Chinese Herbal Medicines 16 (2024) 548–557

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Review

Mechanism of action of protopanaxadiol ginsenosides on hepatocellular carcinoma and network pharmacological analysis

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ARTICLE INFO

Article history:
Received 27 February 2024
Revised 24 March 2024
Accepted 18 June 2024
Available online 20 June 2024

Keywords:
anti-hepatocellular carcinoma
ginsenoside Rb_1
ginsenoside Rd
ginsenoside Rg_3
ginsenoside Rg_5
ginsenoside Rh_2
ginsenoside Rk_1
network pharmacology
protopanaxadiol ginsenosides

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies globally, posing a major challenge to global health care. Protopanaxadiol ginsenosides (PDs) have been believed to significantly improve liver diseases. PDs, such as Rg_3, have been developed as a new class of anti-cancer drugs. Ginsenosides Rb_1, Rd, Rg_3, and Rh_2 exhibit effective anti-inflammatory and anti-tumor activities. Studies have confirmed that PDs could be used to treat HCC. However, the mechanism of action of PDs on HCC remains unclear. In the study, we reviewed the anti-HCC effects and mechanisms of PDs including Rb_1, Rd, Rg_3, Rg_5, Rh_2, Rk_1, and Compound K (CK). Then, we searched for relevant targets of PDs and HCC from databases and enriched them for analysis. Subsequently, molecular docking was simulated to reveal molecular mechanisms. We found that PDs may treat HCC through multiple signaling pathways and related targets. PDs could inhibit the proliferation, invasion, and metastasis of HCC while promoting apoptosis and inducing differentiation. In conclusion, this review and network pharmacological analysis might offer a direction for in-depth research on related mechanisms. These insights will aid in the direction of further pharmacological studies and the development of safe and effective clinical drugs.

Contents

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https://doi.org/10.1016/j.chmed.2024.06.002
1674-6384/

1. Introduction

Hepatocellular carcinoma (HCC) refers to malignant tumors originating in hepatocytes that account for approximately 90% of all primary liver cancers (Llovet et al., 2021), which is the third deadliest malignancy worldwide and a significant complication in patients with chronic liver disease and hepatitis cirrhosis (Bruix, Boix, Sala, & Llovet, 2004). HCC development is associated with four primary factors: viral infections, hereditary diseases that disrupt intracellular pathways, chemical toxins, and metabolic syndrome (Klungboonkrong, Das, & McLennan, 2017), many cellular phenomena, such as tumor microenvironment, inflammation, oxidative stress, and hypoxia, work with various molecular events to promote tumorigenesis, development, and metastasis (Aravalli, Cressman, & Steer, 2013). Traditional treatments for HCC include radical or palliative hepatectomy, radioactive seed implantation, transarterial chemoembolization (TACE), radiofrequency ablation (RFA), and liver transplantation (Liu et al., 2016). Although these methods effectively address local lesions, they may not completely eliminate residual cancer cells, potentially leading to tumor recurrence and metastasis (Xie et al., 2018). Therefore, the application of traditional Chinese medicine (TCM) could illuminate a novel pathway for the treatment and prognosis of HCC.

Ginseng Radix et *Rhizoma* (roots of *Panax ginseng* C. A. Mey, Renshen in Chinese, GRR.) is a king of herbs in TCM that nourishes the five internal organs, including the liver, kidneys, lungs, heart and stomach. Protopanaxadiol ginsenosides (PDs), as the primary pharmacological components of GRR, seasonal variations of PDs and protopanaxatriol ginsenosides (PTs) were found to be similar, but more PDs was always measured (Kim, Kim, Raña, & Han, 2018). PDs have been found to have various pharmacological effects, including antioxidant (Yao et al., 2019), anti-inflammatory (Kan et al., 2023; Lee, 2021), and anti-tumor properties (Aravalli, Cressman, & Steer, 2013). The difference between PDs and protopanaxatriol ginsenosides (PTs) lies in the substitution on the C-6 of B-ring. In general, PDs have stronger antitumor activity than PTs (Tong, Song, Zhang, Xu, & Liu, 2022). Extensive researchers have clearly demonstrated that PDs have promising therapeutic effects on HCC. For example, ginsenoside compound K (CK) downregulates Bcl-2 expression, inhibits activation of the ERK pathway, and causes mitochondrial apoptosis in HCC (Bai et al., 2022). Ginsenoside Rh_2 promotes apoptosis in HCC (Zhang et al., 2019). PDs have also been shown to have excellent anti-HCC effects while significantly reducing toxic side effects (Hwang, Hong, Moon, Yoon, & Park, 2022). For example, ginsenoside Rg_3 could alleviate nausea, vomiting, as well as bone marrow suppression caused by chemotherapy in patients (Wan et al., 2021).

Network pharmacology is a bioinformatics-based research strategy aimed at identifying drug actions and facilitating drug discovery (Udrescu, Ardelean, & Udrescu, 2022; Niu et al., 2021). In this paper, we reviewed the mechanism of action of several reported PDs on HCC. The structures of Rh_2, Rg_3, Rd, Rb_1, CK, Rk_1, and Rg_5 were shown in Fig. 1. Importantly, network pharmacology tools were used to analyze the relationship between chemical components and disease-related targets, providing a reference for further pharmacological studies and clinical applications, as well as drug development for TCM in the treatment of HCC.

2. Anti-HCC activities of PDs

2.1. Ginsenoside Rh_2

Ginsenoside Rh_2 is an important component in GRR. As a representative component of anti-HCC, Rh_2 has received widespread attention to date. It has demonstrated the ability to inhibit prolif-

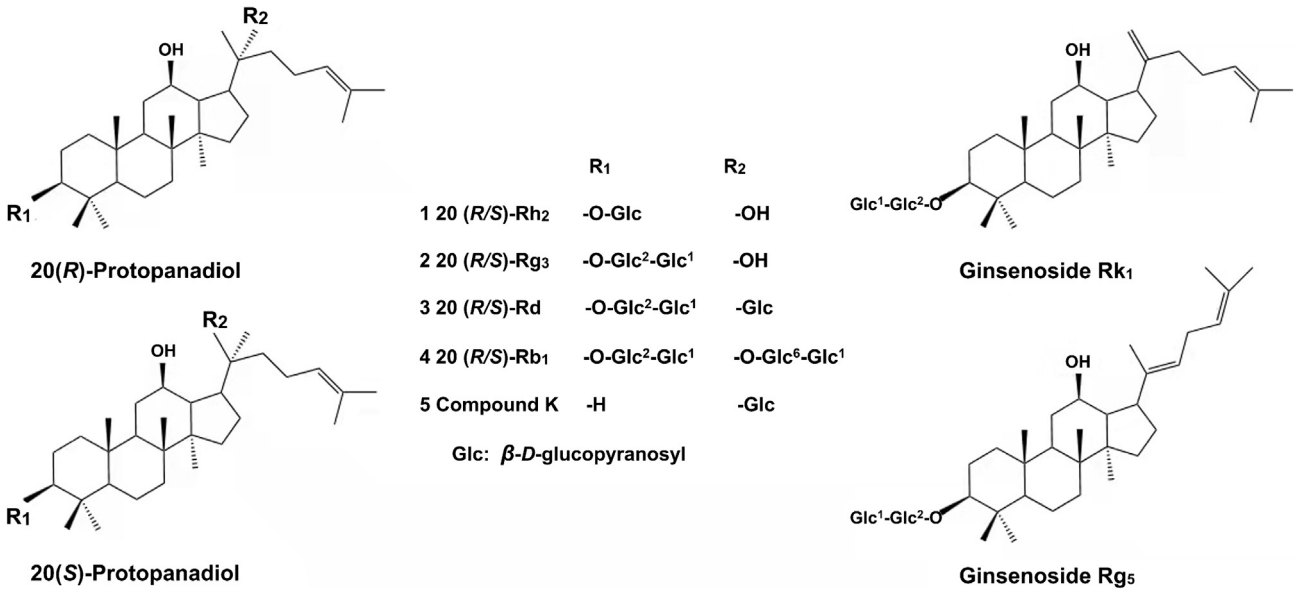

Fig. 1. Protopanaxadiol ginsenoside chemical structures.

eration, invasion, and metastasis, induce cell cycle arrest and differentiation, as well as alleviate the adverse effects of chemotherapy or radiotherapy (Li et al., 2020).

Shi et al. found that Rh_2 could inhibit the proliferation of HepG2, promote apoptosis and inhibit metastasis (Shi et al., 2016). Rh_2 could promote increased expression of downstream target genes Bcl-2-associated X (Bax), and downregulation of cyclin D1, B-cell lymphoma-2 (Bcl-2), Matrix metalloproteinase-3 (MMP3) expression, inhibiting the proliferation of HepG2, metastasis, and promoting apoptosis. Rh_2 is also associated with increased levels of caspase-3, caspase-6, and poly (ADP-ribose) polymerase proteins (Zhang et al., 2019). Furthermore, Rh_2 may induce apoptosis through direct activation of the mitochondrial pathway, as it triggers the release of mitochondrial cytochrome *c* and the activation of caspase-3 and Bax (Park, Kim, Kim, & Kang, 2012). Guo et al. found that Rh_2 promoted the translocation of BCL2 antagonist/killer (Bak) and Bax, rapidly inducing mitochondrial cytochrome *c* release and subsequent caspase activation (Guo et al., 2012). Similarly, during apoptosis induced by ginsenoside Rh_2, caspase-3 protease is activated by the Bcl-2 insensitive pathway (Park, Lee, Oh, Kim, & Lee, 1997).

Signal transducer and activator of Transcription3 (STAT3), a transcriptional signal transducer that translates cytokine stimulation into specific gene expression. STAT3 normally acts as a cellular regulator that controls cell proliferation and apoptosis, but under the regulation of oncogenic factors, STAT3 is continuously activated, contributing to uncontrolled cell growth (Yang et al., 2019). Rh_2 has been shown to inhibit recombinant human membrane-anchored protein (Annexin A2), thereby suppressing STAT3 activity in HepG2 cells. The inhibition of STAT3 activity leads to negative regulation of the four vascular endothelial growth factors (Wang, Chen, Zhang, Li, & Jin, 2021). Moreover, Rh_2 binding to Annexin A2 reduces the interaction between Annexin A2 and NF-κB (Nuclear factor kappa-B) p50 subunit, resulting in decreased *trans*-activation activity of NF-κB and subsequently reduced growth and migration ability of HCC (Wang et al., 2017).

Heat shock protein HSP 90-alpha (HSP90A) is frequently overexpressed in human HCC cells, is targeted by Rh_2 to inhibit cell cycle and growth. This interference with the HSP90A-Cdc37 chaperone system by Rh_2 leads to cell cycle arrest at the G0-G1 phase (Chen et al., 2021). In addition, Rh_2 induces cell differentiation, resulting in downregulation of telomerase activity, which may play a crucial role in carcinogenesis and cell immortalization (Zeng & Tu, 2004a). Treatment of SMMC-7721 cells with Rh_2 resulted in an increase in G1 phase and a decrease in S and G2/M phase cells, primarily in the G1 phase. Rh_2 also attenuated the expression of positive regulatory factors, such as Cyclin D1 and Cyclin E, and increased the expression of negative regulatory factors, such as P16 protein and P21 gene in SMMC-7721 cells. Furthermore, Rh_2 induced normalization of SMMC-7721 cell differentiation (Zeng & Tu, 2004b).

2.2. Ginsenoside Rg_3

Ginsenoside Rg_3 has been developed into a new class of anticancer drugs and plays a vital role in the prevention and treatment of cancer. Its suggested mechanisms include apoptotic induction, as well as the inhibition of proliferation, metastasis, and angiogenesis (Sun et al., 2017).

Ginsenoside Rg_3 is known for inhibiting the proliferation of HCC cells and promoting their apoptosis, synergizing with antihepatocyte drugs for better anticancer effects. The combination of Rg_3 with oxaliplatin enhances antitumor effects by regulating the expression of proliferating cell nuclear antigen (PCNA) and CyclinD1 (Shan et al., 2019). Additionally, sorafenib, a multikinase inhibitor for cellular cancer therapy, inhibits growth and angiogenic signaling pathways (Sim & Knox, 2018), but also activates the PI3K/Akt signaling pathway and generates drug resistance, combining 20(*S*)-Rg_3 can produce synergistic anticancer effects by inhibiting the PI3K-Akt signaling pathway (Lu, Fei, & Zhang, 2018). Furthermore, the combination of Rg_3 with doxorubicin or adriamycin may synergistically kill HCC cell lines (Zhou, Wang, & Yan, 2014). Rg_3 in combination with TACE may also prolong survival (Zhou et al., 2016). In addition, the TRAIL pathway is a potential therapeutic target for anticancer drugs due to selective cytotoxicity in cancer cells. Rg_3 in combination with TRAIL can further promote specificity against HCC cells (Lee et al., 2013).

Consistently, one aspect of the anti-cancer properties of Rg_3 is its anti-angiogenic effect (Nakhjavani, Smith, Townsend, Price, & Hardingham, 2020). This action is known to limit metastasis and promote survival by decreasing the overexpression of vascular endothelial growth factor (VEGF) specifically in tumors of HCC (Zhou, Wang, & Yan, 2014). Microvessel density is a reliable marker for measuring tumor angiogenic activity and metastasis, and Rg_3 has demonstrated efficacy in inhibiting the activation of microtumor angiogenesis *in vivo*, making it a promising adjuvant in the treatment of HCC (Hu et al., 2019).

Rg_3 has demonstrated efficacy as a multi-targeted antitumor agent in the treatment of HCC. It is believed to induce apoptosis by activating the mitochondrial pathway (Park, Kim, Kim, & Kang, 2012), promoting the expression of caspase-3 and Bax, while inhibiting the expression of anti-apoptotic genes Bcl-2 and Bcl-XL via a cystein-dependent apoptotic pathway mediated by mitochondria (Jiang, Chen, Chen, & Zheng, 2011; Zhang et al., 2012). Rg_3 has also been shown to play a key role in regulating several anti-metastatic effects, inhibiting the migration and invasion of HCC cells by upregulating the expression of Rho GTPase Activating Protein 9 (ARHGAP9) protein (Sun et al., 2019), and significantly reducing the expression of PCNA and TNF protein (Li et al., 2005). Moreover, Rg_3 can decrease the expression of Na^+/H^+ exchanger 1 (NHE1) by integrally inhibiting epidermal growth factor-epidermal growth factor receptor-Extracellular signal-regulated kinase 1/2-hypoxia inducible factor 1 subunit alpha (EGF-EGFR-ERK1/2-HIF-1α) signal axis in HCC, making it a highly effective multi-targeted antitumor agent for the treatment of HCC (Li et al., 2018).

2.3. Ginsenoside CK

Ginsenoside CK, is produced by intestinal bacteria during the metabolism of GRR PDs (Zhang et al., 2018). In the tumor microenvironment, hypoxia and excessive activation of the NF-κB signaling pathway cause uncontrolled cell growth and have been identified as key inducers of EMT in HCC, and CK reverses hypoxia-induced Epithelial-mesenchymal transition (EMT) in HCC by blocking communication between HIF-1α and NF-κB signaling pathways (Zhang, Ma, & Fan, 2021), CK strongly attenuates colony formation, adhesion, and invasion of HCC cells *in vitro* and significantly inhibits spontaneous HCC metastatic growth *in vivo* (Ming et al., 2011). In addition, the interaction between Annexin A2 and CK prevented the joint of Annexin A2 and NF-κB p50 subunits, their nuclear co-localization, which attenuated the activation of NF-κB and its downstream gene expression, subsequently activated caspase 9 and caspase 3 (Wang et al., 2019). In conclusion, CK can inhibit spontaneous HCC metastatic growth by suppressing the NF-κB signaling pathway (Ming et al., 2011).

CK inhibits STAT3 protein expression, decreases the DNA-binding capacity of STAT3, and contributes to elevated levels of ERS-related proteins, these findings suggest that CK induces ERS and apoptosis by inhibiting STAT3 in HCC (Zhang et al., 2018). Furthermore, CK induces the Bid-mediated mitochondrial pathway in HCC cells, indicating that CK may be a viable drug for Bid targeting.

Full-length Bid migrates from the nucleus to the mitochondria during cytotoxic apoptosis, triggering the release of cytochrome *c* and promoting cytotoxic apoptosis (Song et al., 2010). Other studies have shown that CK upregulates Fas, the Bax/Bcl-2 ratio, and promotes caspase-9 and caspase-3 protein expression while inhibiting Akt phosphorylation. This research implies that CK suppresses HCC cell proliferation and triggers apoptosis via Fas and mitochondria-mediated cystathione-dependent pathways (Zheng et al., 2014). In addition, Shin et al. found that CK also induces apoptosis by inhibiting glycolysis and AKT/mTOR/c-Myc signaling in HCC cells (Shin et al., 2021). It is also possible that CK-induced apoptosis is associated with the MAPK signaling pathway and JNK phosphorylation (Igami, Shimojo, Ito, Miyazaki, & Kashiwada, 2015; Kim, Yuan, Chung, & Chung, 2009).

2.4. Ginsenosides Rk_1 and Rg_5

Ginsenosides Rk_1 and Rg_5 have received significant attention in recent years due to their high oral bioavailability (Gao et al., 2022). Research has shown that Rk_1 and Rg_5 may trigger apoptosis in HCC cells by activating the MAPK and NF-κB signaling pathways, as well as inhibiting the expression of anti-apoptotic proteins in the mitochondrial apoptosis pathway (Chen, Lv, Li, & Jin, 2021). Rk_1 and Rg_5 also induce apoptosis in HCC cells by inducing cell cycle arrest. Rk_1 was first shown to inhibit glutamine (GSH) metabolism in HCC and down-regulates GLS1 (glutaminase) expression, thereby reducing GSH production and stimulating ROS accumulation to induce apoptosis (Lu et al., 2022), In addition, Rg_5 blocks the cell cycle of SK-HEP-1 cells at the G1/S transition by reducing cyclin E-dependent kinase activity, which is associated with increased levels of p21Cip/WAF1 protein and decreased levels of cyclin E, cyclin dependent kinase 2 (CDK2) and cell division cycle 25A (CDC25A) proteins (Lee, Lee, Kim, Park, & Lee, 1997).

Kim et al. discovered that Rk_1's antitumor activity involves synergistic inhibition of telomerase and induction of apoptosis. Rk_1 significantly inhibits telomerase activity and reduces cell growth, while causing morphological changes (Kim et al., 2008). Following Rk_1 has antitumor activity, and its mode of action for 48 h of treatment in HepG2 cells involves coordinated inhibition of telomerase and induction of apoptosis, and Rk_1 in combination with autophagy inhibitors can enhance antitumor activity. Ko et al. found that autophagy acts as a survival mechanism against Rk_1-induced apoptosis in HepG2 cells (Ko, Kim, Park, Park, & Yang, 2009). The results supported the use of autophagy inhibitors in combination with Rk_1 as an effective anti-cancer regimen in HepG2 cells.

2.5. Ginsenosides Rb_1 and Rd

Chronic inflammation is often accompanied by an increase in matrix metalloproteinase (MMP) production and subsequent degradation of the extra cellular matrix. The ginsenoside Rb_1 efficiently suppresses TNF-α-elicited MMP-9 generation in HepG-2 cells (Sun, Yang, Or, Luo, & Li, 2022).

Rd suppresses HepG2 cell migration and invasion by reducing MMP-1, MMP-2, and MMP-7 expression, inhibiting ERK and p38 MAPK phosphorylation, preventing AP-1 activation, and promoting adherent plaque formation to block MAPK signaling (Yoon, Choi, Cha, & Lee, 2012).

Overall. the mechanism of PDs in treating HCC is mainly in inhibition of proliferation, induction of apoptosis, inhibition of invasion and metastasis, normalizing the divergence and blocking the cell cycle. The specifically regulated up- and down-regulated proteins are shown in Fig. 2.

3. Network pharmacology analysis

In order to further explore the relevant targets and mechanisms of PDs on HCC, we used network pharmacology as an adjunct study for further validation. Overall, the structures and properties of seven target components (Rh_2, Rg_3, Rd, Rb_1, CK, Rk_1, and Rg_5) were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Genetic targets for these seven ginsenoside components (Probability > 0) were acquired from five disease databases, including Drugbank, GeneCards, OMIM, TTD, and PharmGKB, to identify HCC disease-related targets, and R language was used to analyze the drug and disease-associated targets. The network map of ginsenoside components and core targets was created using cytoscape. TSV files were obtained from the STRING network database (https://cn.string-db.org/cgi/) with a score > 0.4, and disconnected nodes in the network were hidden. The node information between the major targets was retained to receive interactions between each disease target. Finally, R language was utilized for the complete molecular docking of the aforementioned ginsenosides as well as the core target results.

Anti-hepatocellular carcinoma activity of protopanaxdiol ginsenosides

	Inhibition of proliferation	Induction of apoptosis	Inhibition of invasion and metastasis	Normalizing the divergence	Blocking the cell cycle
UP	Bax P21/P53	Cytochrome capase-3 caspase-6 caspase-9 AMPK Bax ERS ROS GLS1	ARHGAP9	P21 P16	HSP90A P21/WAF1
DOWN	AMPK STAT3 VEGF NF-κ B CyclinD1 Bcl-2 Bcl-XL MMP3 MMP2/MMP9	P13K/Akt/Bcl-2 NF-κ B AKT/mTOR/c-Myc	MMP2/MMP9 MMP1 MMP2 MMP7 NF-κ B PCNA TNF EGF-EGFR-ERK1/2-HIF	CyclinD1 Cyclin E	CyclinE CDK2 CDC25A

Fig. 2. Anti-HCC activity of protopanaxadiol ginsenosides.

3.1. Methods

3.1.1. PDs related targets screening

The canonical SMILES of seven target components (Rh_2, Rg_3, Rd, Rb_1, CK, Rk_1, and Rg_5) were got from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Swiss target prediction (http://www.swisstargetprediction.ch/) was employed to identify targets for these ginsenosides in Homo sapiens. Different conformations of ginsenosides were considered in the Swiss target prediction process.

3.1.2. HCC disease targets screening

Five disease databases, including Drugbank (https://go.drugbank.com/), GeneCards (https://www.genecards.org/), OMIM (https://www.omim.org/), TTD (https://db.idrblab.net/web/), and PharmGKB (https://www.pharmgkb.org/), were utilized to acquire HCC disease-related targets. The genes obtained from these databases were interlaced using the R language, and a Venn diagram was created.

3.1.3. Network construction

Core target genes were acquired by intersecting component-related target genes with disease-related genes using the R language. These genes were then visualized using Cytoscape software (version 3.9.1) to map regulatory networks of both component and core targets.

To receive the disease target Protein-Protein Interaction (PPI) network, we used the STRING network database (https://cn.string-db.org/cgi/) and set the organism to "Homo sapiens". The resulting PPI network was then converted to a TSV file with a score greater than 0.4, with disconnected nodes hidden in the network. This file was also visualized using Cytoscape software (version 3.9.1), where the degree of a node was defined as the number of other nodes directly connected to it. The core targets were identified as those in the top 20 based on their degree value, which was calculated using network analysis from the Cytoscape plugin. The higher the degree value of a node, the more important it is considered within the PPI network.

3.1.4. GO and KEGG pathway enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using R-x64-4.1.3 and the DOSE Bioconductor package. The three categories of GO were Biological Processes (BP), Cellular Components (CC), and Molecular Function (MF), and statistical significance was defined as a P-value < 0.05. The GO enrichment and KEGG pathways were ranked based on their P-values.

3.1.5. Molecular docking simulations

The 2D molecular structures of various compounds were retrieved from the PubChem database and energy minimized using Chem3D software to generate mol2 files. In addition, protein

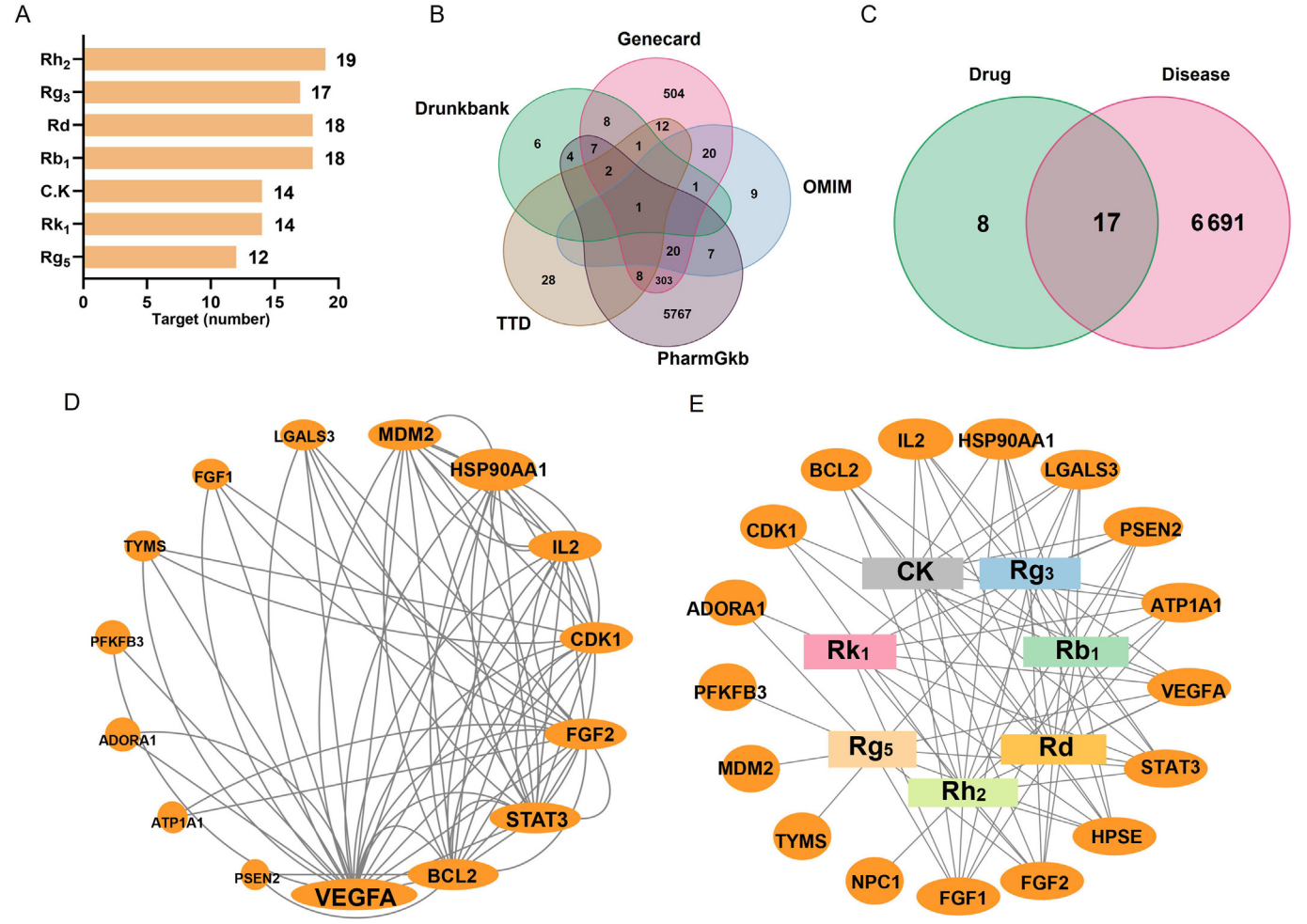

Fig. 3. Component and disease target screening, regulatory network construction, PPI network construction. (A), Number of PDs targets. (B), Venn diagram of HCC disease targets. (C), Intersection of drug components and disease targets. (D), Component and disease target regulatory network. (E), Network construction of Component and disease target.

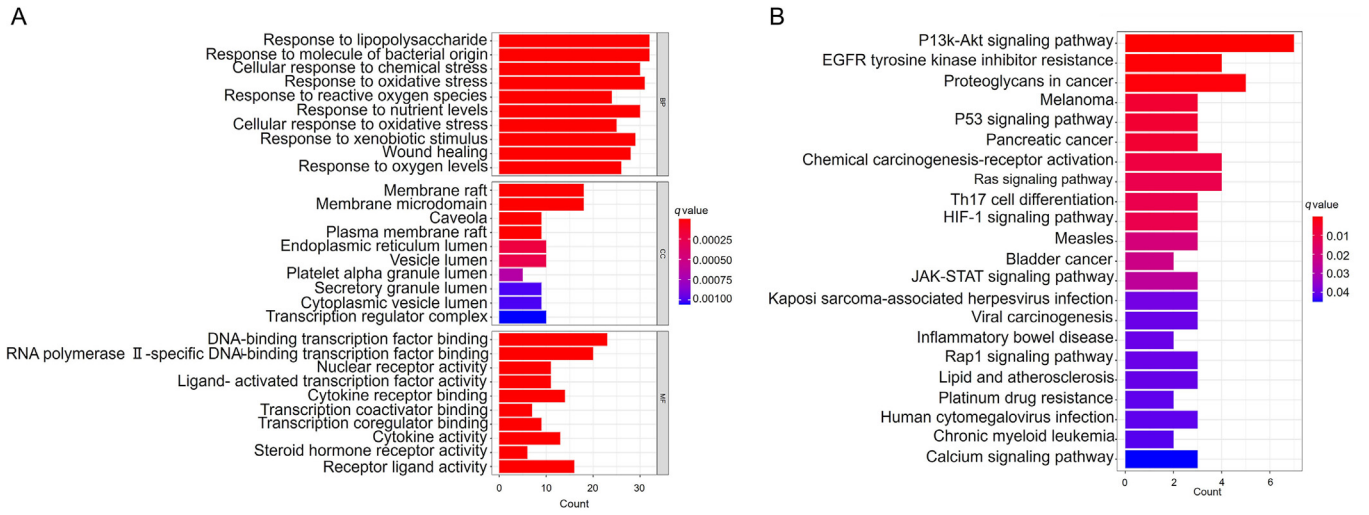

Fig. 4. GO and KEGG enrichment analysis. (A), GO enrichment analysis. (B), KEGG enrichment analysis.

receptor PDB files were obtained from the AlphaFold Protein Structure database (https://www.alphafold.ebi.ak.uk). Autodock Vina was used to perform many-to-many molecular docking, and the resulting data was organized using Microsoft Excel software. Finally, GraphPad Prism 9 was used to create visual heat maps, and compounds were selected for visualization and analysis based on optimal results with disease targets.

3.2. Results

3.2.1. Components and disease-related targets

The two-dimensional structural formula of the above ginsenosides was drawn with KingDraw chemical structure editor (Fig. 1), a total of seven ginsenoside targets (Rh_2, Rg_3, Rd, Rb_1, CK, Rk_1 and Rg_3) were identified from the Swiss Target Prediction Database (Probability > 0). After screening, each ginsenoside target was obtained (Fig. 3A). To collect disease-associated target genes for HCC, information from reputable sources such as Drugbank, GeneCards, OMIM, TTD, and PharmGKB were collected for a total of 8, 504, 9, 28, and 5, 767 targets, respectively. The correlation of HCC disease targets in these five databases was illustrated using a Venn diagram (Fig. 3B).

3.2.2. Compounds regulation network and PPI network

The HCC disease targets were matched with the protein targets of the above-mentioned 7 ginsenosides to generate cross-targets of 17 components and disease targets (Fig. 3C), these included Interleukin 2 (IL2) (PDB ID P14784). Heat shock protein 90 alpha family class A member 1 (HSP9OAA1) (PDB ID P07900). Advanced glycation end-product receptor (3LGALS3) (PDB ID P17931). Presenilin 2 (PSEN2) (PDB ID P49810). ATPase Na^+/K^+ transporting subunit alpha 1 (ATP1A1) (PDB ID P05023). Vascular endothelial growth factor A (VEGFA) (PDB ID P42574). STAT3 (PDB ID P40763). Heparanase (HPSE) (PDB ID Q9Y251). Fibroblast growth factor 2 (FGF2) (PDB ID P09038). Fibroblast growth factor 2 (FGF1) (PDB ID P05230). NPC intracellular cholesterol transporter 1 (NPC1) (PDB ID 035604). Thymidylate synthetase (TYMS) (PDB ID P04818). MDM2 proto-oncogene (MDM2) (PDB ID Q00987). 6-phospho fructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) (PDB ID Q16875). Adenosine A1 receptor (ADORA1) (PDB ID P30542). CDK1 (PDB ID P06493), BCL2 (PDB ID P10415), We then used these 17 intersection targets to create a composite intersection target network by Cytoscape (3.9.2) software, we constructed a regulatory network where the more connections between compounds and predicted protein targets, the greater the linear density and the higher the correlation (Fig. 3D). We identified targets with high correlation with ginsenosides, such as HSP9OAA1, VEGFA, STAT3, FGF2, MDM2, CDK1, and BCL2. This further confirms that ginsenosides can target disease genes involved in regulating the HCC cell cycle, promoting apoptosis, inhibiting growth, differentiation, and proliferation of cancer cells.

A network-based analysis of protein–protein interactions (PPI) was conducted using the STRING database, with a threshold interaction score of > 0.4. The resulting network was visualized using Cytoscape software (Fig. 3E). Core HCC targets identified included VEGFA, BCL2, STAT3, FGF2, CDK1, and MDM2. These targets were found to be highly relevant for the development and progression of this cancer.

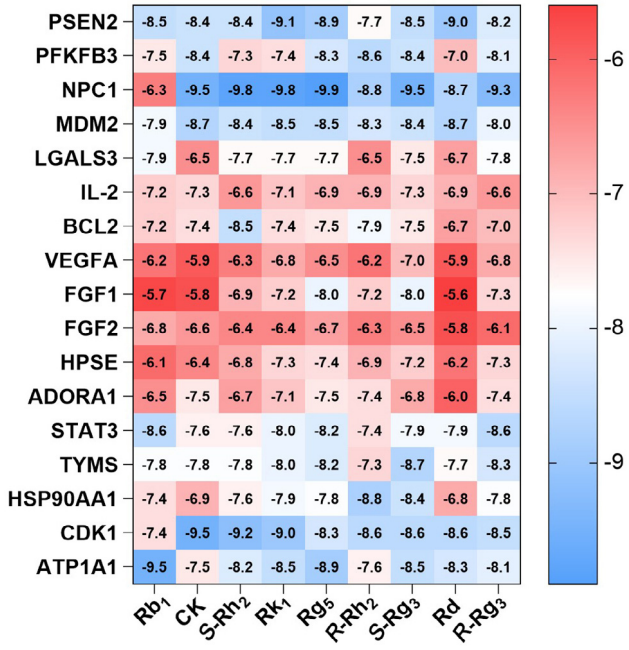

Fig. 5. Heat map of PDs docking results with core target molecules.

3.2.3. GO and KEGG pathway enrichment analysis

To explore its potential therapeutic role in HCC, we conducted GO enrichment analysis and KEGG pathway analysis using the DOSE Bioconductor sotware package in the R language. The GO enrichment analysis revealed significant enrichment in biological processes such as the responding to lipopolysaccharide, bacterial molecules, cellular responses to chemical stress, and DNA-binding transcription factor binding (Fig. 4A). Analysis of the KEGG

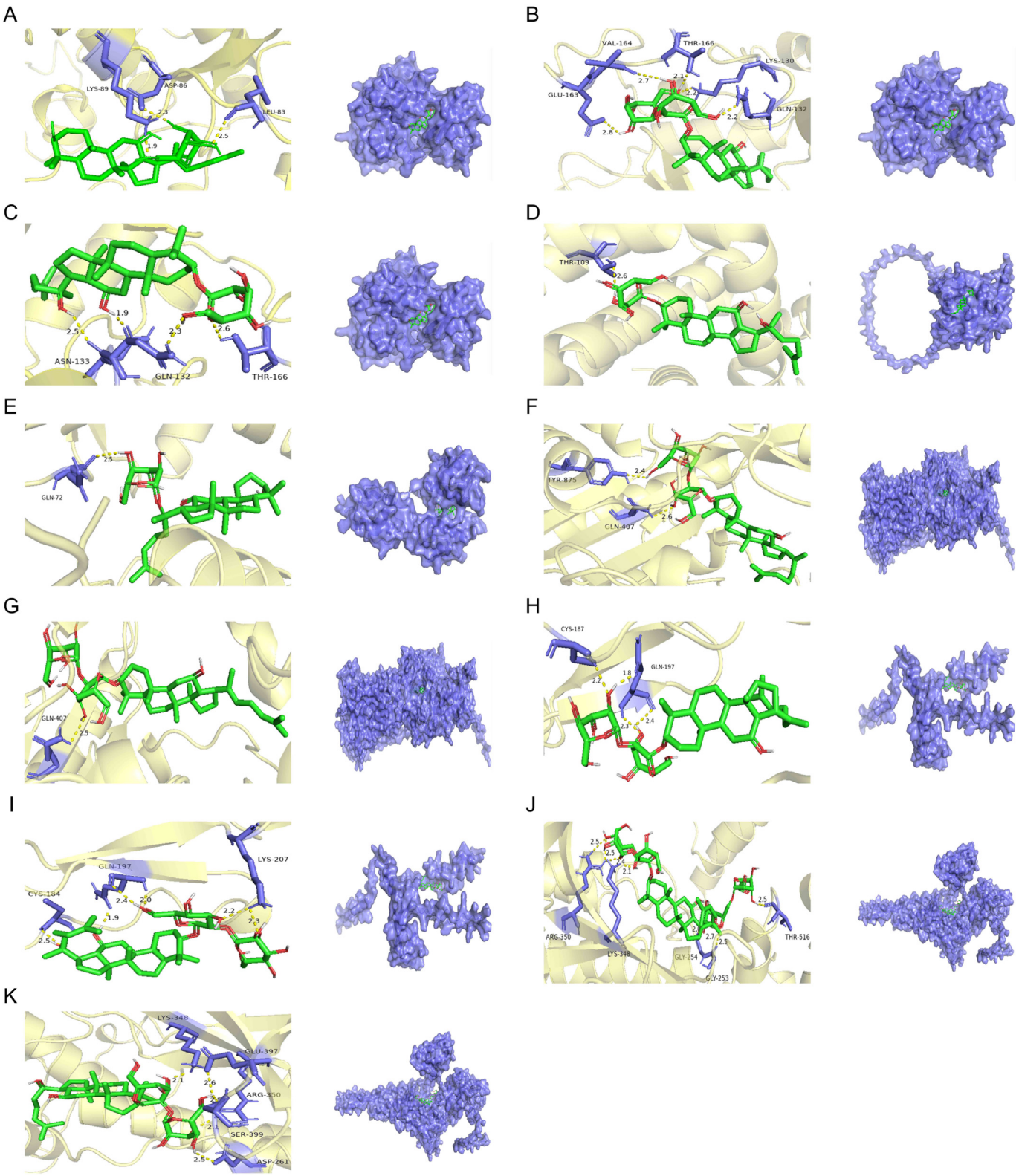

Fig. 6. Molecular docking results of target genes and PDs. Molecular docking results of CDK1 with CK, Rk_1 and Rh_2 (A–C), BCL2 with *S*-Rh_2 (D), MDM2 with CK (E), NPC1 with Rk_1 and Rg_5 (F–G), VEGFA with Rk_1 and *S*-Rg_3 (H–I), STAT3 with Rb_1 and *R*-Rg_3 (J–K).

pathway showed significant enrichment in pathways such as the PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, proteoglycans in cancer, and Ras signaling pathway (Fig. 4B).

3.2.4. Molecular docking simulations analysis

To further investigate the potential binding of PDs to core targets, all of the above ginsenosides were molecularly bulk-docked to 17 core targets using Autodock Vina for multiple-to-many molecular dockings. The results demonstrated that all core targets exhibited strong binding affinity to their corresponding chemicals, with binding energy of less than −5 kcal/mol, as illustrated in (Fig. 5). Notably, CDK1, MDM2, NPC1, BCL2, VEGFA and STAT3 were found to exhibit significant binding activity to ginsenosides. Specifically, the CDK1 (Cyclin-dependent kinase 1) target gene played a crucial role in cell cycle regulation during mitosis and meiosis, and exhibited strong binding affinity to CK, Rk_1, and *S*-Rh_2 (Fig. 6-A-C). The BCL2 protein family, which regulates cell apoptosis, exhibited remarkable binding activity with S-Rh_2 (Fig. 6D). MDM2, an E3 Ubiquitin protein ligase that negatively regulates tumor suppressor p53, was observed to exhibit robust binding activity with CK (Fig. 6E). NPC1, which plays a vital role in the excretion of cholesterol from the endosomal/lysosomal compartment, demonstrated a notable binding affinity to Rk_1 and Rg_5 (Fig. 6F–G). Furthermore, VEGFA, the main driver of physiological and pathological angiogenesis, exhibited strong binding activity with Rk_1 and *S*-Rg_3 (Fig. 6H–I). Finally, STAT3 regulated the expression of immune factors and recruits immunosuppressive cells to establish a tolerant tumor microenvironment with significant binding activity to Rb_1, *R*-Rg_3 (Fig. 6J–K). The molecular docking results showed that the target genes VEGFA, STAT3, CDK1, NPC1, BCL2 and MDM2 displayed high binding energy to ginsenosides, involving pathways such as PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, proteoglycans in cancer.

4. Discussion

HCC is the most prevalent tumor in the world and is responsible for over 500 000 deaths annually (Wu & Yang, 2020). *Ginseng Radix* et *Rhizoma* possesses a broad spectrum of pharmacological effects. We reviewed the potential mechanism of action of commonly used PDs in the treatment of HCC. PDs (Rh_2, Rg_3, Rd, Rb_1, CK, Rk_1, and Rg_5) can inhibit the proliferation, differentiation, migration, and metastasis of HCC cells, normalize their differentiation, and impede the cell cycle. The mitochondrial transduction pathway is the most prevalent apoptotic mechanism (Li, et al., 2018), and Rh_2, Rg_3, and CK can activate the mitochondrial pathway to induce apoptosis (Guo et al., 2012; Park, Kim, Kim, & Kang, 2012; Park, Lee, Oh, Kim, & Lee, 1997; Zhang et al., 2012; Zhang et al., 2019; Zheng et al., 2014), activate Cytochrome, promote the expression of capase-3, caspase-6, and caspase-9, and promote apoptosis of HCC cells. In addition, the above ginsenosides promote the expression of Bax, inhibit the expression of Bcl-2 and Bcl-XL (Hong, Baatar, & Hwang, 2021; Jiang, Chen, Chen, & Zheng, 2011; Shi et al., 2016), and promote apoptosis by inhibiting PI3K/Akt, NF-κB, and AKT/mTOR/c-Myc signaling pathways (Chen et al., 2021; Igami, Shimojo, Ito, Miyazaki, & Kashiwada, 2015; Ming et al., 2011; Wang et al., 2017). Moreover, Rg_3 may play an auxiliary therapeutic role in reducing the toxic side effects of anti-HCC drugs and work synergistically in the management of HCC (Huang et al., 2022).

Tumor invasion is strongly correlated with the activity of proteases that target extracellular matrix and basement membrane proteins. Metal ion-dependent MMPs are known to play a significant role in tumor cell invasion and metastasis (Wang et al., 2021), PDs have been found to inhibit the expression of MMPs, thereby suppressing HCC invasion and metastasis (Ming et al., 2011; Yoon, Choi, Cha, & Lee, 2012). Ginsenoside Rb_1 and Rd significantly reduce MMP protein expression and hinder HepG2 cell migration and invasion (Sun, Yang, Or, Luo, & Li, 2022; Yoon, Choi, Cha, & Lee, 2012). Besides, Rg_3 has been shown to upregulate ARHGAP9 protein (Li et al., 2005), suppress PCNA and TNF protein expression, and inhibit the EGF-EGFR-ERK1/2-HIF signaling axis in HCC through overall inhibition (Li et al., 2018). Rg_5 has been shown to block the cell cycle of cells at the G1/S transition, downregulate Cyclin E, CDK2, and CDC25A, and increase p21 protein levels (Lee, Lee, Kim, Park, & Lee, 1997). Rh_2 inhibits the cellular regulator STAT3 and leads to negative regulation of the four VEGFs (Yang et al., 2019), decreases NF-κB activity (Wang et al., 2017) to inhibits HCC proliferation, and Rh_2 has been found to target HSP90A, which is known to cause cell cycle arrest (Chen et al., 2021). Besides, Rh_2 causes cell differentiation and downregulates telomerase activity and attenuates the expression of Cyclin D1, Cyclin E, and increases the expression of P16 and p21 in cells, additionally, Rh_2 induces a normalization of cell differentiation (Zeng & Tu, 2004b).

In order to support and complement the above, we searched for drug and disease related targets from reliable databases and enriched them for analysis. By conducting network pharmacology and molecular docking, we identified 17 core targets, including but not limited to IL2, HSP9OAA1, LGALS3, PSEN2, ATP1A1, VEGFA, STAT3, HPSE, FGF2, FGF1, NPC1, TYMS, MDM2, PFKFB3, ADORA1, CDK1, and BCL2, and comprehensively assessed their interactions. The pathways involved include the P13K-AKT signaling pathway (Xie et al., 2019), EGFR tyrosine inhibitor resistance (Yang et al., 2018), and proteoglycans in cancer (Baghy, Tátrai, Regős, & Kovalszky, 2016), which responds to extracellular signals to promote metabolism, proliferation, cell survival, growth, and angiogenesis. These results provide further evidence that PDs have a potential role in inhibiting the HCC cell proliferation, invasion, and metastasis, promoting tumor cell apoptosis, inducing differentiation, and arresting cell cycle. Our findings are consistent with relevant literature reviews.

5. Conclusion

The development and manifestation of HCC involves complex processes, and TCM seeks ways and means to combat this disease. Nevertheless. Treatment of HCC is a prolonged process, and its clinical efficacy requires long-term exploration. Therefore, a comprehensive and in-depth investigation of the mechanism of action of PDs on HCC is necessary to advance the development of the basic theories of TCM. This will further benefit the development of future anti-tumor drugs. therefore, it is essential to continue research on the mechanism of action of PDs on HCC, promoting in-depth investigations and breakthroughs of the fundamental theories of TCM, thereby facilitating the development of more potent antitumor drugs.

CRediT authorship contribution statement

Yue Zhou: Conceptualization, Methodology, Validation, Data curation, Formal analysis, Visualization, Writing – original draft. **Zi Wang:** Validation, Writing – review & editing. **Shen Ren:** Validation, Writing – review & editing. **Wei Li:** Funding acquisition, Project administration, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the grants of National Natural Science Foundation of China (No. 82104465), Changchun Science & Technology Development Plan (No. 21ZGY11), and the Scientific Research Planning Project of Jilin Provincial Department of Education (No. JJKH20220371KJ).

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