Study on the Potential Mechanism of Spleen and Stomach Peiyuan Prescription for Gastric Cancer Based on Network Pharmacology and Bioinformatics

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Introduction. To explore the potential mechanism of spleen-stomach Peiyuan prescription in patients with gastric cancer based on network pharmacology and bioinformatics.

Methods. TCMSP database was searched to screen the effective components and targets of spleen-stomach Peiyuan prescription. Using Uniprot database to retrieve the human genetic disease corresponding to the target and complete the naming; The main targets of gastric cancer were obtained by using GeneCards, TTD and OMIM databases, and the intersection targets of drug targets and gastric cancer were obtained by drawing Wayne diagram. Collate the data and use Cytoscape3.7.2 software to construct the network of "Chinese traditional medicine components of spleen and stomach prescription-gastric cancer target", analyze the interaction between proteins, draw the interaction network diagram (PPI) between different proteins, complete the enrichment of gene ontology (GO), Kyoto gene and KEGG, and use AutoDock software to verify the molecular docking.

Results. Combined with OB and DL, the effective components of the above six drugs (42 in total) were screened out, including quercetin, kaempferol, isorhamnetin and luteolin. 4757 targets of gastric cancer and 55 targets of spleen and stomach Peiyuan prescription were screened out, and 181 targets of intersection protein were obtained. Different targets can interact with each other, and have anti-inflammatory, anti-cancer cell proliferation and anti-angiogenesis effects. The molecular functions involved in the signal pathway of Spleen

and Stomach Peiyuan Prescription in gastric cancer include the combination of protein and enzyme. In terms of pathways, the enrichment results of key targets are more reflected in cancer-related signaling pathways and PI3K-AKT signaling pathways; The downstream of hormone-like signaling pathway is usually related to the regulation of mitogen-activated protein kinases (MAPKs) activity. The results of molecular docking showed that ATK1 had better binding stability with stigmasterol and β -sitosterol and naringin with IL-6 and AKT1, respectively.

Conclusion. The mechanism of Spleen-stomach Peiyuan Prescription in the treatment of gastric cancer is complicated, and it can provide new ideas and methods for the follow-up research through multi-genes and multi-channels.

Keywords. Spleen and stomach peiyuan recipe; Gastric cancer; Mechanism of action; Network pharmacology; Bioinformatics; Molecular docking

INTRODUCTION

Gastric cancer can occur in different age groups, it is a malignant tumor originating from gastric mucosa epithelium, and the pathological type is mainly adenocarcinoma [1-2]. At present, the diagnosis rate of early gastric cancer is relatively low, and most patients do not have obvious symptoms in the onset stage, which increases the difficulty of clinical diagnosis and treatment [3]. Surgical treatment, targeted therapy and radiotherapy and chemotherapy are all commonly used treatment measures for gastric cancer, which can delay the development of the disease and prolong the life span of patients, and most patients can benefit from it. However, the overall treatment effect of the above treatment methods was not ideal, and the 5-year survival rate was only 27.4% [4]. With the continuous development of TCM, TCM is often used as an adjuvant drug for tumor treatment, which can consolidate the clinical treatment effect under the guidance of TCM theory [5-6]. The spleen and stomach cultivation prescription is the experience prescription summarized on the theory of Li Dongheng's spleen and stomach disease. It is composed of Atractylodes, Liu Jinu, Astragalus, white peony root, Xiangfu and Guizhi, which can

play the effect of nourishing qi and nourishing the spleen and stomach. Previous studies have shown that [7], spleen and stomach culture formula can treat gastric cancer well, which can quickly improve the symptoms of patients and delay the development of the disease, but the mechanism has not been clear about [8]. This study mainly investigated the underlying mechanism of the network-based pharmacology and bioinformatics analysis in GC patients, as reported below.

1. DATA AND METHODS

1.1 Screening of relevant targets of spleen and stomach culture sources

Databases of PubMed, Ovid, TCMSP, Embase, Web of Science (WOS), Cochrane Library, China CNKI (CNKI) and Wanfang were searched to screen the active components and targets of spleen and stomach culture sources. Find active ingredients in different sources of spleen and stomach, set screening conditions: 30% oral availability (OB), drug-like (DL) 0.18 to obtain corresponding protein targets; search known database to further supplement unsuccessful targets. After screening, the standardized analysis of the resulting compound protein targets was completed and entered into the Uniprot protein database to complete the regulatory [9].

1.2 Screening and drawing of related targets in gastric cancer

(1) Screening of targets related to gastric cancer. Using GeneCards, TTD and OMIM databases, the search term was Gastric cancer, obtained the main target of gastric cancer, and screened the repeated gene [10]; (2) Wayn diagram. Wayn diagram to obtain the intersection of drug targets and gastric cancer. Through the online drawing tool Venny2.1.0 software, the selected spleen and stomach culture source targets and gastric cancer targets were summarized, and the common target [11] can be obtained with the help of this software.

1.3 Construct a network of "active component-target" for treating and stomach

According to the "active ingredient-target" of traditional Chinese medicine in the culture formula of the spleen and stomach, Organizing the data and using the

Cytoscape3.7.2 software, Construct a network of "spleen and stomach source Chinese medicine ingredients-gastric cancer target", Analyze the interactions between the proteins; To search the String target database, Enter the known protein targets into them, Using the CytoNCA plug-in, Using three parameters C, BC and CC, Determine the most core gene and protein interaction targets (i. e., the nodes with> twice the DC, BC and CC means are regarded as important protein targets and active small molecules in the network); Set the biological species as the human, Further to determine the interactions between the known intersection proteins, Network of action (PPI) [12].

1.4 Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment GO

Association with gene function [13]; KEGG can accurately analyze gene function. GO and KEGG enrichment analysis of the spleen and stomach sources used for gastric cancer through the DACID database [14]. Explore the main molecular biological processes and signaling pathways of key targets, so as to further explore the possible mechanism of action.

1.5 Molecular docking and validation

For molecular docking verification and molecular docking technology, the interaction of active components of gastric cancer was further analyzed for analysis of the top four targets in the network diagram. At the same time, the AlphaFold database was searched to download the three-dimensional spatial structure of the target proteins of gastric cancer; explore the pharmacophore in ZINC database, download the three-dimensional spatial structure of the active component of the spleen and stomach, and use Chem 3D21.0 software. After the above operation, complete the protein receptor hydrogenation, charge calculated by Autodock Tools software, and save the [15] in Pdbqt. The Grid Box software is used to set the size of protein receptor grid and the binding point of small molecule ligand protein receptor; the threshold is set in vina software: 20 models, binding energy 5.0 kJ / moL to obtain the corresponding binding site and binding energy. Pymol software.

2 RESULTS

2.1 Prediction of active components and gene targets of spleen and stomach culture formula

By searching the above database, the relevant drug ingredients of the spleen and stomach culture source prescription were obtained. Among them, there are 55 kinds of atractylodes, 104 kinds of incense components, 87 kinds of astragalus, 46 kinds of Liu Jinu and 220 kinds of cassia branch. Combining OB and DL selected the active ingredients of the above 6 drugs (42 kinds in total), including quercetin, kaempferol, rhamphone and luteolin, as shown in Table 1.

Table 1 Analysis of Effective Components of Spleen and Stomach Peiyuan Prescription

NO.	Traditional Chinese medicine name	effective constituent	OB（%）	DL
M1	Rhizome of large-headed atractylodes	8β-ethoxy atractylenolide III	49.62	0.69
M2	Rhizome of large-headed atractylodes	12-senecioyl-2E，8E，10E-atractylentriol	58.96	0.32
M3	Rhizome of large-headed atractylodes	14-acetyl-12-senecioyl-2E，8E，10E-atractylentriol	36.63	0.26
M4	Rhizome of large-headed atractylodes	14-acetyl-12-senecioyl-2E，8Z，10E-atractylentriol	35.53	0.36
M5	Rhizome of large-headed atractylodes	α-Amyrin	44.35	0.41
M6	Rhizome of large-headed atractylodes	3β-acetoxyatractylone	36.92	0.38
M7	Astragalus mongholicus	1，7-Dihydroxy-3，9-dimethoxy	57.63	0.45

		pterocarpene		
M8	Astragalus mongholicus	5'-hydroxyiso-muronulatol-2', 5'-di-O-glucoside	60.51	0.41
M9	Astragalus mongholicus	3，9-di-O-methylnissolin	54.46	0.32
M10	Astragalus mongholicus	Formononetin	48.51	0.26
M11	Astragalus mongholicus	FA	45.53	0.56
M12	Astragalus mongholicus	Bifendate	36.82	0.51
M13	Astragalus mongholicus	Isoflavanone	43.56	0.46
M14	Astragalus mongholicus	Jaranol	41.88	0.52
M15	Astragalus mongholicus	Isomucronulatol-7, 2'-di-O-glucosiole	45.09	0.55
M16	Astragalus mongholicus	Mairin	43.83	0.34
M17	Astragalus mongholicus	Quercetin	73.96	0.59
M18	Radices paeoniae alba	Sitosterol	30.82	0.52
M19	Radices paeoniae alba	Benzoyl paeoniflorin	74.69	0.43
M20	Radices paeoniae alba	Kaempferol	33.32	0.37
M21	Radices paeoniae alba	Beta-sitosterol	45.63	0.41
M22	Radices paeoniae alba	Mairin	36.94	0.43
M23	Radices paeoniae alba	（+）-Catechin	36.91	0.61
M24	Radices paeoniae alba	Paeoniflorgenone	38.52	0.43
M25	Radices paeoniae alba	albiflorin_qt	87.52	0.53
M26	Rhizoma cyperi	Sitosterol	68.54	0.46
M27	Rhizoma cyperi	Chryseriol	66.62	0.49
M28	Rhizoma cyperi	8-Isopentenyl-kaempferol	53.24	0.52
M29	Rhizoma cyperi	Beta-sitosterol	43.69	0.41
M30	Rhizoma cyperi	Khellol glucoside	46.62	0.32
M31	Rhizoma cyperi	Stigmasterol glucoside_qt	50.92	0.46
M32	Rhizoma cyperi	Luteolin	51.43	0.39

M33	Rhizoma cyperi	Luteolin	52.19	0.52
M34	Cassia twig	Peroxyergosterol	54.33	0.43
M35	Cassia twig	ent-Epicatechin	56.71	0.46
M36	Cassia twig	Sitosterol	43.36	0.32
M37	Cassia twig	（+）-Catechin	39.68	0.55
M38	Cassia twig	Taxifolin	40.53	0.56
M39	Artemisia anomala	3，4-di-O-caffeoylquinic acid	43.36	0.52
M40	Artemisia anomala	Ermanin	45.69	0.49
M41	Artemisia anomala	EUPATORIN	50.32	0.31
M42	Artemisia anomala	Beta-sitosterol	51.15	0.34

2.2 Spleen and stomach Peiyuan prescription is used as an intersection target in the treatment of gastric cancer

By searching the above database, 4757 gastric cancer targets and 55 source targets of spleen and stomach were selected. After integrating the target information in the database, 181 intersection protein targets were obtained and Wayn diagram is shown in Figure 1.

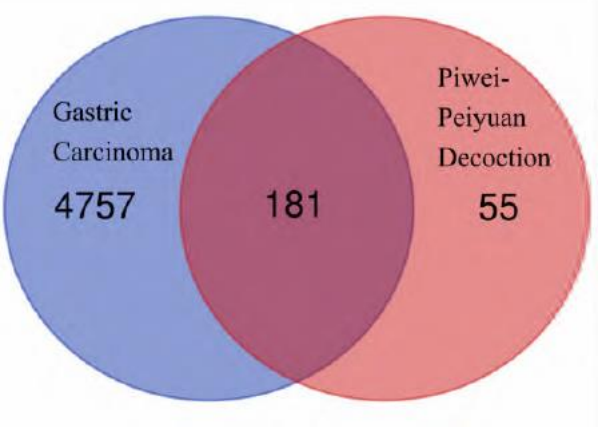

Figure 1 The intersection targets of the spleen and stomach culture sources in the treatment of gastric cancer

2.3 Network map of active components and targets for gastric stomach treatment with

spleen and stomach cultivation method

The effective components of the spleen and stomach source prescription for treating gastric cancer were determined, and the target network map was drawn. It can be seen from the figure that different targets can interact with anti-inflammation, inhibition of cancer cell proliferation and inhibition of neoangiogenesis. The common signaling pathways include JUN, IL-1, Blc 2 and AKT 1, as shown in Figure 2 and Figure 3.

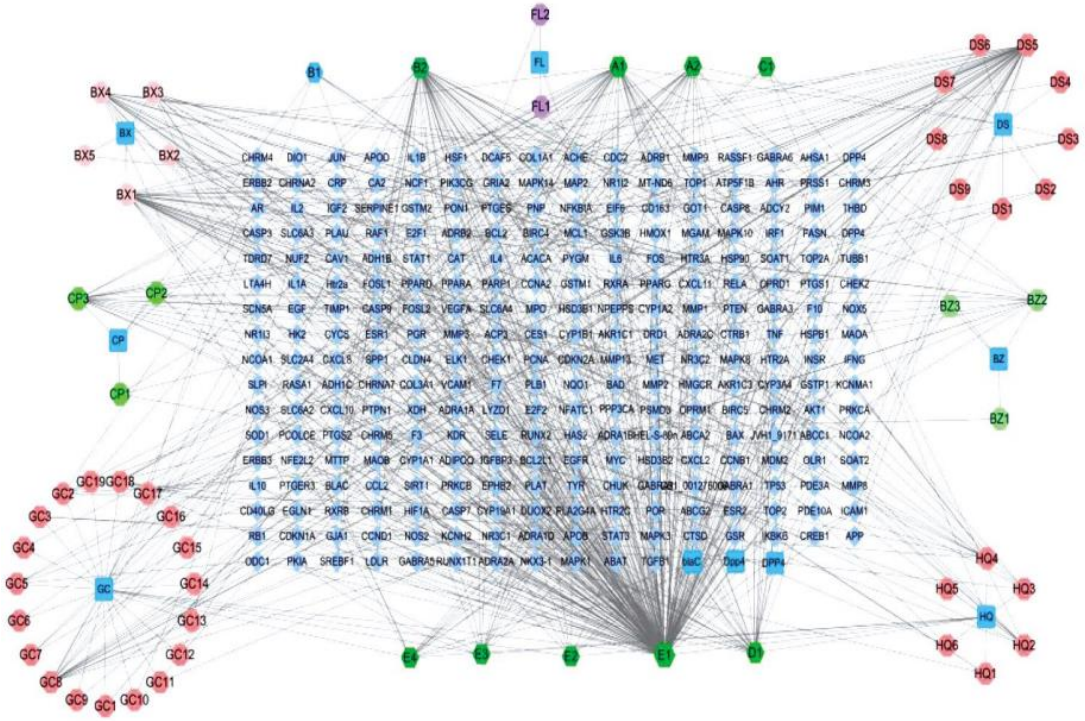

Figure 2 PPI network diagram of the active ingredient of the spleen and stomach culture source square

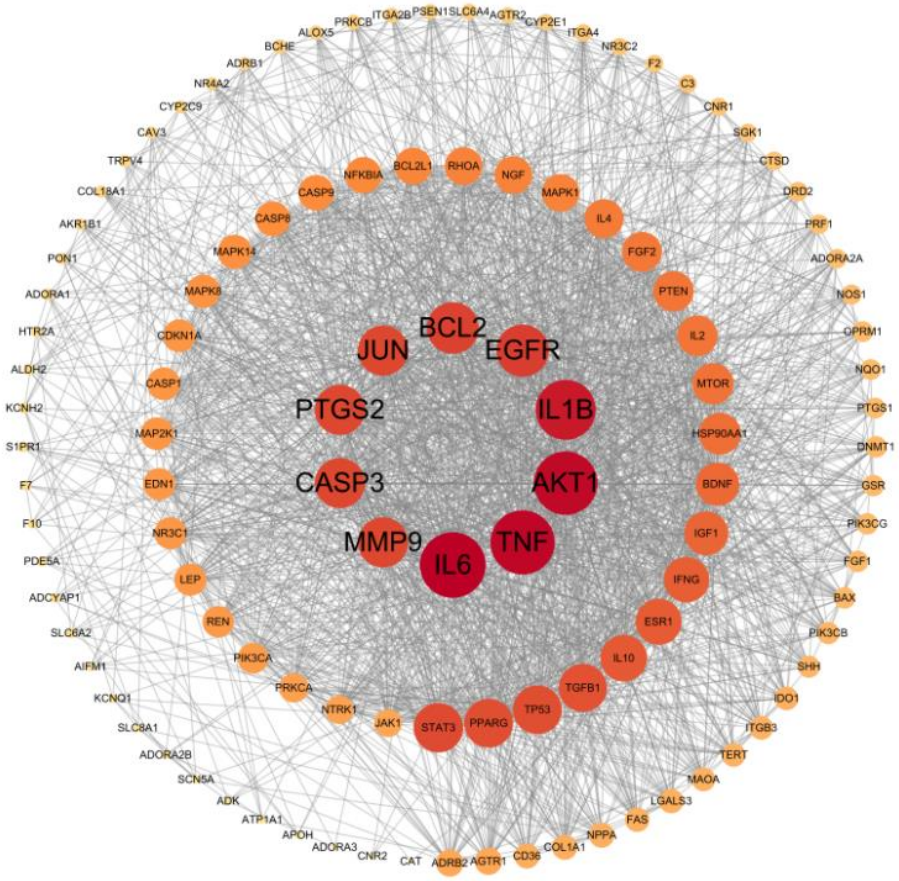

Figure 3 Network diagram of target-signaling pathways

2.4 Enrichment analysis of the GO and KEGG pathways

From the above analysis, it can be seen that the spleen and stomach culture formula is used in gastric cancer, has a relatively wide distribution, and contains molecular functions including the combination of proteins and enzymes. In terms of pathways, the enrichment of key targets is more reflected in cancer-related signaling and PI3K-AKT signaling; downstream of hormone-like signaling is usually associated with regulation of mitogen-activated protein kinases (MAPKs) activity in Figures 4 and 5.

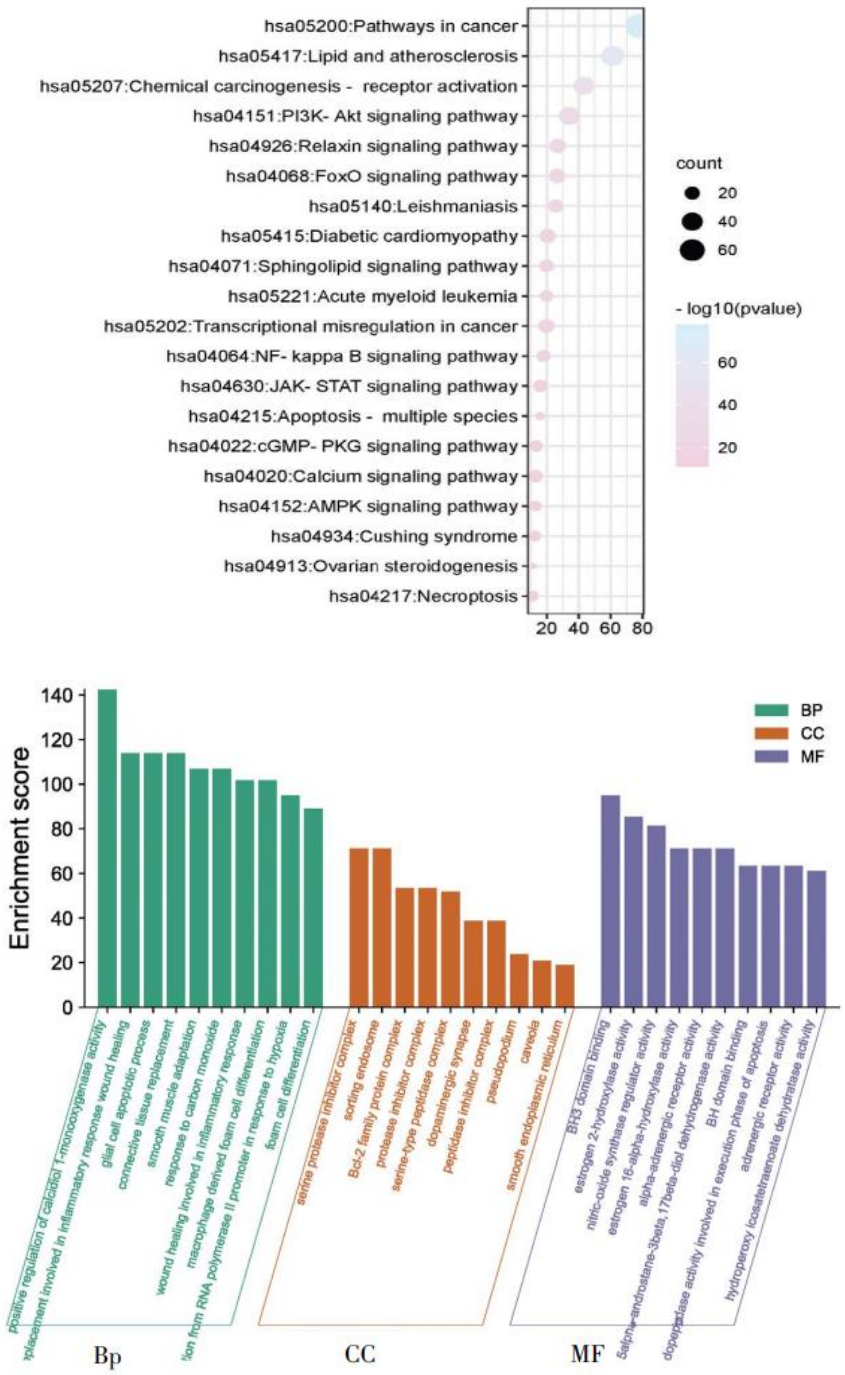

Figure 4 Source square of spleen and stomach culture was used for gastric cancer enrichment analysis diagram

2.5 spleen and stomach culture method for molecular docking results of gastric cancer

Based on the above results, the core targets JUN, IL-1, Blc 2 and AKT 1 were selected for docking with quercetin, stigmasterol, naringenin and β -sitosterol. The

results showed that the docking fraction of binding energy <-5 kcal / mol showed that the compound and the target were well bound, and ATK 1 combined with stigmasterol and β -sitosterol, naringenin and IL-6 and AKT 1, see Table 2 and Figure 5.

Table 2. Results of molecular docking

Target spot	binding energy（kcal/moL）			
	Target spot	Naringenin	Stigmasterol	β-Sitosterol
AKT1	-5.57	-7.26	-8.46	-4.62
TP53	-6.19	-5.59	-7.24	-6.09
TNF	-3.53	-5.62	-6.39	-6.31
IL-6	-4.32	-6.29	-6.51	-5.23

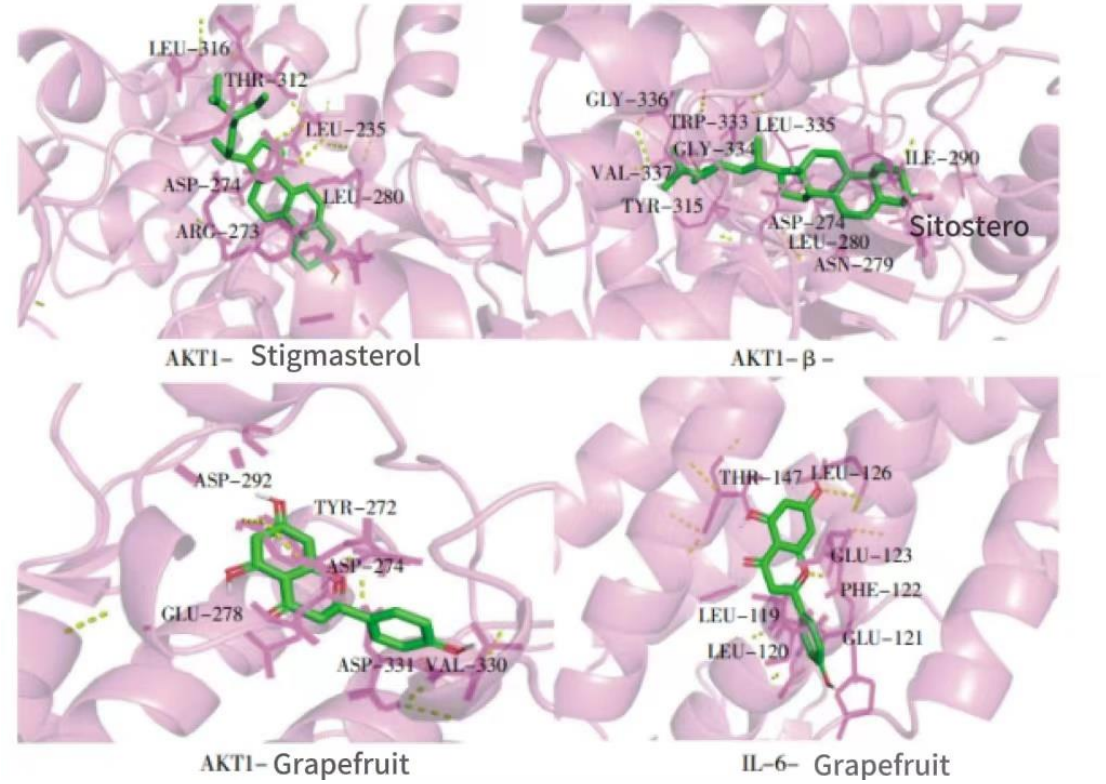

Figure 5 Source method of spleen and stomach for gastric cancer

3 DISCUSSION

As an important treatment method for gastric cancer patients, surgery and

radiotherapy and chemotherapy can prolong the life of patients, but the above methods have obvious disadvantages and poor therapeutic effect for patients [16]. According to the traditional Chinese medicine, the occurrence of tumor is caused by the imbalance of Yin, Yang and qi, coupled with the invasion of external evil. With the extension of the course of the disease, the immune function of the body is suppressed, which further aggravates the development of the tumor, leading to qi deficiency, and qi deficiency can also aggravate the disease, thus forming a vicious circle [17]. Therefore, according to the pathogenesis of gastric cancer, the TCM concept of qi deficiency with Yin deficiency, Yin deficiency and the severity of the disease, and the therapeutic intervention of "fuzheng and dispelling evil" is advocated. In this study, the active components (42) of the above 6 drugs were selected by OB and DL, including quercetin, kaempferol, rat and luteolin; 4757 targets for gastric cancer, 55 targets and 181 intersection protein targets for anti-inflammation, promote cell proliferation, anti-tumor and inhibit angiogenesis, including JUN, IL-1, Blc 2, AKT 1, etc., showing the feasibility and effectiveness of gastric cancer. The spleen and stomach cultivation source prescription is the clinical experience prescription, which is on the basis of "nourishing qi and blood and strengthening the foundation cultivation yuan", with the principle of "nourishing the midway, nourishing and nourishing the day after tomorrow". According to the characteristics of gastric cancer, the treatment of this disease should be mainly "invigorating the spleen and replenishing qi, strengthening the soil and cultivating the source", especially for gastric cancer with weak spleen and stomach, its effect is better [18].

The spleen and stomach culture prescription is mainly composed of atractylodes, Liu Jinu, Astragalus, white peony root, fragrant attachment, Guizhi and other drugs. In the prescription, atractylodes has the effect of dryness and dampness benefiting water and invigorating spleen and replenishing qi; Liu Jinu can play the effect of breaking blood and reducing swelling; Astragalus can play the effect of nourishing blood and reducing water and reducing swelling; white peony root has the effect of nourishing blood and suppressing liver and Yang; the fragrance can relieve liver and

play the effect of relieving qi; and the effect of relieving qi and relieving qi [19]. In this study, the spleen and stomach spleen were used for the molecular functions involved in gastric cancer, including the combination of protein and enzymes. In terms of pathways, the enrichment results of key targets are more reflected in cancer-related signaling pathway and PI3K-AKT signaling pathway; the downstream of hormone-like signaling pathway is usually related to the regulation of MAPKs activity; molecular docking results show that ATK 1 binds to stigmasterol, naringenin, IL-6 and AKT 1. The results show that the spleen and stomach source could treat gastric cancer, and the mechanism may be related to PI3K-AKT and AKT and MAPKs signaling, and stigmosterol and naringenin are the main components. Analysis reasons: Polymasterol, as a commonly used anti-tumor auxiliary component, has good free radical clearance function and can resist inflammation and apoptosis; naringenin can play antibacterial, anti-inflammatory and antioxidant effects, regulate related signaling pathways, and affect the proliferation, metabolism, apoptosis and metastasis of gastric cancer and other digestive tract tumor cells. According to the advantages of spleen and stomach cultivation, the potential chemical components, small molecule protein and signaling pathway of gastric cancer were obtained, providing a theoretical basis for further experimental research and clinical application of this method at the molecular biology level [20].

To sum up, the mechanism of spleen and stomach cultivation in gastric cancer is complex, and it can provide new ideas and methods for follow-up research.

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