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Isoliensinine Suppresses Colorectal Tumorigenesis by Targeting PPP2R1A to Activate AMPK Signaling Pathway

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ABSTRACT: Colorectal cancer, characterized by its invasive behavior, frequent recurrence, and limited responsiveness to standard treatment, necessitate the advancement of innovative therapeutic approaches. Isoliensinine (ISO), a natural bisbenzylisoquinoline alkaloid, has demonstrated broad antitumor activity. However, its efficacy and underlying mechanisms in colorectal cancer therapy remain unclear and require further investigation. In our study, ISO demonstrates robust anti-colorectal cancer activity in both *in vitro* and *in vivo*. Through limited proteolysis-mass spectrometry (Lip-MS) screening, PPP2R1A was identified as a direct binding target of ISO, which was validated by bio-layer interferometry assays. Molecular dynamics simulations delineated the specific binding mode between ISO and PPP2R1A. Genetic knockdown of PPP2R1A attenuated colorectal cancer proliferation and partially abolished the antitumor effects of ISO. Integrated proteomic and phosphoproteomic analyses further revealed that ISO activates the AMPK signaling pathway following binding PPP2R1A. These results collectively establish ISO as a promising therapeutic agent for colorectal cancer treatment.

Keywords: Colorectal cancer; Isoliensinine; PPP2R1A; AMPK signaling pathway

1. Introduction

Colorectal cancer (CRC) ranks as the third most prevalent malignancy globally and the second leading cause of cancer-related mortality, with its incidence showing an upward trend in recent years^[1]. The persistently increasing incidence and mortality rates of CRC have established it as a major public health challenge^[2]. Most patients present no obvious symptoms during early stages and are typically diagnosed at advanced stages, resulting in poor treatment outcomes and low 5-year survival rates^[3]. Currently, the severe toxic side effects associated with radiotherapy or chemotherapy and tumor drug resistance remain primary causes of treatment failure and unfavorable prognosis^[4,5]. Therefore, identifying novel molecular targets and developing innovative therapeutic strategies have become crucial for improving treatment efficacy.

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Interventions targeting post-translational protein phosphorylation have emerged as viable anti-cancer strategies. Protein phosphatase 2A (PP2A), a crucial serine/threonine phosphatase, participates extensively in biological processes including cell cycle regulation, signal transduction, cellular differentiation, and DNA repair by counterbalancing kinase activities^[6,7]. As the scaffolding subunit of PP2A, PPP2R1A regulates holoenzyme complex stability and substrate specificity^[8]. Mutations and functional abnormalities of PPP2R1A have been implicated in various cancers such as endometrial carcinoma, ovarian cancer, breast cancer, and gastrointestinal stromal tumors^[9-11]. AMPK, a well-known and highly conserved energy sensor, has been demonstrated to function as a downstream substrate of PP2A in colorectal cancer (CRC). Moreover, the development of small-molecule inhibitors targeting the PP2A-AMPK regulatory axis has been shown to effectively suppress CRC progression^[12,13]. A thorough investigation of PPP2R1A-mediated AMPK activation in CRC will not only shed light on novel mechanisms underlying CRC pathogenesis and progression, but also facilitate the development of targeted therapeutics based on this pivotal molecular target.

In recent years, natural plant-derived compounds have garnered significant attention as potential agents for cancer treatment. Isolinsinine (ISO), a natural bisbenzylisoquinoline alkaloid extracted from lotus plumule, exhibits significant anti-tumor activities^[14]. Accumulating evidence has demonstrated the anti-cancer effects of ISO across various malignancies. Studies have reported that ISO induces apoptosis in human triple-negative breast cancer cells through ROS generation and activation of p38 MAPK/JNK pathways^[15]. In gastric cancer, it suppresses tumor cell proliferation and migration by targeting TGFBR1 to modulate the TGF- β -Smad signaling pathway^[16]. For cervical cancer, ISO induces cell cycle arrest and apoptosis via inhibition of the AKT/GSK3 α pathway^[17]. Moreover, ISO has shown similar inhibitory effects in other solid tumors including prostate cancer, lung adenocarcinoma, and hepatocellular carcinoma^[18-20]. These findings position ISO as a promising candidate for clinical translation in cancer therapy. Notably, while existing studies have reported that ISO can enhance the therapeutic sensitivity of drug-resistant colorectal cancer cells when used in combination with other anticancer agents^[21-23], the precise pharmacological targets and mechanisms of ISO in CRC treatment remain to be uncovered.

In this study, we validated the significant anti-tumor effects of ISO in CRC through comprehensive *in vivo* and *in vitro* experiments. PPP2R1A was identified as the direct binding target of ISO by using Lip-MS. Knockdown experiments in HCT116 and SW480 cell lines further testified PPP2R1A as ISO's key functional target. Finally, phosphoproteomics analysis provided in-depth characterization of potential downstream signaling mechanisms regulated by both ISO and PPP2R1A, thereby offering robust experimental evidence for elucidating ISO's anti-CRC mechanisms.

2. Materials and methods

2.1 Cell culture and reagents

ISO was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China) and dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution. The human colorectal cancer (CRC) cell lines HCT116 and SW480 were acquired from the Cell Bank of the Chinese Academy of Sciences (Shanghai,

China). The human normal colonic epithelial cell line NCM460 was purchased from Cellcook (Guangzhou, China). HCT116 cells were cultured in McCoy's 5A medium (KeyGEN, China), SW480 cells were maintained in Leibovitz's L-15 medium (KeyGEN, China), and NCM460 cells were grown in RPMI-1640 medium (KeyGEN, China). Both media were supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, USA) and 1% penicillin-streptomycin (HyClone, USA). All cell lines were authenticated by Short Tandem Repeat (STR) profiling and were routinely tested for mycoplasma contamination using PCR-based assays, with all results confirmed as negative. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO₂. Adherent cells were harvested using 0.25% trypsin (Thermo Fisher Scientific, USA) and passaged accordingly. The culture medium was replaced every three days. And cells were incubated with various concentrations of ISO for experimental treatments. The stock solution was diluted in the respective culture media such that the final DMSO concentration was maintained at 0.1% (v/v) across all treatment groups.

2.2 Cell viability assay

CRC cells were seeded into 96-well plates at a density of 1×10^5 cells per well and incubated for 24 hours. After cell adhesion, the cells were treated with ISO. Subsequently, 10 µL of CCK-8 reagent (Abbkine, China) was added to 100 µL of culture medium in each well, and the plates were further incubated at 37 °C for 2 hours. Absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, USA).

2.3 Colony formation assay

CRC cells were treated with ISO in 12-well plates. After treatment, the cells were harvested and reseeded into new 12-well plates at a density of 5×10^2 cells per well. The cells were cultured for two weeks to allow colony formation. The cells were washed with phosphate-buffered saline (PBS; KeyGen, China), fixed with 4% paraformaldehyde (PFA; Biosharp, China), and stained with crystal violet (Beyotime, China). Colonies were imaged, and the number of clones was quantified using ImageJ software.

2.4 Migration assay

CRC cells were initially treated with ISO in 6-well plates. The cells were then harvested and seeded into transwell chambers (Corning, China) with an 8.0 µm PET membrane at a density of 5×10^4 cells per well. After 48 hours, the migrated cells were washed with PBS, fixed with 4% PFA, and stained with crystal violet. Images were captured using a Leica inverted microscope at 200× magnification, and the number of migrated cells was quantified with ImageJ software.

2.5 Cell cycle analysis

CRC cells were fixed with 70% cold ethanol at 4°C overnight. Subsequently, the cells were resuspended in FxCycle™ PI/RNase Staining Solution (Thermo Fisher Scientific, USA) and incubated for 30 minutes at room temperature. DNA content was analyzed by flow cytometry using FACS (FACSCelesta, Becton Dickinson, USA). The cell cycle distribution was evaluated with FlowJo software (version 10.8.1).

2.6 Apoptosis analysis

Cell apoptosis was assessed using an Annexin V-AbFlour™ 647/PI Apoptosis Detection Kit (Abbkine, China). Briefly, CRC cells were washed with ice-cold PBS and resuspended in 100 µL of 1× Annexin V binding buffer. The cell suspension was then incubated with 4 µL of Annexin V-AbFlour™ 647 and 2 µL of propidium iodide (PI) for 15 minutes at room temperature in the dark. After incubation, 400 µL of 1× binding buffer was added, and samples were gently mixed. Apoptotic cells were quantified by flow cytometry and data were analyzed using FlowJo software (version 10.8.1).

2.7 Caspase activity assay

Caspase-3 and caspase-9 activities were measured using commercial colorimetric assay kits (Abbkine, China). Cells were lysed in 50 µL of lysis buffer on ice for 20 minutes and centrifuged at $16,000 \times g$ for 30 minutes at 4 °C. The supernatant was collected and incubated with 50 µL of caspase-3 substrate (Ac-DEVD-pNA) or caspase-9 substrate (Ac-LEHD-pNA) at 37 °C for 2 hours in the dark. Absorbance was measured at 405 nm. Caspase activity was normalized to the total protein concentration in the cell lysates.

2.8 Tumor xenograft model

Subcutaneous xenograft model was established using male BALB/c nude mice (4 weeks old, 18g–22 g) obtained from the Laboratory Animal Center of Fujian University of Traditional Chinese Medicine. All animals were housed under specific pathogen-free (SPF) conditions. The experimental procedures were approved by the Ethics Committee of the Fujian University of Traditional Chinese Medicine (Approval Number: 2025023).

HCT116 cells were harvested, washed twice with PBS, and resuspended in a 1:1 mixture of Matrigel (Corning, USA) and PBS at a density of 1×10^5 cells per 200 µL. The cell suspension was subcutaneously injected into the right axillary region of each mouse. Tumor size was measured regularly with calipers, and tumor volume was calculated using the formula: $\text{Volume} = (\text{Length} \times \text{Width}^2) / 2$. When the tumor volume reached approximately 50 mm³, the mice were randomly divided into four groups ($n = 6$ per group) and received daily intraperitoneal injections of either the vehicle (10% DMSO and 90% of 20% SBE-β-CD in saline) or ISO at doses of 2.5, 5, or 10 mg/kg. Each dose of ISO was prepared by dissolving the compound in the aforementioned vehicle, and the injection volume for each individual mouse was precisely adjusted according to its most recent body weight to ensure accurate dosing. The treatments were continued for 18 consecutive days, during which tumor dimensions and body weights were recorded every three days throughout the experiment. At the end of the study, all mice were euthanized, and tumors as well as major organs (heart, liver, spleen, lungs, and kidneys) were collected for further analysis.

2.9 Serum biochemical analysis

At the conclusion of the 18-day treatment period, peripheral blood samples were collected from the anaesthetised mice to ensure animal welfare and minimise distress. The obtained blood was allowed to clot at room temperature for 30 minutes, followed by centrifugation at 3000 rpm for 15 minutes at 4 °C to isolate the serum. The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and

alkaline phosphatase (ALP), along with the concentration of serum creatinine (Cr), were quantified using commercial assay kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's protocols. All biochemical parameters were measured using an automated microplate reader, and the results were expressed as U/L for enzyme activities and $\mu\text{mol/L}$ for Cr concentration.

2.10 Limited proteolysis-mass spectrometry (LiP-MS)

LiP-MS analysis was performed by Shanghai Genechem Co., Ltd. (Shanghai, China). Protein was extracted from HCT116 cells through three cycles of freeze-thawing in liquid nitrogen. Protein concentration was determined using a bicinchoninic acid assay (BCA assay kit; Beyotime, China). Extracted proteins were incubated with 10 $\mu\text{mol/L}$ ISO for 10 minutes at room temperature. Subsequently, proteinase K (Sigma-Aldrich, USA) was added for limited proteolysis, followed by incubation at room temperature for 5 minutes. The reaction was terminated by heating at 98 °C for 3 minutes. Then, 4% sodium deoxycholate (Sangon, China) was added and the samples were heated again at 98 °C for 3 minutes. 40 mM tris (2-carboxyethyl) phosphine hydrochloride (Sigma-Aldrich, USA) and 100 mM iodoacetamide (Sigma-Aldrich, USA) were added. After incubation in a boiling water bath for 5 minutes, an appropriate amount of 250 mM hydroxypropyl- β -cyclodextrin (Sangon, China) and 100 μL of 100 mM ammonium bicarbonate (Sigma-Aldrich, USA) were introduced. Trypsin (Promega, USA) was then added to digest the samples overnight at 37 °C. Digestion was stopped by adding trifluoroacetic acid to reduce the pH below 2. The samples were separated using an Easy-nLC system (Thermo Fisher Scientific, USA) and analyzed with a Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific, USA).

2.11 Cellular thermal shift assay (CETSA)

For the in-cell CETSA experiment, cells were treated with or without ISO (40 $\mu\text{mol/L}$) for 3 hours. The cells were harvested and equally distributed into 12 aliquots in PBS, heated at designated temperatures (37°C–72 °C) for 3 minutes, and subsequently cooled on ice. The samples were lysed via three rapid freeze–thaw cycles using liquid nitrogen. Following ultracentrifugation at 20,000 g for 20 minutes at 4 °C, the supernatants were collected for Western blot analysis. Similarly, for the recombinant protein CETSA assay, purified human PPP2R1A proteins (wild-type and T144A) were incubated with or without ISO (40 $\mu\text{mol/L}$) for 1.5 hours at 25 °C. Subsequent procedures were identical to those described above, with the exception that the freeze–thaw cycles using liquid nitrogen were omitted, as the recombinant protein was already in a cell-free solution.

2.12 Molecular docking and Molecular dynamics simulation

ISO (CAS No. 6817-41-0) was docked to human PPP2R1A (PDB ID: 1B3U) using CB-Dock2, which added hydrogens, assigned charges and removed water molecules. Binding affinities were calculated, and the top complex was analyzed via PLIP and visualized with PyMOL v2.6. Molecular dynamics simulations were performed using GROMACS v2022.03 with AMBER99SB-ILDN and GAFF force fields. The complex was solvated in a dodecahedron box with the TIP3P water model, maintaining a minimum distance of 1.2 nm to the box edges, and neutralised with 0.154 M Na^+/Cl^- ions. Following steepest descent energy minimisation ,

the system was equilibrated in NVT and NPT ensembles for 100 ps each. A 100 ns production run was conducted at 300 K and 1 bar using a 2 fs time step, with long-range electrostatics treated via the Particle Mesh Ewald (PME) method. Binding free energy (ΔG_{total}) and per-residue decomposition were calculated using the MM/PBSA method via MMPBSA.py v.16.0. A total of 200 snapshots were extracted from the final 20 ns of the stable trajectory for analysis, where the total energy was determined by the sum of gas-phase molecular mechanics (ΔG_{gas}) and solvation free energy ($\Delta G_{\text{solvation}}$): $\Delta G_{\text{total}} = \Delta G_{\text{gas}} + \Delta G_{\text{solvation}}$.

2.13 Expression and purification of recombinant PPP2R1A protein and its mutants

The DNA coding sequences of human PPP2R1A and mutants were subcloned into the pET-28a expression vector. The plasmids were transformed into *E. coli* BL21 (DE3) cells (Solarbio, China) for recombinant protein expression. Bacterial cultures were grown until the OD₆₀₀ reached 0.6–0.8, followed by induction with 0.5 mM IPTG (Solarbio, China) at 20 °C for 16 hours with shaking at 220 rpm. After induction, the cells were harvested by centrifugation and lysed using a high-pressure homogenizer. The recombinant proteins were applied to Ni-NTA. Bound proteins were eluted with a stepwise imidazole gradient (0, 25, 50, and 500 mM). Target protein fractions were concentrated using a 35 kDa molecular weight cut-off ultrafiltration tube (Millipore, USA) and buffer-exchanged into PBS. The purity of the recombinant proteins was assessed via SDS-PAGE followed by staining with Coomassie Brilliant Blue (CBB; Beyotime, China).

2.14 Biolayer interferometry (BLI)

Proteins were biotinylated using biotin reagent (Genemore, China) and subsequently desalted using PD MiniTrapTM G-25 desalting columns (Genemore, China). The biotinylated proteins were diluted in PBS buffer containing 0.5% DMSO (MedChemExpress, China), 0.01% Tween-20 (Biofroxx, Germany), and 0.01% bovine serum albumin (BSA; Sigma, USA), and dispensed into black 96-well plates. Super streptavidin (SSA) high-binding biosensors (Fortebio, USA) were pre-hydrated in 200 μ L of sample diluent for 10 minutes, followed by loading with the biotinylated proteins. The biosensors were then incubated with various concentrations of ISO prepared in the same PBS-based assay buffer containing 0.5% DMSO, 0.01% Tween-20, and 0.01% BSA to record association and dissociation curves. Binding kinetics parameters were derived from the sensorgrams, and the equilibrium dissociation constant (KD) was calculated. All data were acquired and processed using the Octet analysis software.

2.15 shRNA transduction by lentivirus

Three shRNA sequences targeting human PPP2R1A were designed and individually cloned into the GV493 lentiviral vector (GeneChem, China), which contains GFP and puromycin resistance genes. To generate stable PPP2R1A knockdown cell lines, HCT116 and SW480 cells were transduced with the respective lentiviral constructs for 72 hours. Transductants were selected for 2 weeks using puromycin (MedChemExpress, China) at 1 μ g/mL for HCT116 and 2 μ g/mL for SW480. The target sequences of the three shRNAs used for PPP2R1A knockdown are listed below.

#1:GACTGTGTGAACGAGGTGATT.

#2: TGCCAATGTCCGCTTCAATG.

#3: CTGGCTTGTGGATCATGTATA.

2.16 High-content screening (HCS) for cell growth

After shRNA transduction for 72 hours, the CRC cells were seeded into 96-well plates at a density of 2×10^3 cells per well in 100 μ L of medium. Cell growth was monitored every day for 5 days using the MuviCyte Live Cell Image System.

2.17 Cell proliferation assay

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) assay. At 72 hours after shRNA transduction, CRC cells were seeded into 96-well plates at a density of 2×10^3 cells per well. After cell adhesion, 10 μ L of CCK-8 reagent was added to each well at 0, 24, 48, 72, and 96 hours post-seeding. The plates were incubated at 37 °C for 2 hours, and the absorbance at 450 nm was measured using a microplate reader.

2.18 Proteomics and phosphoproteomics

ISO-treated, PPP2R1A knockdown and control HCT116 cells were utilized for the mass spectrometry in PTM Bio (Hangzhou, China). Briefly, the cells were lysed with lysis buffer. The concentration of protein was determined with BCA kit according to manufacturer's instructions. Then, the protein samples underwent trypsin digestion and affinity enrichment. Finally, the treated samples were applied to Mass spectrometer adhering to the guidelines provided by the manufacturer. The data were processed using Spectronaut (v.18). For the identification phase, the False Discovery Rate (FDR) for proteins, peptides, and PSMs was strictly controlled at $< 1\%$. For the differential screening phase, significantly altered proteins and phosphorylation sites were identified using a cut-off of a fold change > 1.5 or < 0.67 , and a P -value < 0.05 .

2.19 Western blotting

Cells were lysed using WB/IP lysis buffer (Beyotime, China) supplemented with protease and phosphatase inhibitors (Beyotime, China). Protein concentration was determined with a bicinchoninic acid (BCA) assay. Equal amounts of protein (40 μ g per lane) were separated by SDS-PAGE and transferred onto PVDF membranes (Millipore, USA). The membranes were blocked with a protein-free rapid blocking buffer for 15 minutes at room temperature and then incubated overnight at 4 °C with primary antibodies. After washing, the membranes were incubated with HRP-conjugated secondary antibodies for 1 hour at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection kit (Beyotime, China) and quantified with ImageJ software. All the information of antibodies used in the experiments are listed in Supplementary table S1.

2.20 Data sources and bioinformatic analysis

To evaluate the protein expression profile of PPP2R1A in colorectal cancer (CRC), proteomic data were retrieved and analysed from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) via the UALCAN portal. The expression levels were quantified as Z-values, representing standard deviations from the median across the sample set. A total of 100 normal tissues and 97 primary tumour tissues were included in the

comparison. Statistical differences between the two groups were assessed using an unpaired two-tailed Student's t-test, with a *P*-value of less than 0.05 considered statistically significant.

2.21 Statistical analysis

Statistical analyses were executed using GraphPad Prism 9. Continuous data are presented as mean±SD. Pairwise comparisons were conducted using the independent-samples Student's t-test, while comparisons across three or more groups employed one-way ANOVA. A two-sided *p*-value < 0.05 was considered statistically significant.

3. Results

3.1 ISO inhibits the viability, proliferation, and migration of CRC cells

To investigate the anti-CRC effects of ISO, we evaluated its efficacy from different aspects *in vitro*. First, the CCK-8 assay was performed to examine the effects of various concentrations of ISO on the viability of NCM460, HCT116, and SW480 cells. The results showed that ISO treatment significantly reduced the viability of CRC cells in a dose-dependent manner, with lower toxicity in normal colonic epithelial cells. The half-maximal inhibitory concentrations (IC₅₀) of ISO in NCM460, HCT116, and SW480 cells were determined to be 97.46 µmol/L, 29.29 µmol/L, and 46.62 µmol/L, respectively, indicating its selective inhibitory effect on cell viability (Figure 1A). Based on these findings, concentrations of 10, 20, and 40 µmol/L were selected for subsequent experiments. Then, a colony formation assay was conducted to assess the effect of ISO on the proliferative capacity of CRC cells. After ISO treatment, both CRC cell lines exhibited a significant decrease in the number of colonies compared to the control group, demonstrating that ISO markedly suppresses cell proliferation (Figure 1B, C). Moreover, cell cycle progression revealed an increase in the proportion of cells in the G0/G1 phase and a decrease in the S phase population (Figure 1D, E), which was consistent with the results that ISO inhibited cell proliferation. To further elucidate the molecular mechanisms underlying this G0/G1 arrest, the expression of key cell cycle regulatory proteins was examined. Western blot analysis demonstrated that ISO treatment downregulated the levels of Cyclin D1 and CDK4, while upregulating the expression of P21 (Figure 1F-1I). These results suggest that ISO-induced G0/G1 phase arrest is mediated through the P21-dependent inhibition of the Cyclin D1/CDK4 complex. Furthermore, Transwell migration assays were carried out to evaluate the influence of ISO on cell migration. The results revealed that a considerable number of cells migrated to the lower chamber in the control group, whereas ISO treatment significantly reduced the number of migrated cells (Figure 1J, K). In summary, these results indicate that ISO (Figure 1L) significantly inhibits the viability and proliferation of CRC cells and also strongly impairs their migratory ability.

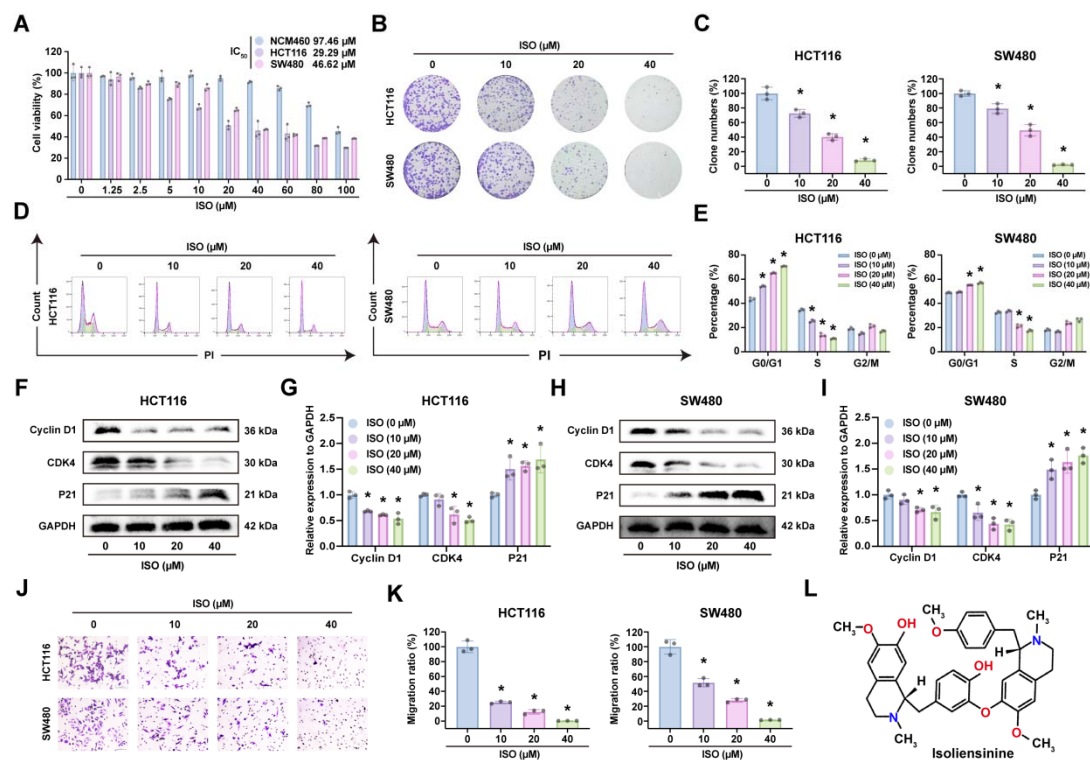


Fig. 1. ISO exerted inhibitory effects on the viability, proliferation, and migratory capacity of CRC cells. (A) Cell viability of NCM460, HCT116, and SW480 cells treated with different concentrations of ISO was assessed by CCK-8 assay. (B, C) Colony formation assays were performed to evaluate the proliferative capacity of HCT116 and SW480 cells after different concentrations of ISO treatment. (D, E) Cell cycle progression in HCT116 and SW480 cells was analyzed by PI staining and flow cytometry after different concentrations of ISO treatment. (F-I) Western blot analysis of cell cycle-related protein expression in HCT116 and SW480 cells following ISO treatment. (J, K) Cell migration ability was determined by Transwell assay in HCT116 and SW480 cells following different concentrations of ISO exposure. (L) Chemical structure of ISO. Data are presented as mean $\pm$ SD ($n=3$) in three independent experiments. * $P < 0.05$ versus control group.

3.2 ISO inhibits the proliferation of CRC cells by regulating apoptosis

To study the mechanism by which ISO inhibits CRC cell proliferation, we performed PI staining to assess cell apoptosis. Annexin V/PI staining displayed that ISO treatment significantly increased the percentage of apoptotic cells (Figure 2A, B). We further examined the activity of caspase-3 and caspase-9, which are closely associated with apoptosis, and found that ISO treatment markedly enhanced the activity of both caspases (Figure 2C, D). To further validate the mechanism of ISO-induced apoptosis in CRC cells, Western blot analysis showed that ISO treatment up-regulated the expression of Cleaved caspase-3 and Bax, while down-regulating the expression of Bcl-2 (Figure 2E-2H). Collectively, these results indicate that ISO inhibits the proliferation of CRC cells by modulating cell cycle progression and promoting apoptosis.

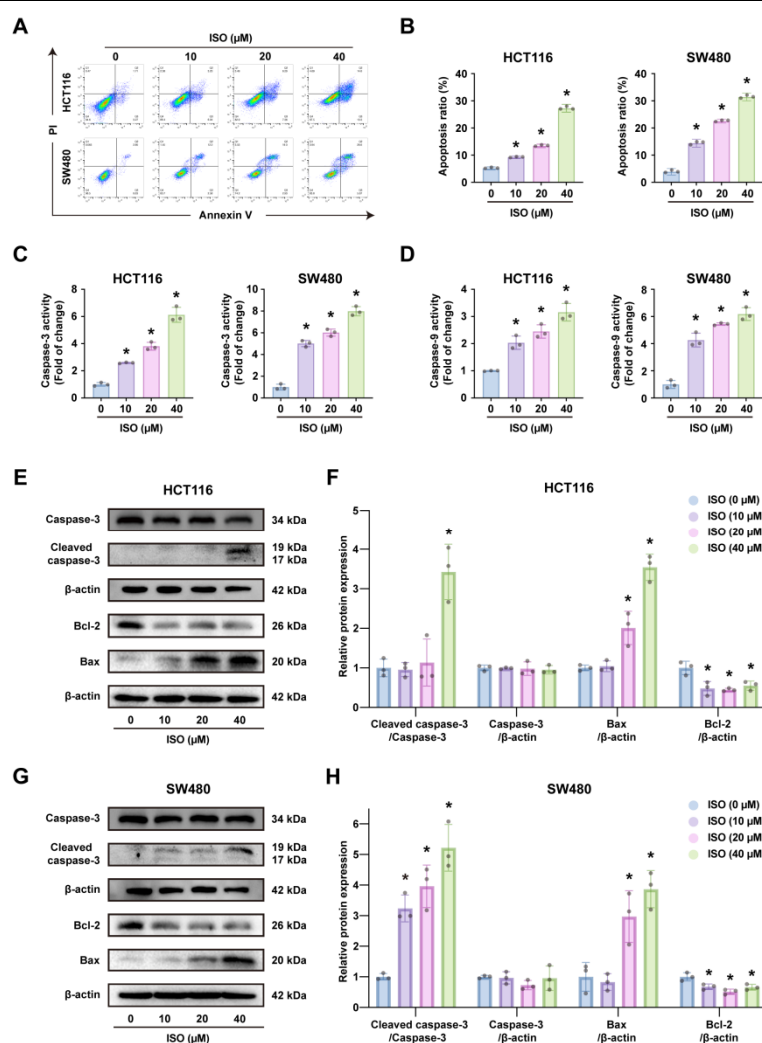


Fig. 2. ISO suppresses the proliferation of CRC cells via mediation of apoptosis. (A, B) Apoptosis was assessed by Annexin V-AbFlour™ 647/PI staining and flow cytometry. (C, D) Enzymatic activities of caspase-3 and caspase-9 were measured in ISO-treated cells. (E-H) Western blot analysis of apoptosis-related protein expression in HCT116 and SW480 cells following ISO treatment. Data are representative of three independent experiments and presented as mean $\pm$ SD. * P < 0.05 versus control group.

3.3 ISO inhibits the proliferation of CRC cells in vivo

For the purpose of evaluating the anti-CRC effect of ISO *in vivo*, a subcutaneous xenograft model was established in nude mice by inoculating HCT116 cells (Figure 3A). The results showed that ISO treatment significantly reduced tumor size and weight compared to the control group (Figure 3B, C). The tumor growth curves further confirmed that ISO treatment effectively suppressed the growth of CRC cells (Figure 3D). Immunohistochemical analysis displayed a significant decrease in Ki67-positive cells after ISO treatment, indicating that ISO inhibits the proliferation of CRC cells *in vivo* (Figure 3E, F). To assess potential toxic side effects of ISO, body weight changes were monitored throughout the treatment period. No significant differences in body weight were observed between the ISO-treated and control groups (Figure 3G). Moreover, no notable alterations were found in organ indices (Figure 3H). And these quantitative data were further supported by H&E staining, which suggested a favorable safety profile of ISO (Figure 3I). Consistent with these findings, serum biochemical analysis revealed that the levels of ALT, AST, ALP, and CR showed no statistical significance between the ISO-treated and control groups, indicating that ISO induces no detectable

hepatotoxicity or nephrotoxicity.(Figure 3J–3M). ISO treatment exhibited significant anti-tumor effects *in vivo* without apparent toxicity. These results are consistent with the *in vitro* findings, underscoring the potential of ISO as a promising therapeutic agent against CRC.

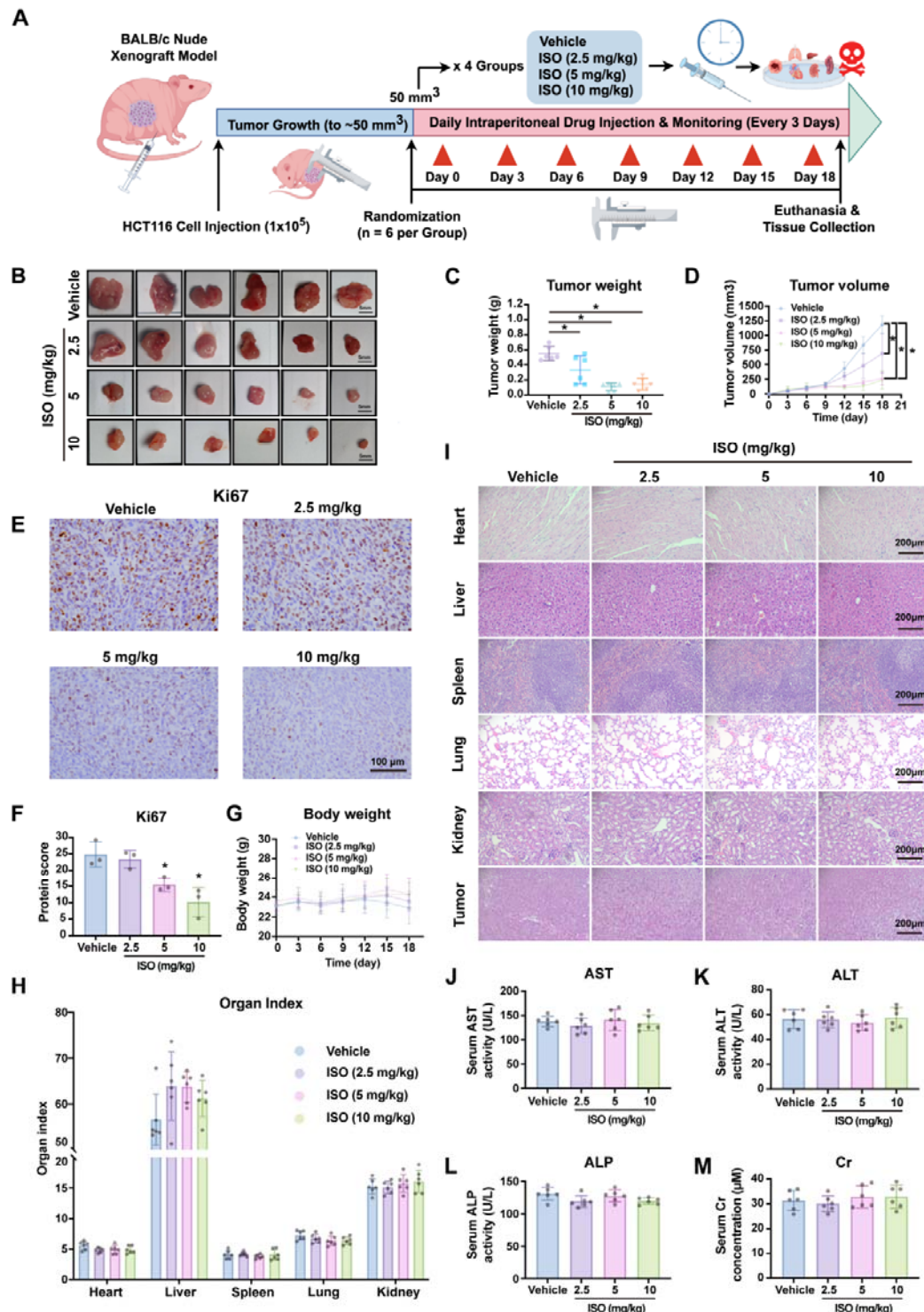


Fig. 3. ISO inhibits tumor growth in a mouse xenograft model. (A) Schematic representation of the xenograft model design and the 18-day ISO treatment schedule. (B) Representative images of dissected subcutaneous tumors from nude mice. (C) Weight of the isolated tumor tissues after different concentrations of ISO treatment ($n=6$). (D) Tumor growth curves during the course of ISO administration. (E, F) Representative images of tumor stained for Ki67 and its protein score following ISO treatment. (G) Body weight changes of nude mice during the treatment period. (H) HE staining of key organs to assess ISO-induced pathological changes. (I) No significant alterations were observed in the organ indices of major organs following ISO treatment. (J–M) Serum biochemical analysis of AST, ALT, ALP, and Cr levels. Data are presented as mean $\pm$ SD. * $P < 0.05$ versus vehicle group.

3.4 PPP2R1A is a potential binding target of ISO in CRC

In order to uncover the specific target through which ISO exerts its anti-CRC effects, we performed Lip-MS to isolate and identify ISO-bound proteins and identified PPP2R1A as a potential candidate by differential peptides analysis (Figure 4A and S1). CETSA confirmed that ISO treatment significantly enhanced the thermal stability of PPP2R1A in cells, indicating a potential direct binding interaction (Figure 4B, C). To further characterize the binding mode, we conducted a 100 ns molecular dynamics simulation. Multiple simulation metrics indicated stable binding between ISO and PPP2R1A (Figure S2). Analysis of the binding pocket revealed the formation of multiple hydrogen bonds, with a binding energy of -8.5 kcal/mol, suggesting a strong interaction (Figure 4D). Energy decomposition using the MM/GBSA method identified key residues contributing to the binding free energy, including THR144 (-2.947 kcal/mol), ARG182 (-1.711 kcal/mol), and LYS187 (-1.212 kcal/mol), which likely stabilize the ligand via hydrophobic interactions or hydrogen bonding with THR144 exhibiting the strongest binding affinity (Figure 4E). Guided by these results, we expressed and purified recombinant wild-type PPP2R1A and its corresponding point-mutant proteins at these three residues *in vitro* (Figure 4F). And the following BLI assays showed that ISO binds to wild-type PPP2R1A directly with a dissociation constant of 1.1×10^{-5} M, whereas a significantly increased K_D values for all mutants compared to the wild-type protein, indicating reduced binding affinity upon mutation. Notably, the T144A mutation resulted in the most pronounced reduction in binding affinity compared to other mutants, identifying it as the critical residue for ISO recognition (Figure 4G-4J). Consequently, CETSA showed that ISO increased the thermal stability of wild-type PPP2R1A but had a negligible effect on the T144A mutant, confirming the essential role of THR144 in ISO-mediated stabilisation (Figure 4K-4M). Finally, Western blot analysis revealed that ISO did not alter total PPP2R1A protein levels in a dose- or time-dependent manner, suggesting it modulates PPP2R1A function rather than its expression or degradation (Figure 4N-4Q). These experimental findings are consistent with the MD simulations and collectively demonstrate that residues THR144 are critical for the ISO-PPP2R1A interaction.

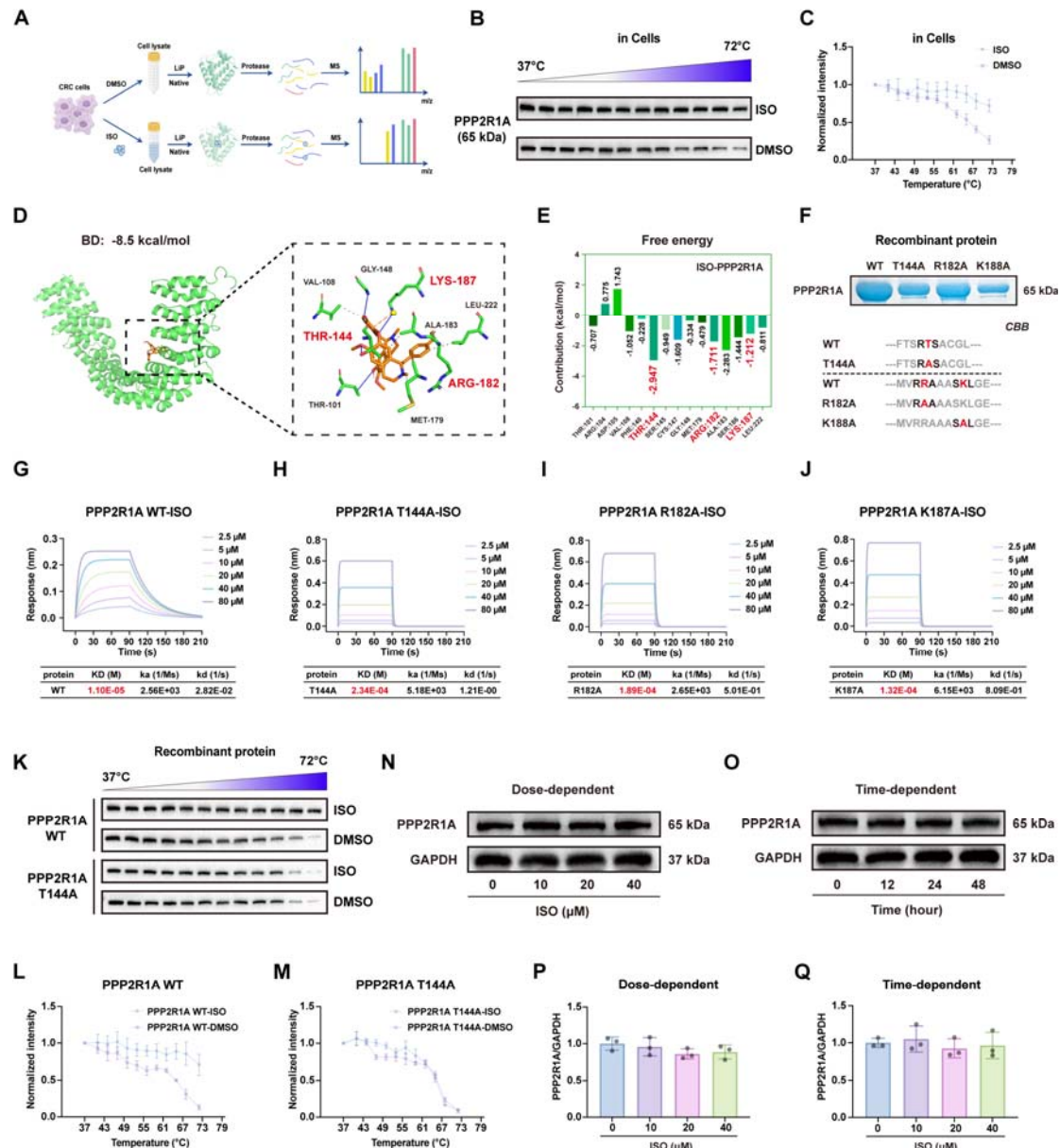


Fig. 4. Identification of PPP2R1A as a direct target of ISO. (A) Schematic diagram illustrating the principle and workflow of LiP-MS. (B-C) ISO (40 μ mol/L) treatment increases the thermal stability of PPP2R1A in living cells in a temperature-dependent manner, as determined by CETSA. (D) Interaction interface between ISO and PPP2R1A, with blue solid lines representing hydrogen bonds, gray dashed lines indicating hydrophobic interactions, and an orange dashed line denoting a π -cation interaction. (E) Amino acid residue free energy contribution plot for the ISO-PPP2R1A complex obtained from MD simulations. (F) SDS-PAGE analysis of purified recombinant protein. (G-J) BLI analysis of the interaction between ISO and recombinant PPP2R1A proteins (wild-type and three site-specific mutants). (K-M) CETSA evaluates the thermal stability of recombinant wild-type and T144A mutant PPP2R1A proteins. (N-Q) ISO treatment has no significant effect on total PPP2R1A protein levels in HCT116 cells across various concentrations and time points. Data are presented as mean $\pm$ SD. * $P < 0.05$ versus control group.

3.5 Knockdown of PPP2R1A significantly suppresses CRC proliferation

To assess the clinical relevance of PPP2R1A in colorectal cancer, we first analysed its protein expression levels using the CPTAC database. The results showed that PPP2R1A protein expression was significantly downregulated in primary CRC tumors compared to normal tissues ($P < 0.0001$) (Figure S3). This clinical observation suggests that CRC cells may have a specific dependency on the remaining PPP2R1A pool for maintaining malignant growth. To verify the role of PPP2R1A in CRC development, we knocked down

PPP2R1A expression in HCT116 and SW480 cells. The knockdown efficiency was confirmed in both cell lines (Figure S4). The effect of PPP2R1A knockdown on cell growth was assessed using high-content imaging analysis, which revealed a significant reduction in the number of GFP-positive cells in both HCT116 and SW480 after PPP2R1A depletion (Figure 5A). We further evaluated cell viability using the CCK-8 assay. The results demonstrated that PPP2R1A knockdown reduced cell viability at multiple time points after reseeding (Figure 5B). Additionally, colony formation assays showed that sh-PPP2R1A-transfected cells formed significantly fewer colonies compared to sh-NC controls, indicating impaired proliferative capacity (Figure 5C, D). Furthermore, cell cycle was detected by PI staining and flow cytometry. PPP2R1A knockdown increased the proportion of HCT116 cells in G0/G1 phase while reducing the S phase population. In SW480 cells, a decrease in S phase cells was observed (Figure 5E, F). These results suggest that PPP2R1A depletion inhibits CRC cell growth by perturbing cell cycle progression. Taken together, these findings indicate that PPP2R1A plays a critical role in regulating CRC proliferation.

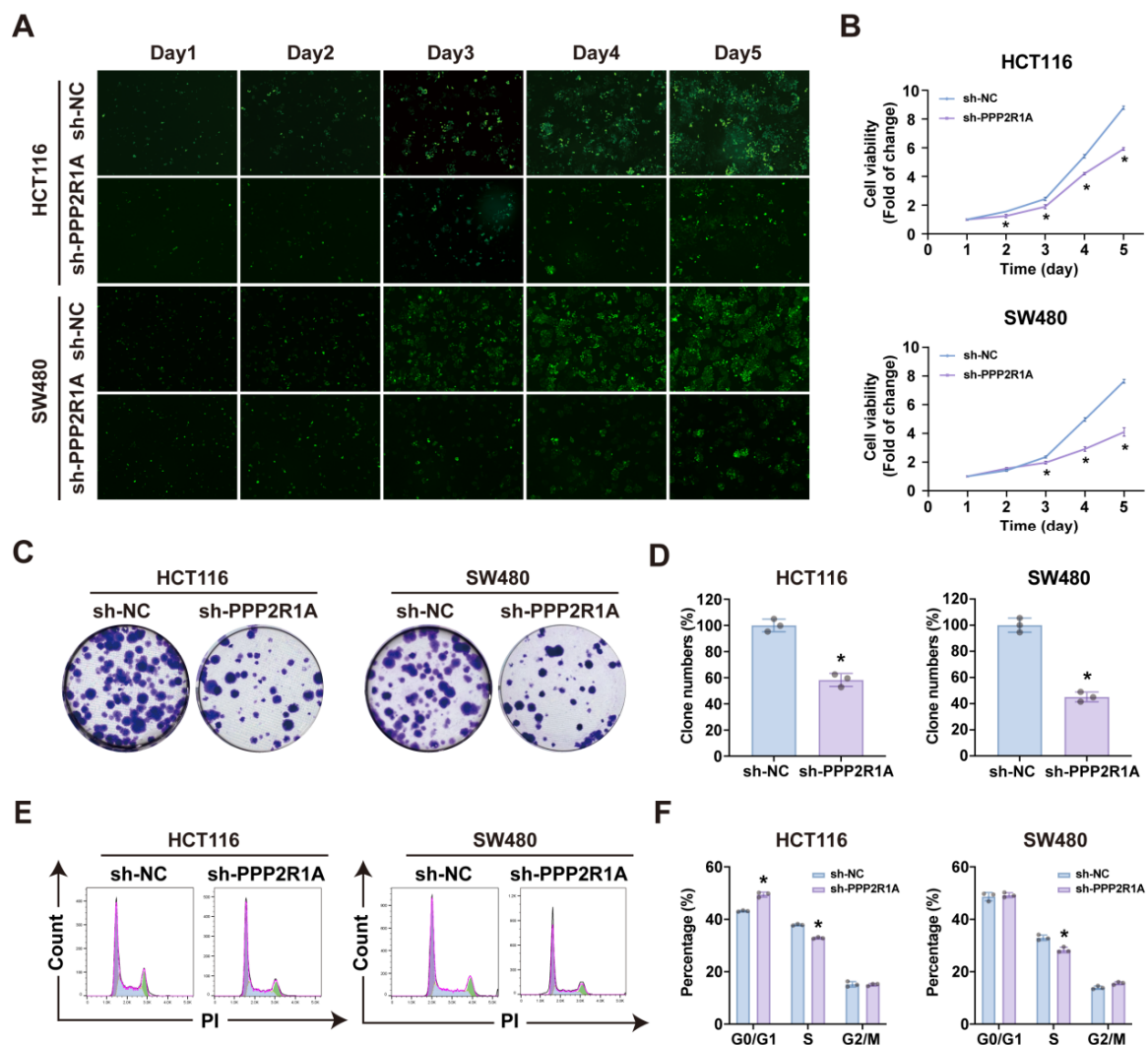


Fig. 5. The depletion of PPP2R1A results in a significant suppression of CRC proliferation. (A) Representative images of CRC cell growth after lentivirus-mediated PPP2R1A knockdown were captured using a high-content imaging system. (B) The effect of PPP2R1A knockdown on cell viability was assessed by CCK-8 assay. (C) Colony formation assay was performed to evaluate the colony-forming capacity of PPP2R1A-knockdown CRC cells. (D) Cell cycle distribution was analyzed by PI staining and flow cytometry following PPP2R1A knockdown. Data are presented as mean $\pm$ SD (n=3) in three independent experiments. * $P < 0.05$ versus sh-NC group.

3.6 The anti-CRC effects of ISO depend on PPP2R1A

Given PPP2R1A's anti-CRC role, we hypothesized that ISO might exert its efficacy through binding to PPP2R1A. Using CCK-8 assay, we found that knockdown of PPP2R1A in both HCT116 and SW480 cells diminished sensitivity to ISO treatment (Figure 6A). Transwell migration assays confirmed that PPP2R1A knockdown suppressed the migratory capacity of CRC cells and abolished the inhibitory effect of ISO (Figure 6B, C). Annexin V staining followed by flow cytometry revealed that PPP2R1A knockdown in CRC cells rendered them resistant to ISO-induced apoptosis (Figure 6D, E). Consistent with this, increased activity of caspase-3 and caspase-9 was observed after PPP2R1A knockdown. However, compared to ISO-treated wild-type cells, additional ISO treatment in the PPP2R1A knockdown cells did not result in a further increase in caspase activity, but led to a further reduction (Figure 6F, G). Overall, these results demonstrate that PPP2R1A is essential for mediating the anti-tumor effects of ISO in CRC.

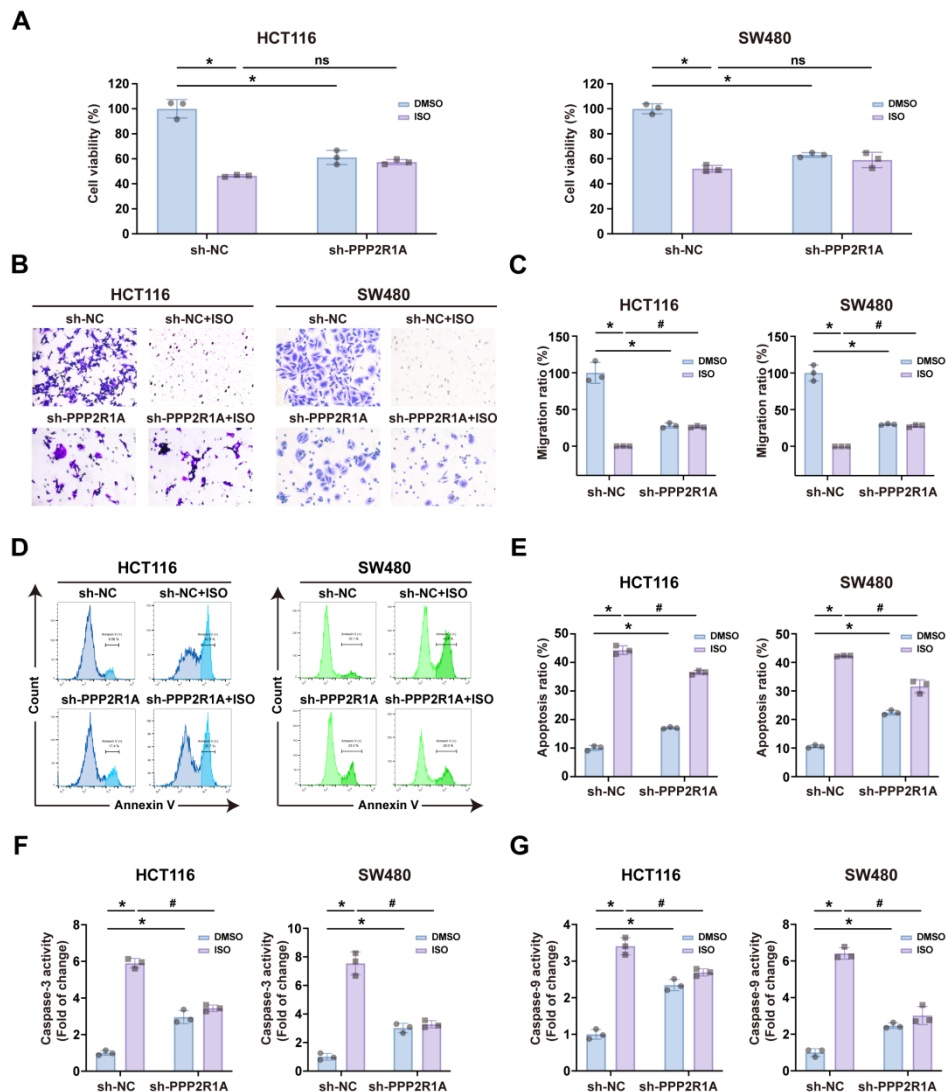


Fig. 6. PPP2R1A knockdown attenuates the anti-tumor effects of ISO in CRC cells. CRC cells were treated with ISO (40 $\mu\text{mol/L}$) for 48 hours following PPP2R1A knockdown (A) Cell viability was assessed by CCK-8 assay to evaluate the impact of PPP2R1A knockdown on ISO-induced cytotoxicity. (B, C) Cell migration was determined by Transwell assay to examine the effect of PPP2R1A knockdown on ISO-mediated migration inhibition. (D-G) Apoptosis was analyzed by Annexin V-AbFlourTM 647 staining and flow cytometry and enzymatic activities of caspase-3 and caspase-9 to investigate the role of PPP2R1A in ISO-triggered apoptosis. Data are presented as mean $\pm$ SD ($n=3$) in three independent experiments.

* $P < 0.05$ versus sh-NC group, # $P < 0.05$ versus ISO-treated sh-NC group, ns means no significance.

3.7 ISO suppresses CRC by mediating PPP2R1A-AMPK-mTOR axis

PPP2R1A can activate diverse downstream pathways by interacting with different regulatory subunits. To determine which pathway is modulated upon ISO binding to PPP2R1A and contributes to its anti-CRC effects, we performed proteomic and phosphoproteomic analyses of HCT116 cells treated with ISO and those with PPP2R1A knockdown (Figure 7A, B). ISO treatment resulted in 2,104 differentially expressed proteins and 2,221 differentially phosphorylated sites, while PPP2R1A knockdown led to 549 differentially expressed proteins and 1,215 altered phosphorylation sites. Through intersection analysis, we identified 245 overlapping differentially expressed proteins and 965 common differentially phosphorylated proteins (Figure 7C). Subsequent KEGG pathway enrichment analysis of these overlapping entries revealed significant enrichment of the AMPK signaling pathway in both differential protein and phosphoprotein datasets (Figure 7D). Based on these bioinformatic findings, we proposed a mechanistic model wherein ISO targets PPP2R1A to trigger the AMPK-mTOR signalling cascade, while the knock-down of PPP2R1A is expected to attenuate this drug-induced response. (Figure 7E). Western blot validation further demonstrated that both ISO treatment and PPP2R1A knockdown increased the ratio of p-AMPK to total AMPK and reduced the ratio of p-mTOR to total mTOR, without affecting total AMPK and mTOR expression, indicating activation of AMPK and inhibition of mTOR. Moreover, PPP2R1A knockdown abrogated AMPK activation and mTOR inhibition induced by ISO (Figure 7D-7M). These findings indicate that ISO binding to PPP2R1A activates the AMPK/mTOR axis, thereby inhibiting CRC proliferation.

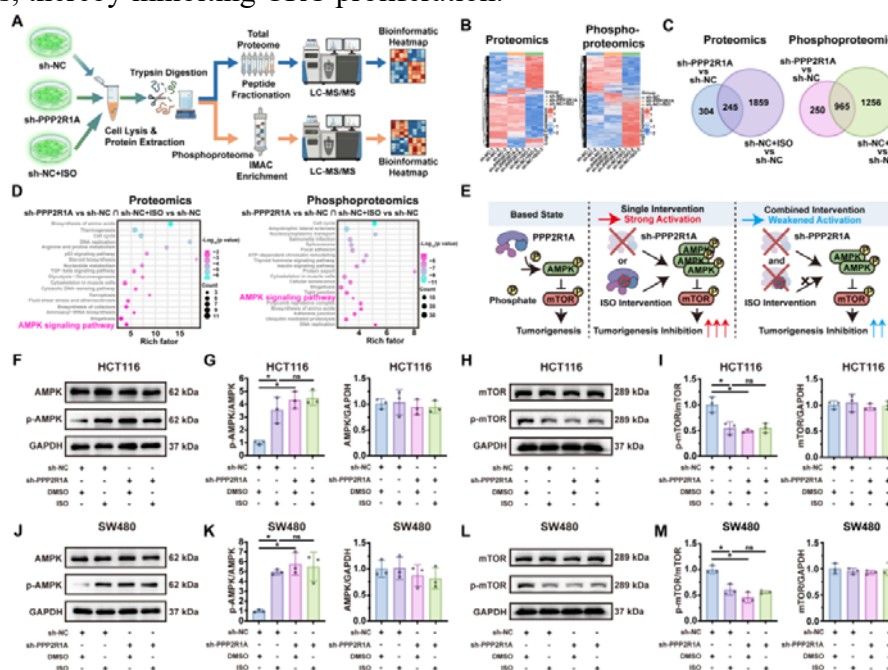


Fig. 7. ISO exerts its anti-tumor effects against CRC via the PPP2R1A-AMPK signaling axis. (A) Schematic workflow of the proteomic and phosphoproteomic profiling in HCT116 cells. (B) Cluster analysis of differentially expressed proteins and differentially expressed phosphoproteins identified from proteomic and phosphoproteomic profiling. (C) Venn diagrams showing the overlap of common differentially expressed proteins and differentially expressed phosphoproteins across the sh-PPP2R1A vs sh-NC and sh-NC+ISO and sh-NC. (D) KEGG pathway enrichment analysis of overlapping common differentially expressed proteins and differentially expressed phosphoproteins. (E) Mechanistic model illustrating that ISO-induced anti-tumour activity is mediated via the PPP2R1A-AMPK-mTOR axis. (F-M) Western blot analysis verifying the phosphorylation status of AMPK and mTOR in HCT116 and SW480 cells following ISO (40 μ mol/L) treatment and PPP2R1A knockdown. Data are presented as mean $\pm$ SD (n=3) in three independent experiments. * P < 0.05 versus sh-NC group, ns means no significance.

4. Discussions

Globally, CRC constitutes approximately one in ten newly diagnosed cancer cases annually, and its prevalence continues to climb^[24]. This disease imposes significant physical suffering and psychological burden on patients. Current treatment strategies primarily include surgical resection, radiotherapy, chemotherapy, targeted therapy, and immunotherapy; however, each strategy has its own limitations. Therefore, developing novel therapeutic approaches is of great importance. Alkaloids, as natural products, have demonstrated remarkable efficacy against various types of tumors and are considered a promising alternative strategy for cancer treatment^[25]. In our study, we identified ISO, a natural bisbenzylisoquinoline alkaloid, which exhibited anti-CRC effects both *in vitro* and *in vivo* by inhibiting tumor proliferation and migration, inducing cell cycle arrest, and promoting apoptosis. Furthermore, using Lip-MS technology, we identified PPP2R1A as a direct binding target of ISO. This interaction was validated via BLI and CETSA. Molecular dynamics simulations elucidated the binding mode between ISO and PPP2R1A, which was further confirmed through binding site mutation experiments. Genetic knockdown of PPP2R1A and subsequent drug rescue assays affirmed that ISO exerts its anti-CRC activity by directly binding to PPP2R1A. Integrated proteomic and phosphoproteomic data demonstrated that ISO's binding to PPP2R1A ultimately modulates the AMPK signaling pathway. Our findings provide a mechanistic foundation for the potential therapeutic application of ISO in CRC treatment.

ISO has been demonstrated to possess various biological activities, including antioxidant, anti-inflammatory, and anticancer effects^[14]. It has also been reported to be effective in the treatment of type 2 diabetes, cognitive dysfunction, osteoarthritis, and several types of tumors[26-30]. However, its role in the treatment of CRC remains largely unexplored. In our study, we demonstrated that isolieninine inhibited the proliferation of CRC cells, as evidenced by cytotoxicity measurement, colony formation and cell cycle detection. Transwell migration results confirmed that isolieninine suppressed the migratory capacity of CRC cells. Furthermore, Apoptosis induction by isolieninine was verified through Annexin V/PI staining, caspase-3/9 activity assays, and an increased Cleaved caspase-3/caspase-3 ratio. *In vivo*, we established a subcutaneous xenograft model of CRC in mice. Tumor growth curves and final tumor weights indicated that isolieninine significantly inhibits tumor formation. Moreover, isolieninine exhibited a favorable safety profile, as supported by body weight monitoring and vital organ indices. Collectively, isolieninine demonstrated dose-dependent anti-CRC effects both *in vivo* and *in vitro*.

However, unlike its well-established therapeutic efficacy in other cancer types such as breast cancer and gastric cancer, ISO has only been reported to exert anti-tumor effects in colorectal CRC when used in combination therapies^[21,22]. Therefore, investigating the inhibitory effect of ISO as a monotherapy in CRC is of particular significance. Our results demonstrate that ISO alone not only exerts significant antitumor effects against CRC but also reveal the identification of its direct binding target-PPP2R1A using Lip-MS technology *in vivo*. Moreover, the direct interaction between ISO and PPP2R1A was confirmed using BLI and CETSA. PPP2R1A serves as the scaffold subunit of PP2A, playing an essential role in assembling the regulatory and

catalytic subunits into the holoenzyme^[31]. PP2A acts as a crucial “molecular switch” that precisely modulates numerous core cellular processes by dephosphorylating specific proteins^[32]. To further elucidate the binding mode between PPP2R1A and ISO, we performed molecular dynamics simulations, which revealed that ISO interacted with PPP2R1A via multiple hydrogen bonds and salt bridges. Mutation of three key residues—threonine 144, arginine 182, and lysine 187—significantly reduced the binding affinity. Notably, arginine 182 has been demonstrated to be critical for PP2A activity in other cancers^[33,34]. These results strongly suggest that ISO disrupts PP2A function through allosteric inhibition.

In cancer, dysfunction of PPP2R1A directly leads to impaired activity of the PP2A holoenzyme, preventing it from concisely dephosphorylating oncoproteins, thereby promoting uncontrolled cell proliferation and tumorigenesis^[35]. In our experiments, knockdown of PPP2R1A in CRC cell lines resulted in suppressed proliferation, as demonstrated by CCK-8 assays, colony formation assays, high-content imaging of cell proliferation and cell cycle test. These findings are consistent with observations of PPP2RR1A in other cancers^[36,37]. Furthermore, comparison between ISO-treated wild-type cells and those treated with both PPP2R1A knockout and ISO showed significant differences in proliferation, migration, or apoptosis, suggesting that ISO exerts its anti-tumor effects specifically through targeting PPP2R1A. Integrated proteomic and phosphoproteomic analyses revealed that the AMPK pathway was significantly enriched among the differentially expressed proteins in both PPP2R1A-knockout and ISO-treated groups. Western blot analysis confirmed activation of the AMPK pathway, which aligns with previous reports indicating that AMPK activation suppresses CRC progression^[38,39]. AMPK has been established as a downstream target regulated by PP2A, which, as a phosphatase, directly modulates the phosphorylation levels of AMPK to control the activation status of the AMPK signaling pathway^[40,41]. The natural compound ISO binds to PPP2R1A, a regulatory subunit of PP2A, thereby inhibiting PP2A activity and subsequently activating the AMPK pathway, ultimately exerting a suppressive effect on CRC. However, we have not yet validated pharmacological effects of the PPP2R1A binding-deficient mutants in response to ISO treatment *in vivo*, including assessments of apoptosis, migration, proliferation, and AMPK pathway activation. Further experiments are warranted to elucidate how ISO exerts its anti-tumor effects *in vivo* through binding to PPP2R1A.

5. Conclusion

In our study, we found that ISO effectively ameliorated CRC progression both *in vivo* and *in vitro*. The anti-tumor activity of ISO primarily depends on its binding to PP2R1A and subsequent activation of the AMPK pathway. These findings underscore the therapeutic rationale for employing ISO in the treatment of CRC.

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Conflict of Interest

The authors declare no conflict interest.

References

- [1] X. Zhao, H. Feng, Y. Wang, et al., Septin4 promotes cell death in human colon cancer cells by interacting with BAX, *International Journal of Biological Sciences* 16 (2020) 1917-1928.<http://doi.org/10.7150/ijbs.44429>.
- [2] Z. Cao, J. Lin, G. Fu, et al., An integrated bioinformatic investigation of mitochondrial energy metabolism genes in colon adenocarcinoma followed by preliminary validation of CPT2 in tumor immune infiltration, *Frontiers in Immunology* Volume 13 - 2022 (2022).<http://doi.org/10.3389/fimmu.2022.959967>.
- [3] S. Wei, J. Chen, Y. Huang, et al., Identification of hub genes and construction of transcriptional regulatory network for the progression of colon adenocarcinoma hub genes and TF regulatory network of colon adenocarcinoma, *Journal of Cellular Physiology* 235 (2020) 2037-2048.<http://doi.org/https://doi.org/10.1002/jcp.29067>.
- [4] A. Farhana, A.E. Koh, S. Kothandan, et al., Treatment of HT29 Human Colorectal Cancer Cell Line with Nanocarrier-Encapsulated Camptothecin Reveals Histone Modifier Genes in the Wnt Signaling Pathway as Important Molecular Cues for Colon Cancer Targeting, *International Journal of Molecular Sciences* 22 (2021).<http://doi.org/10.3390/ijms222212286>.
- [5] J. Li, S. Qin, R.-H. Xu, et al., Effect of Fruquintinib vs Placebo on Overall Survival in Patients With Previously Treated Metastatic Colorectal Cancer: The FRESCO Randomized Clinical Trial, *JAMA* 319 (2018) 2486-2496.<http://doi.org/10.1001/jama.2018.7855>.
- [6] David L. Brautigan, C. Farrington, G. Narla, Targeting protein phosphatase PP2A for cancer therapy: development of allosteric pharmaceutical agents, *Clinical Science* 135 (2021) 1545-1556.<http://doi.org/10.1042/CS20201367>.
- [7] S. Hao, H. Song, W. Zhang, et al., Protein phosphatase 2A inhibition enhances radiation sensitivity and reduces tumor growth in chordoma, *Neuro-Oncology* 20 (2018) 799-809.<http://doi.org/10.1093/neuonc/nox241>.
- [8] Y. Wang, G. Chiappetta, R. Guérois, et al., PPP2R1A regulates migration persistence through the NHSL1-containing WAVE Shell Complex, *Nature Communications* 14 (2023) 3541.<http://doi.org/10.1038/s41467-023-39276-w>.
- [9] D.C. Nagendra, J. Burke Iii, G.L. Maxwell, et al., PPP2R1A mutations are common in the serous type of endometrial cancer, *Molecular Carcinogenesis* 51 (2012) 826-831.<http://doi.org/https://doi.org/10.1002/mc.20850>.
- [10] M. Shih Ie, P.K. Panuganti, K.T. Kuo, et al., Somatic mutations of PPP2R1A in ovarian and uterine carcinomas, *Am J Pathol* 178 (2011) 1442-1447.<http://doi.org/10.1016/j.ajpath.2011.01.009>.
- [11] M. Toda-Ishii, K. Akaike, Y. Suehara, et al., Clinicopathological effects of protein phosphatase 2, regulatory subunit A, alpha mutations in gastrointestinal stromal tumors, *Mod Pathol* 29 (2016) 1424-1432.<http://doi.org/10.1038/modpathol.2016.138>.
- [12] C. Dai, X. Zhang, D. Xie, et al., Targeting PP2A activates AMPK signaling to inhibit colorectal cancer cells, *Oncotarget* 8 (2017) 95810-95823.<http://doi.org/10.18632/oncotarget.21336>.
- [13] Q. Zuo, L. Liao, Z.-T. Yao, et al., Targeting PP2A with lomitapide suppresses colorectal tumorigenesis through the activation of AMPK/Beclin1-mediated autophagy, *Cancer Letters* 521 (2021) 281-293.<http://doi.org/https://doi.org/10.1016/j.canlet.2021.09.010>.
- [14] Y. Cheng, H.-L. Li, Z.-W. Zhou, et al., Isoliensinine: A Natural Compound with “Drug-Like” Potential, *Frontiers in Pharmacology* Volume 12 - 2021 (2021).<http://doi.org/10.3389/fphar.2021.630385>.
- [15] X. Zhang, X. Wang, T. Wu, et al., Isoliensinine induces apoptosis in triple-negative human breast cancer cells through ROS generation and p38 MAPK/JNK activation, *Scientific Reports* 5 (2015) 12579.<http://doi.org/10.1038/srep12579>.
- [16] J. Hu, S. Dai, M. Yuan, et al., Isoliensinine suppressed gastric cancer cell proliferation and migration by targeting TGFBR1 to regulate TGF- β -smad signaling pathways, *Frontiers in Pharmacology* Volume 15 - 2024 (2024).<http://doi.org/10.3389/fphar.2024.1438161>.

- [17] H.L. Li, Y. Cheng, Z.W. Zhou, et al., Isoliensinine induces cervical cancer cell cycle arrest and apoptosis by inhibiting the AKT/GSK3 α pathway, *Oncol Lett* 23 (2022) 8.<http://doi.org/10.3892/ol.2021.13126>.
- [18] C.M. Liu, Z. Wu, B. Pan, et al., The antiandrogenic effect of neferine, liensinine, and isoliensinine by inhibiting 5- α -reductase and androgen receptor expression via PI3K/AKT signaling pathway in prostate cancer, *Pharmazie* 76 (2021) 225-231.<http://doi.org/10.1691/ph.2021.1301>.
- [19] C. Chen, C. Shu, D. Shen, et al., Isoliensinine exerts antitumor effects in lung adenocarcinoma by inhibiting APEX1-driven ROS production, *Frontiers in Pharmacology Volume 16 - 2025* (2025).<http://doi.org/10.3389/fphar.2025.1555802>.
- [20] X. Xu, W. Li, Y. Zhou, et al., Induction of UBQLN1-mediated PGC1 α stability by isoliensinine overcame hypoxia-induced resistance in liver cancer cells, *BioFactors* 51 (2025) e70008.<http://doi.org/https://doi.org/10.1002/biof.70008>.
- [21] P. Manogaran, N.M. Beeraka, C.-Y. Huang, et al., Neferine and isoliensinine enhance ‘intracellular uptake of cisplatin’ and induce ‘ROS-mediated apoptosis’ in colorectal cancer cells – A comparative study, *Food and Chemical Toxicology* 132 (2019) 110652.<http://doi.org/https://doi.org/10.1016/j.fct.2019.110652>.
- [22] P. Manogaran, B. Somasundaram, V.P. Viswanadha, Reversal of cisplatin resistance by neferine/isoliensinine and their combinatorial regimens with cisplatin-induced apoptosis in cisplatin-resistant colon cancer stem cells (CSCs), *Journal of Biochemical and Molecular Toxicology* 36 (2022) e22967.<http://doi.org/https://doi.org/10.1002/jbt.22967>.
- [23] P. Manogaran, A. Anandan, V. Vijaya Padma, Isoliensinine augments the therapeutic potential of paclitaxel in multidrug-resistant colon cancer stem cells and induced mitochondria-mediated cell death, *Journal of Biochemical and Molecular Toxicology* 37 (2023) e23395.<http://doi.org/https://doi.org/10.1002/jbt.23395>.
- [24] E. Dekker, P.J. Tanis, J.L.A. Vleugels, et al., Colorectal cancer, *The Lancet* 394 (2019) 1467-1480.[http://doi.org/https://doi.org/10.1016/S0140-6736\(19\)32319-0](http://doi.org/https://doi.org/10.1016/S0140-6736(19)32319-0).
- [25] K. Olofinson, H. Abrahamse, B.P. George, Therapeutic Role of Alkaloids and Alkaloid Derivatives in Cancer Management, *Molecules* 28 (2023).<http://doi.org/10.3390/molecules28145578>.
- [26] G. Shu, L. Yue, W. Zhao, et al., Isoliensinine, a Bioactive Alkaloid Derived from Embryos of *Nelumbo nucifera*, Induces Hepatocellular Carcinoma Cell Apoptosis through Suppression of NF- κ B Signaling, *Journal of Agricultural and Food Chemistry* 63 (2015) 8793-8803.<http://doi.org/10.1021/acs.jafc.5b02993>.
- [27] X. Yang, M. Huang, J. Yang, et al., Activity of Isoliensinine in Improving the Symptoms of Type 2 Diabetic Mice via Activation of AMP-Activated Kinase and Regulation of PPAR γ , *Journal of Agricultural and Food Chemistry* 65 (2017) 7168-7178.<http://doi.org/10.1021/acs.jafc.7b01964>.
- [28] Z. Zhang, J. Ma, Y. Yi, et al., Isoliensinine suppresses chondrocytes pyroptosis against osteoarthritis via the MAPK/NF- κ B signaling pathway, *International Immunopharmacology* 143 (2024) 113589.<http://doi.org/https://doi.org/10.1016/j.intimp.2024.113589>.
- [29] J.-Q. Li, X.-H. Ma, H. Dai, et al., Isoliensinine ameliorates cognitive dysfunction in A β 13/D-gal-induced Alzheimer’s disease-like mice by inhibiting the calcium signaling pathway, *Journal of Ethnopharmacology* 344 (2025) 119567.<http://doi.org/https://doi.org/10.1016/j.jep.2025.119567>.
- [30] M.-J. Wu, Y.-T. Chang, T.-Y. Chuang, et al., Targeting mitophagy using isoliensinine as a therapeutic strategy for renal cell carcinoma treatment, *Free Radical Biology and Medicine* 233 (2025) 132-147.<http://doi.org/https://doi.org/10.1016/j.freeradbiomed.2025.03.037>.
- [31] H. Ronk, J.S. Rosenblum, T. Kung, et al., Targeting PP2A for cancer therapeutic modulation, *Cancer Biol Med* 19 (2022) 1428-1439.<http://doi.org/10.20892/j.issn.2095-3941.2022.0330>.
- [32] X. Wu, S. Huang, J. Gao, et al., AKR1B10 dictates c-Myc stability to suppress colorectal cancer metastasis via PP2A nitration, *Science Advances* 11 eadv6937.<http://doi.org/10.1126/sciadv.adv6937>.
- [33] D. Haesen, L. Abbasi Asbagh, R. Derua, et al., Recurrent PPP2R1A Mutations in Uterine Cancer Act through a Dominant-Negative Mechanism to Promote Malignant Cell Growth, *Cancer Research* 76 (2016) 5719-5731.<http://doi.org/10.1158/0008-5472.CAN-15-3342>.
- [34] J. Stewart, D.B. Krastev, R. Brough, et al., PPP2R1A mutations cause ATR inhibitor sensitivity in ovarian clear cell carcinoma, *Oncogene* 44 (2025) 618-629.<http://doi.org/10.1038/s41388-024-03265-0>.

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- [35] J. Sangodkar, C.C. Farrington, K. McClinch, et al., All roads lead to PP2A: exploiting the therapeutic potential of this phosphatase, *The FEBS Journal* 283 (2016) 1004-1024.<http://doi.org/https://doi.org/10.1111/febs.13573>.
- [36] Q. Kang, D. Ma, P. Zhao, et al., BRG1 promotes progression of B-cell acute lymphoblastic leukemia by disrupting PPP2R1A transcription, *Cell Death & Disease* 15 (2024) 621.<http://doi.org/10.1038/s41419-024-06996-w>.
- [37] G. Cheng, L. Wusiman, D. Song, et al., Silencing PPP2R1A inhibits the progression of gastric cancer cells, *Journal of Cancer Research and Clinical Oncology* 151 (2025) 142.<http://doi.org/10.1007/s00432-025-06177-y>.
- [38] H. Yan, R. Talty, C.H. Johnson, Targeting ferroptosis to treat colorectal cancer, *Trends in Cell Biology* 33 (2023) 185-188.<http://doi.org/https://doi.org/10.1016/j.tcb.2022.11.003>.
- [39] D. Wang, Y. Zhou, L. Hua, et al., The role of the natural compound naringenin in AMPK-mitochondria modulation and colorectal cancer inhibition, *Phytomedicine* 131 (2024) 155786.<http://doi.org/https://doi.org/10.1016/j.phymed.2024.155786>.
- [40] W.-M. Han, Y.-X. Hong, G.-S. Xiao, et al., NMDARs activation regulates endothelial ferroptosis via the PP2A-AMPK-HMGB1 axis, *Cell Death Discovery* 10 (2024) 34.<http://doi.org/10.1038/s41420-023-01794-3>.
- [41] J. Xie, S. Xiong, J. Yu, et al., *Prunella vulgaris* polyphenols alleviate liver injury-uveitis comorbidity by regulating acylcarnitine via the S100A9-PP2A-AMPK pathway, *Phytomedicine* 141 (2025) 156675.<http://doi.org/https://doi.org/10.1016/j.phymed.2025.156675>.