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Original Article

Brucea Javanica Oil Emulsion Injection inhibits proliferation of pancreatic cancer via regulating apoptosis-related genes

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Abstract: Brucea Javanica Oil Emulsion Injection (BJOEI) has been proven to have extensive anti-tumor effects. But the anti-cancer mechanisms need further exploration. So, the aim of this study was to investigate the role and mechanisms of BJOEI on pancreatic cancer using network pharmacology and experimental validation. Disease targets were obtained from the GSE101448 dataset in the Gene Expression Omnibus (GEO) database. Eight active ingredients were identified following a comprehensive literature search. The target genes of BJOEI were obtained from the SwissTarget Prediction database. The core targets of BJOEI and the involved signaling pathways were determined using the compound-target network, protein-protein interaction (PPI) network, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. GO and KEGG enrichment analyses of 50 potential overlapping genes indicated that BJOEI exerted therapeutic effects on pancreatic cancer through the apoptotic pathway. *In vitro* experiments further revealed that BJOEI could suppress cell growth and invasion, arrest cells at the S stage, and cause cell apoptosis in three pancreatic cell lines. Additionally, BJOEI inhibited tumor growth *in vivo*. Among the 15 key genes regulating apoptosis, 11 were upregulated, while 4 were downregulated. PPARG emerged as a core target in bioinformatics analysis. The ability of PPARG to regulate apoptosis was validated by Western Blot. Our findings verified that BJOEI could regulate apoptosis-related genes, especially PPARG, thereby inducing apoptosis and inhibiting proliferation in pancreatic cancer cells. BJOEI can impede pancreatic cancer progression and induce cell apoptosis. The underlying mechanism appears to be closely associated with the regulation of apoptosis-related genes.

Keywords: Pancreatic cancer, Brucea Javanica Oil Emulsion Injection, network pharmacology, apoptosis, PPARG

Introduction

Despite notable advances, pancreatic cancer exhibits the highest fatality rate among cancers, with an overall five-year survival rate of around 7% [1]. Pancreatic ductal adenocarcinoma (PDAC), the predominant subtype, exhibits a 5-year survival rate of only 6-8%, primarily attributed to limited treatment modalities [2]. Surgical resection is the only potentially curative treatment; however, up to 80% of patients are in advanced stage or complicated with metastatic disease at initial diagnosis, precluding surgical intervention. Therefore, it is important to identify novel anti-tumor drugs and therapeutic targets for pancreatic cancer.

As an essential part of complementary and alternative medicinal systems, traditional Chinese medicine has emerged as a pivotal domain of investigation and clinical application in cancer management [3, 4]. Chinese medicine compound preparations have multi-target and multi-level characteristics. Brucea Javanica Oil Emulsion Injection (BJOEI), derived from the *Brucea javanica* after petroleum ether desulfurization and ethyl acetate extraction, is a novel anti-cancer drug. Its principal components include unsaturated fatty acids, demonstrating a distinct affinity for tumor cells [5]. BJOEI have been proven to inhibit cell proliferation [6], induce tumor cell apoptosis [7], and mitigate resistance to chemotherapy drugs [8]. In China,

BJOEI is widely applied as an adjuvant therapy drug for lung and gastrointestinal cancers [9, 10]. However, the precise mechanisms of BJOEI on pancreatic cancer remains unclear.

Network pharmacology studies can provide a novel understanding of the complex relationship between drugs and disease-related target pathways. The traditional single-target, single-drug research model is gradually evolving into a network-targeted multi-component treatment model, which is also in line with the current multi-target, multi-level trajectory of anti-tumor strategies. Moreover, the pharmacological disease-gene-target-drug survey pattern can more effectively reveal the intricate connections between drug constituents and diseases, providing a more convenient and effective learning platform for understanding the mechanism of action of drugs. Surveillance, Epidemiology, and End Results (SEER) database analysis identified race as one of the factors influencing clinical features and survival outcomes in patients with pancreatic neuroendocrine tumors [11].

In this study, we found that BJOEI could regulate apoptosis-related genes, especially peroxisome proliferator-activated receptor-gamma (PPARG), thereby inducing apoptosis and inhibiting proliferation in pancreatic cancer cells. These findings provide valuable insights into the clinical management of patients with pancreatic cancer. Significantly, genes enriched in the apoptosis pathway may serve as a potential target for the treatment of pancreatic cancer. The schematic representation of our research is depicted in **Figure 1**.

Materials and methods

BJOEI acquisition

BJOEI, provided by Guangzhou Baiyunshan Mingxing Pharmaceutical Co., Ltd (approval number: Z44021325), was obtained from *Brucea Javanica* after petroleum ether deestification, followed by extraction with ethyl acetate or chloroform.

Active components and predicted targets of BJOEI

Through a comprehensive literature survey [12-18], 8 active components of BJOEI were screened. All compounds were fed into the PubChem database (https://pubchem.ncbi.nlm.nih.gov) to obtain their 2D chemical structures. These structures were inputted into The SwissTargetPrediction database (http://www.swisstargetprediction.ch/) for target genes prediction, with a probability threshold > 0.

Disease-related targets screening

The keywords "pancreatic cancer, human" were entered into the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The entry criteria were set as follows: (1) tissue samples taken from the human pancreas; (2) a sample size ≥ 30; (3) data collected within the past 5 years; and (4) both tumor and non-tumor pancreatic tissue samples available. GSE101448 complied with all criteria. Disease-related target genes were screened using the "GEO2R" function in the GEO database with selection criteria of $P < 0.05$ and $|\log2FC| > 1$.

Drug-disease-related target screening

The BJOEI target genes and pancreatic cancer target genes were matched to obtain the overlapping targets, and the intersecting genes were displayed using Venn 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html) to determine the effective targets.

Construction of BJOEI-active ingredients-targets network

Multiple active compounds and relevant primary genes were introduced into the Cytoscape software 3.9.1 for the construction of a BJOEI-active elements-objects network. Nodes in this network represented components and targets, while edges symbolized the associations between drugs with active elements and ingredients with target genes. This network collectively illustrates the intricate interplay between BJOEI and its target genes.

Protein-protein interaction (PPI) analysis

The protein-protein interaction (PPI) network was generated using the STRING11.5 database (https://cn.string-db.org/) based on overlapping genes to elucidate the association between the functional proteins. The screening

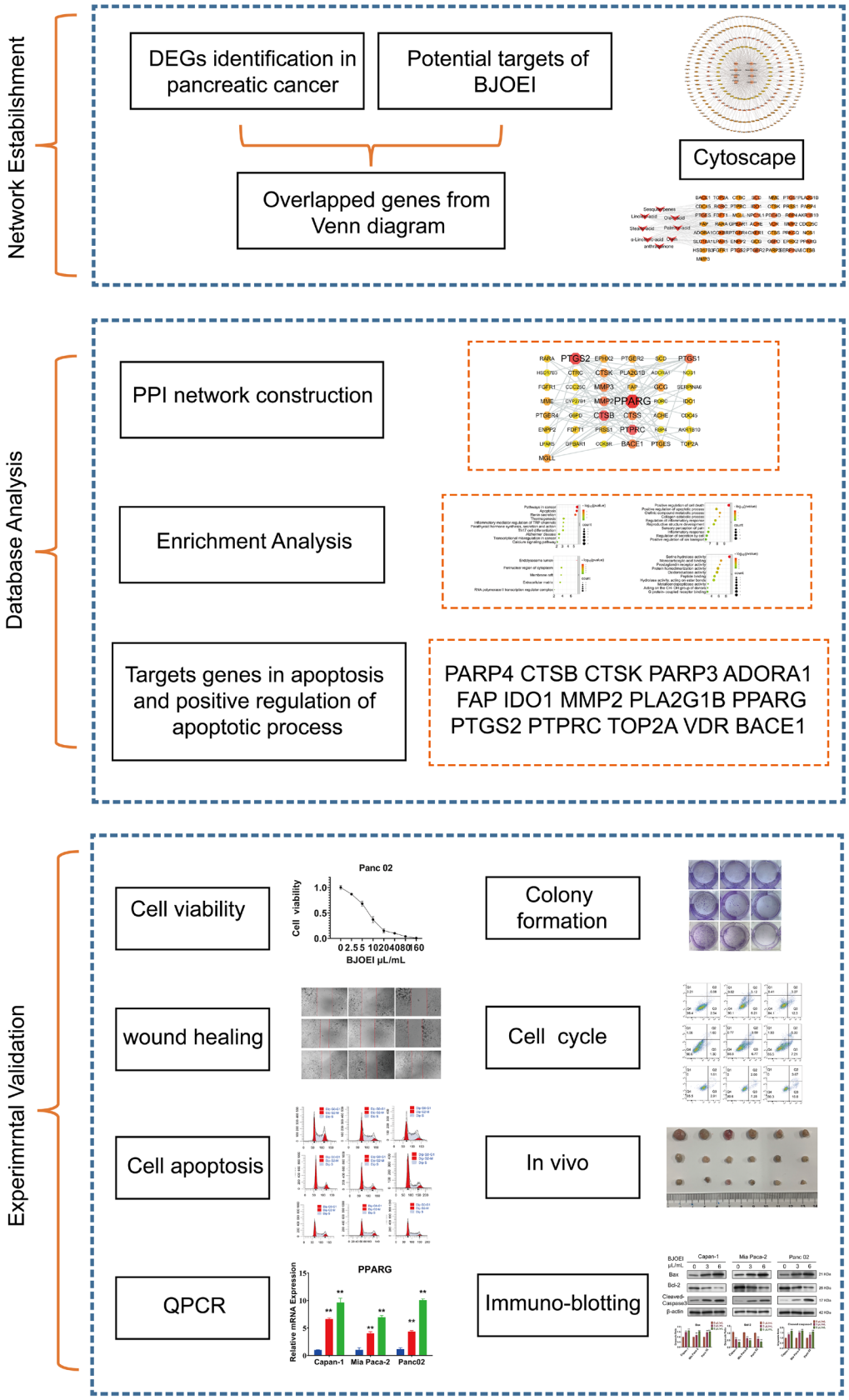

Figure 1. Graphical abstract.

criteria were set as "Homo sapiens" and "medium confidence", and the disconnected nodes were concealed in the network to obtain the PPI network. The key topological parameter and degree described the most important nodes in the network. Higher quantified values of topological parameters indicate greater importance of nodes [19].

Potential pathways of BJOEI in pancreatic cancer

Gene Ontology Biological Processes (GOBP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using Metascape online tool (https://metascape.org/) to elucidate gene function and signaling pathways.

Cell lines

Three cell lines, Mia PaCa-2, Panc02, and Capan-1, were procured from the American Type Culture Collection (ATCC, USA). Panc02 and Mia PaCa-2 cells were cultured in Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), and 100 μg/ml of penicillin and streptomycin (Gibco) each in a humid environment containing 5% CO_2. Capan-1 cells were cultured under identical conditions described above, except that the base medium was Iscove's Modified Dulbecco's Medium (Gibco).

Cell viability assay

Enhanced Cell viability was quantified using Cell Counting Kit-8 (Beyotime, China), following the manufacturer's instructions [20]. Briefly, cells (1×10^4/well) were seeded into 96-well plates for 24 h, and subsequently exposed to escalating concentrations (0, 2.5, 5, 10, 20, 40, 80, and 160 μl/mL) of BJOEI. After 48 h of incubation, a sterile CCK-8 solution was added to each well and incubated for 30 min at 37°C. The absorbance was measured through a microplate reader (SpectraMax ABS plus, USA) at 450 nm. Linear regression was applied to determine the IC50 values.

Clonogenic assay

A clonogenic assay was carried out to assess cell sensitivity to BJOEI [21]. Pancreatic cancer cells were seeded (1×10^3/well) into 12-well plates for one day, followed by exposure to increasing concentrations (0, 3, 6 μL/mL) of BJOEI for 10 days. After fixation, cells were stained with 0.1% crystal violet (Beyotime, China) for 30 mins, and colonies with over 50 cells were calculated using an Olympus CKX53 microscope (Olympus, Japan).

Wound healing assay

A conventional wound healing assay was utilized [22]. Approximately 1×10^6 cells were seeded in a well. The next day, a scratch was created on the cell monolayer using a pipette tip while being held vertically and not tilted. Subsequently, the cells were washed with PBS thrice, the scratched cells were removed, and serum-free culture medium was added. The cells were then incubated in a 37°C, 5% CO_2 incubator. Finally, images were collected at 0 h, 24 h, and 48 h.

Cell apoptosis assay

Cell apoptosis was measured using an apoptosis reagent (Beyotime, China) [23]. Briefly, cancer cells (1×10^5/well) were seeded in a 6-well plate for one day and then treated with varying doses of BJOEI (0, 3, 6 μL/mL) for 2 days. Then, the cells were treated with Annexin V-FITC and propidium iodide solution for 20 mins and analyzed using a Cyto FLEXS flow cytometer (Beckman COULTER, USA).

Cell cycle analysis

Cell Cycle reagent (Beyotime, Chia) was utilized as described previously [24]. Briefly, cells were treated with BJOEI at different concentrations for 48 h, followed by fixation with chilled 70% ethanol for one day. Subsequently, the cells were dyed with propidium iodide for 30 min in the dark and analyzed using ModfitLT 5 software (Verity Software House, USA).

Real-time PCR

Total RNA was extracted from experimental cells using an RNA Isolation Kit with Spin Column (Beyotime, China). The BeyoFast™ SYBR Green One-Step qRT-PCR Kit (Beyotime, China) was employed for continuous reverse

transcription and fluorescence quantitative PCR using LightCycler 96 Real-Time PCR system (Roche, Switzerland). All experiments were repeated thrice. The sequences of gene primers are provided in Table S1.

Immunoblot

Total protein extraction from cells was conducted using the Radio Immunoprecipitation Assay (RIPA) lysis buffer and BCA kit (Beyotime). Following SDS-PAGE separation, protein bands were electro-transferred onto polyvinylidene difluoride (PVDF) membranes (0.45 μm, Beyotime). Samples were then incubated in 5% skimmed milk in 1×TBST (1 h), followed by overnight incubation at 4°C with primary antibodies, including β-actin (Beyotime, China), BAX rabbit (BBI, China), BCL2 (BBI, China), and CASP3 (BBI, China), PPARG (Beyotime, China), and GW9662 (Sigma-Aldrich, USA). Samples were then washed and incubated with HRP-labeled goat anti-rabbit IgG (H+L) (Beyotime, China) for 1 h at room temperature. The bands were visualized using an ECL Kit (Beyotime, China), while ImageJ.JS (http://cnij.imjoy.io/) was utilized for quantification.

Animal models

Female C57BL/6 mice, aged 4 weeks, were sourced from HFK Bio-Technology (China). The animals were housed in a pathogen-free environment with a temperature of 22 ± 1°C, a humidity of 50 ± 1%, and a 12 h light/dark cycle. The mice had *ad libitum* access to water and food. A 7-day acclimatization period was provided for all animals. Assays involving animals were approved by the Experimental Animal Ethics Committee of Anhui Medical University (approval number LLSC20221106).

To establish subcutaneous tumor models, 1×10^6 Panc02 cells were injected subcutaneously into the right flank of each animal. After one week, tumor-harboring animals were divided into three groups (n = 6 mice in each group): a control and two BJOEI treatment groups. The BJOEI-treated mice received an intraperitoneal injection of BJOEI (25 mL/kg or 50 mL/kg) every day, while the control group received equivalent volumes of PBS once daily. Mouse weights and tumor sizes were measured at 3-day intervals. Vernier caliper was used to measure the tumor size, which was calculated with the following formula: $1/2\ a\times b^2$, where a and b are the long and short diameters, respectively. After 18 days of treatment, the animals were euthanized by CO_2 inhalation [25], followed by tumor extraction and weighing.

Immunohistochemistry

Tumor tissues were subjected to formalin fixation, dehydration, paraffin-embedding, and then sectioned at a thickness of 4 μm. Following deparaffinization, hydration, and microwave-based antigen retrieval, the sections were incubated with 3% H_2O_2 for 1 h and then blocked with 5% bovine serum albumin (Gentihold, China) for 20 min. The sections were subsequently incubated with primary antibody anti-Ki-67 (Beyotime, China; overnight at 4°C) and secondary antibody IgG H&L (HRP) (Beyotime, China; 60 min at room temperature). Diaminobenzidine and hematoxylin were utilized for development and counterstaining, respectively. An Olympus inverted fluorescence microscope (Olympus) was used to image five randomly selected high-power fields (400×) per specimen. Finally, ImageJ was used for quantification.

Statistical analysis

GraphPad Prism (version 9.0, USA) software was used to perform statistical analyses. Measurement data were demonstrated as mean ± standard deviation based on three independent assays. Shapiro-Wilk test and QQ plot were used to assess the data distribution. Data conforming to a normal distribution were analyzed using t-tests or one-way ANOVA followed by Dunnett's multiple comparison test [19]. $P < 0.05$ was considered statically significant.

Results

Chemical components and potential targets of BJOEI

Through a literature review, we identified eight compounds of BJOEI, including oleic acid, linoleic acid, olein, palmitic acid, sesquiterpenes, stearic acid, α-Linolenic acid, and anthraquinone. Comprehensive information and 2D structure of these 8 mixtures were retrieved from the PubChem database, and the results are summarized in Table S2. Furthermore, the

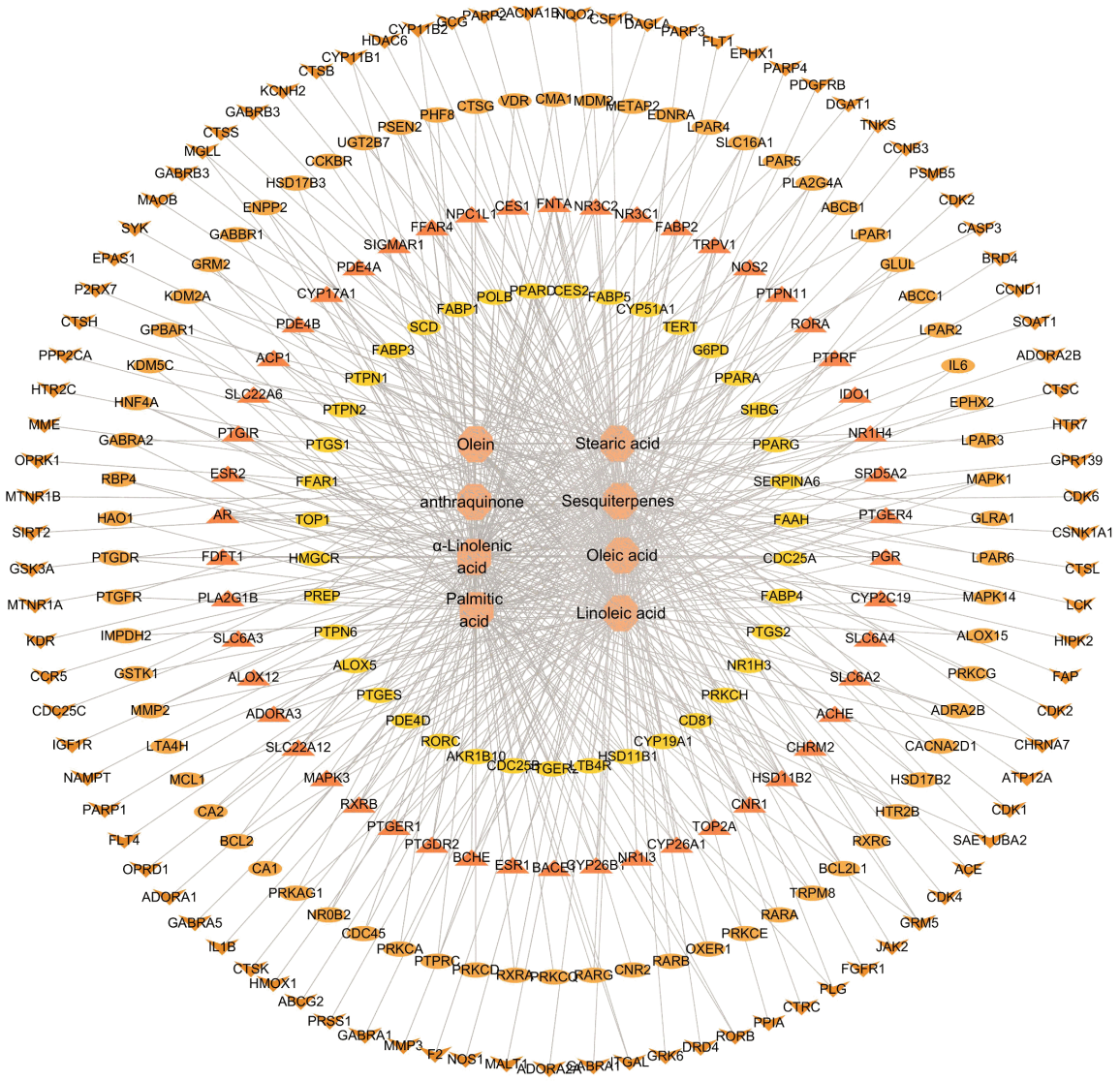

Figure 2. The "Compounds-targets" network.

target prediction of these 8 active components was carried out in the SwissTarget Prediction database, yielding 250 predicted target genes after deduplication. The drug components and action targets were visualized using the Cytoscape software (**Figure 2**).

Identification of potential targets for BJOEI treatment in pancreatic cancer

Total RNA expression data from 24 normal tissue samples and 19 pancreatic tumor tissue samples were obtained from GSE101448 in the GEO database. A total of 2573 differential genes were obtained from GSE101448 from both normal and pancreatic tumor tissues. The obtained differential genes and 250 drug component prediction target genes were then incorporated into Venn 2.1.0 software for plotting and analysis. After intersecting the two, we obtained 50 disease-drug component common targets. Details about these 50 overlapping target genes are listed in Table S3. The 50 target genes and 8 active ingredients were used to form an "overlapping target gene-corresponding compound of BJOEI" network diagram (**Figure 3A**).

PPI network construction and analysis

We subjected the 50 overlapping genes to the STRING database to obtain the PPI data. We

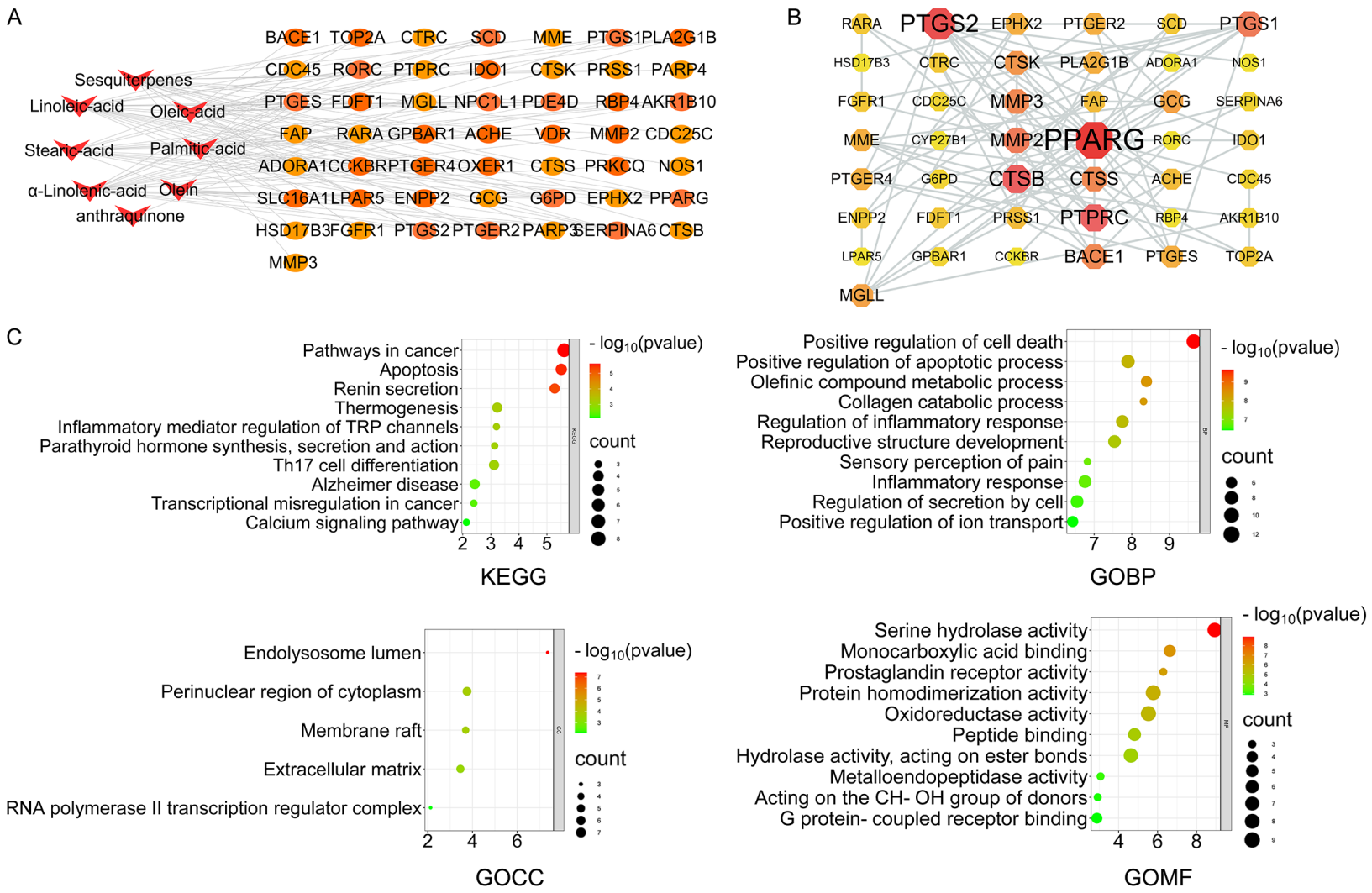

Figure 3. Screening key targets and functional analysis. A. Overlapping target gene-corresponding compound network of BJOEI; B. PPI network diagram of candidate genes; C. KEGG pathway of BJOEI and GO biological processes, GO cell components, GO molecular functions.

then constructed and visualized the PPI network, comprising 50 nodes and 105 edges, using Cytoscape (**Figure 3B**). A higher degree indicates a larger node size, with red indicating the highest degree and yellow indicating the lowest degree. The top node based on degree values was PPARG, suggesting a pivotal role of PPARG within the PPI network pertaining to pancreatic cancer.

Potential pathways influenced by BJOEI in pancreatic cancer

Fifty overlapping genes were entered into the Metascape platform for the GO and KEGG pathway analyses. The top 2 pathways in the KEGG pathway analysis included pathways in cancer and apoptosis. Additionally, GO analysis revealed targets related to biological processes, particularly the positive regulation processes encompassing cell death and the apoptotic process, both of which emerged as top hits based on *P*-value < 0.05. The targets and rankings of cell components and molecular functions are shown in **Figure 3C**.

Cytotoxic impact of BJOEI on pancreatic cancer cells

The CCK8 test demonstrated a dose-dependent suppression of Capan-1, Panc02, and Mia PaCa-2 cells by BJOEI compared with that in the control cells (**Figure 4A**). The IC_{50} values of BJOEI on Panc02, Capan-1, and Mia Paca-2 cells were 7.7 μl/mL, 7.3 μL/mL, and 6.243 μL/mL, respectively, at 48 h. Furthermore, increasing BJOEI levels inhibited colony formation ($P < 0.05$ vs. control; **Figure 4B**).

Next, we examined the effect of BJOEI on pancreatic cancer cell migration using wound healing assays. BJOEI was able to inhibit the migratory abilities of cancer cells compared with the control group ($P < 0.05$; **Figure 5**).

BJOEI induced cell cycle arrest and cell apoptosis

Cells administered with BJOEI exhibited S phase arrest (**Figure 6A**). Moreover, the apoptotic rate was significantly correlated with BJOEI concentration, suggesting BJOEI-induced pancreatic cancer cell apoptosis ($P < 0.05$ vs. control; **Figure 6B**). Apoptosis-related proteins, such as BAX, BCL2, and cleaved caspase3, were confirmed using immunoblotting. Our results showed that BJOEI upregulated BAX and cleaved caspase3 expression and downregulated BCL2 expression (**Figure 7**).

BJOEI inhibited tumor growth in vivo

To examine BJOEI's anticancer properties in pancreatic cancer *in vivo*, we established a Panc02 cell tumor model in female C57BL/6 mice. Following tumor formation after 7 days of cell injection, animals were randomized into respective groups. No toxicity was observed throughout the study. Remarkably, the average tumor size exhibited slower growth after BJOEI treatment compared to that in the control group (**Figure 8A** and **8B**). Histological examination revealed distinctive architectural alterations, including poor gland generation, predominant solid tumor growth, and substantial neutrophil infiltration in the control group compared to the BJOEI-treated mice, highlighting BJOEI's potential to restrain neoplastic progression. Consistent with this, immunohistochemistry analysis demonstrated a reduction in the number of tumor cells expressing the proliferation marker Ki-67 after BJOEI treatment compared with controls (**Figure 8C**). These findings demonstrated that BJOEI effectively inhibited Panc02-derived tumor growth in mice.

Apoptosis genes played an essential role in BJOEI inhibiting pancreatic cancer

The genes enriched to apoptosis pathway and positive regulation of apoptotic processes were validated by RT-PCR (**Figure 9**). These 15 genes are listed in Table S4. Following BJOEI treatment, the mRNA expression of PPARG, CTSB, PARP3, PLA2G1B, FAP, ADORA1, PTPRC, VDR, PARP4, IDO1, and CTSK were found to be upregulated, while the expression of MMP2, PTGS2, BACE1, and TOP2A were downregulated.

Based on the PPI network and the pathway enrichment analyses, the top target PPARG was selected for subsequent experimental verification. Immunoblot further demonstrated that BJOEI could upregulate PPARG (**Figure 10A**). We used PPARG inhibitor, GW9662, to study the effect of BJOEI on pancreatic cell apoptosis. Compared with the control group, the addi-

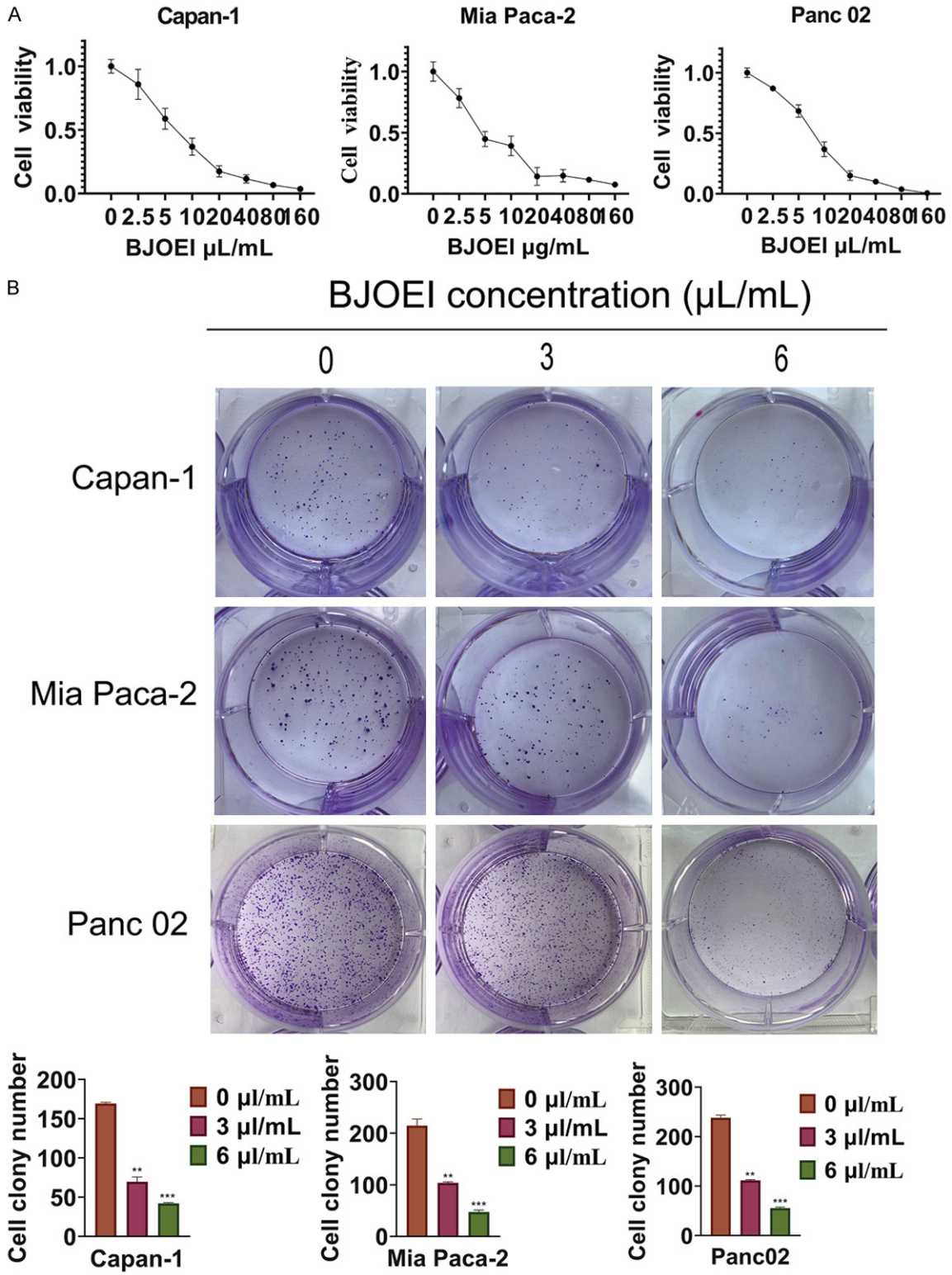

Figure 4. BJOEI suppressed pancreatic cancer cell growth *in vitro*. A. BJOEI inhibited pancreatic cancer viability in a dose-dependent manner; B. BJOEI significantly inhibited the migration of Capan-1, Mia Paca-2 and Panc02 cells. N = 3; ns, not significant; $^{**}P < 0.01$, $^{***}P < 0.001$, vs. 0 group.

tion of GW9662 partially rescued BJOEI's pro-apoptotic effect on these three BJOEI cell lines (**Figure 10B**). These findings were consistent with the results from bioinformatics analysis.

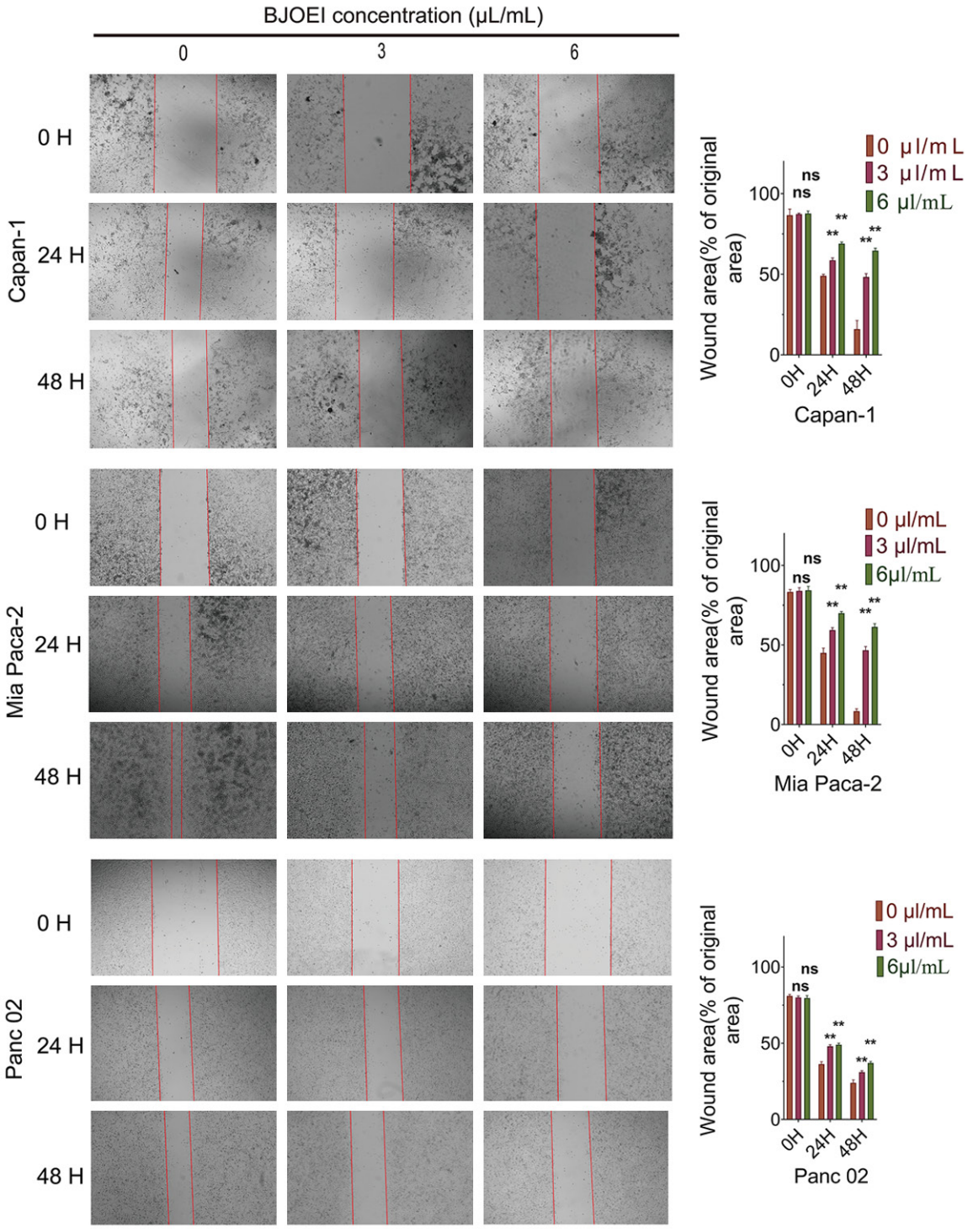

Figure 5. BJOEI suppressed pancreatic cancer cell growth *in vitro*. BJOEI significantly inhibited migration of Capan-1, Panc02, and Mia Paca-2 cells. N = 3; ns, not significant; $^{**}P < 0.01$, vs. 0 group.

Discussion

Traditional Chinese medicine has been utilized in China for thousands of years to prevent and treat malignant tumors at diverse stages, including pancreatic cancer. In traditional Chinese medicine, the etiology and pathogenesis of pancreatic cancer are often related to

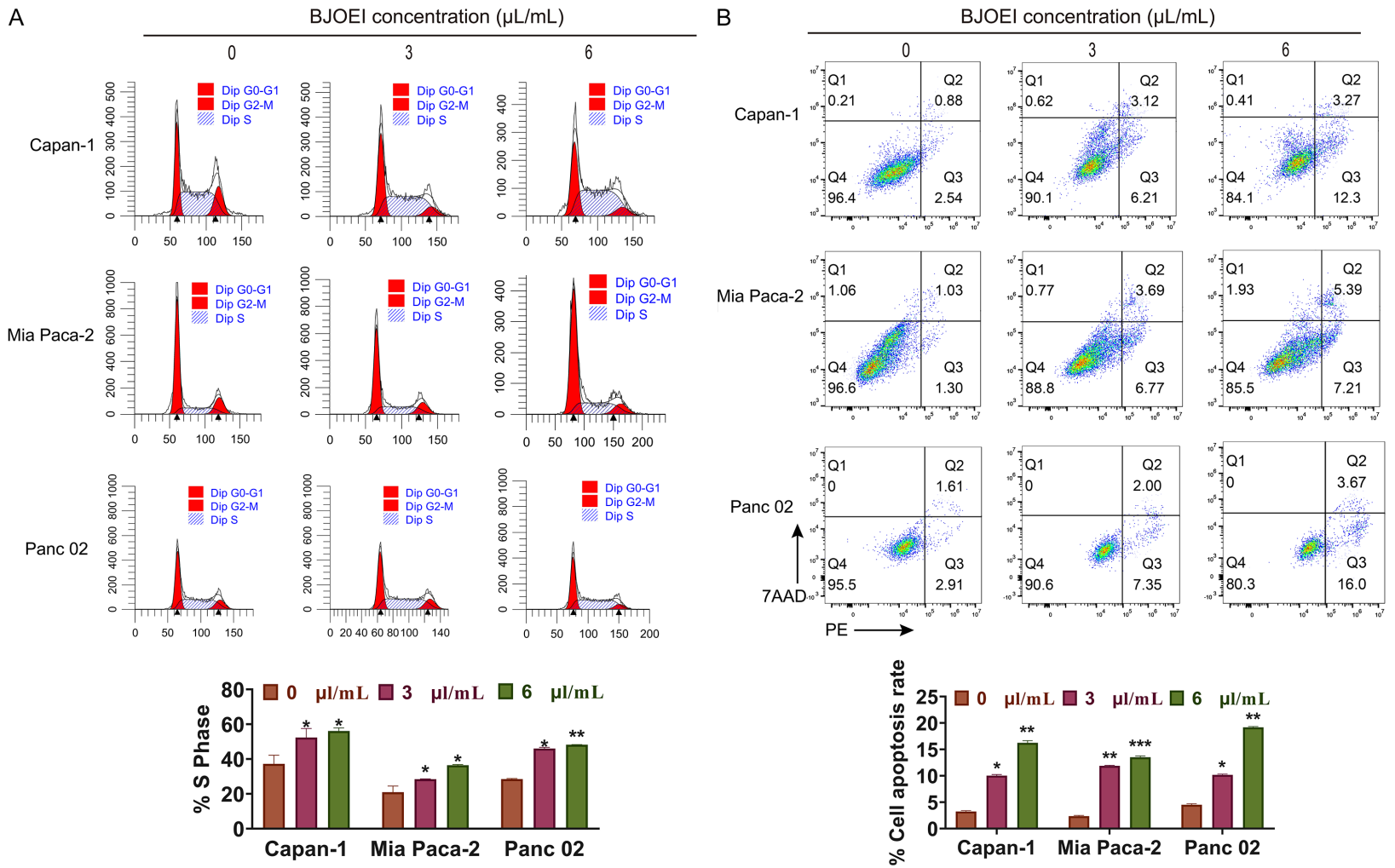

Figure 6. BJOEI induced cell cycle arrest in the S phase and caused apoptosis. A. BJOEI arrested Panc02, Capan-1, and Mia PaCa-2 cells in the S phase; B. BJOEI induced cell apoptosis. N = 3; *P < 0.05, **P < 0.01, ***P < 0.001, vs. 0 group.

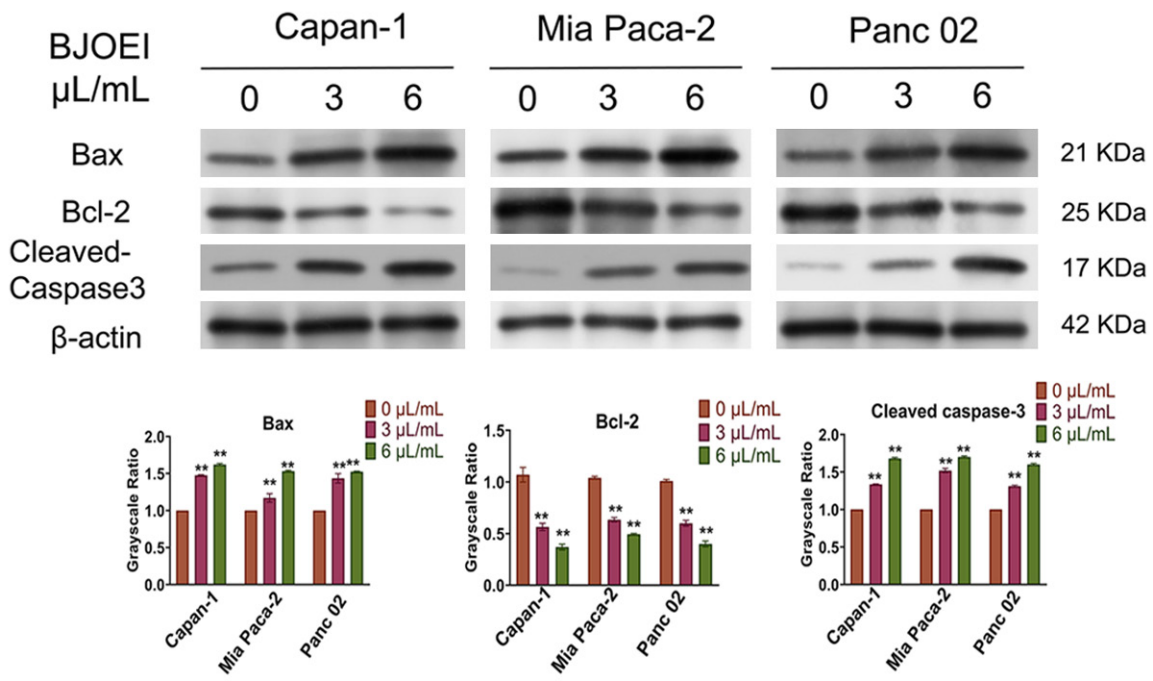

Figure 7. Protein expression in Panc02, Mia paca-2 and Capan-1 cells after BJOEI treatment. BJOEI upregulated BAX and cleaved caspase 3 but downregulated BCL2 expression. N = 3; $^{**}P < 0.01$, vs. 0 group.

"dampness, heat, phlegm, and blood stasis", whereby the disease emergence is attributed to the gradual accumulation of pathogens and toxins over time [26]. BJOEI can guard the zhengqi and warmth, disperse the knots, and contribute to the flow of qi and blood circulation, threby treating pancreatic cancer. Modern pharmacological research has also highlighted the anti-tumor effects of BJOEI [27, 28].

Multiple active ingredients in BJOEI exhibit a synergistic anti-tumor effect [29]. BJOEI consists of 85% triglycerides and 10% oleic acids, interlaced with saturated and unsaturated fatty acids along with triterpene alcohols. Notably, unsaturated fatty acid components, such as oleic acid and linoleic acid, exhibit a specific affinity for tumor cell membranes, with a significant anti-tumor activity [8]. Triglycerides have an indirect anti-cancer activity and can be hydrolyzed into oleic acid, subsequently exerting anti-tumor activity. Oleic acid can decrease insulin secretion and enhance sensitivity, thereby potentially curbing PDAC through attenuation of hyperinsulinemia, leading to DNA damage and, in turn, tumor growth [30]. In contrast, Palmitic acid, the most common saturated free fatty acid, causes lipotoxicity and apoptosis of non-fat cells when at full capacity [31]. Additionally, accumulation of palmitic acid can conduce pancreatic β-cell dysfunction and cardiac myocyte apoptosis [31]. Sesquiterpenes, such as alantolactone, can sensitize human pancreatic cancer cells to EGFR inhibitors by inhibiting STAT3 signaling [32]. Emodin, a natural anthraquinone derivative, can inhibit pancreatic cancer epithelial-mesenchymal transition [33]. As a composite formulation of bioactive constituents, BJOEI would potentially attract widespread attention in the years ahead. However, the mechanism of BJOEI in pancreatic cancer is still not completely understood.

KEGG analysis revealed that cancer and apoptosis pathways exhibited the highest number of genes enriched in tumor-associated signaling pathways, underscoring their pivotal role in mediating the effects of BJOEI on pancreatic cancer. Subsequent *in vitro* experiments revealed that BJOEI suppressed pancreatic cancer cell growth and aggression, increased cell apoptosis, and induced S-phase cell cycle arrest. Immunoblotting further corroborated BJOEI's pro-apoptotic influence, as evidenced by modulations in BAX, BCL2, and caspase3 expressions, thereby confirming the regulation of apoptosis. BJOEI reduced cell apoptosis by

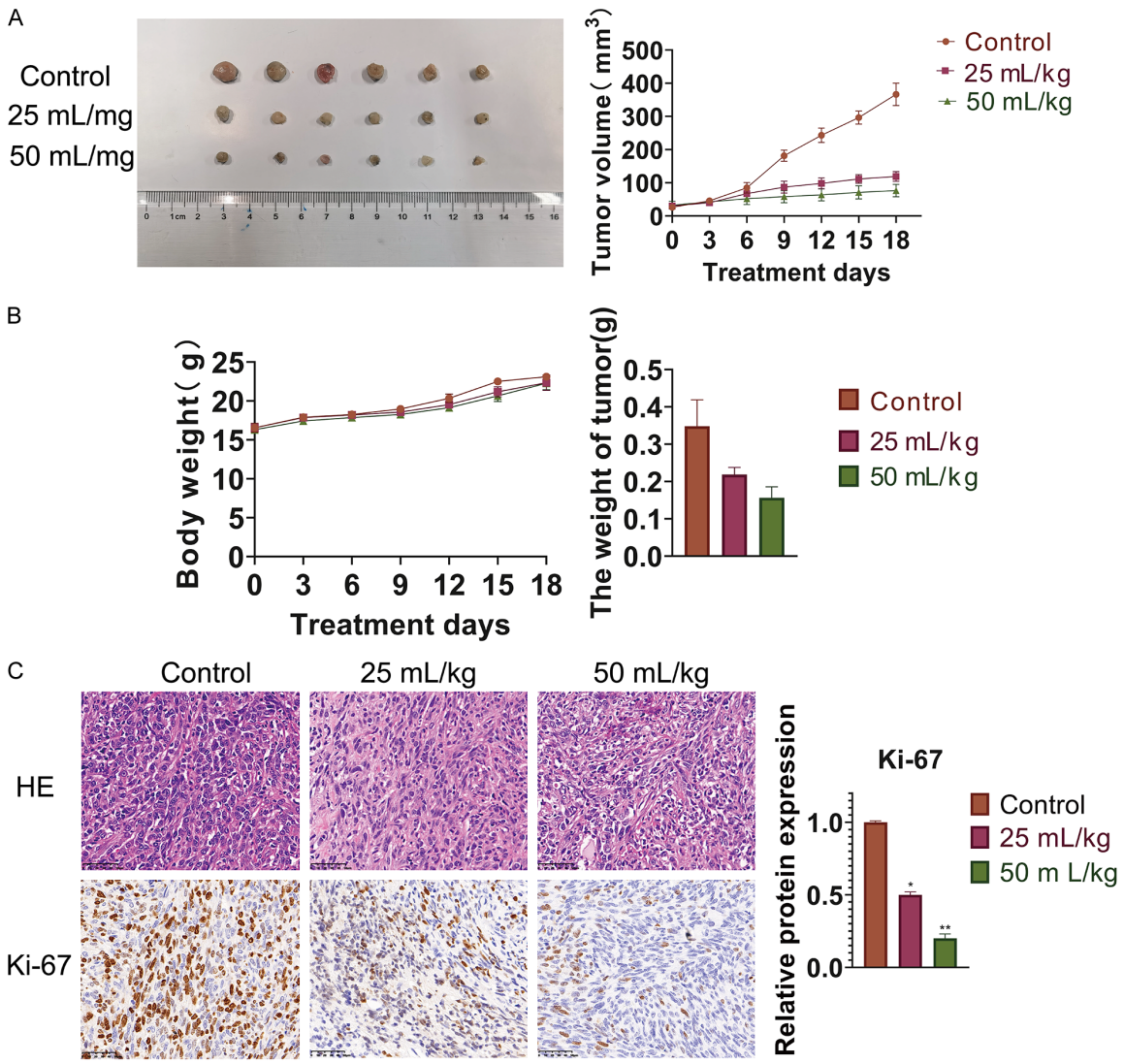

Figure 8. BJOEI suppressed tumor growth in mice. A. BJOEI suppressed panc02 mouse xenograft tumor growth; B. BJOEI did not affect mouse weight; C. BJOEI suppressed Ki-67 expression in tumor tissues. Magnification: 50×. N = 3; **P* < 0.05, ***P* < 0.01, vs. control group.

regulating apoptosis-related proteins, therefore suppressing tumor growth. Furthermore, qPCR results demonstrated that BJOEI could upregulate VDR, CTSK, PARP4, FAP, CTSB, ADORA1, PARP3, PTPRC, IDO1, PPARG, and PLA2G1B while downregulating TOP2A, PTGS2, BACE1, and MMP2. These results indicate that BJOEI exerts therapeutic effects on pancreatic cancer by modulating apoptosis-related genes.

The induction of tumor cell death is an effective strategy to control tumor development. Apoptosis involves the activation, expression, and regulation of a series of genes. The Bcl-2 family, encompassing anti-apoptotic (e.g., Bcl-2) and proapoptotic (e.g., Bax) proteins, plays a crucial role in the process of cell apoptosis [34, 35], regulating and activating cysteine protease caspases, as well as leading to apoptosis [35]. Multiple genes and pathways can regulate the expression of Bcl-2 family genes. Our RT-PCR results demonstrated that the expression of apoptosis-related genes, including PPARG, CTSB, PARP3, PLA2G1B, FAP, ADORA1, PTPRC, VDR, PARP4, IDO1, CTSK, MMP2, PTGS2, BACE1, and TOP2A, which play crucial roles in modulating the Bcl-2 family genes, were regulated in pancreatic cell lines upon BJOEI treatment. Among them, the PPI results suggested that PPARG may play a key

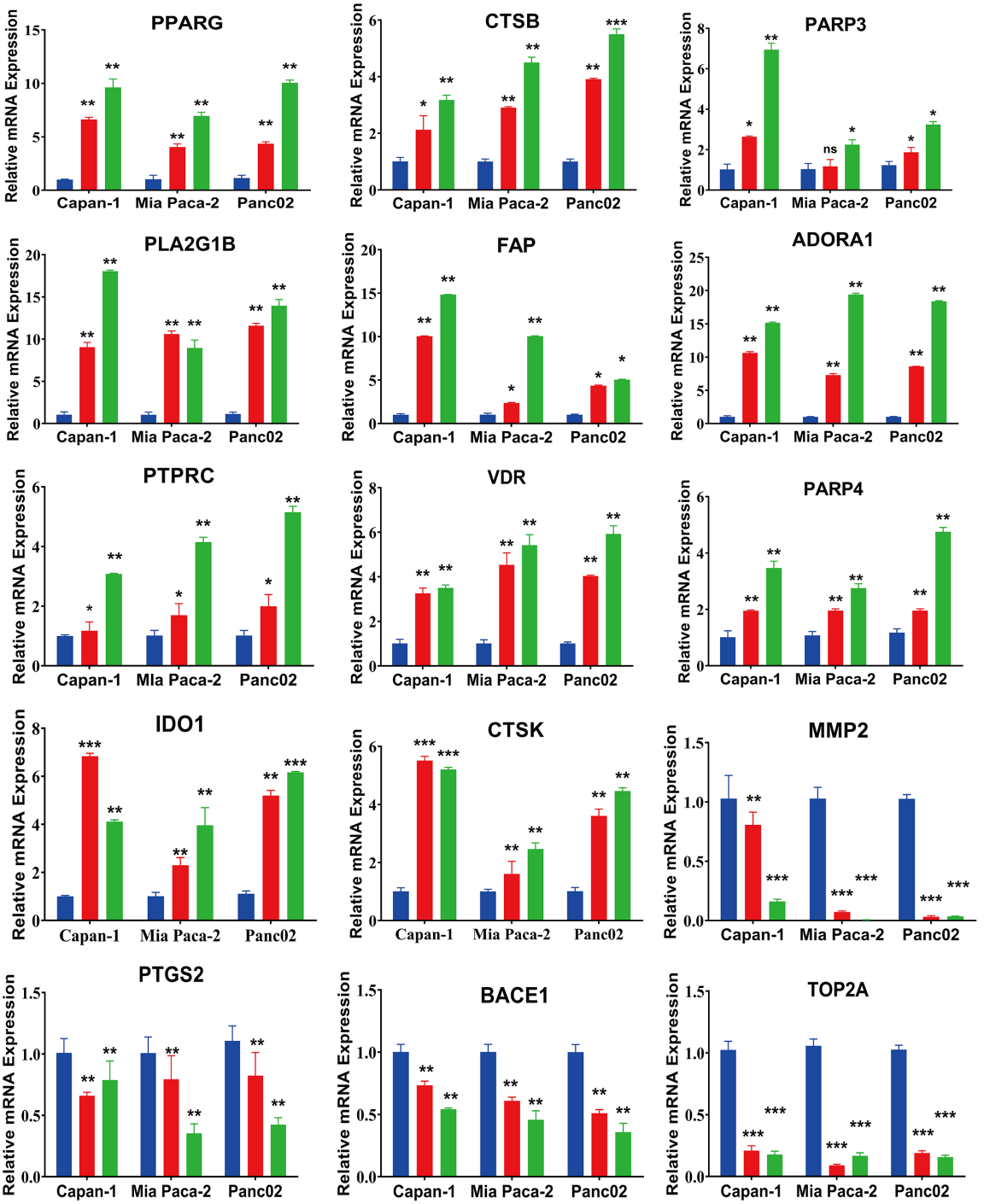

Figure 9. BJOEI modulated apoptotic pathway genes. The mRNA expression of PPARG, CTSB, PARP3, PLA2G1B, FAP, ADORA1, PTPRC, VDR, PARP4, IDO1, CTSK, were upregulated, while those of MMP2, PTGS2, BACE1 and TOP2A were downregulated. N = 3; $*P < 0.05$, $**P < 0.01$, vs. control group.

role in the anti-pancreatic cancer process, prompting further experimental verification. Immunoblot analysis showed an increase in PPARG expression after BJOEI treatment. We then used a PPARG inhibitor, GW9662, to study the effect of BJOEI on pancreatic cell apoptosis. It was found that GW9662 partially rescued the pro-apoptotic effect of BJOEI on these three

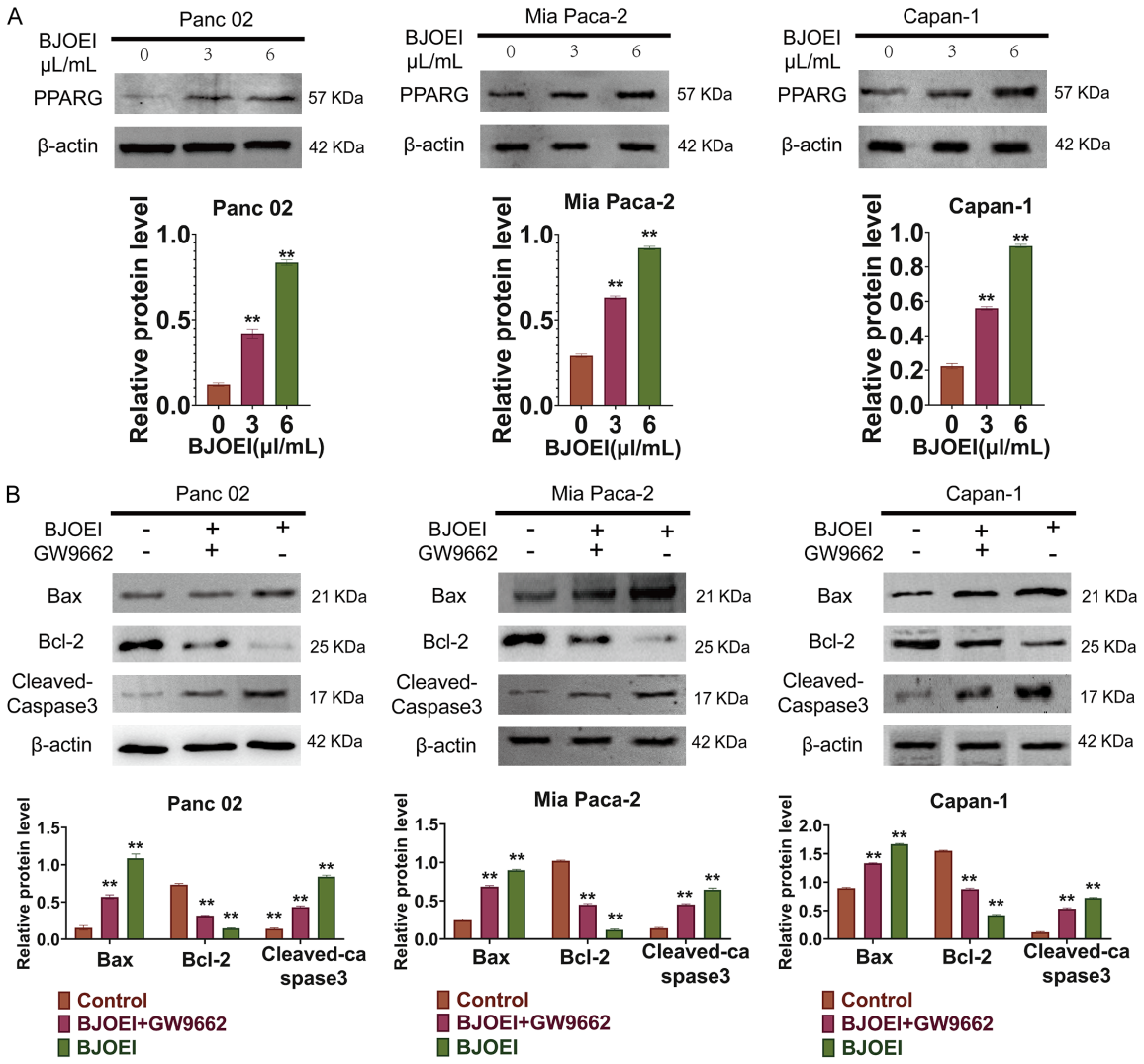

Figure 10. BJOEI inhibited the apoptosis of pancreatic cancer by activating PPARG, a key gene in the apoptotic pathway. Pancreatic cancer cells were pre-treated with 2 μM GW9662 for 6 h, followed by 6 μL/mL BJOEI treatment for 48 h, then the protein expression was assessed by Western blotting. A. BJOEI upregulated the expression of PPARG; B. Compared with the control group, the addition of GW9662 partially rescued BJOEI's pro-apoptotic effect on these three BJOEI cell lines. N = 3; $^{**}P < 0.01$, vs. 0 group.

BJOEI cell lines compared with the control group [36]. PPARG, a transcriptional factor, has been reported to be a key regulator of cellular energy metabolism. Our finding aligns with previous reports showing that PPARG inhibition could promote proliferation and inhibit apoptosis through the Bcl-2/Caspase3 pathways [37, 38]. These findings verify that BJOEI can regulate apoptosis-related genes, especially PPARG, thereby inducing apoptosis and inhibiting proliferation of pancreatic cancer cells.

However, this study has several limitations. First, the *in vivo* effect of BJOEI on pancreatic cancer needs to be further explored. Second, we haven't verified all the genes that related to apoptosis. Finally, a comprehensive examination of the potential side effects of BJOIE (e.g., the effect on the digestive system) needs to be explored. Future research should shed light on BJOEI's role in pancreatic cancer while illuminating its mechanism of action.

Conclusions

Incorporating network pharmacology and experimental methods, this study has explored the mechanism underlying pancreatic cancer attenuation through BJOEI intervention. Our findings suggest that BJOEI can suppress the

progression of pancreatic cancer and cause cell apoptosis. The mechanism might be associated with the modulation of apoptosis-related genes.

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Disclosure of conflict of interest

None.

Abbreviations

BJOEI, Brucea Javanica oil emulsion injection; GEO, Gene Expression Omnibus; GEPIA, Gene Expression Profiling Interactive Analysis; CTSK, Cathepsin K; PLA2G1B, Phospholipase A2 Group IB; PARP3, Poly (ADP-Ribose) Polymerase Family Member 3; CTSB, Cathepsin B; IDO1, Indoleamine 2,3-Dioxygenase 1; ADORA1, Adenosine A1 Receptor; PPARG, Peroxisome proliferator-activated receptor gamma; PARP4, Poly (ADP-Ribose) Polymerase Family Member 4; TOP2A, DNA Topoisomerase II Alpha; VDR, Vitamin D Receptor; FAP, Poly (ADP-Ribose) Polymerase; PTGS2, Prostaglandin-Endoperoxide Synthase 2; BACE1, Beta-Secretase 1; MMP2, Matrix Metallopeptidase 2; PTRPC, Protein Tyrosine Phosphatase Receptor Type C; GOBP, Gene Ontology Biological Processes; GOCC, GO cell components; GOMF, GO molecular functions; KEGG, Kyoto Encyclopedia of Genes and Genomes; PDB, Protein Data Bank; ATCC, American Type Culture Collection; CCK-8, Cell Counting Kit-8; RT-PCR, Real-time PCR.

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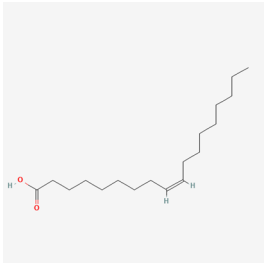
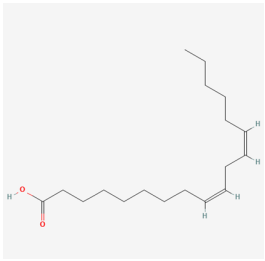
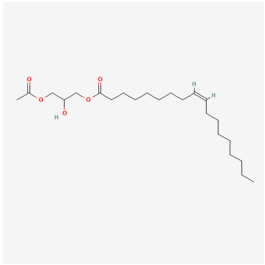
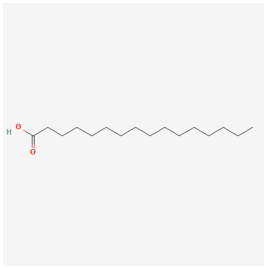
Table S1. The sequences of the primers for genes

Gene	Forward	Reverse
PLA2G1B	AGACACATGACAACTGCTATGA	AGAGCACGAGTATGAATAGGTG
PTPRC	AATCCTCTTGCTCTGTGCCTCTTTG	GAACACTCACCTTCACCTGCCTTC
PARP4	TTTCGTACAATGGTGTCGACTA	GAAAAGGCGACTCATTCTCATC
PRRP3	CTCACACTTTTACACCGTCATC	CACTTGAGAAGCTGGTAGTCTC
CTSK	TTCCAGTTTTACAGCAAAGGTG	CGTTGTTCTTATTTCGAGCCAT
CTSB	ATACTCAGAGGACAGGATCACT	ATCTTTTCCCAGTACTGATCGG
VDR	AAAGGTCATTGGCTTTGCTAAG	CTTGACTTCAGCAGTACGATCT
IDO1	CTGCCTGATCTCATAGAGTCTG	TTGTGGTCTGTGAGATGATCAA
PPARG	AGATCATTTACACAATGCTGGC	TAAAGTCACCAAAAGGCTTTCG
PTGS2	TGTCAAAACCGAGGTGTATGTA	AACGTTCCAAAATCCCTTGAAG
FAP	AGCAGTGGTCGGAATGTTCAAGTG	ATGTCTCGCCTCCTCTGTCTTCTG
MMP2	ATTGTATTTGATGGCATCGCTC	ATTCATTCCCTGCAAAGAACAC
TOP2A	AAGATTCATTGAAGACGCTTCG	GCTGTAAAATGCCATTTCTTGC
ADORA1	CCTATGTTTGGCTGGAACAATC	GTACTCCATGCTGATGACCTTC
BACE1	CAGACAAGTTCTTCATCAACGG	GTCTGCTTTACCAGAGAGTCAA

Table S2. Information about the 8 active compounds of BJOEI

PubChem CID	COMPOUND	Canonical SMILES	STRUCTURE
Oleic acid	445639	CCCCCCCCC=CCCCCCCCC(=O) O	
Linoleic acid	5280450	CCCCCC=CCC=CCCCCCCCC(=O) O	
Olein	45102063	CCCCCCCCC=CCCCCCCCC(=O) OCC(COC(=O) C) O	
Palmitic acid	985	CCCCCCCCCCCCCCCC(=O) O	

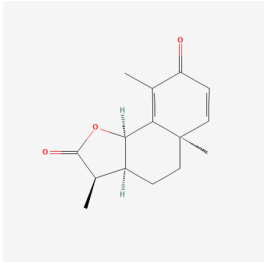
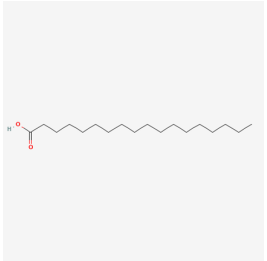
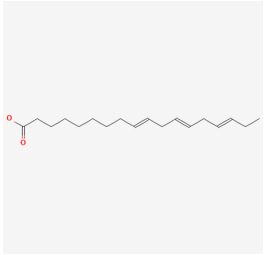
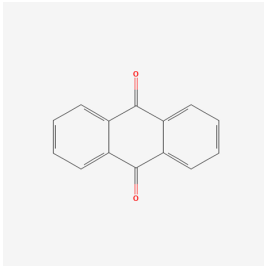
Sesquiterpenes	667450	CC1C2CCC3(C=CC(=O) C(=C3C2OC1=O) C) C	
Stearic acid	5281	CCCCCCCCCCCCCCCCCC(=O) O	
α-Linolenic acid	5280934	CCC=CCC=CCC=CCCCCCCCC(=O) O	
Anthraquinone	6780	C1=CC=C2C(=C1)C(=O)C3=CC=CC=C3C2=O	

Table S3. Basic information of the 50 genes

Gene names	Protein names	Gene related pathways
PPARG	Peroxisome proliferator-activated receptor gamma	PIP3 activates AKT signaling
SCD	Stearoyl-CoA Desaturase	Metabolism of steroids
PTGS1	Prostaglandin-Endoperoxide Synthase 1	Arachidonic acid metabolism
PTGES	Prostaglandin E Synthase	Arachidonic acid metabolism
PDE4D	Phosphodiesterase 4D	GPCR downstream signalling
RORC	RAR Related Orphan Receptor C	Gene expression (Transcription)
AKR1B10	Aldo-Keto Reductase Family 1 Member B10	Visual phototransduction
PTGER2	Prostaglandin E Receptor 2	Arachidonic acid metabolism
PTGS2	Prostaglandin-Endoperoxide Synthase 2	Arachidonic acid metabolism
SERPINA6	Serpin Family A Member 6	Metabolism of steroid hormones
G6PD	Glucose-6-Phosphate Dehydrogenase	KEAP1-NFE2L2 pathway
NPC1L1	NPC1 Like Intracellular Cholesterol Transporter 1	Digestion and absorption
PLA2G1B	Phospholipase A2 Group IB	Glycerophospholipid biosynthesis
BACE1	Beta-Secretase 1	Alzheimer's disease and miRNA effects
TOP2A	DNA Topoisomerase II Alpha	Transport of the SLBP independent Mature mRNA
ACHE	GeneCards Symbol: ACHE	Glycerophospholipid biosynthesis
PTGER4	Prostaglandin E Receptor 4	ADORA2B mediated anti-inflammatory cytokines production
IDO1	Indoleamine 2,3-Dioxygenase 1	NAD de novo biosynthesis II (from tryptophan)
FDFT1	Farnesyl-Diphosphate Farnesyltransferase 1	Superpathway of cholesterol biosynthesis
OXER1	Oxoeicosanoid Receptor 1	GPCR downstream signalling
SLC16A1	Solute Carrier Family 16 Member 1	Transport of inorganic cations/anions and amino acids/oligopeptides
CCKBR	Cholecystokinin B Receptor	Cholecystokinin B Receptor
ENPP2	Ectonucleotide Pyrophosphatase/Phosphodiesterase 2	Metabolism of water-soluble vitamins and cofactors
GPBAR1	G Protein-Coupled Bile Acid Receptor 1	ADORA2B mediated anti-inflammatory cytokines production
RBP4	Retinol Binding Protein 4	Visual phototransduction
MMP2	Matrix Metallopeptidase 2	Matrix metalloproteinases
PRKCQ	Protein Kinase C Theta	Prolactin Signaling
LPAR5	Lysophosphatidic Acid Receptor 5	GPCR downstream signaling
VDR	Vitamin D Receptor	Gene expression (Transcription)
HSD17B3	Hydroxysteroid 17-Beta Dehydrogenase 3	Androstenedione and testosterone biosynthesis and metabolism p.1
CDC45	Cell Division Cycle 45	Activation of the pre-replicative complex
PTPRC	Protein Tyrosine Phosphatase Receptor Type C	Activation of cAMP-Dependent PKA
RARA	Retinoic Acid Receptor Alpha	RNA Polymerase I Promoter Opening
EPHX2	Epoxide Hydrolase 2	Arachidonic acid metabolism
PRSS1	Serine Protease 1	Cobalamin (Cbl, vitamin B12) transport and metabolism
CTSK	Cathepsin K	Gene expression (Transcription)
ADORA1	Adenosine A1 Receptor	Purinergic signaling
CDC25C	Cell Division Cycle 25C	Defective Intrinsic Pathway for Apoptosis 25C
CTSS	Cathepsin S	Innate Immune System

CTSB	Cathepsin B	Collagen chain trimerization
PARP3	Poly (ADP-Ribose) Polymerase Family Member 3	Overview of interferons-mediated signaling pathway
PARP4	Poly (ADP-Ribose) Polymerase Family Member 4	SARS-CoV-1 Infection
FAP	Fibroblast Activation Protein Alpha	Extracellular region
CTRC	Chymotrypsin C	Urokinase-type plasminogen activator (uPA) and uPAR-mediated signaling
MMP3	Matrix Metallopeptidase 3	Matrix metalloproteinases
FGFR1	Fibroblast Growth Factor Receptor 1	Apoptotic Pathways in Synovial Fibroblasts
NOS1	Nitric Oxide Synthase 1	Superpathway of L-citrulline metabolism
MME	Membrane Metalloendopeptidase	Innate Immune System
MGLL	Monoglyceride Lipase	Glycerophospholipid biosynthesis
GCG	Glucagon	ADORA2B mediated anti-inflammatory cytokines production

Table S4. Fifteen genes relate to apoptosis for pancreatic cancer treatment

PARP4	CTSB	CTSK	PARP3	ADORA1
FAP	IDO1	MMP2	PLA2G1B	PPARG
PTGS2	PTPRC	TOP2A	VDR	BACE1