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Study On The Mechanism Of Jiedu Sanjie Recipe In The Treatment Of Liver Cancer Based On Network Pharmacology

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Abstract: Objective: to study the mechanism of action of Jiedu Sanjie Recipe in the treatment of Liver Cancer through network pharmacology. Method: Collect and screen the active ingredients of Jiedu Sanjie Recipe through the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP), and search for the human target protein corresponding to the active ingredients in the database; screen out the target set of liver cancer-related diseases through GeneCards and OMIM database; construct a Venn diagram to obtain the key target of Jiedu Sanjie Recipe for treating liver cancer; use Cytoscape 3.8.0 software to construct drug-component-target-pathway network; use Cytoscape software and BisoGenet plug-in to construct protein interaction network, use CytoNCA plug-in and R language to perform network topology analysis, gene ontology functional annotation (GO) and KEGG rich Collect pathway analysis to understand the possible biological processes and pathways of Jiedu Sanjie Decoction in treating liver cancer. Results: A total of 89 active ingredients of Jiedu Sanjie Prescription, 170 targets and 1885 liver cancer targets were obtained; After mapping, 114 potential targets of Jiedu Sanjie Recipe for liver cancer are obtained, and the core active ingredients are quercetin, luteolin, kaempferol, wogonin, baicalein, etc.The core targets NTRK1, TP53, CUL3, ESR1, MCM2, etc., involving lipids and atherosclerosis, chemical carcinogenesis-receptor activation signaling pathways, Kaposi's sarcoma-associated herpes virus infection, fluid shear stress and atherosclerosis Cirrhosis, hepatitis C, AGE-RAGE signaling pathway, hepatitis B, human cytomegalovirus infection, cancer proteoglycan, TNF signaling pathway, IL-17 signaling pathway, etc.Conclusion: This study reveals the mechanism of the multi-component, multi-target, and multi-channel treatment of liver cancer by "Knowing Du Sanjie Decoction", and provides a basis for the clinical application of Chinese medicine prescriptions for liver cancer.

Keywords: Jiedu Sanjie Recipe; Liver Cancer; Internet Pharmacology; Mechanism of Action

Introduction

Liver cancer is a common malignant tumor in China, and in recent years, the incidence rate of liver cancer ranks the 4th and the fatality rate ranks the 2nd in China, which is a serious threat to the life and health of our people [1]. At present, the treatment of hepatocellular carcinoma by western medicine is mainly based on drug, immunization and surgery, which is not very effective for the long-term prognosis of patients. Traditional Chinese medicine has reliable efficacy and unique advantages in the clinical practice of treating hepatocellular carcinoma. With years of clinical experience, Professor. Hu Zhenbin created an empirical formula "Jiedu Sanjie Recipe" for hepatocellular carcinoma, which has been applied for many years in the Department of Liver Diseases of the First Affiliated Hospital of Guangxi University of Traditional Chinese Medicine. In this study, we investigated the mechanism of detoxification and knot-dissolving formula in hepatocellular carcinoma by using network pharmacology to provide further evidence to support the clinical application of detoxification and knot-dissolving formula.

1. Material And Methods

1.1 Screening Of Active Ingredients And Collection Of Target Data Of Jiedu Sanjie Recipe

The TCM Systematic Pharmacology Database and Analysis Platform (TCMSP) was searched to obtain Radix Bupleuri, Hedysarum Multijugum Maxim, Chuanxiong Rhizoma, Angelicae Sinensis Radix,Pseudobulbus Cremastrae Seu Pleiones, Agrimonia Eupatoria, Polyporus Umbellatus (Pers) Fr, Coicis Semen, Hedyotis Diffusae Herba, and Scutellariae Barbatae Herba in Jiedu Sanjie Recipe for their chemical composition and their associated ADME parameters. Based on the oral bioavailability (OB) OB ≥ 30% and drug-likeness (DL) DL ≥ 0.18,

the target proteins of the active ingredients of each traditional Chinese medicine were retrieved from TCMSP, de-emphasized and then integrated, and the data of the active ingredients and targets of the traditional Chinese medicines in Jiedu Sanjie Recipe were finally obtained.

1.2 Acquisition Of Information On Liver Cancer Targets

Based on GeneCards (https://www.genecards.org) and OMIM (https://www.omim.org) databases, we searched for liver cancer-related targets using “Liver cancer” as the keyword, de-emphasized them and integrated them to obtain a set of liver cancer targets.

1.3 Construction Of A Visualization Network For Jiedu Sanjie Recipe And Hepatocellular Carcinoma

Using R software, the above obtained targets of Jiedu Sanjie Recipe were mapped with liver cancer related targets to obtain the potential action targets of Jiedu Sanjie Recipe for the treatment of liver cancer, and the Wayne diagram was plotted. The dataset of active ingredients of traditional Chinese medicines and their target proteins were imported into Cytoscape 3.8.0 software, and the traditional Chinese medicine-active ingredient-target network was constructed using Cytoscape 3.8.0 software.

1.4 Target Network Analysis

Cytocsape 3.8.0 software and its BisoGenet plug-in were used to import common target genes to construct a drug-disease related PPI network, and CytoNCA plug-in was used to analyze the network topology as follows:1）Extraction of key network: The Degree Centrality (DC) values of the network targets were statistically analyzed, and the DC values were ranked according to the DC values from the largest to the smallest, and the target proteins with higher number of connecting nodes and tighter interactions were screened to construct the target interactions network, and the core network was constructed according to the distribution of the Betweenness Centrality (BC) values.Then, according to the distribution of the Betweenness Centrality (BC) value of each target, statistical analysis was performed again to obtain the core target, and network topology analysis was performed to construct the core network (see Figure 4);2）In order to investigate the physiological functional process of Jiedu Sanjie Recipe, the constructed core network was subjected to more GO enrichment analysis and KEGG pathway enrichment analysis by utilizing the R language and Bioconductor platform (http://bioconductor.org/biocLite.R), so as to explore the physiological functional process. R package installation in R language includes

#install.packages(“colorspace”),#install.packages(“stringi”),#install.packages(“ggplot2”),#install.

packages(“BiocManager”),#BiocManagerinstall(“DOSE”，#BiocManager install(“clusterProfiler”)， #BiocManager install(“enrichplot”) Get GO enrichment analysis table, KEGG enrichment analysis pathway plot, and get GO, KEGG enrichment analysis bubble and barplot were performed to investigate the biological process and signaling pathway of Jiedu Sanjie Recipe in the treatment of hepatocellular carcinoma.

2. Result

2.1 Bioinformatic Data Of Jiedu Sanjie Recipe

Through screening, the results of the chemical constituents of the herbs in the formula for detoxification and dispersal of knots were as follows: 349 from Radix Bupleuri, 89 from Hedysarum Multijugum Maxim, 189 from Chuanxiong Rhizoma, 125 from Angelicae Sinensis Radix,18 from Pseudobulbus Cremastrae Seu Pleiones,41 from Agrimonia Eupatoria,31 from Polyporus Umbellatus(Pers)Fr,38 from Coicis Semen,37 from Hedyotis Diffusae Herba,94 from Scutellariae Barbatae Herba. A total of 89 active ingredients were obtained after de-weighting, and 170 targets of action were obtained by excluding duplicate targets of action in the active ingredient targets and excluding non-human targets of action through standardization in the UniProt database (https://www.uniprot.org/).

2.2 Hepatocellular Carcinoma Target Data

Based on the international bioinformatics databases, a total of 16804 GeneCard and 450 OMIM targets were retrieved for allergic rhi-

nitis, and 1885 targets were screened and de-emphasized. The names of the retrieved targets were standardized to facilitate further research.

2.3 Wayne's Diagram Of Common Target Genes

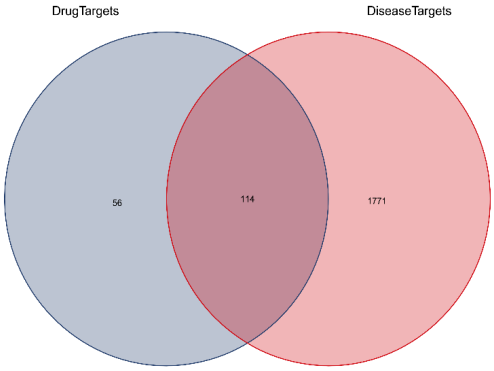

Figure 1 Wayne's diagram of the active ingredient targets of Jiedu Sanjie Recipe and liver cancer targets

Note: 170 active ingredient target genes and 1885 hepatocellular carcinoma target genes take the intersection to get 114 common target genes

2.3.1 Traditional Chinese Medicine-active Ingredient-target Network

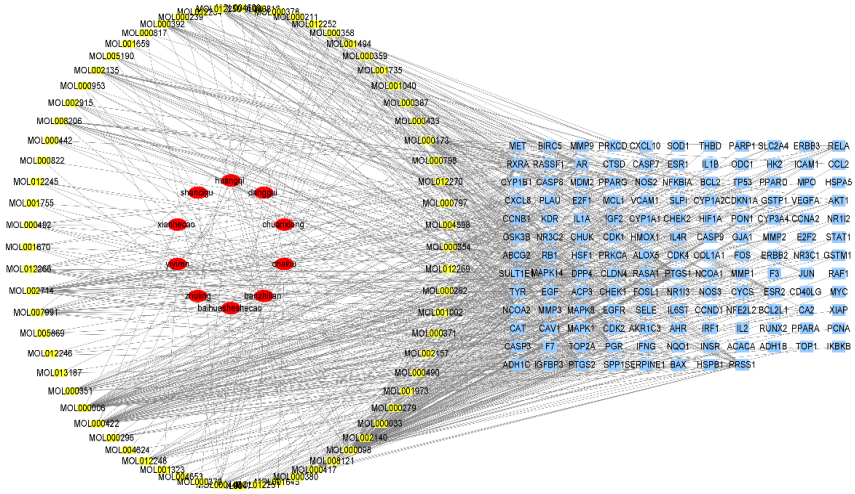

Figure 2 Chinese medicine-active ingredient-target network diagram

Note: The red circle in the figure indicates the traditional Chinese medicine, the square octagon indicates the active ingredient of the traditional Chinese medicine, and the blue rhombus indicates the target of the active ingredient.

Table 1 Ranking of degree values of active ingredients of Jiedu Sanjie Recipe

Ingredient ID	Ingredient Name	Source	Degree Value
MOL000098	Quercetin	Radix Bupleuri、Hedysarum Multijugum Maxim、Scutellariae Barbatae Herba、Agrimonia Eupatoria、Hedyotis Diffusae Herba	107
MOL000006	Luteolin	Scutellariae Barbatae Herba、Agrimonia Eupatoria	47
MOL000422	Kaempferol	Radix Bupleuri、Hedysarum Multijugum Maxim、Agrimonia Eupatoria	39
MOL000173	Wogonin	Scutellariae Barbatae Herba	30

MOL002714	Baicalein	Scutellariae Barbatae Herba	24
MOL000354	Isorhamnetin	Radix Bupleuri、Hedysarum Multijugum Maxim	19
MOL002135	Myricanone	Chuanxiong Rhizoma	17
MOL000392	Formononetin	Hedysarum Multijugum Maxim	17
MOL012250	7-hydroxy-5,8-dimethoxy-2-phenyl-chromone	Scutellariae Barbatae Herba	16
MOL000351	Rhamnazin	Scutellariae Barbatae Herba	16
MOL000378	7-O-methylisomucronulatol	Hedysarum Multijugum Maxim	16
MOL000417	Calycosin	Hedysarum Multijugum Maxim	16

As analyzed by Cytoscape software, the active ingredient Quercetin (107) possessed the most targets, followed by Luteolin (47), Kaempferol (39), Wogonin (30), Baicalein (24).From the perspective of the target of action, the top 5 targets of the linkage were Prostaglandin G/H Synthase 2 (PTGS2), Prostaglandin G/H Synthase 1 (PTGS1), Nuclear Receptor Coactivator 2 (NCOA2),Recombinant Protease, Serine 1 (PRSS1), Progesterone Receptor (PGR).

2.4 Network Topology Analysis, GO Enrichment Analysis And KEGG Enrichment Analysis

2.4.1 Network Topology Analysis

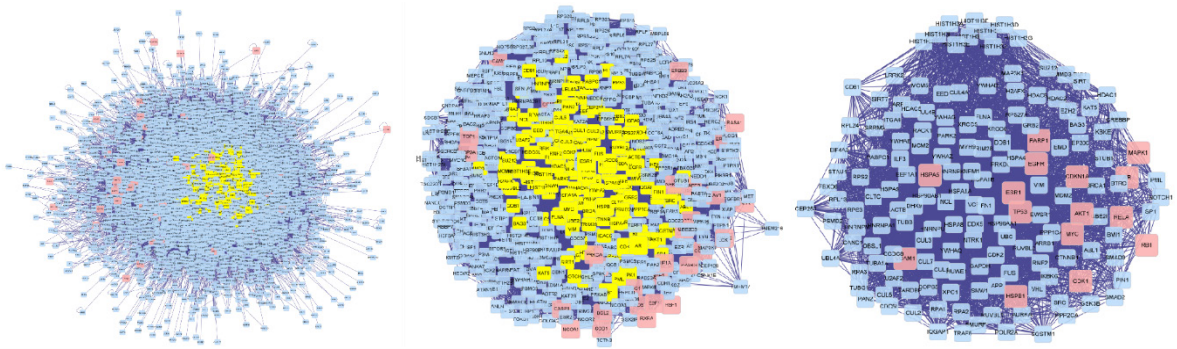

Figure 3 Network topology diagram

As shown in Figure 3, 114 common targets were uploaded to BisoGenet to construct a protein interaction network, where nodes denote proteins and edges denote correlations, involving a total of 5411 nodes and 134106 edges. Degree≥100 was selected as the key target by the number of gene interconnections, at this time, there were 663 nodes, and then the core target was screened by BC≥600 to construct the core network, and the top 5 targets were ranked by BC≥600.The core network was constructed, and the top 5 were: neurotrophic receptor tyrosine kinase 1 (NTRK1), tumor protein 53 (TP53), Cullin-3 (CUL3), estrogen receptor 1 (ESR1), and microchromosome maintenance complex component 2 (MCM2).

2.4.2 GO Enrichment Analysis

GO includes biological processes, molecular functions and cellular composition [2]. The GO analysis was performed on the core network, and the bar charts and barplot plots of the GO functional enrichment analysis of the top 10 molecular functions, cellular components, and bioprocesses were obtained after running the core network in R language, see Fig. 4. The results show that the therapeutic effects of detoxification and dispersal formula for liver cancer treatment in biological processes mainly involve cellular response to chemical stress, response to oxidative stress, response to drugs, response to nutrient levels, response to metal ions, response to lipopolysaccharides, response to molecules of bacterial origin, response to steroid hormones, cellular response to oxidative stress, response to reactive oxygen species, and so on; Cellular components are mainly involved in membrane rafts, membrane microregions, membrane zones, transcriptional regulatory com-

plexes, RNA polymerase II transcriptional regulatory complexes, organelle outer membranes, plasma membrane caveolae-like invaginations, protein kinase complexes, plasma membrane rafts, and cell cycle protein-dependent protein kinase holoenzyme complexes; The molecular functions mainly involve DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, ubiquitin-like protein ligase binding, cytokine receptor binding, nuclear receptor activity, ligand-activated transcription factor activity, transcriptional cofactor binding, repressor of transcription factor binding, steroid hormone receptor, and transcriptional coactivator binding.

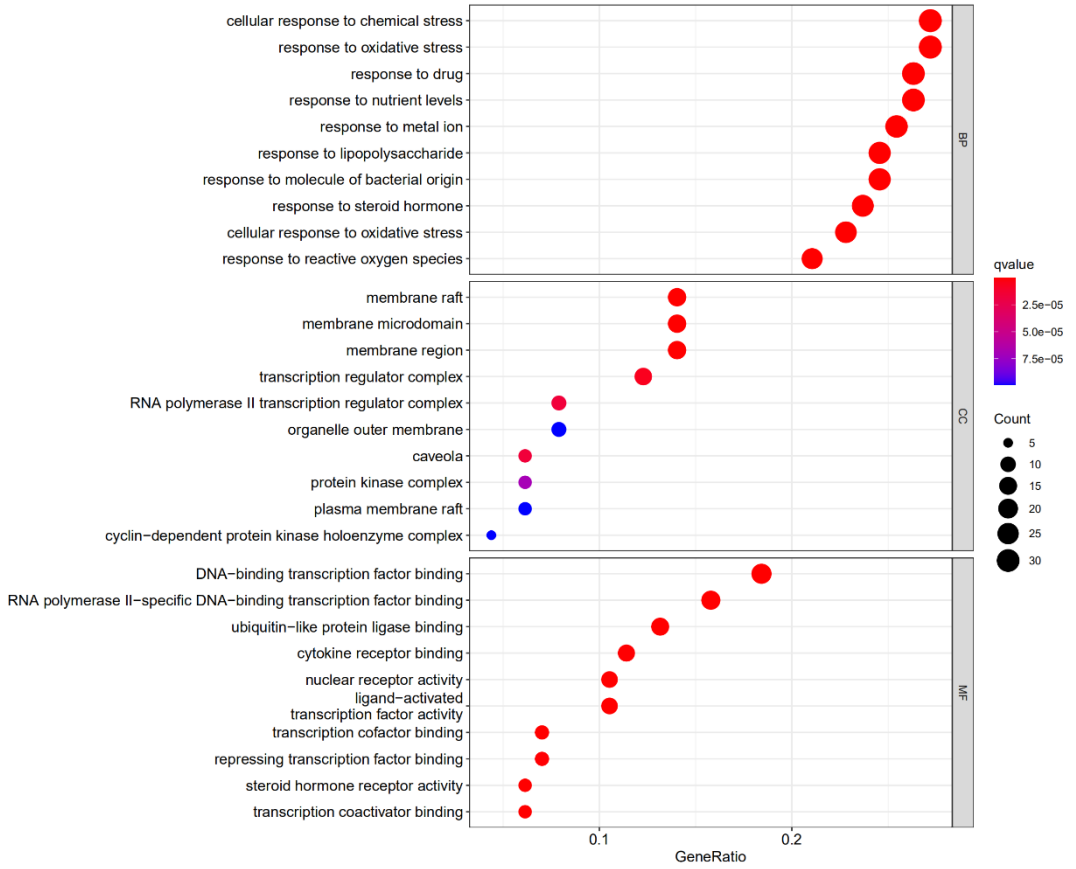

Figure 4 Functional enrichment of GO gene, a core target of Jiedu Sanjie Recipe for hepatocellular carcinoma treatment

Note: The size of the bubble area represents the number of genes enriched, and the color of the bubble represents the degree of significance, with the color ranging from blue to red, and the redder the color, the more significant the degree of significance. The redder the color, the more significant the degree.

2.4.3 KEGG Enrichment Analysis

The 114 intersecting targets were subjected to KEGG pathway enrichment analysis using the R language program, and 158 pathways were obtained after the run, and the results of the top 30 in terms of significance were obtained as bubble diagrams, see Fig. 5, with the vertical coordinates denoting the names of the pathways, the horizontal coordinates denoting the number of enriched genes, and the colors denoting the p-values, with the smaller p-values the redder the color and the higher the significance, and the bigger p-values the bluer the color, and the higher-significance network maps of the KEGG pathways and the associated target genes were obtained at the same time, see Fig. 6.

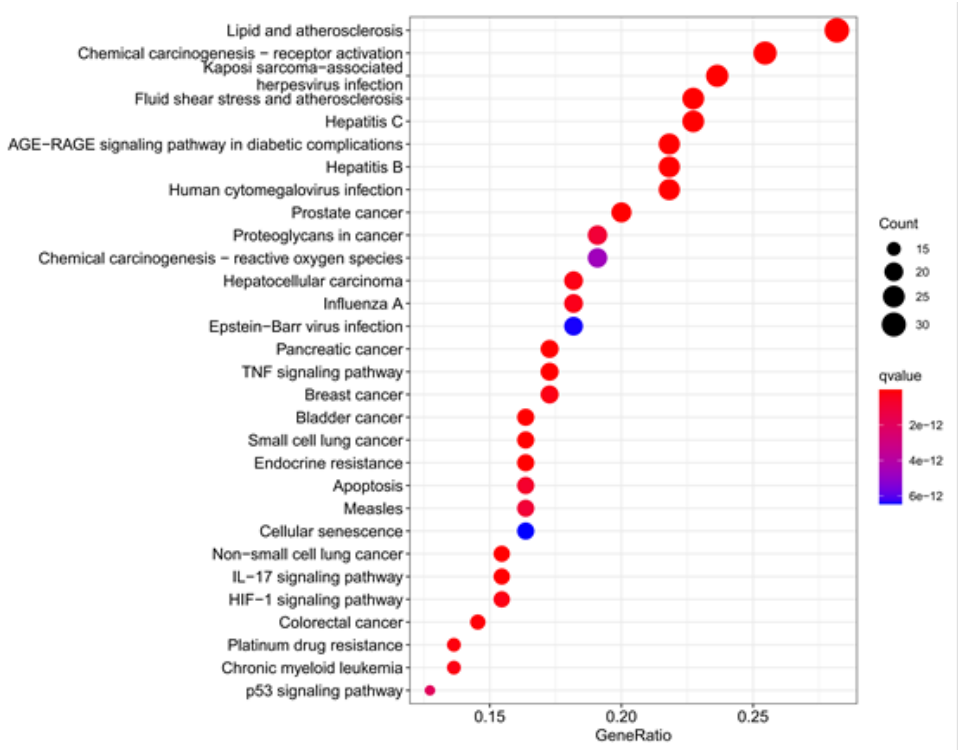

Figure 5 KEGG Enrichment Bubble Plot

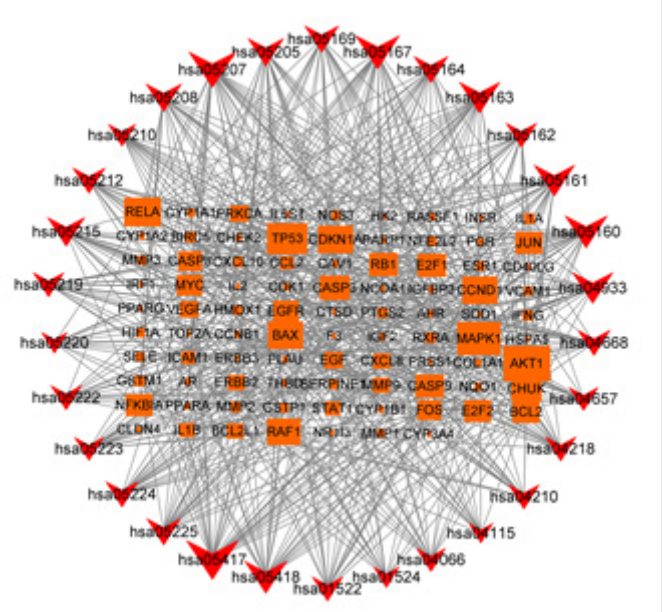

Figure 6 Relevant target gene-KEGG pathway map

The results showed that the key gene target pathways of Jiedu Sanjie Recipe for hepatocellular carcinoma mainly involved lipids and atherosclerosis, chemical carcinogenesis-receptor activation signaling pathway, Kaposi's sarcoma-associated herpesvirus infection, fluid shear stress and atherosclerosis, hepatitis C, AGE-RAGE signaling pathway, hepatitis B, human cytomegalovirus infection, proteoglycans in cancer, TNF signaling pathway, and IL-17 signaling pathway.

3. Conclusion

Currently, the pathogenesis of liver cancer is complicated. The vast majority of liver cancer patients have a history of chronic liver

disease, and the liver tissues of these patients are affected by various factors for a long period of time causing inflammation, which gradually develops into hepatic fibrosis, cirrhosis, and then develops into liver cancer. This paper analyzes the mechanism of Jiedu Sanjie Recipe for the treatment of hepatocellular carcinoma based on the method of network pharmacology, and provides a scientific basis for the subsequent in-depth study of Jiedu Sanjie Recipe.

The active ingredients Quercetin, Lignans, Kaempferol, Wogonin and Baicalein have the most abundant targets. Among the potential compounds, Quercetin is a common component of Radix Bupleuri, Hedysarum Multijugum Maxim, Scutellariae Barbatae Herba, Hedyotis Diffusae Herba and Hedyotis Diffusae Herba, which can exert anti-inflammatory effects by balancing Th1/Th2 and inhibiting the formation of antigen-specific antibodies [3], and also inhibit the proliferation of human hepatocellular carcinoma cells by inducing a TP53-non-dependent G2/M cell-cycle block as well as by promoting apoptotic cell death [4];

The Lignans enriched in Scutellariae Barbatae Herba and Agrimonia Eupatoria can inhibit the growth of hepatocellular carcinoma cells and exert antitumor effects by inhibiting the proliferation of hepatocellular carcinoma cells, blocking the growth cycle of hepatocellular carcinoma cells, inhibiting their angiogenesis, and inducing apoptosis of the cells [5]; Kaempferol is present in Radix Bupleuri, Hedysarum Multijugum Maxim, and Agrimonia Eupatoria, and can induce apoptosis in human hepatocellular carcinoma HepG2 cells via the mitochondrial pathway and the endoplasmic reticulum stress pathway [6]; Wogonin can exert antitumor effects by inhibiting the proliferation of human hepatocellular carcinoma cells [7]; Flavopiridol can inhibit the migration of human hepatocellular carcinoma cells and can cause them to undergo autophagy thus achieving anti-tumor effects [8].

NTRK1, TP53, CUL3, ESR1, and MCM2 were the target proteins with the highest number of connected nodes and the strongest interactions in the network topology analysis, in which the signaling pathways mainly involved in high-affinity nerve growth factor (NTRK1) were related to cell proliferation, inflammatory response, and inhibition of apoptosis; Tumor protein 53 (TP53), also known as P53, is an oncogene that is mainly involved in mitochondrial autophagy apoptosis in quercetin-regulated hepatic steatosis process [9]; Cullin 3(CUL3)plays an important role in a variety of cellular physiological processes, including cell cycle,cell proliferation and differentiation, and signal transduction; Microchromosome maintenance complex component 2 (MCM2) plays an important role in DNA replication and is mainly involved in suppressing tumor cell proliferation [10].

GO analysis shows that the cellular sites where the effect of detoxification and dispersal formula is exerted are structurally and functionally coherent, and that it exerts its therapeutic effect on hepatocellular carcinoma by participating in complex biological processes such as protein metabolism regulation process, enzyme-receptor binding process, and cellular response to chemical stresses.

The treatment of hepatocellular carcinoma by Jiedu Sanjie Recipe contains multiple pathways, which are related to lipid and atherosclerosis, chemical oncogenic-receptor activation signaling pathway, Kaposi's sarcoma-associated herpesvirus infection, hepatitis C, AGE-RAGE signaling pathway, TNF signaling pathway, and IL-17 signaling pathway according to KEGG enrichment analysis. Among other things, AGE is associated with a variety of diseases including diabetes, vascular disease, aging, kidney disease and tumors. Numerous studies have shown that AGE can induce a series of inflammatory responses by activating NF-κB, causing it to rapidly phosphorylate, and by regulating gene transcription. In addition, signaling pathways related to cell proliferation and apoptosis are activated through the binding of AGE and RAGE to participate in tumor cell proliferation, apoptosis, autophagy, invasion and distant metastasis [11]. Jiedu Sanjie Recipe can also play a therapeutic role by regulating TNF signaling pathway, IL-17 signaling pathway, HIF-1 signaling pathway, p53 signaling pathway and so on. The TNF signaling pathway regulates important biological activities such as immune response, inflammatory response, apoptosis and tumorigenesis. It has been found that the interaction of TNF-α with its ligand TNFR is involved in the development of cancer disease. IL-17 is an inflammatory factor secreted by Th17, which can activate a variety of immune cells to secrete inflammatory regulatory factors and play an important role in the body's immune response, which can lead to sustained inflammation and liver damage, so elevated IL-17 is positively correlated with the occurrence and development of hepatocellular carcinoma [12]. Hypoxia-inducible factor (HIF-1) consists of two subunits, α and β, and is a transcriptional regulator expressed in the cell nucleus. HIF-1α is highly expressed in a variety of tumors, and positively correlates with the hypoxic level of tumors, which promotes the regulation of the homeostasis of the intra-tumor environment by VEGF in the hypoxic microenvironment of tumors, stimulates the growth of tumor blood vessels, and promotes the massive growth and proliferation of

tumor cells [13]. Inhibition of the activity of the P53 signaling pathway can maintain the activation of various tumor cytological behaviors such as malignant proliferation, apoptosis inhibition, and invasion and metastasis in hepatocellular carcinoma cells, which in turn provide opportunities for hepatocellular carcinoma development [14]. It can be concluded that Jiedu Sanjie Recipe can play an anti-tumor role by inhibiting the expression of multiple pathways and blocking their signaling pathways.

In summary, relevant basic research in modern medicine has shown that Radix Bupleuri, Hedysarum Multijugum Maxim, Angelicae Sinensis Radix, Coicis Semen, Hedyotis Diffusae Herba and Pseudobulbus Cremastrae Seu Pleiones have powerful anti-tumor effects. Based on network pharmacodynamics, this study explored the potential mechanism of Jiedu Sanjie Recipe for the treatment of hepatocellular carcinoma, which, to a certain extent, illustrated that Jiedu Sanjie Recipe can treat hepatocellular carcinoma with multi-targets and multi-pathways, and provided the basis for in-depth elucidation of the material basis and mechanism of action of Jiedu Sanjie Recipe in the treatment of hepatocellular carcinoma, which provides more possibilities for the treatment of hepatocellular carcinoma in clinic.

References

[1] Guidelines for diagnosis and treatment of primary liver cancer in China (2019 edition) [J]. Journal of Clinical Hepatology,2020,36(02):277-292.

[2] REN Shuang, NI Li-qiang, MENG Di, et al. Mechanisms of Cortex phellodendri-Herba tuberculate speranskia in treatment of rheumatoid arthritis based on network pharmacology [J].

Chinese Journal of New Drugs and Clinical Remedies,2019,38(12):757-766.

[3] Mlcek J, Jurikova T, Skrovankova S, et al. Quercetin and Its Anti-Allergic Immune Response.

[J]. Molecules (Basel, Switzerland),2016,21(5):623.

[4]WANG Zi-xuan, ZHOU Jing, TANG Yue, et al. Quercetin induces P53-independent G2/M arrest and apoptosis in cancer cells [J]. Chinese Journal of Pharmacology and Toxicology,2018,32(10):790-796.

[5]Yang Peiwei, Zhang Shuhui. Advances in the study of the effect and mechanism of Luteolin on

hepatocellular carcinoma [J]. Pharmacology and Clinics of Chinese Materia Medica,2018,34(01):190-193.

[6]Guo Haiqing, Liu Yali, Ren Feng, et al. Research on the effect of kaempferol on HepG2 apoptosis and its mechanism [J]. Beijing Medical Journal,2021,43(09):899-904.

[7]Zhong Nan, Li Qin, Feng Yanhong, et al. Research advances in wogonin's anti-tumor effects [J]. Clinical Misdiagnosis & Mistherapy,2019,32(11):112-116.

[8]LEI Boting, MA Chaoying. Research Progress on Anti-Hepatocarcinoma of Berberine [J]. Chinese Archives of Traditional Chinese Medicine,2014,32(02):254-256.

[9]Liu Peiyi. Mitochondrial homeostasis and mitophagy in hepatic steatosis: regulation of quercetin through Frataxin [D]. Huazhong University of Science and Technology,2018.

[10].Deng M , Sun J , Xie S , et al. Inhibition of MCM2 enhances the sensitivity of ovarian cancer

cell to carboplatin[J]. Molecular Medicine Reports, 2019, 20(3).

[11]ZHANG Xia, LIU Jian-ying. AGES-RAGE system and anti-tumor effect of metformin [J]. Chinese Journal of New Drugs,2014,23(04):441-444.

[12]LU Gan-shan, SHI Guang-ying, QI Ying-chao, et al. Determination Analysis of Serum IGF-1, IL-17 and AFP Levels of Primary Liver Cancer Patients [J]. World Latest Medicine Information,2018,18(46)：8-9.

[13]ZHANG Geng, LIU Guangzhao, FENG Guiyin, et al. Correlation Analysis on the Relationship between Pain and Serum HlF-1 and VEGF Levels in Patients with Primary Liver Cancer [J]. Hebei Medicine,2019,25(03):615-618.

[14]CAO Jian, ZHU Xiao-ran, YANG Zhen-huan, et al. An exploration on mechanisms of Qinggan Huayu Granule in treating liver cancer based on network pharmacology [J]. Chinese Traditional and Herbal Drugs,2021,52(07):2039-2052.

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