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Erianin inhibits the progression of pancreatic cancer by directly targeting AKT and ASK1

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Abstract

Background Pancreatic cancer is a malignant tumor of the digestive tract with a high mortality rate. Erianin has antitumor activity, but the regulatory targets and mechanism of action in pancreatic cancer are unclear. The objective of this study was to evaluate the anti-pancreatic cancer activity of Erianin and explore its underlying mechanisms.

Methods A network pharmacology approach was used to investigate the mechanism of action of Erianin in pancreatic cancer cells. Cell proliferation was analyzed using CCK8, colony-formation, and EdU proliferation assays. Cell migration was evaluated through wound healing and transwell assays, as well as determination of the protein expression levels of EMT markers and β-catenin. Apoptosis and the cell cycle were measured using flow cytometry and JC-1 staining, respectively. The protein expression levels of p-Rb, CyclinB1, P21, Cleaved-PARP, and Cleaved-Caspase3 were assessed using western blotting. RNA sequencing (RNA-seq) and bioinformatics analyses were performed to elucidate the mechanism underlying the action of Erianin in pancreatic cancer. Western blotting was used to examine the expression levels of key proteins in the AKT, JNK, and p38 MAPK signaling pathways. Molecular docking and CETSA were used to test hypotheses. The tumor-suppressive ability of Erianin in vivo was assessed using a tumor-bearing assay in nude mice.

Results Network pharmacology revealed that Erianin inhibited pancreatic cancer through multiple pathways. Erianin significantly inhibited pancreatic cancer cell proliferation and migration while promoting intracellular ROS and inducing apoptosis. Mechanistically, Erianin inhibited pancreatic cancer cell proliferation by regulating the AKT/FOXO1 and ASK1/JNK/p38 MAPK signaling pathways. In vivo experiments showed that Erianin inhibited subcutaneous tumor growth and promoted tumor tissue apoptosis in nude mice.

Conclusions The component-target-pathway network revealed that Erianin exerted anti-cancer effects through multiple components, targets, and pathways. Erianin inhibited the proliferation and migration of pancreatic cancer

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cells and induced apoptosis through the AKT/FOXO1 and ASK1/JNK/p38 MAPK signaling pathways. These results indicate that Erianin is a promising agent for pancreatic cancer treatment.

Keywords Pancreatic cancer, Erianin, AKT signaling pathway, JNK/p38 signaling pathway, Molecular mechanism

Background

Pancreatic cancer is a common digestive tract tumor with a high degree of malignancy [1]. The disease progresses without typical symptoms, which makes early diagnosis difficult. Pancreatic cancer has an extremely high mortality rate, with a 5-year survival rate of less than 10%, and is expected to become the second deadliest cancer worldwide by 2030 [2, 3]. Moreover, pancreatic cancer is resistant to chemoradiotherapy and has limited treatment options, posing great challenges to clinical treatment [4]. Consequently, the identification of more effective molecular therapeutic targets for pancreatic cancer and development of novel drug candidates are essential.

An imbalance in the PI3K/AKT signaling pathway is a critical regulator of tumor aggressiveness. Angiogenesis provides oxygen and nutrients for tumor growth, and inhibition of AKT phosphorylation can inhibit angiogenesis in pancreatic cancer [5]. Furthermore, forkhead box protein O1 (FOXO1) functions as a downstream molecular effector of the AKT pathway and is an important cell cycle-associated transcription factor [6]. P38 and JNK are two major subfamily members of the mitogen-activated protein kinase (MAPK) signaling pathway and play essential roles in the regulation of intracellular metabolism, gene expression, cell growth, differentiation, and apoptosis [7]. JNK and p38 play important roles in the malignant phenotype of various cancers [8]. JNK is highly activated in pancreatic cancer cells [9] and ASK1 overexpression plays a crucial role in pancreatic cancer development by promoting cell proliferation [10]. Therefore, it is vital to evaluate whether the AKT/FOXO1 and JNK/p38 MAPK signaling pathways play a role in anti-pancreatic cancer effects.

Erianin, a natural biphenyl compound derived from the Chinese herbal medicine Dendrobium nobile, is a prominent chemical component. Initial reports have shown that Erianin can be used as an antipyretic and analgesic drug [11]. Recent studies have demonstrated remarkable anticancer activity of Erianin. For instance, Erianin can increase the expression of BAX and Caspase-3, inhibit tumor cell proliferation, and promote apoptosis [12]. Additionally, Erianin exerts its anticancer effects by activating Ca2+/CaM signaling, increasing Ca2+and Fe2+levels, inducing ferroptosis, and inhibiting cell migration in lung cancer cells [13]. While the pharmacological effects of Erianin and the molecular mechanisms of its antitumor activity and effects on natural immunity have been reviewed and systematically described [14], the effect of Erianin and its molecular mechanism of action on pancreatic cancer remain to be explored.

In this study, we investigated the effects of Erianin on pancreatic cancer cells and its potential mechanism of action. Our data revealed that Erianin significantly inhibited the proliferation and migration of pancreatic cancer cells by inducing cell cycle arrest and apoptosis. Mechanistically, Erianin directly bound to AKT and ASK1 and inhibited the progression of pancreatic cancer by regulating the AKT/FOXO1 and JNK/p38 MAPK signaling pathways. In conclusion, Erianin exhibited potent anticancer efficacy both in vivo and in vitro and has great potential as an effective therapeutic agent for pancreatic cancer patients.

Materials and methods

Prediction of drug targets for Erianin

The SMILES chemical structure of Erianin was obtained using PubChem (https://pubchem.ncbi.nlm.nih.gov/). Subsequently, the SMILES structure was uploaded to the Swiss Target Prediction database (http://www.swisstargetprediction.ch/) to identify Erianin targets.

Prediction of targets for pancreatic cancer

Pancreatic cancer-related targets were searched using the GeneCards (https://www.genecards.org/), OMIM (https://www.omim.org/), TTD (https://db.idrblab.net/ttd/), and DisGeNET (https://www.disgenet.org/home/) databases.

Construction of a protein-protein interaction (PPI) network and gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) enrichment analyses

A PPI network of intersecting targets between Erianin and pancreatic cancer cells was constructed using the STRING database (https://string-db.org/). The minimum required interaction score, ranging from 0 to 1, was set to >0.9. The PPI network was further calculated, and hub genes were identified and visually analyzed using Cytoscape 3.7.2 software. The ClusterProfiler package of R3.18 was used to perform KEGG enrichment analyses.

Molecular docking and cellular thermal shift assay (CETSA)

The 3D structure of Erianin was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov). AutoDock Vina was used to dock the target proteins with Erianin. After exposure to DMSO or Erianin for 48 h, cells were collected, washed with PBS containing protease inhibitors, aliquoted into 7 PCR tubes, and heated in a thermal

cycler (T100, BioRad, Hercules, CA, USA) from 37 °C to 55 °C for 4 min to denature proteins. The cells were then subjected to three freeze-thaw cycles with liquid nitrogen, and centrifuged at 12,500 × g for 20 min at 4 °C. The supernatant was boiled in a loading buffer for western blot analysis.

Cell lines and cell culture

Human pancreatic cancer cell lines (SW1990 and L3.7) were kindly donated by Tianjin Medical University Cancer Institute & Hospital, Tianjin, China. Cell lines were cultured in DMEM and RPMI 1640 supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell viability analysis

Pancreatic cancer cells were seeded at a density of 5,000 cells per well in 96-well plates and treated with different concentrations of Erianin (Yuanye Biotechnology, Shanghai, China) at various time points. Following treatment, 10 μL CCK8 solution (#C0039, Beyotime Biotechnology, Shanghai, China) was added to each well and the plates were placed in the dark at 37 °C for 2 h. Absorbance was measured at 450 nm using a microplate reader.

Clone formation assay

SW1990 and L3.7 cells (500 cells/well) were seeded in 6-well plates and maintained overnight. The cells were exposed to DMSO or Erianin for 48 h, and then cultured in fresh medium for 14 d. The cell colonies were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Colonies containing more than 50 cells were manually counted.

5-ethynyl-29-deoxyuridine (EdU) assay

EdU assay was performed according to the EdU cell proliferation kit instructions (#C0078S, Beyotime Biotechnology, Shanghai, China). Cells (2×10^4 cells/well) were seeded in 24-well plates and exposed to Erianin for 48 h. Cells were incubated with 200 μL EdU reagent (10 μM) per well for 1 h, fixed with 4% paraformaldehyde for 15 min, and perforated with 0.3% TritonX-100 for 15 min. The Click Additive Solution and DAPI were added to the cells and incubated in the dark for 30 min. The results were captured using a fluorescence microscope.

Tumor cell migration assays

For the wound-healing assays, pancreatic cancer cells were seeded in 6-well plates and scratched with a sterile plastic pipette tip (200 μL) when the monolayer reached approximately 90% confluence. The cells were then washed thrice with PBS and treated with Erianin for 48 h. Images were captured using a light microscope at 0 and 48 h after scratching. The results were quantitatively analyzed using ImageJ software.

For the transwell assay, 1×10^4 cells in 200 μL serum-free medium were placed in the upper chambers, and the lower chambers were filled with a medium containing 20% FBS (total volume 600 μL). The cells were treated with Erianin for 48 h, fixed with 4% paraformaldehyde for 30 min, and stained with 0.1% crystal violet for 30 min. Transwells were photographed and analyzed under a microscope. The migration ability of the pancreatic cancer cells was quantified as the mean number of cells in three randomly selected fields.

Flow cytometry analysis of apoptosis and cell cycle

The proportion of apoptotic cells was assessed using the Annexin V-FITC/PI Apoptosis Detection Kit (#An0050, Zeta life, USA). SW1990 and L3.7 cells were treated with Erianin for 48 h. Treated cells (1×10^6) were centrifuged and re-suspended in 1× cold binding buffer. Subsequently, 5 μL each of Annexin V-FITC and PI were added and incubated for 15 min at room temperature in the dark.

The cell cycle was assessed using the Cell Cycle Assay Kit (#E-CK-A351, Elabscience Biotechnology, Wuhan, China). Treated cells (5×10^5) were fixed with absolute ethanol and stored at -20 °C overnight. RNase A Reagent (100 μL) was added, the cells were re-suspended in a 37 °C water bath for 30 min, and 400 μL PI Reagent (500 μg/mL) was added and mixed thoroughly. Cells were incubated at 4 °C in the dark for 30 min. Stained cells were detected using an flow cytometer and analyzed using FlowJo 10.8 software.

JC-1 staining

Cells (2×10^4 cells/well) were seeded in 12-well plates and exposed to Erianin for 48 h. The mitochondrial membrane potential in SW1990 and L3.7 cells was determined via a JC-1 fluorescent probe (#C2006, Beyotime Biotechnology, Shanghai, China). Cells were incubated with JC-1 working solution for 20 min at 37 °C, followed by at least three washes with JC-1 buffer solution. Cells were photographed using fluorescence microscopy; the red fluorescence intensity represents the level of mitochondrial destruction.

ROS detection

For detection of total ROS in cells, SW1990 and L3.7 cells were treated with Erianin for 48 h and then incubated with the DCFH-DA (#S0033S, Beyotime Biotechnology, Shanghai, China) for 30 min at 37 °C. The cells were then washed thrice with FBS-free medium. Finally, fluorescence was observed and analyzed.

Western blotting

Total protein was extracted using RIPA buffer and quantified using the BCA Protein Assay Kit (#PC0020, Solarbio, Beijing, China). Each lane was loaded with 20 μg of protein, and the proteins were separated using SDS-PAGE and transferred onto 0.22 μM PVDF membranes. The membrane was blocked using a 5% skim milk solution for 1 h at room temperature, followed by incubation with different primary antibodies and corresponding HRP-labelled secondary antibodies. Target protein expression was detected using an enhanced chemiluminescence reagent. The primary antibodies used were: N-cadherin (#22018-1-AP), E-cadherin (#20874-1-AP), β-catenin (#51067-2-AP), Vimentin (#10366-1-AP), β-ACTIN (#81115-1-RR), BCL2 (#68103-1-Ig), BAX (#50599-2-Ig), ASK1 (#28201-1-AP), p-ASK1 (#28846-1-AP), P38 (#14064-1-AP), p-P38 (Thr180/Tyr182, #28796-1-AP), these antibodies were purchased from Proteintech (Wuhan, China). Cleaved-Caspase3 (Asp175, #9664), AKT (#9272), p-AKT (#4060), Foxo1 (#2880), p-Foxo1 (#84192), p-Rb (Ser807/811,#8516), CyclinB1 (#4138), P21 (#2947), Cleaved-PARP (Asp214, #9541), JNK (#9252), and p-JNK (#4668), the above antibodies were purchased from Cell Signaling Technology (Beverly, California, USA).

RNA-seq and pathway enrichment analysis

Total RNA was isolated from SW1990 cells treated with either 0 or 100 nM Erianin for 48 h using TRIzol Reagent (#15596026, Invitrogen, Waltham, MA, USA). Subsequent RNA sequencing was performed using an Illumina NovaseqTM 6000 (Illumina, Beijing, China). Raw reads were filtered and cleaned using FASTq. Sequences were aligned to the GRCh38 reference genome using STAR. The gene expression levels were quantified using FPKM. Differentially expressed genes (DEGs) were identified using the Deseq2 package with a p-value < 0.05. Enrichment analysis was performed using the ClusterProfiler software package. R software was used for visualization.

Animal experiments

Female BALB/c nude mice (4–6 week) were purchased from Tianjin Jinke Bona Biotechnology Co., LTD (Tianjin, China). The Laboratory Animal Ethics Committee approved the animal experiments, which were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Statistical analysis

Significant differences between two groups were analyzed using an unpaired t-test, and differences between more than two groups were analyzed using a one-way ANOVA. Statistical analyses were performed using GraphPad Prism 9.5 software. Statistical significance was set at $P<0.05$.

Results

Identification of Erianin and pancreatic cancer-related targets and construction of ER-PC intersection network

The structure of Erianin is shown in Fig. 1A. After overlapping, 105 Erianin targets were identified from the Swiss Target Prediction database. A total of 4,131 pancreatic cancer targets were obtained from the above four databases after removing overlapping targets. A total of 75 common targets (Fig. 1B) were screened based on the Venn diagram, and an ER-PC-target network diagram (Fig. 1C) was generated using Cytoscape software. In total, 77 nodes and 150 edges were identified (Fig. 1D). These targets were filtered according to the cytoHubba analysis, and 16 key target genes were obtained for Erianin treatment of pancreatic cancer (Fig. 1E). KEGG enrichment analysis showed that the key targets were mainly enriched in cellular senescence, focal adhesion, cell cycle, PI3K-AKT signaling pathway, MAPK signaling pathway, and FOXO signaling pathway (Fig. 1F, Supplementary Table S1). These results revealed the signaling pathways and biological processes of Erianin against pancreatic cancer, showing that its tumor suppression is multi-targeted and multi-pathway.

Erianin suppresses pancreatic cancer cell proliferation

To clarify whether Erianin affects the proliferation of pancreatic cancer cells, two pancreatic cancer cell lines (SW1990 and L3.7) were treated with different concentrations of Erianin for 48 h. CCK8 results showed that Erianin significantly inhibited the proliferative viability of pancreatic cancer cells in a dose-dependent manner (Fig. 2A-B). The 48-h inhibitory concentration 50 (IC50) values of SW1990 and L3.7 cells were 472.8 and 101.0 nM, respectively (Fig. 2C-E). Based on these results, Erianin concentrations approaching the IC50 value were used for subsequent experiments. Morphological observations showed that in both the SW1990 and L3.7 cell lines, the number of shrunken, rounded, and detached cells significantly increased with Erianin treatment (Fig. 2F). To further demonstrate the inhibitory effects of Erianin on pancreatic cancer cells, we performed colony formation and EdU proliferation assays. Compared to the control group, there was a significant reduction in the number of clones and actively dividing cells after 48 h of Erianin treatment, indicating the potent inhibitory capacity of Erianin (Fig. 2G-J). These experiments demonstrated that Erianin significantly inhibited the proliferative ability of pancreatic cancer cells in vitro.

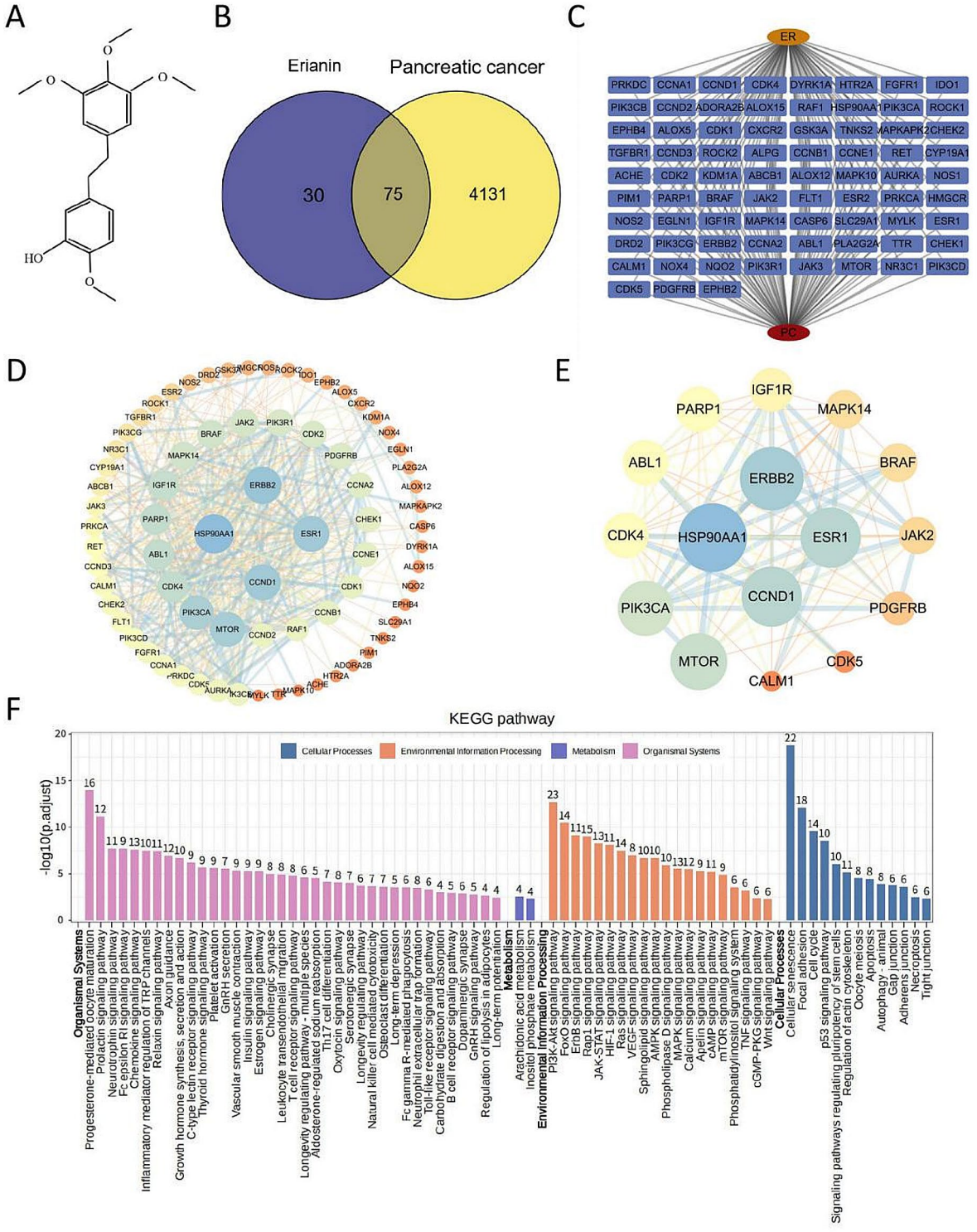

Fig. 1 Intersection targets and bioinformatic analysis between ER and PC. (**A**) Chemical structure of Erianin. (**B**, **C**) 75 intersection targets of ER and PC. (**D**) The PPI network of ER and PC intersection targets. (**E**) Key targets of ER for treating PC based on cytoHubba analysis. (**F**) KEGG pathway analysis

Erianin inhibits cell migration and induces G2/M phase arrest in pancreatic cancer cells

To further investigate the inhibitory effect of Erianin on pancreatic cancer cells, cell wound-healing assays were performed to analyze the effect of Erianin on the migratory ability of pancreatic cancer cells. After 48 h, the migrated cells in the control group had almost completely filled the scratch area. However, in the Erianin-treated

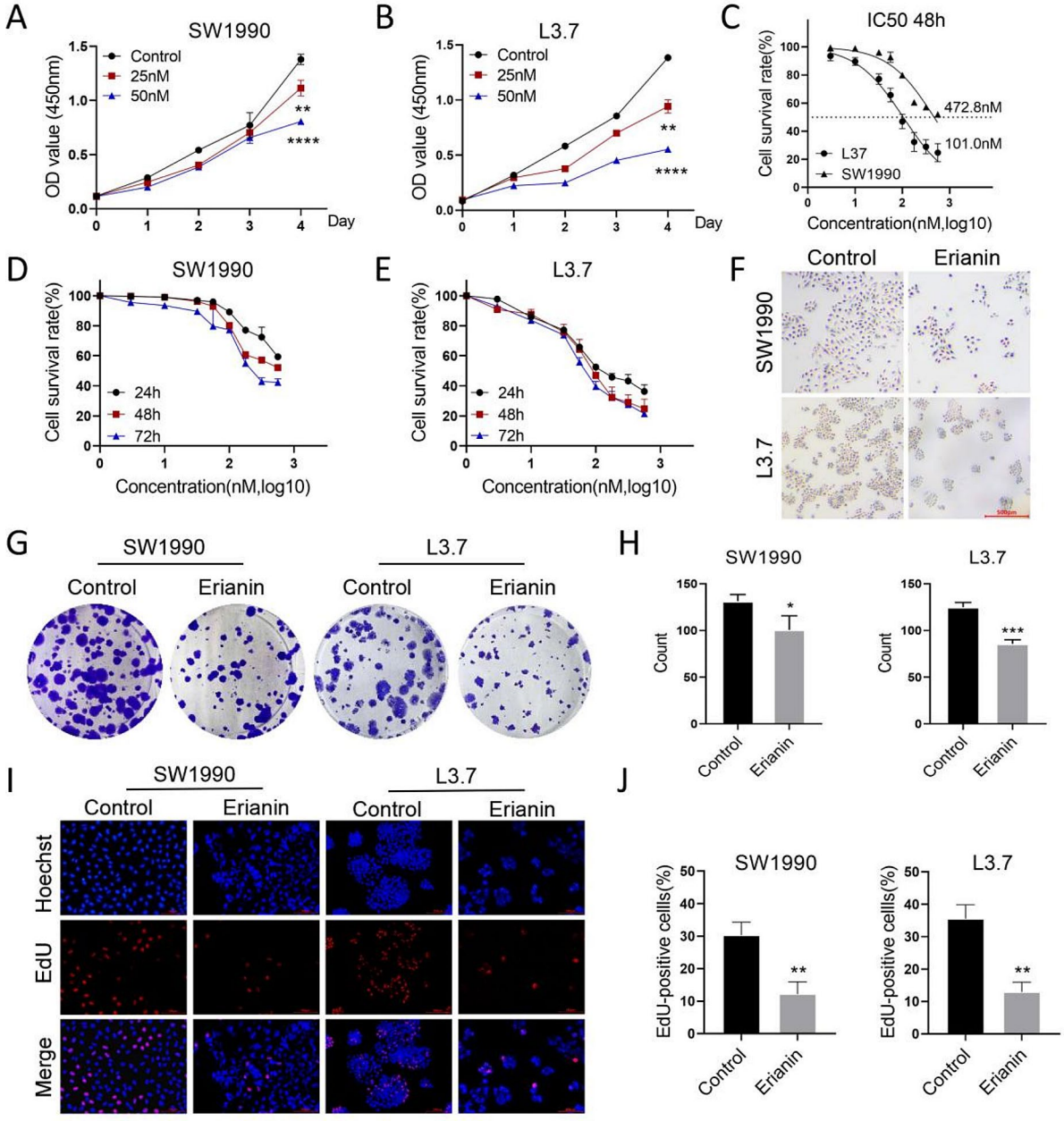

Fig. 2 Erianin effectively suppresses the proliferation of PC cells. (**A**, **B**) Cell viability determined by CCK8 assay for SW1990 and L3.7 cells treated with Erianin (0, 25, 50nM) for 24,48,72 and 96 h. (**C**) IC50 values of the SW1990 and L3.7 cells after Erianin exposure for 48 h. (**D**, **E**) Cell viability determined by CCK8 assay for SW1990 and L3.7 cells treated with Erianin. (**F**) Cell morphology of SW1990 and L3.7 cells treated with Erianin for 48 h. (**G**) Cell colony formation assays of SW1990 and L3.7 cells exposed to Erianin for 14 day. (**H**) The number of colonies of those in Fig. 1G. The data are presented as means ± SD, $n = 3$, $^{*}P < 0.05$ vs. control group. (**I**, **J**) EdU proliferation assays of SW1990 and L3.7 cells exposed to Erianin for 48 h

group, minimal migration distance was observed for SW1990 and L3.7 cells, indicating that Erianin significantly decreased the migratory ability of pancreatic cancer cells (Fig. 3A-B). Transwell assays also showed that Erianin significantly inhibited the migration of SW1990 and L3.7 cells (Fig. 3C-D). Western blotting showed that Erianin significantly down-regulated the expression of N-cadherin, vimentin, and β-catenin, while E-cadherin was up-regulated (Fig. 3E).

In addition, we investigated whether Erianin affected the cell cycle of pancreatic cancer cells. Flow cytometric analysis revealed that Erianin induced G2/M phase arrest (Fig. 3F-G). Western blotting showed that the expression of P21 was elevated, whereas the expression of p-Rb and the CDK regulator CyclinB1 was decreased after treatment with Erianin for 48 h (Fig. 3H). These findings further confirmed that Erianin suppressed

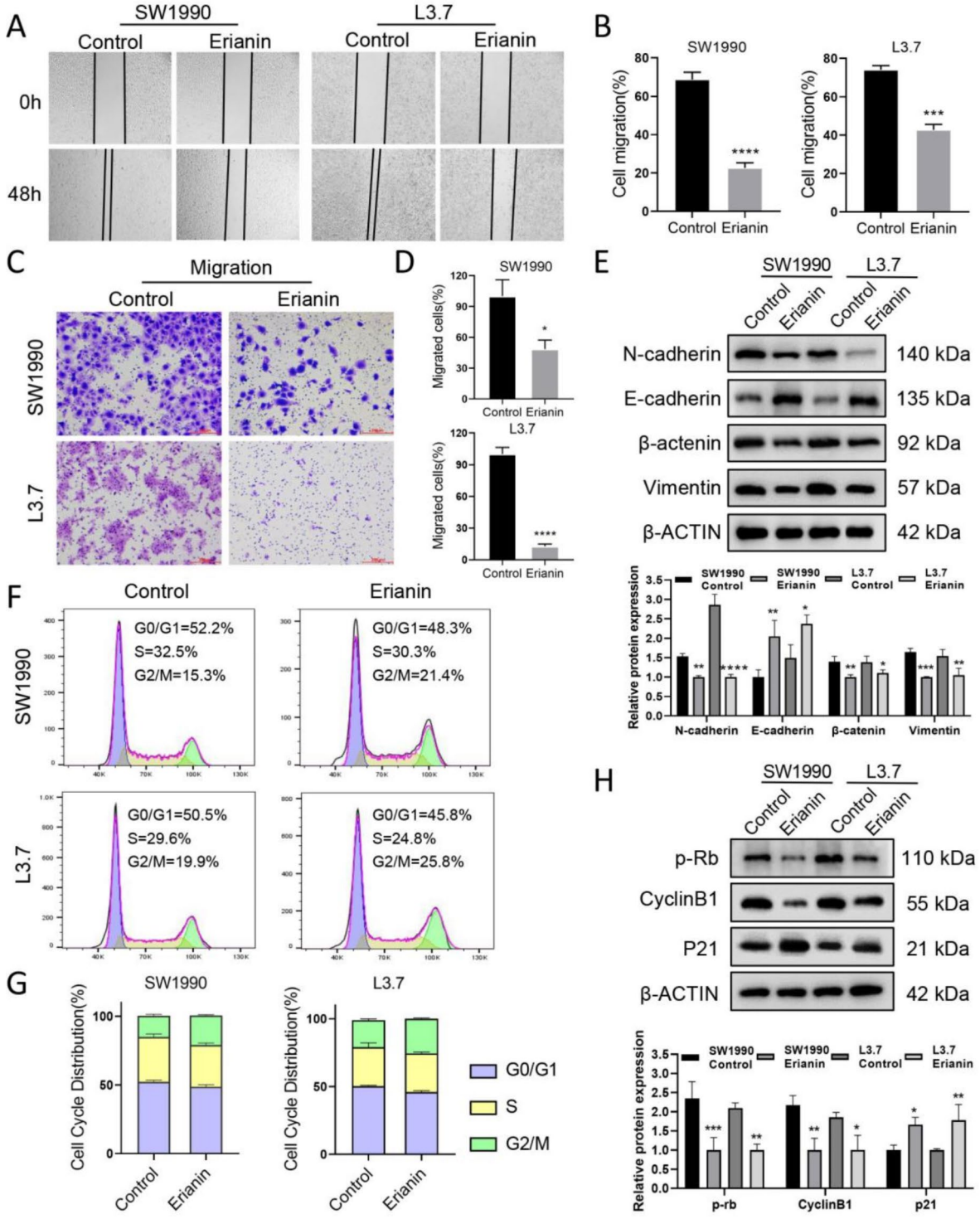

Fig. 3 Erianin suppresses migration and cell cycle of PC cells. (**A**, **B**) Wound-healing ability of SW1990 and L3.7 cells with Erianin for 48 h. (**C**, **D**) The effect of Erianin on the migration in SW1990 and L3.7 cells. (**E**) Expression of N-cadherin, E-cadherin, β-catenin, and vimentin in SW1990 and L3.7 cells by western blotting. β-actin served as a loading control. (**F**, **G**) Cell cycle distribution as analyzed by flow cytometry. (**H**) Western blotting analyses of the expression of the cell cycle regulators phosphorylated Rb (p-Rb), Cyclin B1, and P21 in SW1990 and L3.7 cells. β-actin served as a loading control

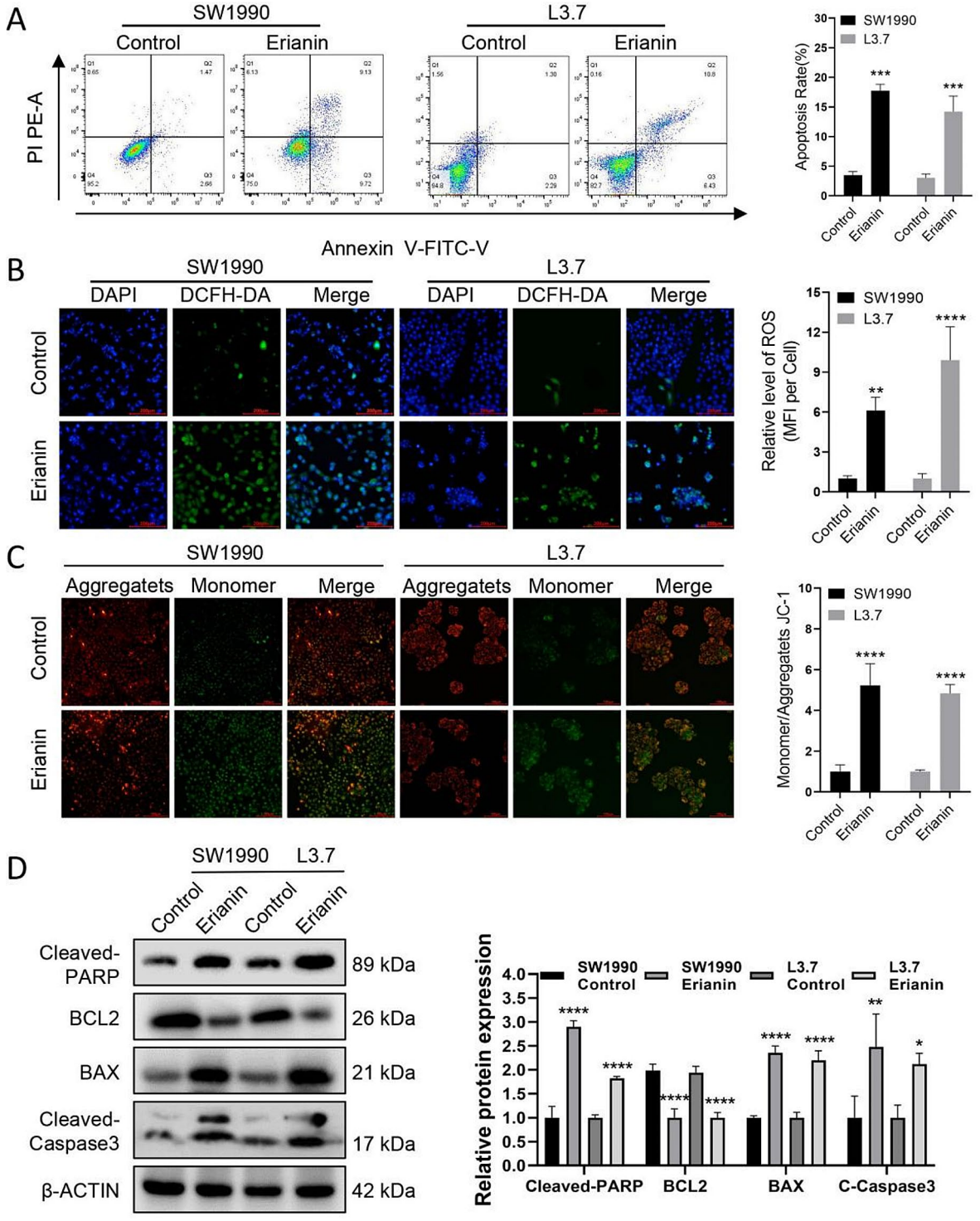

Fig. 4 Erianin promotes apoptosis of PC cells. (**A**) Apoptosis of PC cells after Erianin for 48 h measured by flow cytometry. (**B**) ROS of PC cells after Erianin for 48 h. (**C**) JC-1 staining of PC cells after Erianin for 48 h. (**D**) Expression of Cleaved-PARP, BCL2, BAX and Cleave-Caspase3 in SW1990 and L3.7 cells by western blotting. β-ACTIN served as internal standard

epithelial-mesenchymal transition(EMT), inhibited pancreatic cancer cell migration, and arrested the cell cycle.

Erianin promotes apoptosis in pancreatic cancer cells

To elucidate the mechanisms underlying Erianin-induced cytotoxicity in pancreatic cancer cells, we first investigated apoptosis. Flow cytometric analysis revealed

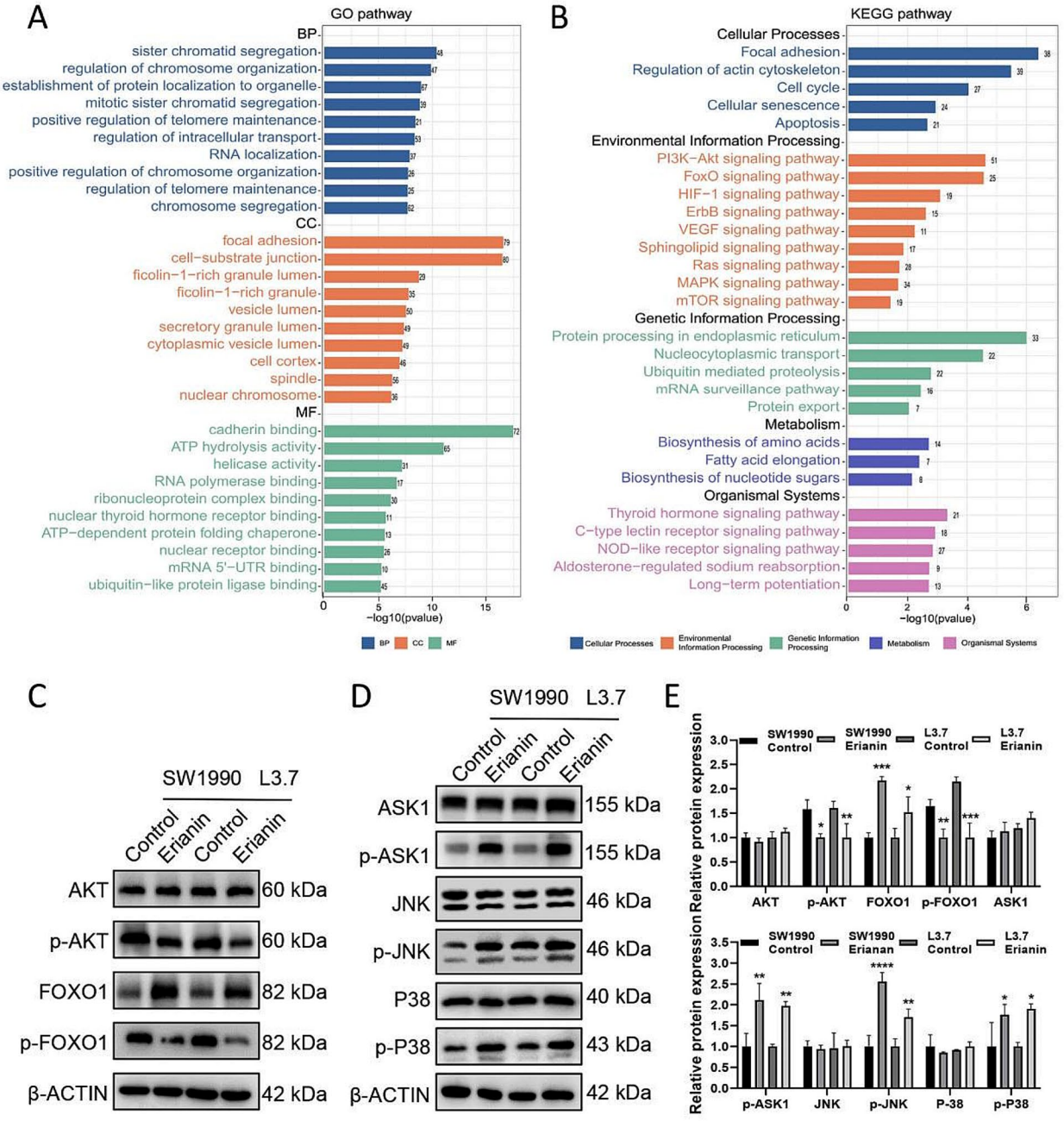

Fig. 5 Enrichment analysis and mechanism of Erianin inhibition in pancreatic cancer cells. (**A**, **B**) GO and KEGG pathway analysis (**C-E**) Expression of AKT and JNK/p38 MAPK pathway. β-ACTIN served as internal standard

that Erianin significantly increased the apoptotic rate of SW1990 and L3.7 cells (Fig. 4A). Correspondingly, the production of reactive oxygen species (ROS) increased after Erianin treatment and the mitochondrial membrane potential of the cells was significantly reduced (Fig. 4B-C). After Erianin treatment, the protein expression of BCL-2 and apoptosis-related proteins Cleaved-PARP, BAX, and Cleaved-Caspase3 increased (Fig. 4D). These results suggested that Erianin promoted ROS production and induced apoptosis via the mitochondrial pathway.

Erianin inhibits the AKT/FOXO1 pathway and activates the ASK1/JNK/p38 MAPK pathway in pancreatic cancer cells

To further explore the potential molecular mechanisms of Erianin in pancreatic cancer, we performed RNA Sequencing analysis to identify DEGs under Erianin

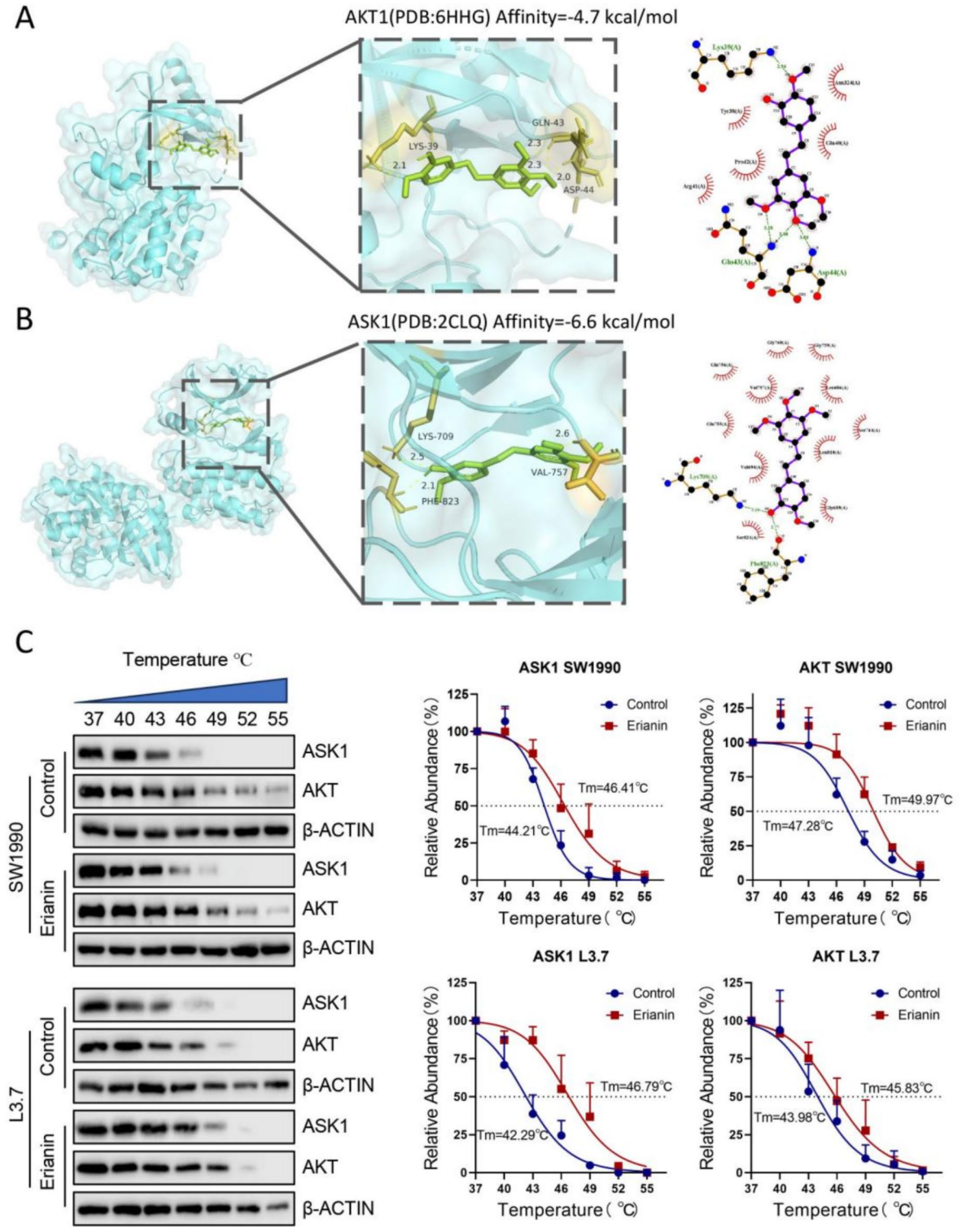

Fig. 6 Molecular docking and CETSA results of Hub protein and ER. (**A**, **B**) Molecular docking between ER complexed with AKT and ASK1. (**C**) Cellular thermal shift assay between Erianin AKT and ASK1. The curve was fitted using GraphPad Prism 9.5

stimulation in SW1990 cells (Supplementary Table S2). GO enrichment analysis showed that Erianin-associated DEGs were enriched in important biological processes, such as focal adhesion, cadherin binding, cell-substrate junction, and ATP hydrolysis activity (Fig. 5A, Supplementary Table S3). Consistent with the ER-PC-target network, KEGG pathway enrichment analysis also showed that Erianin-associated DEGs were enriched in

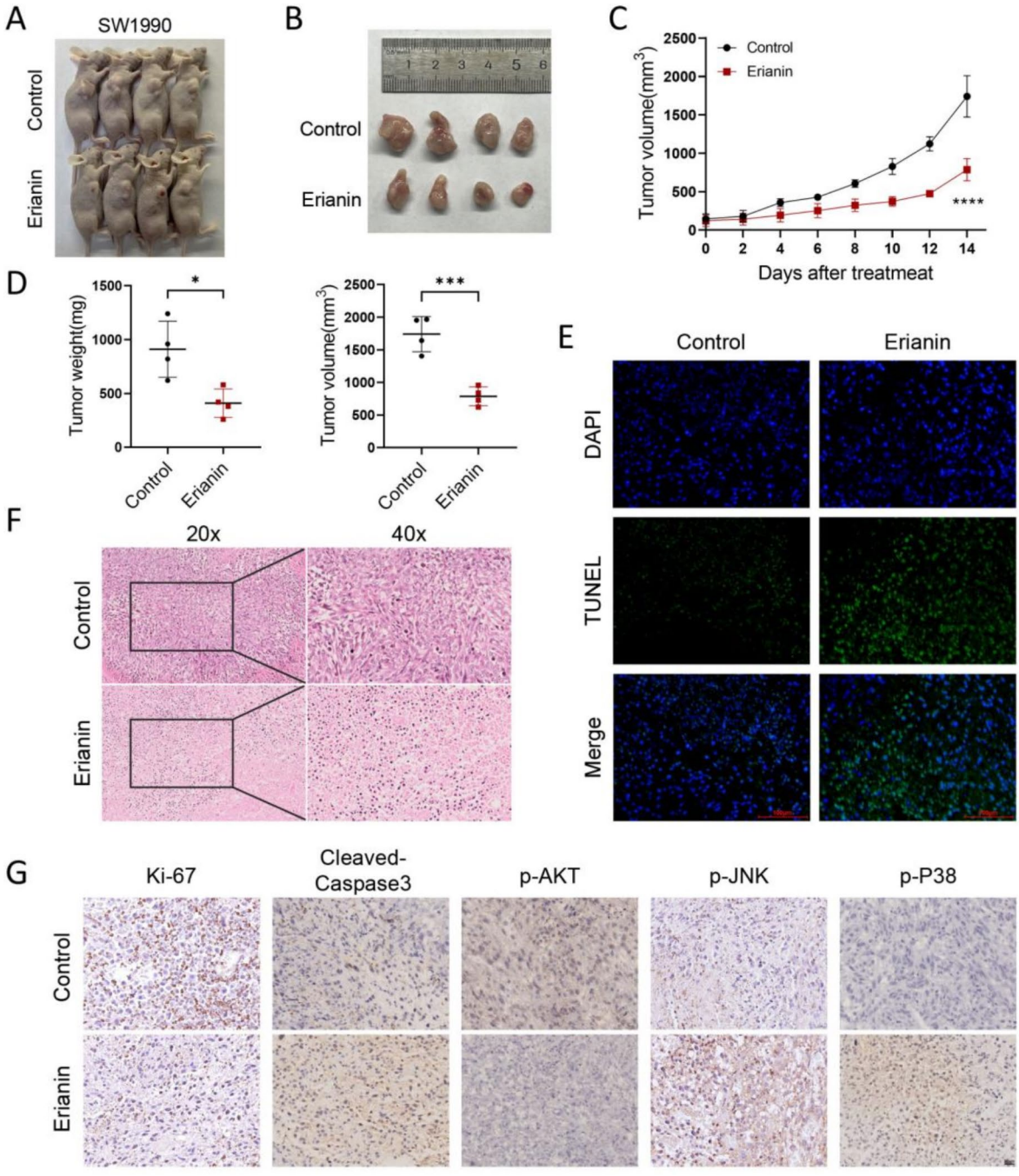

Fig. 7 Erianin exhibits anti-cancer effects in vivo. (**A**)Xenograft model in nude mice. (**B**, **D**) Tumor volume and weight of SW1990 xenograft tumors after Erianin treatment for 14 days. (**C**) Growth curve of the tumor volumes measured on the indicated days. Error bars represent the mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, unpaired t-test. (**E**) TUNEL staining was used to detect apoptosis in tumour tissues. (**F**) HE staining was used to observe the changes in the morphology and structure of the tumour tissue. (**G**) IHC was used to detect the expression levels of Ki-67, Cleaved-Caspase3 and the phosphorylation levels of AKT, JNK, and P38 in tissue cells

the PI3K-AKT, FOXO, and MAPK signaling pathways, suggesting that these pathways may play key roles in the anti-pancreatic cancer effects of Erianin (Fig. 5B, Supplementary Table S4). Western blotting showed that Erianin significantly downregulated the phosphorylation level of AKT and the phosphorylation and total expression level of FOXO1 (downstream protein of AKT). In addition, Erianin significantly upregulated the phosphorylation of the MAPK pathway-related proteins ASK1, p38, and JNK in pancreatic cancer cells (Fig. 5C-E). Together, these results confirm that Erianin inhibited AKT/FOXO1 and

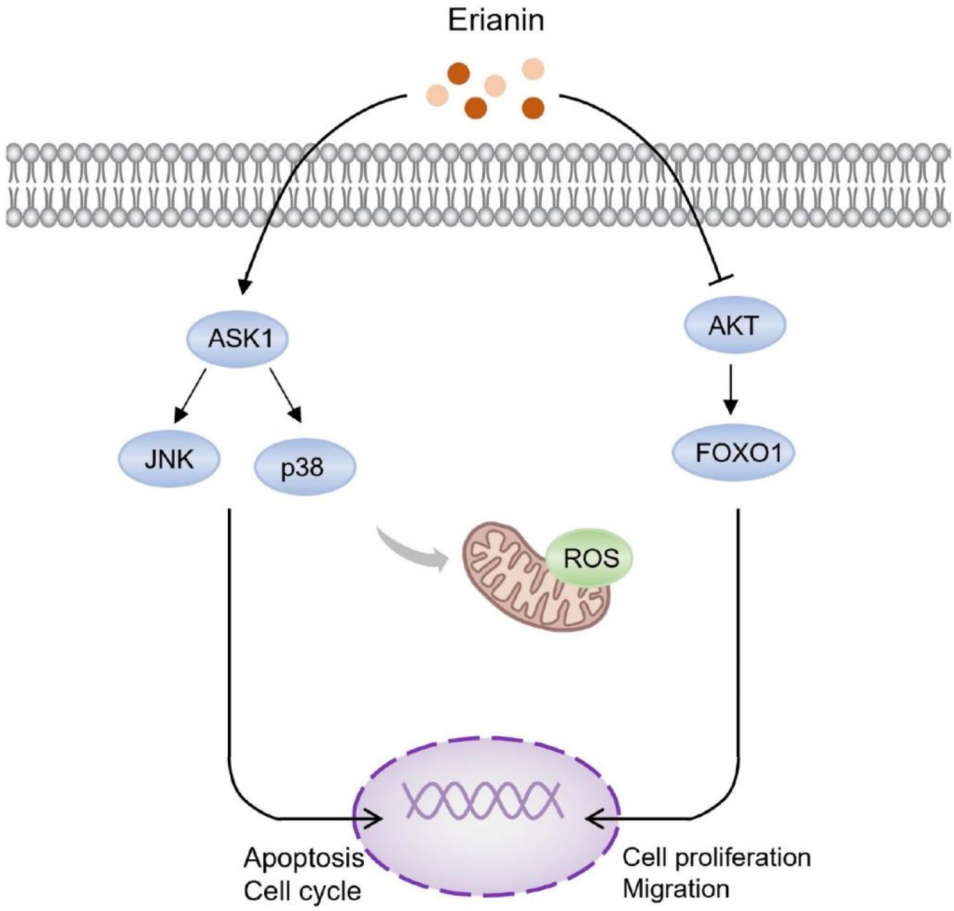

Fig. 8 The potential mechanism for the treatment of pancreatic cancer with Erianin

activated the ASK1/JNK/p38 MAPK signaling pathway in pancreatic cancer cells.

Erianin binds to target proteins with better thermal stability

We analyzed whether Erianin could bind to AKT or ASK1 proteins. Molecular docking predictions showed that Erianin has potential binding affinity with ASK1 and AKT, with binding energies of -6.6 kcal/mol and −4.7 kcal/mol, respectively (Fig. 6A-B). Cellular thermal shift assay(CETSA) experiments showed that Erianin shifted the melting curve of ASK1 to the right (Fig. 6C), implying that Erianin binds directly to ASK1. The same trend was observed for AKT. The results showed that Erianin binds to ASK1 and AKT with better protein thermal stability.

Erianin inhibits tumorigenicity of pancreatic cancer in vivo

To further determine whether Erianin inhibited the tumorigenicity of pancreatic cancer, we constructed a subcutaneous xenograft tumor model using SW1990 cells and BALB/c nude mice. The results showed that Erianin inhibited tumor growth in tumor-bearing nude mice, and the tumor size and weight were significantly lower than those in the control group (Fig. 7A-D). In addition, HE and TUNEL staining showed that Erianin significantly induced apoptosis in tumor tissues (Fig. 7E-F). IHC showed increased phosphorylation levels of JNK and P38 and cleaved-caspase3, and decreased phosphorylation levels of AKT and ki-67 in the Erianin group (Fig. 7G). In conclusion, these findings demonstrate that Erianin inhibited tumor growth in pancreatic cancer in vivo.

Discussion

Changes in cell signaling are the main mechanisms employed by cells in the development and progression of multiple cancers [15]. AKT and MAPK pathways are important signal transduction pathways in a variety of malignancies because they are critically involved in tumor cell growth, metabolism, proliferation, invasion, and apoptosis [16, 17]. For instance, the AKT/FOXO1 and MAPK pathways act as tumor factors in lung, colorectal, breast, bladder, and prostate cancer [18–23]. Both pathways are expected to be useful in cancer treatment, especially for pancreatic cancer.

Natural products and their derivatives, which are characterized by structural diversity and high biological activity, are important sources for drug discovery and development. A total of 75% of antitumor compounds currently used to treat human cancers are natural products [24]. With advancements in natural compound extraction and separation technologies, a growing number of compounds with anti-pancreatic cancer activities have been identified in plants [25–29]. For instance, CK21 is a novel pro-drug of triptolide that exerts potent anti-proliferative effects on human pancreatic tumors by inhibiting the NF-κB pathway, ultimately leading to mitochondrial-mediated tumor cell apoptosis [25]. DT-13 inhibits proliferation and induces apoptosis of pancreatic cancer cells by activating the AMPK/mTOR signaling pathway and suppressing p70 S6K [29].

In recent years, the tumor-suppressive effects of Erianin have been confirmed in studies on various diseases such as liver [30, 31], lung [13], breast [32, 33], and colorectal cancer [34]. These findings illustrate that Erianin exhibits different targets and phenotypes in diverse tumor cell types. Consistent with previous reports, we found that Erianin suppressed the proliferation, migration, and EMT of pancreatic cancer cells and promoted apoptosis at nanomolar concentrations. Further experiments revealed that Erianin inhibited AKT/FOXO1, while activating the ASK1/JNK/p38 MAPK pathway. Our results are consistent with those of previous reports showing that Erianin promotes apoptosis in tumor cells by inhibiting AKT and activating the MAPK pathway [35, 36]. However, another study found that Erianin inhibited p38 phosphorylation to prevent human oral cancer cell proliferation, while JNK phosphorylation was also activated [37]. This discrepancy may be because JNK and p38 signaling outcomes depend on cell types and contexts. In our study, ASK1 activated the MAPK pathway to exert antitumor effects. Our findings further refine the mechanism of action of Erianin in various tumors. Therefore, Erianin may have a bidirectional regulatory role; it is necessary to explore in depth the role of Erianin in tumors in future studies.

Molecular docking techniques use receptors and ligands of known structures to identify intermolecular interactions, which can identify the binding of traditional Chinese medicine active ingredients to potential targets [38]. Molecular docking results indicated that Erianin could bind directly to AKT and ASK1 proteins. This was verified by CETSA experiments. The results indicated that the thermodynamic stability of the target protein was significantly improved and that Erianin bound tightly to the target proteins. These results emphasize the potential mechanism of Erianin for treating pancreatic cancer via AKT and ASK1 pathway (Fig. 8).

Conclusions

In conclusion, Erianin inhibited the proliferation and migration of pancreatic cancer cells and induced apoptosis by targeting the AKT/FOXO1 and ASK1/JNK/p38 MAPK pathways. Our study provides a rationale for the use of Erianin as a potential agent for pancreatic cancer treatment.

Abbreviations

ER	Erianin
PC	Pancreatic cancer
DMSO	Dimethyl sulfoxide
ROS	Reactive oxygen species
DEGs	Differently expressed genes
FBS	Fetal bovine serum
PBS	Phosphate buffer saline
CETSA	Cellular thermal shift assay
EdU	5-Ethynyl-29-Deoxyuridine
HE	Hematoxylin and eosin
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling
IHC	Immunohistochemistry staining

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12935-024-03533-9.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Author contributions

RL., M.Q., Z.Y. and H.W.: Designed the research. RL, MQ, XD, MZ, ZG, YW, HM, MZ, QZ, ZY: Performed the experiments and generated data. RL, MQ, ZY, XD: Significantly contributed to the manuscript drafting. HW, MQ, ZY: Funding acquisition, editing, and supervision. All authors have read and approved the final manuscript.

Data availability

All data generated in this study are included in this published article (and its supplementary files). The RNAseq dataset of this article is available at the NCBI GEO repository (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE269714).

Declarations

Ethics approval and consent to participate

All animal experiments were approved by the Ethics Committee for Animal Experimentation at the Nankai University.

Competing interests

The authors declare no competing interests.

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