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Prediction of the Mechanism of Jinlingzi Powder in the Intervention of Gastric Cancer Based on Transcriptomics and Network Pharmacology

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Abstract: *Traditional Chinese medicine (TCM) has unique advantages in alleviating gastric cancer (GC), and Jinlingzi Powder is a common drug used in TCM treatment. However, the potential mechanism for its treatment of GC is unclear. The purpose of this study is to integrate transcriptomics, network pharmacology and molecular docking to investigate the active components and targets of Jinlingzi Powder's intervention in GC and related pathways, in order to provide a basis for further revealing its mechanism of action and developing Jinlingzi Powder. We obtained the potential targets of Jinlingzi Powder and GC-related genes from public database. Then we identified and visualized Potential targets and signaling pathways through bioinformatics analysis, including protein-protein interaction (PPI), Gene Ontology (GO) functional enrichment analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Subsequently, molecular docking was performed to further validate these findings. Resultly, the results showed that potential targets including IL6, PTGS2, MMP9, HMOX1, MYC, CHRM3, TOP2A, CA2, and KCNMA1 were the therapeutic targets of Jinlingzi Powder for gastric cancer. The functional enrichment analysis indicate that through synergistically regulating some biological pathway, such as inflammatory response, cellular response to tumor necrosis factor, AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, TNF signaling pathway, MicroRNAs in cancer, Pathways in cancer, etc. , which have therapeutic effects on gastric cancer. In addition, the molecular docking results showed that the compounds had good binding activity to the action target in vivo. In Conclusion, this study comprehensively describes the potential targets and molecular mechanisms of Jinlingzi Powder for the treatment of gastric cancer. It also provides promising avenues for revealing the treatment of diseases by TCM through scientific basis and therapeutic mechanisms.*

Keywords: *IBS-D; Tongxie Yaofang; Sijunzi Decoction; Meta-analysis*

1. Introduction

Nowadays, gastric cancer (GC) is still one of the important global diseases, with an estimated number of more than 1 million new cases per year [1]. As a heterogeneous and highly aggressive malignancy, most gastric cancer patients are diagnosed at an advanced stage, and 25-50% of patients develop metastases during the course of the disease, making the mortality rate remain high [2]. According to statistics, gastric cancer ranks the fifth in incidence and the third in mortality among malignant tumors in the world [3]. Although surgical resection, radiotherapy, chemotherapy, targeted therapy, immunotherapy and other therapies are widely used in clinical practice, the 5-year survival rate is still less than 30% [1]. The development of gastric cancer and the associated therapeutic processes have brought about tremendous economic and social implications, as well as posing a great challenge to healthcare systems worldwide [4]. However, the proposed multimodal treatment approach has become a cornerstone for screening, diagnosis, staging, treatment, and support of gastric cancer patients while increasing survival rates [5]. Traditional Chinese medicine (TCM), as one of the multimodal therapies, has a history of several thousand years. According to relevant studies, Chinese medicine, guided by a holistic concept, can effectively inhibit the invasion and metastasis of gastric cancer cells through local treatment or regulation of systemic status, which largely improves the prognosis of gastric cancer patients and enhances their quality of life [6,7]. Studies have shown that gastric cancer has different subtypes and presents different characteristics, including clinical features,

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genetics, morphology, epidemiology and extension characteristics. Its development is closely related to high-risk factors such as diet, alcohol consumption, smoking, family history, Helicobacter pylori infection and Epstein-Barr virus (EBV) infection [8].

Therefore, single-target drugs are no longer adequate for this complex multifactorial disease and there remains an urgent need to develop new therapeutic agents and strategies to improve the efficacy against this deadly disease. With the in-depth understanding of the mechanism of drug action, it has been found that drugsexert their effects by regulating multiple protein targets rather than a single target [9]. Better therapeutic results can be achieved through multi-target therapy with a single drug interacting with multiple targets at the same time or multiple drug combinations [10]. As a multidisciplinary approach integrating pharmacology, bioinformatics, and systems biology, which considers both drug action and side effects, network pharmacology can explore the mechanism of drug on diseases in a systematic and holistic manner, providing a new approach to drug discovery for complex diseases, which is consistent with the integrity and systematicness of traditional Chinese medicine (TCM) treatment of diseases [11]. It not only improves the global understanding of drug targets, but also suggests new therapeutic targets and approaches and provides a deeper understanding of drug action [12].

Transcriptomics refers to the study of gene transcription and transcriptional regulation in cells at the overall level. It is the study of gene expression from the RNA level, and the transcriptome, the sum of all RNAs that can be transcribed from a living cell, is an important tool for the study of cellular phenotype and function. The transcriptional process of synthesizing RNA using DNA as a template is the first step in gene expression and also the key link in gene expression regulation. By gene expression, we mean the whole process of transforming genetic information carried by a gene into a recognizable phenotype, which can be used to study gene expression, gene function, structure, and screening of differentially expressed genes after drug intervention.

Molecular docking is a process based on computer simulation technology that simulates the geometric structure of molecules and intermolecular forces by chemometric methods to study intermolecular interactions and discover low-energy binding patterns between ligands and receptors, which can further validate the results of the study.

TCM has unique advantages in disease mitigation, such as reducing recurrence, improving symptoms, enhancing quality of life, reversing multidrug resistance, and prolonging survival [13]. Zheng Ya et al. showed that the enrichment of the targets of action of Jinlingzi Powder was significant in cancer, with a total of 20 cancer-related pathways involving cancer in several tissues and organs of the body, as well as cancer choline and central carbon metabolism[14]. Dai et al. showed that semi-biomimetic extraction of Jinlingzi Powder and found that the extracts all exhibited some anticancer activity [15]. However, the mechanism of Jinlingzi Powder in the treatment of gastric cancer is still unclear, so its application is greatly limited.

In this study, transcriptomics combined with network pharmacology and molecular docking was used to provide a comprehensive description of its possible mechanism of action. This approach is expected to improve the accuracy of network pharmacology active compound screening and target identification and provide potential therapeutic targets for further clinical and basic research.

2. Materials and methods

2.1. Databases and software

(1) TCMSP, the pharmacology database and analysis platform for Chinese medicine systems (http: //tcmspw.com/index.php); (2) UniProt database (https//www.uniprot.org/); (3) GEO, the high-throughput gene expression database (https: //www.ncbi.nlm.nih.gov/); (4) Venny 2.1.0 platform (https://bioinfogp.cnb.csic.es/tools/venny/index.html); (5) STRING website (https://cn.string-db.org/); (6) DAVID database (https://david.ncifcrf.gov/); (7) Microbiology letter platform (http://www.bioinformatics.com.cn/); (8) R software; (9) Cytoscape 3.9.1 software; (10) PyMOL 4.3.0 software (https://pymol.org/); (11) CSP PDB database (https://www.rcsb.org/); (12) AutodockTools (http://mgltools.scripps.edu/downloads); (13) PLIP platform (https://github.com/pharmai/plip); (14) AutoDock Vina 1.1.2 program.

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2.2. Acquisition of active ingredients and drug targets of Jinlingzi Powder

The constituent drugs of Jinlingzi Powder, Melia toosendan and corydalis tuber, were searched separately in the TCMSP database, and compounds with good pharmacokinetic properties were screened according to the ADME system (distribution, absorption, metabolism, and excretion), with parameters including oral bioavailability (OB) and Drug-Likeness (DL) . OB ≥ 30%; DL ≥ 0. 18 were used as screening conditions to obtain the active ingredients of each drug, and then their corresponding drug targets were obtained from the active components. The obtained drug targets were entered into the UniProt database to find their corresponding gene abbreviations for subsequent analysis. Finally, Cytoscape 3.9.1 software was used to construct drug-component-target network diagram.

2.3. Acquisition of gastric cancer disease targets

We downloaded transcriptomic gastric cancer data from GEO database, searched expression profiling microarray data with "gastric cancer" as keyword, selected "Series" as entry type, "expression profiling by array" as study type, and "homo sapiens" as tissue source, and downloaded the microarray with GSE118916 and the gene annotation file with GPL15207. The microarray data were obtained from the study of Li L et al. [16], and the samples included human gastric cancer (n=15) and human normal stomach tissue (n=15), and the data were screened using the "limma" package of R software. The gene expression data of normal and gastric cancer groups were further screened to obtain differential expression genes (DEGs), up-regulated genes and down-regulated genes with |logFC|>1, adjusted P<0. 05, and displayed in Volcano Plot and heat map, and the differential genes were subjected to GO enrichment analysis and KEGG pathway enrichment analysis were performed on the differential expression genes.

2.4. Mapping the common targets of Jinlingzi Powder and gastric cancer

We used the Venny2.1.0 platform, the drug targets obtained in "2. 2" and the disease targets obtained in "2. 3" were mapped, and the common drug-disease targets were obtained as the intersection gene.

2.5. Construct drug-component-intersection target-disease network diagram

We selected intersection targets and their ccorresponding information such as active ingredients of drugs, names of traditional Chinese medicines, names of diseases, etc., and imported Cytoscape 3.9.1 software, built drug-component-common target-disease network diagram, made the connection between each component and target more clear and intuitive.

2.6. Protein-protein interaction (PPI) network topology analysis

PPI network topology analysis was performed on intersecting genes by STRING platform, and Centiscape2. 2, a plug-in in Cytoscape 3.9.1 software, was used to filter the core targets based on the values of degree, closeness, and betweenness three values to filter the top five core targets.

2.7. GO function and KEGG pathway enrichment analysis

DAVID database was used for GO function enrichment analysis and KEGG pathway enrichment analysis of intersecting bases. The obtained results were visualized in the microbiology platform according to PValue ranking.

2.8. Mapping pathway-target network

The results of KEGG pathway enrichment analysis were sorted according to PValue values, and the top 20 pathways and targets in terms of significance were mapped into a pathway-target network using Cytoscape 3.9.1 software.

2.9. Molecular docking

The target crystal structures of the target proteins PTGS2, IL6 and MMP9 are obtained from the Protein Data Bank (PDB) RCSB, and the structures of the compounds Isocorypalmine and Quercetin

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are downloaded from the Pubchem database. PyMOL (version 4.3.0) software is used to separate the original ligands from the protein structures, dehydrate and remove organic matter, and AutodockTools is used to hydrogenate, check the charge, assign the atom type to AD4 type, calculate the gasteiger, and construct a docking grid box for the protein structure. In addition, the chemical composition (small molecule ligands) should be determined Root, and the twistable bonds of the ligands should be selected in AutodockTools. Finally, in AutodockTools, the protein structure and the format of small molecule ligands should be changed from ".PDB" to ".PDBQT" for further docking. After docking with Vina, the protein-molecular combination scores of PTGS2-Isocorypalmine, MMP9-Quercetin and IL6-Quercetin were calculated, and the results were analyzed and visualized using the PLIP online website for force analysis.

3. Results

3.1. Acquisition of active ingredients and drug targets of Jinlingzi Powder

The database retrieved 33 active ingredients of Melia toosendan, and 6 active ingredients corresponding to 171 action targets were obtained after screening conditions; 77 active ingredients of Corydalis tuber were retrieved, and 49 active ingredients corresponding to 1052 action targets were obtained after screening conditions. After target de-duplication, 205 targets were identified, and MOL000098 (quercetin) was found to be the common active ingredient of the two drugs after screening. After numbering them, Cytoscape 3.9.1 software was used to construct drug-component-target network diagrams (Figure 1), and some active components and targets with high oral bioavailability and drug-likeness properties were selected for display (Table 1).

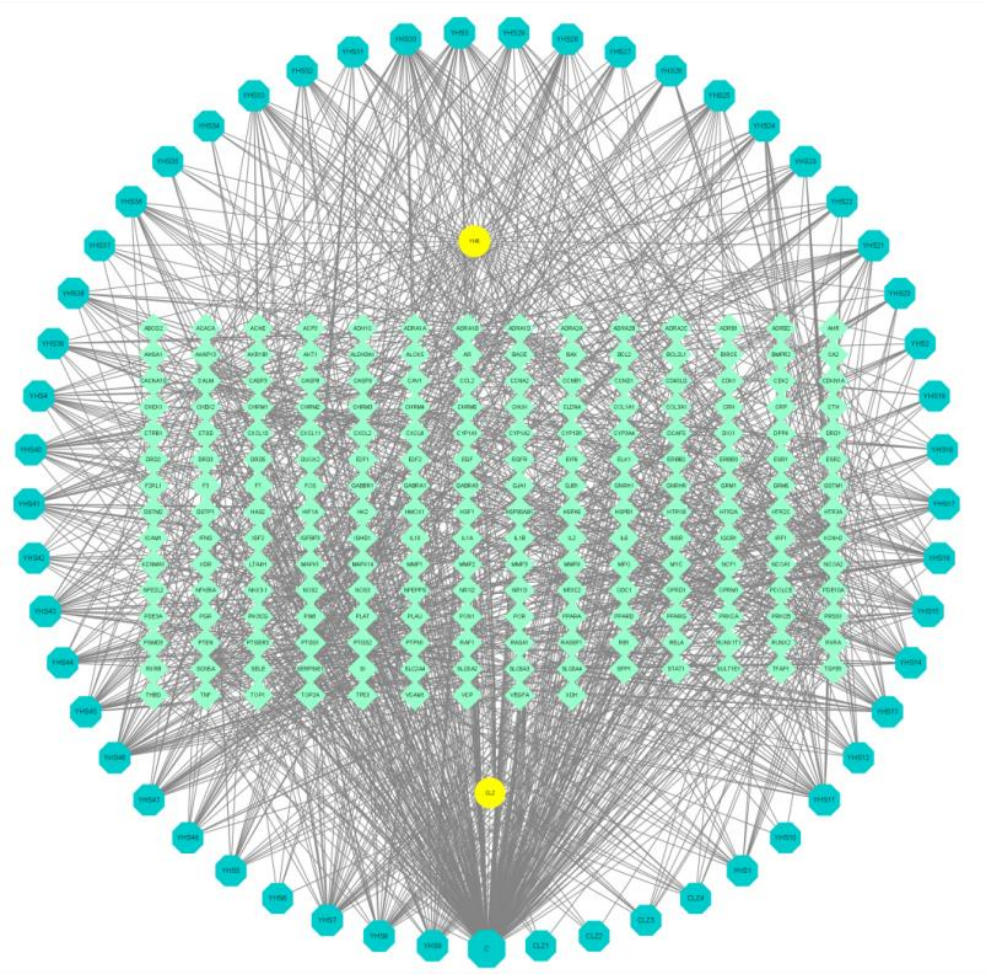

Figure 1: Drug-component-target network diagram. Yellow circles represent the drugs Melia toosendan and Corydalis tuber, green diamonds represent genes, and blue hexagons represent drug active components

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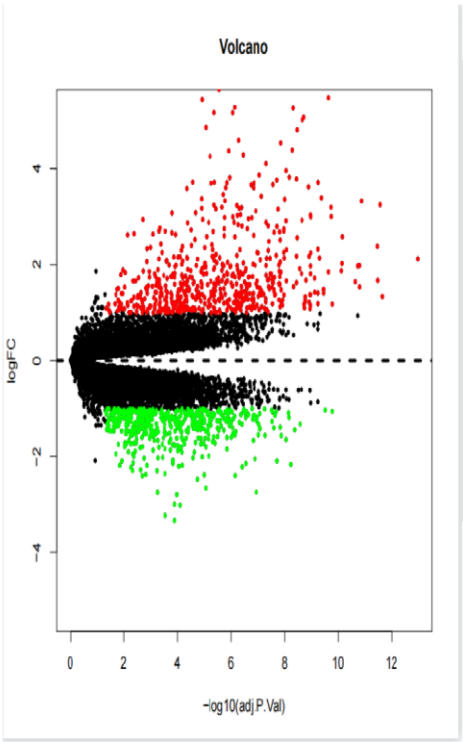
Table 1: Partial active components and targets of Jinlingzi Powder

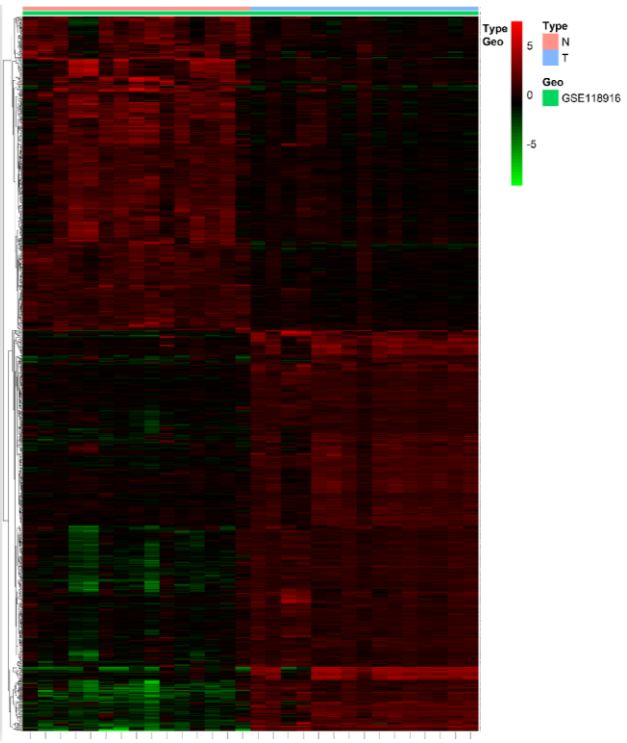
Mol ID	Short Name	Molecule Name	OB%	DL	Drug	Gene Symbol
MOL002058	CLZ5	40957-99-1	57.2	0.62	Melia toosendan	KCNH2, SCN5A,F2RL1,PTGS 2
MOL002056	CLZ4	(E)-3-[(2S,3R)-2-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-3-methylol-2,3-dihydrobenzofuran-5-yl] acrolein	54.74	0.4	Melia toosendan	PTGS1,KCNH2,PTGS2,PRSS1,
MOL000098	C	quercetin	46.43	0.28	Melia toosendan,Corydalis Tuber	PTGS1,PPARG,PTGS2,EGFR
MOL001495	CLZ2	Ethyl linolenate	46.1	0.2	Melia toosendan	PTGS1,NCOA2
MOL002045	CLZ3	Stigmasterol	43.41	0.76	Melia toosendan	PGR
MOL004193	YHS14	Clarkeanidine	86.65	0.54	Corydalis Tuber	PTGS1,DRD1,CHRM3,KCNH2
MOL001460	YHS3	Cryptopin	78.74	0.72	Corydalis Tuber	PTGS1,DRD1,CHRM3,KCNH2
MOL004234	YHS42	2,3,9,10-tetramethoxy-13-methyl-5,6-dihydroisoquinolino [2,1-b] isoquinolin-8-one	76.77	0.73	Corydalis Tuber	NOS2,PTGS1,KCNH2,PRSS1
MOL004071	YHS11	Hyndarin	73.94	0.64	Corydalis Tuber	DRD1,CHRM3,KCNH2,PTGS2
MOL000791	YHS47	bicuculline	69.67	0.88	Corydalis Tuber	PTGS1,KCNH2,SCN5A,PTGS2

3.2. Acquisition of gastric cancer disease targets



A

Figure A shows the volcano Plot, red dots represent up-regulated genes and green dots represent down-regulated genes.



B

Figure B shows the clustering heat map, the horizontal coordinates represent the samples, the vertical coordinates represent the genes, red represents high expression and green represents low expression.

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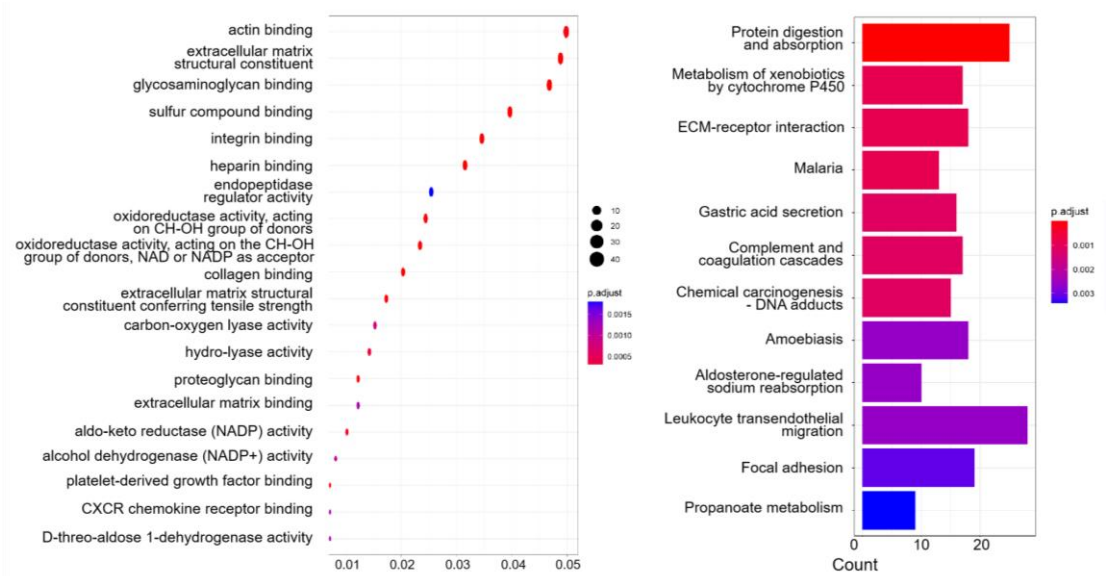

Figure 2: Analysis of the visualization results for differential genes

After analyzing the microarray of GSE118916 and the gene annotation file numbered GPL15207, a total of 1080 differential genes were obtained from normal group and gastric cancer tissue, including 606 up-regulated genes and 474 down-regulated genes. The Volcano Plot (Figure 2A) and clustering heat map (Figure 2B) were plotted for the differential genes, and GO (Figure 2D) and KEGG (Figure 2E) analyses were also performed. The GO enrichment analysis indicated that the differential genes were mainly involved in such biological processes as actin binding, extracellular matrix structural constituent, glycosaminoglycan binding, etc. The KEGG pathway enrichment analysis revealed that the differential genes were mainly involved in several gastric cancer-related pathways such as Protein digestion and absorption, Metabolism of xenobiotics by cytochrome P450, ECM-receptor interaction, and Gastric acid secretion.

3.3. Mapping the common targets of Jinlingzi Powder and gastric cancer

The 1080 microarray datasets of gastric cancer differential genes were intersected with 205 drug targets of Jinlingzi Powder by STRING platform, and 34 intersected targets were obtained (Figure 3).

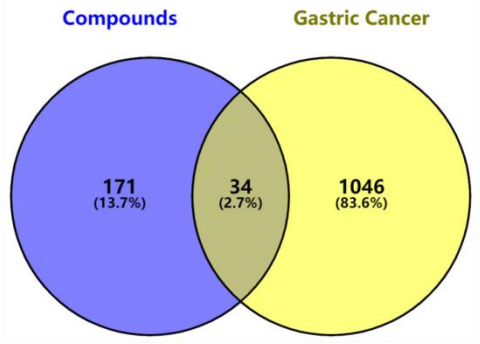

Figure 3: Venn diagram of drug-disease common targets of Jinlingzi Powder for the treatment of gastric cancer.The blue circle is the drug-acting targets of Jinlingzi Powder, the yellow circle is the transcriptomic differential genes of gastric cancer, and the intersection of the two circles is the intersection targets

3.4. Construction of drug-component-ntersection target-disease network diagram

We organized the relevant data and drew the drug-component-common target-disease network diagram of Jinlingzi powder in treating gastric cancer (Figure 4). The graph involves 8 nodes and 247

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edges. The network diagram was analyzed and ranked according to the degree values. The results showed that the top targets were quercetin, CHRM3, TOP2A, CA2, Isocorypalmine, Corydine, Capaurine, (S)-Scoulerine, KCNMA1, and bicuculline. It is thus speculated that the above listed components and targets may be relevant to the treatment of gastric cancer with Jinlingzi Powder.

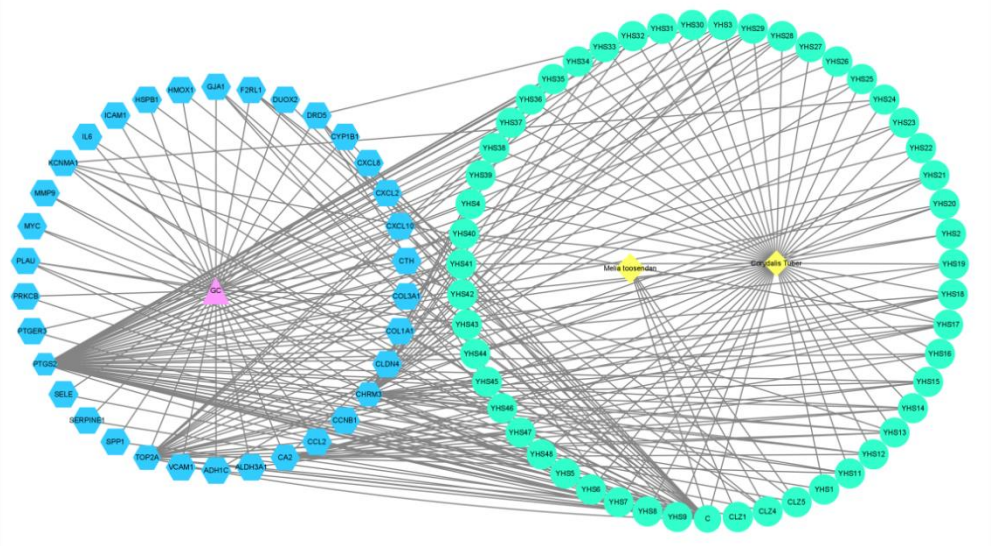

Figure 4: Drug-component-intersection target-disease network diagram

3.5. PPI network topology analysis

A PPI network graph involving 34 nodes and 142 edges was finally obtained by searching the STRING online database through the web page, uploading intersection targets, selecting Homo sapiens for species, setting the confidence level to >0. 4, and selecting all channels (Figure 5). Five core targets (Table 2) were identified, including IL6, PTGS2, MMP9, HMOX1, and MYC.

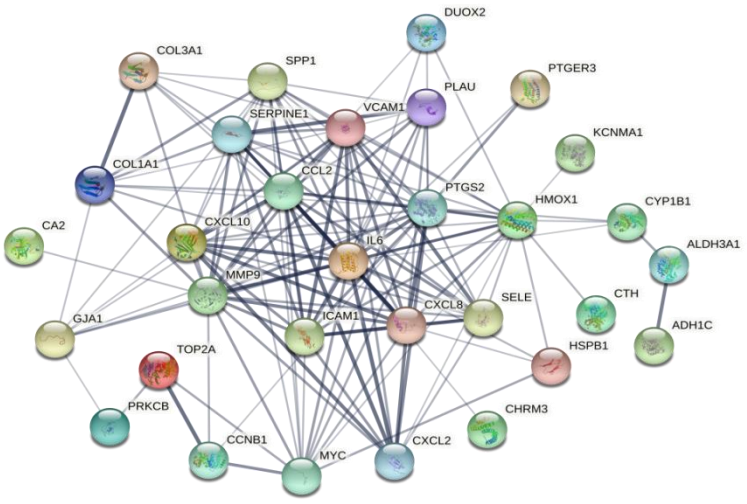

Figure 5: PPI network diagram of the intersectional targets of Jinlingzi Powder for the treatment of gastric cancer

Table 2: Core target-related data obtained by PPI network analysis screening

Betweenness unDir	Closeness unDir	Degree unDir	name	selected	shared name
176.843673	0.025641026	22	IL6	FALSE	IL6
112.0198857	0.024390244	20	PTGS2	FALSE	PTGS2
92.90559996	0.022727273	19	MMP9	FALSE	MMP9
159.6365301	0.022727273	18	HMOX1	FALSE	HMOX1
60.86436341	0.02	13	MYC	FALSE	MYC

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3.6. GO function and KEGG pathway enrichment analysis

A total of 178 entries were obtained for GO function enrichment analysis of the intersection targets, including 144 for Biological Process (BP), 20 for Cellular Component (CC), and 17 for Molecular Function (MF). The top 10 items in BP, CC, and MF analyses were visualized, arranged according to their PValue (Figure 6A). 42 pathways were obtained from the KEGG pathway enrichment analysis, and the top 20 pathways were visualized according to the PValue (Figure 6B). Through GO functional enrichment analysis, it can be concluded that Jinlingzi Powder may achieve the purpose of treating gastric cancer by regulating the aforementioned biological processes. As shown in Figure 6B, KEGG pathway enrichment analysis shows that the process of treating gastric cancer with Jinlingzi Powder may be related to AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, African trypanosomiasis, Malaria, IL-17 signaling pathway, Amoebiasis, NF-kappa B signaling pathway, Lipid and atherosclerosis, Fluid shear stress and atherosclerosis, Human cytomegalovirus infection, etc.

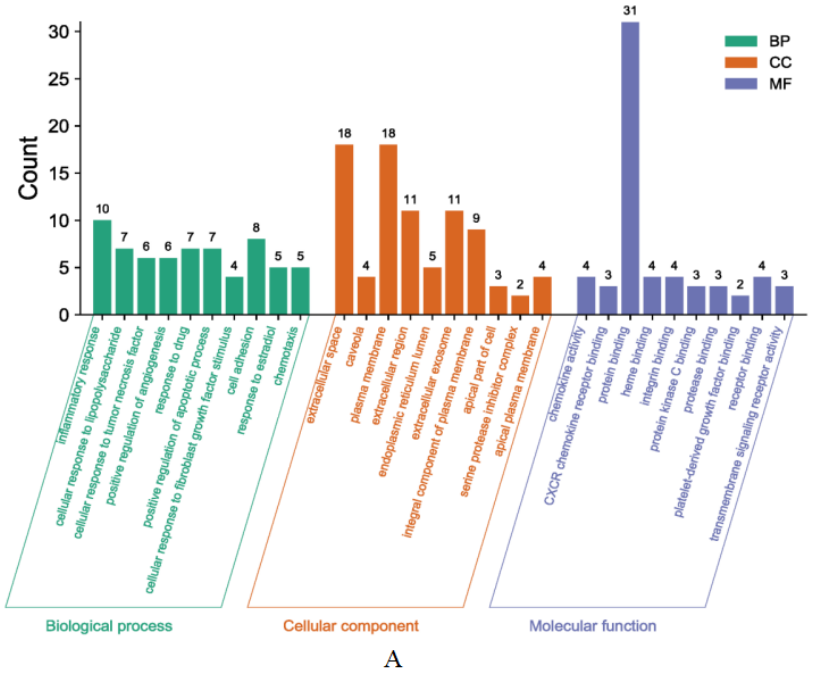

Figure 6: A shows results of GO enrichment analysis. B shows results of KEGG analysis

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3.7. Mapping the pathway-target network

The top 20 pathways according to the KEGG pathway enrichment results and their target relationships were visualized (Figure 7). The diagram involves 4 nodes and 13 edges. As can be seen from the figure, the more critical targets involved in the pathway are IL6, CXCL8, PRKCB, ICAM1, CCL2, etc. , suggesting that it may play a key role in the treatment of gastric cancer by Jinlingzi Powder.

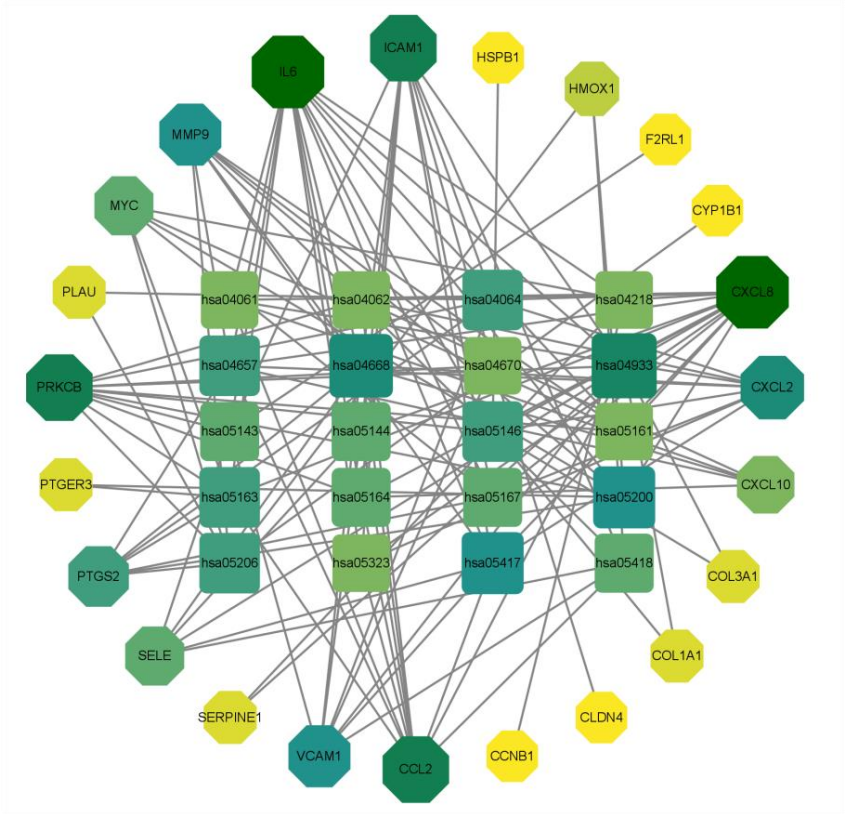

Figure 7: Pathway-target network diagram

3.8. Molecular docking results

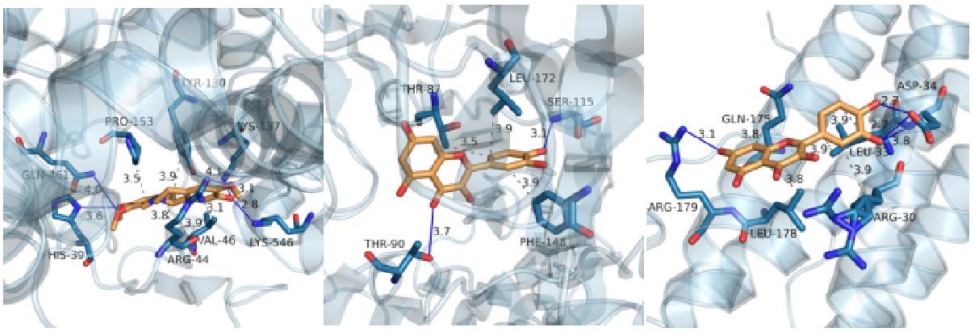

Figure 8: 3D pattern diagram of molecular docking between active ingredient and target of Jinlingzi Powder for the treatment of gastric cancer (partial)

The average binding energy between the target protein PTGS2 and the compound Isocorypalmine were -8.8 kcal/mol (Figure 8A), between MMP9 and compound Quercetin were -7.3 kcal/mol (Figure 8B), and between IL6 and compound Quercetin were -7.1 kcal/mol (Figure 8C). Generally, if the binding energy between ligand and target protein is less than 0, the ligand and receptor protein can bind

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spontaneously. If the binding energy is <-5.0 kcal/mol, it indicates that the active ingredient has a strong affinity activity with the target. Therefore, all three compounds have strong affinity activity with the target protein. Two-dimensional force analysis using ligplus software also yielded consistent results.

4. Discussion

The incidence of gastric cancer is extremely high worldwide, and the incidence of gastric cancer in China is much higher than that in many other countries. TCM plays an important role in the prevention and treatment of tumors, mainly in the mechanisms of action that enhance the body's immune function, inhibit tumor cells division and proliferation, accelerate tumor cells apoptosis, and reverse multi-drug resistance of tumor cells [17]. However, the composition of traditional Chinese medicines is complex, and it is difficult to elucidate their mechanisms of action due to the diversity of herbal components and action targets, thus lacking reliable scientific evidence to verify their effectiveness [18]. In recent years, with the rapid development of high-throughput technology and bioinformatics, virtual pharmacology studies are considered to be the fastest and most effective screening method for early studies of drug effectiveness, solving the problem of multi-component, multi-target, and complex diseases in TCM [19]. In this study, the drug-component-intersection target-disease network diagram and the associated PPI network diagram were constructed, along with GO and KEGG enrichment analysis, to systematically elucidate the mechanism of action of Jinlingzi Powder in the treatment of gastric cancer. Molecular docking was used to verify the correspondence between targets and components, and good docking scores reflected the effectiveness of the components.

According to the network pharmacology research, there are 55 active ingredients in Jinlingzi Powder, and the main active ingredients including quercetin, (S)-Scoulerine, Isocorypalmine, leonticine, Hyndarin, etc. Haghi A et al. found that quercetin inhibits cell growth and induces apoptosis, necrosis and autophagy through and anti-Helicobacterial activity for the treatment of gastric cancer [20]. Wangchuk P et al. found that (S)-Scoulerine achieved the treatment of cancer and inflammation through the inhibition of acetylcholinesterase, TNF-α, and Helicobacter pylori [21]. Although the TCMSP database shows that Isocorypalmine, leonticine, and Hyndarin all have anti-cancer effects, their related mechanisms of action are unclear. This study shows that the above drug components may achieve their effects in the treatment of gastric cancer through IL6, PTGS2, MMP9, HMOX1, MYC, and other targets, which may provide a mechanism of action The present study showed that the above drug components may be used to treat gastric cancer through IL6, PTGS2, MMP9, HMOX1, MYC and other targets, which can provide reference for research on its action mechanism.

The target screening by PPI network topology analysis diagram, drug-component-intersection target-disease network diagram, and pathway-target network diagram suggested that Jinlingzi Powder for the treatment of gastric cancer might be closely related to such targets as IL6, PTGS2, MMP9, HMOX1, MYC, CHRM3, TOP2A, CA2, and KCNMA1. Huang SP et al. found that IL-6 expression was significantly elevated in gastric cancer tissues, which could promote angiogenesis and affect tumor cells adhesion and invasion by inducing the synthesis of vascular endothelial growth factor [22]. IL-6 was found to be one of the most important cytokine families in tumorigenesis and metastasis and was highly expressed in gastric cancer tissues [23]. Meanwhile, a study found that the PD-L1 glycosylation process mediated by IL-6 triggered by STT3A not only upregulated the expression level of PD-L1 in tumor tissues, but also reduced the detection rate of PD-L1, resulting in some patients missing the opportunity of immune-checkpoint-blockade (ICB) therapy for a more prolonged survival benefit [24]. Sun WH et al. found that COX-2 (PTGS2) was not expressed in normal gastric mucosal tissues but was significantly more expressed in gastric cancer tissues [25], possibly by promoting angiogenesis and lymph node metastasis [26], immune escape [27], altering telomerase activity [28], acting on adhesion factors [29], inhibiting apoptosis [30] and romoting cell proliferation [31] are among the mechanisms that promote the development of gastric cancer. Torii A et al. found that MMP-9, a protease closely related to gastric cancer invasion and metastasis, caused gastric cancer invasion and metastasis by degrading the extracellular matrix [32]. Ren QG et al. found that HMOX1 expression was elevated in gastric cancer tissues and played a crucial role in the development of gastric cancer. It is closely related to malignant apoptosis, immune escape and oxidative stress, etc. And low HMOX1 expression promotes gastric cancer cells apoptosis, inhibits their proliferation and invasion, improves overall patient survival, and may become an important target for the treatment of gastric cancer [33]. The MYC gene consists of three paralogs, C-MYC, N-MYC and L-MYC, and is one of the most frequently dysregulated driver genes in human cancer that can disrupt the microenvironment of tumor cells and evade the immune response [34]. Liu M et al. found that MYC is highly expressed in gastric cancer tissues, that its expression level is negatively correlated with clinical outcome, and that the development of gastric cancer requires a novel mechanism of transcriptional repression dependent on c-Myc target genes, providing a potential new

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strategy for targeted therapy of gastric cancer [35]. CHRM3 is one of the M receptors, a G protein-coupled receptor involved in fluid-secreting exocrine gland cells, consisting of seven transmembrane structural domains that are expressed in a variety of tumor cells and can be linked to a variety of signaling pathways to play a role in promoting tumor cell proliferation and metastasis [36]. Sorokin M et al. found that CHRM3 is also closely related in immunotherapy of gastric cancer [37]. Wang Y et al. found that TOP2A is a key gene in gastric carcinogenesis [38] and can inhibit the proliferation, migration and invasion of gastric cancer cells and promote apoptosis by decreasing TOP2A expression [39]. CAII has been shown to be associated with gastrointestinal tumors, with much less expression in malignant tissues and a negative decrease with increasing tumor malignancy, suggesting that they may be associated with tumor progression and invasion [40]. Ma G found that KCNMA1 is a key oncogene in gastric carcinogenesis and its hypermethylation is an independent factor affecting the prognosis of gastric cancer patients [41]. In conclusion, in terms of action targets, Jinlingzi Powder for gastric cancer may be related to oxidative stress, cell proliferation and apoptosis, immune response, and inhibition of angiogenesis. Molecular docking also verified that the main active ingredients of Jinlingzi Powder for the treatment of gastric cancer all had good binding ability with their targets, which further improved the credibility of the results of this network pharmacology study.

The top ranking entries of GO functional enrichment analysis results suggest that Jinlingzi Powder for gastric cancer is mainly related to inflammatory response, cellular response to tumor necrosis factor, etc. Inflammatory response has been an important influencing factor in tumorigenesis and development, and the tumor microenvironment mediated by inflammatory cells is an indispensable player in tumor formation, promoting proliferation, survival and migration of tumor cells [42]. Tumor cells are also involved in the innate immune system through some signaling molecules such as selectins, chemokines and their receptors in order to invade, migrate and metastasize [43]. Not only that, Balkwill F found that TNF is an important inflammatory cytokine that causes hemorrhagic necrosis of tumor tissue by increasing vascular permeability and causing other types of cells in the blood to spill through the cell membrane, resulting in the loss of a large number of red blood cells in the blood vessels [44]. It is thus hypothesized that Jinlingzi Powder may achieve the treatment of gastric cancer by reducing the inflammatory response and inducing the cellular response to tumor necrosis factor, among other ways.

The KEGG pathway enrichment analysis revealed that the process of Jinlingzi Powder for gastric cancer mainly involved AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, MicroRNAs in cancer, Pathways in cancer, etc. It was found that RAGE is a factor that regulates cancer cells invasion and metastasis, that RAGE expression is closely related to invasion and metastasis of gastric cancer [45], and that the AGE-RAGE system is a new target for the treatment of various tumors [46]. The TNF-α/IL-33/ST2L signaling-mediated epithelial-stromal interaction was found to play a key role in the progression of gastric cancer and provided the rationale for targeting this pathway for the treatment of gastric cancer metastasis [47]. Immunotherapy offers new hope for gastric cancer patients, macrophages infiltrating gastric cancer tissues regulate PD-L1 expression by releasing the pro-inflammatory cytokine TNF-α, which regulates PD-L1 expression by activating NF-kB and STAT3 signaling pathways, and the expression of TNF-α has important prognostic value in gastric cancer[48]. MicroRNAs are stably present in serum and tissues and are key molecule in post-transcriptional regulation of gene expression, which can regulate gastric cancer cells survival and apoptosis, cell proliferation, invasion and metastasis, and is the hub of gene regulation in the process of gastric cancer development and progression [49], and microRNA can affect the sensitivity of gastric cancer patients to chemotherapy through multiple pathways, and microRNA-based therapy is considered a suitable approach to overcome chemotherapy resistance in gastric cancer patients [50]. Pathways in cancer play different roles in different types of tumors, and in gastric cancer, the synergistic dysregulation of multiple Pathways in cancer may activate signature overgrowth, which in turn leads to the development of gastric cancer [51]. In conclusion, Jinlingzi Powder may play a role in the treatment of gastric cancer through the frontal regulation of immune, drug resistance, multi-target and multi-pathway pathways.

The molecular docking results showed that the binding energy between all three compounds and the target protein was less than 5 kJ/mol, indicating that these core compounds have high binding activity to the receptor protein, further validating the results of the network pharmacology study.

However, there are limitations in the network pharmacology analysis, which in most studies used two parameters of OB and DL compounds from the herbal database, while low DL compounds were often ignored. In subsequent studies, these data need to be further experimentally validated at the cellular, animal, and molecular levels to provide an experimental basis for the treatment of gastric cancer with Jinlingzi Powder.

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5. Conclusions

In conclusion, this study integrated transcriptomics, network pharmacology and molecular docking to preliminarily explore the multiple targets, biological functions, signaling pathways and possible mechanisms of action of Jinlingzi Powder for the treatment of gastric cancer, and validated the results by molecular docking. The results showed that Jinlingzi Powder may act through AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, MicroRNAs in cancer, Pathways in cancer on IL6, PTGS2, MMP9, HMOX1, MYC, CHRM3, TOP2A, CA2, and KCNMA1, suggesting that Jinlingzi Powder has the potential of multi-component, multi-target and multi-pathway synergistic treatment for gastric cancer. The results of the study initially validated and predicted the molecular mechanism of anti-gastric cancer with Jinlingzi Powder, but further experimental validation is needed. The results provide a basis for further exploring the mechanism of action of Jinlingzi Powder in the treatment of gastric cancer, and also provide a reference for studying the more complex mechanism of action of this Chinese herbal compound.

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