

Original Article

Natural product 2-dihydroailanthone suppresses colorectal cancer via targeting integrin $\alpha 3$

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Abstract

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, necessitating the discovery of novel therapeutic agents. Here, we report a natural small molecule, 2-dihydroailanthone (2-DAIL), as a promising candidate for CRC treatment. First, our results demonstrate that 2-DAIL exhibits significant anti-CRC activity *in vitro* and *in vivo*. Then, we find that 2-DAIL directly binds to integrin alpha-3 (ITGA3) revealed by stable isotope labeling by amino acids in cell culture coupled with thermal proteome profiling (SILAC-TPP). Additionally, the RNA sequencing data obtained from CRC cells and tumor tissues suggest that 2-DAIL blocks the PI3K/AKT signaling pathway mediated by ITGA3 inhibition. Collectively, 2-DAIL exerts its anti-CRC effects, at least partially, by binding to and inhibiting the function of ITGA3, thereby blocking the activation of the PI3K/AKT signaling pathway, which leads to CRC cell growth inhibition. Our study provides a promising drug candidate for the treatment of CRC and suggests the potential of 2-DAIL in treating other diseases linked to ITGA3 dysfunction.

Key words 2-dihydroailanthone, colorectal cancer, ITGA3 inhibitor, PI3K/AKT pathway

Introduction

Colorectal cancer (CRC), one of the malignancies with the highest incidence and mortality rates globally, faces significant challenges in clinical management due to its complex pathogenesis and acquired chemoresistance [1,2]. Integrin receptors, as pivotal regulators of tumor cell-matrix interactions, orchestrate critical biological processes, including extracellular matrix signaling, transduction, and cytoskeletal remodeling, also playing a significant role in CRC [3,4]. Current integrin-targeted therapeutics in clinical development face substantial limitations, with most candidates remaining in early-stage clinical trials and exhibiting suboptimal therapeutic efficacy [5]. This therapeutic impasse underscores the urgent need for novel agents.

Terpenoids are a large family of plant-derived natural products

characterized by a diverse array of unique chemical structures and prominent biological activities [6–8]. 2-Dihydroailanthone (2-DAIL), a terpenoid compound, first isolated from *Ailanthus altissima* (Mill.) Swingle exhibits excellent anti-tumor activity *in vitro*. However, current research on its role in CRC remains insufficient. Elucidating its therapeutic mechanisms in CRC holds significant importance for addressing current therapeutic challenges and advancing the development of lead compounds.

This study comprehensively evaluated the therapeutic efficacy of 2-DAIL in CRC and investigated its underlying molecular mechanisms. We first assessed the anti-tumor activity of 2-DAIL and demonstrated that it exerts significant therapeutic effects against CRC. Mechanistically, integrin alpha-3 (ITGA3) was identified as a direct molecular target of 2-DAIL, mediating its inhibitory effects on

CRC progression. Furthermore, RNA sequencing analysis revealed that 2-DAIL suppresses CRC development by inhibiting the ITGA3-mediated PI3K/AKT signaling pathway. Collectively, these findings provide a comprehensive evaluation of the pharmacological efficacy of 2-DAIL in CRC and elucidate its potential molecular mechanism, highlighting 2-DAIL as a promising therapeutic candidate for CRC treatment.

Materials and Methods

Materials and chemicals

Ailanthus altissima (Mill.) Swingle utilized in this study was sourced from Sichuan Neatus Pharmaceutical (Chengdu, China). It was authenticated by Professor Wang Guangzhi, who is affiliated with Chengdu University of Traditional Chinese Medicine (CDUTCM). Subsequently, 2-DAIL was isolated from this material and identified using spectral evidence from NMR (Supplementary Figures S1 and S2). The compound sample is currently stored at the Herbarium of CDUTCM with the collection number of 20250617/CDCM/SCA.

Cell culture

Colorectal cancer cell lines (CT26 and SW620) were obtained from the Cell Bank of Type the Culture Collection of the Chinese Academy of Sciences China (Shanghai, China). Each cell line was cultivated in DMEM or RPMI-1640 media (Gibco, Waltham, USA) containing 10% fetal bovine serum (FBS; Excell, Montevideo, Uruguay) and 1% penicillin-streptomycin (Servicebio, Wuhan, China). The cells were incubated at 37°C in a humidified incubator with 5% CO₂.

CKK-8 assay

Cells were seeded into 96-well plates at a density of 8.0×10^3 cells/well in 100 μ L of complete medium. SW620 cells were treated with 2-DAIL at concentrations of 1, 4, 7, 10, 13, 16, 19, 22, 25, and 28 μ M, while CT26 cells were treated with 2-DAIL at concentrations of 0.94, 1.88, 3.75, 7.5, 15, 30, 60, and 120 μ M. All experimental conditions were replicated in quintuplicate and incubated for either 48 or 72 h. Cell viability was assessed using the Cell Counting Kit-8 (Dingyou Shengwu, Chengdu, China). The absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Waltham, USA).

Live-cell imaging

CT26 and SW620 cells were seeded in 12-well plates at a density of 1.0×10^5 cells per well. SW620 cells were treated with 2-DAIL at concentrations of 5, 10, and 20 μ M, while CT26 cells were treated with 2-DAIL at concentrations of 10, 20, and 40 μ M. Live-cell imaging was conducted using an Incucyte S3 automated imaging system (Sartorius, Göttingen, Germany) maintained at 37°C and 5% CO₂. Images were automatically acquired at 12 h intervals over a 48-h observation period to capture dynamic changes.

Colony formation assay

CT26 (2000 cells/well) and SW620 (5000 cells/well) cells were seeded into separate 6-well plates containing RPMI-1640 or DMEM (Gibco) supplemented with 10% FBS and 1% penicillin-streptomycin. SW620 cells were exposed to 2-DAIL at 5, 10, and 20 μ M, whereas CT26 cells received 10, 20, and 40 μ M of the compound. Following 48 h of treatment, the drug-containing medium was

aspirated, and the cells were gently washed three times with pre-warmed PBS to eliminate residual compounds. The washed cells were maintained in serum-free medium, with fresh medium replacement every 3 days. Colony growth was monitored throughout the 14 days incubation. Cells were fixed with 4% paraformaldehyde at room temperature for 15 min, rinsed three times with PBS, and stained with 0.1% crystal violet (1 mL/well, 30 min). The plates were thoroughly rinsed with ultrapure water until background clearance was achieved, air-dried at room temperature, and imaged for documentation.

Wound healing assay

CT26 and SW620 cells were seeded in 96-well plates at a density of 5.0×10^3 cells per well and cultured in RPMI-1640 or DMEM, both supplemented with 10% FBS and 1% penicillin-streptomycin, until they reached approximately 85% confluency. A sterilized 96-well Incucyte S3 automated wounding device was applied to create uniform scratches in accordance with the manufacturer's instructions. The plates were gently washed three times with ice-cold PBS to remove dislodged cells. SW620 cells were exposed to 2-DAIL at 5, 10, and 20 μ M, whereas CT26 cells received 10, 20, and 40 μ M of the compound. The plates were then transferred to a live-cell imaging system, which maintained at 37°C in a humidified atmosphere containing 5% CO₂. At each time point (0, 6, 12, and 24 h), four non-overlapping fields per well were imaged at predefined coordinates using a 4 $\times$ objective.

Cell cycle analysis

SW620 cells in the logarithmic growth phase were seeded in 6-well plates at a density of 2.0×10^6 cells per well. After a 12-h adhesion period, the medium was replaced by complete medium. Experimental groups: 2-DAIL (serially diluted from 120 mM DMSO stock). Control groups: Vehicle (DMSO, $\leq 0.1\%$ v/v). Treatments were conducted in triplicate biological replicates for 24 h. Cells were washed twice with ice-cold PBS and detached using 0.25% EDTA-free trypsin (Servicebio). Following centrifugation at 300 g for 5 min at 4°C, the pellets were resuspended in pre-chilled 70% ethanol and fixed overnight at 4°C. Fixed cells were then centrifuged at 300 g for 5 min, washed twice with PBS, and stained with the cell cycle analysis kit (Beyotime Biotechnology, Shanghai, China). Cell sorting was performed using a flow cytometer (BD, Franklin Lakes, USA). A minimum of 1.0×10^4 events per sample were acquired. The cell cycle distribution was analyzed using FlowJo software.

Cell apoptosis assay

SW620 cells were seeded in a 6-well plate and incubated with 2-DAIL. After a brief trypsinization, the cells were harvested and washed with PBS buffer. Subsequently, the cells were stained with 5 μ L of Annexin V-FITC (Beyotime Biotechnology) and 10 μ L of PI (Beyotime Biotechnology) for 20 min and immediately analyzed by flow cytometry.

Western blot analysis

Total proteins from SW620 and tumor tissue were extracted with RIPA lysis buffer (Beyotime Biotechnology). The protein concentration was quantified with the BCA kit (Jiangsu CoWin Biotech, Taizhou, China). Then, a total of 30 μ g of protein was separated using 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis (Servicebio), followed by transfer to 0.22- μ m

polyvinylidene fluoride (PVDF) membranes (Merck Millipore, Massachusetts, USA). The membranes were then blocked with a 5% BSA solution (Beyotime Biotechnology), followed by overnight incubation with primary antibodies at 4°C and a subsequent 2-h incubation with species-specific secondary antibodies (Proteintech, Wuhan, China) at room temperature. The enhanced chemiluminescence (ECL) reagent from the Chemiluminescent Imaging System (MiniChem, Beijing, China) was utilized, protein bands on the membranes were detected. Subsequently, quantitative assessment of protein expression and data visualization were performed using ImageJ software.

Establishment and administration regimen of the tumor-bearing mouse model

SPF-grade BALB/c mice (6–8 weeks old) weighing approximately 18–22 g were purchased from Mouse Daqiang Biotechnology (Chengdu, China), with laboratory animal license SCXK (Beijing) 2024-0001. This experiment adhered to animal welfare and ethics standards and received approval from the Laboratory Animal Ethics Committee of CDUTCM (Registration Number: 2025081). All mice began modeling after one week of adaptive feeding. The colorectal cancer model was established by injecting 5.0×10^5 CT26 cells into the left flank of BALB/c mice. After three days, all mice with tumors were randomly divided into 4 groups ($n = 6$) and treated with PBS, 5-fluorouracil (5-FU; 30 mg/kg; MedChemExpress, Monmouth Junction, USA), or 2-DAIL (15 mg/kg, 30 mg/kg) via intraperitoneal injection. After the experiment, each mouse was euthanized after weighing. The tumor along with heart, liver, spleen, lung, and kidney tissues were completely excised. The organ index (organ weight/body weight $\times 100\%$) was calculated after weighing, and the tumor and some organs were fixed in 4% paraformaldehyde for future analysis.

H&E staining

Following the experiment, tumor tissues and visceral organs were fixed in a 4% paraformaldehyde buffer solution for over 24 h. After thorough rinsing with PBS, the tissues underwent histological sectioning. Subsequent operations were carried out according to the instruction manual. After applying a neutral resin mounting medium for coverslips, the integrity of the sections was assessed microscopically. Once the mounting medium had dried, whole-slide images were acquired using a digital pathology slide scanning system for pathological analysis.

Immunohistochemistry assay

After deparaffinization and hydration, the tissue sections were immersed in citrate buffer for antigen retrieval and treated with 3% H_2O_2 to inactivate endogenous peroxidase. The sections were then blocked with 3% BSA for 30 min and incubated overnight with anti-Ki67 or anti-p-PI3K antibodies (Cell Signaling Technology, Massachusetts, USA). Following DAB staining, the stained sections were examined under a microscope (Soptop IRX50; Sunny Optical Technology, Ningbo, China).

SILAC cell labeling assay

The SILAC labeling efficiency was quantified after 6 to 8 cell generations using LC-MS/MS (Thermo Fisher Scientific). A total of 200 μg of protein was reduced with 20 mM dithiothreitol (DTT; Sigma-Aldrich, St Louis, USA) at 30°C for 2 h, followed by

alkylation with 50 mM iodoacetamide (IAM; Sigma-Aldrich) in the dark for 30 min, and subsequently subjected to overnight acetone precipitation. The resulting pellets were washed, dissolved in 8 M urea (Sigma-Aldrich), and buffer-exchanged into 50 mM NH_4HCO_3 (Sigma-Aldrich) using a 10-kDa ultrafiltration system (Thermo Fisher Scientific). Digestion with 0.5 $\mu g/\mu L$ trypsin (Promega, Madison, USA) was performed at 37°C for 20 h. The peptides were desalted using a C_{18} column (Thermo Fisher Scientific), lyophilized, and analyzed by nano-LC-MS/MS. The labeling efficiency exceeded 95%, as indicated by the heavy/light ratio.

Thermal proteome profiling sample preparation and analysis

After 6–8 generations of SILAC labeling with an efficiency exceeding 95%, as verified by LC-MS/MS, heavy and light-labeled cells at 70%–80% confluency were treated with 20 μM 2-DAIL or DMSO for 2 h at 37°C. Subsequently, the cells were washed with ice-cold PBS, trypsinized, and centrifuged at 800 g for 5 min. The pellets were then resuspended in PBS containing protease and phosphatase inhibitors, followed by three freeze-thaw cycles using liquid nitrogen and 37°C water bath after 3 min of thermal shock at 50°C. After centrifugation at 12,000 g for 20 min at 4°C, the supernatants were collected, and the protein concentrations were normalized to 2 $\mu g/\mu L$ using the BCA assay. Equal amounts of light and heavy lysates (200 μg total) were combined, processed according to the SILAC cell labeling assay protocol, and analyzed via LC-MS/MS. The raw files were searched against the UniProt database using Proteome Discoverer 3.1.

Target validation and molecular docking

The potential targets (top 5) identified through SILAC-based thermal proteome profiling (TPP) were structurally characterized as follows: (1) Protein structures were retrieved from the Protein Data Bank (<https://www.rcsb.org/>) and GeneCards (<https://www.genecards.org/>), prioritizing complexes with a resolution of ≤ 2.5 Å. The specific identifiers are as follows: CD44 (4PZ4), IGTA3 (AF-P26006-F1), Histone H4 (5YE3), Integrin $\beta 1$ (1PT6), and Histone H2B1A (3X1V). (2) Ligand preparation involved sketching 2-DAIL and energetic minimization using the MMFF94 method and subsequent processing in Schrödinger LigPrep. (3) For molecular docking, Schrödinger Glide was employed, with binding pockets defined via SiteMap and a 5-Å extension grid. The docking parameters included van der Waals scaling of 0.8 and electrostatic scaling of 0.15. The complexes were evaluated based on Glide Score and hydrogen-bond interactions, with visualization performed using Maestro and PyMOL 2.5.

Molecular dynamics simulation

Following molecular docking, the resulting complexes were subjected to molecular dynamics (MD) simulations. Using the methodology previously described [9], MD simulations were performed using GROMACS with the AMBER99SB force field and the SPC water model for a duration of 50 ns. The simulation results were analyzed for root mean square deviation (RMSD), root mean square fluctuation (RMSF), and solvent accessible surface area (SASA).

Cell thermal stability analysis

When cell confluency reached 70%–80%, the culture medium was discarded, and the cells were washed three times with ice-cold PBS.

Following trypsinization, the cells were collected by centrifugation at 800 *g* for 5 min at 4°C in 10-mL tubes. The cell pellets were lysed in 2 mL of NP-40 buffer (Beyotime Biotechnology) with gentle pipetting, followed by centrifugation at 12,000 *g* for 20 min at 4°C. The supernatant was divided equally into two aliquots: (1) an experimental group treated with 40 μ M 2-DAIL and (2) a blank group incubated with vehicle control. Both groups were incubated at 37°C for 2 h. Subsequently, samples were transferred to PCR tubes and subjected to thermal gradient treatment at temperatures of 36.4°C, 39.9°C, 43.7°C, 47.5°C, 51.3°C, 55.1°C, 58.9°C, and 62.4°C for 3 min, followed by a 3-min equilibration at room temperature. After centrifugation at 12,000 *g* for 20 min at 4°C, the supernatants were mixed with 5 $\times$ loading buffer, denatured at 95°C for 10 min, and analyzed by western blot analysis.

Drug affinity responsive target stability

Upon reaching 70%–80% confluency, the cells were washed thrice with ice-cold PBS after the removal of the medium. Following trypsinization, the cells were pelleted by centrifugation at 800 *g* for 5 min at 4°C and subsequently lysed in RIPA buffer. The lysate was divided into three experimental groups: (1) Control group received PBS only; (2) Protease-digested group treated with protease at 1:1500 dilution; (3) Drug-treated groups pre-incubated with 2-DAIL at concentrations of 20 and 40 μ M for 1 h at room temperature. Protease was added to Groups 2 and 3 excluding the Control, followed by incubation at 37°C for 15 min. The reactions were terminated by adding 5 $\times$ loading buffer and denaturing at 95°C for 10 min. The samples were analyzed using western blot analysis.

Surface plasmon resonance analysis

Recombinant human ITGA3 protein (Cat# RPB289Mu01; Cloud-Clone Corp., Wuhan, China) was immobilized onto a CM5 sensor chip (Cytiva, Marlborough, USA) through amine coupling in sodium acetate buffer (pH 4.0). 2-DAIL was dissolved in PBS containing 5% DMSO to create concentration gradients. Utilizing a multi-cycle kinetics mode, serially diluted samples were injected over the protein-functionalized chip surface at 25°C, with PBS and 5% DMSO used as the running buffer. Binding kinetics were recorded using a Biacore T200 instrument (Cytiva), and equilibrium dissociation constants (K_D) were calculated with Biacore Evaluation Software (v3.2).

Knockdown and overexpression of target genes

Twenty-four hours prior to transfection, SW620 cells were trypsinized, counted, and seeded into 6-well plates at a density of 1.5×10^5 cells per well in 2 mL of complete medium. Following an overnight incubation, transfection was initiated when the cells reached 60% confluency. Target-specific siRNA designed and synthesized by Genepharma (Shanghai, China) was diluted to a concentration of 20 μ M in RNase-free water. The sequence details of siRNAs targeting the human ITGA3 gene are provided in [Supplementary Table S1](#). For each well, 75 pmol of siRNA was mixed with 42.5 μ L of buffer, followed by the addition of 7.5 μ L siRNA-mate Plus transfection reagent (Genepharma). After gentle pipetting, the resulting complexes were immediately added dropwise to the wells in accordance with the manufacturer's protocol. Cells were harvested at 24 h transfection for RT-PCR and western blot analysis.

SW620 cells (2.0×10^5 cells/well) were seeded in 6-well plates with 2 mL of complete medium. Upon reaching 40%–60%

confluency, the cells were infected with lentivirus (20 μ L, 1×10^8 TU/mL) in the presence of 5 μ g/mL polybrene (Sigma-Aldrich). After 48 h, the medium was replaced by fresh medium containing 2.5 μ g/mL puromycin for selection. Following a 48-h antibiotic treatment, surviving cells were trypsinized, serially diluted, and plated into 96-well plates via limited dilution. Monoclonal cell populations were expanded to establish stable overexpressing cell lines, which were validated by RT-PCR and western blot analysis.

RNA-seq analysis

For RNA-seq transcriptome sequencing, the cells were cultured in a 6-cm dish. When 60%–70% confluency was reached, cells were treated with 10 μ M 2-DAIL for 12 h, followed by lysis with Trizol reagent (Vazyme) to isolate total RNA. Total RNA purity and concentration were evaluated, and agarose gel electrophoresis was used to evaluate integrity. In addition, the construction and sequencing of RNA-seq gene libraries were performed by Brainstorm Biotechnology (Nanjing, China). Differentially expressed genes (DEGs) were identified using the DESeq 2 R package. Differentially expressed genes with Foldchanges ≥ 1 and *p*-correction value < 0.05 were considered significant. Data visualization was performed in R software using the cluster Profiler R package.

Statistical analysis

The data are represented as the mean with standard deviation (SD). A one-way analysis of variance (ANOVA) was performed for data analysis with a statistical significance set at $P < 0.05$. Statistically significant differences between the control and test groups were assessed using GraphPad Prism 7 for correlation tests.

Results

2-DAIL inhibits the growth and migration of CRC cells *in vitro*

2-DAIL ([Figure 1A](#)) demonstrated potent anti-proliferative effects against colorectal cancer cells. The compound exhibited IC_{50} values of 23.2 μ M (CT26, 72 h) and 8.5 μ M (SW620, 72 h), as determined by CCK-8 assays ([Figure 1B,C](#)). Morphological analysis ([Figure 1D](#) and [Supplementary Figure S3](#)), wound healing ([Figure 1E](#) and [Supplementary Figure S4](#)), and clonogenic assays ([Figure 1F](#) and [Supplementary Figure S5](#)) further confirmed its inhibitory activity. Flow cytometry revealed that 2-DAIL at a concentration of 5 μ M induced G0/G1 phase arrest in SW620 cells ([Figure 1G](#)), accompanied by significant apoptosis ([Figure 1H](#)). Western blot analysis further demonstrated that 2-DAIL affected the expressions of apoptosis-related proteins in SW620 cells ([Figure 1I](#)). These findings establish that 2-DAIL has potential for the treatment of CRC.

2-DAIL exhibits anti-CRC activity *in vivo*

To further validate the anti-CRC activity of 2-DAIL *in vivo*, we constructed a BALB/c mouse transplant tumor model through the subcutaneous injection of CT26 colorectal cancer cells. The anti-tumor activity of 2-DAIL was systematically evaluated *in vivo* ([Figure 2A](#)). The experimental results showed that 2-DAIL (15 mg/kg) could significantly inhibit tumor growth, with a notable reduction in tumor volume observed after 15 days of administration ([Figure 2B,C](#)). Safety evaluations indicated that 2-DAIL had no significant toxic damage in the main organ indexes or histopathology, which was significantly better than that of the 5-FU group ([Figure 2D,E](#)) and no significant effects on body weight, food intake,

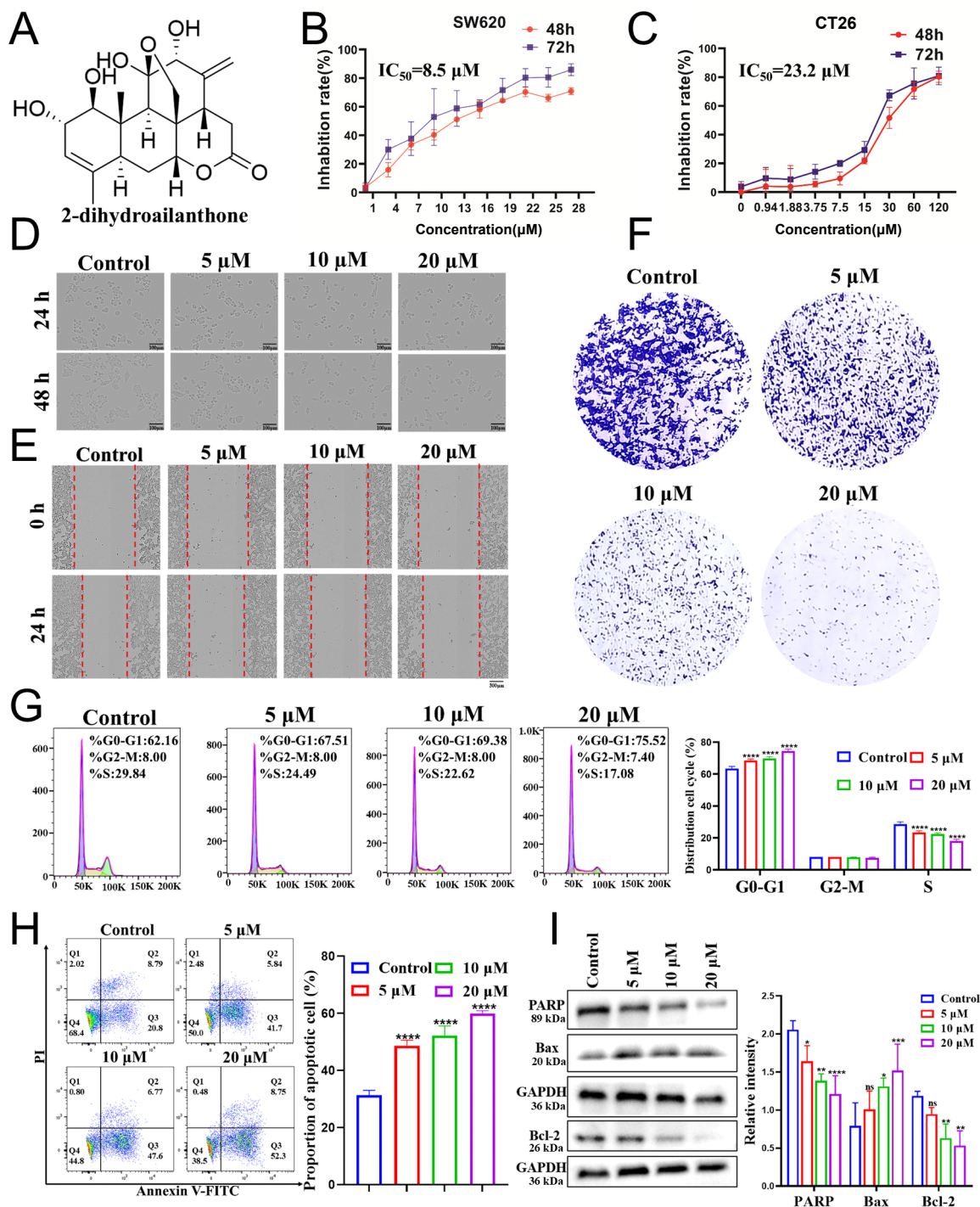


Figure 1. 2-DAIL inhibits growth and migration of CRC cells *in vitro* (A) Chemical structure of 2-DAIL. (B) Inhibitory effect of 2-DAIL on cell viability in SW620 cells. (C) Inhibitory effect of 2-DAIL on cell viability in CT26 cells. (D) Cell morphology experiment confirms 2-DAIL-mediated inhibition of SW620 cell proliferation. (E) Scratch assay confirms 2-DAIL-mediated inhibition of migration in SW620 cells. (F) Colony formation assay confirms 2-DAIL-mediated inhibition of SW620 cell proliferation. (G) 2-DAIL induces G0/G1 phase cell cycle arrest in SW620 Cells. (H) 2-DAIL induces apoptosis in SW620 cells. (I) Western blot analysis reveals that 2-DAIL modulates apoptosis-related protein expressions in SW620 cells. Data are displayed as the mean $\pm$ SD ($n = 3$), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ vs control group. ns, no significant difference.

or water intake (Figure 2F). These results suggested that 2-DAIL has both high potency against CRC and low systemic toxicity, indicating its potential for further development as a treatment candidate.

ITGA3 is a direct target of 2-DAIL

To further elucidate the potential mechanism by which 2-DAIL inhibits the proliferative activity of SW620 cells, we screened and

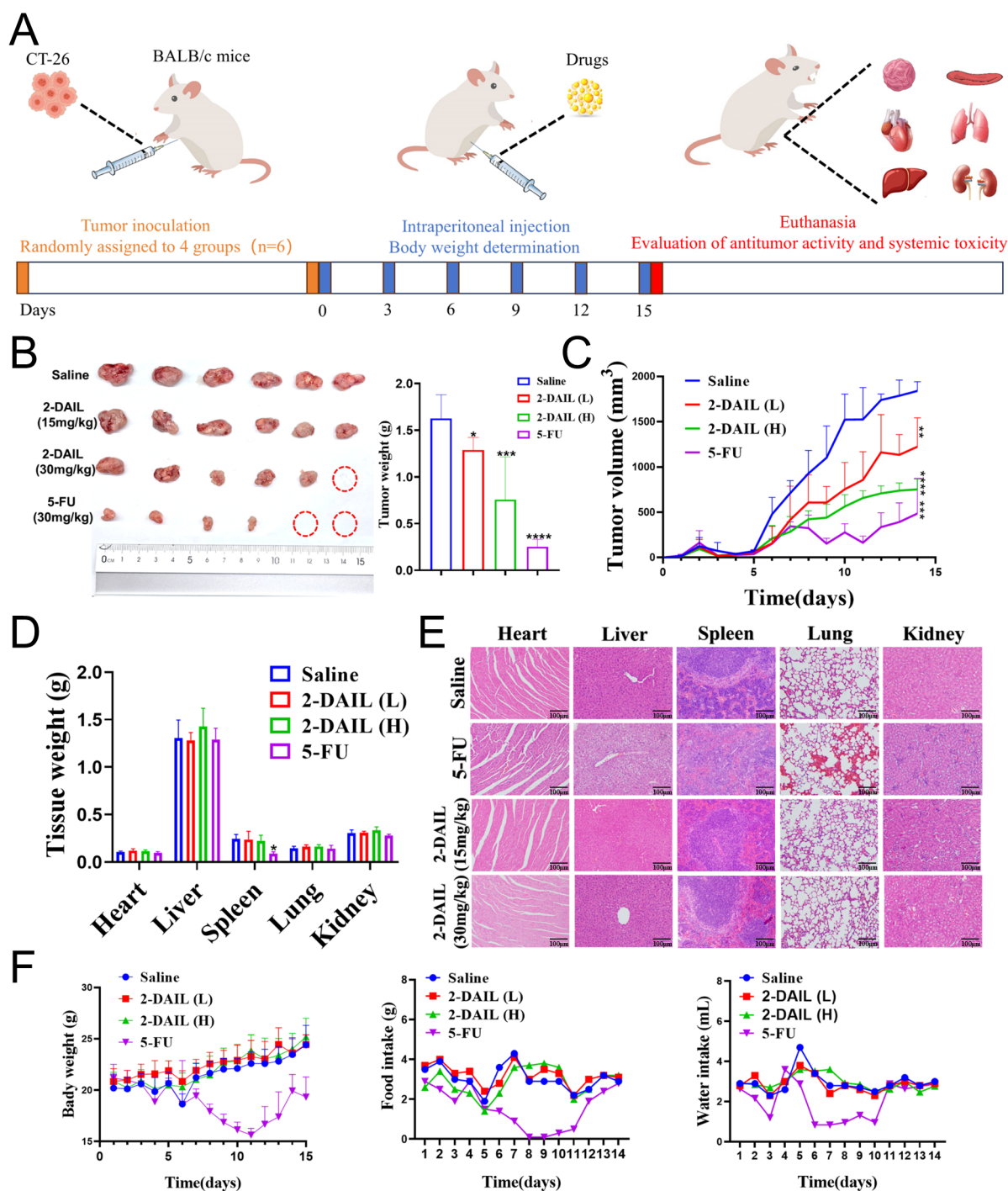


Figure 2. 2-DAIL exhibits anti-CRC activity *in vivo*. (A) Diagram of the experimental protocol of the mouse. (B) Tumor size. (C) Tumor volume. (D) Organ index. (E) H&E staining results. (F) Statistical results of mouse weight, diet and water intake. Data are displayed as the mean $\pm$ SD ($n = 3$), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs control group.

identified the targets of 2-DAIL using SILAC-TPP technology. A schematic representation of the SILAC-TPP methodology is provided in Figure 3A. Subsequently, we identified the differentially expressed proteins resulting from heat stimulation treatment through protein spectroscopy, yielding the top five significantly differentiated proteins based on their weight/hydrogen ratio

(Supplementary Figure S6). Coupled with molecular docking analysis, we discovered that ITGA3, which ranked second, exhibited the highest binding potential (Figure 3B,C). The MD simulation results revealed that the RMSD of the complex reached a stable plateau after 30 ns, with an average value of 1.57 nm. In contrast, the RMSD of the apo-protein (ITGA3) continued to

fluctuate, exhibiting a higher average value of 1.64 nm (Figure 3D). RMSF analysis indicated that the ligand 2-DAIL exerted a stabilizing effect on specific amino acid residues within ITGA3, as evidenced by a decrease in their RMSF values (Figure 3E). Consistent with these findings, SASA analysis demonstrated a reduction in the solvent accessible surface area of the complex compared to ITGA3 alone (Figure 3F), suggesting a more compact protein structure upon ligand binding. Further validation through cellular thermal shift assay (CETSA) and drug affinity responsive target stability (DARTS) indicated that 2-DAIL could enhance ITGA3's thermal or enzymatic stability (Figure 3G,H). Additionally, surface plasmon resonance (SPR) further confirmed that 2-DAIL specifically binds to ITGA3 with a dissociation equilibrium constant of $K_D = 9.01 \mu\text{M}$ (Figure 3I).

The anti-CRC activity of 2-DAIL is mediated by ITGA3

ITGA3 is highly expressed in a variety of tumors, and its expression level is directly linked to the risk of metastasis, which is significantly negatively correlated with patient survival [10,11]. To validate the role of 2-DAIL in interfering with ITGA3, we employed small interfering RNA (siRNA) and lentiviral infection techniques to modulate the expression of ITGA3 and observe its effects on cell phenotype. Prior to the experiments, we confirmed the silencing and overexpression efficiency of ITGA3 following siRNA and lentiviral infection. The results indicated that the transfection of four siRNAs significantly reduced the mRNA expression of ITGA3, with siRNA-3135 and siRNA-3511 exhibiting the highest silencing efficiency, which was further validated at the protein level (Figure 4A). Subsequently, the overexpression efficiency of the lentivirus was also verified at both the mRNA and protein expression levels (Figure 4B). ITGA3 was silenced and overexpressed using the aforementioned transfection methods. By observing its effects on the proliferation (Figure 4C,D), migration (Figure 4F), and apoptosis of SW620 cells (Figure 4E), we found that silencing of ITGA3 reduced the cell proliferation viability of SW620 cells, while the effect of 2-DAIL treatment in overexpressing cells was reversed. Similar results were obtained from the colony formation assay. The apoptosis experiments revealed that the number of apoptotic SW620 cells increased after ITGA3 silencing. However, no significant difference in apoptosis was observed after overexpression of ITGA3 compared to the blank control group. Notably, the number of apoptotic cells significantly increased after administering 2-DAIL to ITGA3-over-expressing cells. ITGA3 typically forms a heterodimer with ITGB1. Co-immunoprecipitation (Co-IP) experiments revealed that 2-DAIL did not significantly disrupt their interaction (Supplementary Methods and Supplementary Figure S7). These results provide evidence that 2-DAIL acts as an inhibitor of ITGA3.

2-DAIL inhibits PI3K-AKT signaling pathway downstream of ITGA3

To further reveal the underlying mechanism, RNA-seq was conducted. Specifically, a total of 3176 differentially expressed genes were identified by analyzing the differences with $|\log_2\text{Fold-Change}| > 0.5$ and $p\text{-value} < 0.05$, including 1701 upregulated genes and 1475 downregulated genes. The analysis of these differential genes showed that compared to the control group, there was a significant perturbation following 2-DAIL treatment, which notably reversed the expression of tumor-related genes (Figure 5A,B). Our research suggests that 2-DAIL may exert its effects through the PI3K/

AKT signaling pathway (Figure 5C,D). To elucidate the role of ITGA3 in tumor progression, we retrieved clinical data from The Cancer Genome Atlas (TCGA) database. Our analysis revealed that ITGA3 expression was significantly upregulated in tumor tissues compared to normal tissues (Figure 5E). Furthermore, survival analysis demonstrated that patients with high ITGA3 expression exhibited markedly reduced survival rates (Figure 5F).

2-DAIL suppresses PI3K/AKT signaling pathway by inhibiting ITGA3

The dysregulated activation of the PI3K/AKT signaling pathway plays a critical role in driving tumorigenesis and disease progression [12–14]. Previous studies have demonstrated that ITGA3 drives the proliferation and migration of thyroid cancer cells through cooperative activation of MET-mediated ERK and PI3K/AKT signaling pathways, suggesting that the ITGA3-MET axis is a potential therapeutic target [15]. Induced collagen crosslinking stiffens the extracellular matrix (ECM), promotes focal adhesion assembly, enhances PI3K activity, and triggers oncogene-driven invasion in epithelial cells [16,17]. In this study, western blot analysis demonstrated that 2-DAIL exerted anti-CRC activity through PI3K/AKT signaling pathways (Figure 6A,B) in SW620 cells, which was further corroborated by RNA sequencing results (Figure 6C). Compared to the clinically utilized AKT inhibitor Capivasertib (Abmole, Houston, USA), 2-DAIL exhibited superior inhibitory efficacy against SW620 cell proliferation (Supplementary Figure S8). *In vitro* rescue experiments showed that knockdown of ITGA3 significantly decreased p-PI3K and p-AKT protein levels, whereas overexpression of ITGA3 markedly increased them. Subsequent treatment with 2-DAIL reversed this increase, again significantly reducing the levels of both phosphoproteins. Collectively, these findings provide compelling evidence that 2-DAIL modulates the PI3K/AKT pathway through ITGA3. *In vivo* studies also confirmed that 2-DAIL reduced p-PI3K protein levels (Figure 6D), and western blot analyses were conducted to verify the PI3K/AKT signaling pathway in tumors (Figure 6E).

These data indicate that 2-DAIL inhibits ITGA3, thereby suppressing the activation of the PI3K/AKT pathway, which in turn inhibits the proliferation and migration of SW620 cells.

Discussion

CRC is a common malignancy of the gastrointestinal system [18]. In recent years, both the incidence and mortality rate of CRC have shown an upward trend primarily attributed to lifestyle and dietary habits [19]. The lack of obvious early symptoms, coupled with the tendency for misdiagnosis of other conditions such as hemorrhoids, results in 70% of patients being diagnosed at an advanced stage [20]. Clinically, cisplatin, pentafluorouracil, and other drugs are commonly used to treat bowel cancer. However, these chemotherapy agents are associated with serious toxic side effects and long-term drug resistance [21]. In recent years, targeted therapy has shown significant efficacy in tumor treatment, offering considerable advantages in terms of low toxicity and reduced side effects [22]. Therefore, there is an urgent need to develop innovative drugs that exhibit substantial efficacy and precise targets in the treatment of CRC.

It has been extensively reported that quassinoids exhibit anti-colorectal cancer activity, including triterpenoids. A previous study has demonstrated that ailanthone suppresses human colorectal cancer cell activity by inhibiting the STAT3 signaling pathway,

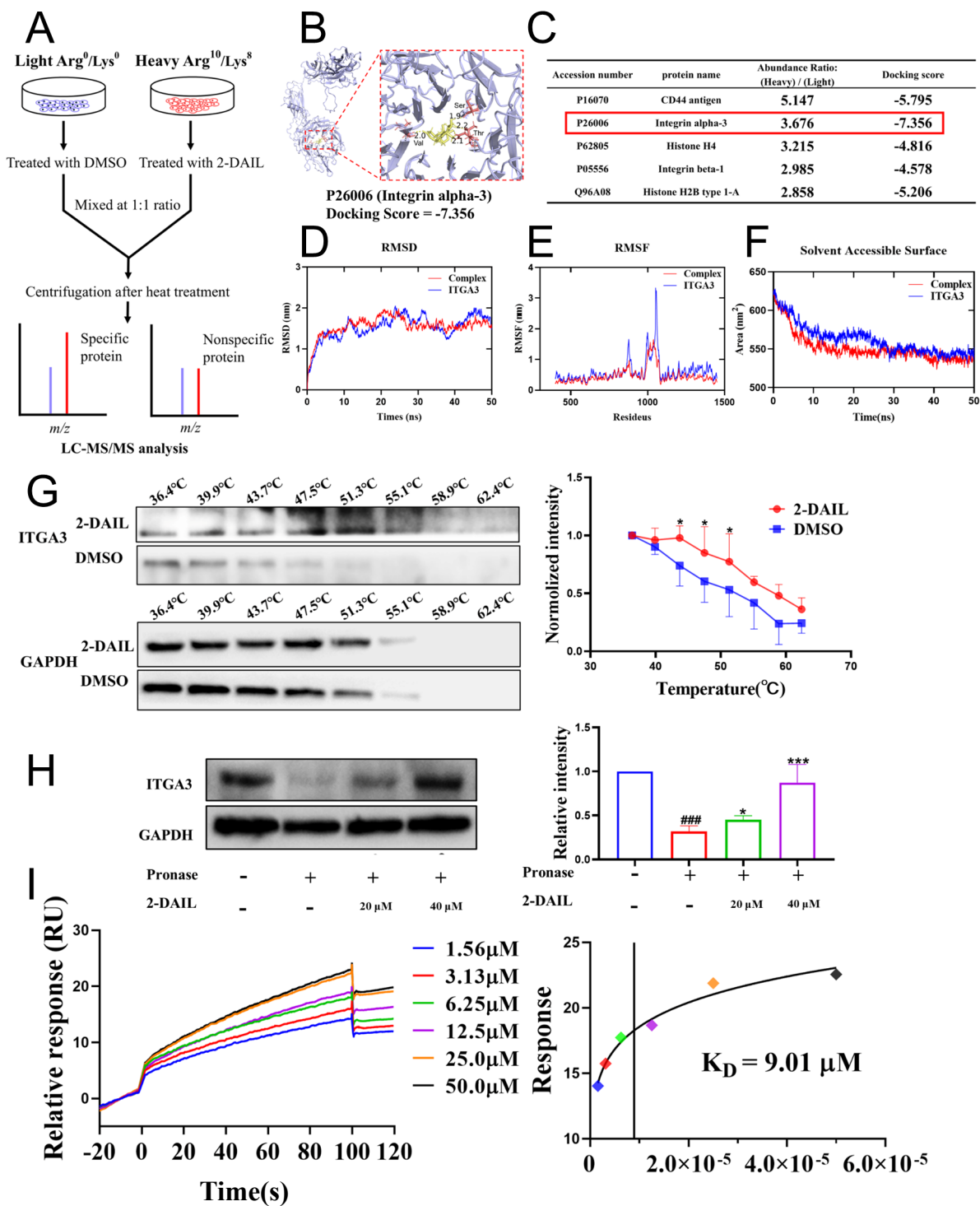


Figure 3. 2-DAIL directly targets ITGA3 (A) SILAC-TPP experimental flow chart. (B) Molecular docking reveals that 2-DAIL forms hydrogen bonds with multiple amino acid residues of ITGA3. (C) Identification of top 5 ranked targets and their docking scores. (D) RMSD analysis reveals enhanced stability of the protein backbone in the 2-DAIL-ITGA3 complex compared to free ITGA3. (E) RMSF analysis demonstrates that 2-DAIL stabilizes dynamic residues in ITGA3. (F) SASA analysis reveals enhanced compactness of the protein complex. (G) CETSA confirms that 2-DAIL significantly enhances the thermal stability of ITGA3. Data are displayed as the mean $\pm$ SD ($n = 3$), $*P < 0.05$ vs control group. (H) DARTS validates 2-DAIL as a potent stabilizer of ITGA3 proteolytic stability. Data are displayed as the mean $\pm$ SD ($n = 3$), $***P < 0.001$ vs control group; $*P < 0.05$, $***P < 0.001$ vs model group. (I) SPR analysis demonstrates strong binding affinity between 2-DAIL and ITGA3.

thereby attenuating tumor proliferation and survival [23]. Ma *et al.* [24] elucidated through network pharmacology, bioinformatics

analysis, and experimental validation that Ailanthone impedes PI3K/AKT pathway activation, leading to suppression of CRC cell

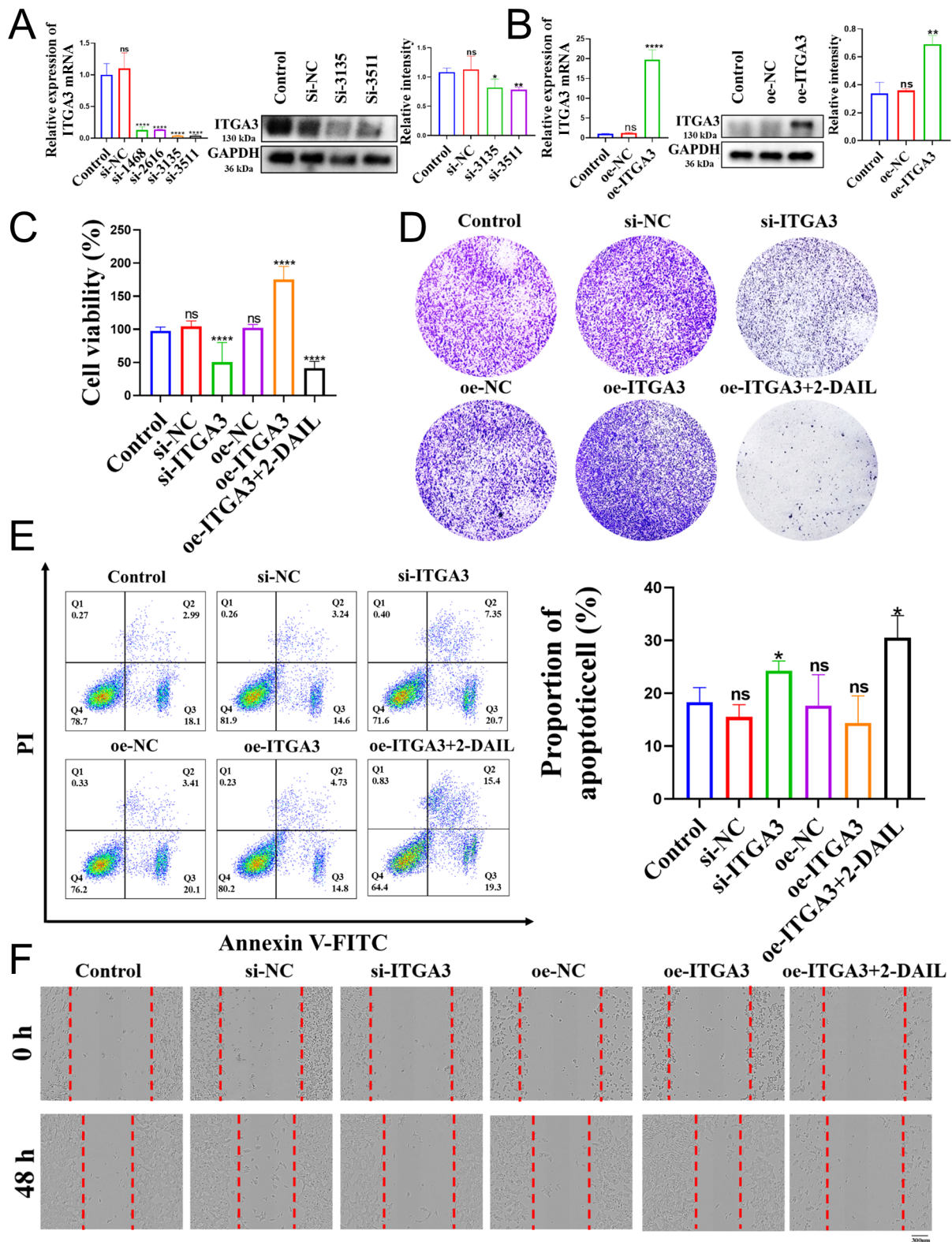


Figure 4. The anti-CRC activity of 2-DAIL is mediated by *ITGA3* (A) Reverse transcription PCR and western blot analyses confirm *ITGA3* knockdown at mRNA and protein levels. (B) Reverse transcription PCR and western blot analyses confirm *ITGA3* overexpression at mRNA and protein levels. (C) CCK-8 assays demonstrate that *ITGA3* modulation significantly affects SW620 cell proliferation. (D) Colony formation reveal that *ITGA3* modulation alters SW620 cell proliferation. (E) Flow cytometry demonstrates that *ITGA3* modulation alters SW620 cell apoptosis. (F) Scratch assay reveals that *ITGA3* modulation alters SW620 cell migration. Data are displayed as the mean $\pm$ SD ($n = 3$), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs control group. ns, no significant difference.

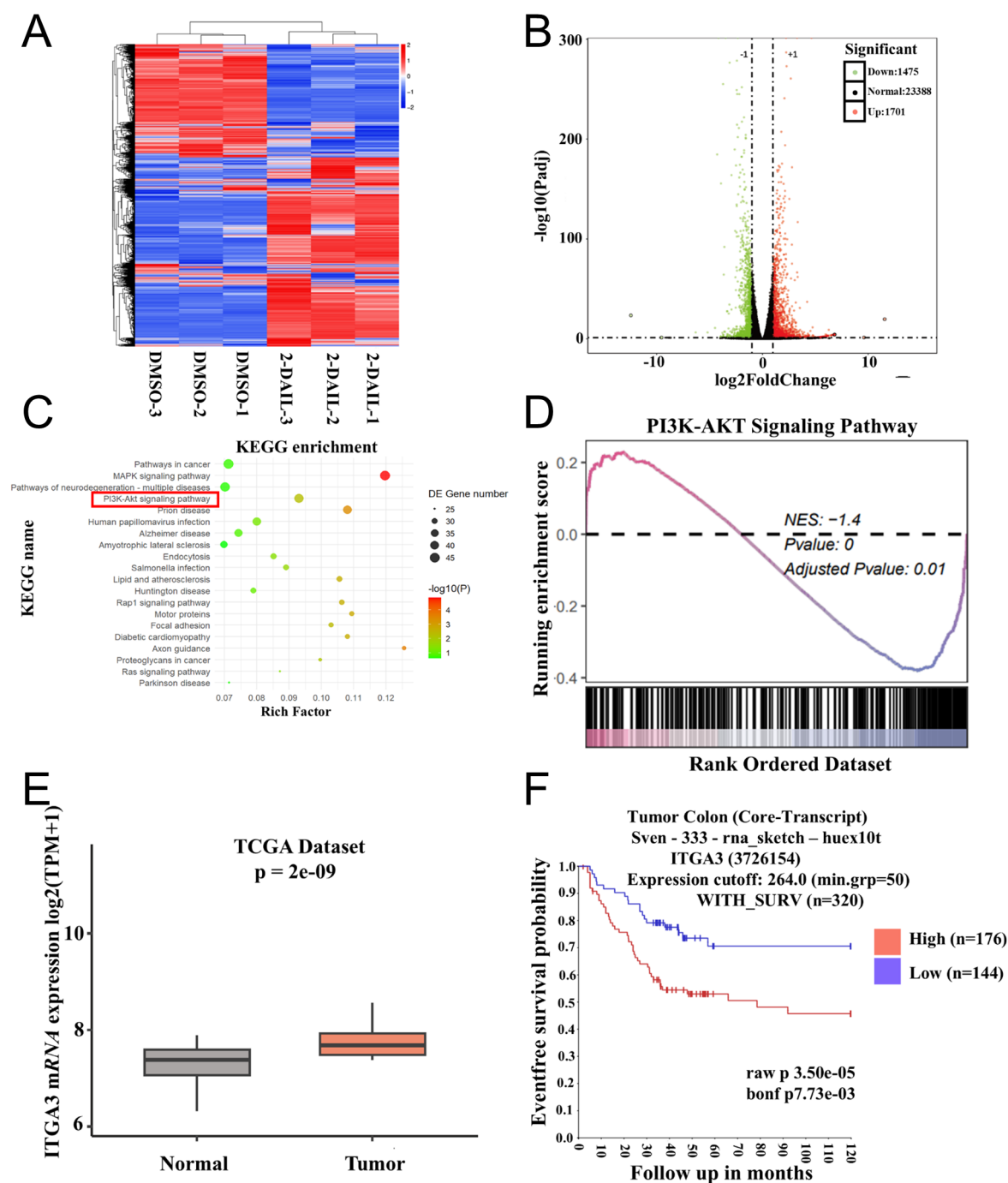


Figure 5. 2-DAIL suppresses PI3K/AKT signaling pathway, a downstream of ITGA3 (A,B) Heat maps and volcano maps. (C) KEGG pathway enrichment analysis revealed significant enrichment of the PI3K/AKT signaling pathway in CRC cell models. (D) GSEA analysis was performed to assess PI3K/AKT pathway enrichment. (E) Box-and-Whisker Plot results demonstrated that ITGA3 expression is significantly elevated in tumor tissues compared to normal tissues. (F) Kaplan-Meier analysis reveals that higher ITGA3 expression correlates with reduced survival in patients.

proliferation and metastasis. Wei *et al.* [25] identified quassinoid derivatives as potent inhibitors of CRC via reversible blockade of protein biosynthesis, achieving greater than 80% tumor growth suppression in preclinical models. Huynh *et al.* [26] reported that quassinoid glaucarubinone concurrently inhibits HIF-1 α and β -catenin by targeting the PAK1-dependent pathway, disrupting

hypoxia adaptation and Wnt-driven proliferation in CRC. It has been revealed that Brusatol upregulates ARRDC4 expression via modulation of the PI3K/YAP1/TAZ axis, effectively suppressing CRC growth and metastasis [27]. Nevertheless, these reported findings warrant further investigation to elucidate the molecular targets of such quassinoids.

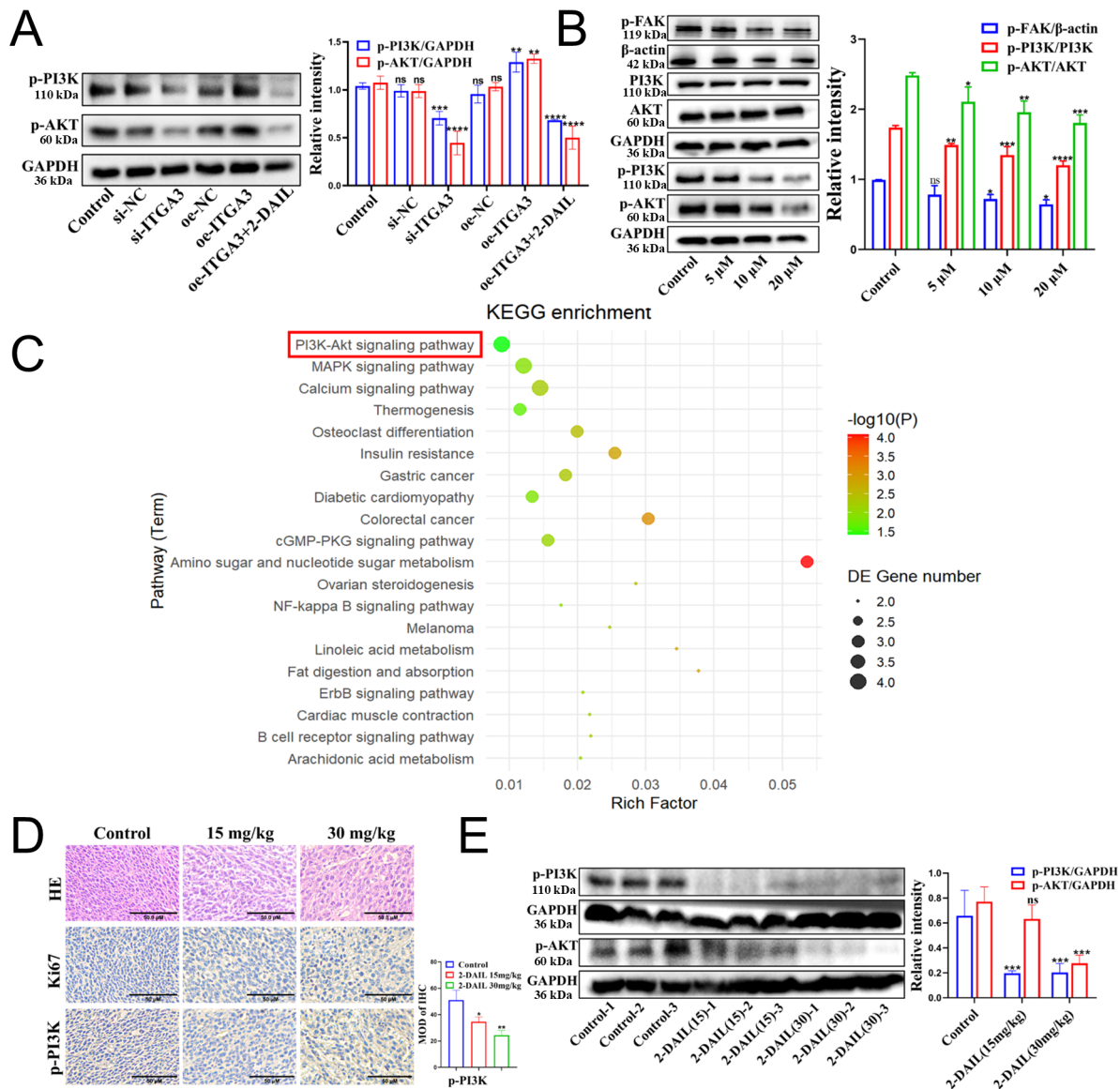


Figure 6. The inhibition of 2-DAIL on PI3K/AKT signaling pathway is mediated by ITGA3 (A) Knockdown and overexpression experiments demonstrated that 2-DAIL directly mediates the PI3K/AKT signaling pathway. (B) Western blot analysis demonstrated that 2-DAIL exerts anti-tumor effects in SW620 cells via the FAK/PI3K/AKT signaling pathway. (C) RNA-Seq data analysis of tumor tissues demonstrated significant enrichment of the PI3K/AKT signaling pathway. (D) H&E staining, Ki67 and p-PI3K IHC staining of tumor tissues in colon cancer model. (E) Western blot analysis demonstrated that 2-DAIL exerts anti-tumor effects *in vivo* via the PI3K/AKT signaling pathway. Data are displayed as the mean $\pm$ SD ($n = 3$), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs control group. ns, no significant difference.

Previous study has demonstrated that 2-DAIL significantly inhibits the growth of MCF-7 human breast cancer cells and U251 human glioma cells [28]. However, the research on 2-DAIL remains in its early stages, and its specific mechanism of action is still unclear. Our study reveals that 2-DAIL inhibits the proliferation and migration of CT26 and SW620 cells in a dose-dependent manner, while also affecting the cell cycle of SW620 cells and promoting apoptosis. Furthermore, *in vivo* studies indicate that 2-DAIL possesses a superior safety profile than ailanthone and can be a candidate molecule warranting in-depth pharmacological investigation and exploration of novel targets among quassinoids.

Integrins are cell surface adhesion receptors that bind to

extracellular matrix proteins such as fibronectin and laminin as well as chemokines secreted by tumor cells, thereby activating the PI3K/AKT pathway [29]. Upon binding to the ECM, tumor cell surface integrin receptors recruit focal adhesion kinase (FAK) and Src family kinases, which phosphorylate the p85 regulatory subunit of PI3K, catalyzing the generation of PIP_2 [30]. PIP_2 functions as a second messenger, recruiting AKT to the cell membrane where it is phosphorylated by PDK1 [31]. Subsequent AKT phosphorylates pro-apoptotic proteins including Bad and FoxO, inhibiting their function and thus inhibiting apoptosis in tumor cells [32]. At the same time, AKT activates mTOR, promoting ribosome biosynthesis and the expression of cyclins, such as Cyclin D1, thus accelerating

the G1/S phase transition, and facilitating tumor cell growth and proliferation [33]. Furthermore, AKT promotes cancer cell invasion by activating NF- κ B, inducing the expression of HIF-1 α and VEGF, and promoting tumor angiogenesis [34]. At the same time, the integrin-PI3K-AKT axis increases cytokines such as IL-10 and INF- γ through NF- κ B, recruiting myeloid-derived suppressor cells and tumor-associated macrophages to form an immunosuppressive microenvironment [35,36]. Integrin $\alpha 3$ subunits typically heterodimerize with other integrin receptors to mediate functional interactions. 2-DAIL may exert its inhibitory effects by occupying critical binding sites within the extracellular domain of integrin $\alpha 3$, thereby inducing steric hindrance that directly blocks heterodimerization with other integrins [37]. Furthermore, studies demonstrate that integrin glycosylations participate in ligand recognition processes [38]. 2-DAIL might modulate the glycosylation microenvironment on $\alpha 3$ surfaces, indirectly altering the binding affinity of other integrins. This selective blockade could disrupt $\alpha 3$ -mediated cell-matrix adhesion and downstream signaling cascades, ultimately suppressing integrin $\alpha 3$ -dependent cellular functions such as migration and transduction. However, most existing studies are limited to the cellular level, and *in vivo* experimental data to support this conclusion are lacking. Future research could employ stable *ITGA3*-knockdown or overexpressing mouse models and more cell models to further validate these findings.

In the field of oncology therapeutics, Cilengitide targeting integrin receptors demonstrated promising activity in preclinical studies and early-phase clinical trials [39,40], however, it ultimately failed to meet primary endpoints in Phase III trials, highlighting the need for a critical focus on the development of *ITGA3* inhibitors. 5-FU, a standard first-line chemotherapy for CRC, offers proven efficacy compared with 2-DAIL. However, its utility is often limited by significant toxicity and the emergence of resistance. In contrast, 2-DAIL operates through a distinct molecular mechanism. Future studies will prioritize evaluating 2-DAIL in combination therapies, where it may sensitize tumors to conventional agents such as 5-FU or overcome specific resistance mechanisms, potentially achieving enhanced efficacy without compounding toxicity. Notably, 2-DAIL demonstrated superior inhibitory activity compared to Capiivasertib in SW620 cells, suggesting its potential clinical utility for AKT-driven malignancies with heterogeneous genetic alterations. However, current safety profiling of 2-DAIL remains incomplete, necessitating systematic toxicological investigations to elucidate its adverse event profile. Therefore, its primary potential may reside not as a stand-alone replacement but as a complementary agent within a broader therapeutic strategy. Our study demonstrates that 2-DAIL can target *ITGA3* to inhibit the activation of PI3K/AKT signaling pathway, thereby inhibiting the proliferation, migration, and apoptosis of SW620 cells.

In conclusion, 2-DAIL, isolated from *Ailanthus altissima*, exhibits potent anti-CRC activity and demonstrates a favorable safety profile. Mechanistic investigations identified *ITGA3* as a direct molecular target of 2-DAIL. Targeting *ITGA3* with 2-DAIL suppressed PI3K/AKT signaling in CRC cells, thereby inhibiting CRC progression. These findings highlight 2-DAIL as a promising candidate for the development of novel CRC therapeutics.

Supplementary Data

Supplementary data are available at *Acta Biochimica et Biophysica Sinica* online.

Data Availability

The raw RNA-seq data generated from SW620 cells and tumor tissues in this study have been deposited in the NCBI Sequence Read Archive (SRA) under the BioProject accession numbers PRJNA1393597 and PRJNA1395783.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Morgan E, Arnold M, Gini A, Lorenzoni V, Cabaasag CJ, Laversanne M, Vignat J, *et al.* Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. *Cut* 2023, 72: 338–344
- Wang Z, Dan W, Zhang N, Fang J, Yang Y. Colorectal cancer and gut microbiota studies in China. *Cut Microbes* 2023, 15: 2236364
- Biagiotti G, Purić E, Urbančić I, Krišelj A, Weiss M, Mravljak J, Gellini C, *et al.* Combining cross-coupling reaction and Knoevenagel condensation in the synthesis of glyco-BODIPY probes for DC-SIGN super-resolution bioimaging. *Bioorg Chem* 2021, 109: 104730
- Nasimi Shad A, Moghbeli M. Integrins as the pivotal regulators of cisplatin response in tumor cells. *Cell Commun Signal* 2024, 22: 265
- Bian Z, Yang F, Xu P, Gao G, Yang C, Cao Y, Yao S, *et al.* LINC01852 inhibits the tumorigenesis and chemoresistance in colorectal cancer by suppressing SRSF5-mediated alternative splicing of PKM. *Mol Cancer* 2024, 23: 23
- Ali M, Chen SR, Hwang TL, Duh TH, Salendra L, Niaz SI, Cheng PW, *et al.* New diterpenoids with anti-inflammatory activity from *callicarpa rubella* collected in vietnam. *Phytochem Anal* 2025, 36: 1605–1613
- Lin JL, Chen L, Wu WK, Guo XX, Yu CH, Xu M, Nie GB, *et al.* Single-cell RNA sequencing reveals a hierarchical transcriptional regulatory network of terpenoid biosynthesis in cotton secretory glandular cells. *Mol Plant* 2023, 16: 1990–2003
- Pastuñá-Fasso JV, Espinosa de los Monteros-Silva N, Quiroz-Moreno CD, Medina-Villamizar EJ, Sosa-Pozo G, Cisneros-Pérez PA, Proaño-Bolaños C, *et al.* Untargeted characterization and biological activity of amazonian aqueous stem bark extracts by liquid and gas chromatography-mass spectrometry. *Phytochem Anal* 2025, 36: 1153–1167
- Liu M, Huo X, Li C, Hu Y, Lei H, Wang D, Zhu L, *et al.* Oxypeucedanin hydrate alleviates rheumatoid arthritis by inhibiting the TLR4-MD2/NF- κ B/MAPK signaling axis. *Acta Biochim Biophys Sin* 2024, 56: 1789–1801
- Du Q, Wang W, Liu T, Shang C, Huang J, Liao Y, Qin S, *et al.* High expression of integrin $\alpha 3$ predicts poor prognosis and promotes tumor metastasis and angiogenesis by activating the c-Src/extracellular signal-regulated protein kinase/focal adhesion kinase signaling pathway in cervical cancer. *Front Oncol* 2020, 10: 36
- Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic

- Mark M, Molina H, *et al.* Tumour exosome integrins determine organotropic metastasis. *Nature* 2015, 527: 329–335
12. Song X, Li Y, Li Y, Li J, Li D, Kou X, Liu Y, *et al.* Molecular mechanisms of ferroptosis induced by *Eleutherococcus senticosus* in glioblastoma. *Phytochem Anal* 2025, 36: 2065–2079
 13. Fontana F, Giannitti G, Marchesi S, Limonta P. The PI3K/Akt pathway and glucose metabolism: a dangerous liaison in cancer. *Int J Biol Sci* 2024, 20: 3113–3125
 14. Liu FT, Chen YH, Wang WY, Zhang JN, Sun RB, Zhang XR, Han YT, *et al.* A comprehensive analysis of volatile and nonvolatile components in *Berberis fortunei* and its inhibition against HT29 colorectal cancer cells through GC-IMS, LC-QTOF-MS, and docking-based network analysis. *Phytochem Anal* 2025, 36: 2349–2361
 15. Lan Y, Liu D, Liang B, Song X, Xie L, Peng H, Guo H, *et al.* ITGA3–MET interaction promotes papillary thyroid cancer progression via ERK and PI3K/AKT pathways. *Ann Med* 2025, 57: 2483379
 16. Huang Y, Zhao H, Zhang Y, Tang Y, Shi X, Jiang S, Pu W, *et al.* Enhancement of zyxin promotes skin fibrosis by regulating FAK/PI3K/AKT and TGF- β signaling pathways via integrins. *Int J Biol Sci* 2023, 19: 2394–2408
 17. Zhong T, Zhang W, Guo H, Pan X, Chen X, He Q, Yang B, *et al.* The regulatory and modulatory roles of TRP family channels in malignant tumors and relevant therapeutic strategies. *Acta Pharm Sin B* 2022, 12: 1761–1780
 18. Eng C, Yoshino T, Ruiz-García E, Mostafa N, Cann CG, O'Brian B, Benny A, *et al.* Colorectal cancer. *Lancet* 2024, 404: 294–310
 19. Song M, Chan AT, Sun J. Influence of the gut microbiome, diet, and environment on risk of colorectal cancer. *Gastroenterology* 2020, 158: 322–340
 20. Guo CG, Ma W, Drew DA, Cao Y, Nguyen LH, Joshi AD, Ng K, *et al.* Aspirin use and risk of colorectal cancer among older adults. *JAMA Oncol* 2021, 7: 428–435
 21. Ardil B, Alper M. Potential cancer treatment effects of brusatol or eriodictyol combined with 5-fluorouracil (5-FU) in colorectal cancer cell. *Naunyn Schmiedebergs Arch Pharmacol* 2022, 395: 1109–1123
 22. Li Q, Geng S, Luo H, Wang W, Mo YQ, Luo Q, Wang L, *et al.* Signaling pathways involved in colorectal cancer: pathogenesis and targeted therapy. *Sig Transduct Target Ther* 2024, 9: 266
 23. Ding H, Yu X, Yan Z. Ailanthone suppresses the activity of human colorectal cancer cells through the STAT3 signaling pathway. *Int J Mol Med* 2022, 49: 21
 24. Ma S, Guo X, Han R, Meng Q, Zhang Y, Quan W, Miao S, *et al.* Elucidation of the mechanism of action of ailanthone in the treatment of colorectal cancer: integration of network pharmacology, bioinformatics analysis and experimental validation. *Front Pharmacol* 2024, 15: 1355644
 25. Wei N, Burnett J, Crocker DL, Huang Y, Li S, Wipf P, Chu E, *et al.* Quassinoid analogs exert potent antitumor activity via reversible protein biosynthesis inhibition in human colorectal cancer. *Biochem Pharmacol* 2023, 212: 115564
 26. Huynh N, Beutler JA, Shulkes A, Baldwin GS, He H. Glaucarubinone inhibits colorectal cancer growth by suppression of hypoxia-inducible factor 1 α and β -catenin via a p-21 activated kinase 1-dependent pathway. *Biochim Biophys Acta* 2015, 1853: 157–165
 27. Huang QH, Zhang J, Cho WCS, Huang Y, Yang W, Zuo Z, Xian YF, *et al.* Brusatol suppresses the tumor growth and metastasis of colorectal cancer via upregulating ARRDC4 expression through modulating PI3K/YAP1/TAZ pathway. *Phytomedicine* 2023, 109: 154567
 28. Wang R, Xu Q, Liu L, Liang X, Cheng L, Zhang M, Shi Q. Antitumor activity of 2-dihydroailanthone from the bark of *Ailanthus altissima* against U251. *Pharm Biol* 2016, 54: 1641–1648
 29. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SFT, *et al.* Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* 2009, 139: 891–906
 30. Wasik A, Ratajczak-Wielgomas K, Badzinski A, Dziegiel P, Podhorska-Okolow M. The role of periostin in angiogenesis and lymphangiogenesis in tumors. *Cancers* 2022, 14: 4225
 31. Leroux AE, Biondi RM. The choreography of protein kinase PDK1 and its diverse substrate dance partners. *Biochem J* 2023, 480: 1503–1532
 32. Pahlavani HA. Exercise-induced signaling pathways to counteracting cardiac apoptotic processes. *Front Cell Dev Biol* 2022, 10: 950927
 33. Ma L, Zhang R, Li D, Qiao T, Guo X. Fluoride regulates chondrocyte proliferation and autophagy via PI3K/AKT/mTOR signaling pathway. *Chem-Biol Interact* 2021, 349: 109659
 34. Li Y, Xia J, Jiang N, Xian Y, Ju H, Wei Y, Zhang X. Corin protects H2O2-induced apoptosis through PI3K/AKT and NF- κ B pathway in cardiomyocytes. *Biomed Pharmacother* 2018, 97: 594–599
 35. Zhou X, Fang D, Liu H, Ou X, Zhang C, Zhao Z, Zhao S, *et al.* PMN-MDSCs accumulation induced by CXCL1 promotes CD8 $^{+}$ T cells exhaustion in gastric cancer. *Cancer Lett* 2022, 532: 215598
 36. Lin JP, Huang MH, Sun ZT, Chen L, Lei YH, Huang YQ, Qi M, *et al.* Periplocin inhibits hepatocellular carcinoma progression and reduces the recruitment of MDSCs through AKT/NF- κ B pathway. *Life Sci* 2023, 324: 121715
 37. Brown AC, Dysart MM, Clarke KC, Stabenfeldt SE, Barker TH. Integrin $\alpha 3 \beta 1$ binding to fibronectin is dependent on the ninth type III repeat. *J Biol Chem* 2015, 290: 25534–25547
 38. Eto DS, Jones TA, Sundsbak JL, Mulvey MA, Isberg RR. Integrin-mediated host cell invasion by type 1-piliated uropathogenic *Escherichia coli*. *PLoS Pathog* 2007, 3: e100
 39. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer* 2010, 10: 9–22
 40. Girnius N, Henstridge AZ, Marks B, Yu JK, Gray GK, Sander C, Zervantonakis IK, *et al.* Cilengitide sensitivity is predicted by overall integrin expression in breast cancer. *Breast Cancer Res* 2024, 26: 187