MMP function.

Apoptosis is an important homeostatic mechanism, which involves the activation, expression, and regulation of a series of genes, and has potential therapeutic significance.[33] Therefore, inducing tumor
cell apoptosis is one strategy to develop anticancer drugs. We confirmed that PSO significantly increased the apoptosis rate of OS cells by flow cytometry. Caspase3 is the main executor in the process of apoptosis and plays a role in the early stage of apoptosis. Bcl-2 family proteins are key regulators of mitochondria-mediated apoptosis and include antiapoptotic members such as Bcl-2 and proapoptotic members such as Bax. In this study, we found that PSO significantly increased the apoptosis rate of OS cells, promoted the protein levels of Bax, and cleaved caspase-3, while reducing and downregulating Bcl-2 levels. These results further indicated that PSO could affect OS cells through the apoptotic pathway.

To explore the potential mechanism of the antitumor effect of PSO in OS cells, we performed RNA sequence analysis, which showed significant changes in PI3K/Akt and FAK signaling in PSO-treated 143B, MG63 cells. Further GESA and KM analysis revealed important DEGs, such as ITGB1, PIK3CD, MAPK3 and PRKCA, which are often associated with tumors. Many studies have confirmed that ITGB1 is responsible for Focal adhesion kinase (FAK) activation and Integrin ligand adhesion triggers an increase in FAK tyrosine (Tyr) 397 phosphorylation and FAK is a marked effect on tumor cell survival, migration, invasion, angiogenesis, and metastasis. In addition, after ITGB1 activates Tyr397 phosphorylation, FAK can form a complex with Src, which binds to the P85 subunit of PI3K, which can further activate downstream Akt and regulate cell proliferation and cycle. Therefore, it is necessary to inhibit the expression of ITGB1. Several studies have revealed that repression of ITGB1 expressions can attenuate Metastasis and invasiveness of tumor. In this study, we confirmed that PSO inhibited FAK expression and downstream PI3K/Akt phosphorylation. Further molecular docking assays demonstrated that PSO targeted ITGB1 for decreasing FAK activation. Our Western blot results supported the conclusion of our RNA sequence analysis, showing that PSO treatment reduced the expressed level of ITGB1, FAK and p-FAK and ratio levels of p-PI3K /PI3K, and p-Akt/Akt in OS cells. Therefore, our study suggests that PSO therapy may inhibit the growth and progression of osteosarcoma by down-regulating ITGB1 expression via the FAK and PI3K/Akt signaling pathways.

5 | Conclusion

In conclusion, this study suggests that Psoralidin has a significant inhibitory effect on osteosarcoma, and Psoralidin may down-regulate ITGB1 expression through FAK and PI3K/Akt signaling pathway to play its anti-osteosarcoma role. ITGB1 may be a potential therapeutic site for osteosarcoma. Psoralidin is an excellent candidate for targeted anti-osteosarcoma drugs.

Declarations

Disclosure

Ethics approval and consent to participate

All animal experiments were approved by the Animal Care and Use Organization Committee IACUC of Chongqing Medical University.
Consent for publication

All authors are in agreement with the content of the manuscript and approved the final version of the manuscript.

Availability of data and materials

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

Conflict of interests

The authors have no conflict of interest.

Authors’ contribution

SC, SL, and BC completed the experiment and wrote the manuscript. CD, PX, and YL helped in preparing the figures. XL and LW participated in animal experiments. CZ and WH refined and arranged the contents of the manuscript. All authors approved the final manuscript.

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Not applicable

References


Figures

A

Chemical structure of PSO

Figure 1

Chemical structures of PSO
Figure 2

PSO inhibits OS cell proliferation in vitro. 143B and MG63 cells were treated with PSO with various dosages and the cell viabilities were examined by CCK8 assay (A, B). The effect of PSO on the proliferation of human OS cells were detected by crystal violet staining (C-F) and EdU staining assay (G-J). Western blot analysis showed PSO down-regulated PCNA (K-N). PSO significantly inhibited the proliferation ability of OS cells (**P<0.01, vs the control group, n=3).
PSO inhibits the migration and invasion of OS cells. The effects of PSO on the migration abilities of human OS cells were detected by wound healing test (A-D, ×100) and transwell assay (E-H, crystal violet staining, ×100). The effects of PSO on the invasive abilities of human OS cells were detected by transwell assay (I-L, crystal violet staining, ×100). The effect of PSO on the protein level of...
MMP-2, MMP-9, and Snail (Western Blot) (M-P). PSO significantly inhibited the migration and invasion of OS cells (**P<0.01, vs the control group, n=3).

Figure 4
PSO induces OS cell cycle arrest at G0/G1 phase. Effect of PSO on the cell cycle of human OS cells was detected by flow cytometry assay (A–D). (*P<0.05, **P<0.01, vs the control group, n=3)
Figure 5

Effect of PSO on the apoptosis of human OS cells was detected by flow cytometry (A-D). Then the expression levels of apoptosis-related proteins Bax, Bcl-2, and cleaved Caspase 3 were detected by Western blotting (E-H). PSO induced OS cells cell cycle arrest. PSO significantly promoted the apoptosis of OS cells (*P<0.05, **P<0.01, vs the control group, n=3).
Figure 6

OS cells were treated with 30μM PSO for 24 h, and the global changes in gene expression were analyzed by RNA-seq. Heatmaps and Volcano maps show differentially expressed genes in OS cells treated with PSO compared to the untreated control (A-D). The intersection of different osteosarcoma cells with identical differences in gene expression (E, F). Gene Ontology (GO) was presented through the biological process, cellular component, and molecular function (G). (*P<0.05)
Figure 7

Altered signaling pathways in PSO-treated OS cells according to KEGG enrichment analysis (A). GSEA enrichment plots in PSO-treated OS cells (B). Venn interaction on DEGs from selected top 5 pathways (C). The K-M curve represents the effect of different genes on the overall survival of OS patients (D). 2D and 3D molecular structures of PSO, stable complex and docking pocket of PSO with ITGB1 (E). Protein expression of ITGB1, PIK3CD, MAPK3, and PRKCA with or without PSO treatment (F-H).
Figure 8

Western blotting analysis of FAK, p-FAK, PI3K, p-PI3K, AKT, and p-AKT was performed on OS cells treated with PSO (A-D). PSO treatment inhibits the FAK and PI3K-Akt signaling pathways (*p < 0.05).
Figure 9

PSO inhibits OS cells growth and metastasis in tumor animal model (A). The effect of PSO on tumor volume and mouse weight (B, C). The effect of PSO on the tumor was detected by HE and immunohistochemistry (D). PSO inhibited tumor development in vivo (vs the CMC group, n=5).

Supplementary Files

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