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Prediction of potential mechanisms of rhubarb therapy for colorectal cancer based on network pharmacological analysis and molecular docking

Fan Yang, MM[a], Xinghua Li, MD[a], Yujie Zhang, MD[a], Yun Ren, MD[a], Jiao Zhang, MM[a], Keyuan Xiao, MM[a,*]

Abstract

The objective of this study was to investigate the potential targets and mechanism of *Rheum palmatum* L in the treatment of colorectal cancer based on the network pharmacology and molecular docking, which could provide the theoretical basis for clinical applications. The potential components were screened using TCMSP database and articles. The gene targets of colorectal cancer were screened through the Genecards database and Online Mendelian Inheritance in Man database. Then, the common targets of components and colorectal cancer were used to construct the network diagram of active components and targets in Cytoscape 3.7.0. The protein-protein interaction (PPI) diagram was generated using String database, and the targets were further analyzed by gene ontology and Kyoto Encyclopedia of Genes and Genomes. Molecular docking between gene targets and active components was analyzed via AutoDock, and visualized through PyMol. Among this study, main targets might be TP53, EGF, MYC, CASP3, JUN, PTGS2, HSP90AA1, MMP9, ESR1, PPARG. And 10 key elements might associate with them, such as aloe-emodin, beta-sitosterol, gallic acid, eupatin, emodin, physcion, cis-resveratrol, rhein, crysophanol, catechin. The treatment process was found to involve nitrogen metabolism, p53 signaling pathway, and various cancer related pathway, as well as the AGE-RAGE signaling pathway, estrogen signaling pathway, interleukin-17 signaling pathway and thyroid hormone signaling pathway. The molecular docking was verified the combination between key components and their respective target proteins. Network pharmacological analysis demonstrated that *R palmatum* was could regulated p53, AGE-RAGE, interleukin-17 and related signaling pathway in colorectal cancer, which might provide a scientific basis of mechanism.

Abbreviations: BP = biological process, CC = cell component, CRC = colorectal cancer, EGF = epidermal growth factor, GO = gene ontology, IL-17 = interleukin-17, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function, MMP9 = matrix metalloproteinase 9, PPI = protein-protein interaction, ROS = reactive oxygen species, TCM = traditional Chinese medicine.

Keywords: colorectal cancer, network pharmacology, pathway, *Rheum palmatum* L

1. Introduction

Colorectal cancer (CRC), is a highly invasive and lethal type of gastrointestinal cancer.[1] Among all cancers worldwide, CRC ranks third in terms of morbidity, and mortality accounts for approximately 10%.[2] The early symptom of CRC was neglected, including hematochezia, abdominal pain, diarrhea, changes in bowel habits. Currently, the more common treatment for CRC patients included reoperation, chemotherapy, radiotherapy, targeted therapy or a combination of therapies. But after treating, the prognosis for CRC patients remains poor.[3,4] Moreover, long-term use of these therapies can lead to severe side effects, such as nausea and vomiting, oral ulcers, diarrhea, hepatotoxicity, myelosuppression and immunosuppression.[3] It is common to use traditional Chinese medicine (TCM) combined with Western medicine in clinical practice, which could reduce toxicity and enhance effects.[5] TCM were applying in CRC to anti-tumor through promoted cancer cell apoptosis, inhibited cancer metastasis, and reduced drug resistance and side effects and regulated intestinal flora.[6] However, the specific mechanism underlying TCM was still not clear.

Rheum palmatum L. *Syst. Ed*, a TCM, widely using for centuries, have broader range of beneficial effects such as antioxidant, anti-inflammatory, antimicrobial and cardioprotective

FY and XL contributed equally to this work.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

The present study is a Bioinformatics-based analysis, so ethical and consent permission is unnecessary.

[a] Changzhi People's Hospital, The Affiliated Hospital of Shanxi Medical University, Changzhi, Shanxi Province, P.R. China.

** Correspondence: Keyuan Xiao, Changzhi People's Hospital, The Affiliated Hospital of Changzhi Medical College, Changzhi, Shanxi Province 046000, P.R. China (e-mail: xky1551395@163.com).*

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activities. It contains many chemical ingredients such as emodin, aloe-emodin. These components have shown pharmacological effects in various cancers, including CRC, hepatocellular carcinoma, and lung cancer.[7–9] Some studies has reported that emodin reversed 5-Fu resistance in CRC via downregulation of PI3K/Akt signaling pathway and aloe-emodin suppressed epidermal growth factor (EGF)-induced neoplastic cell transformation by inhibiting the ERK/MSK1 and AKT/GSK3β signaling pathways.[10,11] Some studies have reported the efficacy of prescription about *R palmatum L* in treating CRC. Dahuang Fuzi Baijiang decoction could prevent tumor progression by modulating adaptive immunity, and Dahuang Zhechong Pill could suppress liver metastasis of colorectal cancer.[12,13] But the core targets and specific mechanism of *R palmatum* is still not clear for CRC.

Network pharmacology can guide and assist in drug repositioning and predict the mechanism and metabolic characteristics of CHMs.[14] The components of CHMs were screened using network analysis and the binging target was visualizing through Autodock. These techniques help us better comprehend the molecular mechanism of diseases from a multidimensional perspective.[15] In this study, utilizing the network pharmacology and molecular docking, it was explored including the crucial components of *R palmatum*, the core targets of CRC and *R palmatum*, and the molecular biological mechanism for CRC, which would be a scientific reference in clinic.

2. Materials and methods

2.1. The collection and screening of active ingredients and targets with R palmatum L

According to TCMSP database (https://old.tcmsp-e.com/tcmsp.php),[16] the ingredients of *R palmatum* L were collected. The potential active ingredients of *R palmatum* L with DL L 0.18 and OB ≥ 30 were selected for CRC. In addition, the other active ingredients with *R palmatum* L that were searched from articles in PubMed database were enrolled, including emodin, cis-resveratrol, physcion, emodin 8-O-β-D-glucoside, gallic acid, chrysophanol 8-O-β-D-glucoside, aloe-emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside,

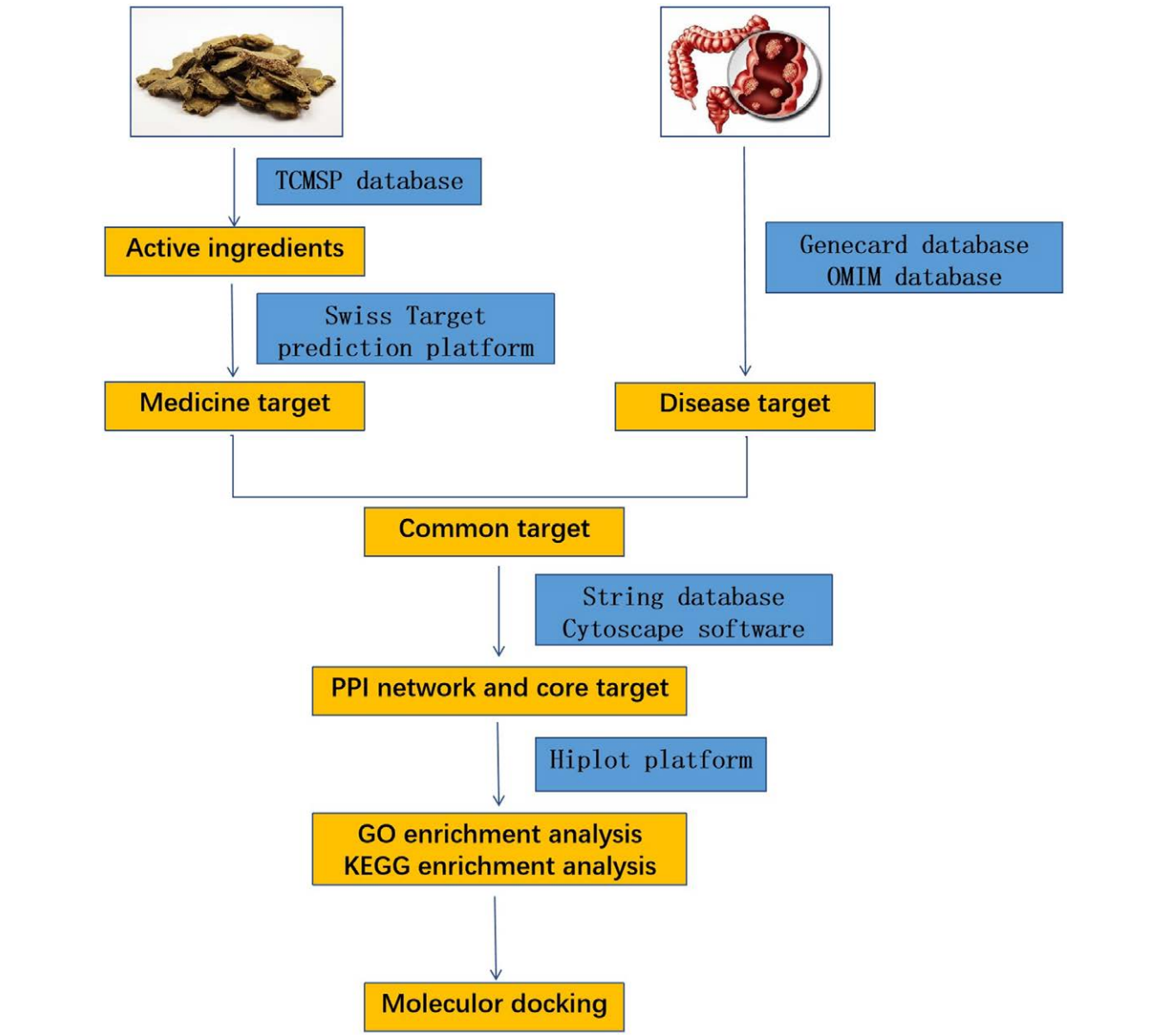

Figure 1. Flow diagram of the study.

chrysophanol.[17–22] The SMILES of potential active ingredients was obtained from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/).[23] The potential targets of the active components were predicted from TCMSP database and Swiss Target Prediction database (http://www.Swisstargetprediction.ch/).[24] After removing the repetitive targets, the targets in Swiss database registered as potential active ingredients with probability of 0.5.

2.2. The acquisition of core targets in CRC

The gene of CRC was obtained after searching "colorectal cancer" in Online Mendelian Inheritance in Man database[25] (https://omim.org/) and Genecards database (https://www.genecards.org/).[26] Then, the targets of CRC were screened after removing the reduplicated targets of 2 databases.

2.3. Network construction between the active components of R palmatum L and the targets of CRC

The intersection was analysis between core targets of active components in *R palmatum* L and related targets in CRC, which constructed Venn diagram (http://www.ehbio.com/test/venn/#/). Then, the network diagram of active components of *R palmatum* L and common targets was exported from Cytoscape 3.7.0, and analyzed by topological property.

2.4. The network construction of PPI

The interaction function of protein to protein was analyzed by String database (https://string-db.org/).[27] After restricting species of "homosapiens," the mutual target was entered into String database with mediumconfidence = 0.4 of the minimum interaction score. Then, the PPI network diagram was constructed after hiding the unrelated interaction. The database imported into Cytoscape3.7.0 (www.cytoscape.org/)[28] was subjected to topology analysis. Finally, the top 10 gene targets were screened after sequencing by degree.

Table 1

The potential active components of *Rheum palmatum* L.

MOL_ID	Molecule_name	OB	MW	DL
MOL000471	Aloe-emodin	83.38	270.25	0.24
MOL002293	Sennoside D_qt	61.06	524.50	0.61
MOL002235	Eupatin	50.80	360.34	0.41
MOL002276	Sennoside E_qt	50.69	524.50	0.61
MOL000096	(–)-Catechin	49.68	290.29	0.24
MOL002251	Mutatochrome	48.64	552.96	0.61
MOL002268	Rhein	47.07	284.23	0.28
MOL002281	Toralactone	46.46	272.27	0.24
MOL002288	Emodin-1-O-β-D-glucopyranoside	44.81	432.41	0.80
MOL002280	Torachrysone-8-O-D-(6'-oxayl)-glucoside	43.02	480.46	0.74
MOL002259	Physciondiglucoside	41.65	608.60	0.63
MOL000358	Beta-Sitosterol	36.91	414.79	0.75
MOL002297	Daucosterol_qt	35.89	386.73	0.70
MOL002303	Palmidin A	32.45	510.52	0.65
MOL002260	Procyanidin B-5,3'-O-gallate	31.99	730.67	0.32
MOL000554	Gallic acid-3-O-(6'-O-galloyl)-glucoside	30.25	484.40	0.67
MOL000472	Emodin	24.40	270.25	0.24
MOL001229	Cis-resveratrol	41.13	228.26	0.11
MOL000476	Physcion	22.29	284.28	0.27
MOL002243	Emodin 8-O-β-D-glucoside	27.06	432.41	0.80
MOL000513	Gallic acid	31.69	170.13	0.04
MOL002360	Chrysophanol-8-O-β-D-glucoside	20.85	416.41	0.77
MOL002241	Aloe-emodin-8-O-β-D-glucoside	9.04	432.41	0.81
MOL002366	Physcion-8-O-β-D-glucoside	18.31	446.44	0.82
MOL001729	Chrysophanol	18.64	254.25	0.21

2.5. The core component analysis of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway

The common targets were analyzed by GO including biological process (BP), molecular function (MF) and cell component (CC), and the pathway was analyzed by KEGG. The GO and KEGG was analyzed in the Hiplot platform (https://hiplot-academic.com/). The bubble chart of the top 20 entries with *P* value < .05 were drawn in R 4.0.3 software.

2.6. Molecular docking validation

By restricting "homosapiens" with an acceptable resolution of <2.5, suitable target protein structures were screened in the PDB database (https://www.rcsb.org/).[29] The SDF files of ingredients were download from the PubChem database. The target proteins were dehydrogenated in AutoDock.[30] The size and position of the box were adjusted to optimal conformation of ligands and receptors outputting in "pdbqt" format. The molecular docking results visualized using PyMol 2.4.0.[31] And the docking results of core components and target was showed by heat map. The flow chartis shown in Figure 1.

3. Result

3.1. Screening of active components in R palmatum L

The active components were screened by OB ≥ 30% and DL ≥ 0.18 in TCMSP database.[32] And some active components of *R palmatum* L that could obliterate CRC cell were added in reported studies, such as emodin, cis-resveratrol, physcion, emodin 8-O-β-D-glucoside, gallic acid, Chrysophanol 8-O-β-D-glucoside, aloe-emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside, chrysophanol. All the components were in Table 1.

3.2. The prediction targets of active components and the network construction and analysis

The 110 targets of active components were predicated by TCMSP database and Swiss Target Prediction database. The 10,660 targets of CRC were screened by OMIN database and GeneCards database. Then 87 mutual targets were drawn in Veen diagram (Fig. 2A). The network diagram of "antitumor component—targets" of *R palmatum* L was constructed

Table 2

The degree of potential active component with *Rheum palmatum* L.

Name	Degree
Emodin	35
Beta-sitosterol	26
Gallic acid	20
Aloe-emodin	20
Cis-resveratrol	18
Eupatin	13
Physcion	10
Daucosterol_qt	9
Toralactone	7
Rhein	7
Crysophanol	7
(–)-Catechin	7
Emodin-8-O-β-D-glucoside	2
Chrysophanol-8-O-β-D-glucoside	2
Emodin-1-O-β-D-glucopyranoside	1

by Cytoscape3.7.0 (Fig. 2B). A total of 102 interactions was obtained, including 15 compounds and 87 targets were shown as quadrate and roundness, respectively. In the network diagram, the emodin, beta-sitosterol, gallic acid, aloe-emodin, cis-resveratrol, eupatin, physcion, rhein, crysophanol, catechin had higher degree (Table 2).

The common targets and the CRC defined species as "Homo sapiens" and mediumconfidence as 0.4, and hid the unconnected interactions in the String database. Then, the PPI network diagram was constructed (Fig. 2C). The average degree of common targets and CRC was 12.815 using Cytoscape 3.7.0 and NetworkAnalyer. Then, the 34 targets which the degree value was higher than 12.815 were displayed in different sizes and colors according to the degree value in the PPI network diagram (Fig. 2D). After ranking the 34 targets, the top 10 targets were TP53, EGF, MYC, CASP3, JUN, PTGS2, HSP90AA1, MMP9, ESR1, PPARG.

3.3. The GO enrichment and KEGG enrichment analysis

The function of gene production was described by GO enrichment analysis, which consisted of BP, MF and CC. In this study, the result of GO enrichment analysis gathered 3221 BPs, 389 MFs, 239 CCs, and KEGG analysis collected 237 related pathways. The bubble chart was drawn by the top 20 items and KEGG enrichment analysis according to the *P* value screening with *P* va.5 and ranked from smaller to larger. The size of bubble represented the number of genes in GO and KEGG enrichment, with the large bubble representing the more genes. The size of *P* value was correlated with red with the smaller *P* value representing the tight correlation. Then, the bar chart of 20 items was present according to the *P* value (Fig. 3).

The results of GO enrichment explained that the treatment process was involved in response to lipopolysaccharide, response to drug, response to steroid hormone, and tightly related with carbonate dehydratase activity, nuclear receptor activity, ligand-activated transcription factor activity, hydrolyase activity, steroid hormone receptor activity (Fig. 3). This function and process was carried out from membrane raft, membrane microdomain, membrane region. The results of KEGG enrichment analysis suggested that the mechanism of CRC was correlated with the pathways included nitrogen metabolism, p53 signaling pathway, some cancer related pathway (colorectal cancer, small cell lung cancer, breast cancer, thyroid cancer), AGE-RAGE signaling pathway, estrogen signaling pathway, interleukin-17 (IL-17) signaling pathway and thyroid hormone signaling pathway (Fig. 4A).

3.4. Molecular docking results

Molecular docking was performed to analyze how the core compounds interacted with the core targets. The docking results were combined and displayed using heatmaps. PyMol

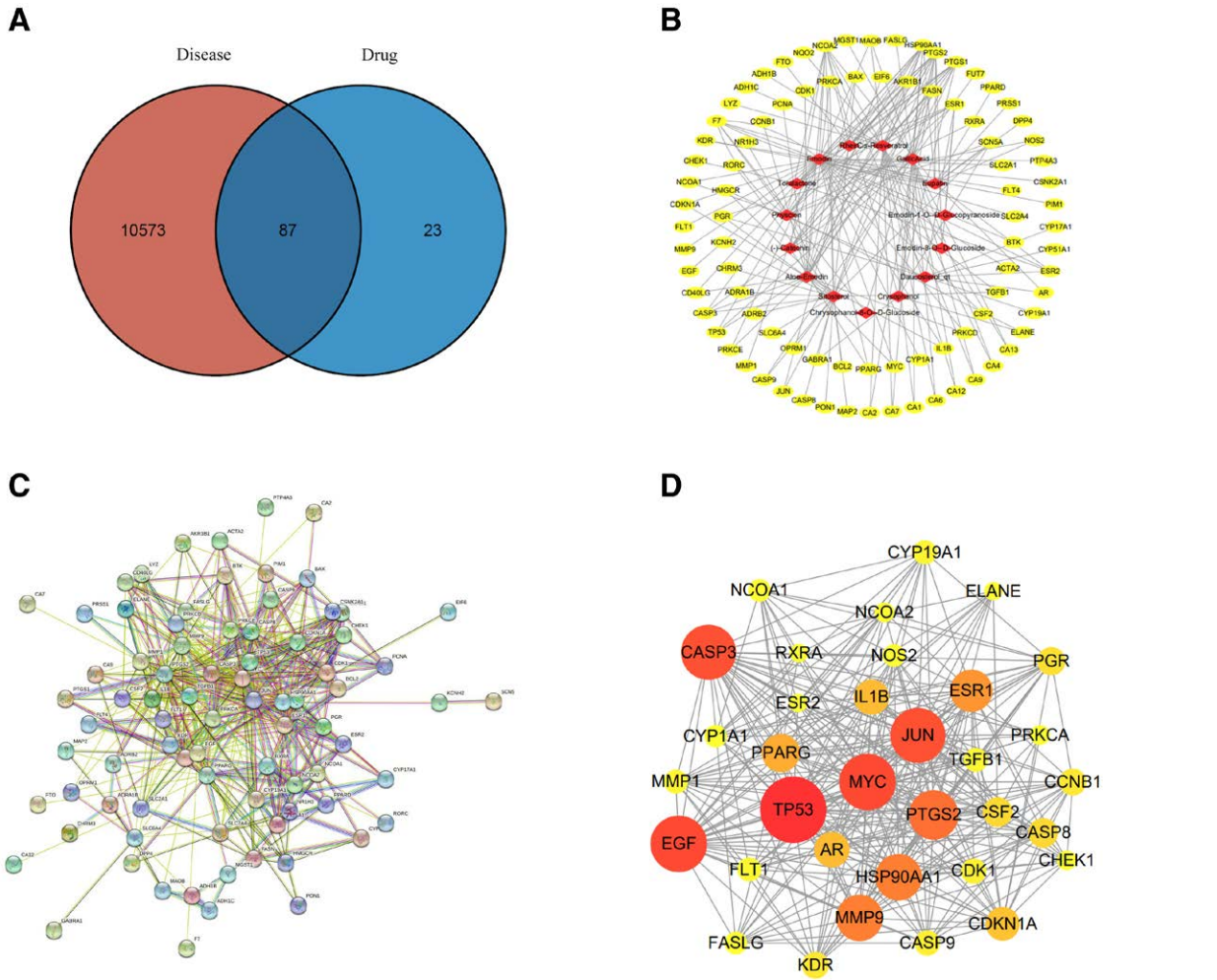

Figure 2. (A) Venn diagram of *Rheum palmatum* L targets and CRC disease-related targets. (B) Drug-ingredient-target-disease network diagram. (C) PPI network diagram of candidate genes. (D) Topological analysis diagram of the candidate genes. CRC = colorectal cancer, PPI = protein-protein interaction.

was used to visualize the top-ranked results. A docking score lower than −4.25 kcal/mol indicates some binding activity, while a score lower than −7.0 kcal/mol indicates strong binding activity. The present results showed that the average docking score was −7.51 kJ/mol, among which the results with docking score ≤ −7.0 kJ/mol accounted for 78%, reflecting that the compounds in the Rheum officinale pairs had good binding ability to the core target according to the scoring results. Heatmaps showed all the scoring results (Fig. 4B). Some molecular docking results were visualized byusing PyMol 2.4.0, as shown in Figure 5.

4. Discussion

CRC is a complex tumor involving multiple carcinogenic signaling pathways, the molecular mechanisms of which are not fully understood.[33] The prognosis of patients is difficult to achieve satisfactory prognosis treating by surgery, radiotherapy and chemotherapy.[34] Researches have suggested that the TCMs have good anti-tumor effects and regulate the immune microenvironment.[35,36] And in clinic, TCMs with small side effect could prolong the survival time and improve the life quality of patients.[36] However, TCMs have multi-targets and multi-pathways in treatment of CRC. And the specific mechanism of TCMs were not understood. Therefore, it is necessary to predict mechanisms of TCMs and provide theoretical basis for clinic.

In this study, 15 active ingredients were screened from *R palmatum*, to determine the correlation between the ingredients and clinical efficacy. Using network pharmacology, we identified 87 targets associated with these active ingredients. Through network construction analysis, we identified 10 key targets, including TP53, EGF, MYC, CASP3, JUN, PTGS2, HSP90AA1, MMP9, ESR1, PPARG, which play an important role in this study. TP53 is a crucial molecule in the occurrence and development of colorectal cancer.[37] Research has indicated that upregulation of TP53 can impede the trans-activation of TCF4, provoke endoplasmic reticulum stress disorder in cancerous cells, and facilitate the demise of colon cancer cell.[38] The EGF receptor (EGFR, ErbB-1, HER1) expressed in numerous tissues, plays various roles during development, in healthy individuals, and in different pathological conditions, including cancer.[39] After binding with EGFR, it escalates EGFR kinase activity and triggers several signaling pathways, thereby managing cell survival and metabolism.[40,41] HSP90AA1, a member of genetically conserved heat shock protein family, downregulated HSP90AA1 to restrain the malignant biological behavior of CRC.[42] HSP90, a vital regulator of tumor development and metastasis, was suggested that it may be an independent prognostic factor for CRC patients.[43] MMP9, a zinc-dependent endopeptidase, instigates gelatin and collagen degradation during tissue remodeling, thereby encouraging tumor invasion and metastasis.[44] Studies have indicated that MMP9 expression is a natural biological means to inhibit CAC by

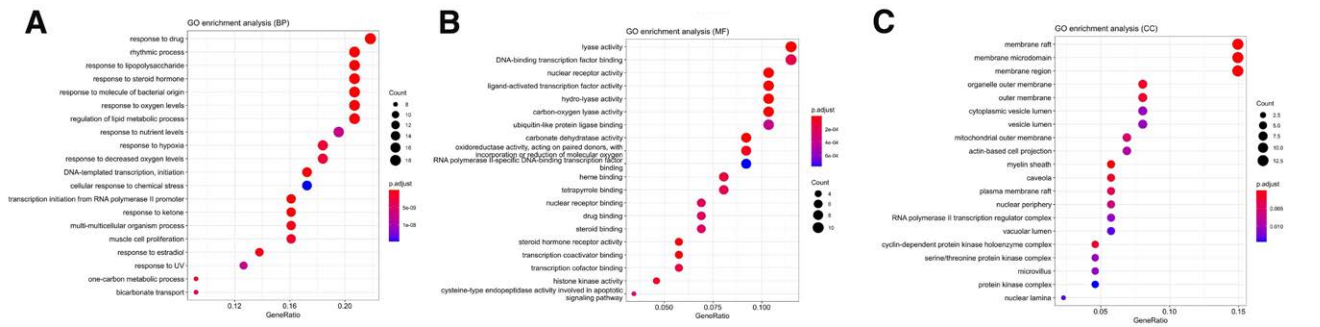

Figure 3. GO enrichment analysis pathway diagram. (A) Enrichment of GO biological process. (B) Enrichment of GO molecular function. (C) Enrichment of GO cellular component. GO = gene ontology.

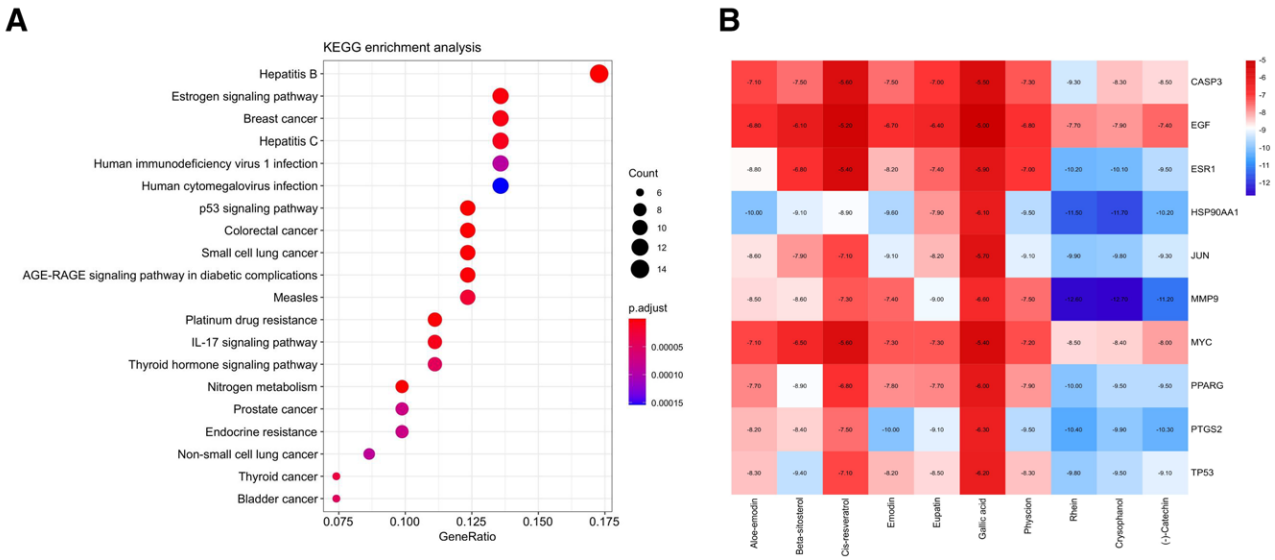

Figure 4. (A) KEGG pathway enrichment diagram. (B) Molecular docking heat map. KEGG = Kyoto Encyclopedia of Genes and Genomes.

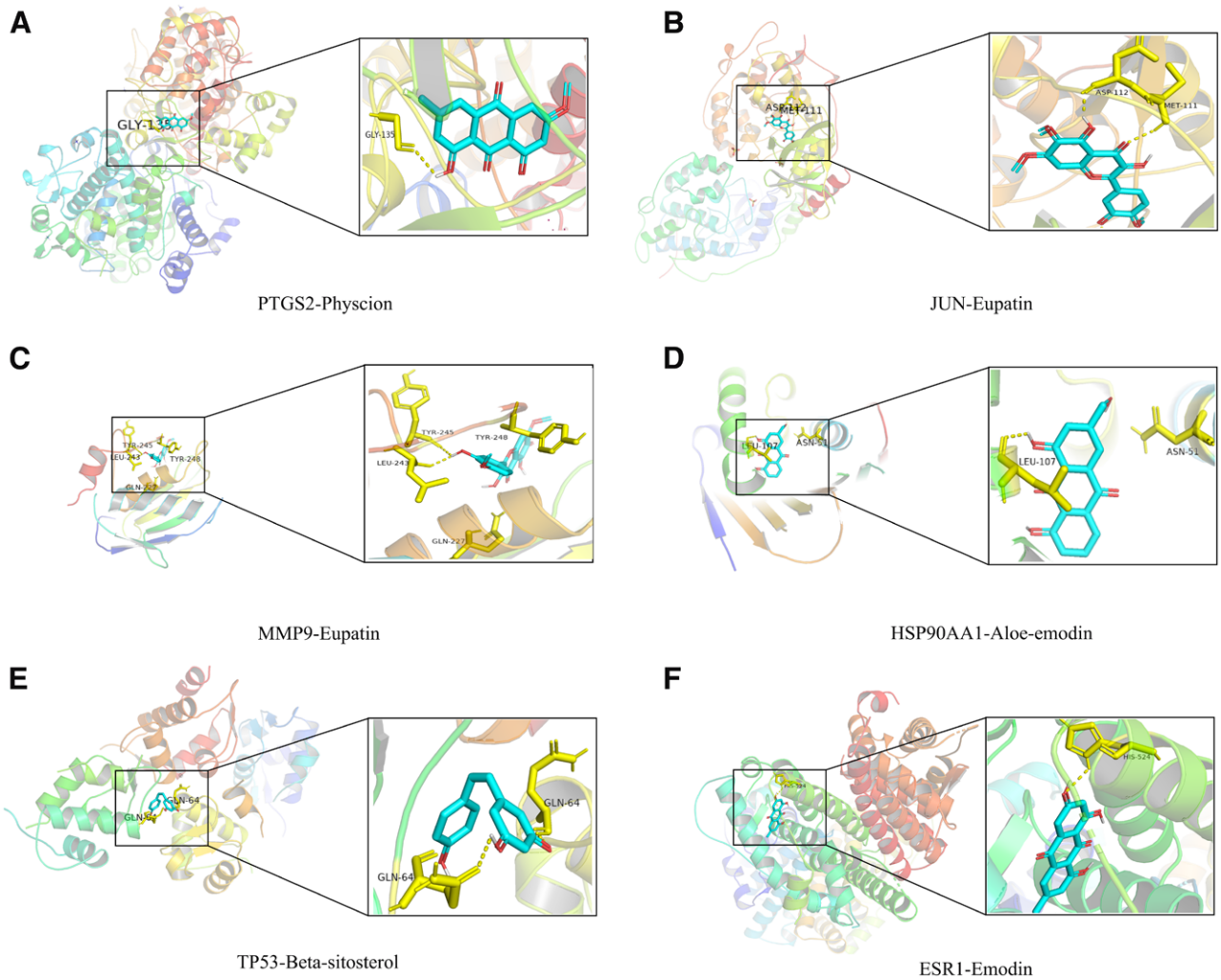

Figure 5. Schematic diagram of molecular docking.

restricting reactive oxygen species (ROS) accumulation and DNA damage in the colon.[45] The MYC gene encodes a transcription factor protein, which plays a role in regulating important processes such as cell growth, proliferation and apoptosis.[46] Studies have shown that the abnormal expression of MYC is closely linked to the development and prognosis of CRC, with high levels of MYC associated with increased tumor aggressiveness and metastasis.[47]

Based on the core targets, we have identified 10 key elements that are associated with them. These components can serve as the fundamental active ingredients in the treatment of CRC using the Rheum officinale pair. Studies have shown that emodin can inhibit the proliferation and invasion of CRC cells by targeting ACSL4, as well as reduce the secretion of VEGF and the expression of VEGFR1 and VEGFR2.[48] Furthermore, it can also inhibit cancer cells growth by regulating the Bcl-2/Bax ratio, reducing M2-like macrophages and affecting mitochondrial apoptosis pathway.[49,50] Beta-Sitosterol inhibits the metastasis of CT26/luc colon cancer by inhibiting MMP-9 and inducing an anti-tumor Th1 immune response.[51] Moreover, beta-sitosterol can induce the apoptosis of CRC cells by promoting the production of short-chain fatty acids by intestinal flora.[52] Gallic acid can inhibit the progression of CRC by binding to targets associated with iron death and regulating the expression of the corresponding protein.[53] The experimental results also shown that gallic acid exhibits obvious inhibitory activity on CRC cells and can inhibit the growth of CRC cells by disrupt mitochondrial function.[54] Aloe-emodin could induce the production of ROS to improving apoptotic. In rectal cancer cells, aloe-emodin can generate ROS and increase the level of cytoplasmic Ca^{2+}, which leading to elevated ER stress and the expression level of apoptosis-related caspase protein.[55] Resveratrol activates tristetraprolin, upregulates the expression of miR-200c in CRC, and inhibits the proliferation and metastasis of CRC cells.[56] Eupatin significantly inhibits the expression of iNOS and COX-2 in LPS-induced macrophages and microglia, as well as the production of nitrite, demonstrating a notable anti-inflammatory effect and protection of the intestinal microenvironment from inflammation.[57] And the *R palmatum* L was applied in CRC.

GO enrichment analysis reveals that the treatment of CRC with Rheum officinale primarily involves the regulation of nutrients, vascular lesions, and oxidative substance metabolism. This process is influenced by various organelles, hormone levels, and steroid metabolism, and encompasses multiple pathways associated with inflammation and metabolic processes. These finding indicate that TCMs achieve therapeutic effects on CRC through multiple components, targets, pathways, and biological mechanisms. KEGG enrichment analysis identifies the p53 signaling pathway is the core signaling pathway of rhubarb in the treatment of colorectal cancer. p53, a tumor suppressor, plays a crucial role in tumor occurrence and development, involving to regulating of cell cycle arrest, DNA repair, antioxidant effect, anti-angiogenesis effect, metabolism, autophagy, aging and apoptosis, among others.[58] p53 and its pathway-related

genes p16 and p21 are involved in cell cycle checkpoint regulation affecting the cell cycle process by influencing CyclinD1/Cyclin-dependent kinases4 genes.[59] Advanced glycation end products (AGE) and its receptor (RAGE) was associated with various pathological conditions, such as diabetes, cancer.[60] Some research reported that AGE are possibly involved in colorectal carcinogenesis, and AGE-RAGE signaling could considerably influence apoptosis, autophagy, and necroptosis.[60,61] IL-17, a pleiotropic proinflammatory cytokine, prevent cancer cells from immune surveillance and regulate tumor angiogenesis, which play an important role in metastasis and prognosis of CRC.[62,63] And its signaling pathway could promote tumor growth. In addition, activation of the IL-17 signaling pathway by the CXCL17-GPR35 axis affects drug resistance and colorectal cancer tumorigenesis.[64,65]

In this study, although targets and signaling pathways of *Rheum* officinale for CRC were potentially identified, there are certain limitations that need to be acknowledged. These limitations are as follows: Firstly, as the database slowly update, the data in this article may not timely updated; Second, the network pharmacology were used for the prediction of the target and mechanism, but database cannot replace the experimental verification. In this study, the components of *Rheum* and the targets of CRC was verified using molecular docking, but still lack of the verification by experiments.

5. Conclusions

In conclusion, the integration of network pharmacology and molecular docking revealed that the function of *Rheum* officinale was related to the core targets such as TP53, EGF, MYC, CASP3, JUN, PTGS2, HSP90AA1, MMP9, and others. The active ingredients mainly regulate BPs related to the response to lipopolysaccharides, drugs, and steroid hormone levels for CRC. The main pathways involved in the modulation include AGE-RAGE signaling pathway, estrogen signaling pathway, IL-17 signaling pathway and the TP53 signaling pathway, which are crucial for CRC treatment. In this study, This comprehensive analysis of the multi-component, multi-target, functional, and signaling pathway of Rheum officinale provides valuable insights for CRC treatment and can serve as a reference in clinic.

Author contributions

Conceptualization: Keyuan Xiao.
Data curation: Xinghua Li, Jiao Zhang.
Formal analysis: Yun Ren.
Funding acquisition: Keyuan Xiao.
Visualization: Xinghua Li.
Writing – original draft: Fan Yang, Xinghua Li, Keyuan Xiao.
Writing – review & editing: Fan Yang, Xinghua Li, Yujie Zhang, Yun Ren, Jiao Zhang, Keyuan Xiao.

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