Network Pharmacology Based Study on the Mechanism of Mongolian Medicine *Valeriana officinalis* L. on Liver Cancer

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Abstract [**Objectives**] To explore the mechanism of Mongolian medicine *Valeriana officinalis* L. on liver cancer by network pharmacology. [**Methods**] The HERB database of *V. officinalis* L. was searched, and the Uniprot database was used to normalize and standardize the targets. Liver cancer targets were searched through GeneCards, DISGENET, and other databases. Venny website was used to obtain the intersection target of valerian active ingredients and liver cancer disease. The protein-protein interaction (PPI) network of the intersection targets was analyzed by STRING database, and the PPI network was constructed by Cytoscape software. The David database was used for GO functional annotation and KEGG pathway enrichment analysis to obtain the relevant pathways in the treatment of liver cancer with Mongolian medicine *V. officinalis*. The corresponding chemical components, targets and pathways of liver cancer were imported into Cytoscape software to construct the network topology of "chemical component-disease-target-pathway". According to the analysis results, the potential of the active components in *V. officinalis* as a therapeutic drug for liver cancer was evaluated, and the correlation between the results of network pharmacology analysis and clinical treatment of liver cancer was discussed, which provided a reference for clinical application. [**Results**] A total of 13 kinds of chemical components and 108 drug disease intersection target genes were screened, and the core genes acting on diseases were caffeic acid, perillyl acetate, (+)-alpha-Terpineol, eucalyptol, *etc.*; GO functional enrichment analysis involved 389 items of biological processes, 62 items of cellular components and 120 items of molecular functions. Enrichment analysis of KEGG signaling pathways screened out chemical carcinogenesis-receptor activation, cancer pathways, prolactin signaling pathways, proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance and other signaling pathways. [**Conclusions**] The mechanism of Mongolian medicine *V. officinalis* on liver cancer was studied by network pharmacology. It was found that it can inhibit the proliferation of liver cancer cells from multiple targets and pathways. This is expected to provide a theoretical basis for further basic experimental research.

Key words Network pharmacology, Mongolian medicine *Valeriana officinalis* L., Liver cancer, Mechanism of action

1 Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor and the fourth leading cause of death worldwide. Liver cancer (LC) is the main histopathological type (75% - 85%). According to the latest data, the annual number of new cases of liver cancer in China is 389 000, ranking fourth in malignant tumors; the annual number of deaths is 336 000, ranking second in malignant tumors, and the disease burden is heavy[1]. The clinical treatment of tumors mainly includes surgery, chemotherapy, radiotherapy, immunomodulation therapy, traditional Chinese medicine therapy and others (laser, microwave, *etc.*). However, the increasing drug resistance and side effects of drugs have brought troubles to clinical treatment. Therefore, it is urgent to research and develop more effective drugs for the treatment of liver cancer. Mongolian medicine *Valeriana officinalis* L. is a perennial herb belonging to *Valeriana* of the family Valerianaceae. Its roots and rhizomes are widely used in traditional medicine. It is bitter in taste, cold in nature, enters the heart and liver meridians, and has various pharmacological effects such as tranquilization, spasmolysis and analgesia[2]. In addition, modern studies suggest that *V. officinalis* has pharmacological effects such as anti-cancer, improving insomnia, anti-depression and anxiety, neuroprotection, anti-epilepsy and so on[3]. The *V. officinalis* extract has anti-tumor effect and can inhibit the growth of cancer cells and induce apoptosis of cancer cells, but the pathway and molecular biological mechanism of *V. officinalis* extract in promoting apoptosis have not been clearly clarified.

Network pharmacology is a new bioinformatics technology, and it reveals the mechanism of action and the material basis of drug efficacy by constructing and analyzing the complex network relationship among drugs, components and targets. This method is particularly suitable for the study of traditional Chinese medicine (TCM), which often contains multiple components that may act through multiple pathways and targets[4]. Therefore, this study intends to use the network pharmacology method to construct the network of "Mongolian medicine *V. officinalis* - *V. officinalis* active component - target" from the two aspects of disease target and Mongolian medicine *V. officinalis* active component target, to explore the anti-LC mechanism of Mongolian medicine *V. officinalis*, and to lay a foundation for the discovery of new pharmacodynamic targets and the verification and further study of the therapeutic effect of traditional Chinese medicine pharmacodynamic substances[5].

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2 Data and methods

2.1 Screening of active components of *V. officinalis* The known active components of *V. officinalis* were collected by consulting the HERB database[6]. According To Lipinski's five principles (MW≤500, logP≤5, hydrogen bond acceptor nOH≤10, hydrogen bond donor nOHNH≤5), the active components of *V. officinalis* were further screened, and the target sites of active components were predicted according to Swiss Target Prediction Database. Drug-component-target network mapping was performed using Cytoscape 3.7.1 software[7]. The pubchemID of the relevant component were copied and pasted into an organic small molecule bioactivity database (PubChem, https://pubchem.ncbi.nlm.nih.gov/), and then continued to carry out logP≤5 according to Lipinski's five principles; relevant components were screened out when nOHNH≤5. The screened active components were screened in Pubchem, and the determined compound structure was screened in Swiss ADME. The criteria were set: gastrointestinal absorption was set as high (the component had high oral bioavailability), and three or more of the five prediction settings were "Yes"[8]. The SMILES number of each component was obtained from PubChem, and then entered the SMILES number in the SwissTargetPrediction database to search the target of the active component of *V. officinalis*, and screened the result of Probability > 0.1[9].

2.2 Screening of disease candidate targets The database DisGeNET (https://www.disgenet.org/), GeneCards (https://www.genecards.org/), with "liver cancer" as the keyword, to find genes and targets related to liver cancer. After screening and weight removal, the relevant target information of LC was finally obtained, and the intersection of drug and disease target was obtained through Venny 2.1.0, so as to obtain the key target of anti-LC of Mongolian medicine *V. officinalis*[10].

2.3 Construction of protein-protein interaction (PPI) network Venny software was used to compare the active ingredient targets of *V. officinalis* with the gene targets of liver cancer, and to find out the intersections between them, which were potential targets. In order to ensure the reliability of the interaction, the target of *V. officinalis* active components and liver cancer was imported into the STRING database (http://string-db.org/), the species was set as "human", and the confidence level was set as 0.7. Cytoscape 3.10.0 software was used for topological analysis, and a PPI network diagram with key targets as the core was constructed according to the Degree value, and 12 PPI core gene targets were obtained[11].

2.4 GO biological function and KEGG pathway enrichment analysis DAVID database was used for GO functional enrichment analysis Biological Process (BP), Cellular Component (CC), Molecular Function (MF) and KEGG pathway enrichment analysis. The results were plotted as a bar graph and a bubble graph[12].

3 Results and analysis

3.1 Screening of active components Taking the conditions set in Section **2.1** as the screening conditions, 117 chemical components related to Mongolian medicine *V. officinalis* were retrieved from HERB database, and 13 related active components were obtained (Table 1). The active components were screened to the relevant targets, and 217 related targets of Mongolian medicine *V. officinalis* were finally obtained after summarization and deduplication.

Table 1 Active components of Mongolian medicine *Valeriana officinalis* after screening

No.	Compound	Standard linear symbol
xie 1	Actinidine	C[C@H]1CCc2c1cncc2C
xie 2	(+)-alpha-Terpineol	CC1=CC[C@@H](CC1)C(O)(C)C
xie 3	CID 44630107	O[C@H]1C[C@@H]2C(C1(C)CC2)(C)C
xie 4	[(3R,4S)-4,7,7-trimethyl-3-bicyclo[2.2.1]heptanyl] 3-methylbutanoate	CC(CC(=O)O[C@@H]1CC2C([C@]1(C)CC2)(C)C)C
xie 5	Caffeic Acid	OC(=O)/C=C/c1ccc(c(c1)O)O
xie 6	Camphor	O=C1CC2C(C1(C)CC2)(C)C
xie 7	Eucalyptol	CC12CCC(CC1)C(O2)(C)C
xie 8	Fenchone	O=C1C2(C)CCC(C1(C)C)C2
xie 9	Ledum camphor	C[C@@H]1CCC2C1[C@H]1[C@H](C1(C)C)CC[C@@]2(C)O
xie 10	Linalool, (+/-)-	C=CC(CCC=C(C)C)(O)C
xie 11	Patchouli alcohol	C[C@H]1CC[C@@]2([C@@]3([C@H]1C[C@H](C2(C)C)CC3)C)O
xie 12	Perillyl acetate	CC(=O)OCC1=CCC(CC1)C(=C)C
xie 13	(3R)-4-methylidene-1-(propan-2-yl)bicyclo[3.1.0]hexan-3-ol	CC(C12CC2C(=C)[C@@H](C1)O)C

3.2 Construction of target network Using the database GeneCards, with "liver cancer" as the key word, a total of 1916 genes related to LC were screened from the GeneCards database, and 3 296 LC corresponding target genes were obtained after screening with Relevance scores > 30 (median) as the limiting condition. The above results were used as the disease candidate genes in this experiment, and were intersected with the candidate genes of Mongolian medicine *V. officinalis*, and 108 potential targets were obtained (Fig. 1). Through network analysis, it can be intuitively shown that the network contains 122 nodes and 226 edges. The light blue pattern represents the active component, the red pattern represents the Mongolian medicine *V. officinalis* (EF), and the dark blue pattern represents the target point. The node size represents the value size, and the larger the node, the larger the value (Fig. 2). Through network topology analysis, the average values of BC, CC, EC and degree (BC > 220, CC > 0.36, EC > 0.06, de-

gree > 6) of the active components were used for screening. The top five components of the degree value were as follows: Caffeic Acid, 4-(1-methylethenyl)-1-cyclohexene-1-methanol acetate (Perillyl acetate), (+)-alpha-Terpineol, Linalool, (+/ −)-) and Ledum camphor, suggesting that it is in the core position in the network and is regarded as the core component. Therefore, Mongolian medicine *V. officinalis* has the characteristics of multi-component and multi-target in the prevention of LC.

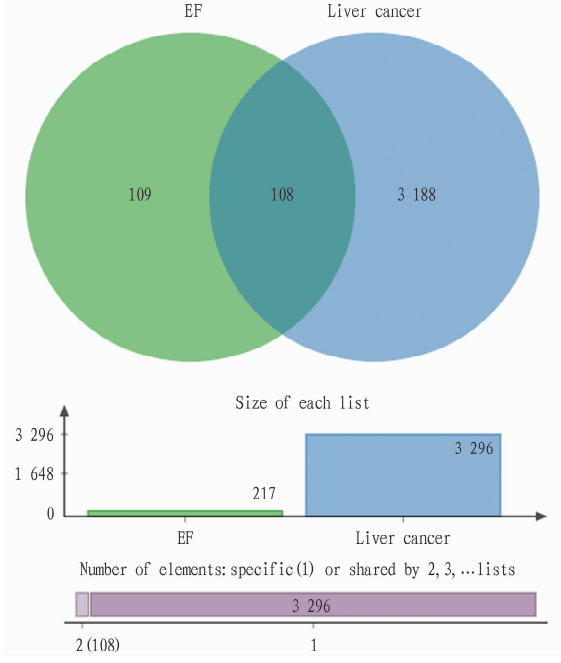

Fig. 1 Venn diagram for component target of *Valeriana officinalis*-LC disease target

3.3 Construction of PPI network The common targets of Mongolian medicine *V. officinalis* and LC were input into the String database to obtain the PPI network of Mongolian medicine *V. officinalis* and LC. The lines between the nodes in the figure indicate that there is interaction between them. The different colors indicate different types of interaction. The more lines, the closer the interaction. The number of nodes is 108, the number of edges is 338, and the average node degree is 6.26. The average local clustering coefficient was 0.5 (Fig. 3A). Through barplot, it can be seen more intuitively that the more nodes connected to each node, the more critical the role of the node in the network[13], and 12 PPI core gene targets were obtained, of which the top five nodes with the highest correlation value were *STAT3*, *EGFR*, *IL1B*, *ESR1* and *PTGS2*. These results suggest that these genes may be potential targets for the treatment of LC with Mongolian medicine *V. officinalis*.

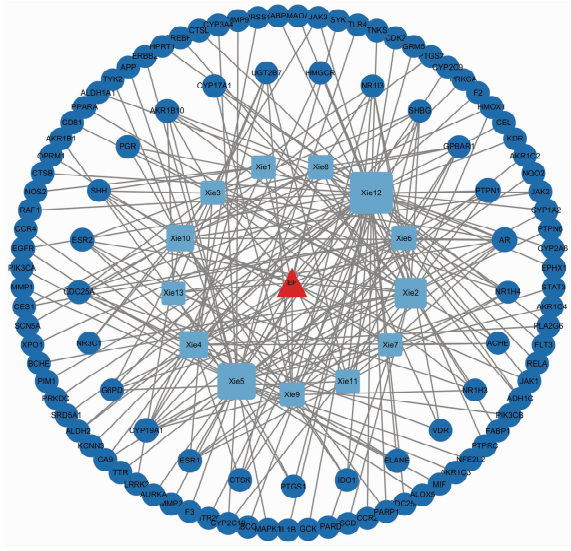

Fig. 2 Network diagram of *Valeriana officinalis*-active components of *Valeriana officinalis*-target

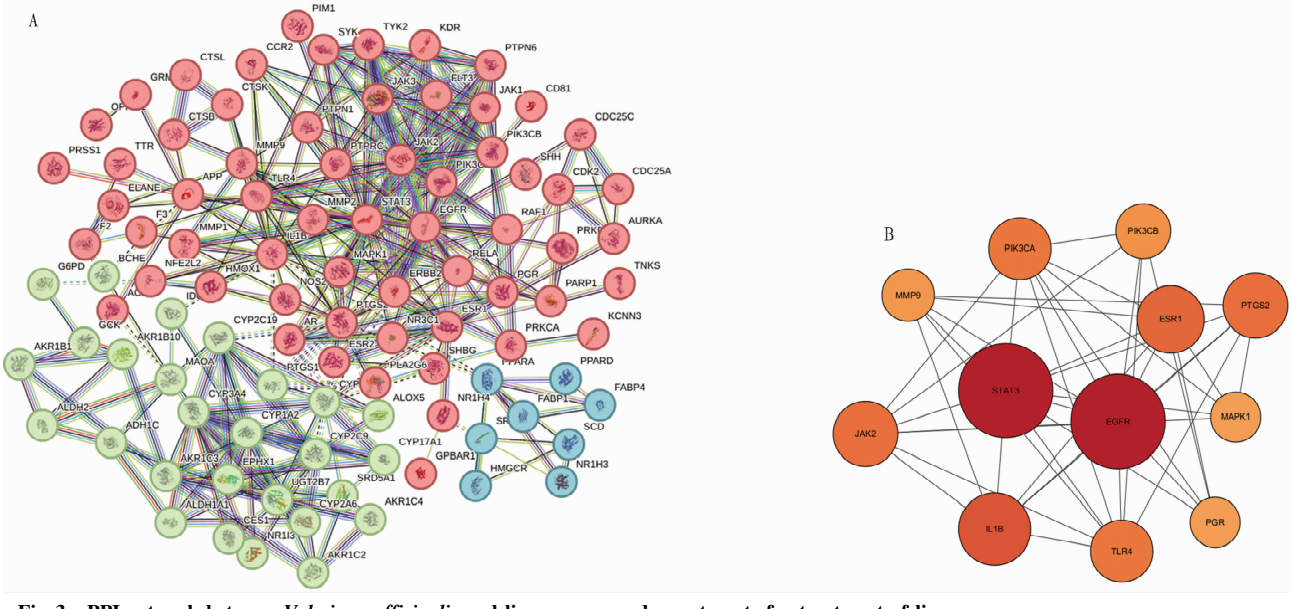

Fig. 3 PPI network between *Valeriana officinalis* and liver cancer and core targets for treatment of liver cancer

3.4 GO biological function and KEGG pathway enrichment analysis The DAVID database was used to select the species parameter as Homo sapiens, and the GO function and KEGG signaling pathway enrichment analysis were carried out on the intersection targets of Mongolian medicine *V. officinalis* in the treatment of LC, and 389 entries of biological processes were obtained, including JAK-STAT-mediated growth hormone receptor signaling pathway, progesterone metabolism, intracellular receptor signaling pathway and so on. There were 62 entries of cellular components, including receptor complex, cytoplasmic perinuclear region, endoplasmic reticulum membrane, *etc.*, and 120 entries of molecular functions, including retinal dehydrogenase activity, bile acid binding, estrogen response element binding, *etc.*, which were sorted in descending order according to the number of enriched genes, and the top 10 items of each category were selected to plot a bar graph (Fig. 4). There were 141 KEGG signaling pathways enriched, and after deleting the irrelevant pathways, which were mainly chemical carcinogen-receptor activation, cancer morbidity pathway, prolactin signaling pathway, *etc.* The first 20 pathways were selected to draw the bubble map, in which the size of the bubble represented the number of targets enriched by the entry, and the larger the bubble, the more targets enriched; the color of the bubble represents the size of the *P* value, and the redder the color, the smaller the *P* value (Fig. 5).

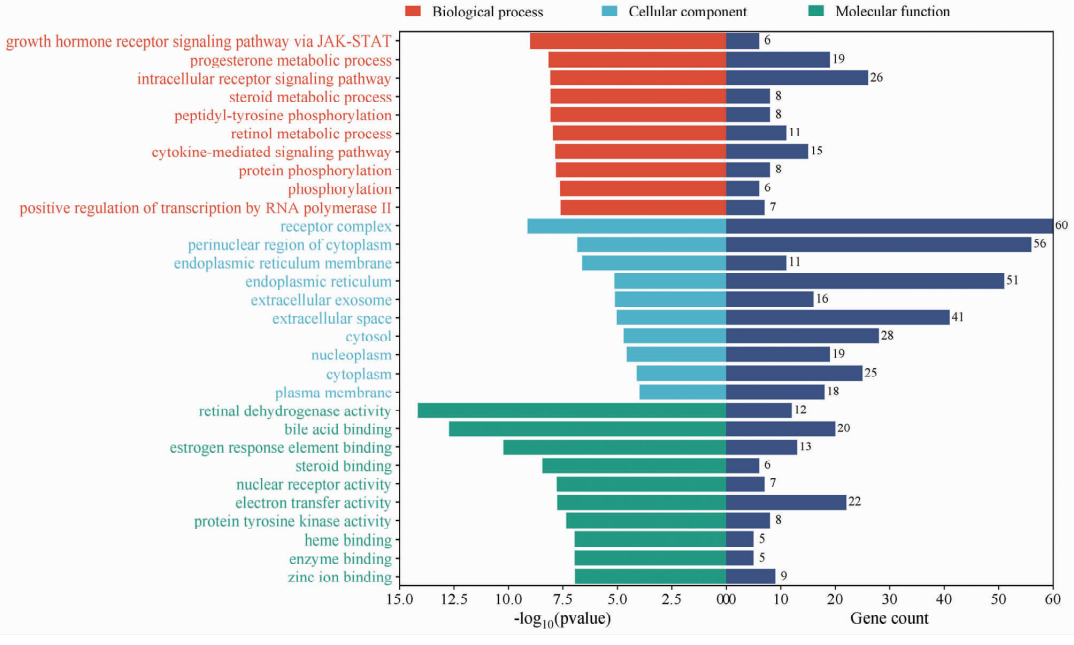

Fig. 4 GO functional enrichment analysis

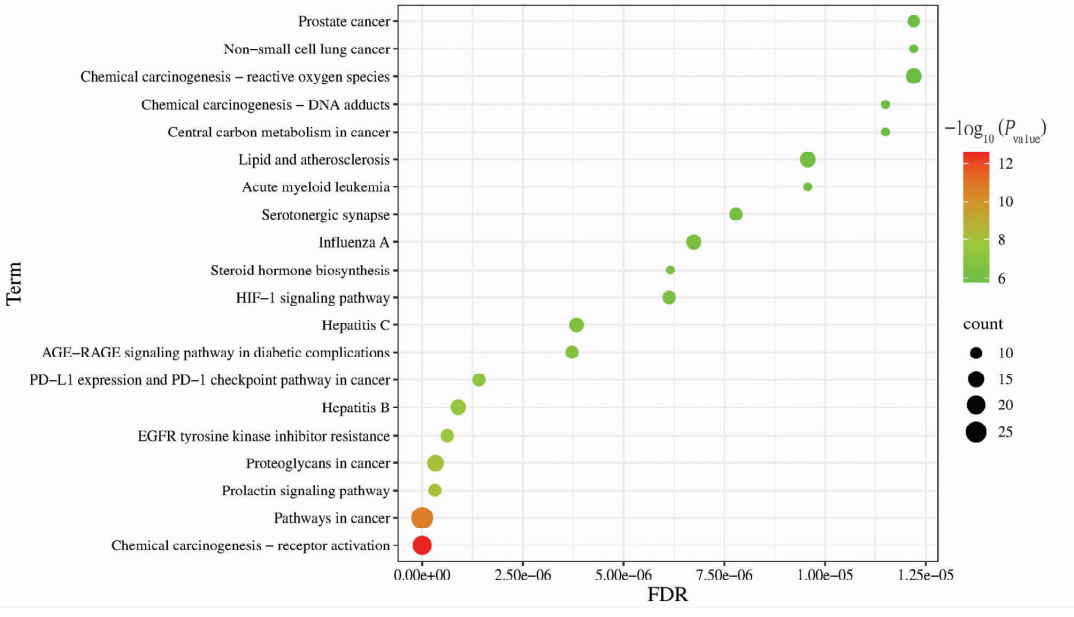

Fig. 5 KEGG pathway enrichment analysis

4 Discussion

Iridoids are abundant constituents of the genus *Valeriana*. A new class of valerian triesters, valtrate, acevaltate and didrovaltrat, was first isolated from the rhizomes of *V. officinalis* by Thies P W *et al.* in 1966. It has been confirmed that this component is the sedative and hypnotic active component in *V. officinalis*, and the iridoid compounds in this genus have been widely concerned and found one after another[14]. It is found that the iridoid compounds in valerian, such as Valepotriates and Valerenic acid, are the effective anti-tumor components[15]. Due to the limitation of detection technology, all active components of *V. officinalis*, including trace components, have not been detected, and the anti-LC bioactive components of *V. officinalis* and its mechanism of action have not been fully explained. Therefore, it is necessary to further isolate and identify bioactive components with clear physiological activity and effective therapeutic or auxiliary anti-LC effect from Mongolian medicine *V. officinalis*[16].

In this study, the anti-LC mechanism of Mongolian medicine *V. officinalis* was discussed based on network pharmacology. The results showed that there were 13 potential active components and 108 potential targets in Mongolian medicine *V. officinalis*, which indicated that Mongolian medicine *V. officinalis* might have synergistic effects of multi-components and multi-targets. Based on the PPI network, the effective components of anti-LC were screened, and the effective components of LC (Caffeic Acid, Perillyl acetate, (+)-alpha-Terpineol, *etc.*) were obtained. According to the degree value calculated by the software, 12 core targets were selected, and these nodes played a key role in the PPI network graph. In order to explore the core targets of Mongolian medicine *V. officinalis* against LC, the targets with greater significance included STAT3, EGFR, IL1B, ESR1, PTGS2, *etc.*[17]. STAT3, EGFR and IL1B have the largest nodes in PPI, which may be the important targets of anti-LC of Mongolian medicine *V. officinalis*. STAT3, named signal transducer and activator of transcription 3 in Chinese, is an important molecule, which plays a key role in cell signal transduction and gene expression regulation. The gene for STAT3 is located on chromosome 17 and encodes a protein that is a member of the STAT protein family. STAT3 plays important roles in a variety of physiological and pathological processes, including but not limited to cell proliferation, differentiation, apoptosis, and immune response. STAT3 activation is aberrant in many types of cancer and is strongly associated with tumor initiation, progression, invasion, and metastasis. The continuous activation of STAT3 can promote the proliferation of tumor cells, inhibit apoptosis, promote angiogenesis and immune escape, so STAT3 has become an important target for cancer therapy[18]. In this study, the target GO pathway enrichment of Mongolian medicine *V. officinalis* against LC showed that the anti-LC BP of Mongolian medicine *V. officinalis* was mainly through JAK-STAT-mediated growth hormone receptor signaling pathway, progesterone metabolism process, intracellular receptor signaling pathway and so on. CC mainly includes receptor complex, cytoplasmic perinuclear region, endoplasmic reticulum membrane, *etc.* MF mainly includes retinal dehydrogenase activity, bile acid binding, estrogen response element binding, and so on. KEGG signaling pathway enrichment analysis showed that it mainly involved chemical carcinogen-receptor activation, cancer morbidity pathway, and prolactin signaling pathway. In summary, the studies on the active components and pharmacological effects of *V. officinalis* provide theoretical support for the anti-LC effect, and lay a foundation for the subsequent pharmacodynamic evaluation and serum pharmacochemistry studies. Future research can build on this foundation with a view to developing more effective drugs and treatments[19].

Mongolian medicine *V. officinalis* is a traditional medicine, and its network pharmacology study can reveal its functional components and protein targets for the treatment of LC. In the study of network pharmacology, the components of *V. officinalis* that may be effective in the treatment of LC can be screened out through databases and software, and their possible targets can be predicted[20]. However, there are some shortcomings in network pharmacology research. For example, the data in the current database may not be comprehensive, the possible interactions between traditional Chinese medicine compound drugs and drugs are complex, and the confirmation of specific targets requires further experimental studies[21]. In addition, the pharmacodynamic material basis of traditional Chinese medicine is not necessarily a single chemical component, but the overall effect of multiple chemical components, which brings challenges to the study of network pharmacology[22]. In order to ensure the accuracy of network pharmacology prediction, later studies need to be confirmed by experimental verification, including *in vitro* and *in vivo* experiments on the active ingredients of Mongolian medicine *V. officinalis* to verify its efficacy in the treatment of LC, as well as in-depth study of its mechanism of action. Through a series of studies, we can further promote the development and utilization of Mongolian medicine *V. officinalis*, and provide more treatment options for the clinical treatment of liver cancer.

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