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Potential mechanism of Shugan Jieyu capsule in the treatment of hepatitis B based on network pharmacology

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Author contributions

Jing-Wen Ke performed the data analyses and wrote the manuscript; Jun-Xiu Tao contributed significantly to the analysis and manuscript preparation; Ming-Zhong Xiao helped perform the analysis with constructive discussions. All the authors reviewed the final manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

SGJY, shugan jieyu capsule; PPI, protein-protein interaction; HBV, hepatitis B virus; NAs, nucleos(t)ide analogs; TCMSP, traditional chinese medicine systems pharmacology; TCMID, traditional chinese medicine integrated database; OB, oral bioavailability; DL, drug-likeness; OMIM, online mendelian inheritance in man database; MF, molecular function; CC, cell component; EGFR, epidermal growth factor receptor.

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Abstract

Objective: In this study, the effective components and related targets of Shugan Jieyu Capsule (SGJY) in the treatment of hepatitis B were determined to explore the mechanism of SGJY in the treatment of hepatitis B. **Methods:** In this study, the effective components and targets of SGJY, and the related targets of hepatitis B were searched, and obtained the targets of SGJY in the treatment of hepatitis B according to the principle of Venn diagram. To build a protein-protein interaction network, String database was used, Cytoscape (3.7.2) software was used for topology analysis, R (4.0.5) software was used for go analysis, KEGG pathway enrichment analysis, and study of putative signaling pathways to determine how they could work. **Results:** SZJY was used to predict a total of 11 Chinese herbal components and 85 associated targets for the treatment of hepatitis B. 34 important targets were examined, including AKT1, EGFR, and 10 important pathways were examined, including proteoglycans in cancer and the PI3K/Akt signaling pathway. **Conclusion:** SGJY in the treatment of hepatitis B mainly inhibits the secretion of HBsAg and HBeAg by affecting PI3K-Akt and proteoglycans in cancer, and inhibits the progression of liver cancer.

Keywords: network pharmacology; Shugan Jieyu capsule; acanthopanax senticosus; hypericum perforatum; hepatitis B

Introduction

Hepatitis B is a widespread viral disease that is brought on by the hepatitis B virus. Hepatitis B causes abnormal immune response in the body, thus destroying liver cells and producing inflammatory necrosis, and is a major factor in the development of liver cirrhosis and potentially liver cancer, accounting for 60-80% of primary liver cancers worldwide [1]. Clinical treatment of hepatitis B has a variety of treatments such as peginterferon, orally administered nucleos(t)ide analogs (NAs), anti-inflammatory liver protection, among which Chinese medicine has significant efficacy in liver protection and anti-inflammation, antioxidation and anti-fibrosis [2, 3]. In order to better understand its potential mechanism of action for treating hepatitis B, this research examines the active ingredients, targets, and critical pathways of Shugan Jieyu Capsule (SGJY) from the perspective of network pharmacology.

Materials and Methods

Screening for SGJY ingredient targets

Oral bioavailability (OB) ⩾ 30% and drug-likeness (DL) ⩾ 0.18 were used as screening criteria to screen for targets of corresponding ingredients in the TCMSP (https://tcmspw.com/tcmsp.php) [4] and TCMID (http://www.megabionet.org/tcmid/) [5] searches for the components of SGJY, Hyperici perforati Herba, and Acanthopanacis Senticosi Radix Et Rhizoma Seu Caulis. The active ingredients of SGJY were further complemented by searching the paper. The SDF structure diagram from the Swiss Target Prediction database (http://www.swisstargetprediction.ch) was used to determine the expected targets for the active components [6].

Screening of hepatitis B-related genes

The keywords “hepatitis B” were searched in the OMIM database (https://www.omim.org) [7], and the GeneCards (https://www.genecards.org) [8]. Hepatitis B-related targets were obtained after combined screening.

Identification of possible SGJY targets for the treatment of hepatitis B

The screened targets of SGJY and hepatitis B were entered into the Venny 2.1, and obtain drug-disease common targets. Using Cystoscape 3.7.2, a drug-active ingredient-target network diagram was created. The drug’s constituent parts were ranked according to degree value [9].

Topological analysis and PPI network construction

In order to build a protein interaction network - PPI network, the discovered drug-disease common target genes were submitted to the String database (https://string-db.org/cgi/input.pl) [10]. Homo sapiens was chosen as the species. The PPI network was exported into Cystoscape 3.7.2, and NetworkAnalyzer was used to do a topological study [9]. Genes with scores higher than the average were chosen as core targets after the genes were sorted according to degree values.

GO functional enrichment and KEGG pathway enrichment analysis

Utilizing R 4.0.5 software, GO and KEGG analysis (enrichment analysis) is carried out. To describe the cellular, molecular, and biological functions of gene targets, GO functional analysis is used. Signaling pathways that are enriched for popular pharmacological and disease targets can be found using KEGG enrichment analysis. Visualize the first 10 pathways, then choose the path with a corrected P value less than 0.05.

Results

Search the TCMSP and TCMID databases for the SGJY active substances. More specifically, the 373 targets of Hyperici perforati Herba and the 324 targets of Acanthopanacis Senticosi Radix Et Rhizoma Seu Caulis of these active components were attained. In the end, a total of 209 targets were obtained after de-duplication (Table 1).

The database yielded a total of 12249 hepatitis B-related genes, of which 11775 were found in the GeneCards database and 474 in the OMIM database. 1885 genes associated to hepatitis B were obtained after the repeat value was removed.

Compared with drug and disease targets, there are 85 repeated targets, that is, the common targets of SGJY and hepatitis B (Figure 1). These targets may serve as SGJY's prospective hepatitis B therapeutic targets and can be used to further examine the drug's mechanism of action. The active ingredient and target information were incorporated into Cytoscape 3.7.2 to create a drug-active ingredient-target network diagram in order to more intuitively depict the complicated relationships between herbal medicines, components, and their related targets (Figure 2). The four substances with the highest echelons were luteolin, quercetin, kaempferol, and sitosterol, which may be the core components of the drug for hepatitis B disease. The top four target genes with the highest values were CYP19A1, ESR1, AR, ACHE.

In the PPI network built with 85 common targets (Figure 3). There are 661 edges and 85 nodes, with an average node degree of 15.6; the edges reflect the connections between proteins, and the nodes represent the target proteins. 34 genes with above-average scores were identified as the core targets by topological analysis of the PPI networks. Therefore, the AKT1, EGFR, PPARG, SRC, MPO, ABCB1, F2, ABCG2, PTGS2, ESR1, PIK3R1, ABL1, MMP9, AR, MAPK14, KDR, MCL1, PARP1, NR3C1, HIF1A, IGF1R, PGR, CYP19A1, CHEK1, CDK6, PTPN1, CHEK2, MMP2, TOP2A, MET, CDK2, PLG, CDK1, and CCNA2 genes may play the most important roles in the treatment of hepatitis B in SGJY.

GO enrichment analysis of the SGJY-hepatitis B common targets. A total of 1045 biological process (BP) terms ($P < 0.01$), 35 cell component (CC) terms and 95 molecular function (MF) terms were found. The bubble chart displayed the top ten GO phrases in each category (Figure 4). The top BP terms are mainly involved in cellular response to chemical stress, response to oxidative stress, cellular response to oxidative stress, regulation of inflammatory response, and cellular response to reactive oxygen species pathway. The top CC terms are mainly including transferase complex, transferring phosphorus－containing groups, membrane raft, membrane microdomain, and membrane region. The enriched MF terms mainly include protein serine/threonine kinase activity, protein tyrosine kinase activity, RNA polymerase II－specific DNA－binding transcription factor binding, nuclear receptor activity, and ligand－activated transcription factor activity.

The 166 pathways contained a substantial enrichment of the 85 targets ($P < 0.05$). Figure 5 displays the top 10 paths. The “prostate cancer” and other hepatitis B-unrelated pathways were omitted. In the end, 6 pathways were selected: proteoglycans in cancer, endocrine resistance, cell cycle, EGFR tyrosine kinase inhibitor resistance, and PI3K-Akt signaling pathways.

Table 1 The active ingredients of SGJY

Mol ID	Molecule name	OB	DL
MOL000006	Luteolin	36.16	0.25
MOL000073	Ent-epicatechin	48.96	0.24
MOL000098	Quercetin	46.43	0.28
MOL000358	Sitosterol	36.91	0.75
MOL000359	Gamma-sitosterol	36.91	0.75
MOL000422	Kaempferol	41.88	0.24
MOL000492	(＋)-catechin	54.83	0.24
MOL001494	10,13-octadecadienoicacid ethyl ester	42	0.19
MOL001558	Sesamin	56.55	0.83
MOL009047	Pinoresinol dimethyl ether	33.29	0.62
MOL009622	28-isofucosterol	43.78	0.76

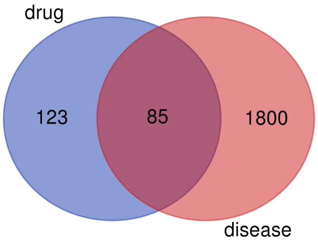


Figure 1 SGJY active ingredients targets and hepatitis B-related targets.

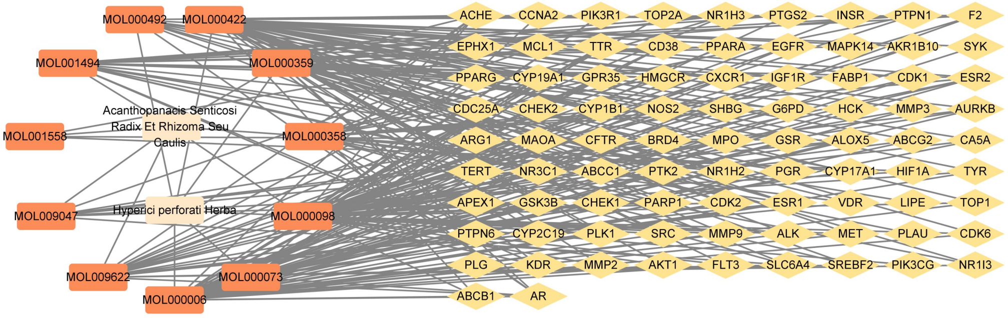


Figure 2 Drug-active ingredient-target network diagram

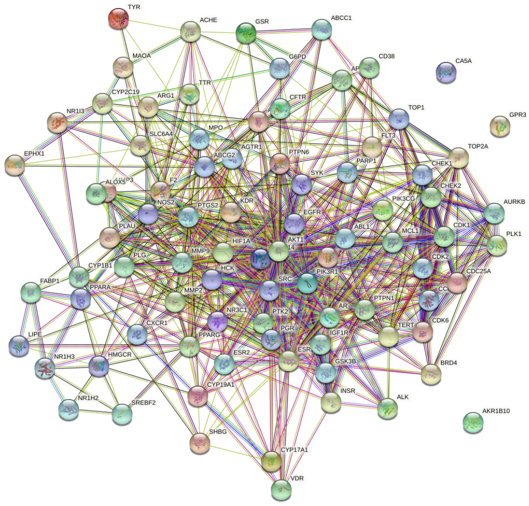
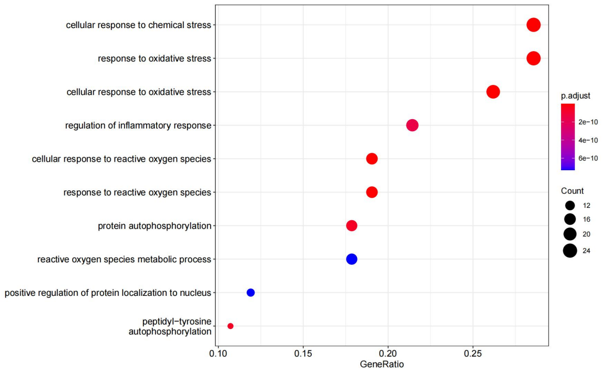
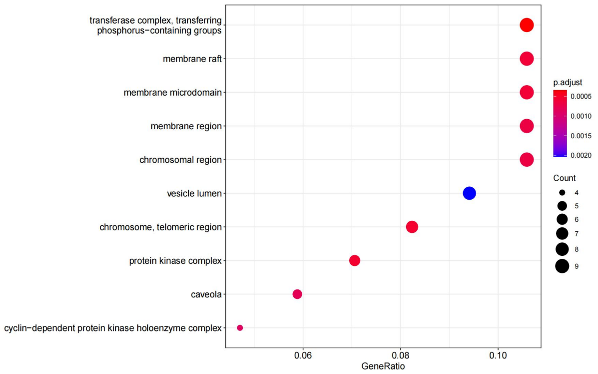


Figure 3 PPI network of SGJY and hepatitis B shared targets.





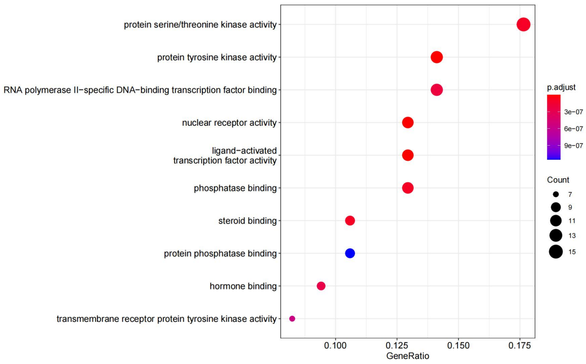


Figure 4 Top SGJY terms for hepatitis B treatment in GO.

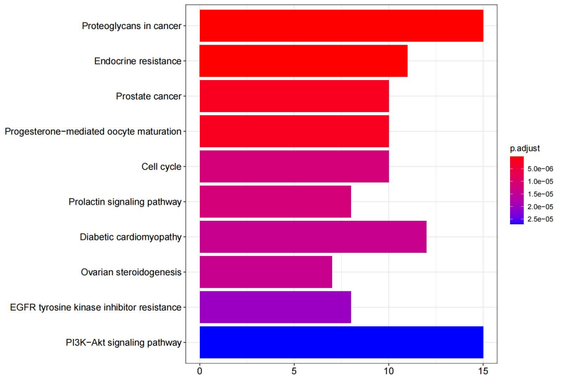


Figure 5 Top SGJY pathways for treating hepatitis B according to KEGG.

Analysis

Hepatitis B is one of the most common infectious diseases in clinical practice and remains a worldwide public health problem. The main ingredients of SGJY are Hyperici perforati Herba, and Acanthopanacis Senticosi Radix Et Rhizoma Seu Caulis, there are also numerous pharmacological studies on these two drugs. Several studies have shown that Hyperici perforati Herba has good antidepressant, antiviral and antitumor effects [11]. HQ Xiao studied the aqueous and alcoholic extracts of Hypericum perforatum and discovered that the extracts could successfully stop HBsAg and HBeAg from being secreted by cells, thus exerting anti-hepatitis B virus effects [12]. JZ Pan and others integrated relevant animal experiments at home and abroad to prove the significant hepatoprotective effect of Acanthopanax [13]. Clinically, studies have also demonstrated the therapeutic effect of Acanthopanax injection on hepatitis B [14].

The active ingredients of SGJY for hepatitis B mainly include luteolin, quercetin, kaempferol, and β-sitosterol. It has been demonstrated that the common polyphenolic bioflavonoid lignans possesses superior anti-inflammatory, neuroprotective, antioxidant, anti-anxiety, and cancer prevention properties [15]. Some researchers isolated the anti-HBV active component from Dictyostelium and analyzed it, and found that lignans were the strongest active component, and then further verified through in vitro experiments that lignans may have a therapeutic impact on hepatitis B by preventing the release of HBsAg and HBeAg by HepG2.2.15 cells.s [16]. In addition, it has been found that lignocaine can reduce intracellular ROS levels and thus alter intracellular redox status, and regulate mitochondrial levels to inhibit hepatitis B virus [17]. β-sitosterol and quercetin are widely present within antiviral herbal medicines. A study was conducted using a validated HPTLC method on Guiera senegalensis J.F- a plant with anti-HBV efficacy. In the analysis of its bioactive components, the second-most prevalent component in GSLE, -sitosterol, was discovered, amply supporting its hepatoprotective and anti-HBV potential, and quercetin was also found to play a role in this drug in attenuating the oxidative mechanisms of cells [18]. Mohammad K. Parvez conducted cell culture and molecular docking studies on phytopharmaceuticals with hepatitis B virus inhibitory potential and demonstrated that both β-sitosterol and quercetin have high anti-hepatitis B virus potential [19]. Minghao Liu et al found that quercetin could increase PI3K and AKT1 expression and decrease NF-KB p65 expression in liver tissues and inhibit inflammatory response through regulation of PI3K/AKT pathway [20].

Among the potential targets screened, the key targets were AKT1 and epidermal growth factor receptor (EGFR), respectively. AKT1 plays a crucial role in the control of numerous biological processes, including cell development, proliferation, and differentiation. It is a key downstream target in the PI3K-Akt signaling pathway [21]. EGFR, a receptor tyrosine kinase, is a representative factor in the tumor microenvironment. It has been found that there is overexpression of EGFR in hepatocellular carcinoma cells, and the regulation of EGFR phosphorylation status alters intracellular signaling, thus affecting the development and prognosis of liver disease [22].

From the results, it can be concluded that 15 targets are involved in both Proteoglycans in cancer and PI3K-Akt signaling pathway. The therapeutic effect of SGJY on hepatitis B may be achieved mainly through these two pathways. The findings indicated that activation of the PI3K-Akt pathway might suppress the transcription of the HBV pgRNA and 2.4-kb mRNA and regulate HBV DNA cell replication, indicating that the PI3K-Akt signaling system controls HBV infection and is crucial for treating hepatitis B [23]. Additionally, there is evidence to suggest that elevated PI3K-Akt signaling activity shields cells from Fas-mediated apoptotic signaling, preventing Fas from inducing apoptosis in hepatocytes [24].

Molecular docking studies revealed that the binding energy of AKT1 protein docked with luteolin, quercetin, and kaempferol were all less than -9.0 kcal-mo-1 [25], suggesting that one of the possible mechanisms for the treatment of hepatitis B with SGJY is that the binding of quercetin, lignocaine, and kaempferol to AKT1 protein inhibited its phosphorylation activation and partially blocked the PI3K-AKT signaling pathway, thereby inhibiting the secretion of HBsAg and HBeAg. And through the proteoglycan pathway in cancer to inhibit the progression of hepatitis B to hepatocellular carcinoma and improve the disease prognosis. In summary, SGJY has a variety of targets and pathways by which it can affect the treatment of hepatitis B. The clinical evidence, however, is insufficient and needs more investigation.

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