

A network pharmacology approach to explore Dahuang on colon cancer

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Abstract To investigate the mechanism of action of Rhubarb (Dahuang) in the treatment of colon cancer by network pharmacological method. TCMSP database was used to screen the active components and action targets of Rhubarb, Genecards database was used to screen the relevant action targets of colon cancer, Venny 2.1.0 software was used to make the intersection of Rhubarb and colon cancer, and the potential targets of Rhubarb for the treatment of colon cancer were obtained. Cytoscape 3.7.2 software and the STRING database were used to construct the component-target network of Rhubarb active components and colon cancer related targets. The important targets were obtained by analyzing the network, and the GO and KEGG pathway enrichment analysis was performed on them using Metascape database. Sixty active ingredients including EUPATIN, Mutatochrome, Physciondiglucoside, Procyanidin B-5,3'-o-gallate, rhenin were screened from Rhubarb, which could act on 61 colon cancer related targets. Among them, 13 targets, including PTGS2, IL1B and Bax, are important targets, which may be involved in various biological processes such as the response to the regulation of colon cancer nutritional level, the homeostasis of multicellular organisms and the regulation of peptide secretion through the regulation of PI3K and other pathways. Rhubarb can participate in the treatment of colon cancer through multi-target and multi-pathway.

1 Introduction

With the development of big data, network pharmacology has a profound influence on the prediction of the mechanism of Chinese herbal medicine against disease. As a traditional Chinese medicine, Rhubarb has many pharmacological effects. Colon cancer as one of the common cancers, in addition to the traditional chemotherapy has no other way of treatment. However, the harm of chemotherapy to human body can not be ignored. In this study, network pharmacological methods were used to explore the mechanism of Rhubarb in the treatment of colon cancer, revealing the complex molecular mechanism of Rhubarb in the treatment of colon cancer at the level of target and pathway, and providing new ideas and new directions for the study of the mechanism of action of Rhubarb in the treatment of colon cancer.

2 Methods and results

2.1 Potential active components of Rhubarb and their action targets

System in Chinese medicine pharmacological analysis platform (TCMSP, <http://lsp.nwu.edu.cn/tcmsp.php>)^[1] to retrieve the Rhubarb potential active ingredients, in accordance with the oral bioavailability (OB) 30% or

higher, classes, medicinal standard screening (DL) of 0.18 or higher^[2-3]. TCMSP database was used to search the action targets of the active ingredients obtained in the previous step, and the targets were mapped into standard gene names (gene IDs) through Uniprot database (<https://www.uniprot.org>) to obtain the active ingredients and action targets of Rhubarb. A total of 16 active ingredients were screened out (Table 1).

Table1. Basic information of active ingredients in Rhubarb

TCMSP NUMBER	INGREDIENTS	OB%	DL%
MOL002235	EUPATIN	50.8	0.41
MOL002251	Mutatochrome	48.64	0.61
MOL002259	Physciondiglucoside	41.65	0.63
MOL002260	Procyanidin B-5,3'-O-gallate	31.99	0.32
MOL002268	rhein	47.07	0.28
MOL002276	Sennoiside E _{qt}	50.69	0.61
MOL002280	Torachryson-8-O-beta-D-(6'-oxayl)-glucoside	43.02	0.74
MOL002281	Toralactone	46.46	0.24
MOL002288	Emodin-1-O-beta-D-glucopyranoside	44.81	0.8
MOL002293	Sennoiside D _{qt}	61.06	0.61
MOL002297	Daucosterol _{qt}	35.89	0.7
MOL002303	palmitin A	32.45	0.65
MOL000358	beta-sitosterol	36.91	0.75
MOL000471	aloe-emodin	83.38	0.24

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MOL000554	gallic acid-3-O-(6'-O-galloyl)-glucoside	30.25	0.67
MOL000096	(-)-catechin	49.68	0.24

2.2 Target of Rhubarb in the treatment of colon cancer

Colon cancer related target genes were retrieved from GeneCards database (<https://www.genecards.org>)^[4]. Then, the target gene of colon cancer was mapped to the target gene of Rhubarb active ingredient to screen, and the intersection was taken to obtain the potential target of Rhubarb in the treatment of colon cancer, and the Venny diagram was made. A total of 92 active component targets of Rhubarb and 1669 targets of colon cancer were retrieved, and 61 targets were obtained by mapping them together, which were potential target genes of Rhubarb for the treatment of colon cancer (Table 2, Fig.1).

Table2. Potential target genes of Rhubarb in treatment of colon cancer

Name of gene target					
ADH1C	RRM1	AR	F3	CDKN1A	EGF
NR3C1	SLC16A7	ESR2	ARG1	TNFSF15	MMP1
PTGS2	MTR	PRSS1	CASP3	TP63	PPARG
CTSD	IDH1	KDR	SLC6A4	FASN	CYP1A1
HDC	TF	ESR1	BCL2	PCNA	CSF2
PTGS1	GPT	MMP9	BAX	MYC	ACTA2
BCHE	SDHA	RXRA	CASP9	IL1B	BTK
ALDH2	PGR	ADRB2	CASP8	PRKCD	SLC2A4
MMP12	NR3C2	JUN	PRKCA	CCNB1	FLT4
CAT	NOS2	CHEK1	PON1	FLT1	SLC2A1
FASLG					

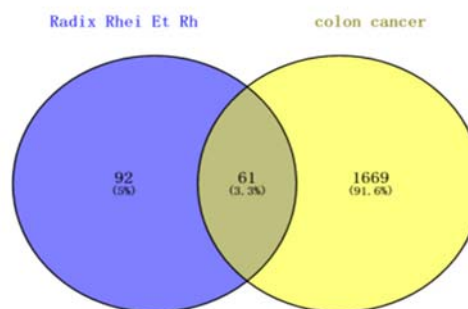


Figure 1. Venny plot of Rhubarb active ingredient target and colon cancer target

2.3 Protein interaction networks and important targets

STRING database ([HTTPS://String-DB.org](https://string-db.org)) is a database of known and data-predicted protein-protein interactions (PPI) that can be used to bring the resulting Rhubarb potential targets for colon cancer into the STRING platform. The species was set as "Homo sapiens", the confidence was set as "0.4" for screening, and the remaining parameters were kept as default. TSV file of protein interaction information of target gene was obtained, and PPI network was constructed by String database and Cytoscape software^[5-6]. Cytoscape software was used to render PPI network and perform cluster analysis of important gene modules. All the default parameters in Mcode application were executed to obtain the densely connected network modules (modules) in PPI network. Targets contained in the Module were important targets of Rhubarb in the treatment of colon cancer. Figure 2 is a PPI network diagram, which contains 61 Nodes and 373 Edges. Figure 3 shows subcluster B (score 9), which contains 13 Nodes and 54 Edges, and 13 core genes are obtained, including PTGS2, IL1B, Bax, NR3C1, NOS2, CSF2, CASP3, GPT, CTSD, MYC, PPARG, JN, EGF.

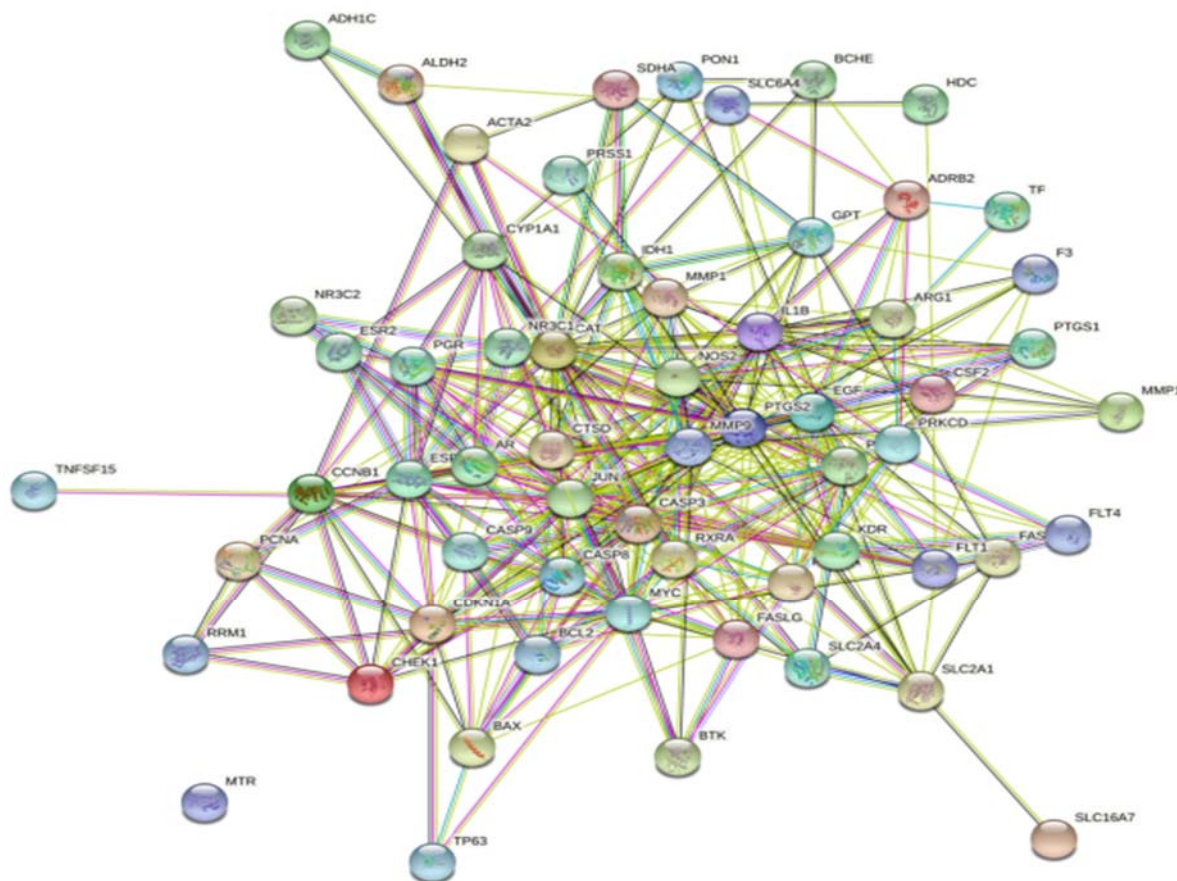


Figure 2. PPI network interaction diagram

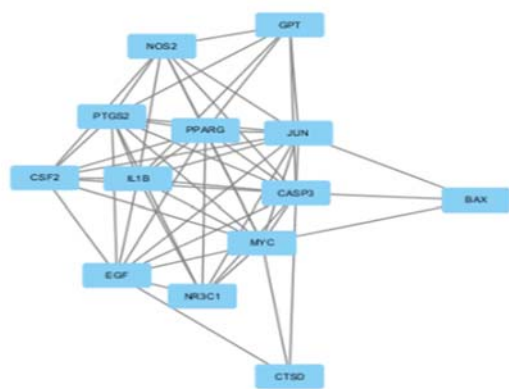


Figure 3. Subcluster B: Core gene

performed for gene annotation, and the results were visualized.

Metascape was used to carry out GO enrichment analysis and KEGG pathway enrichment analysis of 13 important targets of Rhubarb in the treatment of colon cancer. The enrichment results were sorted according to P value, and COUNT value represented the number of genes enriched in this pathway. GO enrichment analysis was carried out in three aspects of biological process enrichment, molecular function analysis and cell component analysis. The results are shown in Figure 4 ~ 7. According to the P-value and COUNT value of KEGG pathway analysis, PI3K plays a key role in the top 15 pathways related to Rhubarb in the treatment of colon cancer, as shown in Figure 4. Enrichment of GO biological processes mainly involves responses to nutrient levels, homeostasis in multicellular organisms, regulation of peptide secretion, regulation of protein secretion, and response to peptide hormones, etc., as shown in Figure 5. The molecular function analysis of GO mainly involves the molecular functions of the ciliary body, outer plasma membrane, cytoplasmic cystic cavity, cystic cavity, endoplasmic reticulum and other parts, as shown in Figure 6. The analysis of GO cell components mainly involves receptor ligand activity, cytokine receptor binding, cytokine activity, DNA-binding transcriptional activator activity, RNA polymerase specificity, hormone activity, etc., as shown in Figure 7.

2.4 Ontology analysis and KEGG pathway annotation and enrichment analysis

Gene Ontology (GO) analysis is a general method to annotate Gene products and their functional characteristics^[7-8]. GO analysis can be divided into biological process (BP) and cellular component according to different functions (CC), Molecular Function (MF). Import the intersection target into the Metascape database ([HTTPS://metascape.org](https://metascape.org)), select the species type as "Homo sapiens", then select "Custom Analysis" and use the default setting as the filter condition. Go analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation and enrichment analysis were

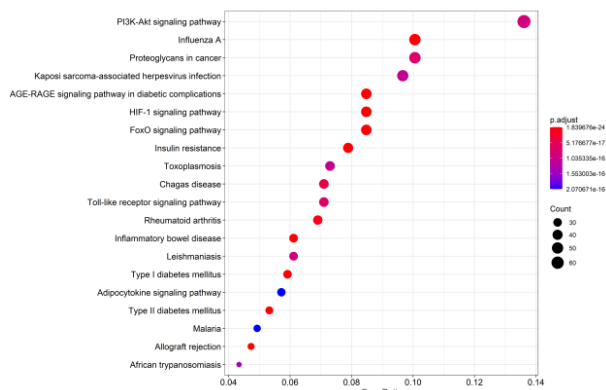


Figure 4. Bubble diagram of KEGG analysis

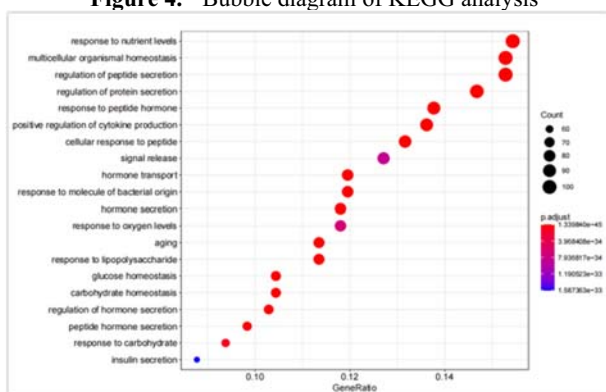


Figure 5. Bubble diagram of GO biological process

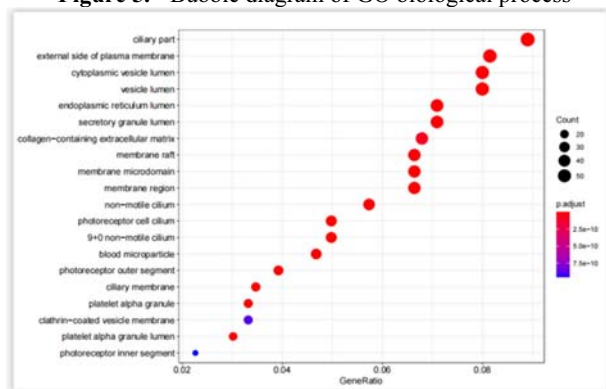


Figure 6. Bubble diagram of GO molecular function analysis

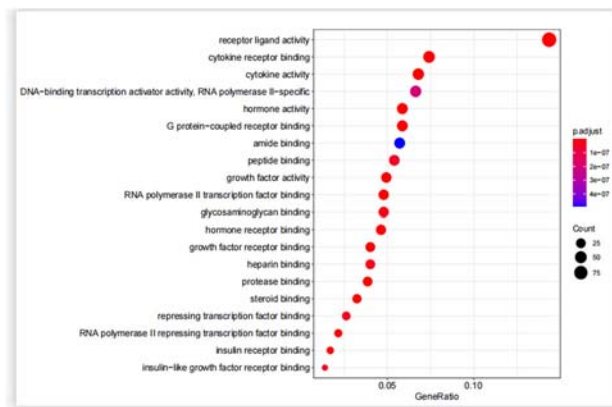


Figure 7. Bubble diagram of GO cell component analysis

2.5 Rhubarb active ingredient - colon cancer - KEGG action network

Cytoscape 3.7.2 software was used to construct the Rhubarb active ingredient - colon cancer - KEGG action network. In this network, Node represents the active component of Rhubarb and its target protein acting on colon cancer, while Edge represents the interaction between active component and target protein, as shown in Figure 8^[9].

3 Conclusions

Network pharmacology plays an important role in analyzing the pharmacological action of traditional Chinese medicine and predicting the effect of treating diseases. Through the network pharmacological study of Rhubarb in this study, it was found that Rhubarb can participate in the treatment of colon cancer through multi-target and multi-pathway. Pharmacology research through the network, KEGG pathway analysis showed that PI3K play a key role, GO enrichment of biological process shows mainly involved in response to the nutrition level, multicellular organisms, homeostasis, peptide secretion regulation, molecular function analysis showed mainly involves the ciliary body, GO cell component analysis showed that mainly involves receptor ligands.

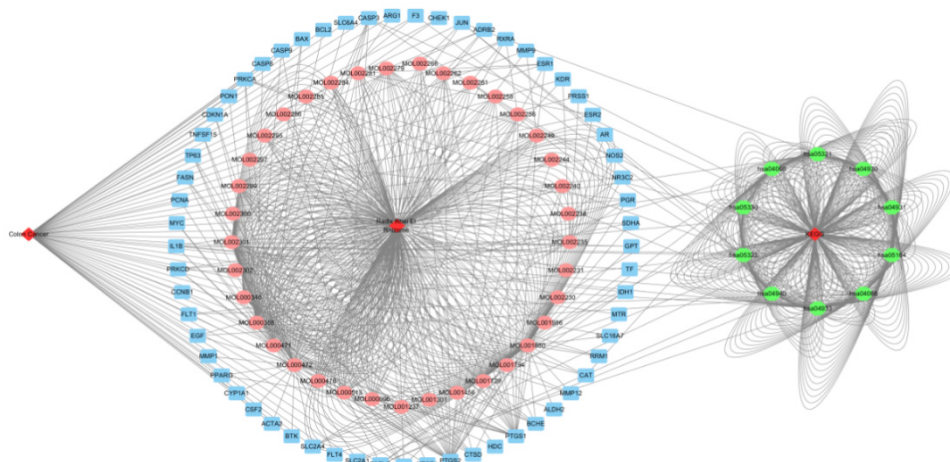


Figure 8. Rhubarb active ingredient - colon cancer-KEGG action network

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