

Original Research

Ginkgolide B Inhibits Colorectal Cancer Growth via Regulation of the PI3K/AKT/MAPK Pathways: A Comprehensive Analysis Using Network Pharmacology, Molecular Docking, and Verification in Cellular and Animal Models

Qi Liu^{1,2}, Xin Sun², Aikelimu Aisikaer³, Nyamsambuu Altannavch², Maonan Wang², Gang Yin^{2,4,5}, Guang Shu^{2,*}, Qing Xiao^{1,*}

¹Department of Pathology, The Third Xiangya Hospital, Central South University, 410013 Changsha, Hunan, China

²Department of Pathology, Xiangya Hospital, School of Basic Medical Sciences, Central South University, 410013 Changsha, Hunan, China

³Department of Pathology, School of Pharmacy, Xinjiang Medical University, 830011 Urumqi, Xinjiang, China

⁴School of Basic Medical Sciences, Central South University, 410013 Changsha, Hunan, China

⁵National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, 410013 Changsha, Hunan, China

*Correspondence: shuguang78@csu.edu.cn (Guang Shu); 522402330@qq.com (Qing Xiao)

Academic Editor: Amancio Carnero Moya

Submitted: 18 June 2026 Revised: 4 August 2026 Accepted: 21 August 2026 Published: 16 October 2026

Abstract

Background: Ginkgolide B (GB), a bioactive constituent isolated from *Ginkgo biloba*, has shown antitumor activity in several malignancies. However, the molecular mechanisms underlying GB action against colorectal cancer (CRC) remain incompletely understood. Thus, this study aimed to systematically investigate the potential molecular mechanisms and therapeutic effects of GB on CRC using an integrated approach. **Methods:** Potential therapeutic targets of GB against CRC were identified by integrating GB-related and CRC-associated genes. Protein–protein interaction (PPI) networks and functional enrichment analysis (FEA) identified key targets and pathways. Interactions between GB and hub proteins were assessed by docking. The antitumor effects of GB were evaluated in CRC cells using assays for cell proliferation, colony formation, and apoptosis, as well as Western blotting. These data were validated in an MC38 syngeneic subcutaneous tumor model. **Results:** A total of 288 target proteins were shared between the 522 predicted GB targets and the 4199 CRC-associated targets. FEA indicated that these targets were primarily associated with the phosphatidylinositol 3-kinase (PI3K) / protein kinase B (AKT) and mitogen-activated protein kinase (MAPK) axes. Docking predicted favorable binding of GB to several hub proteins, with AKT1 showing the strongest predicted affinity. Experimental validation demonstrated that GB inhibited CRC cell proliferation and colony formation, promoted apoptosis, decreased B-cell lymphoma 2 (BCL-2), increased BCL-2-associated X protein (BAX) and Cleaved Caspase-3, and reduced the phosphorylation of AKT and extracellular signal-regulated kinase (ERK). Notably, GB significantly suppressed tumor growth and reduced Ki-67 expression in the MC38 syngeneic subcutaneous tumor model. **Conclusions:** GB exhibits antitumor activity against CRC by inhibiting proliferation, promoting apoptosis, and inhibiting the PI3K/AKT and MAPK pathways. The study provides a mechanistic basis for the anticancer effects of GB and supports further investigation of its potential as a therapeutic candidate for CRC.

Keywords: ginkgolide B; colorectal neoplasms; phosphatidylinositol 3-kinases; proto-oncogene proteins c-akt; MAP kinase signaling system; apoptosis

1. Introduction

Colorectal cancer (CRC) is a relatively common gastrointestinal malignancy worldwide and continues to be linked to significant rates of occurrence and death [1,2,3,4]. It ranks as the 3rd most frequently identified cancer and the 2nd highest contributor to cancer-related deaths globally, posing a significant risk to public health [5,6,7]. The standard approach to clinical management includes surgical removal, chemotherapy, radiotherapy, and therapies aimed at specific molecular targets. Although these approaches have improved patient survival, chemotherapy and radiotherapy are frequently associated with considerable toxicity, while targeted therapies are often limited by acquired resistance

and high treatment costs [8,9]. Therefore, there is a pressing need to develop antitumor drugs, particularly those that can be used as adjuvant treatments with fewer adverse effects.

Traditional Chinese medicine (TCM) has been widely used as an adjunctive approach in cancer treatment owing to its multi-component, multi-target, and multi-pathway pharmacological characteristics [10,11]. *Ginkgo biloba*, a medicinal plant with a history of clinical use spanning more than 2000 years, has attracted considerable interest because of its diverse pharmacological activities [12,13]. Ginkgolide B (GB), a diterpene lactone isolated from *Ginkgo biloba* leaves, is one of its key bioactive constituents [14]. GB has demonstrated platelet-activating fac-



tor (PAF) antagonistic, antioxidant, anti-inflammatory, and neuroprotective effects [15,16]. More recently, GB has also shown antitumor activity by inhibiting cancer cell proliferation and metastasis through multiple signaling pathways [17,18,19,20]. However, the molecular basis by which GB modulates CRC progression remains insufficiently defined, which hinders its further translational and clinical application.

Network pharmacology (NP) is a systems biology-based approach that enables comprehensive analysis of drug–target–disease interactions and is particularly suitable for investigating the multi-target mechanisms of TCM [21,22,23,24,25]. Docking complements this strategy by predicting the binding interactions between small molecules and protein targets, providing structural evidence to support target identification [26,27,28]. Here, NP, docking, and experimental validation were integrated to explore the effects of GB on CRC. The antitumor activity of GB was assessed using Cell Counting Kit-8 (CCK-8), colony formation assay (CFA), flow cytometry (FCM), and Western blot (WB) analyses, and its efficacy was further evaluated in a mouse syngeneic allograft model. These findings provide insight into the anti-CRC effects of GB and may support its future development as a therapeutic candidate. The study flowchart is illustrated in Fig. 1.

2. Materials and Methods

2.1 Reagents

GB (purity $\geq 99.96\%$; Catalog No. HY-N0784) was purchased from MedChemExpress (MCE, NJ, USA). A DMSO-based stock solution was prepared and kept at -20°C . Before each experiment, it was diluted with the culture medium to the required working concentrations.

2.2 Screening and Standardization of Potential Targets of Ginkgolide B

Potential GB targets were investigated using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP; accessed in December 2025), SwissTargetPrediction (STP; accessed in December 2025), Search Tool for Interactions of Chemicals (STITCH) (accessed in December 2025), and HERB database (HERB) (accessed in December 2025). “Ginkgolide B” was used as the search term, and all retrieved targets were collected. Protein names were converted into gene symbols using the Universal Protein Resource (UniProt) database (accessed in December 2025). Duplicate entries and non-human targets were discarded, yielding the final predicted GB targets.

2.3 Collection of CRC-Related Targets

CRC-associated targets were retrieved from GeneCards and Online Mendelian Inheritance in Man (OMIM). In GeneCards, “colorectal cancer” was used as the search term, using the criterion of Relevance Score ≥ 20 for identifying candidate targets. The same keyword was

utilized to search the OMIM database. Results from the two databases were merged and deduplicated to establish the CRC-associated target dataset.

2.4 Acquisition of Drug–Disease Intersection Targets

The predicted GB targets and CRC-linked targets were imported into Venny 2.1.0 (<https://bioinfogp.cnb.csic.es/tools/venny/>) to identify shared genes. The resulting intersection targets were regarded as candidate targets of GB against CRC and were visualized using a Venn diagram.

2.5 PPI Networks and Identification of Core Targets

Shared targets were imported into STRING, using *Homo sapiens* as the species and 0.4 as the minimum required interaction score. Other parameters were set to default settings [29,30]. The protein–protein interaction (PPI) network was imported into Cytoscape 3.8.0. Network topological parameters were calculated using the NetworkAnalyzer plugin, and core targets were identified according to node degree.

2.6 GO and KEGG Enrichment

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment was undertaken using the Metascape platform. Results were limited to *Homo sapiens*, with $p < 0.01$ as the threshold, and were visualized as bubble plots and bar charts using the platform (<https://www.bioinformatics.com.cn/>) to represent the GO biological process (BP), cellular component (CC), and molecular function (MF) categories and KEGG pathways.

2.7 Molecular Docking

The structure of ginkgolide B was obtained from PubChem (CID: 6324617). High-resolution crystal structures of the selected core target proteins (SRC, PIK3CA, AKT1, HSP90AA1, and ESR1) were downloaded from the Protein Data Bank (PDB IDs: 8BQ3, 8EXL, 3QKL, 1YC1, 1SJ0) [29,31,32].

Protein structures were preprocessed using AutoDockTools 1.5.7. The docking grid was placed on the co-crystallized ligands with dimensions of $20 \times 20 \times 20 \text{ \AA}$ and a grid spacing of $1.0 \text{ \AA}$. Docking reliability was evaluated by redocking the native ligands, with a heavy-atom root mean square deviation (RMSD) $\leq 2.0 \text{ \AA}$ considered acceptable. Docking was performed in AutoDock Vina 1.1.2 using an exhaustiveness value of 8. Each ligand was docked independently three times, and the highest-ranked binding pose was selected for further analysis. Binding affinity was assessed according to the predicted binding energy, where values < -5.0 and < -7.0 kcal/mol represented favorable and strong binding, respectively. Visualizations and evaluations were performed using PyMOL 2.5.0.

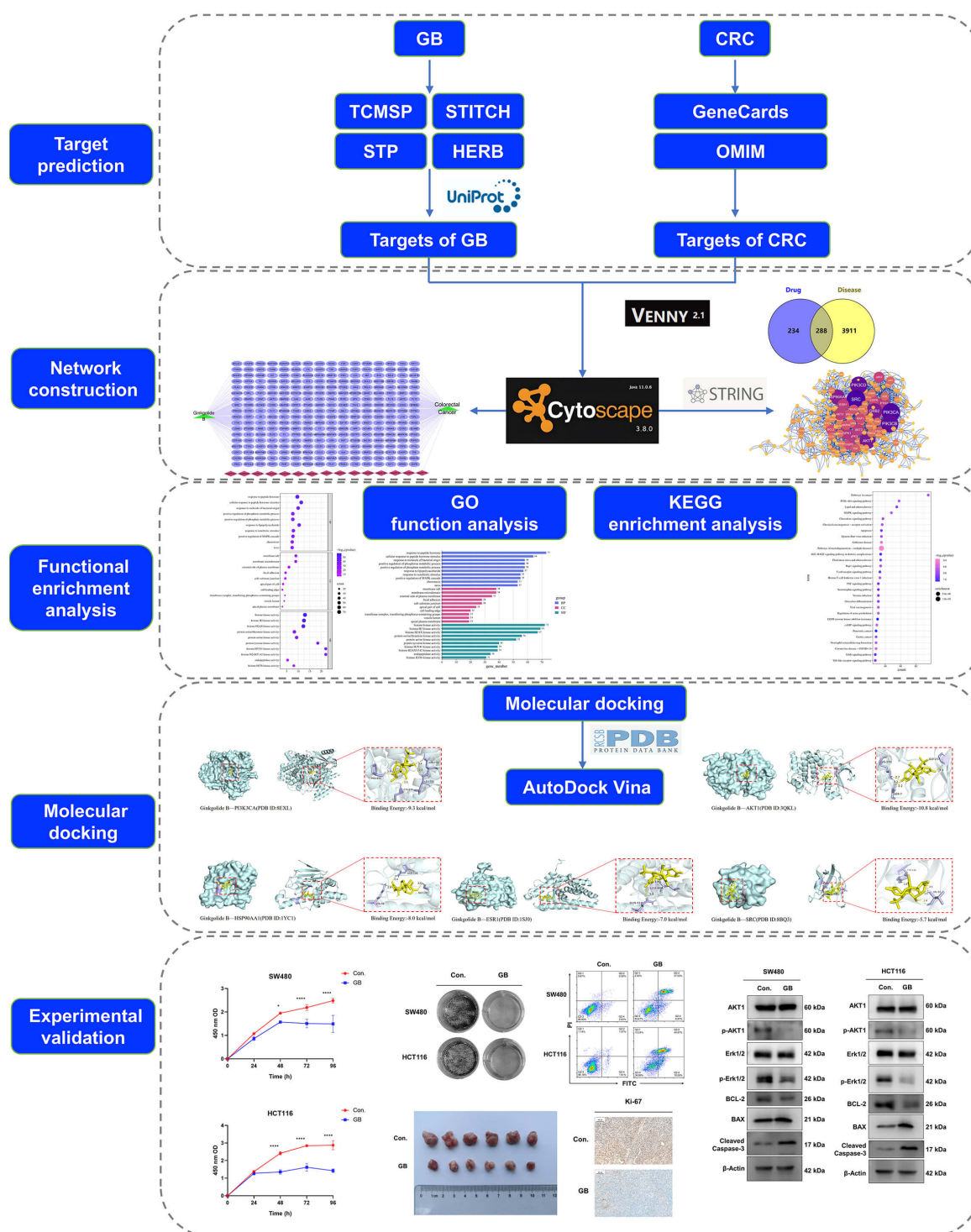


Fig. 1. Flowchart showing the integrated NP, molecular docking, and experimental validation approach. NP, network pharmacology.

2.8 Cell Culture

The human colonic epithelial cell line NCM460 (RRID: CVCL_0460) and the human CRC cell lines SW480 (RRID: CVCL_0546) and HCT116 (RRID:

CVCL_0291) and the mouse colon cancer cell line MC38 (RRID: CVCL_B288) were employed for various analyses in this study. HCT116 and SW480 cells were obtained from the ATCC, whereas NCM460 cells and MC38 cells

were kindly provided by Professor Ceshi Chen (Kunming Medical University). All cell lines were authenticated by Short Tandem Repeat (STR) profiling and were mycoplasma-negative. All cell lines used in this study were within 10 passages.

Cells were grown in RPMI 1640 with 10% FBS (Ex-Cell), 100 µg/mL streptomycin, and 100 U/mL penicillin (Solarbio) at 37 °C with 5% CO₂.

2.9 Cell Proliferation Assay

Evaluation of cell proliferation was carried out while employing CCK-8 and CFA assays. For CCK-8 assays, cells (1000/well) were inoculated in 96-well plates and allowed to adhere overnight before treatment with GB at 0, 5, 10, 20, 40, 80, and 160 µM to determine the half maximal inhibitory concentration (IC₅₀). Viability was assessed at 24, 48, 72, and 96 h (n = 3).

For the CFA, 1000 cells were seeded into 12-well plates (n = 3). Colonies were fixed, stained with crystal violet, imaged, and enumerated after 10 days of culture. All experiments were performed a minimum of three times independently.

2.10 Apoptosis Assays

Apoptosis was examined using an Annexin V-FITC/PI Apoptosis Detection Kit (MultiSciences, Cat. No. AP101). Cells (2 × 10⁵/well) were inoculated into 12-well plates and grown for 24 h (n = 3). After treatment for another 24 h, cells were collected, followed by staining and analysis by FCM. The gating strategy for apoptosis analysis was as follows: cells were first gated on FSC-A vs. SSC-A to exclude debris, then on FSC-H vs. FSC-A to exclude doublets, and finally analyzed for Annexin V-FITC and PI staining. Each experiment was performed with three independent biological replicates.

2.11 Western Blotting

Total protein was isolated using RIPA buffer with protease inhibitors and measured using a Bicinchoninic Acid (BCA) protein assay. Proteins (30 µg) were resolved on SDS-PAGE and then transferred onto PVDF membranes. Primary antibodies were β-Actin (HUABIO, EM21002, 1:5000), AKT1 (#75692), phospho-AKT (#9018), ERK1/2 (#4695), Cleaved Caspase-3 (#9661), and phospho-ERK1/2 (#4370) from Cell Signaling Technology (CST, 1:2000); and BCL-2 (12789-1-AP) and BAX (50599-2-Ig) from Proteintech (1:500). Secondary antibodies were anti-mouse IgG (1:10,000, #511103) or anti-rabbit IgG (1:10,000, #511203) from ZEN-BIOSCIENCE. Detection of protein bands was performed while employing an enhanced chemiluminescence (ECL) kit (Biosharp, BL520A) and visualized with a ChemiDoc XRS+ imaging system (Bio-Rad).

2.12 Animal Experiments

To evaluate GB antitumor activity *in vivo*, male C57BL/6 (6-week-old) mice were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China). Mice were specific pathogen-free (SPF) and were housed under a 12 h light/dark cycle at 22 ± 2 °C and 50 ± 10% humidity with free access to food and water. After acclimatization for 1 week, mice were subcutaneously injected with 2 × 10⁶ MC38 cells suspended in Matrigel. When tumors became palpable, mice were randomly allocated to control and GB treatment groups (n = 6/group) using a random number table. The experimental unit was the individual mouse. The sample size was determined using the resource equation method. GB (20 mg/kg) was administered intraperitoneally (*i.p.*) once daily from day 5 for 14 consecutive days [33,34]. No animals were excluded from the analysis. To minimize potential confounders, mice were housed under the same environmental conditions, and tumor inoculation, drug administration, and tumor measurement were performed by the same investigator. The primary outcome measure was tumor weight at the end of the experiment. Blinding procedures: group allocation, drug administration, tumor measurement, and data analysis were performed blindly. On completion of the experiment, animals were euthanized by cervical dislocation, and tumors were excised, weighed, and photographed. Statistical assumptions were assessed before analysis, including normality and variance homogeneity. All animal procedures were approved by the Institutional Animal Care and Use Committee of Central South University (Ethics Approval Number: CSU-2026-0306) and were conducted in accordance with the ARRIVE guidelines and the NIH Guide for the Care and Use of Laboratory Animals.

2.13 Immunohistochemistry (IHC)

The IHC assays and evaluations were conducted in strict adherence to the previously established protocol [35]. The primary antibody Ki-67 was purchased from Servicebio (GB111499).

2.14 Statistical Analysis

All cellular experiments used three biological replicates. Statistical analyses were conducted using GraphPad Prism 8.0.2. Data are reported as mean ± standard deviation (SD) and were compared using unpaired two-tailed *t*-tests (two groups) and one-way or two-way ANOVA, followed by Tukey's multiple-comparison test (more than two groups). The statistical methods and sample sizes are specified in the corresponding figure legends. For animal experiments, each group contained six mice. A two-sided *p* < 0.05 was considered statistically significant.

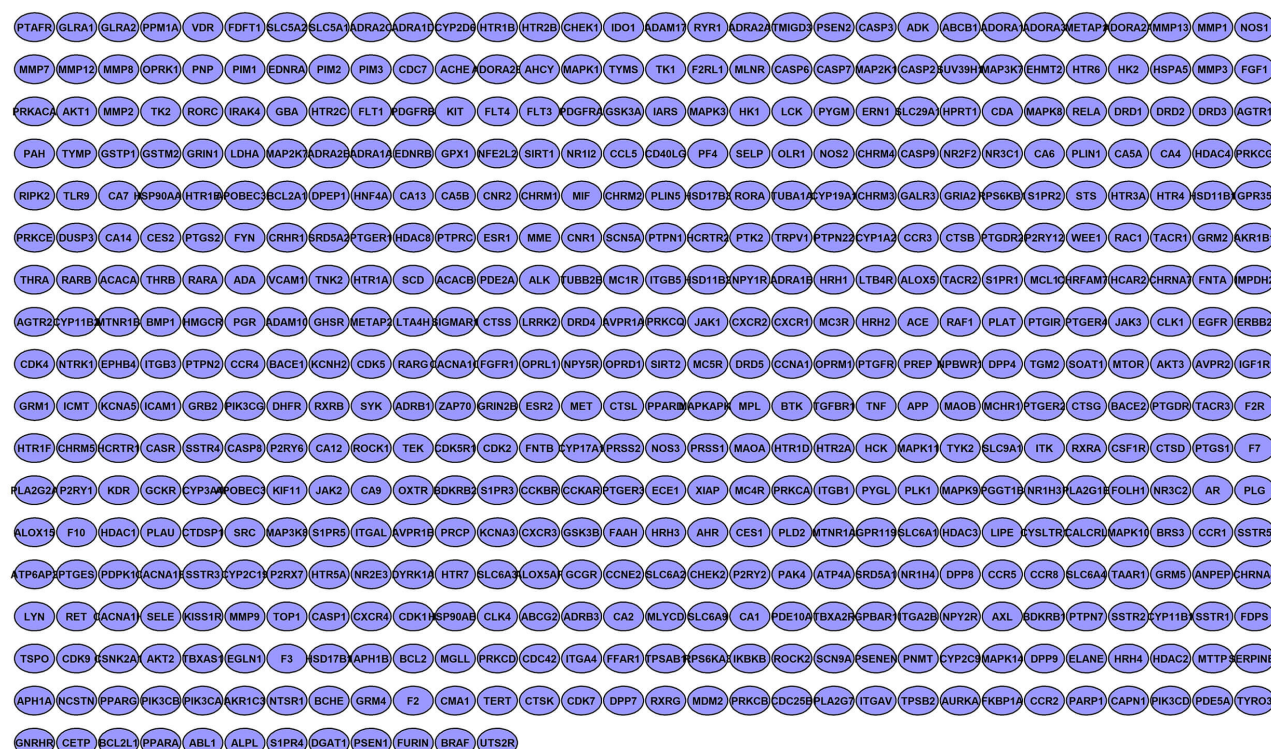


Fig. 2. Ginkgolide B component targets. A total of 522 potential targets were screened, including MAPK1, MAPK3, AKT1, and TNF.

3. Results

3.1 Identification of Candidate Targets of Ginkgolide B

Potential targets of GB were retrieved from the TCMSP, STP, STITCH, and HERB databases and standardized using the UniProt database. After removing duplicates and non-human entries, 522 potential GB targets were identified (Fig. 2 and **Supplementary Table 1**). These targets included several proteins involved in cell signaling and inflammatory responses, such as MAPK1, MAPK3, AKT1, and tumor necrosis factor (TNF).

3.2 Collection of Results of Colorectal Cancer-Related Targets

A total of 4167 CRC-associated targets were retrieved from GeneCards and 199 from OMIM. After integrating the datasets and removing duplicate entries, a final CRC-associated target set comprising 4199 genes was established (**Supplementary Table 2**).

3.3 Screening Results of Drug–Disease Intersection Targets

To identify potential therapeutic targets of GB against CRC, the 522 predicted GB targets were intersected with the 4199 CRC-associated targets using Venny. This analysis identified 288 overlapping targets (Fig. 3A and **Supplementary Table 3**). A compound–target interaction network was then developed using these shared targets (Fig. 3B).

3.4 Construction and Analysis Results of the PPI Network

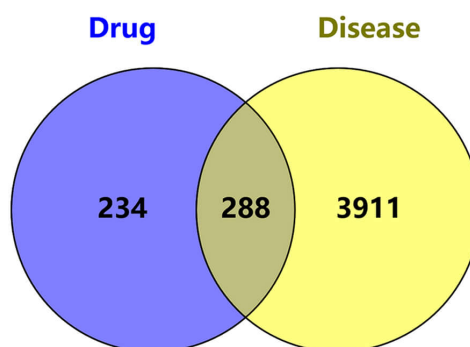
A PPI network containing 288 nodes and 919 edges, with an average node degree of 6.4, was constructed. The enrichment p -value was $<1.0 \times 10^{-16}$, demonstrating that the interactions among the proteins were markedly higher than expected by chance.

The network was visualized using Cytoscape (Fig. 4A,B). Node size and color intensity indicate the degree values. A larger degree value means the node has more connections in the network and a more important biological role. Hub genes were defined as the top 10 nodes ranked by degree centrality. The top 10 hub genes screened according to the degree value were: *SRC*, *AKT1*, *ESR1*, *PIK3CA*, *HSP90AA1*, *MAPK3*, *MAPK1*, *EGFR*, *TNF*, and *PIK3CB*.

3.5 GO Functional Enrichment Analysis (FEA) Results

GO analysis of the 288 overlapping targets using the Metascape platform identified 2961 BP terms, 103 CC terms, and 310 MF terms. Terms with $p < 0.01$ were selected for further visualization [36]. The enriched BP terms largely involved responses to peptide hormones, responses to molecules of bacterial origin, positive regulation of phosphorus metabolic processes, responses to lipopolysaccharide, responses to xenobiotic stimuli, positive regulation of the MAPK cascade, and chemotaxis/taxis (Fig. 5A,B) [37,38,39,40,41].

A



B

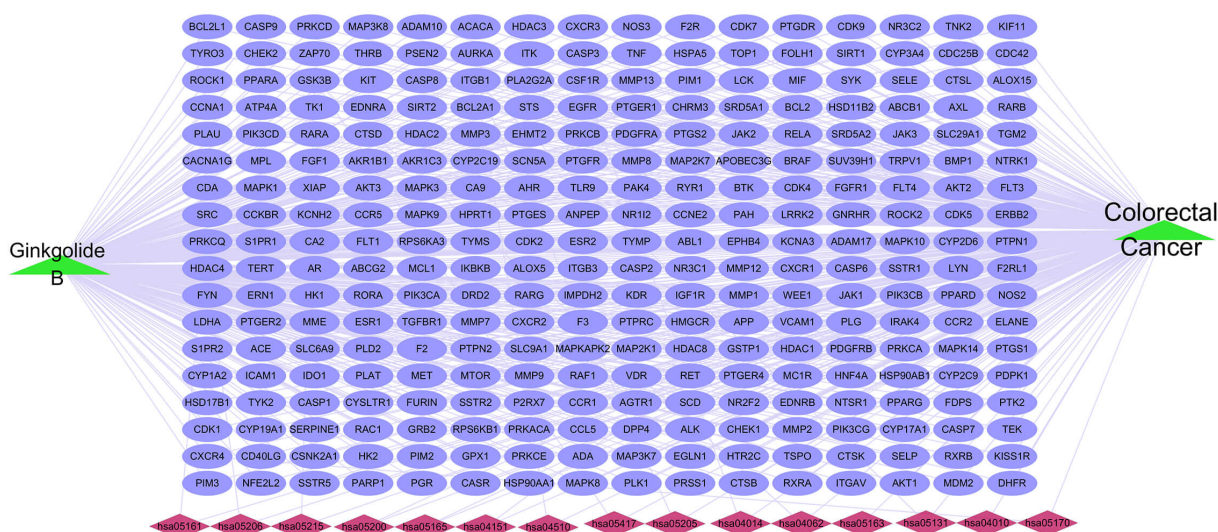


Fig. 3. Drug–disease intersection targets. (A) Venn diagram comparing 522 predicted GB targets and 4199 CRC-associated targets. (B) Compound–target interaction network constructed from the 288 overlapping targets. GB, Ginkgolide B; CRC, colorectal cancer.

3.6 KEGG Pathway Enrichment Analysis (PEA) Results

KEGG PEA was performed using Metascape ($p < 0.01$). A total of 29 significantly enriched pathways were identified (Fig. 6). The major enriched pathways were associated with cancer, PI3K/AKT signaling, lipid and atherosclerosis, MAPK signaling, chemokine signaling, chemical carcinogenesis–receptor activation, and apoptosis [42,43,44,45,46]. The findings suggest that the anticancer activity of GB against CRC is largely mediated by its ability to modulate classical signaling pathways associated with tumor development, such as PI3K/AKT and MAPK.

3.7 Molecular Docking Results

The hub proteins identified by PPI analysis together with key targets reported in the literature (SRC, PIK3CA, AKT1, HSP90AA1, and ESR1) were selected for molecular docking with GB. Docking analysis showed that binding energies for GB were below -5.0 kcal/mol for all selected targets, indicating favorable binding affinity. Among them, AKT1 showed the strongest predicted in-

teraction with GB (-10.8 kcal/mol), followed by PIK3CA (-9.3 kcal/mol), HSP90AA1 (-8.0 kcal/mol), ESR1 (-7.0 kcal/mol), and SRC (-5.7 kcal/mol) (Fig. 7A–E). The docking model showed that GB occupied the active-site pocket of AKT1 and formed multiple hydrogen-bond interactions with residues GLY157, LYS276, and SER-7, together with hydrophobic interactions, supporting stable binding between GB and AKT1.

3.8 GB Inhibits Proliferation and Promotes Apoptosis via the PI3K/AKT and MAPK Axes in CRC Cells

To evaluate the regulatory effects of GB on CRC cell growth, the IC_{50} values of GB were first determined in NCM460, SW480, and HCT116 cells using concentrations of 0, 5, 10, 20, 40, 80, and 160 μ M ($n = 3$). GB showed a substantially higher IC_{50} value in NCM460 cells than in the two CRC cell lines (Supplementary Fig. 1A).

Based on these results, SW480 and HCT116 cells were exposed to 30 μ M GB for 24, 48, 72, and 96 h ($n = 3$). GB treatment significantly reduced the viability of both CRC cell lines relative to the untreated control (Fig. 8A). CFA

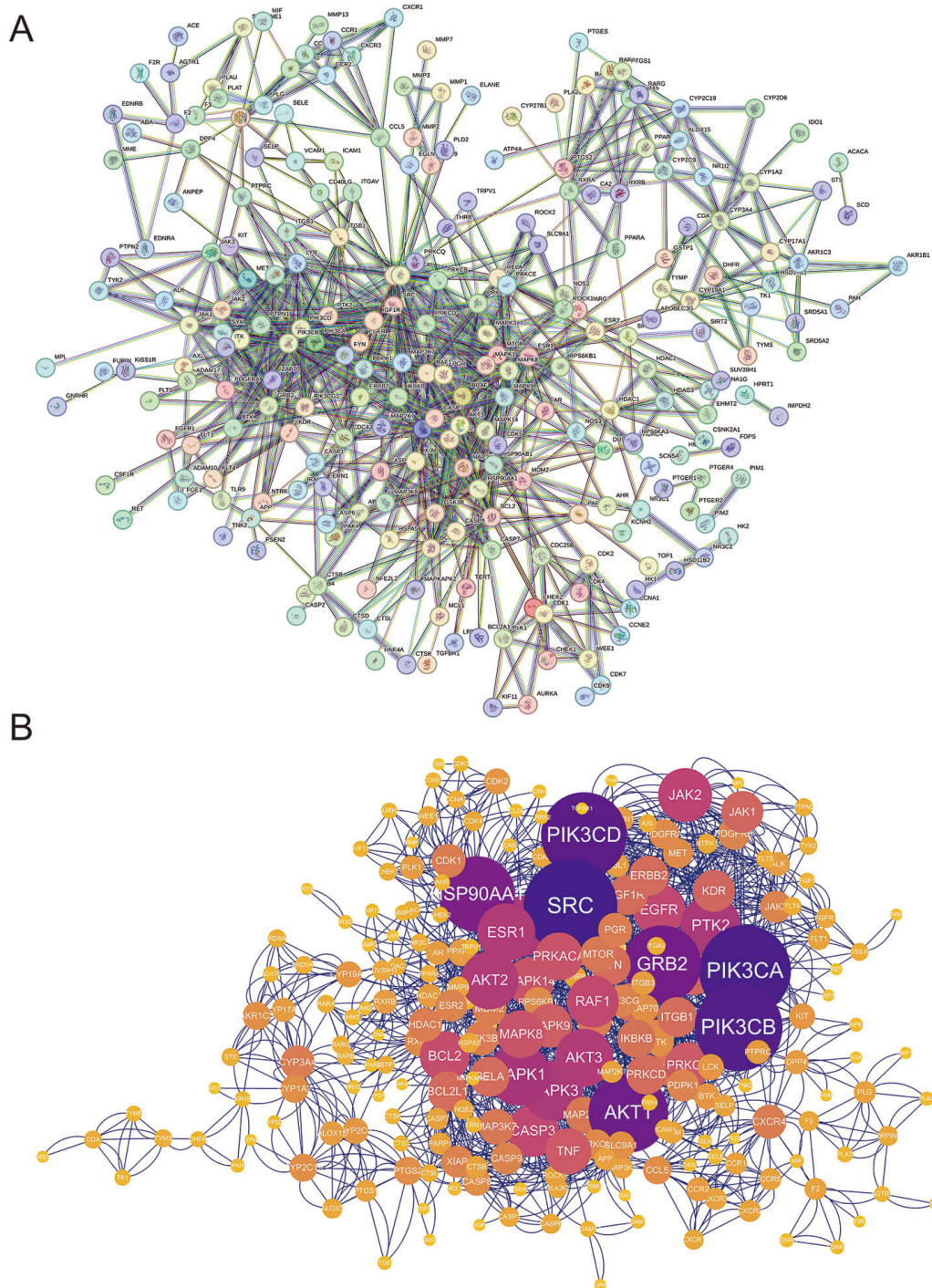


Fig. 4. PPI network analysis. (A) PPI network where proteins are indicated as nodes and interactions as edges. (B) PPI network of potential targets. Node size/color represents degree centrality. PPI, protein–protein interaction.

further showed that treatment with 30 μ M GB for 10 days decreased the colonies formed by SW480 and HCT116 cells ($n = 3$, Fig. 8B). Flow cytometric analysis showed that treatment with 30 μ M GB for 24 h increased apoptosis in both CRC cell lines ($n = 3$, Fig. 8C).

To investigate the molecular changes associated with GB treatment, the levels of apoptosis-associated proteins and components of the PI3K/AKT and MAPK axes were

analyzed by WB. Treatment with 30 μ M GB for 24 h increased the levels of Cleaved Caspase-3 and BAX while reducing those of BCL-2. Furthermore, GB treatment reduced the phosphorylation levels of AKT and ERK1/2 without altering the corresponding total protein levels (Fig. 8D and **Supplementary Fig. 1B,C**).

Further evaluation of the antitumor activity of GB was carried out in MC38 syngeneic subcutaneous tumor model.

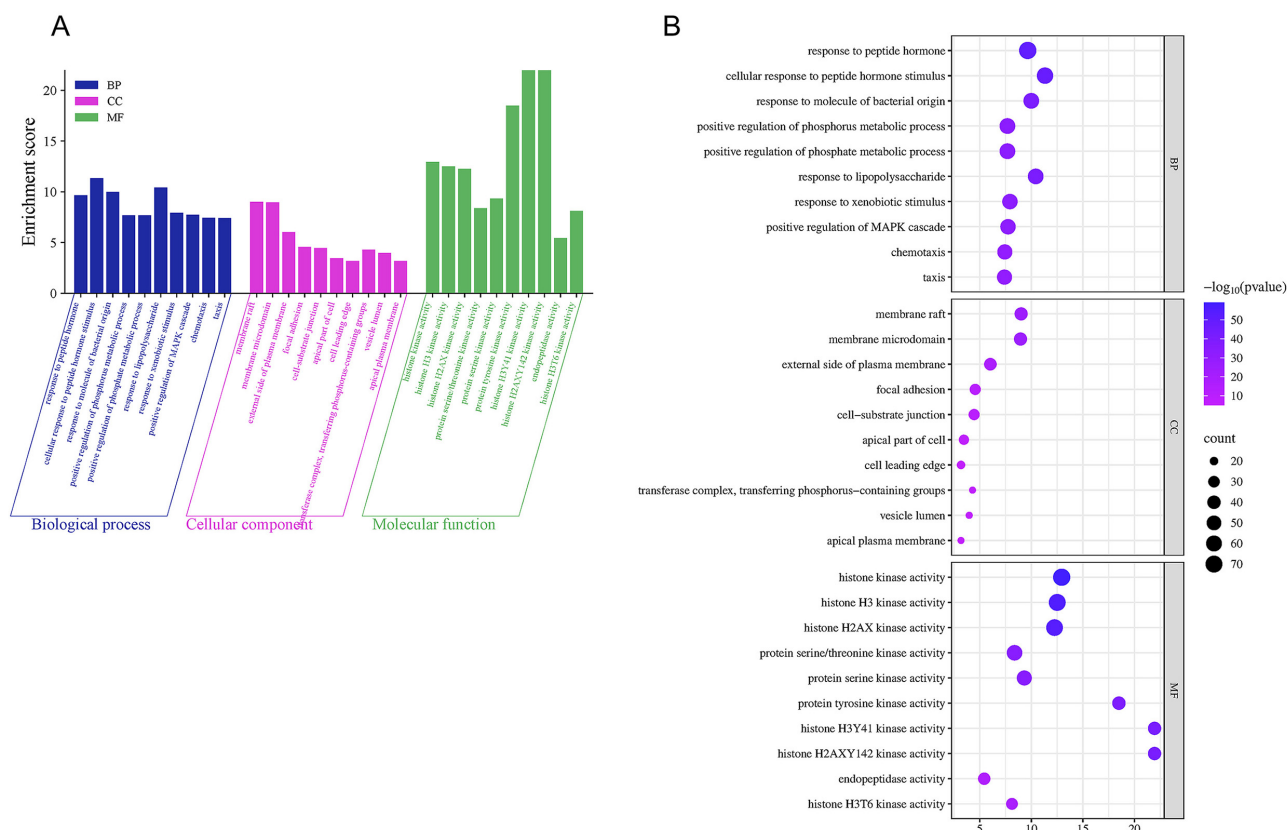


Fig. 5. GO enrichment analysis. (A) GO enrichment analysis of potential targets. GO terms (BP/CC/MF) are arranged in descending order of count. (B) Bubble chart visualization of significantly enriched GO terms. Bubble size represents the gene number, and color intensity reflects the p -value. GO, Gene Ontology.

Mice received daily i.p. injections of GB (20 mg/kg) for 14 days ($n = 6/\text{group}$). Compared with the control group, GB treatment significantly reduced tumor weight (Fig. 8E,F). Immunohistochemical staining demonstrated reduced Ki-67 expression following GB treatment ($n = 3$, Fig. 8G).

4. Discussion

CRC develops and advances through a complex pathological process characterized by the progressive accumulation of genetic alterations and dysregulation of various signaling pathways that collectively drive tumor initiation and progression [47]. Although advances in therapeutic interventions have improved patient outcomes, treatment efficacy remains limited by drug resistance and treatment-related toxicity. Thus, there is continued interest in identifying novel agents that target multiple oncogenic pathways while maintaining favorable safety profiles. Natural products derived from TCM have attracted considerable attention because they frequently exert pharmacological effects through multiple molecular targets and signaling networks [48]. This multi-target mode of action is particularly attractive for complex diseases such as CRC, in which aberrant signaling involves numerous interconnected molecular pathways.

In this study, an integrated strategy was used to investigate the antitumor activity of GB against CRC. NP identified 288 overlapping targets between GB and CRC, and PPI networks highlighted SRC, PIK3CA, AKT1, HSP90AA1, and ESR1 as major hub proteins within the interaction network. FEA further indicated that these targets were associated with the PI3K/AKT and MAPK axes, suggesting involvement of these cascades in the effects of GB. Docking predicted favorable binding affinities between GB and several hub proteins, with AKT1 showing the strongest predicted interaction (-10.8 kcal/mol). Although docking analysis cannot confirm direct target engagement, it provides structural evidence supporting the potential interaction of GB with these candidate proteins.

The computational predictions were further supported by experimental observations. GB inhibited the proliferation of SW480 and HCT116 cells, promoted apoptosis, increased the levels of Cleaved Caspase-3 and BAX, reduced those of BCL-2, and decreased phosphorylation of ERK and AKT. These molecular changes were accompanied by significant inhibition of tumor growth in the MC38 mouse model, together with reduced Ki-67 levels in tumor tissues. The consistency between the computational analyses and the experimental findings strengthens the overall



Fig. 6. KEGG pathway analysis of GB candidate targets identified from CRC-related proteins. KEGG pathway enrichment analysis of 288 overlapping targets ($p < 0.01$). Enriched pathways are represented by nodes. Gene count is indicated by node size; p -value is indicated by color intensity. KEGG, Kyoto Encyclopedia of Genes and Genomes.

evidence supporting the antitumor activity of GB and suggests that modulation of the MAPK and PI3K/AKT pathways may contribute to its biological effects. Rather than relying on a single experimental approach, the present study integrated multiple complementary methods to evaluate the potential mechanisms underlying the antitumor activity of GB.

Previous studies have also reported anticancer activities of GB in several malignancies. Lu et al. [17] demon-

strated that GB inhibited the epithelial–mesenchymal transition and promoted pyroptosis in gastric cancer through the AKT/mTOR signaling pathway. Shao et al. [20] reported that GB suppressed PI3K/AKT signaling, promoted apoptosis, and inhibited the growth of non-small cell lung cancer cells. Our findings extend these observations to CRC and further support the possibility that suppression of PI3K/AKT signaling represents a common mechanism underlying the anticancer activity of GB. Although different

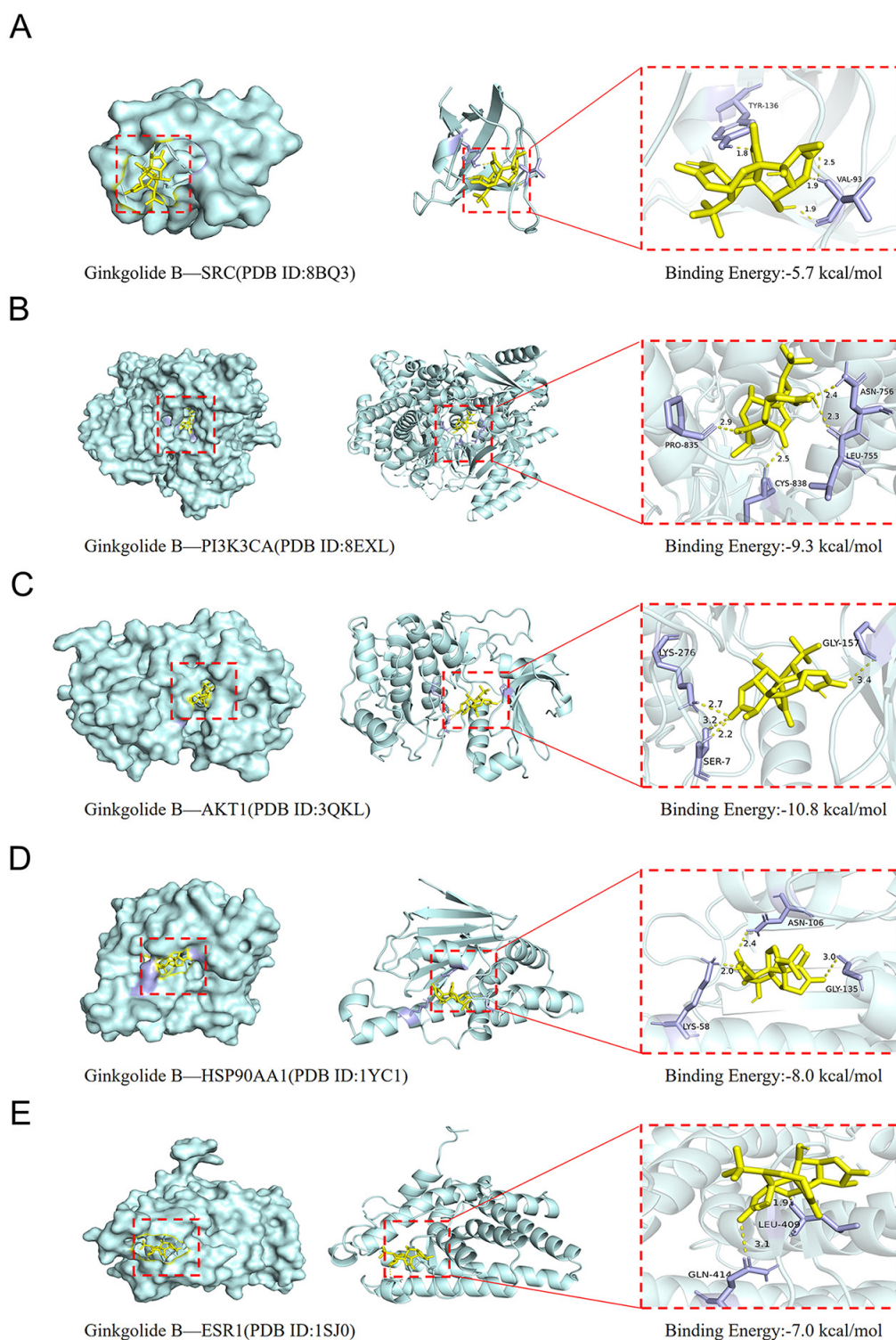


Fig. 7. Molecular docking. (A–E) 3D and 2D diagrams of GB binding with SRC, PIK3CA, AKT1, HSP90AA1, and ESR1, respectively. Binding energies (kcal/mol) are: SRC (−5.7), PIK3CA (−9.3), AKT1 (−10.8), HSP90AA1 (−8.0), and ESR1 (−7.0). AKT1 exhibits the strongest binding affinity. GB forms hydrogen bonds with GLY157, LYS276, and SER-7 residues of AKT1.

tumor types exhibit distinct molecular characteristics, the consistency of these findings suggests that the biological effects of GB may not be restricted to a single cancer type. In addition to GB, other ginkgolides exhibit distinct phar-

macological properties. Ginkgolide A has been reported to protect against cardiovascular injury and promote cardiomyocyte differentiation from pluripotent stem cells [49], whereas ginkgolide C possesses anti-inflammatory activ-

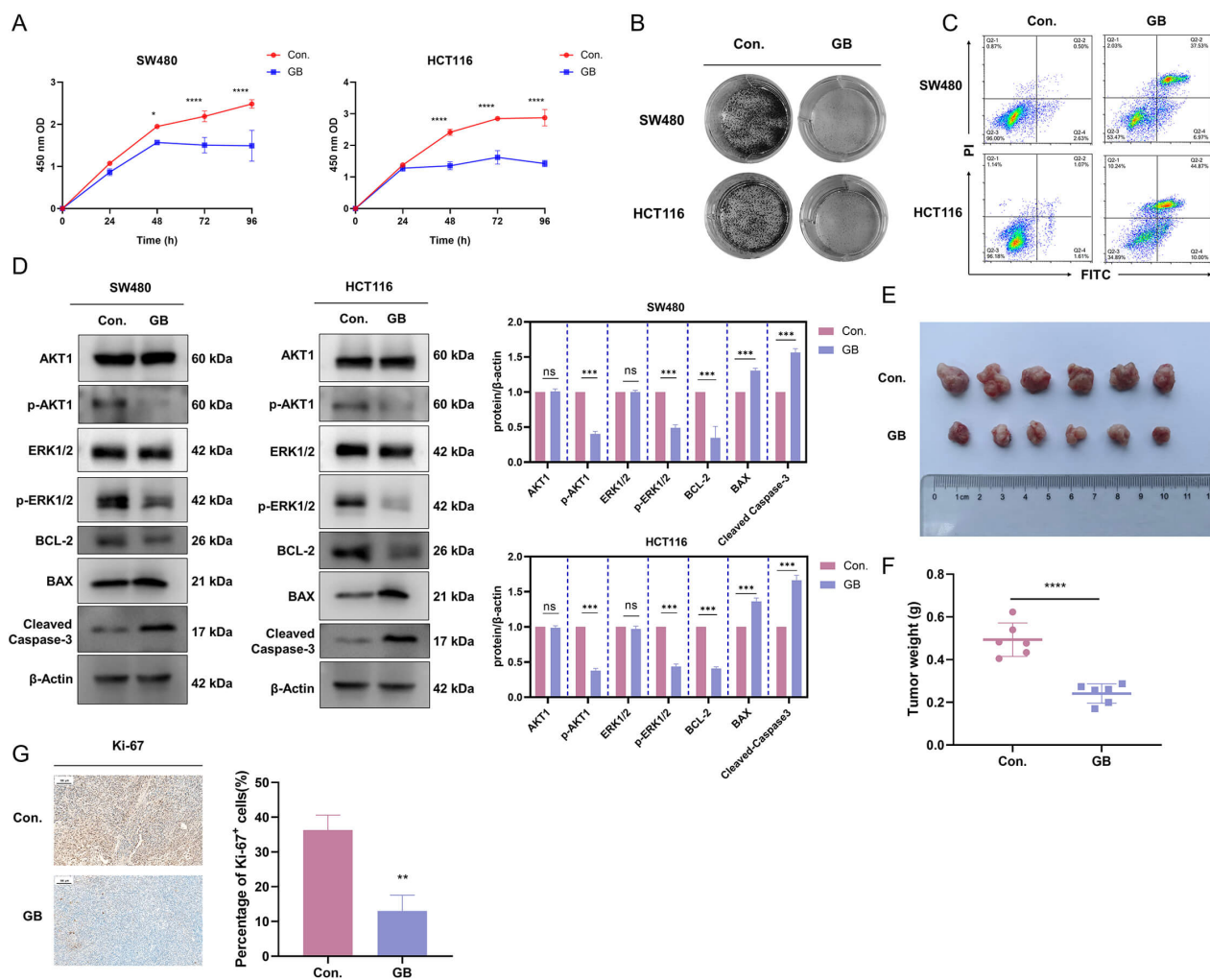


Fig. 8. GB inhibits the proliferation of CRCs. (A) CCK-8 assay. HCT116 and SW480 cells were treated with GB (30 μ M) for 0, 24, 48, 72, and 96 h (n = 3). Cell viability was measured at each time point. (B) CFA. Cells were exposed to GB (30 μ M) for 10 days (n = 3). (C) FCM analysis of apoptosis. HCT116 and SW480 cells treated with GB (30 μ M) for 24 h (n = 3) were treated with Annexin V-FITC/PI and evaluated by FCM. (D) WB analysis. SW480 and HCT116 cells were subjected to treatment with GB (30 μ M) for 24 h. Detection of protein levels of BAX, BCL-2, Cleaved Caspase-3, p-AKT1, AKT1, p-ERK1/2, and ERK1/2 was carried out while employing WB. β -actin represented the loading control. (E,F) *In vivo* MC38 syngeneic subcutaneous tumor model. MC38 cells were subcutaneously injected into C57BL/6 mice (n = 6/group). Mice received i.p. injections of GB (20 mg/kg) daily for 14 days, and tumor weight was recorded at the end of the experiment. (G) Ki-67 IHC staining of subcutaneous tumor tissues from mice in control and GB treatment groups (n = 3). Scale bar = 100 μ m. ns, not significant, * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

ity and protects against myocardial and cerebral ischemia-reperfusion injury [50]. Together, these observations highlight the broad biological activities of ginkgolides and support further investigation of this class of natural compounds for therapeutic development.

Previous research has shown that persistent phosphorylation of AKT and ERK contributes to cetuximab resistance, whereas inhibition of endocytosis using prochlorperazine reduces activation of both pathways [51]. In our study, GB likewise reduced the phosphorylation levels of AKT and ERK in CRC cells. Although these observations do not establish that GB can overcome resistance to anti-

EGFR therapy, they raise the possibility that GB may have value as part of combination therapeutic strategies. Further investigations using drug combination experiments and mechanistic studies will be necessary to determine whether GB enhances the efficacy of existing targeted therapies or delays the development of therapeutic resistance [52].

5. Limitations

There are several limitations to the study. First, the NP analysis relied on publicly available databases and therefore reflects computational predictions rather than experimentally verified target interactions. Second, although molecu-

lar docking suggested favorable binding between GB and several candidate proteins, direct target engagement was not experimentally confirmed. Third, mechanistic conclusions were based primarily on changes in protein phosphorylation, and additional studies using genetic or pharmacological manipulation of the MAPK and PI3K/AKT pathways are required to establish causal relationships. Finally, the *in vitro* experiments were performed in human CRC cell lines, whereas the *in vivo* studies used the MC38 syngeneic subcutaneous tumor model. Although this model preserves an intact immune microenvironment, species-specific differences may influence drug responses and signaling activity. Future studies should validate these findings using patient-derived xenograft (PDX) models, humanized mouse models, or additional preclinical systems that more closely recapitulate human CRC biology. Such studies will help further define the therapeutic potential of GB and facilitate its translation toward clinical application.

6. Conclusions

NP, docking, and experimental validation were integrated for the investigation of the antitumor effects of GB against CRC. GB inhibited CRC cell proliferation by negatively regulating the PI3K/AKT and MAPK axes. These findings provide evidence that GB is a promising candidate for CRC therapy and establish a foundation for future mechanistic and preclinical studies.

Abbreviations

GB, Ginkgolide B; CRC, Colorectal Cancer; TCM, Traditional Chinese Medicine; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; STP, SwissTargetPrediction; STITCH, Search Tool for Interactions of Chemicals; HERB, HERB database; UniProt, Universal Protein Resource; GeneCards, GeneCards database; OMIM, Online Mendelian Inheritance in Man; BP, Biological Process; CC, Cellular Component; MF, Molecular Function; TNF, Tumor Necrosis Factor; SRC, SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase; ESR1, Estrogen Receptor 1; HSP90AA1, Heat Shock Protein 90 Alpha Family Class A Member 1.

Availability of Data and Materials

Publicly available databases used in this study include TCMSP (<https://tcm-sp-e.com/>), SwissTargetPrediction (<http://www.swisstargetprediction.ch>), STITCH (<http://stitch.embl.de>), HERB (<http://herb.ac.cn/>), GeneCards (<https://www.genecards.org/>), OMIM (<https://omim.org/>), STRING (<https://string-db.org/>), and the RCSB PDB (<http://s://www.rcsb.org/>). All datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

Author Contributions

Conceptualization: QX and GS; Data accumulation, writing, and original draft preparation: QL and XS; Figure preparation: QL, AA and NA; Data curation and Writing – review & editing: MW and GY; Supervision and resources: QX and GY. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

For animal studies, all experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of Central South University (Ethics Approval Number: CSU-2026-0306) and were conducted in accordance with the ARRIVE guidelines and the NIH Guide for the Care and Use of Laboratory Animals.

Acknowledgment

We appreciate the assistance from the members of the Yin laboratory who were actively involved in this work.

Funding

This work was supported by the National Natural Science Foundation of China (No.82372711, No.82303495), Natural Science Foundation of Hunan Province (2023JJ40910).

Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used Grammarly in order to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/FBL55020>.

References

- [1] Xiong L, Wei Y, Jia Q, Chen J, Chen T, Yuan J, et al. The application of extracellular vesicles in colorectal cancer metastasis and drug resistance: recent advances and trends. *Journal of Nanobiotechnology*. 2023; 21: 143. <https://doi.org/10.1186/s12951-023-01888-1>
- [2] Wang K, Fu S, Dong L, Zhang D, Wang M, Wu X, et al. Periplocin suppresses the growth of colorectal cancer cells by triggering LGALS3 (galectin 3)-mediated lysophagy.

- Autophagy. 2023; 19: 3132–3150. <https://doi.org/10.1080/15548627.2023.2239042>
- [3] Zhou H, Zhu L, Song J, Wang G, Li P, Li W, et al. Liquid biopsy at the frontier of detection, prognosis and progression monitoring in colorectal cancer. *Molecular Cancer*. 2022; 21: 86. <https://doi.org/10.1186/s12943-022-01556-2>
 - [4] Wang C, Hong H, Zhou L, Chu F, Chen X. BRD4-S Drives Colorectal Cancer Progression via DDX27-Regulated Splicing and MAPK Signaling Activation. *Frontiers in Bioscience (Landmark Edition)*. 2025; 30: 46158. <https://doi.org/10.31083/FB L46158>
 - [5] Tao XY, Li QQ, Zeng Y. Clinical application of liquid biopsy in colorectal cancer: detection, prediction, and treatment monitoring. *Molecular Cancer*. 2024; 23: 145. <https://doi.org/10.1186/s12943-024-02063-2>
 - [6] Liu Y, Liu Z, Hu Y, Ling Y, Qing S, Liu Y, et al. Caffeine enhances antitumor T-cell activity by suppressing kynurenine pathway in colorectal cancer. *Nature Communications*. 2025; 16: 5906. <https://doi.org/10.1038/s41467-025-60958-0>
 - [7] Mao T, Qin F, Zhang M, Li J, Li J, Lai M. Elevated serum β -hydroxybutyrate, a circulating ketone metabolite, accelerates colorectal cancer proliferation and metastasis via ACAT1. *Oncogene*. 2023; 42: 1889–1899. <https://doi.org/10.1038/s41388-023-02700-y>
 - [8] Johdi NA, Sukor NF. Colorectal Cancer Immunotherapy: Options and Strategies. *Frontiers in Immunology*. 2020; 11: 1624. <https://doi.org/10.3389/fimmu.2020.01624>
 - [9] Hu F, Song D, Yan Y, Huang C, Shen C, Lan J, et al. IL-6 regulates autophagy and chemotherapy resistance by promoting BECN1 phosphorylation. *Nature Communications*. 2021; 12: 3651. <https://doi.org/10.1038/s41467-021-23923-1>
 - [10] Wang S, Fu JL, Hao HF, Jiao YN, Li PP, Han SY. Metabolic reprogramming by traditional Chinese medicine and its role in effective cancer therapy. *Pharmacological Research*. 2021; 170: 105728. <https://doi.org/10.1016/j.phrs.2021.105728>
 - [11] Lu F, Wang D, Li RL, He LY, Ai L, Wu CJ. Current strategies and technologies for finding drug targets of active components from traditional Chinese medicine. *Frontiers in Bioscience (Landmark Edition)*. 2021; 26: 572–589. <https://doi.org/10.52586/4968>
 - [12] Liu Y, Ye J, Fan Z, Wu X, Zhang Y, Yang R, et al. Ginkgetin Alleviates Inflammation and Senescence by Targeting STING. *Advanced Science (Weinheim, Baden-Wurtemberg, Germany)*. 2025; 12: e2407222. <https://doi.org/10.1002/adv.202407222>
 - [13] Sun S, Li Y, Chu L, Kuang X, Song J, Sun C. Full-length sequencing of ginkgo transcriptomes for an in-depth understanding of flavonoid and terpenoid trilactone biosynthesis. *Gene*. 2020; 758: 144961. <https://doi.org/10.1016/j.gene.2020.144961>
 - [14] Xie Q, Li H, Lu D, Yuan J, Ma R, Li J, et al. Neuroprotective Effect for Cerebral Ischemia by Natural Products: A Review. *Frontiers in Pharmacology*. 2021; 12: 607412. <https://doi.org/10.3389/fphar.2021.607412>
 - [15] Xia SH, Fang DC. Pharmacological action and mechanisms of ginkgolide B. *Chinese Medical Journal*. 2007; 120: 922–928.
 - [16] Qin H, Jiang Y, Wang Y, Guo R, Feng J, Yu H, et al. Preparation, purification and pharmacological effects of the ginkgolide B: A review. *Fitoterapia*. 2026; 191: 107231. <https://doi.org/10.1016/j.fitote.2026.107231>
 - [17] Lu X, Zhang Y, Wang R, Li Z. Ginkgolide B Inhibits EMT and Promotes Pyroptosis in Gastric Cancer via AKT/mTOR Pathway. *Drug Design, Development and Therapy*. 2025; 19: 2491–2502. <https://doi.org/10.2147/DDDT.S485240>
 - [18] Kawasaki K, Kasamatsu A, Ando T, Saito T, Nobuchi T, Nozaki R, et al. Ginkgolide B Regulates CDDP Chemoresistance in Oral Cancer via the Platelet-Activating Factor Receptor Pathway. *Cancers*. 2021; 13: 6299. <https://doi.org/10.3390/cancer s13246299>
 - [19] Lai DW, Chu PY, Sheu ML, Tsai YC, Lee YH, Liu SC, et al. The potential immunotherapy effect of Ginkgolide B thwarts oral squamous cell carcinoma progression by targeting the SREBP1/KLK8/CCL22 axis. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*. 2025; 136: 156249. <https://doi.org/10.1016/j.phymed.2024.156249>
 - [20] Shao L, Song Z, Cong J, Ma J, Du A. Ginkgolide B inhibits non-small cell lung cancer cells by modulating the integrin β 3 pathway. *Toxicology and Applied Pharmacology*. 2026; 511: 117806. <https://doi.org/10.1016/j.taap.2026.117806>
 - [21] Nogales C, Mamdouh ZM, List M, Kiel C, Casas AI, Schmidt HHHW. Network pharmacology: curing causal mechanisms instead of treating symptoms. *Trends in Pharmacological Sciences*. 2022; 43: 136–150. <https://doi.org/10.1016/j.tips.2021.11.004>
 - [22] Zhou W, Zhang H, Wang X, Kang J, Guo W, Zhou L, et al. Network pharmacology to unveil the mechanism of Muluodan in the treatment of chronic atrophic gastritis. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*. 2022; 95: 153837. <https://doi.org/10.1016/j.phymed.2021.153837>
 - [23] Wu P, Chen L, Xu YX, Ren YY, Dong XM, Fan JX, et al. Decoding herbal medicine: AI-powered omics and network pharmacology. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*. 2025; 148: 157453. <https://doi.org/10.1016/j.phymed.2025.157453>
 - [24] Zhao L, Zhang H, Li N, Chen J, Xu H, Wang Y, et al. Network pharmacology, a promising approach to reveal the pharmacology mechanism of Chinese medicine formula. *Journal of Ethnopharmacology*. 2023; 309: 116306. <https://doi.org/10.1016/j.jep.2023.116306>
 - [25] Xiao Q, Yan Y, Zhao Z, Zhang X, Jiang T, Kong F. A Preliminary Investigation of the Anti-*Salmonella Enteritidis* Potential of Quercetin in Chickens Using Network Pharmacology, Molecular Docking, and In Vitro Antibacterial Assays. *Current Issues in Molecular Biology*. 2026; 48: 409. <https://doi.org/10.3390/cimb48040409>
 - [26] Li T, Guo R, Zong Q, Ling G. Application of molecular docking in elaborating molecular mechanisms and interactions of supramolecular cyclodextrin. *Carbohydrate Polymers*. 2022; 276: 118644. <https://doi.org/10.1016/j.carbpol.2021.118644>
 - [27] Bai G, Pan Y, Zhang Y, Li Y, Wang J, Wang Y, et al. Research advances of molecular docking and molecular dynamic simulation in recognizing interaction between muscle proteins and exogenous additives. *Food Chemistry*. 2023; 429: 136836. <https://doi.org/10.1016/j.foodchem.2023.136836>
 - [28] Bisht A, Tewari D, Kumar S, Chandra S. Network pharmacology, molecular docking, and molecular dynamics simulation to elucidate the mechanism of anti-aging action of *Tinospora cordifolia*. *Molecular Diversity*. 2024; 28: 1743–1763. <https://doi.org/10.1007/s11030-023-10684-w>
 - [29] Zhang T, Wang T, Yu K, Huang C, Bao K. Aspartame and ischemic stroke: unraveling the molecular link through network toxicology and molecular docking analysis. *Scientific Reports*. 2025; 15: 23871. <https://doi.org/10.1038/s41598-025-08898-z>
 - [30] Luo Y, Liu C, Liu J, Wang H, Fu Y, Liu Y. Exploration of Liuwei Dihuang Pill on periodontitis based on network pharmacology and molecular docking. *Medicine*. 2025; 104: e42466. <https://doi.org/10.1097/MD.00000000000042466>
 - [31] AlKharboush DF, Khayat MT, Jamal A, El-Araby ME, Awaji AA, Khan MI, et al. Exploring a kinase inhibitor targeting PI3KCA mutant cancer cells. *Journal of Biomolecular Structure & Dynamics*. 2026; 44: 779–796. <https://doi.org/10.1080/07391102.2025.2502137>
 - [32] Jeyaraj G, Yang B, Sathishkumar K, Chokkakula S, Almutairi BO, Xie W. Pharmacogenomic and *in silico* identification of

- isoform-selective AKT inhibitors from *Pithecellobium dulce* for precision cancer therapy. *Frontiers in Pharmacology*. 2026; 16: 1744408. <https://doi.org/10.3389/fphar.2025.1744408>
- [33] Zheng PD, Mungur R, Zhou HJ, Hassan M, Jiang SN, Zheng JS. Ginkgolide B promotes the proliferation and differentiation of neural stem cells following cerebral ischemia/reperfusion injury, both *in vivo* and *in vitro*. *Neural Regeneration Research*. 2018; 13: 1204–1211. <https://doi.org/10.4103/1673-5374.232476>
- [34] Zhang XY, Xu H, Ren XW, Li QX, Hong C, Chen YF, et al. Nuclear localization of platelet activating factor receptor accounts for microglial phagocytosis in ischemic stroke. *Neurobiology of Disease*. 2025; 213: 107020. <https://doi.org/10.1016/j.nbd.2025.107020>
- [35] Xiao Q, Xiao J, Liu J, Liu J, Shu G, Yin G. Metformin suppresses the growth of colorectal cancer by targeting IN-HBA to inhibit TGF- β /PI3K/AKT signaling transduction. *Cell Death & Disease*. 2022; 13: 202. <https://doi.org/10.1038/s41419-022-04649-4>
- [36] Zhang L, Han L, Wang X, Wei Y, Zheng J, Zhao L, et al. Exploring the mechanisms underlying the therapeutic effect of *Salvia miltiorrhiza* in diabetic nephropathy using network pharmacology and molecular docking. *Bioscience Reports*. 2021; 41: BSR20203520. <https://doi.org/10.1042/BSR20203520>
- [37] Liu N, Pang X, Zhang H, Ji P. The cGAS-STING Pathway in Bacterial Infection and Bacterial Immunity. *Frontiers in Immunology*. 2022; 12: 814709. <https://doi.org/10.3389/fimmu.2021.814709>
- [38] Yu J, Gao X, Shi H, Zhang L, Nie W, Zhang R, et al. Activation of Nuclear Receptor CAR: A Pathway to Delay Aging through Enhanced Capacity for Xenobiotic Resistance. *Advanced Science (Weinheim, Baden-Wurtemberg, Germany)*. 2025; 12: e2416823. <https://doi.org/10.1002/advs.202416823>
- [39] Klomp JA, Klomp JE, Stalneck CA, Bryant KL, Edwards AC, Drizyte-Miller K, et al. Defining the KRAS- and ERK-dependent transcriptome in KRAS-mutant cancers. *Science (New York, N.Y.)*. 2024; 384: eadk0775. <https://doi.org/10.1126/science.adk0775>
- [40] Wang G, Liu Z, Li M, Li Y, Alvi SS, Ansari IA, et al. Ginkgolide B Mediated Alleviation of Inflammatory Cascades and Altered Lipid Metabolism in HUVECs via Targeting PCSK-9 Expression and Functionality. *BioMed Research International*. 2019; 2019: 7284767. <https://doi.org/10.1155/2019/7284767>
- [41] Li X, Du Y, Huang S, Yang Y, Lu D, Zhang J, et al. Exploring the molecular mechanism of glycyrrhetic acid in the treatment of gastric cancer based on network pharmacology and experimental validation. *Aging*. 2023; 15: 3839–3856. <https://doi.org/10.18632/aging.204718>
- [42] Glaviano A, Foo ASC, Lam HY, Yap KCH, Jacot W, Jones RH, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Molecular Cancer*. 2023; 22: 138. <https://doi.org/10.1186/s12943-023-01827-6>
- [43] Deng X, Li Y, Chen Y, Hu Q, Zhang W, Chen L, et al. Paeoniflorin protects hepatocytes from APAP-induced damage through launching autophagy via the MAPK/mTOR signaling pathway. *Cellular & Molecular Biology Letters*. 2024; 29: 119. <https://doi.org/10.1186/s11658-024-00631-4>
- [44] Jia SN, Han YB, Yang R, Yang ZC. Chemokines in colon cancer progression. *Seminars in Cancer Biology*. 2022; 86: 400–407. <https://doi.org/10.1016/j.semcancer.2022.02.007>
- [45] Shizu R, Ishimura M, Nobusawa S, Hosaka T, Sasaki T, Kakizaki S, et al. The influence of the long-term chemical activation of the nuclear receptor pregnane X receptor (PXR) on liver carcinogenesis in mice. *Archives of Toxicology*. 2021; 95: 1089–1102. <https://doi.org/10.1007/s00204-020-02955-4>
- [46] Moyer A, Tanaka K, Cheng EH. Apoptosis in Cancer Biology and Therapy. *Annual Review of Pathology*. 2025; 20: 303–328. <https://doi.org/10.1146/annurev-pathmechdis-051222-115023>
- [47] Wang H, Jiao W, Li D, Yu Z, Luo H, Zhang J, et al. LncRNA HOTTIP modulated by Hedgehog signaling drives colorectal cancer progression by promoting HUWE1-mediated ubiquitin–proteasome degradation of p53. *Cell Death & Disease*. 2025; 16: 502. <https://doi.org/10.1038/s41419-025-07817-4>
- [48] Dang W, Guo P, Song X, Zhang Y, Li N, Yu C, et al. Nuclear Targeted Peptide Combined With Gambogic Acid for Synergistic Treatment of Breast Cancer. *Frontiers in Chemistry*. 2022; 9: 821426. <https://doi.org/10.3389/fchem.2021.821426>
- [49] Xu W, Wu X, Lei Z, Dang X, Shi H, Li J, et al. Ginkgolide A enhances cardiomyocyte differentiation from pluripotent stem cells by targeting cytochrome c to attenuate intrinsic apoptosis. *The Journal of Biological Chemistry*. 2026; 302: 113224. <https://doi.org/10.1016/j.jbc.2026.113224>
- [50] Li B, Zhang B, Li Z, Li S, Li J, Wang A, et al. Ginkgolide C attenuates cerebral ischemia/reperfusion-induced inflammatory impairments by suppressing CD40/NF- κ B pathway. *Journal of Ethnopharmacology*. 2023; 312: 116537. <https://doi.org/10.1016/j.jep.2023.116537>
- [51] Wu C, Liu X, Liu R, Song S, Zheng ZF, Zeng Y, et al. Sustained Endocytosis Inhibition via Locally-Injected Drug-Eluting Hydrogel Improves ADCC-Mediated Antibody Therapy in Colorectal Cancer. *Advanced Science (Weinheim, Baden-Wurtemberg, Germany)*. 2025; 12: e2407239. <https://doi.org/10.1002/advs.202407239>
- [52] Long X, Liu X, Xia W, Liu L, Chen W. Brexpiprazole Prevents the Malignant Progression of Human Colorectal Cancer Cells and Increases Its Sensitivity to EGFR Inhibition. *Frontiers in Bioscience (Landmark Edition)*. 2024; 29: 174. <https://doi.org/10.31083/j.fbl2905174>