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Study on the Mechanism of Action of RoucongrongTang in Treating Functional Constipation based on Network Pharmacology and Molecular Docking

Shuilan Huang[1], Yaping Cui[2,*]

[1]Shaanxi University of Chinese Medicine, Xianyang 712046, Shaanxi, China
[2]Department of Anorectal, Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang 712000, Shaanxi, China
**Correspondence Author*

Abstract: ***Objective: To study the mechanism of action of RoucongrongTang in treating functional constipation (FC) based on network pharmacology and molecular docking technology. Methods: The FC disease targets obtained from TTD, OMIM, GeneCards and other databases were intersected with the active ingredient targets of RoucongrongTang obtained from the Traditional Chinese Medicine Systems Pharmacology Analysis Platform (TCMSP) to obtain the potential targets of RoucongrongTang for treating FC. The STRING platform was used to construct the potential target protein interaction (PPI) mechanism, and Cytoscape software was used for network topology analysis and visualization. Use the Metascape platform to perform GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis to predict the mechanism of RoucongrongTang effect on FC. Obtain FC disease core targets and active ingredient small molecules from the PDB and TCMSP databases respectively, and use AutoDockTools and PYMOL software for molecular docking and visualization. Results: RoucongrongTang contains 132 chemical components such as quercetin and β-sitosterol, as well as key targets such as CASP3, MAPK3, and MMP9. Enrichment analysis predicted 157 signaling pathways including tumor, MAPK, and Pl3K-Akt. Molecular docking results show that there is good binding activity between key targets and active ingredients. Conclusion: Multiple active ingredients in RoucongrongTang act on multiple targets and jointly affect intestinal flora, intestinal smooth muscle, immunity and inflammation by regulating signaling pathways such as tumors, MAPK and PI3KAkt, thereby treating FC.***

Keywords: RoucongrongTang, Functional constipation, Network pharmacology, Molecular docking, Mechanism of action**.**

1. Introduction

Functional constipation (FC) is a common digestive system disease. According to statistics, the global incidence of FC is about 16% [1], which is characterized by difficulty in defecation or poor defecation without obvious organic disease. At present, Western medicine mainly treats FC with laxatives, prokinetic drugs, microecological preparations, biofeedback therapy, and colon hydrotherapy [2,3]. Some patients have no significant improvement in symptoms after treatment, and even develop anal fissures and hemorrhoids. and other complications. Traditional Chinese medicine mainly follows the principle of treating patients with laxatives, but it does not rely solely on laxatives. It has the characteristics of significant curative effect, few side effects and low recurrence rate. In the field of traditional Chinese medicine, RoucongrongTang has been commonly used to treat FC since Huang Yuanyu, a master of traditional Chinese medicine, pointed out in "Four Sacred Heart Sources·Resolution of Miscellaneous Diseases" that RoucongrongTang can treat "people with yang deficiency, soil dampness, and feces like sheep arrows". According to the "Four Sacred Heart Sources", RoucongrongTang has the effects of soothing the liver and moisturizing the intestines, drying dampness and strengthening the spleen. Its composition includes: Roucongrong, hemp seeds, Poria cocos, pinellia, cassia twig, and licorice. Among them, Roucongrong and hemp seeds can moisturize the intestines and relieve constipation without harming the spleen and stomach; Poria cocos can promote dampness and improve the spleen, while Pinellia ternata can dry the soil and reduce the stomach. The combination of the two can help restore the function of soil transportation and make grains With smooth transportation, the waste can be passed smoothly; cinnamon twig can increase liver function and enhance the effect of diarrhea; licorice can cultivate middle qi. The combination of the above medicines can elevate the spleen and lower the stomach, relax the wood qi, moisturize the dry metal, and help the dregs to be discharged smoothly. Strong wood energy helps relieve constipation symptoms. At present, RoucongrongTang has been widely used in clinical treatment. Clinical research by Liu Xianhong et al. [4] found that the total effective rate of RoucongrongTang in treating constipation was significantly better than that of domperidone tablets, and no obvious adverse reactions were found. Research by Gao Qiangqiang et al. [5] found that compared with lactulose in treating functional constipation, RoucongrongTang plus Atractylodes macrocephala is much more effective than lactulose, and the effective rate can reach 91.94%. Comparing the number of beneficial bacteria and harmful bacteria before and after treatment, It was found that the number of beneficial bacteria in the RoucongrongTang plus Atractylodes macrocephala group was significantly higher than that of lactulose, and the number of harmful bacteria was significantly lower than that of lactulose. Modern clinical research has found that RoucongrongTang can effectively improve the symptoms of FC, not only relieving constipation, but also regulating intestinal microorganisms [6,7], stimulating increased intestinal mucosal secretion, promoting intestinal peristalsis, and reducing water reabsorption [8], enhance the self-regulation function of the intestine, thereby achieving the purpose of treating FC.

2. Materials and Methods

2.1 Acquisition and Screening of Active Ingredients and Drug Targets in RoucongrongTang

Search the chemical components and related protein targets of Roucongrong, FuLing, Huomaren Banxia, Guizhi and Gancao from the Traditional Chinese Medicine Systems Pharmacology Analysis Platform [11,12] (TCMSP, http: // tcmspw.com / tcmsp.php). In order to ensure that the obtained oral bioavailability (OB) and drug-likeness (DL) values are meaningful, the chemical components and corresponding targets of the drugs with an OB of not less than 30% and a DL of not less than 0.18 in the obtained results were screened respectively [13,14]. And establish a network of "medicinal materials-chemical ingredients-drug targets". Through the UniProt database (https://www.uniprot.org/), the protein targets and disease targets related to the active ingredients are corrected, and the protein species is set to "homo sapiens (human)" to obtain the standard gene name.

2.2 Retrieval and Acquisition of Functional Constipation Disease Targets

Through GeneCards database (https://www.genecards.org/), TTD database (https://db.idrblab.net/ttd/), OMIM database (https://omim.org/), NCBI gene database (https://www.ncbi. nlm.nih.gov/gene/) and Disgenet database (https://www. disgenet.org/home/), use the keyword "Functional constipation" or "constipation", and set conditions for “gene” and “homo sapiens” to obtain targets related to FC. All the data obtained above are merged and duplicates are eliminated to obtain the total target point of FC.

2.3 Potential Targets for Treating Functional Constipation

Using the Xiantao Academic (https://www.xiantao.love/) online platform, the total drug targets and total disease targets were imported for Venn analysis. By analyzing the intersection targets obtained, potential targets for RoucongrongTang to treat FC can be determined.

2.4 Establishment of Protein Interaction (PPI) Network and Screening of Key Therapeutic Targets

Use FC potential targets to import STRING (https://www.string-db.org/) and limit the species to humans to obtain interaction information between targets. Use Cytoscape (version 3.10.0) to generate the PPI network, and use the plug-in Centiscape 2.2 for data topology analysis, including degree centrality (DC), betweenness centrality (BC), eigenvector centrality (EC) and closeness centrality (CC). Key therapeutic targets are screened out through topological analysis values. PPI network analysis and visualization of key therapeutic targets in Cytoscape (versions 3.7.0 and 3.10.0).

2.5 GO Functional Enrichment and KEGG Pathway Enrichment Analysis

Import the key therapeutic targets obtained in 1.4 into Metascape (https://metasape.org/) for GO (biological process (BP), cellular localization (CC), and molecular function (MF) and KEGG enrichment analysis. Use the obtained The data is visualized through Weishinxin (https://www.bioinformatics. com.cn/). Select the enrichment GO term BP, CC, MF three-in-one histogram, and use the top 10 functional annotation directories respectively; select Pathway enrichment. The bubble chart of the set results shows KEGG enrichment, and the enrichment data uses the top 22 signaling pathways.

2.6 Molecular Docking

In order to verify the accuracy and reliability of the possible key targets of RoucongrongTang in treating FC, the following steps were carried out: sorting out the drug targets with the highest degree values in the active ingredients and PPI data obtained above. Find the protein ID corresponding to the core target in UniProt, use this ID to obtain the 3D structure in PDB (https://www.rcsb.org/), and use PYMOL to remove solvent molecules such as water. Obtain 3D small molecule structures of active ingredients in TCMSP. In AutoDockTools1.5.7 software, proteins and small molecules are fully hydrogenated to generate receptors and ligands respectively, and then the molecular docking results are obtained by running autogrid4 and autodock4. The docking results were visualized using PYMOL software.

3. Results

3.1 Active Ingredients and Drug Targets in RoucongrongTang

Table 1: Some active ingredients and intersection active ingredients retrieved from TCMSP database

TCMSP number	Compound	OB%	DL	Drug Source
MOL000358	beta-sitosterol	36.91	0.75	肉苁蓉、桂枝、半夏
MOL000098	quercetin	46.43	0.28	肉苁蓉、甘草
MOL000359	sitosterol	36.91	0.75	火麻仁、桂枝、甘草
MOL000449	Stigmasterol	43.83	0.76	火麻仁、半夏
MOL005030	gondoic acid	30.7	0.20	火麻仁、半夏
MOL005320	arachidonate	45.57	0.20	肉苁蓉
MOL005384	suchilactone	57.52	0.56	肉苁蓉
MOL007563	Yangambin	57.53	0.81	肉苁蓉
MOL000287	3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid	38.70	0.81	茯苓
MOL000289	pachymic acid	33.63	0.81	茯苓
MOL000290	Poricoic acid A	30.61	0.76	茯苓
MOL001439	arachidonic acid	45.57	0.20	火麻仁
MOL000483	(Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-[2-(4-hydroxyphenyl)ethyl]acrylamide	118.35	0.26	火麻仁
MOL000006	luteolin	36.16	0.25	火麻仁
MOL002776	Baicalin	40.12	0.75	半夏
MOL003578	Cycloartenol	38.69	0.78	半夏
MOL006967	beta-D-Ribofuranoside, xanthine-9	44.72	0.21	半夏
MOL004841	Licochalcone B	76.76	0.19	甘草
MOL004904	licopyranocoumarin	80.36	0.65	甘草
MOL002311	Glycyrol	90.78	0.67	甘草
MOL000492	(+)-catechin	54.83	0.24	桂枝
MOL001736	(-)-taxifolin	60.51	0.27	桂枝
MOL004576	taxifolin	57.84	0.27	桂枝

According to the screening principles of OB≥30% and

DL≥0.18, the active ingredients and corresponding targets of each traditional Chinese medicine in the composition of RoucongrongTang were searched in TCMSP. The results showed that Roucongrong, FuLing, Huomaren Banxia, Guizhi and Gancao contained 6, 15, 6, 13, 7 and 92 active ingredients respectively, for a total of 132 active ingredients. Among them, the 132 active ingredients obtained include 5 intersection active ingredients. Some active ingredients are shown in Table 1.

Target correction was performed in UniProt and duplicate genes were deleted, resulting in 259 drug targets. In order to further understand the interaction between compounds and targets, Cytoscape (version 3.10.0) was used to establish the drug-active ingredient-target network structure, as shown in Figure 1. In this network structure, each node represents a compound or a protein, and the edges represent the interactions between them.

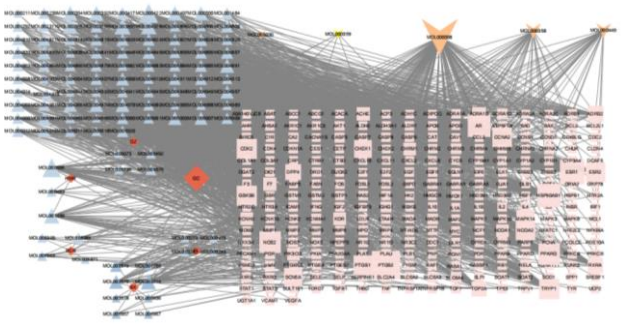

Figure 1: RoucongrongTang "drug-active ingredient-target" network

3.2 Venn Analysis of Functional Constipation Targets and Drug Targets

Figure 2 shows the intersection analysis diagram of RoucongrongTang drug targets and functional constipation targets. It can be seen from the figure that after searching for "Functional constipation" or "constipation" in TTD, OMIM, GeneCards, NCBI gene and DISGENET, 5694 FC target genes were obtained, which are potential targets (intersection targets) of RoucongrongTang in treating FC 204 indivual.

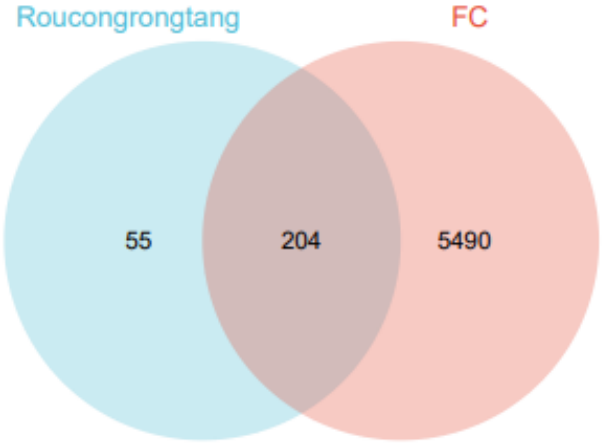

Figure 2: Intersection analysis of drug targets of RoucongrongTang and functional constipation targets

3.3 Establishment of PPI Network and Screening of Key Therapeutic Targets

Set the species in STRING to human, conduct protein interaction analysis on 204 intersection targets, and obtain 3963 target-protein interactions. In Cytoscape (version 3.10.0), the Centiscape 2.2 plug-in was used to perform network topology analysis on the interaction between target proteins BC, CC, DC, and EC, and the obtained values were 200.3267, 0.0025, 39.2376, and 0.0545 respectively. Based on DC, BC, EC and CC values, 35 key therapeutic targets were screened out, with 535 protein-protein interactions. The protein-protein interactions of 35 key therapeutic targets were visualized in Cytoscape (versions 3.7.0 and 3.10.0), and the results are shown in Figure 3. In Figure 3(c), the closer to the center of the circle, the larger the point and the darker the color. The darker the connecting line, the stronger the target-point interaction. Among them, the top 10 targets are CASP3, TNF, PTGS2, IL-6, MAPK3, MMP9, VEGFA, TP53, HIF1A, and STAT3.

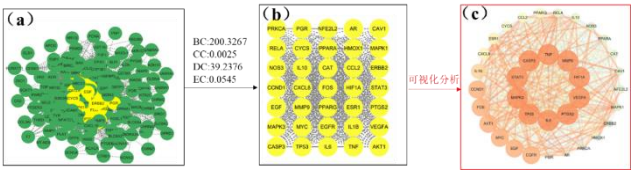

Figure 3: PPI network diagram of protein targets and key therapeutic targets ((a) PPI network diagram of potential targets; (b) PPI network diagram of key therapeutic targets; (c) Visualization of key therapeutic targets)

3.4 GO Functional Enrichment and KEGG Pathway Enrichment Analysis

In Metascape, GO (BP, CC and MF) functional enrichment and KEGG pathway enrichment analysis were performed on key therapeutic targets. Using P<0.01 as the reference standard for the enrichment results, 979 BPs, 27 CCs, 60 MFs and 157 KEGGs in GO were obtained. In the GO functional enrichment analysis (shown in Figure 4), BP is mainly involved in hormone responses, positive regulation of cell migration and movement, inorganic substances, chemical stress, lipids, growth factor responses, epithelial cell proliferation and glandular regulation of body development, etc.; CC mainly involves transcription regulatory complexes, membrane rafts, membrane microdomains, plasma membrane rafts, RNA polymerase II transcription regulator complex, plasma membrane protein complexes, cell-substrate junctions and vesicles Cavity, etc.; MF mainly involves transcription co-regulatory binding, DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, signaling receptor activator activity and regulatory activity, protein domain-specific binding, and chromatin binding., cytokine receptor binding, etc. In the KEGG pathway enrichment analysis, it was found that the main signaling pathways include cell signaling, inflammation, tumors, and infectious diseases. These pathways include MAPK, PI3K-Akt, HIF-1, TNF, IL-17, tumor pathways, and Kaposi's sarcoma-related herpes simplex virus infection. See Figure 5 for specific information. This study also performed a target-pathway mechanism analysis of tumor pathways and the Pl3K-Akt signaling pathway. As shown in Figures 6 and 7, the red parts represent potential targets where RoucongrongTang may have an intervention effect.

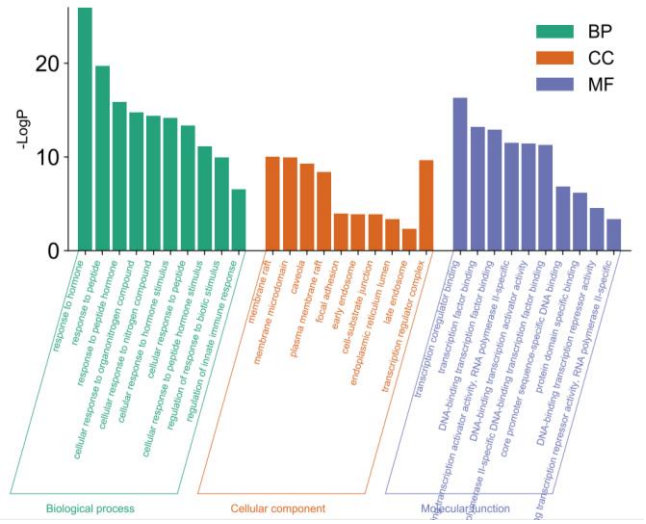

Figure 4: Functional enrichment analysis of GO genes in RoucongrongTang

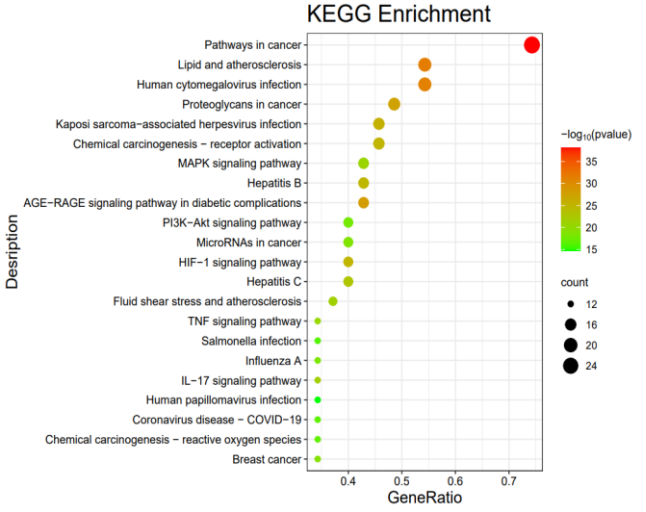

Figure 5: KEGG pathway analysis of RoucongrongTang

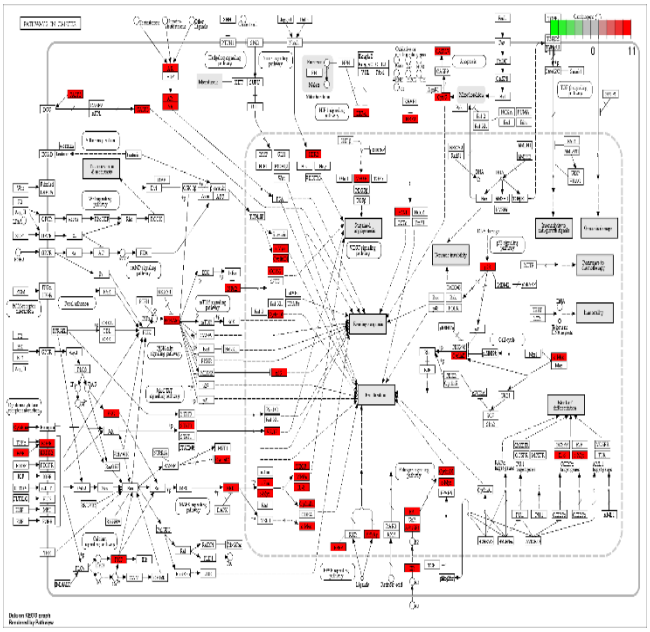

Figure 6: Tumor pathways

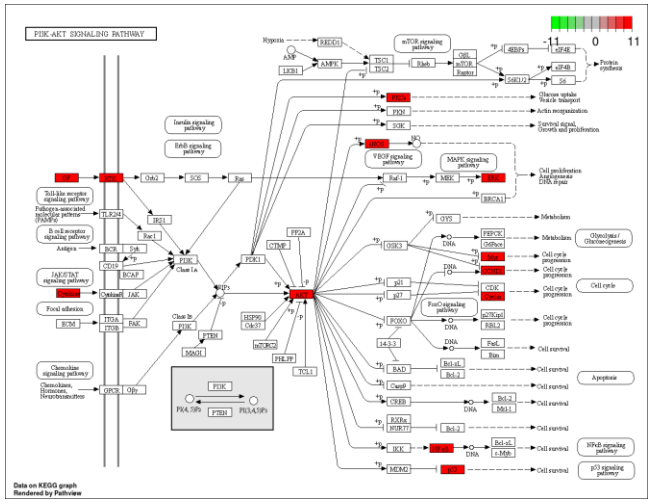

Figure 7: PI3K-Akt signaling pathway

3.5 Molecular Docking Verification

AutoDockTools-1.5.7 software was used when performing molecular docking of active ingredients and key therapeutic targets in RoucongrongTang. Select the active ingredients with the top 6 degree values in RoucongrongTang (quercetin, kaempferol, luteolin, 7-methoxy 2-methyl isoflavone, β-sitosterol, naringenin) before interacting with the protein. 7 key therapeutic targets (CASP3, TNF IL-6, MAPK3, MMP9, VEGFA, HIF1A). The binding energy (a value that reflects the binding strength of the ligand and the receptor) obtained through molecular docking shows that the binding energy of all active ingredients and the target protein is lower than -5.2kcal/mol, indicating that these six active ingredients are key to FC. The target has high biological affinity. The specific binding energy results are detailed in Table 2.

Table 2: Docking binding energy results of active ingredients in RoucongrongTang and core targets

TCM SP number	Chemical Composition	Chinese	Binding Energy(kcal/mol)						
			CASP3 2j32	TNF 5UUI	IL-6 1alu	MAPK3 4qtb	MMP9 6esm	VEGFA 1mkk	HIF1A 4h6j
MOL000098	quercetin	槲皮素	-6.04	-6.06	-5.95	-7.32	-9.45	-6.48	-5.38
MOL000422	kaempferol	山柰酚	-6.61	-6.14	-6.13	-7.19	-9.30	-5.90	-5.52
MOL000006	luteolin	木犀草素	-7.67	-6.47	-6.32	-7.73	-9.72	-6.55	-5.77
MOL003896	7-Methoxy-2-methyl isoflavone	7-甲氧基-2-甲基异黄酮	-6.44	-6.59	-5.74	-8.25	-10.08	-6.80	-5.37
MOL000358	beta-sitosterol	β-谷甾醇	-8.33	-8.12	-7.70	-10.59	-11.63	-7.61	-7.45
MOL004328	naringenin	柚皮素	-7.23	-6.74	-6.48	-7.44	-8.03	-5.65	-5.20

Further comparison found that the MMP9 receptor binds most closely to ligands such as quercetin, kaempferol, luteolin, 7-methoxy-2-methylisoflavone, and β-sitosterol, with an average binding energy of approximately -9.82 KCal/mol. The β-sitosterol ligand binds relatively tightly to all receptors,

with an average binding energy of approximately -8.77KCal/mol. Therefore, taking the docking of MMP9 and active ingredients as an example, PYMOl software was used to visualize the docking results. The model diagram is shown in Figure 8.

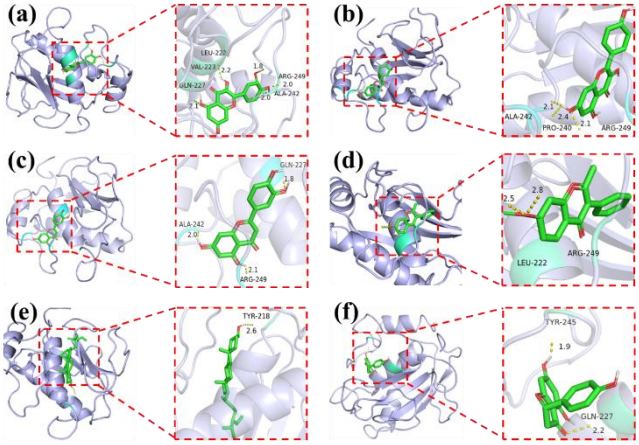

Figure 8: Visualization of target MMP9 and active ingredients ((a) MMP9 and quercetin (b) MMP9 and kaempferol (c) MMP9 and luteolin (d) MMP9 and 7-methoxy-2-methyl Isoflavones (e) MMP9 and β-sitosterol (f) MMP9 and naringenin)

4. Discussion

By screening and analyzing the active ingredients and action targets of RoucongrongTang, it was found that each traditional Chinese medicine contains multiple active ingredients, and different drugs may contain the same active ingredients. In addition, one active ingredient can also act on multiple targets, which reveals the unique characteristics of traditional Chinese medicine compounds. Compared with single compounds, traditional Chinese medicine compounds can regulate diseases through multiple components, multiple targets and multiple pathways. This multiple regulatory mechanism can not only improve drug efficacy, but also reduce the occurrence of adverse reactions and drug tolerance.

This study screened out the active ingredients with the highest degree value in RoucongrongTang, including quercetin, kaempferol, luteolin and β-sitosterol. Among them, quercetin can reduce somatostatin levels and improve gastrointestinal motility in rats by increasing intestinal transit rate, motilin, gastrin, substance P levels and short-chain fatty acid (SCFA) concentration [15]. In addition, quercetin can also regulate the activity of intestinal calcium-activated chloride channels and promote fluid secretion in the ileum of mice [16]. Kaempferol can improve intestinal barrier integrity and inhibit intestinal inflammation, and can also regulate the microbiota of mice fed a high-fat diet [17]. Luteolin can regulate intestinal microbiota and regulate intestinal smooth muscle movement [18], and its absorption is also regulated by the microbiota itself [19]. β-Sitosterol can improve intestinal barrier function and reshape intestinal microbiota, inhibiting the expression of pro-inflammatory cytokines TNF, IL-6 and inflammatory enzyme cyclooxygenase [20]. It is worth noting that there are more than two drugs containing the active ingredients β-sitosterol and quercetin in RoucongrongTang, and they all contain Roucongrong. Studies have found that the total oligosaccharide fraction of Roucongrong can significantly increase the levels of gastrointestinal hormones and intestinal neurotransmitters in constipated mice, and reduce the level of water protein [21]. Although the above active ingredients have varying degrees of effects on intestinal flora, intestinal smooth muscle, immunity and inflammation, there are currently few animal experimental studies on constipation models. Therefore, further research is needed to verify the role and mechanism of these active ingredients in treating constipation.

This study revealed the molecular mechanism of RoucongrongTang in treating functional constipation, and found that CASP3, TNF, IL-6, MAPK3, MMP9 and other genes are the key targets of RoucongrongTang in treating functional constipation. These genes are closely related to the occurrence and development of functional constipation, and they participate in multiple biological processes such as apoptosis, inflammatory response, immune regulation, and signal transduction. Among them, abnormal expression of CASP3 gene can aggravate intestinal mucosal cell apoptosis [22], abnormal expression of TNF and IL-6 genes can induce intestinal inflammatory response and abnormal intestinal peristalsis [23], and abnormal expression of MAPK3 gene can also cause cell signaling. Conduction disorder and abnormal expression of MMP9 gene can cause changes in intestinal tissue structure and abnormal intestinal peristalsis [24]. The above conclusions can provide new targets and directions for the treatment and prevention of constipation, and also provide theoretical basis and scientific support for the clinical application of RoucongrongTang.

GO enrichment analysis found that RoucongrongTang contains genes or proteins that can regulate biological functions such as hormone response, cell migration and movement, and also contains factors related to cell structure and components such as regulating gene expression and intracellular signaling. In addition, RoucongrongTang can also bind to extracellular proteins or enzymes, play a role in regulating molecular functions such as receptor ligand activity and cytokine activity, and can regulate cell function and structure in the cytoplasmic vesicle cavity.

KEGG pathway enrichment analysis showed that RoucongrongTang can jointly treat constipation through multiple pathways, including regulating tumor pathways, MAPK signaling pathways, and PI3K-Akt signaling pathways. By inhibiting the tumor pathway and activating the PI3K-Akt signaling pathway, the number of Cajal interstitial cells can be regulated, thereby affecting colon sensitivity, regulating gastrointestinal motility, and thereby alleviating constipation [25-28]; RoucongrongTang regulates the MAPK signaling pathway to promote intestinal It can improve intestinal peristalsis and increase intestinal water to relieve constipation [29,30]. The above mechanism is basically the same as the confirmed therapeutic mechanism of some compound decoctions for constipation.

Molecular docking results show that the active ingredients have strong binding activity to key therapeutic targets. In particular, various active ingredients have significant binding effects on MMP9 targets, among which β-sitosterol has the most stable binding with MMP9. Since MMP9 plays an important role in the intestine, modulating its activity can lead

to delayed intestinal transit, suggesting that β-sitosterol can be used as a potential drug for the treatment of constipation. Further experiments and research can provide insights into this drug and provide new insights into the drug. More options for treating constipation.

In summary, network pharmacology and molecular docking technology were used to analyze the treatment of FC by RoucongrongTang, and the interrelationships between multiple components, multiple targets, and multiple pathways were found. After research, it was found that the active ingredients in RoucongrongTang, such as quercetin, kaempferol, luteolin and β-sitosterol, interact with the key targets for treating FC, such as CASP3, TNF, IL-6, MAPK3, MMP9, etc. interaction. These effects regulate physiological functions such as cell growth, differentiation, apoptosis, and metabolism by regulating pathways related to cell signaling, inflammation, tumors, and infectious diseases. Therefore, RoucongrongTang can have a therapeutic effect on FC. However, based on the above analysis results, further research is needed on the effect of RoucongrongTang on tumor pathways, MAPK, PI3K-Akt and other signaling pathways, as well as the specific impact of this prescription on intestinal cell function. In addition, this study is limited by problems such as drug databases and disease target research databases, and the results have certain biases. Therefore, the reliability of these conclusions requires further experimental verification.

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