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A Network Pharmacology Approach to Explore the Potential Mechanisms of Huangqin-Baishao Herb Pair in Treatment of Cancer

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Background: The aim of this study was to identify the bioactive ingredients of Huangqin-Baishao herb pair and to reveal its anti-cancer mechanisms through a pharmacology approach.

Material/Methods: Detailed information on compounds in the HQ-BS herb pair was obtained from the Traditional Chinese medicine systems pharmacology (TCMSP) and screened by the criteria of OB ≥30% and DL ≥0.18. A systematic drug targeting model (SysDT) was used for compound targets prediction, and then the targets were analyzed for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. The protein-protein interaction (PPI) network of HQ-BS targets was constructed, after identifying core networks through Cytoscape plugins.

Results: We found 47 bioactive compounds of HQ-BS and 107 human-derived targets. A compound target network and a target signal pathway network were constructed and used for topological analysis. Kaempferol, beta-sitosterol, stigmasterol, wogonin, and oroxylin-a were identified as core compounds and pathways in cancer. The calcium signaling pathway, PI3K-Akt signaling pathway, TNF signaling pathway, chemical carcinogenesis, estrogen signaling pathway, proteoglycans in cancer, HIF-1 signaling pathway, thyroid hormone signaling pathway, VEGF signaling pathway, small cell lung cancer, prostate cancer, colorectal cancer, NOD-like receptor signaling pathway, and T cell receptor signaling pathway were found to be potential signals of HQ-BS in treating cancer. Through PPI network analysis, TNF signaling pathway, tryptophan metabolism, proteoglycans in cancer, cell cycle, and chemical carcinogenesis sub-networks were obtained.

Conclusions: HQ-BS contains various bioactive compounds, including flavonoids, phytosterols, and other compounds, and these compounds can inhibit or activate multiple targets and pathways against cancer.

MeSH Keywords: Computer Communication Networks • Medicine, Chinese Traditional • Molecular Mechanisms of Pharmacological Action • Protein Interaction Maps • Ethnopharmacology

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Background

Cancer is an important public health problem in developing and as well as developed countries, and is now a leading cause of death worldwide. According to newly published cancer statistics data, the cancer death rate rose until 1991, and then fell continuously through 2017 in the USA [1]. Although there have been huge advances in cancer treatment in recent decades, discovering treatments with better efficacy and less toxicity is still an important area of cancer research.

As a major complementary and alternative medicine system, traditional Chinese medicine (TCM) has provided anti-cancer therapy for a long time because of its anti-inflammatory activities and use of abundant ingredients with tumor toxicity and microenvironment regulation. Huang Qin (HQ) refers to the dried root of *Scutellaria baicalensis* and Bai Shao (BS) is derived from *Paeonia lactiflora Pall* in the Chinese Pharmacopoeia (2015). As recorded in the Treatise on Febrile and Miscellaneous Diseases, the 2 herbs are used together at a crude weight ratio of 3: 2 in Huangqin decoction to treat gastrointestinal ailments. Recent research focusing on the anti-cancer effect of Huangqin decoction found Huangqin decoction could ameliorate chemotherapy-induced gastrointestinal toxicity and enhance the therapeutic efficacy of antitumor drugs [2,3]. According to these experimental data, deletion of either HQ or BS eliminated Huangqin decoction's synergistic activity, and deletion of either GC and DZ did not eliminate Huangqin decoction's synergistic activity, indicating HQ and BS are essential in this formula and may have synergistic effects as an herb pair. Furthermore, based on a Chinese patent medicines study, HQ and BS are considered as 2 typical pathogen-eliminating and health-strengthening herbs, which are widely used against cancer [4]. Recent studies showed that HQ inhibited tumor growth and targeted apoptotic pathways, tumor-associated macrophages, MAPK pathway, and PI3K-Akt-mTOR signaling pathway [5]. BS is approved to induce apoptosis in HL-60 leukemic cells [6] and is recognized as a therapeutic agent against cancer cachexia [7].

To understand the anti-cancer mechanisms of HQ-BS herb pair, a network pharmacology approach was employed. Particularly, network pharmacology can help elucidate the interactive relationship between multiple components and multiple targets and investigate multiple molecular mechanisms of HQ-BS herb pair at a network level. Meanwhile, the relationships among compounds, targets, and signal pathways were also investigated. Finally, the multitarget and multipathway mechanisms of the cancer signal pathway were determined for HQ-BS against cancer.

Material and Methods

Active ingredients identification

The Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, *http://ibts.hkbu.edu.hk/LSP/tcmsp.php*) [8] was used for bioactive ingredients identification of HQ-BS. Pharmacokinetic absorption, distribution, metabolism, and excretion (ADME) parameters of each compound in HQ-BS were determined. Bioactive ingredients were screened based on threshold values of OB ≥30% and DL ≥0.18, as recommended by the TCMSP database (Table 1).

Prediction of putative targets of HQ-BS

To identify the potential targets of bioactive ingredients in HQ-BS, a systematic drug targeting model (SysDT) based on RF and SVM methods was proposed, as previously described [9]. The gene name of each target was obtained from the UniProt Knowledgebase (*http://www.uniprot.org/*).

PPI network construction

Protein-protein interactions (PPI) data were obtained from STRING (*https://string-db.org/cgi/input.pl*). We filtered the STRING with threshold 0.4 and constructed a PPI network. Only interactions with weight above the threshold were selected for the newly constructed PPI network.

GO and KEGG pathway enrichment analysis

The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted using the functional annotation tool of DAVID Bioinformatics Resources (*http://david.abcc.ncifcrf.gov/*). Official gene symbols were uploaded and annotations were limited by Homo sapiens and we set the background to be Homo sapiens. With $p<0.05$, we applied a hypergeometric test to obtain enriched GO terms and KEGG pathways.

Network construction

We constructed networks using Cytoscape (version 3.7.1) as follows: (1) the "HQ-BS bioactive compound target network" was built by connecting the HQ-BS ingredients and their targets, and (2) the "compound target signal pathway network" was built by connecting the ingredient targets and relevant signal pathways, and (3) the "PPI network" was constructed by linking targets to other human proteins interacting with them. CytoHubba, a Cytoscape plugin, was applied for calculating topological features of the PPI network, and another Cytoscape plugin, MCODE, was used to identify sub-networks.

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Table 1. Detailed information on active ingredients in HQ-BS herb pair.

Mol ID	Molecule Name	Pubchem Cid	MW	OB (%)	DL	Herb
MOL001689	Acacetin	5280442	284.28	34.97	0.24	Huangqin
MOL000173	Wogonin	5281703	284.28	30.68	0.23	Huangqin
MOL000228	(2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one	821279	270.3	55.23	0.2	Huangqin
MOL002714	Baicalein	5281605	270.25	33.52	0.21	Huangqin
MOL002908	5,8,2'-Trihydroxy-7-methoxyflavone	156992	300.28	37.01	0.27	Huangqin
MOL002909	5,7,2,5-tetrahydroxy-8,6-dimethoxyflavone	44258628	376.34	33.82	0.45	Huangqin
MOL002910	Carthamidin	188308	288.27	41.15	0.24	Huangqin
MOL002911	2,6,2',4'-tetrahydroxy-6'-methoxychaleone	N/A	302.3	69.04	0.22	Huangqin
MOL002913	Dihydrobaicalin_qt	14135323	272.27	40.04	0.21	Huangqin
MOL002914	Eriodyctiol (flavanone)	373261	288.27	41.35	0.24	Huangqin
MOL002915	Salvigenin	161271	328.34	49.07	0.33	Huangqin
MOL002917	5,2',6'-Trihydroxy-7,8-dimethoxyflavone	5322059	330.31	45.05	0.33	Huangqin
MOL002925	5,7,2',6'-Tetrahydroxyflavone	5321865	286.25	37.01	0.24	Huangqin
MOL002926	Dihydrooroxylin A	5316733	286.3	38.72	0.23	Huangqin
MOL002927	Skullcapflavone II	124211	374.37	69.51	0.44	Huangqin
MOL002928	Oroxylin a	5320315	284.28	41.37	0.23	Huangqin
MOL002932	Panicolin	5320399	314.31	76.26	0.29	Huangqin
MOL002933	5,7,4'-Trihydroxy-8-methoxyflavone	5322078	300.28	36.56	0.27	Huangqin
MOL002934	Neobaicalein	124211	374.37	104.34	0.44	Huangqin
MOL002937	Dihydrooroxylin	25721350	286.3	66.06	0.23	Huangqin
MOL000525	Norwogonin	5281674	270.25	39.4	0.21	Huangqin
MOL000552	5,2'-Dihydroxy-6,7,8-trimethoxyflavone	159029	344.34	31.71	0.35	Huangqin
MOL000073	Ent-Epicatechin	182232	290.29	48.96	0.24	Huangqin
MOL000449	Stigmasterol	5280794	412.77	43.83	0.76	Huangqin
MOL001458	Coptisine	72322	320.34	30.67	0.86	Huangqin
MOL001490	Bis[(2S)-2-ethylhexyl] benzene-1,2-dicarboxylate	7057920	390.62	43.59	0.35	Huangqin
MOL001506	Supraene	638072	410.8	33.55	0.42	Huangqin
MOL002879	Diop	33934	390.62	43.59	0.39	Huangqin
MOL002897	Epiberberine	160876	336.39	43.09	0.78	Huangqin
MOL008206	Moslosooflavone	188316	298.31	44.09	0.25	Huangqin
MOL010415	11,13-Eicosadienoic acid, methyl ester	5365674	322.59	39.28	0.23	Huangqin
MOL012245	5,7,4'-trihydroxy-6-methoxyflavanone	26213330	302.3	36.63	0.27	Huangqin
MOL012246	5,7,4'-trihydroxy-8-methoxyflavanone	42608119	302.3	74.24	0.26	Huangqin

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Table 1 continued. Detailed information on active ingredients in HQ-BS herb pair.

Mol ID	Molecule Name	Pubchem Cid	MW	OB (%)	DL	Herb
MOL012266	Rivularin	13889022	344.34	37.94	0.37	Huangqin
MOL001910	11alpha,12alpha-epoxy-3beta-23-dihydroxy-30-norolean-20-en-28,12beta-olide	N/A	470.71	64.77	0.38	Baishao
MOL001918	Paeoniflorgenone	70698143	318.35	87.59	0.37	Baishao
MOL001919	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a] phenanthrene-15,16-dione	9841735	358.52	43.56	0.53	Baishao
MOL001921	Lactiflorin	14605198	462.49	49.12	0.8	Baishao
MOL001924	Paeoniflorin	442534	480.51	53.87	0.79	Baishao
MOL001925	Paeoniflorin_qt	11973336	318.35	68.18	0.4	Baishao
MOL001928	Albiflorin_qt	134761887	318.35	66.64	0.33	Baishao
MOL001930	Benzoylpaeoniflorin	21631106	584.62	31.27	0.75	Baishao
MOL000211	Mairin	64971	456.78	55.38	0.78	Baishao
MOL000422	Kaempferol	5280863	286.25	41.88	0.24	Baishao
MOL000492	(+)-catechin	9064	290.29	54.83	0.24	Baishao
MOL000358	Beta-sitosterol	222284	414.79	36.91	0.75	Huangqin and Baishao
MOL000359	Sitosterol	12303645	414.79	36.91	0.75	Huangqin and Baishao

Results

Identification of bioactive compounds

From the TCMSP database, 143 compounds of Huangqin and 85 compounds of Baishao were obtained. A total of 47 compounds were identified by ADME-related pharmacokinetic parameters, OB and DL (Table 1), and the screening criteria were OB ≥30% and DL ≥0.18. In detail, 34 compounds were only in Huangqin, 11 compounds were only in Baishao, and 2 compounds were both in Huangqin and Baishao (Figure 1A).

Construction of compound target network

After converting the corresponding gene name into Gene Symbol through the UniProt database, deleting duplicate targets, 107 human-derived targets were obtained. Deleting non-target compound, we constructed a visualized compound target network containing 146 nodes and 871 edges (Figure 1B). Through topological analysis, some compounds were characterized as important molecules in compound target network of HQ-BS pair, including kaempferol (Degree: 55), beta-sitosterol (Degree: 50), stigmasterol (Degree: 44), wogonin (Degree: 36), (2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one (Degree: 36), oroxylin-a (Degree: 35), baicalein (Degree: 33), 5,2'-Dihydroxy-6,7,8-trimethoxyflavone (Degree: 32), Skullcap flavone II (Degree: 32), and rivularin (Degree: 31).

Construction of target signal pathway network

To evaluate the potential mechanisms of HQ-BS herb pair pharmacological effects, we used the DAVID web server to analyze target-related signal pathways. The threshold of $P<0.05$ was regarded as a significant signal pathway, which was then used to construct a target signal pathway network (Figure 2A). This network contained 68 nodes and 142 edges. Sorted by degree value, pathways in cancer were identified as the most important signal pathway (Degree: 21), indicating an anti-cancer effect of HQ-BS herb pair. Other related signal pathways included Calcium signaling pathway (Degree: 16), PI3K-Akt signaling pathway (Degree: 13), TNF signaling pathway (Degree: 10), Chemical carcinogenesis (Degree: 9), Estrogen signaling pathway (Degree: 9), Proteoglycans in cancer (Degree: 9), HIF-1 signaling pathway (Degree: 8), Thyroid hormone signaling pathway (Degree: 8), VEGF signaling pathway (Degree: 7), Small cell lung cancer (Degree: 7), Prostate cancer (Degree: 7), Colorectal cancer (Degree: 6), NOD-like receptor signaling pathway (Degree: 6), and T cell receptor signaling pathway (Degree: 6). Some targets were involved in multiple signals. PIK3CG was involved in 12 pathways and TP53 was involved in 7 pathways, suggesting they are potential targets against cancer.

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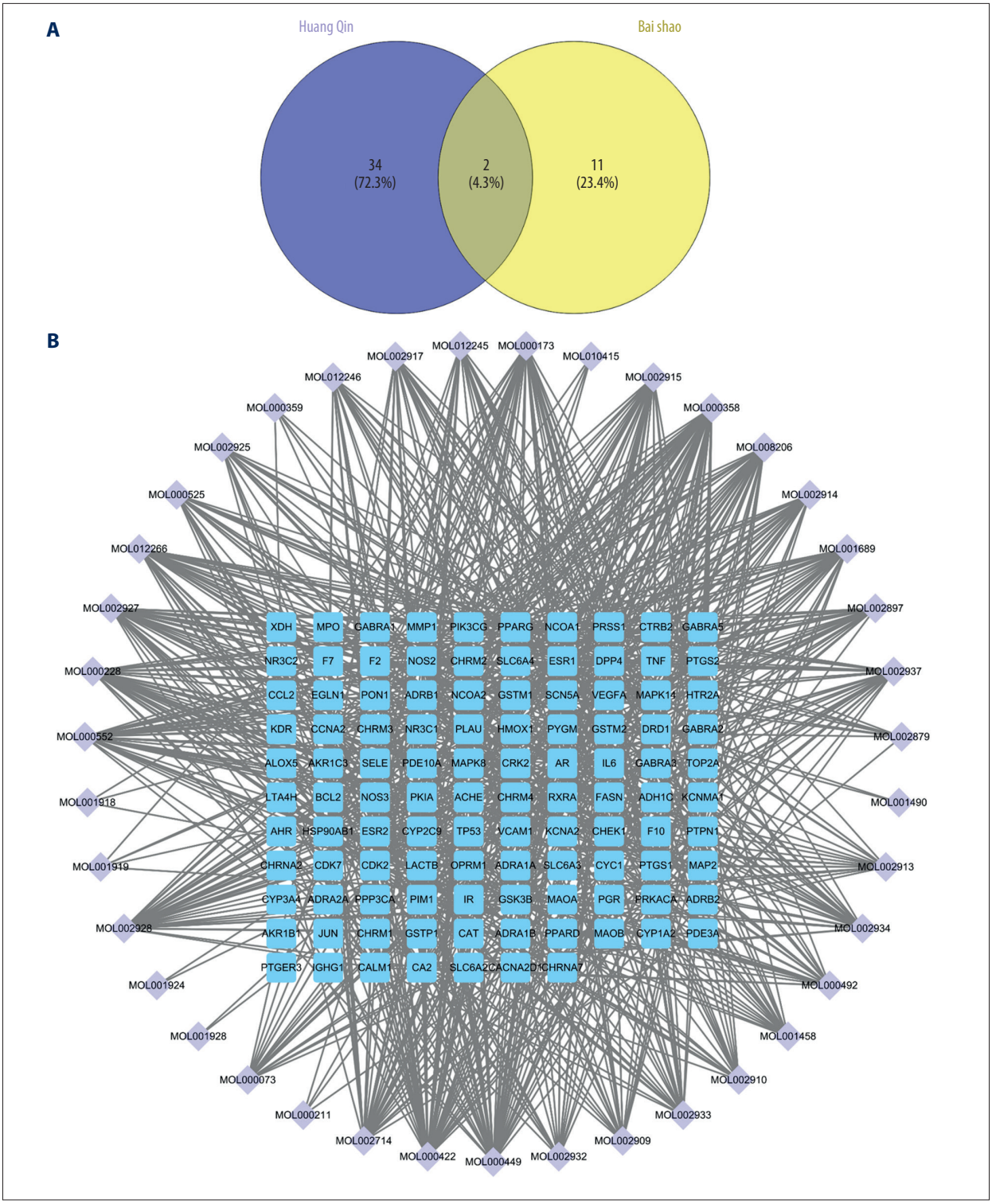

Figure 1. Bioactive compounds screening and compound target network construction. (**A**) The number of bioactive compounds in Huang Qin and Bai Shao are shown in a Venn diagram. (**B**) Compound target network of Huang Qin-Bai Shao herb pair constructed for topological analysis.

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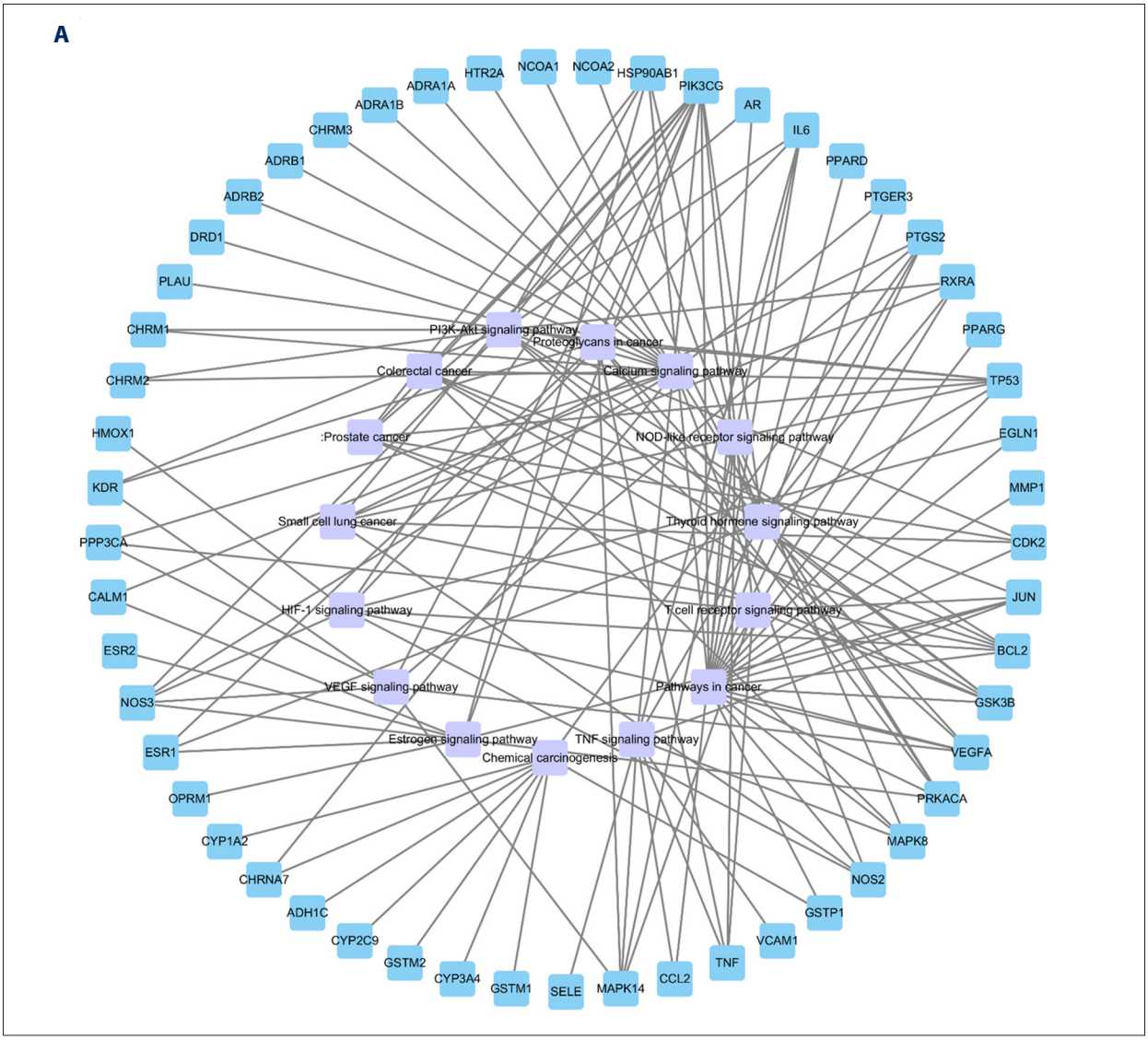
To understand the multiple target effect of HQ-BS herb pair, an ideogram was constructed using the KEGG mapper server (Figure 2B). Red labels represented HQ-BS herb pair-related targets or signal pathways, green labels represented other proteins in cancer pathway, and white labels represented other related signal pathways. HQ-BS herb pair influenced upstream, midstream, and downstream targets of the cancer signal pathway, and also influenced Calcium signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, and Estrogen signaling pathway, which may crosstalk with cancer signal.

Analysis of target protein interaction network (PPI)

We used the STRING online server to construct a PPI network of HQ-BS targets with the combined score >0.4. A network containing 102 nodes and 769 edges was obtained (Figure 3). As shown in Figure 3, each solid circle represented a target protein, and the center of the dot shows the protein structure. In the PPI network, the linkage of each node represented protein homology, gene co-expression, and gene co-evolution. Based on degree values, the top 10 targets in the network were identified: IL6 (degree: 46), TP53 (degree: 44), VEGFA (degree: 43), PTGS2 (degree: 38), JUN (degree: 37), TNF (degree: 37), NOS3 (degree: 37), ESR1 (degree: 36), CAT (degree: 36), and MAPK8 (degree: 35). The more positive the degree value of a target, the more prominent the role of this target in the network, and these may be the key targets for HQ-BS anti-cancer effects.

Identification of PPI sub-network

A Cytoscape plugin, MCODE, was used to identify sub-networks to understand the modulation of HQ-BS herb pair at a PPI network level. As shown in Figure 4, 5 sub-networks were identified. Sub-network 1 contained 19 nodes and 160 edges and



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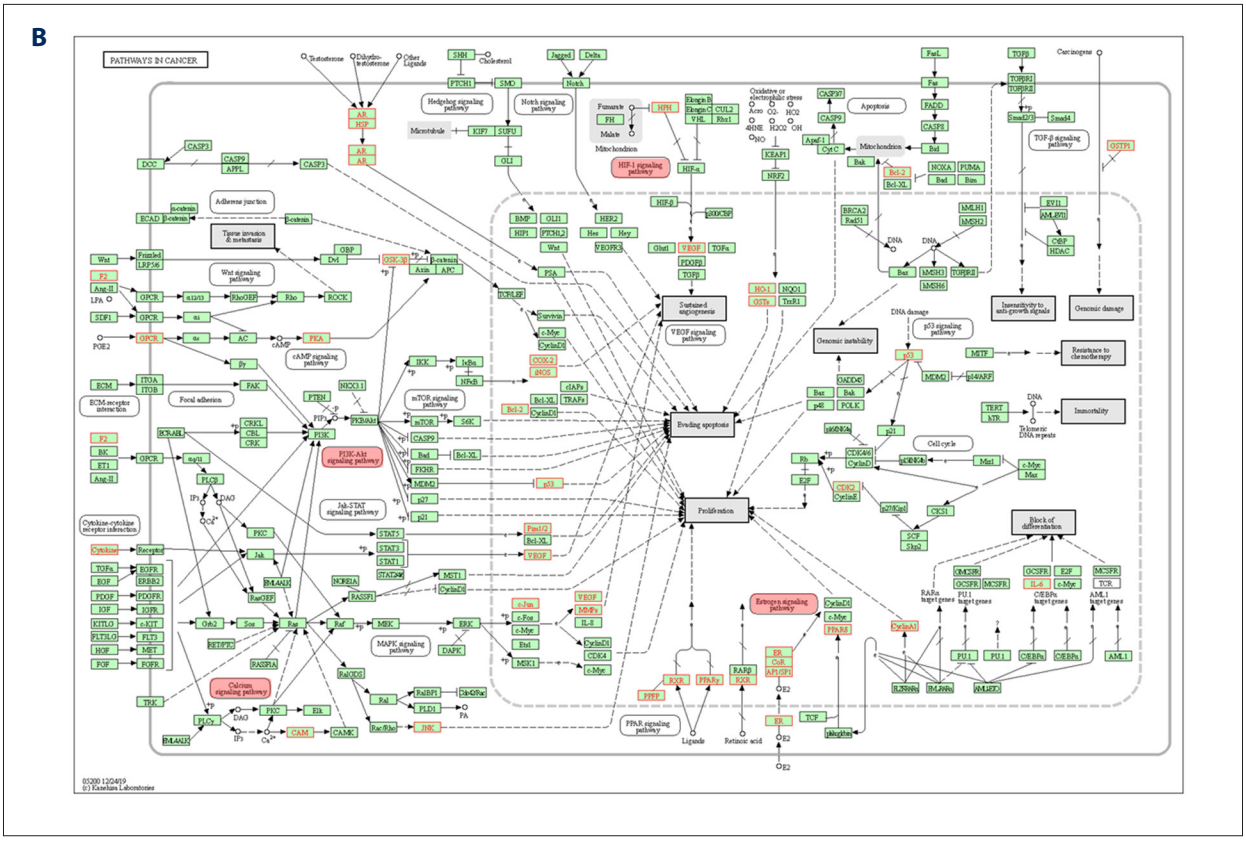

Figure 2. Multiple targets and multiple signal pathways of Huang Qin-Bai Shao (HQ-BS) herb pair against cancer. (**A**) Target KEGG signal pathway network. (**B**) Modulation by HQ-BS herb pair of pathways in cancer. Red labels represent HQ-BS herb pair-related targets or signal pathways, green labels represent other proteins in cancer pathway, and white labels represent other related signal pathways.

was involved in TNF signaling pathway. Sub-network 2 contained 9 nodes and 22 edges and was involved in tryptophan metabolism. Sub-network 3 contained 7 nodes and 14 edges and was involved in proteoglycans in cancer. Sub-network 4 contained 5 nodes and 8 edges and was involved in cell cycle. Sub-network 5 contained 4 nodes and 6 edges and was involved in chemical carcinogenesis.

Discussion

In ancient times, Chinese doctors always used a single herb to treat a specific disease, which was recorded by Shen Nong's Materia Medica. Later, people started to realize that the therapeutic effect of a single herb may be not enough and could have various clinical adverse effects in. Therefore, multi-herb therapy characterized by the combination of 2 or more herbs with more efficacies and less adverse effects have been utilized for thousands of years in China. Herb pairs, which are the most fundamental, simplest, and the most basic composition units of multi-herb therapy, are unique combinations of 2 relatively fixed herbs [10]. Studying the biological mechanisms of herb pairs helps understand compatibility theory of TCM and improves its clinical usage. HQ and BS are 2 typical pathogen-eliminating and health-strengthening herbs which are widely used against cancer. Based on the HQ-BS herb pair, many formulae, including Huangqin decoction [11], are consequently formed, which are all used to treat cancer. Clinical and experimental evidence show that HQ-BS herb pair induces inflammation and oxidative stress of precancerous lesions of colorectal cancer [12,13]. In this study, we employed network pharmacology to reveal the potential biological mechanisms of HQ-BS herb pair against cancer.

Through ADME screening, 47 compounds were recognized as bioactive compounds of HQ-BS herb pair, indicating a multi-component effect. We constructed a compound target network and calculated the degree value of each node. Based on degree value, some ingredients were identified as important in this network, including kaempferol, beta-sitosterol, stigmasterol, wogonin, and oroxylin-a. Many *in vivo* and *in vitro* studies have found that these compounds possess potent anti-cancer pharmacological activity. For example, Kaempferol has a powerful anti-cancer effect against various cancer cell lines [14,15].

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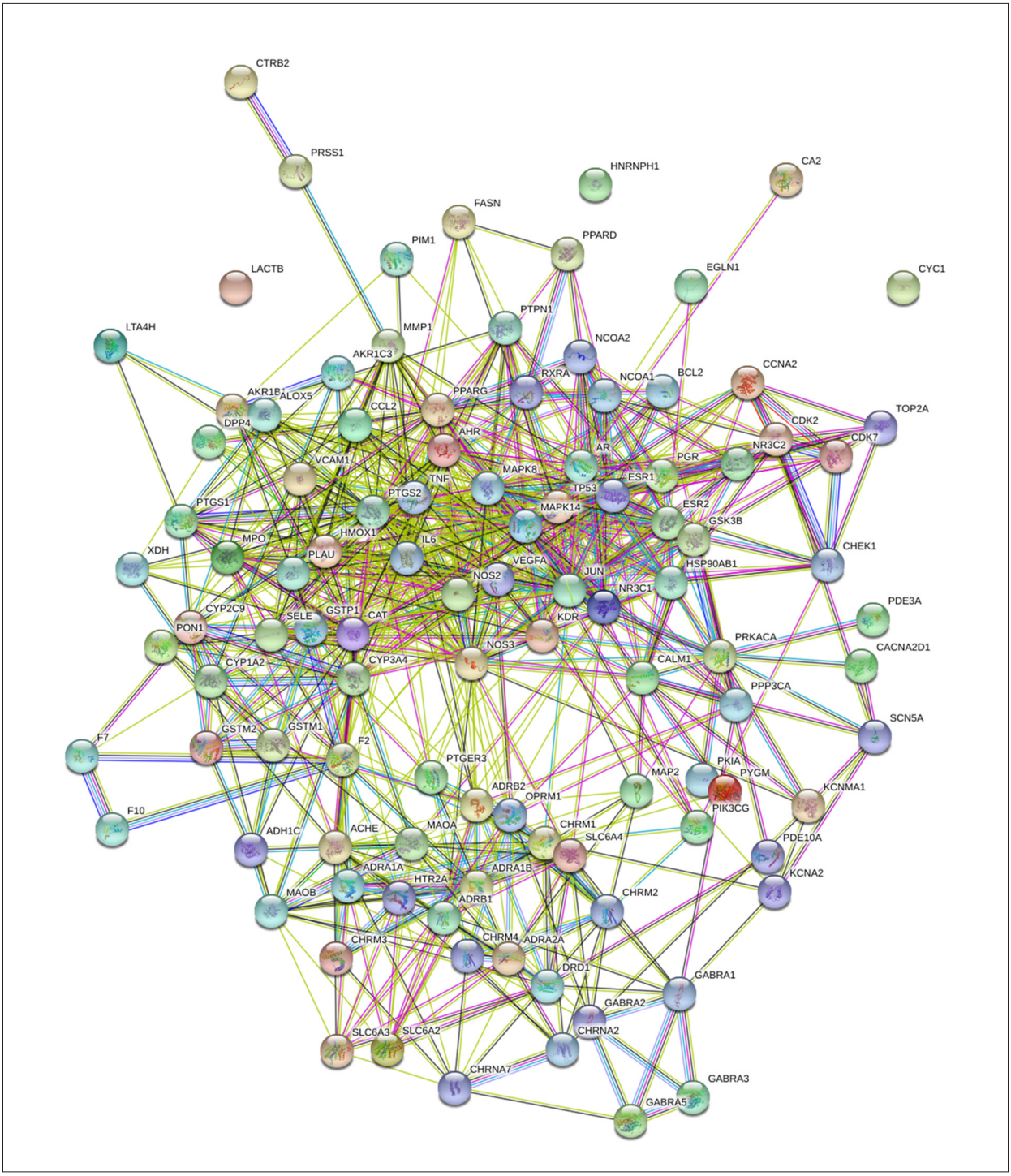

Figure 3. Protein-protein interaction (PPI) networks of Huang Qin-Bai Shao herb pair.

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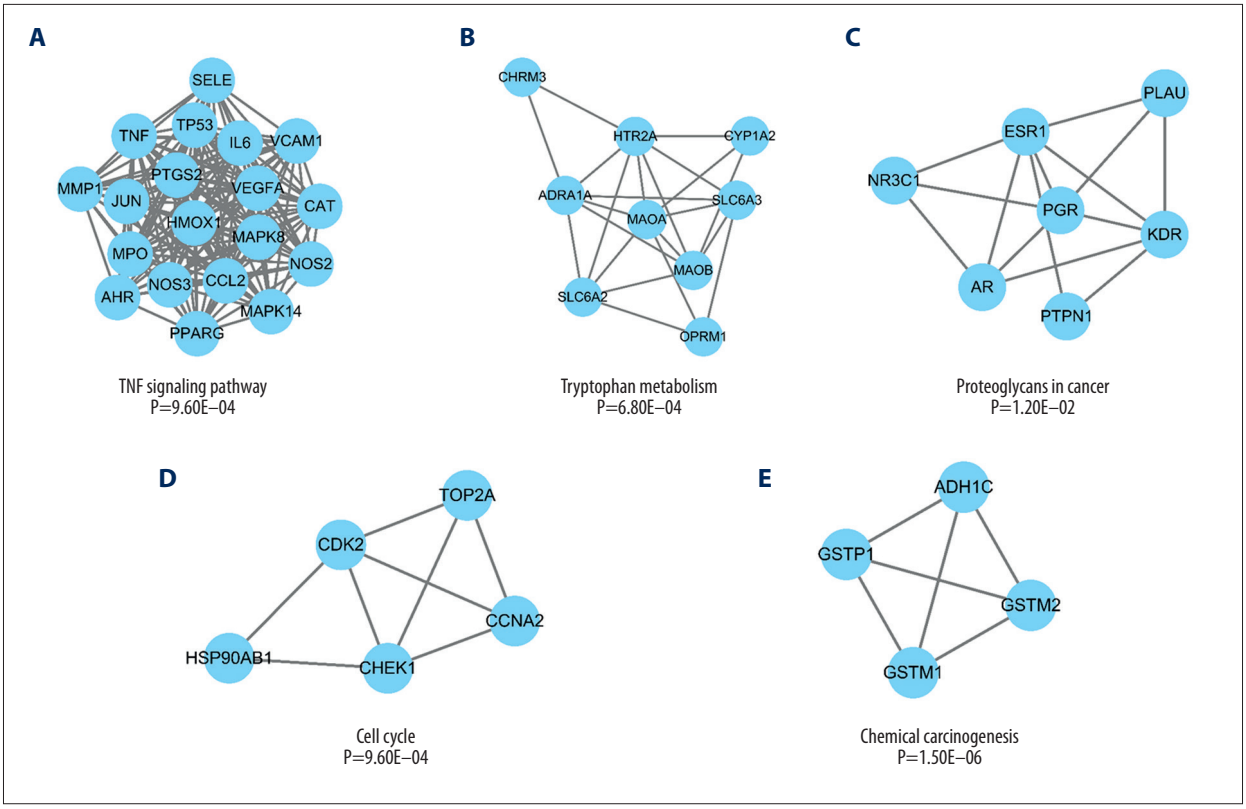

Figure 4. Sub-network of targets protein-protein interaction (PPI) network. (**A**) Sub-network 1 related to TNF signaling pathway. (**B**) Sub-network 2 related to tryptophan metabolism. (**C**) Sub-network 3 related to proteoglycans in cancer. (**D**) Sub-network 4 related to cell cycle. (**E**) Sub-network 5 related to chemical carcinogenesis.

Beta-sitosterol suppresses tumor growth without toxicity in AGS xenograft mouse models and induces apoptosis in human gastric adenocarcinoma cells [16]. Stigmasterol was reported to reduce tumor growth, macrophage recruitment, and tumor angiogenesis in a cholangiocarcinoma xenograft model [17]. Wogonin significantly suppresses inflammation-associated carcinogenesis and tumor development [18]. Oroxylin-a has been reported to have multifunctional roles in anti-cancer effects, and a recent study found that long-term exposure inhibited cell migration via the CCL2 pathway in OSCC cells, without cytotoxic effects [19]. Baicalein was reported to promote MCF-7 breast adenocarcinoma cells apoptosis without toxic properties on normal breast epithelial cells [20]. Rivularin was found to gather in human liver cancer (HepG2) cells, indicating an anti-hepatic carcinoma effect [21]. Skullcap flavone II suppressed cell proliferation in a variety of cancer cell lines, such as HeLa, PC-3, and LNCaP [22,23]. Salvigenin was reported to reduce tumor volume and modulate splenic T regulatory cells [24]. Most of these proven anti-cancer compounds were flavonoids, except for 2 phytosterol compounds – beta-sitosterol and stigmasterol. Kaempferol is derived from BS, beta-sitosterol is derived from both BS and HQ, and other compounds are derived from HQ.

KEGG signal pathway enrichment analysis showed that HQ-BS affects multiple targets and multiple related signal pathways in cancer. Various specific cancer signals were obtained based on P-value, including pathways in cancer, small cell lung cancer, prostate cancer, and colorectal cancer, indicating an anti-cancer effect of HQ-BS herb pair. To evaluate the anti-cancer effect of HQ-BS targets at a network level, we constructed a network according to PPI data and found 5 sub-networks related to TNF signaling pathway, tryptophan metabolism, proteoglycans in cancer, cell cycle, and chemical carcinogenesis, respectively. Tumor necrosis factor (TNF) mediates a variety of cell processes such as inflammation, differentiation, proliferation, and apoptosis. Clinical evidence shows that TNF polymorphisms are associated with susceptibility to cancer, including hepatocellular carcinoma [25], multiple myeloma [26], cervical cancer [27], and colorectal cancer [28]. Tryptophan (Trp) is an essential amino acid that is obtained exclusively from diet and is used for the production of neurotransmitters and neuromodulators. Over 95% of free Trp is a substrate for the kynurenine pathway. Evidence indicates that Trp metabolism is involved in tumor progression by suppressing antitumor immune responses and increasing the malignant properties of cancer cells [29]. Some endogenous tryptophan metabolites, such as melatonin, kynurenines, and serotonin, and bacterial

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tryptophan metabolites, including tryptamine, skatole, indole, and indolic acid were proved to play an important role in regulating the cancer immune system based on data obtained from SPF mice [30]. Our data showed HQ-BS can influence tryptophan metabolism by modulating a network containing 9 proteins. Future studies should assess whether HQ-BS influences tryptophan metabolism and its role in intestinal microbiota homeostasis. Proteoglycans are proteins that are attached by a specific linear carbohydrate chain of the glycosaminoglycan type and a part of the extracellular matrix and cell surfaces. Due to of their interactions with other ECM proteins, growth factors, and receptors, they can activate important cell signaling pathways (e.g., NF-κB, MAPK/β-catenin, Wnt, Hedgehog, TNF, IFN, Erk, FGF, and TGF-β) and their targets are associated with proliferation, angiogenesis, and cell motility [31]. Cell surface proteoglycans are involved in tumor-derived exosome biogenesis through the SDC1-syntenin-alix pathway [32], which mediates interactions of tumor cells and microenvironment, and stimulated tumor growth and development through specific signaling pathways related to metastasis, therapeutic resistance, and immunosuppression [33]. CDK2 is a core regulator of cell cycle through late G1-phase and S-phase. CDK2 is thought to be strongly linked to development of cancer, and accumulating evidence shows that inhibition of CDK2 induces cancer cell apoptosis without normal cell damage [34,35]. TOP2A is a marker of proliferation and chemotherapy resistance in cancer [36]. Several studies have reported that higher expression levels of TOP2A are related to poor cancer prognosis [36,37], and modulating TOP2A resulted in cancer cell apoptosis [38]. Our data show that HQ-BS herb pair can modulate cell cycle via a network containing CDK2 and TOP2A. HQ-BS herb pair also regulated chemical carcinogenesis pathway, indicating an anti-cancer effect.

Conclusions

HQ-BS herb pair contains various bioactive compounds, including flavonoids (e.g., kaempferol and wogonin) and phytosterols (e.g., beta-sitosterol and stigmasterol), and these compounds can interact with multiple targets and pathways. More importantly, HQ-BS herb pair can regulate the TNF signaling pathway, tryptophan metabolism, proteoglycans in cancer, cell cycle, and chemical carcinogenesis pathways to treat cancer. The present study provides evidence and promotes understanding of the multi-compounds and multitarget synergy of traditional Chinese medicine. However, *in vivo* and *in vitro* experiments and clinical investigations should be performed to verify the mechanism of HQ-BS herb pair against specific types of cancer in future studies.

Conflict of interest

None.

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