

Mechanism of the use of four chemotherapeutic drugs for intestinal metaplasia in the treatment of precancerous gastric cancer lesions based on network pharmacology and molecular docking technology

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Abstract: Objective: To use network pharmacology and molecular docking technology to explore material basis and molecular mechanism of *Coix lacryma-jobi*, *Hedyotis diffusa*, *Curcuma zedoaria*, and *Salvia chinensis* on the treatment of pre-cancerous stomach diseases. Our findings provide a theoretical foundation for further clinical research. **Methods:** We searched and screened the targets of four pharmaceutical components for activity against precancerous lesions of gastric cancer (PLGC) using the GeneCards and OMIM network databases. The Chinese medicine composition-target network was constructed using Cytoscape3.7.2 software, and the protein interoperability network of the four drugs for PLGC treatment was constructed using the string data platform. The core target was found by topological analysis. Finally, biopathic and enrichment analyses were carried out on the drug-disease intersection target. **Results:** A total of 19 active ingredients and 123 component targets were collected for four enterolytic drugs. For PLGC, 1487 targets were identified, and 64 targets were collected for pharmaceutical components and diseases. A topological analysis was performed with a value greater than the mean degree (29.0), and 64 key core targets were obtained (including TP53, EGFR, TNF, and VEGFA), and the key targets were screened for TP53, EGFR, TNF, and VEGFA, among others, through network topology and protein interoperability network analyses. GO functional enrichment analysis resulted in 1337 bio-process entries, 46 cell composition entries, and 74 molecular function entries. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis and screening resulted in 254 signaling pathways, including stomach cancer, breast cancer, prostate cancer, non-small cell lung cancer, and colon cancer. **Conclusion:** The four enterolysis drugs may be used to prevent and control PLGC by acting on TP53, EGFR, TNF, and VEGFA targets and relevant gastric cancer, inflammatory, and immune pathways.

Key words: cyber pharmacology, Chinese medicine, precancerous lesions, mechanism studies.

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Abbreviations: ADME, absorption, distribution, metabolism, and excretion; OB, oral bioavailability; DL, drug class; PDB, Protein Data Bank.

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Introduction

Gastric cancer is one of the most common malignant tumors worldwide, and in 2018, more than 1 million new cases were reported worldwide. The incidence and mortality rates attributed to gastric cancer are fifth and third [1], respectively, and this disease has seriously impacted human life and health. Currently, Correa's development model of gastric cancer is widely recognized: chronic superficial gastritis → chronic atrophic gastritis → intestinal metaplasia → intraepithelial neoplasia (dysplasia) → gastric cancer. In the secondary prevention of gastric cancer, the active treatment of precancerous lesions is of great significance [2].

Professor Li Xu is a professor and doctoral advisor of Nanjing University of traditional Chinese medicine and the chief traditional Chinese medicine practitioner of Jiangsu Hospital of traditional Chinese medicine. Professor Xu has been engaged in the clinical teaching and scientific research on traditional Chinese medicine in the context of tumors for nearly 30 years. He has rich clinical experience and unique opinions and methods on the diagnosis and treatment of precancerous lesions of gastric cancer (PLGC).

The four drugs for intestinal chemotherapy were summarized by Professor Li's many years of clinical experience, and the main treatment purpose was to reverse PLGC. The specific composition of the treatment included coix seed, *Hedyotis diffusa*, zedoary turmeric, and Chinese Sage Herb. The dictionary of traditional Chinese medicine records states that coix seeds are sweet, light, and slightly cold in nature. They can localize in the spleen, lungs, and kidney meridians and can eliminate dampness and strengthen the spleen. Coix seed can also relax the tendons, remove arthralgia, clear heat, and expel pus. Modern pharmacological studies have found that coix seeds have anti-tumor [3] and immunoregulatory [4] effects. *Hedyotis diffusa* is cold and tastes bitter and sweet. It targets the heart, liver, and spleen meridians and has the function of clearing heat, eliminating dampness, and detoxification. Pharmacologically, it has anti-tumor [5], antibacterial, and anti-inflammatory [6] effects. Zedoary turmeric is bitter, pungent, and warm. It enters the liver and spleen channels. it removes blood stasis, promoting the circulation of Qi to relieve pain. Chinese Sage Herb is bitter and mild in nature and can cure choking, swelling carbuncle, and scrofula.

Network pharmacology can systematically explore the interaction between drugs, targets, pathways, and diseases. It is particularly suitable for investigating the multi-component and multi-target characteristics of traditional Chinese medicine and the compounds responsible for the medicinal characteristics. This study explores the molecular mechanism and biological effects of four Changhua drugs in the

treatment of PLGC s through network pharmacology. Our findings provide a reference for further research, help the research and development of new drugs, and broaden the prevention and treatment of PLGC using traditional Chinese medicine.

Methods

Screening of active components of four intestinal chemotherapeutic drugs

Based on the pharmacokinetic parameters (absorption, distribution, metabolism, and excretion (ADME)), with “coix seed,” “*Hedyotis diffusa*,” “zedoary turmeric,” and “Shijianchuan” as the keywords, we searched the tcmsp traditional Chinese medicine biological information database (<http://tcmssp.com/tcmssp.php>). The ingredients were identified; active ingredients were screened according to the conditions of oral bioavailability (OB) ≥ 30% and drug class (DL) ≥ 0.18.

Acquisition of action targets of effective components of four intestinal chemotherapeutic drugs

The target proteins corresponding to the active components of “coix seed,” “*Hedyotis diffusa*,” “zedoary turmeric,” and “Shijianchuan” found in the tcmsp database were imported into the UniProt database (<http://www.uniprot.org/>), and the gene names were standardized by inputting the gene name of the target protein.

Prediction of PLGC disease targets

The data on genes related to cancer were collected through the keyword of “cancer” and imported into the UniProt database for gene name standardization and data integration.

Drug disease target gene intersection

After sorting the data, the intersection target genes were constructed by Venny 2.1.0 online software, and the Wayne map was drawn. We used Cytoscape 3.7.2 software to visually analyze the common targets, construct the “disease drug component target” network, and calculate the corresponding relationship nodes.

Construction of a protein–protein interaction (PPI) interaction network

After removing the weight of the action target of the effective components of the four intestinal chemical drugs, the “component disease” target database was established with the disease targets of PLGC. After taking the intersection, the “component disease” mapping file was prepared. Using the common targets and compound components obtained by the intersection, the PPI network of the “four intestinal chemical drugs for PLGC disease targets” was established through the string database. Then, the network was imported into Cytoscape software for

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topological analysis and screened with twice the node degree (degree = 29) to generate the core target map of the “active ingredients.”

Biological pathway and enrichment analysis

The Metascape database was used to analyze the enrichment of biological processes and pathways for the intersection target network between drugs and diseases. According to the enrichment results, the material basis and molecular mechanisms of the four intestinal drugs in the treatment of PLGC were predicted and analyzed.

Component target molecular docking

The processed target protein and compound components were imported into autodock Vina software in “pdbqt” format for molecular docking, and pymol2 3.0 and ligplotv2 24 were used to analyze the interaction mode of the docking results.

Small molecule treatment

We downloaded the compounds in mol2 format from the ZINC database, according to their small molecule CAS numbers, and then imported the information into chembio3d ultra 14.0 for energy minimization. We also used autodocktools-1.5.6 for hydrogenation, charge calculation, and charge distribution assessment, set the rotatable key, and saved the output in “pdbqt” format.

Target protein preparation and treatment

From the Protein Data Bank (PDB) (<http://www.rcsb.org/>), we downloaded the key target proteins (human proteins were preferred, structures with high structural similarity between the original ligand and the active ingredient to be docked were preferred, and structures with high resolution were selected). We imported the protein into PyMOL (2.3.0) to remove the original ligands and water molecules, and then imported the protein into autodocktools (v1.5.6) for hydrogenation, charge calculation, charge distribution, and atomic type designation. Lastly, the output was saved in “pdbqt” format.

Preparation of parameter documents

We took the original protein ligand as the center of the docking box. If there was no original ligand, we took the whole protein as the docking area and set the size of the lattice box to 70 grid × 70 grid × 70 grid (the spacing of each grid point was 0.375 Å). The other parameters were set to their default settings. Center coordinate of TP53 (PDB ID: 2bim) $x=113.117$, Center $y=87.367$, Center $z=22.402$; VEGFA (PDB ID: 1mkk) center coordinate: Center $x=7.467$, Center $y=-0.166$, Center $z=13.089$; IL-6 (PDB ID: 1alu) center coordinate: Center $x=2.615$, Center $y=-19.807$, Center $z=9.103$.

Results

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Effective components of the four intestinal drugs

By using the tcmsp database, the components were searched with “coix seed,” “*Hedyotis diffusa*,” “zedoary turmeric,” and “Chinese Sage Herb” as keywords, and screened under the conditions of oral bioavailability (OB) $\geq 30\%$ and drug class (DL) ≥ 0.18 . Finally, 19 effective components were obtained (Table 1).

PLGC disease targets

We input the keyword “precancerous lesions of gastric cancer” to obtain the action targets related to PLGC and identified a total of 1487 disease targets.

Screening of common targets

Sixty-four common targets were mapped, and the online software Venny 2.1.0 was imported to draw the Wayne diagram, as shown in Figure 1. The mapping rate of the target genes of the four drugs for intestinal chemotherapy on PLGC was 4.1%, suggesting that these drugs may have targets for the treatment of PLGC.

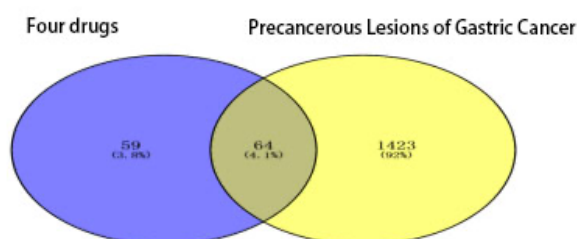


Figure 1 Wayne Diagram

Prediction of component targets of the four intestinal drugs and construction of a component target network diagram

Nineteen active components of the four intestinal drugs were searched, and 123 corresponding targets of their active components were obtained. Gene names were standardized through the UniProt database, and Cytoscape 3 7.2 software was used to construct the network diagram of the “four intestinal chemicals - active components - targets” (Figure 2).

There are 153 nodes in the figure. The yellow squares represent the disease and the four intestinal drugs, green squares represent the active components of the four intestinal drugs, blue squares represent the targets that correspond to the active components, and the edges between the nodes represent the interaction relationship between the drug components and their targets.

Table1 effective components

Mol ID	Molecule Name
MOL001646	2,3-dimethoxy-6-methyanthraquinone
MOL001659	Poriferasterol (4aS,6aR,6aS,6bR,8aR,10R,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,1 2a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropi cene-4a-carboxylic acid
MOL001663	
MOL001670	2-methoxy-3-methyl-9,10-anthraquinone
MOL000449	Stigmasterol
MOL000358	Beta-sitosterol
MOL000098	Quercetin
MOL001323	Sitosterol alpha1
MOL001494	Mandenol (6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18 ,22-hexaene
MOL002372	
MOL002882	[(2R)-2,3-dihydroxypropyl] (Z)-octadec-9-enoate
MOL000359	Sitosterol
MOL008118	Coixenolide
MOL008121	2-Monoolein
MOL000953	CLR
MOL000296	Hederagenin
MOL000906	Wenjine
MOL000915	(1S,10S),(4S,5S)-germacrone-1(10),4-diepoide
MOL000940	Bisdemethoxycurcumin

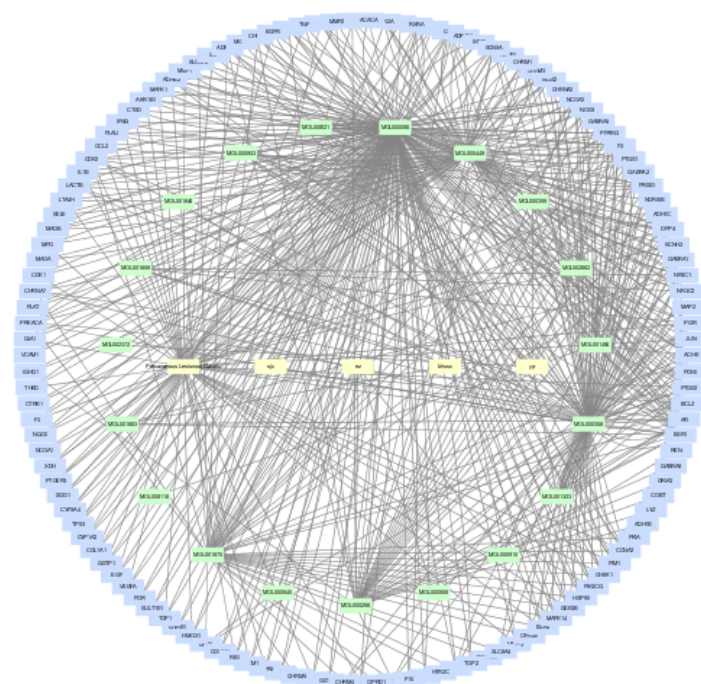


Figure 2 Network relationship of the “four intestinal chemicals - active ingredients - targets.”

PPI network construction and analysis

The PLGC disease targets and effective component targets of the four intestinal chemotherapeutic drugs were combined, and 64 drug components and disease targets were obtained at the intersection. The results

were imported into the string database to establish the PPI network of the “four intestinal chemotherapeutic drugs for PLGC targets” (Fig. 3). Then, the Cytoscape 3.7.2 software was used to conduct a topology analysis. Screens with a value greater than two times (20) the

average degree value were used to obtain the core targets (Fig. 4). The following 10 key targets were obtained: TP53 (tumor suppressor gene p53), VEGFA (vascular endothelial growth factor a), IL6 (interleukin-6), TNF (tumor necrosis factor), mapk1 (mitogen activated protein kinase 1), Jun (proto oncogene), EGFR (human epidermal growth factor receptor), PTGS2 (cyclooxygenase 2), ESR1 (phosphorylated S104), and EGF (epidermal growth factor). The analysis results suggest that the four intestinal chemotherapy PLGC drugs may play a therapeutic role mainly by acting on these 10 targets.

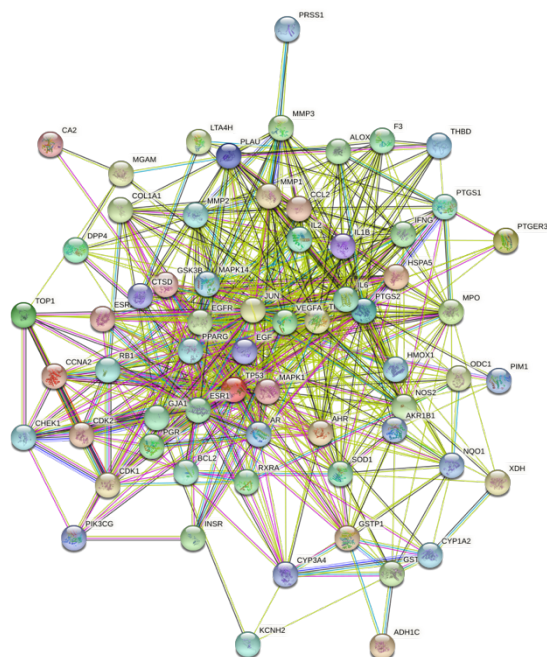


Figure 3 PPI network of the “four intestinal chemotherapeutic drugs for PLGC targets.”

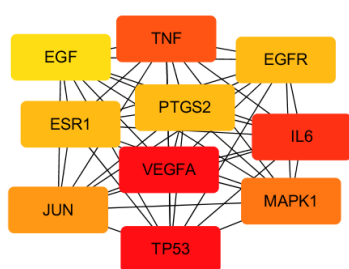


Figure 4 The core targets.

Biological pathway and functional enrichment analysis

The Metascape database was used to analyze the enrichment of biological processes and pathways for the intersection targets of drugs and diseases and to obtain the biological processes and pathways involved in the intersection targets (see Figs. 5, 6 and 7).

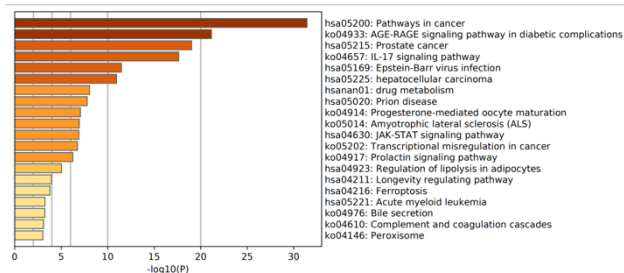


Figure 5 KEGG enrichment analysis.

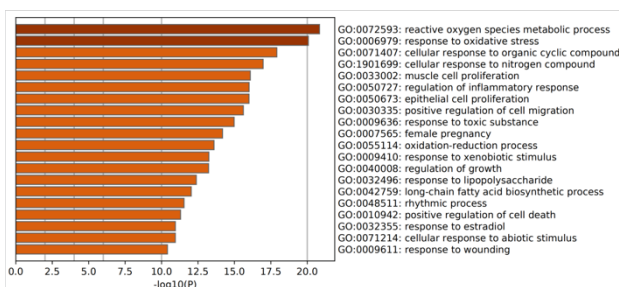


Figure 6 BP(Biological process) enrichment analysis.

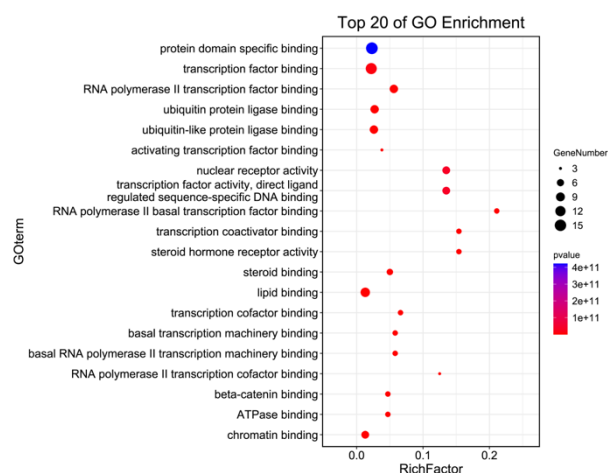


Figure 7 GO(Gene Ontology) enrichment analysis.

Molecular docking results

The three core targets (TP53, VEGFA, and IL6) screened were connected with the three drug components (mol000098, mol000358, and mol000449) with a degree of > 20 by autodock Vina software (see Table 2). Affinity is the strength of the interaction between the interacting molecules; the target and the ligand are more strongly bound if the affinity between them is smaller. It can be seen from Table 2 that the affinity of TP53, VEGFA, and IL6 with the three drug components was negative, indicating that the key components and core targets of the four intestinal drugs have good binding abilities. The combination of β -sitosterol was the best; thus, it can be concluded that β -sitosterol is the core component in the treatment of PLGC. Fig. 8 shows the docking results with the highest absolute affinity value between three target proteins and drugs.

Discussion

In this study, 19 active components, 59 potential targets, and 64 common targets for the prevention and treatment of PLGC were selected by using the method of network pharmacology. Our findings elaborate the multi-component and multi-target action mechanism of the four drugs, so as to provide a scientific basis for research on the treatment of PLGC.

Among the components of traditional Chinese medicine, the most effective targets are mol000098 (quercetin), mol000358 (β -sitosterol), and mol000449 (sitosterol). Quercetin is a polyhydroxyflavone compound, which is one of the strongest anti-tumor components of traditional Chinese medicine [7]. Quercetin can play an anti-tumor role by inhibiting tumor cell proliferation and inducing apoptosis, regulating the expression of tumor suppressor genes and protooncogenes, regulating tumor cell signaling pathways, reversing multidrug resistance, and inhibiting telomerase activity. Notably, quercetin can regulate the STAT3 signal transduction pathway, downregulate the expression of surviving mRNA, induce the apoptosis of human gastric adenocarcinoma cell line SGC7901, inhibit the MAPK/ERK pathway, and regulate the expression of cell cycle related factors through hydrogen bonding to the acyl group of serine [8-11]. β -sitosterol is a kind of phytosterol that widely exists in a variety of cell membranes. It can inhibit the growth of tumor cells and induce tumor cell apoptosis. Its anti-gastric cancer effects may occur through the autophagy-related PI3K/Akt/mTOR pathway [12]. Stigmasterol has anti-tumor and cholesterol lowering pharmacological effects [13]. It can upregulate the expression of Bax, downregulate the expression of anti-Bcl-2, inhibit the proliferation and migration of tumor cells, induce SNU-1 cycle arrest in the G2/M

phase, and inhibit the JAK/STAT signaling pathway [14].

In terms of targets, the four intestinal chemotherapeutic drugs for the treatment of PLGC mainly play a role through TP53, VEGFA, IL6, TNF, mapk1, Jun, EGFR, PTGS2, ESR1, and EGF. TP53 is an important tumor suppressor gene that regulates the cell cycle at G1 and G2/M points. The translated normal (wild-type) p53 protein regulates the growth and proliferation of cells. The protein readily undergoes hydrolysis and has low expression levels. The mutant p53 protein translated after *TP53* gene mutation loses its anti-cancer effect, is characterized by accelerated proliferation and increased deterioration, and induces drug resistance. Mutant p53 is not readily hydrolyzed and accumulated in cancer tissue. Mutant p53 proteins have been found to be highly expressed in gastric cancer and positively correlate with the degree of tumor cell differentiation, TNM stage, and lymph node metastasis in gastric cancer [15]. VEGFA gene is an important member of the PDGF/VEGF family that promotes the proliferation of vascular endothelial cells, induces angiogenesis, and enhances vascular permeability. The expression of the *VEGFA* gene is up-regulated in many tumors. Notably, overexpression of *VEGFA* can activate the p38mapk-hsp27 signaling pathway in gastric tumor cells, promote the phosphorylation of p38, mapkapk, and HSP27 proteins, and promote the proliferation, differentiation, invasion, and metastasis of gastric cancer cells [16]. IL-6 is a growth factor that is produced by activated T cells and fibroblasts and has a wide range of functions. Its receptor (IL-6R) has biological functions in regulating the immune response and tumor cell growth and is highly expressed during carcinogenesis in a variety of tumors. IL-6 mediates cell cycle regulation and tumor angiogenesis by activating JAK / stat and other signaling pathways [17].

Table 2 Docking results of the component targets.

Target protein	PDB ID	component	Affinity (kcal/mol)
TP53	2bim	MOL000098	-7.8
TP53	2bim	MOL000358	-7.6
TP53	2bim	MOL000449	-6.3
VEGFA	1mkk	MOL000098	-7.3
VEGFA	1mkk	MOL000358	-8
VEGFA	1mkk	MOL000449	-7.9
IL6	1alu	MOL000098	-7
IL6	1alu	MOL000358	-7.4
IL6	1alu	MOL000449	-6.4

The GO and KEGG analyses revealed that the four intestinal drugs may be involved in the AGE-RAGE signaling pathway in diabetic complications, through reactive oxygen metabolism, oxidative stress, cell response to organic compounds, cell response to nitrogen compounds, myocyte proliferation, inflammatory response, epithelial cell proliferation, and cell migration. The IL-17 and JAK-STAT signaling pathways play a role in the treatment of PLGC. Molecular docking results showed that the main active components of the four intestinal chemotherapeutic drugs, quercetin, β -sitosterol, and stigmasterol, have good affinity with TP53, VEGFA, and IL6. Further experiments are needed to determine the biological activity of the four Changhua drugs in the treatment of PLGC.

In summary, this study used network pharmacology and molecular docking to determine that the treatment of PLGC with four Changhua drugs has multi-component, multi-target, and multi-channel characteristics. Our results also predict the main active components, core targets, and related pathways of the four Changhua drugs in the treatment of PLGC, to provide a foundation of new ideas for exploring the treatment mechanism of traditional Chinese medicine in the future.

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