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DATABASE ANALYSIS

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Systems Pharmacology-Based Identification of Mechanisms of Action of *Bolbostemma paniculatum* for the Treatment of Hepatocellular Carcinoma

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Traditional Chinese medicine has widely used *Bolbostemma paniculatum* to treat diseases, including cancer, but its underlying mechanisms remain unclear. The present study aimed to elucidate the potential pharmacological mechanisms of "Tu Bei Mu" (TBM), the Chinese name for Bolbostemmatis Rhizoma, the dry tuber of *B. paniculatum*, for the treatment of hepatocellular carcinoma (HCC).

Material/Methods: The active components and putative therapeutic targets of TBM were explored using SwissTargetPrediction, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), and Search Tool for Interactions of Chemicals (STITCH). The HCC-related target database was built using DrugBank, DisGeNet, Online Mendelian Inheritance in Man (OMIM), and Therapeutic Target Database (TTD). A protein–protein interaction network of the common targets was constructed, based on the matches between TBM potential targets and HCC-related targets, using Cytoscape software. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of the cluster networks were used to elucidate the biological functions of TBM.

Results: Pharmacological network diagrams of the TBM compound-target network and HCC-related target network were successfully constructed. A total of 22 active components, 191 predicted biological targets of TBM, and 3775 HCC-related targets were identified. Through construction of an HCC-related target database and a protein–protein interaction network of the common targets, TBM was predicted to be effective in treating HCC mainly through the PI3K-Akt, HIF-1, p53, and PPAR signaling pathways.

Conclusions: The PI3K/Akt, HIF1, p53, and PPAR pathways may play vital roles in TBM treatment of HCC. Also, the potential anti-cancer effect of TBM on HCC appears to stem from the synergetic effect of multiple targets and mechanisms.

MeSH Keywords: **Carcinoma, Hepatocellular • Drugs, Chinese Herbal • Pharmacology**

Abbreviations: **HCC** – hepatocellular carcinoma; **TCMSP** – Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; **TTD** – Therapeutic Target Database; **OMIM** – Online Mendelian Inheritance in Man; **GO** – Gene Ontology; **KEGG** – Kyoto Encyclopedia of Genes and Genomes; **MCODE** – Molecular Complex Detection

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Background

Hepatocellular Carcinoma (HCC) is the sixth most common cancer and fourth most common cause of cancer deaths worldwide [1]. Chronic hepatitis C virus (HCV) infection, chronic hepatitis B virus (HBV) infection, nonalcoholic steatohepatitis, and alcohol abuse are the major risk factors for HCC [2]. Also, obesity, associated with type 2 diabetes, has been shown to increase the risk of HCC [3]. Unfortunately, despite advancements in technology and treatment, HCC remains among the few cancers with an increasing incidence. Its 5-year survival rate is only 18.1% [4]. Surgery is most often not suitable for HCC patients because the tumors are usually at a late stage when diagnosed [5]. Sorafenib has been approved by the Food and Drug Administration (FDA) for HCC treatment, but its survival benefits are reportedly limited and the response rate is also very low [6]. Therefore, more effective treatment strategies for HCC are urgently needed.

The clinical practice of traditional Chinese medicine (TCM) has played an important role in preventing and treating diseases in China for thousands of years. TCM treatments have multicomponent, multitarget, and multi-pathway characteristics [7]. Therefore, exploration of TCM and other natural products as effective and less toxic anti-cancer drugs presents great promise for preventing and treating liver cancer [8]. Bolbostemmatis Rhizoma (Chinese name "Tu Bei Mu", TBM) is the dry tuber of *Bolbostemma paniculatum* (Maxim.) Franquet (Cucurbitaceae). It is mentioned in the Supplement to the Compendium of Materia Medica which was compiled by Zhao Xuemin in the Qing Dynasty. In ancient times, TBM was used to treat diseases such as breast abscess, breast carbuncle, hyperplasia of mammary glands, and dysplasia [9]. It disperses toxins and eliminates the carbuncle swelling. Based on modern pharmacological principles, many chemical constituents in TBM are reported to have significant pharmacological activities, including anticancer, anti-viral, anti-inflammatory, and immunosuppressive activities [10,11]. It is reported that TBM extracts could inhibit the proliferation and mitochondrial metabolic activity of hepatocellular carcinoma cells [12]. The triterpenoid saponins in TBM, known as tubeimosides, can inhibit hepatoma cell growth and proliferation, induce apoptosis, and inhibit migration and angiogenesis, via various signaling pathways [13–16]. However, the potential pharmacological mechanisms underlying these effects of TBM are not completely understood. It is speculated that multicomponent, multitarget pathway characteristics of TBM may help in preventing and treating HCC.

The network-regulatory effect of the cold- and heat-syndrome biomolecular network and cold and heat prescription system of TCM was firstly reported in 2007 [17]. Also, the term "network pharmacology" was proposed for the first time in the same year [18]. Based on the concept of systems biology, network pharmacology integrates many processes, such as high-throughput data integration, database retrieval, data mining, target prediction, and laboratory simulation [19]. In network pharmacology, the interaction between drugs and specific nodes in each network module is analyzed from a systematic point of view, including the "multicomponent-multitarget" mechanisms of TCM or compound prescription. TCM can also independently propose specific disease-related pathways known as "active component-target pathways". Systematic and holistic characteristics of network pharmacology are consistent with the overall view of TCM and the principle of syndrome differentiation and treatment [20]. Therefore, network pharmacology serves to reveal the materialistic basis for the pharmacodynamic effects of TCM and their mechanisms.

In the present study, we used network pharmacology to explore the active components and putative therapeutic targets of TBM to elucidate potential pharmacological mechanisms utilized by TBM in the treatment of hepatocellular carcinoma (HCC).

Material and Methods

Screening of chemical compounds in TBM

The chemical components of TBM were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, *http://www.tcmspw.com/tcmsp.php/*), which is the largest pharmacological data platform for TCM [21]. **Figure 1** shows the schematic of the network pharmacology of TBM for the treatment of HCC; 46 components of TBM were found.

Screening for the active chemical components of TBM

The oral administration route is best for TCM effects in the gastrointestinal tract. Oral bioactivity (OB) is used for determining the dosing regimen. OB signifies the percentage of orally administered drug that reaches the blood circulation [22]. Drug-likeness (DL) is a qualitative concept in drug design that describes and optimizes the pharmacokinetic and pharmaceutical properties of a prospective drug-like compound [23]. In this study, OB and DL were used to screen the bioactive chemical components of TBM. In the screening process, chemical components with OB ≥20% and DL ≥0.18 were chosen for further analysis [24]. Additionally, the putative targets of the active chemical components of TBM were identified using the TCMSP, SwissTargetPrediction, and STITCH. Chemical components lacking target information were excluded.

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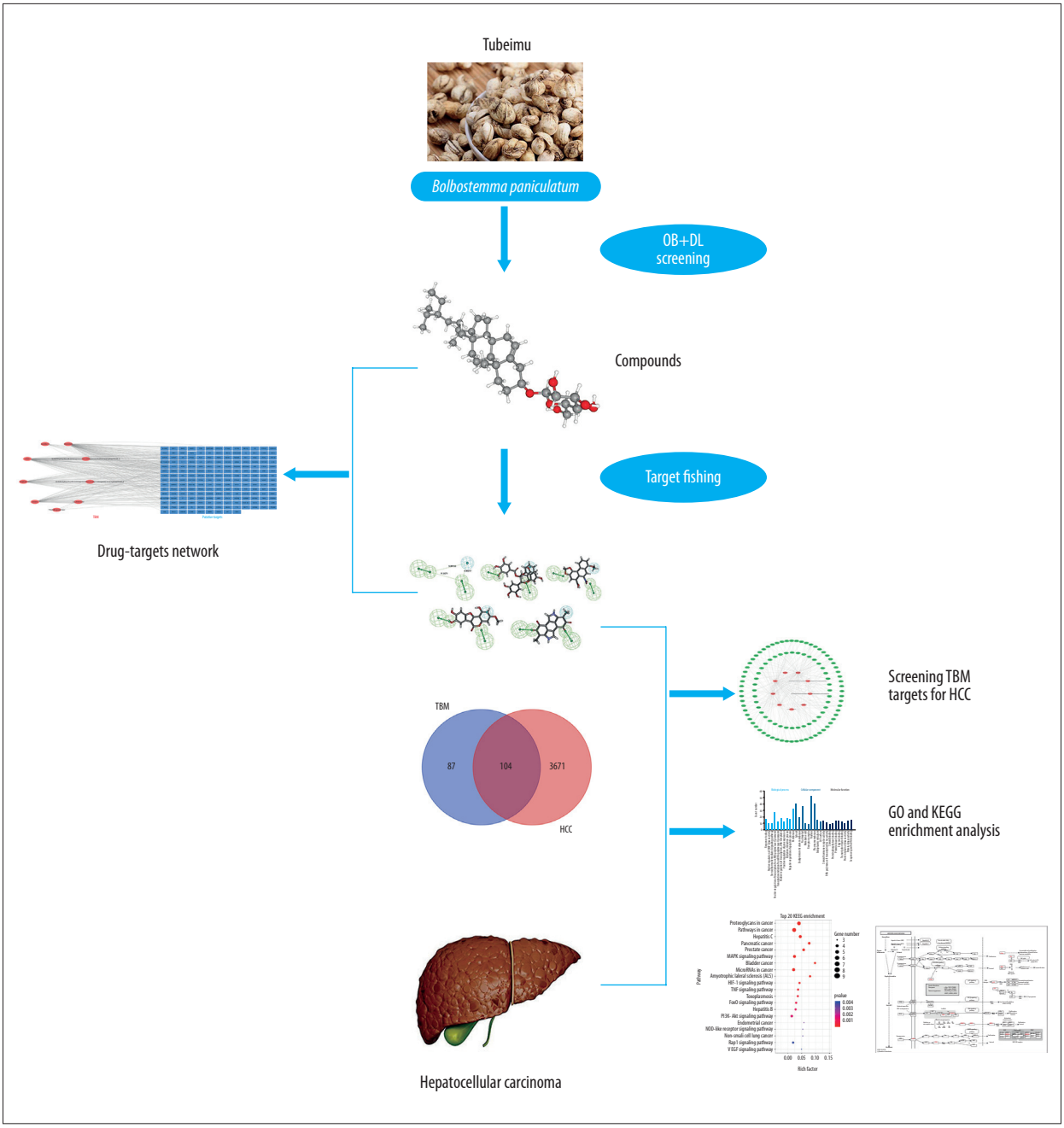

Figure 1. Schematic diagram of the network pharmacology-based analysis for investigating the potential mechanisms of TBM in treating HCC. TBM – Tu Bei Mu; HCC – hepatocellular carcinoma.

Identification of target genes related to the identified chemical components

The relevant targets of the active chemical components of TBM were identified using the TCMSP, SwissTargetPrediction (*http://www.swisstargetprediction.ch/*), and STITCH (*http://stitch.embl.de/*) programs [25]. The TCMSP database offers comprehensive information about the chemical components present in herbs, including chemical structure, drug targets, and their relationships with diseases. Based on the probability value obtained from the cross-validation analysis in the SwissTargetPrediction database, the rank of the targets and the accuracy of the prediction were estimated. Furthermore, to investigate the mode of action of TBM, interactions among the identified protein targets were analyzed using the STITCH database. The target protein names of the TBM bioactive chemical components were acquired and the standard gene names and Uniprot ID of these target proteins were retrieved from

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the UniprotKB (*http://www.uniprot.org/*) database, using the species "*Homo sapiens*" [26].

Collection of targets related to HCC

Targets related to HCC were collected from the OMIM (*http://omim.org/*), Drugbank (*http://www.drugbank.ca*), and DisGeNET (*https://www.disgenet.org/*) databases. OMIM is an information platform for human genes and genetic diseases [27]. Drugbank offers information about the target genes of many FDA-approved drugs [28]. DisGeNET is among the largest publicly available discovery platforms containing collections of genes and their variants associated with human diseases [29]. The database was searched against the keyword "hepatocellular carcinoma" and a total of 3755 HCC-related targets were retrieved, excluding duplicates. The potential active chemical components of TBM were matched against the potential targets related to HCC. The overlapping targets were selected as the components of TBM that have potential bioactivity against HCC.

Analysis of network construction and central network topology

A protein–protein interaction (PPI) network was constructed by processing the common targets using the STRING (*https://string-db.org/*) program [30]. The PPI network was visualized using Cytoscape v3.7.2 software [31,32]. Central network evaluation is a topological method that defines the core central network [33]. Degree centrality (DC), betweenness centrality (BC), and closeness centrality were analyzed using the Network Analyzer plugin in Cytoscape. These were used to measure the topological importance of nodes in the network [34]. The threshold values of the hub nodes in the network analysis were the corresponding median values of each parameter.

Cluster analysis

Cluster analysis is an important classification method and many cluster analysis algorithms have been reported for the PPI network in Cytoscape. In 1 study, the network stability of a module generated using the Molecular Complex Detection (MCODE) algorithm was found to be superior. Hence, MCODE was used to perform cluster analysis for the PPI network [35]. The criteria settings were as follows: node score cutoff=0.2; K-core=2; degree cutoff=2; and max depth=100.

Enrichment analysis of the GO and KEGG pathways

Potential biological processes, cellular components, molecular functions, and pathways related to the overlapping TBM and HCC targets were explored. The Database for Annotation Visualization and Integrated Discovery (DAVID, *http://david.nicifcrf.gov/*), an online platform for high-throughput functional annotation bioinformatics, was used to carry out the functional annotation and enrichment analysis [36]. GO terms with Bonferroni-adjusted *P*-value <0.05 and KEGG pathways with *P*-value <0.05 were considered significant [37].

Results

Target network of TBM compounds

A total of 46 active TBM compounds were identified from the TCMSP database. These were further shortlisted based on the oral bioactivity (OB ≥20%) and drug-likeness (DL ≥0.18) standards. Out of the original 46, only 22 of the active TBM components fulfilled these criteria. Among these, 9 of the bioactive TBM components showed potential to bind to HCC targets while the other 13 did not correspond to any of the HCC targets. After rejecting the duplicates, a total of 191 putative targets for the 9 candidate compounds were identified. Their properties are shown in the **Table 1**.

Target genes related to the identified TBM chemical components

The TBM compound-target network was constructed to elucidate the relationship between the compounds and the potential targets (**Figure 2A**). This network was composed of 200 nodes and 334 edges. Based on DC and BC, it was predicted that sitogluside, beta-sitosterol, sitosterol, and emodin are the principal active compounds that carry out the critical functions of TBM.

GO and KEGG pathway enrichment analyses were further performed to elucidate the characteristics of TBM-related targets. The GO analysis showed that most of the potential targets possess steroid-binding properties. Most of the target proteins were enriched in steroid-related processes, including the regulation of steroid hormone-mediated signaling pathways, drug response pathways, intracellular receptor signaling pathways, and in processes related to positive regulation of cell proliferation. These results suggest that TBM possesses multiple biological synergies (**Figure 2B**). The KEGG enrichment analysis revealed nearly 70 pathways (P<0.05) that could be predicted to be affected by TBM. The top 10 enriched pathways involved neuroactive ligand-receptor interaction, progesterone-mediated oocyte maturation, taste transduction, pathways associated with cancer, the sphingolipid signaling pathway, cell cycle regulation, prostate cancer, the PI3K-Akt signaling pathway, and the hepatitis C and FoxO signaling pathways (**Figure 2C**).

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Table 1. Exploration of the active chemical components of Tu Bei Mu (TBM) through a ligand-based prediction strategy.

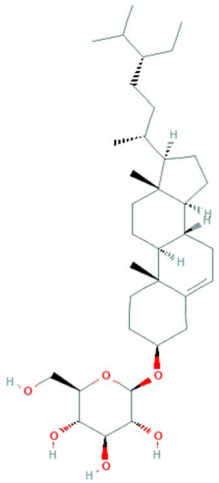
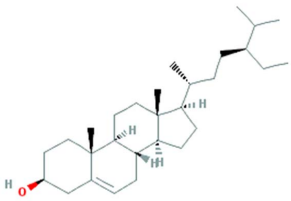
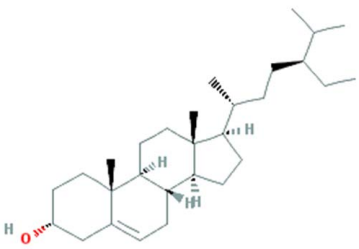
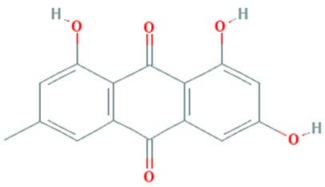
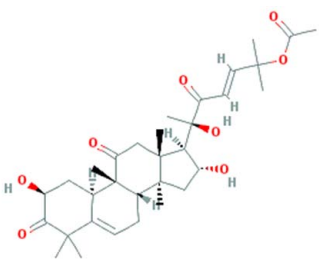
Molecule ID	Molecule name	Structure	OB	DL
MOL000357	Sitogluside		20.63	0.62
MOL000358	beta-sitosterol		36.91	0.75
MOL000359	Sitosterol		36.91	0.75
MOL000472	Emodin		24.40	0.24
MOL002440	Cucurbitacin b		25.90	0.75

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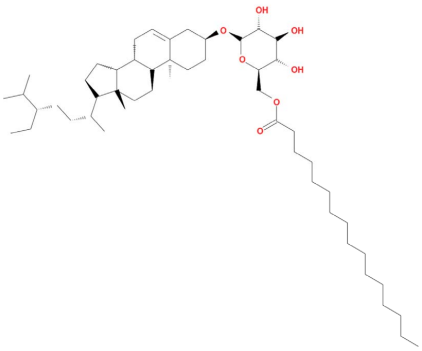
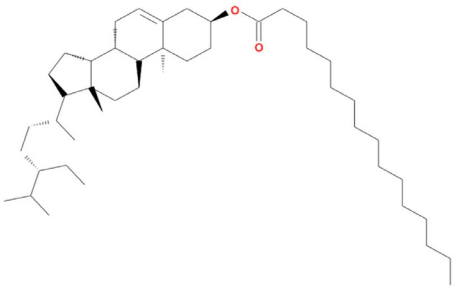
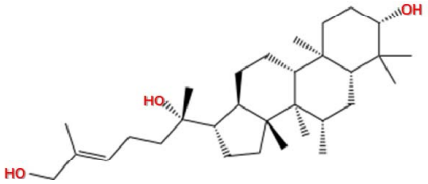
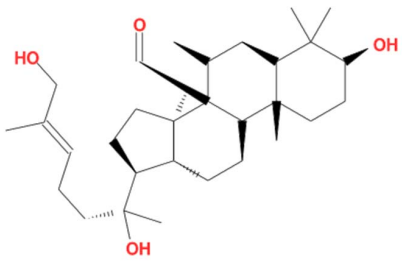
Table 1 continued. Exploration of the active chemical components of Tu Bei Mu (TBM) through a ligand-based prediction strategy.

Molecule ID	Molecule name	Structure	OB	DL
MOL010314	Daucosterol palmitate		25.51	0.19
MOL010315	beta-sitosterol palmitate		30.90	0.41
MOL010324	7β,20,26-trihydroxy-20(s)-24E-dammaragonene-3-O-α-L-arabinopyranose-(l-2)-β-D-(6'-acetyl)-glucopyranoside_qt		28.89	0.79
MOL010328	7β,20,26-trihydroxy-20(s)-24E-dammaragonene-3-O-α-L-(4'-acetyl)-arabinopyranose-(l-2)-β-D-glucopyranoside_qt		28.14	0.80

HCC-related target network

In the present study, 3755 targets related to HCC in the genomic database of human diseases were selected for analysis. Among these, TBM and HCC had 104 common targets (**Figure 3A, 3B**). These common targets also had high confidence scores as per the PPIs generated using the STRING database (**Figure 3C**). The threshold values of the first screening were degree ≥30, closeness ≥0.554, and betweenness ≥141.982. Topological analysis indicated that TP53, CASP3, VEGFA, EGFR, EGF, TNF, STAT3, HSP90AA1, ESR1, PTGS2, AR, PPARG, ERBB2, MAPK14, NR3C1, and CXCR4 were the top shared targets.

GO analysis suggested that the majority of these 16 core targets have protein-binding functions and that they are enriched in the nucleus and cytoplasm (**Figure 3D**). KEGG enrichment

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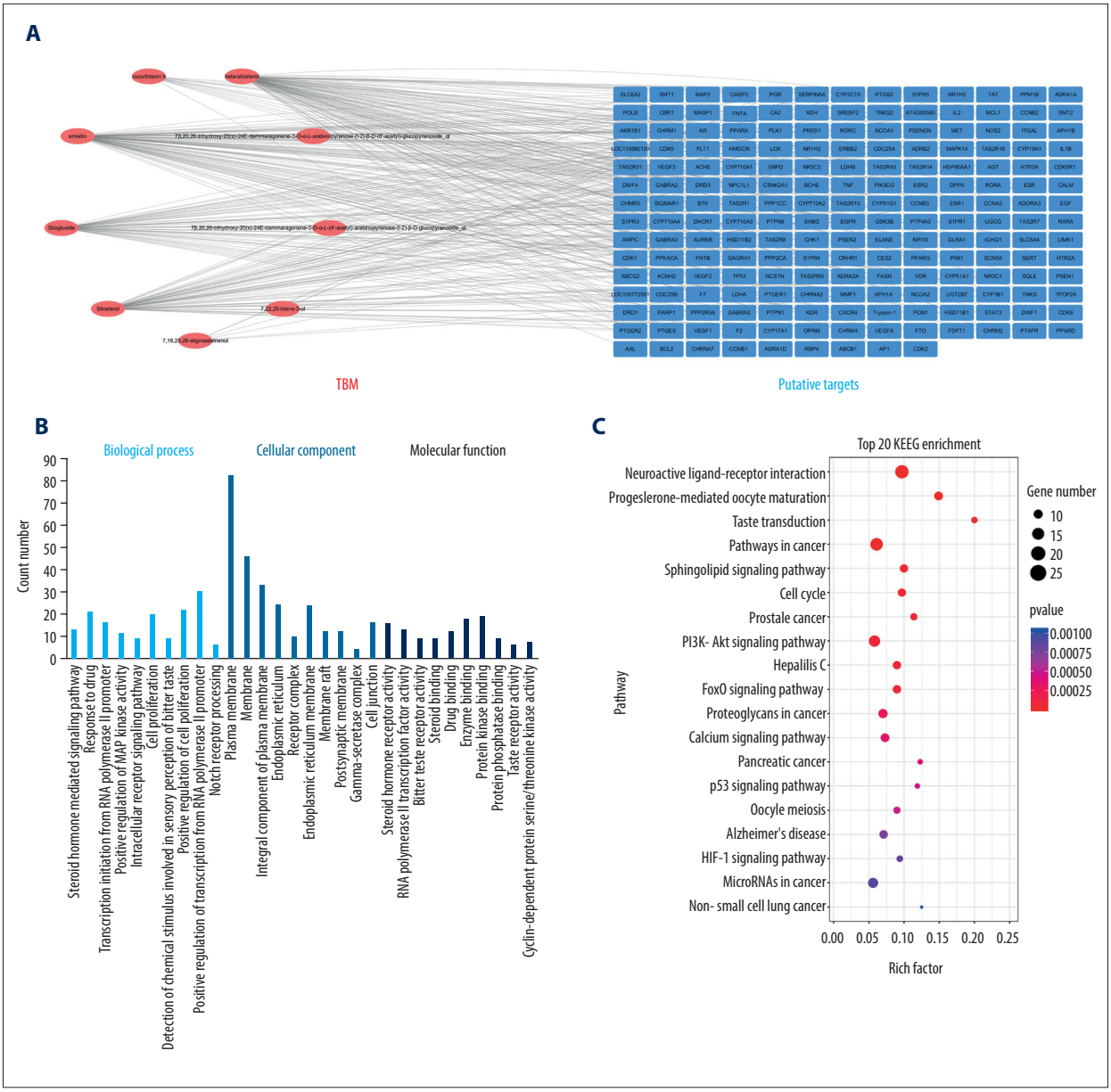

Figure 2. Characteristics of TBM-related targets clarified through GO and KEGG pathway enrichment analysis. (**A**) The component-target network of TBM. (**B**) GO enrichment analysis for potential targets of TBM. (**C**) KEGG enrichment analysis for potential targets of TBM. TBM – Tu Bei Mu; GO – Gene Ontology platform; KEGG – Kyoto Encyclopedia of Genes and Genomes platform.

analysis showed that these targets were enriched in processes related to the positive regulation of nitric oxide biosynthesis, gene expression and transcription from the RNA polymerase II promoter, and negative regulation of the apoptotic process (**Figure 3E**). These results indicate that various biological processes are involved in the multiple synergistic effects of TBM against HCC.

Enrichment analysis of the 104 targets

The PPI network of TBM targets and HCC targets was analyzed using MCODE. The final central PPI network was then classified into 4 clusters (**Figure 4A**). The cluster modules that included targets with enriched biological functions and signaling pathways were used to analyze the integral regulation of TBM in HCC treatment (**Figure 4B, 4C**). The following GO terms were included: (1) peptidyl-tyrosine phosphorylation, nitric oxide biosynthetic process, and MAPK activity; (2) cell division,

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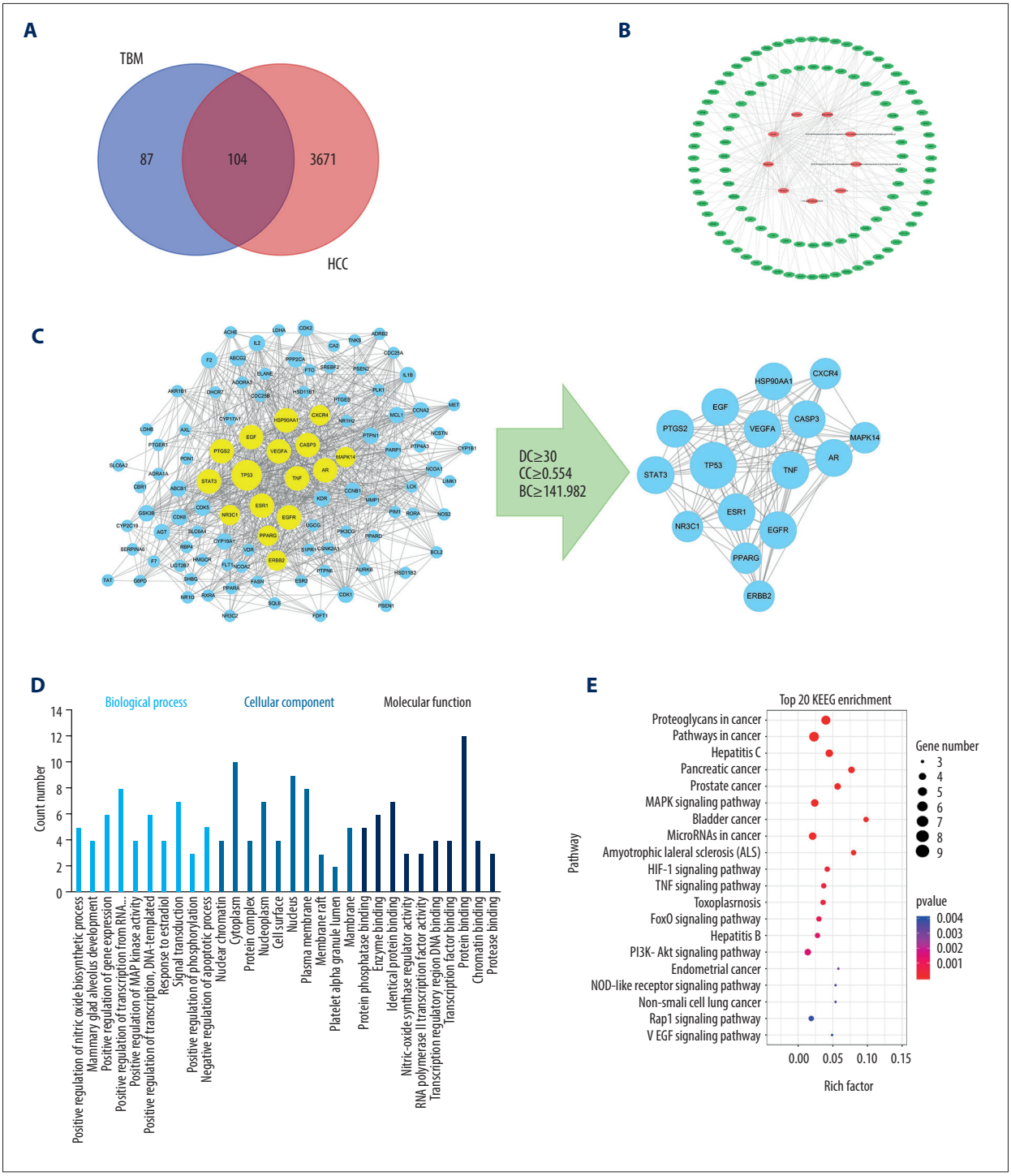

Figure 3. HCC-related target network. (**A**) 104 shared targets between TBM and HCC. (**B**) Component-target network of TBM for the treatment of HCC. The red circles represent components of TBM, and green hexagons represent common targets. (**C**) The process of topological screening of the PPI network. (**D**) GO enrichment analysis for 16 key targets. (**E**) KEGG enrichment analysis for 16 key targets. HCC – hepatocellular carcinoma; TBM – Tu Bei Mu; PPI – protein–protein interaction; GO – Gene Ontology platform; KEGG – Kyoto Encyclopedia of Genes and Genomes platform.

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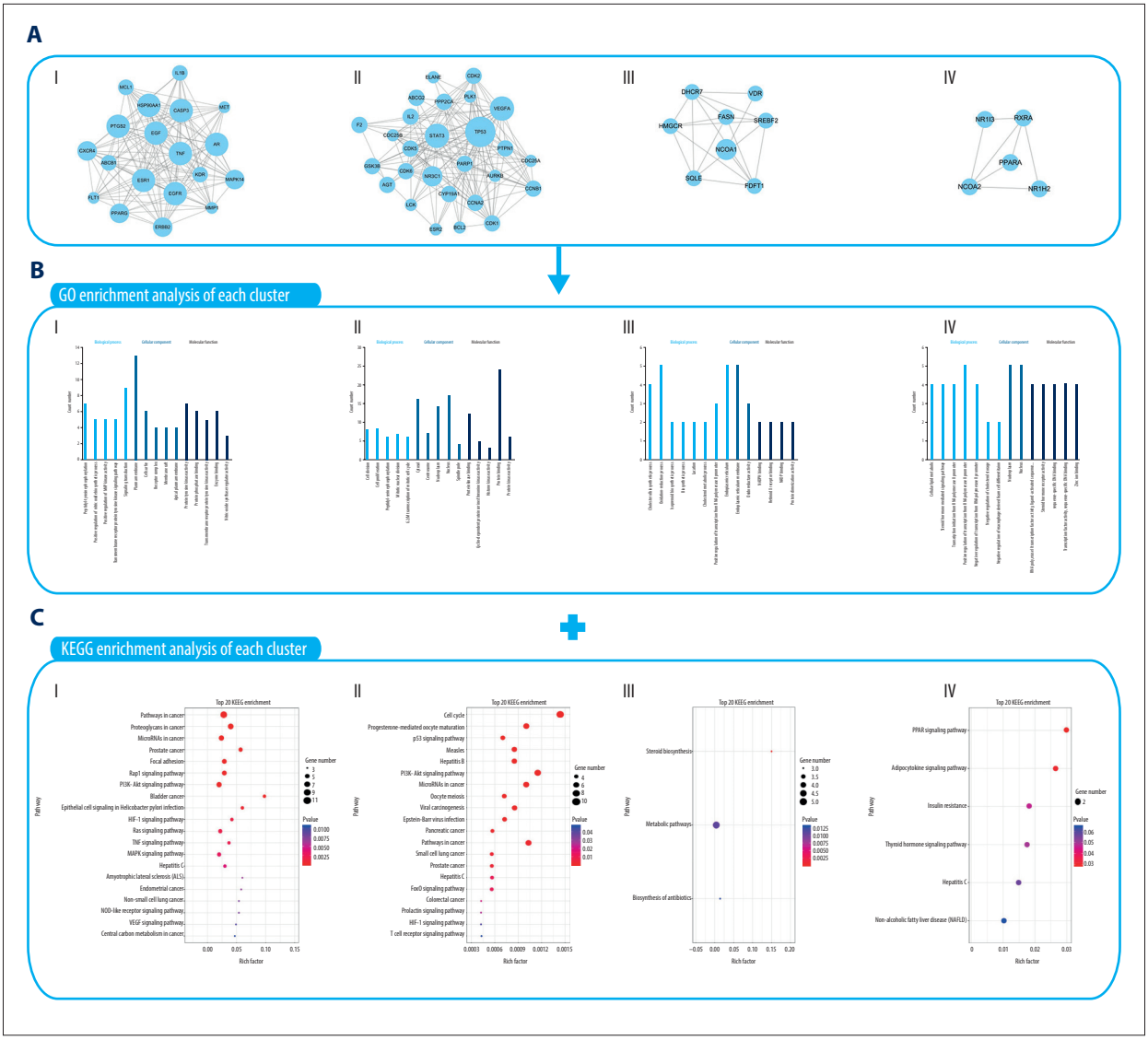

Figure 4. Enrichment analysis of the 104 TBM targets for the treatment of HCC. (**A**) Clusters of the final central PPI network. (**B**) GO enrichment analysis of each cluster. (**C**) KEGG pathway enrichment analysis of each cluster. TBM – Tu Bei Mu; HCC – hepatocellular carcinoma; PPI – protein–protein interaction; GO – Gene Ontology platform; KEGG – Kyoto Encyclopedia of Genes and Genomes platform.

cell proliferation, and G2/M transition of the mitotic cell cycle; (3) cholesterol biosynthetic process, oxidation-reduction process, and isoprenoid biosynthetic process; and (4) cellular lipid metabolic process, steroid hormone-mediated signaling pathway, and transcription initiation from the RNA polymerase II promoter.

The KEGG enrichment analysis revealed that different signaling pathways were enriched in different modules (**Figure 5**). Module 1 was associated with pathways related to cancer, including the PI3K-Akt signaling pathway, hypoxia-inducible factor-1 (HIF-1) signaling pathway, and TNF signaling pathway. Module 2 was associated with the cell cycle and p53 signaling pathways involved in hepatitis B and hepatitis C. Module 3 was associated with steroid biosynthesis and metabolic pathways. Module 4 was associated with the PPAR signaling pathway and adipocytokine signaling pathway, including pathways associated with insulin resistance and nonalcoholic fatty liver disease.

KEGG data analysis also indicated that the key signaling pathways, such as the PI3K-Akt signaling pathway, the HIF-1 signaling pathway, the p53 signaling pathway, and the PPAR signaling pathway, might be the core pharmacological targets of TBM against HCC. Overall, the aforementioned analysis emphasizes a novel strategy for developing drugs against HCC.

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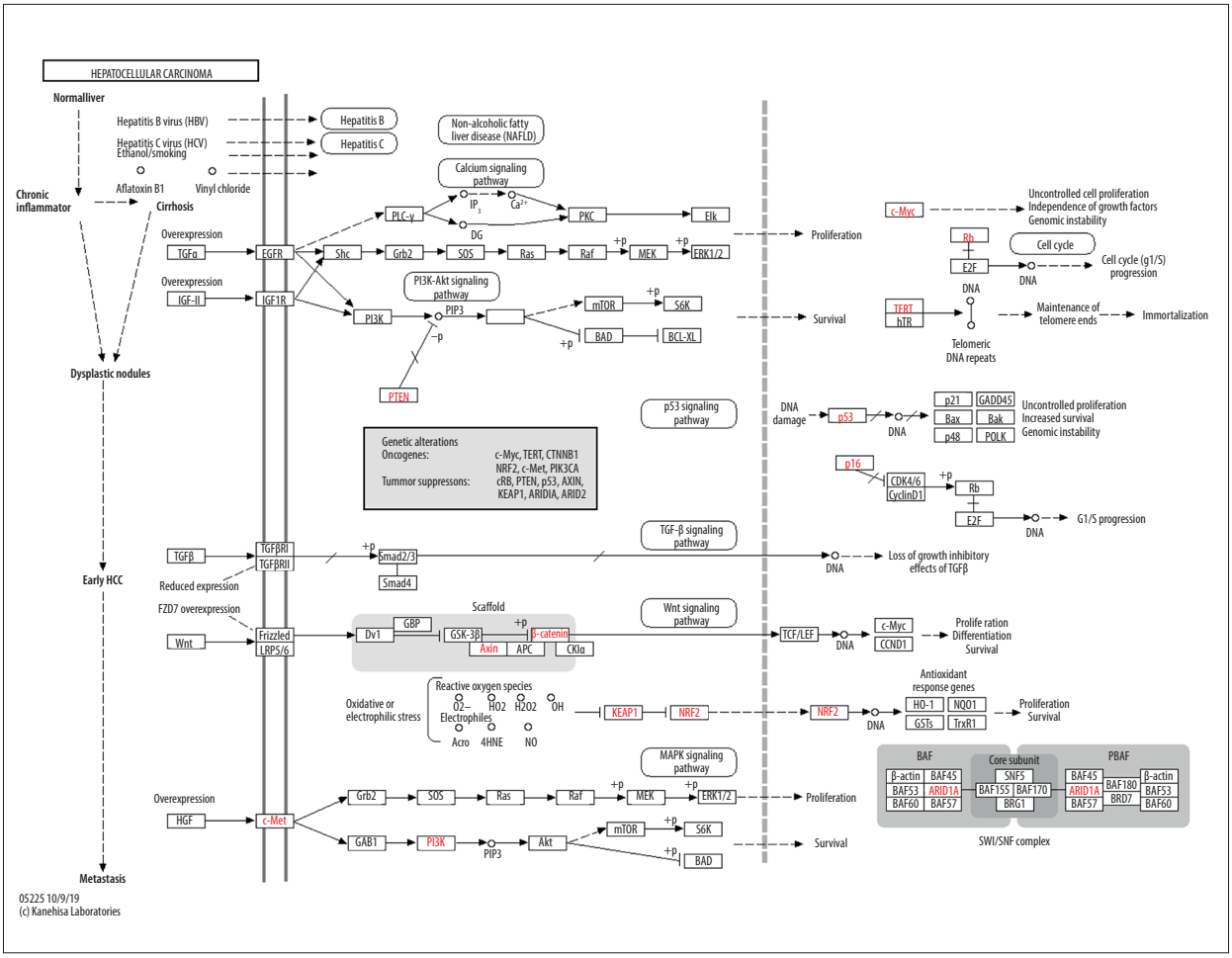

Figure 5. The predicted anticancer pathway of TBM. TBM – Tu Bei Mu.

Discussion

Advanced drug therapy for HCC is still limited [38]. The occurrence and development of HCC is induced by the dysfunction of crucial genes that trigger unrestricted cell growth [39]. Therefore, the use of multitarget compounds in treating liver cancer could be an effective strategy [40]. TBM is used for treating acute mastitis, scrotitis, subcutaneous nodules, sores, warts, snakebites, insect bites, common inflammatory diseases, and tumors, and is also used for detoxification. Its main pharmacologically active components are TBM I, II, III, and V [41,42]. These compounds could inhibit hepatoma cell growth and proliferation, induce apoptosis, and inhibit migration and angiogenesis via various signaling pathways. However, the precise mechanism by which TBM may prevent and treat liver cancer is not clear.

In the present study, a total of 22 active components, 191 predicted biological targets of TBM, and 3775 HCC-related targets were identified through screening and were further analyzed for pathway enrichment. As shown by KEGG enrichment analysis, the function of TBM in preventing and treating HCC could involve the following major pathways.

PI3K-Akt signaling pathway

In several human malignant tumors, the PI3K/Akt/mTOR signaling pathway has been found to be aberrantly activated [43]. Inhibiting any node of the PI3K/Akt pathway can compensate for deficiencies in chemotherapy, radiotherapy, and hormone therapy. This synergistic effect has been closely related to apoptosis resulting from the selective inhibition of PI3K or Akt activity [44]. The PI3K-Akt signaling pathway has also been established in the occurrence and development of HCC. Although it does not play a role in apoptosis, it plays a critical role in cell growth, proliferation, adhesion ability, and expression of cellular adhesion molecules in HCC. Studies have shown that inhibitors of the PI3K signaling pathway increase sensitivity towards radiotherapy-induced tumor cell apoptosis [45]. Radiotherapy could activate the FAK-PI3K/Akt signaling pathway, which enhances the proliferation of radiotherapy-resistant, surviving HCC cells. TBM can inhibit the expression

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of caveolin-1(CAV-1), an oncogenic membrane protein, to initiate the PI3K/Akt signaling pathway, thus inhibiting the metastasis of cancer cells [46].

HIF-1 signaling pathway

Hypoxia-inducible factor-1 (HIF-1) is a key transcription factor that is produced by tumor cells under hypoxic conditions to survive the hypoxic microenvironment. HIF-1 participates in energy metabolism, proliferation, apoptosis, invasion, metastasis, angiogenesis, and resistance to radiotherapy and chemotherapy by activating several downstream target genes in tumor cells [47]. Several studies have shown that changes in HIF-1 expression level are closely related to the proliferation and apoptosis of HCC cells. Additionally, it has been found that HIF-1 can promote cell proliferation through cyclin A and cyclin D [48]. HIF-1 is a direct transcriptional activator of the VEGF pathway under hypoxic conditions. It is reported that the anti-angiogenic effect of polypeptide extract from scorpion venom may be related to reduced levels of PI3K, HIF-1α, VEGF-A, and Akt [49]. Further, knockout of HIF-1α and IL-8 can inhibit angiogenesis in HCC [50]. HIF-1 can also affect the expression of EMT markers, such as cadherin E, cadherin N, and vimentin, by regulating the Snail factor to promote the invasion and metastasis of HCC cells [51]. Compared with other liver diseases, the expression level of HIF-1 is much higher in the serum of liver cancer patients. The HIF-1 serum level could be used as a new marker for the diagnosis and prognosis of liver cancer [52]. It has been reported that unregulated expression of HIF-1 provides a microenvironment conducive to tumors, and potentially increases the chance of recurrence of HCC after tumor resection [53]. Hence, HIF-1 could be used as an indicator of HCC prognosis, but such a use certainly requires further validation [54]. It has been found that reagents such as YC-1, a potential HIF-1 inhibitor, can act on targets by stimulating HIF-dependent P300 dissociation from HIF-1 [55]. The finding that siRNA-based silencing of HIF-1α significantly inhibited the proliferation of hepatoma in hypoxic CBRH-7919 mice through the PI3K/Akt signaling pathway led to the suggestion to focus on this pathway as a potential anti-cancer therapeutic strategy [56].

p53 signaling pathway

Cell tumor antigen p53, an important tumor suppressor gene, encodes for the p53 protein, which is involved in the regulation of the cell cycle, DNA repair, signal transduction, and apoptosis [57,58]. TBM has been reported to initiate apoptosis through a variety of endogenous (mitochondrial) and exogenous (death receptor) pathways to produce broad-spectrum cytotoxic effects on a variety of tumor cells. TBM I has been suggested to induce the exogenous pathway of Fas/FasL/caspase-8 in hepatoma HepG2 cells [59]. Also, TBM has been shown to activate endogenous apoptosis by regulating the Bcl-2 protein [60]. TBM II has been reported to induce G2/M arrest in HepG2 cells. The growth of rabbit VX2 hepatoma was also significantly inhibited by embolization with TBM microcapsules (5.1 mg/kg) [61]. This study suggests that TBM may also inhibit the angiogenesis of cancer cells by regulating necessary proteins related to this process.

PPAR signaling pathway

The PPAR pathway plays an important role in liver lipid metabolism by widely regulating the expression of proteins related to free fatty acid transport and β-oxidation. It also participates in the maintenance of glucose homeostasis [62]. It is involved in the regulation of many diseases, such as diabetes, obesity, and atherosis. However, its role in regulating cell growth, proliferation, and tumorigenesis is still controversial. PPAR-α, an important transcriptional regulatory factor, inhibits inflammation, but the mechanism underlying its regulatory effect in tumor proliferation, occurrence, and development is dubious [63]. It has been shown that endogenous PPAR-α agonists induce hepatocyte hypertrophy in rodents [64]. In transgenic mice, HCV core protein increases the level of circulating nonesterified fatty acids (NEFAs), which activate PPAR-α-mediated hepatogenesis [65]. These findings suggest that the endogenous ligand-activated PPAR-α signaling pathway could be involved in hepatocyte proliferation and carcinogenesis.

Other relevant signaling pathways

Etiological studies of liver cancer, excluding cancers with hepatitis virus infection, suggest that diabetes can increase the risk of liver cancer by 2- to 3-fold [66,67]. Inflammation is central to the immune response in infection and injury. Chronic inflammation, however, aggravates the growth and development of malignant cells, which is closely associated with an increased risk of cancer [68]. TBM exerts an anti-inflammatory immune response. The order of strength of anti-inflammatory and antitumor activities was as follows: TBM III >TBM II >TBM I [69]. This suggests that TBM also plays an important role in regulating inflammation and immune responses. For instance, the hepatitis virus protein HBX plays an important role in the development of HCC by promoting the progression of hepatitis to HCC. Additionally, the enrichment analysis suggests that HBX may also affect other organ tumors, including the occurrence and development of liver cancer.

In general, the network pharmacological analysis revealed possible mechanisms of action of *Bolbostemma paniculatum* in preventing and treating HCC. These findings may provide an important hypothetical basis for future investigations in biological models such as animals by predicting the molecular mechanism underlying HCC treatment.

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Conclusions

The present study predicted the targets of TBM and explored mechanisms that could underlie potential anti-HCC effects. The PI3K/Akt, HIF-1, p53, and PPAR pathways may all play vital roles in TBM treatment of HCC. Our results suggest that the potential anti-cancer effect of TBM on HCC is based on the synergetic effect of multiple targets and mechanisms. However, these hypotheses still must be verified through further laboratory-based experiments.

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Conflicts of interest

None.

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