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Study on the Mechanism of Radix Pseudostellariae and Hedyotis Diffusa in the Treatment of Gastric Cancer Based on Network Pharmacology and Molecular Docking

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Abstract: **Objective**: To analyze the active ingredients and mechanism of action of the drug "Radix Pseudostellariae and Hedyotis Diffusa" in the treatment of gastric cancer using network pharmacology. **Methods**: The investigation was conducted by screening active chemical components and related targets of drug pairs, searching disease targets, constructing drug component-disease target network maps, mapping PPI networks between drugs and diseases, as well as GO functional enrichment analysis and KEGG pathway enrichment analysis. **Results**: There are 290 targets in total, and 10,411 targets for gastric cancer disease after screening, among which 183 are common targets for the treatment of gastric cancer with Radix Pseudostellariae and Hedyotis Piffusa. PPI network shows that AKT1, TP53 and IL-6 are likely to be key proteins for the treatment of gastric cancer with Radix Pseudostellariae and Hedyotis Diffusa. GO functional enrichment analysis shows that the biological process involves KEGG enrichment results involved 469 pathways, mainly including cancer pathway, Hepatitis-B pathway, PI3K-Akt signaling pathway, etc. **Conclusion**: The therapeutic effect of Radix Pseudostellariae and Hedyotis Diffusa on GC through AKT1, TP53, IL-6, VEGFA, JUN and other key targets, the mechanism of action is related to cancer, Hepatitis-B, PI3K-Akt, HIF-1, TNF and other signaling pathways, involved in inflammatory response, immune regulation, vascular proliferation and other processes to play a role in the treatment of gastric cancer, for clinical application lays the theoretical foundation.

1. Introduction

Gastric cancer (GC) is a malignant tumor originating from the epithelial cells of the gastric mucosa, the incidence of which is decreasing worldwide, but its mortality rate is still in the top five malignant tumors[1]. GC belongs to the category of "stomach pain" and "accumulation" in Chinese medicine, which believes that the occurrence of GC is related to internal deficiency of positive Qi, external infection with evil toxins, poor diet and emotional disorders, and so on. The therapy of "supporting the righteousness is to dispel the evil" and "nourishing the righteousness will remove

itself" is emphasized, and the combination of dispelling the evil and supporting the righteousness, on the basis of tonifying the righteousness and combining with drugs with anti-cancer and detoxification effects, can achieve the therapeutic purpose by taking into account both the symptoms and the root cause. Radix Pseudostellariae and Hedyotis Diffusa were derived from complex J Jinguo Weikang Powder. Jinguo Weikang Powder was prepared by Associate Professor Song Jian according to the addition and reduction of JinGuo Weikang capsule, a preparation in the Department of Spleen and Stomach Diseases, the Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine. The formula is composed of seven herbs, namely TengLiGen, BanZhiLian, JuHong, HuangYaoZi and ShouGong. The formula is based on the theory of "stasis and toxicity intermingling" of Professor Shen Shuwen,a national renowned Traditional Chinese medicine(TCM) doctor, for the progressive stage of GC[2]. Radix Pseudostellariae can benefit Qi and strengthen the spleen, produce fluid and moisten the lung. Its aqueous and alcoholic extracts can improve the absorption function of the small intestine and spleen deficiency in rats, and have the effect of enhancing the immunity of the body. Modern research has concluded that Hedyotis Diffusa can exert anti-inflammatory effects by enhancing the phagocytosis of leukocytes, and it has been widely used in the treatment of various cancers in recent years. The two complement each other, and this paper investigates the active components of this pair based on network pharmacology and molecular docking to predict its mechanism of action in the treatment of progressive GC.

2. Material and Methods

2.1 Screening of Drug Active Ingredients and Construction of "Active Ingredient-Target" Network

TCMSP (http: // tcmsp-e.com/tcmsp.php) platform was used to screen the effective components of Radix Pseudostellariae and Hedyotis Diffusa. The active components with Oral bioavailability (OB) ≥30% and Drug likeness (DL) ≥0.18 were screened to predict the corresponding target proteins. The active components and targets of Radix Pseudostellariae and Hedyotis Diffusa were imported into Cytoscape3.7.2 software to construct the "active ingredient-target" network.

2.2 Screening of Disease Targets and Drawing of Venn Diagrams

Using GeneCards (http://www.genecards.org/) database and DrugBank (http://go.drugbank.com) database, GC disease targets were retrieved by "Gastric cancer", and converted into gene names by UniProt database (https://www.uniprot.org/). The targets of drug pairs and disease targets were uploaded to JVENN online mapping software to screen out the common targets of drug pairs and GC.

2.3 The Construction of PPI Network

Import drug-disease common targets into String (https: // string-db.org/) database to obtain the protein-protein interaction (PPI) relationship between the component targets of Radix Pseudostellariae and Hedyotis Diffusa and GC disease targets. The PPI relationship was saved as TSV file and visualized by Cytoscape 3.7.2 software.

2.4 "Active Ingredient-Target-Disease" Network Diagram

Based on the interaction between active component targets and GC targets in Radix

Pseudostellariae and Hedyotis Diffusa, the network diagram of "active component-target and disease" was constructed in Cytoscape3.7.2 software. The Network Analyzer Plugin was selected to analyze the core components in Radix Pseudostellariae and Hedyotis Diffusa.

2.5 GO Analysis and KEGG Pathway Enrichment Analysis

After screening, the common targets of Radix Pseudostellariae and Hedyotis Diffusa with GC may be potential targets for GC treatment, and they were imported into David (https: // david.ncifcrf.gov) database, Gene Ontology (GO) analysis and Kyoto En cyclopedia of Genes and Geomes (KEGG) pathway enrichment analysis were performed.

2.6 Molecular Docking

PDB (http://www.rcsb.org) database and PubChem (http://pubchem.ncbi.nlm.nih.gov) database were used to obtain the structure of target protein and active component, remove water molecules and ligands in the crystal structure of target protein, perform hydrogenation of target protein receptor, calculate charge, set atomic type and other operations through AutoDock, adjust the charge of small molecule ligand and determine root for molecular docking. Pymol was used to visualize the docking method with low binding energy. The binding strength and activity of targets and compounds were evaluated according to binding energy. The smaller the binding energy, the better the binding between them.

3. Results

3.1 Screening of Candidate Compounds and Targets and Construction of "Active Ingredient-Target" Network

Table 1: Basic information of active constituents of Radix Pseudostellariae and Hedyotis Diffusa

MOL ID	TCM	chemical components	OB (%)	DL
MOL001506	Radix Pseudostellariae	Supraene	33.55	0.42
MOL001689	Radix Pseudostellariae	acacetin	34.97	0.24
MOL001790	Radix Pseudostellariae	Linarin	39.84	0.71
MOL000358	Radix Pseudostellariae, Hedyotis Diffusa	beta-sitosterol	36.91	0.75
MOL000006	Radix Pseudostellariae	luteolin	36.16	0.25
MOL006554	Radix Pseudostellariae	friedoolean-14-en-3-beta-ol	38.40	0.77
MOL006756	Radix Pseudostellariae	(3beta,5alpha)-stigmast-7-en-3-ol	37.42	0.75
MOL002464	Radix Pseudostellariae	1-monolinoleoyl-rac-glycerol	37.18	0.30
MOL001646	Hedyotis Diffusa	2,3-dimethoxy-6-methyanthraquinone	34.86	0.26
MOL001659	Hedyotis Diffusa	(3β,22E,24R)-stigmasta-5, 22-dien-3-ol	43.83	0.76
MOL001663	Hedyotis Diffusa	oleanolic acid	32.03	0.76
MOL001670	Hedyotis Diffusa	2-methoxy-3-methyl-9,10-anthraquinone	37.83	0.21
MOL000449	Hedyotis Diffusa	Stigmasterol	43.83	0.76
MOL000098	Hedyotis Diffusa	quercetin	46.43	0.28

After searching and screening in TCMSP database, a total of 14 active ingredients of drug pairs were obtained, as shown in Table 1. After removing the duplicated target proteins, 93 target sites of

Radix Pseudostellariae and 197 target sites of Hedyotis Diffusa were obtained.

Cytoscape3.7.2 software was used to construct the "active ingredient-target" map, as shown in Figure 1. TCMSP database search showed that four components had no corresponding target. The network has 212 nodes and 347 edges. In terms of drug components, quercetin had the largest number of targets (154), followed by luteolin (57), β-sitosterol (38) and Stigmasterol (31). A1 stands for β-sitosterol, which is a common component of Radix Pseudostellariae and Hedyotis Diffusa

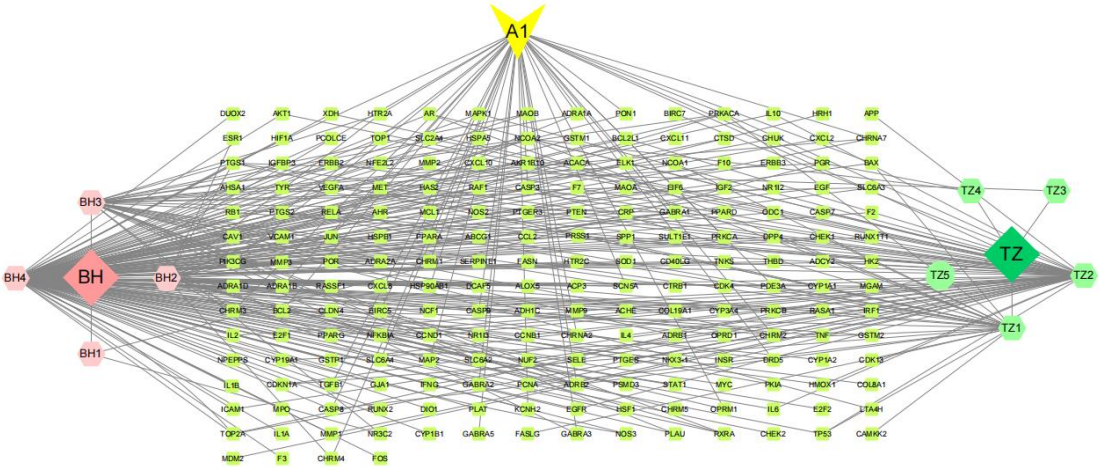


Figure 1: Active Ingredient-Target network

3.2 Screening of Disease Targets and Construction of Venn Diagram

10407 and 32 disease targets of GC were collected from GeneCards and DrugBank databases. The above data were matched and removed by gene names in Uniprot database to obtain 10411 gene names. 216 drug targets and 10411 disease targets were uploaded to JVENN online mapping software, and 183 drug-disease common targets were finally obtained, as shown in Figure 2.

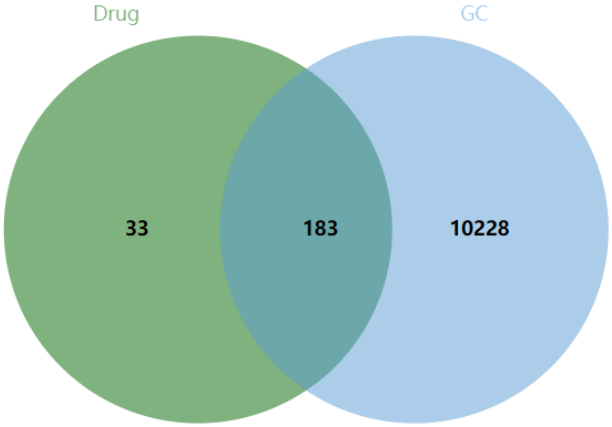


Figure 2: Venn Diagram of the targets of GC and components of Radix Pseudostellariae and Hedyotis Diffusa

3.3 PPI Network Construction

183 drug-disease intersection genes were imported into String database to obtain PPI relationship. Cytoscape3.7.2 software was used to visualize PPI relationship. The network contained 183 nodes

and 3171 edges, and the average degree of target protein was 34.7. Only 65 target proteins with greater than average degree values were shown, as shown in Figure 3. Figure 4 shows the top 10 core targets with degree values. Including protein kinase B1 (AKT1), cell tumor antigen P53 (TP53), tumor necrosis factor (TNF), interleukin-6 (IL-6), vascular endothelial growth factor A (VEGFA), JUN, caspase-3 (CASP3) as the top 7 target proteins of degree value. Therefore, it is speculated that they play an important role in PPI network. It is worth noting that AKT1 has the highest degree value, indicating that it may be an important potential target for the treatment of GC.

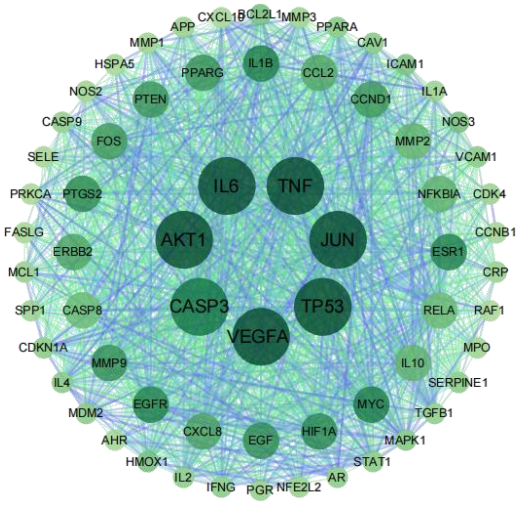


Figure 3: PPI network diagram of Radix Pseudostellariae and Hedyotis Diffusa and gastric cancer

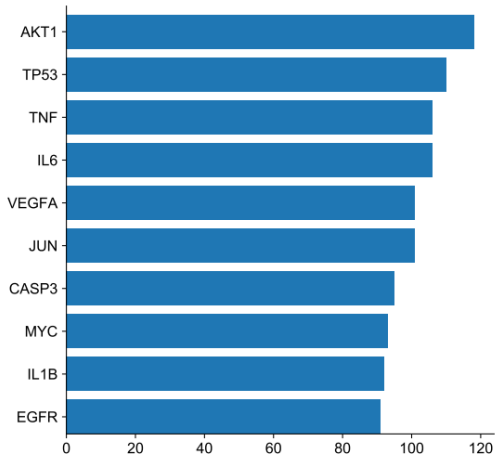


Figure 4: Top 10 core targets by degree value

3.4 Construction of the "active component-target-disease" network

The "Drug component-target-disease" network diagram of Radix Pseudostellariae and Hedyotis Diffusa for GC treatment was constructed with Cytoscape3.7.2 software, as shown in Figure 5. The results showed that quercetin, luteolin, β-sitosterol, stigmasterol and other active components in Radix Pseudostellariae and Hedyotis Diffusa had therapeutic effects on GC.

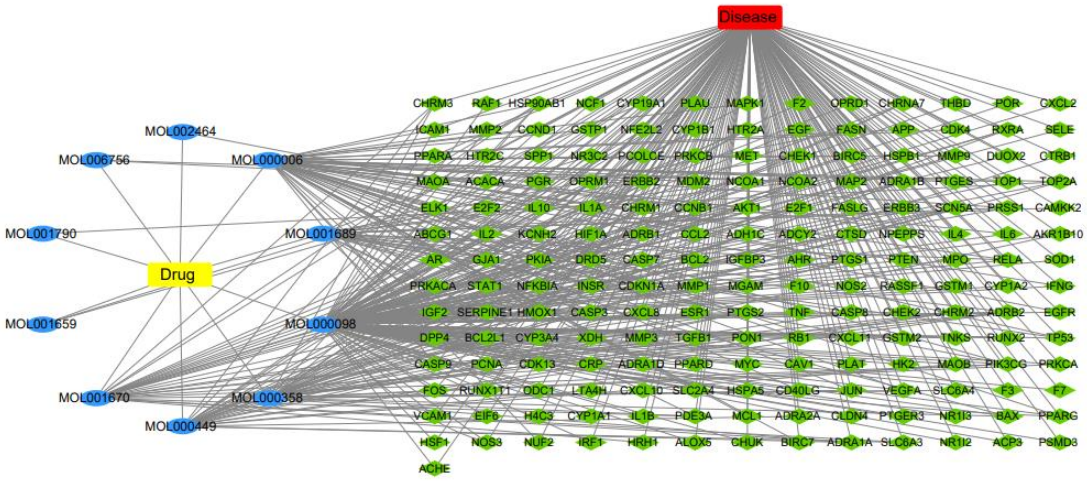


Figure 5: "active component-target-disease" network

3.5 GO Function and KEGG Pathway Enrichment Analysis

The target genes of Radix Pseudostellariae and Hedyotis Diffusa for GC treatment were analyzed by DAVID database, as shown in Figure 6. GO enrichment analysis helped to understand the functional enrichment of gene sets and to obtain the potential functions of genes. The results of the analysis showed that there were 5934 items related to biological process (BP), which mainly involved the reaction of cells to organic circular compounds, lipopolysaccharides, and drugs, etc. Cellular component (CC) mainly involves 449 items, such as membrane raft, cytoplasmic perinuclear region, and outer membrane of organelles. Molecular function (MF) mainly involves 903 items, such as protein domain specific binding, transcription factor binding, and protein homodimerization activity. KEGG pathway functional enrichment analysis is a powerful tool to study metabolic pathways in vivo. A total of 469 signal pathways were obtained, mainly involved in cancer, diabetes and other diseases. Gc-related pathways mainly include cancer, Hepatitis B, phosphatidylinositol 3 kinase (PI3K) -protein kinase B (Akt) signaling pathway, hypoxia inducible factor-1 (HIF-1) pathway, TNF pathway, etc. Based on the p-value, take the top entries to draw the bubble chart, as shown in Figure 7.

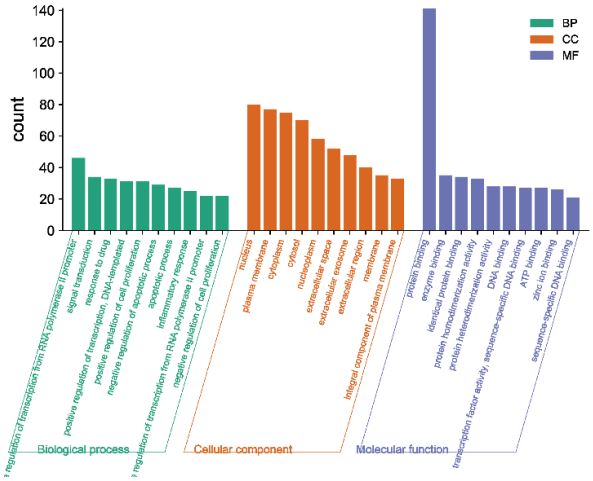


Figure 6: GO enrichment analysis

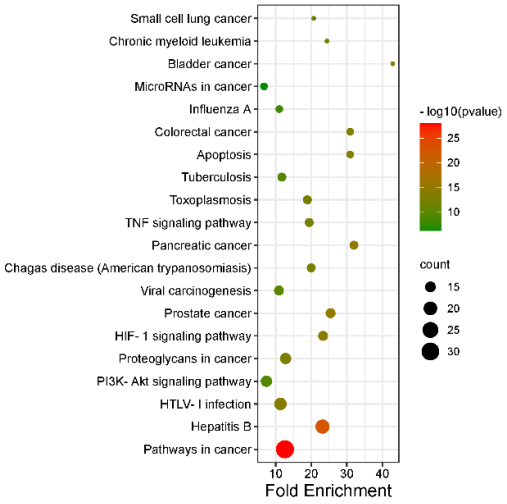


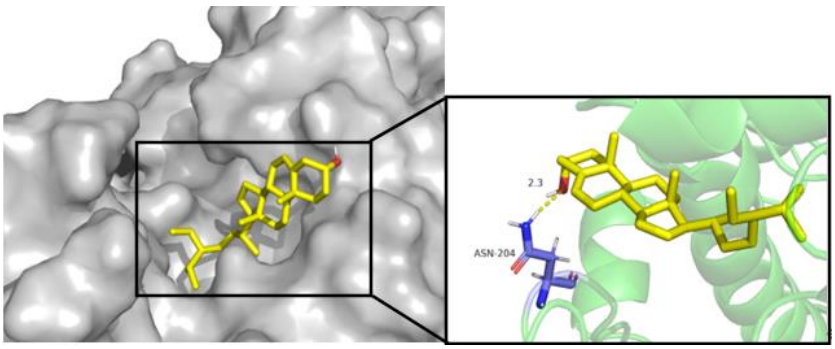
Figure 7: KEGG enrichment analysis

3.6 Molecular Docking Results

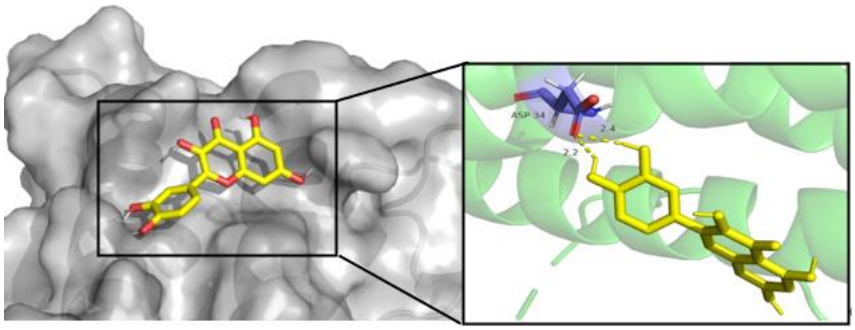
The docking accuracy between candidate compounds quercetin, luteolin, β-sitosterol, stigmasterol and potential target proteins AKT1(PDB ID:5KCV)、TP53(PDB ID:3O96)、TNF(PDB ID:2ZJC)、IL6(PDB ID:6NCO)、VEGFA(PDB ID:6ZFL)、JUN、CASP3(PDB ID:1RHM)was verified by molecular docking of Radix Pseudostellariae and Hedyotis Diffusa, as shown in Table 2.The results showed that the score of the target protein and the corresponding compound molecule was less than -5 kcal/mol, indicating that the target protein and the compound molecule had good binding ability, as shown in Table 2. Pymol software was used to visualize the docking results of the two groups with lower binding energy, as shown in Figure 8.

Table 2: Molecular docking results between active components and core targets

Active component	Binding energy(kcal/mol)				
	AKT1	TP53	TNF	IL6	VEGFA
Quercetin	-5.65	-5.08	-5.12	-7.9	-5.69
Luteolin	-5.51	-6.19	-5.07	-5.12	-5.4
β-sitosterol	-6.79	-7.01	-5.11	-5.28	-5.56
Stigmasterol	-5.21	-5.71	-5.01	-5.21	-5.14



TP53-β-sitosterol



IL6- Quercetin

Figure 8: Molecular docking results

4. Discussion

At present, the main treatment for GC is surgery, combined with radiotherapy, chemotherapy or biological targeting. TCM treatment can reduce postoperative complications and alleviate adverse reactions caused by radiotherapy and chemotherapy. Drinking TCM can strengthen the spleen and stomach, increase appetite, promote digestion and improve the quality of life of patients.Radix Pseudostellariae and Hedyotis Diffusa Oral Chinese medicine can strengthen the spleen and stomach, increase appetite, promote digestion, and improve the quality of life of patients. Radix Pseudostellariae and Hedyotis Diffusa is derived from Jinguo Weikang Powder, which plays a significant effect in the clinical treatment of GC and plays a positive role in the regulation of the prognosis of GC patients[2].

In this study, network pharmacology was used to study the mechanism of action of Radix Pseudostellariae and Hedyotis Diffusa in the treatment of GC, so as to provide theoretical guidance for further clinical research. It was found that quercetin, luteolin, β-sitosterol and stigmasterol were the key components in the treatment of GC. Quercetin has the ability to regulate the proliferation and invasion of GC cells, and its mechanism may be related to the decreased expression of Caveolin (Cav) -1, a GC cell-associated protein[3]. The serum levels of IL-1β and TNF-α in GC patients are high, and they were positively correlated with CEA, CA199, CA125 and other tumor markers. Experiments have found that luteolin and quercetin can reduce the contents of TNF-α, IL-1β and IL-6 by inhibiting lipopolysaccharide (LPS)[4, 5]. β-sitosterol can down-regulate the protein expressions of P-PI3K, p-Akt and p-mTOR in human gastric adenocarcinoma cells (AGS), inducing GC cell autophagy and thus promoting cell apoptosis [6]. Sitosterol and stigmasterol, as common edible plant sterols, inhibit the signaling of anti-tumor cell apoptosis enzyme by reducing the content of cell membrane cholesterol, and enhance the activity of CASP3 to promote the apoptosis of tumor cells [7].

Based on PPI network, AKT1, TP53, IL-6, VEGFA, JUN, TNF and CASP3 were found to be the key targets for GC treatment by Radix Pseudostellariae and Hedyotis Diffusa. AKT plays a crucial role in tumor perfusion and metastasis by protecting the endothelial barrier and preventing abnormal vascular penetration. AKT1, a member of the AKT family, can mediate the vascular growth of tumor cells and inhibit epithelial invasion, endothelial barrier destruction and cancer metastasis[8]. AKT1, which is involved in local tumor growth, can be a valuable target for GC metastasis therapy[9]. TP53 is one of the common tumor suppressor genes, which plays an important role in the regulation of autophagy, apoptosis and DNA repair. TP53 can participate in the tumor immune response by regulating the function of macrophages[10]. Helicobacter pylori(Hp) is the only bacterium that is the ultimate carcinogen in humans, and about 75% of GC is related to Hp infection. Persistent infection with Hp leads to chronic inflammation of the stomach and

duodenum, which affects immune regulation and induces angiogenesis and promotes tumor development[11]. Studies have found that IL-6 plays a key role in differentiation and activation of T lymphocytes, and can also induce proliferation and inhibit apoptosis of tumor cells[12]. Inhibition of IL-6 is effective in the treatment of autoimmune diseases, chronic inflammation and inflammation-related cancer models[13]. TNF-α regulates the immune response. Appropriate amount of TNF-α can inhibit infection, antiviral and tumor occurrence, while excessive TNF-α can promote the release of inflammatory mediators, thus aggravating the inflammatory response after gastric mucosa injury and affecting the occurrence and development of GC[14]. The serum levels of inflammatory cytokines (IL-6, TNF-α, etc.) are positively correlated with the degree of inflammation of gastric mucosa. Chronic inflammation, glandular atrophy, atypical hyperplasia and other changes of gastric mucosa are extremely important in the development and evolution of GC[15]. Hp infection is related to chronic inflammation on the one hand, and to the interaction between TNF-α and cell surface nuclide on the other hand. The localization of cell surface nuclide, as a possible inducing factor in the early stage of cancer, can promote tumor activity[16]. IL-6 can up-regulate the expression of VEGF, which is involved in the regulation of tumor angiogenesis and can bind to vascular endothelial cells to improve vascular permeability[17]. By responding to the signals of carcinogenic factors, AP-1 regulates gene transcription and promotes the invasion and metastasis of tumor cells[18]. JUN is one of the subfamily of transcription factor AP-1, which inhibits the proliferation of gastric cancer cells by inhibiting the phosphorylation of JUN and reducing the activity of AP-1[19]. The Caspases family plays a role in regulating inflammatory response and promoting the apoptosis of inflammatory cells, which is related to tumor and autoimmune diseases, among which Casases-3 plays an irreplaceable role[20].

KEGG pathway enrichment analysis showed that the therapeutic effect of Radix Pseudostellariae and Hedyotis Diffusa on GC was closely related to cancer pathway, Hepatitis B pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway and TNF signaling pathway. Studies have found that regardless of family history of GC, HBV infection and the presence of HBSAG are positively correlated with GC[21]. Chronic HBV infection is carcinogenic and HBV is ubiquitously present in the liver and extrahepatic organs, which can lead to gastric mucosal damage and is related to the clearance of virus by CD8+ cells[22]. In the PI3K/Akt signaling pathway, phosphorylation of related proteins will affect the process of cell protein synthesis and transcription, and CEACAM6, a CEA-related cell adhesion molecule, can induce epithelial mesenchymal transition (EMT)[23, 24]. EMT is a physiological process of embryonic development and tissue regeneration, as well as an important process of cell proliferation and apoptosis, which is often an important reason for the poor prognosis of cancer[25]. HIF-1 can induce RhoE upregulation and promote EMT of AGS cells, along with cytoskeletal remodeling and enhanced cell invasiveness [26]. HIF-1 signaling pathway plays an important role in the adaptation of tumor cells to hypoxia, which can lead to the up-regulation of VEGF and promote the metastasis of GC through the activation of β-catechin [27]. Triple helix collagen (CTHRC) is involved in blood vessel and bone formation, and CTHRC1 is upregulated in patients with GC lymph node metastasis and peritoneal seeding. CTHRC gene up-regulates the expression of CXC chemokine receptor 4 (CXCR4) through HIF-1 signaling pathway, which increases macrophage infiltration and promotes epithelial mesenchymal transformation, leading to poor GC prognosis [28].

In conclusion, quercetin, luteolin, β-sitosterol, stigmasterol and other components of Radix pseudostellariae and Hedyotis pallidariae have therapeutic effects on GC through key targets such as AKT1, TP53, IL-6, VEGFA, JUN, TNF and CASP3. Its mechanism of action in treating gastric cancer is related to cancer, Hepatitis-B, PI3K-Akt, HIF-1, TNF and other signaling pathways. The pharmacological mechanism of Radix Pseudostellariae and Hedyotis Diffusa in the treatment of gastric cancer was verified, which mainly includes several processes such as inflammation, immune

regulation and vascular proliferation and other processes. These results provide a theoretical basis for the clinical application of Radix Pseudostellariae and Hedyotis Diffusa.

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