Clinical Trial/Experimental Study

Medicine

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Exploring the mechanism of Tengli Kangliu Decoction in the prevention and treatment of colorectal cancer precancerous based on network pharmacology

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Abstract

Objective: This study aimed to predict the targets and signaling pathways affected by Tengli Kangliu Decoction (TKD) in the treatment of colorectal cancer (CRC) precursor lesions and to determine TKDs mechanism of action based on previous experimental results using network pharmacology techniques and methods.

Methods: Using the traditional Chinese medicine systems pharmacology database (TCMSP) and UniProt database, the active ingredients and potential targets of TKD were identified. Human colorectal adenoma (CRA) targets were analyzed using the GeneCards database, the Online mendelian inheritance in man (OMIM) database, and the NCBI database. The common targets of drug-disease interactions were input into the String database to construct a protein–protein interaction (PPI) network. These data were then used to construct the network diagram. Gene ontology (GO) function analysis and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis were performed on the target genes. Finally, the component-disease-pathway-target network file was imported into Cytoscape 3.8.0 and used to construct the pathway network diagram.

Results: Compounds with a drug-likeness (DL) score ≥ 0.18 and an oral bioavailability (OB) ≥ 30% were selected as the active constituents of TKD. Two hundred eighty eight chemical constituents were screened and 305 chemical drug targets were predicted. After further screening, 1942 disease-related targets, which are hypothesized to be the main chemical components of TKD, were obtained. When comparing the targets of action and CRA treatment targets, 172 common targets were identified. Using GO enrichment analysis of common targets of drug diseases, 2550 biological processes (BP) were predicted, 164 items of which were related to molecular functioning (MF), and 67 items related to cell composition. KEGG pathway analysis was performed on the common targets of drug diseases, and a total of 178 signaling pathways were enriched.

Conclusion: Using network pharmacology research, this study reports on the synergistic effect of the multiple components of TKD on the multi-target, and multiple pathways of colorectal precancerous lesions. These findings lay a theoretical foundation for further colorectal precancerous lesions research.

Abbreviations: BP = biological processes, CRA = colorectal adenoma, CRC = colorectal cancer, DL = drug-likeness, GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes, MCODE = molecular complex detection, MF = molecular functioning, OB = oral bioavailability, OMIM = Online mendelian inheritance in man, PPI = protein–protein interaction, TCMSP = traditional Chinese medicine systems pharmacology database, TKD = Tengli Kangliu Decoction.

Keywords: colorectal adenomas, network pharmacology, precancerous lesions, Tengli Kangliu Decoction

FL and BC contributed equally to this work.

This study was supported by the Funds for Guiyang Science and technology project (Zhu Ke He Tong[2019]9-2-16); The project of Guizhou Administration of Traditional Chinese Medicine(QZYY2017-105); Project supported by Guizhou University of Traditional Chinese Medicine (Gui Zhong Yi Ke Yuan Nei [2017] 01).

The authors have no conflict of interests to disclose.

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

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How to cite this article: Liu F, Cao B, Zhang H, Zou Q, Liu G, Dong Y, Su D, Ren D-l. Exploring the mechanism of Tengli Kangliu Decoction in the prevention and treatment of colorectal cancer precancerous based on network pharmacology. Medicine 2022;101:46(e31690).

Received: 27 May 2022 / Received in final form: 15 October 2022 / Accepted: 17 October 2022

http://dx.doi.org/10.1097/MD.0000000000031690

1. Introduction

Colorectal cancer (CRC) is a serious threat to human health and survival. In recent years, the incidence of CRC is on the rise. Further, CRC research is a hotspot that attempts to provide solutions to difficulties experienced in the medical field. In the United States alone, approximately 150,000 patients were diagnosed with CRC in 2019, of which, approximately 50,000 died due to tumor progression.[1] Globally, CRC is the third most common malignant tumor and the second leading cause of cancer death.[2] In 2021, in the United States, the incidence of CRC accounted for 8% of the cancer cases, with a similar incidence and mortality observed in men and women.[3] The pathology and development of CRC is a continuous and progressive process (Fig. 1A). A colorectal adenoma (CRA) is recognized as a precancerous lesion of colon cancer. Relevant data indicate that more than 90% of CRC can develop from a colonic polyp, among which, CRA has a higher risk of malignancy.[4,5] It takes 5 to 10 years for a CRA to develop into CRC. Therefore, the prevention and treatment of clinically active CRA are of great significance to prevent the occurrence and development of CRC.

The idea of "preventing disease" in traditional Chinese medicine (TCM) agrees with the idea of disease prevention in western medicine; both of which have shown great value in the prevention and control of CRC. Previous studies have shown that TCM has great advantages in the prevention and control of colorectal precancerous lesions and reducing the incidence of CRC based on the theoretical guidance of prevention before disease.[6] The effect of traditional Chinese medicine on colorectal precancerous lesions is mainly due to its multi-component, multi-target, and multi-channel synergistic activity. Thus, through multi-channel, multi-step, and multi-stage intervention it may be possible to inhibit or delay the onset of cancer.

Based on the concept of "disease-gene-target-drug" multi-level and multi-angle interaction networks, network pharmacology is a new strategy used to study the multi-molecule synergistic effects of traditional Chinese medicine ingredient-targets interactions at the system level. The concept of systematically and comprehensively observing the intervention and influence of drugs on disease networks has been widely used in the study of traditional Chinese medicine mechanisms. Previously, we showed that "Tengli Kangliu Decoction" (TKD), a traditional Chinese medicinal concoction, has a significant beneficial effect in the treatment of colorectal precancerous lesions[7] (Fig. 1B); however, the mechanism of action was unclear. Therefore, based on previous network pharmacology experiments, the current study aims to further explore the mechanism of TKD on precancerous lesions of CRC.

2. Materials and methods

2.1. Screening of the active ingredients and corresponding targets of TKD

The active ingredients and their corresponding targets of TKD were obtained from the database of Traditional Chinese Medicine system pharmacology (TCMSP,http://tcmspw.com/tcmsp.php).[8] Using "Tengligen," "Baihuasheshecao," "Banzhilian," "Chenpi," "Chishao," "Danshen," "Danggui," "Dangshen," "Fuling," "Fuzi," "Huangqi," "Jiezi," "Shanzha," "Shanzhuyu," "Shudi," "Tusizi," "Wumei," "Wuweizi," and "Zexie" as the keywords, data pertaining to the active ingredients of TKD were retrieved and screened by the pharmacokinetic parameters. According to the recommendations detailed by the TCMSP, the oral bioavailability (OB) and drug-likeness (DL) values were used as the indexes for screening the compounds of TKD. Compounds with DL ≥ 0.18 and OB ≥ 30% were selected

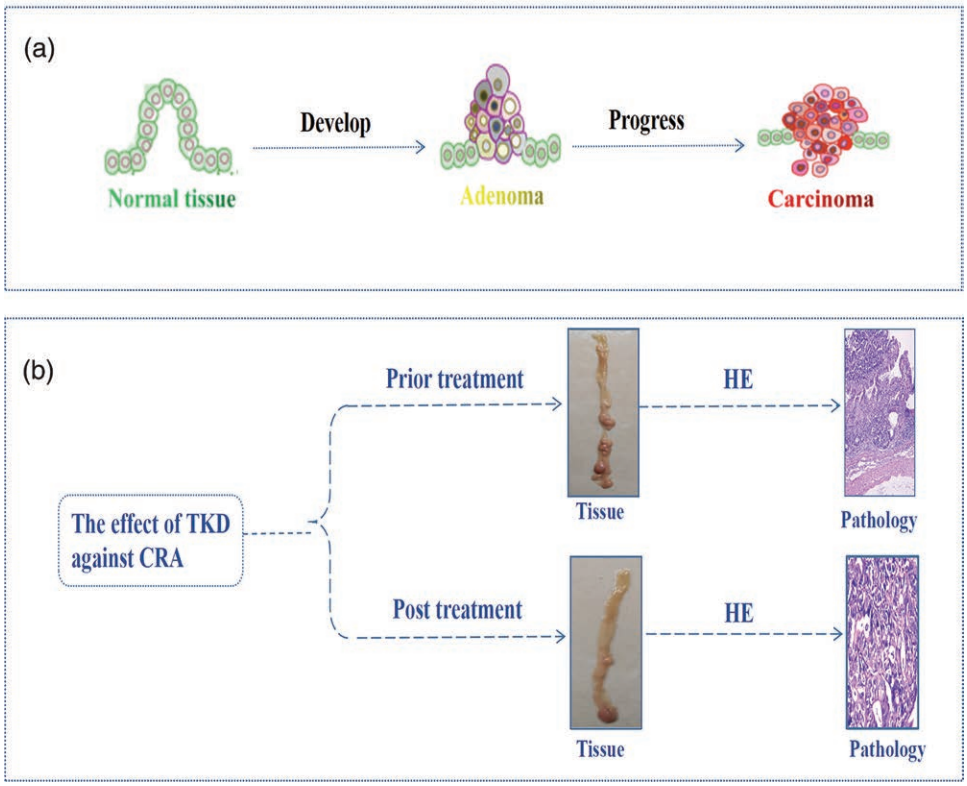

Figure 1. (A) The development of colorectal cancer (CRC). (B) The effect of Tengli Kangliu Decoction (TKD) on prevention and treatment of colorectal adenomas (CRA).

as the active constituents of TKD. Further, the CNKI database (1989-2021) was used to supplement and improve these findings. Based on the Mol ID of the ingredients in the TCMSP database, potential protein targets of chemical constituents were predicted. Using the UniProt database (https://www.uniprot.org),[9] the chemical component target protein name target name was used to identify the corresponding gene name.

2.2. Screening and sorting of CRA targets

Using "colorectal adenoma" and "colorectal adenomas" as the keywords to interrogate the GeneCards[10] database (https://www.genecards.org/),the Online mendelian inheritance in man (OMIM) database[11] (https://www.omim.-org), and the NCBI database[12] (https://www.ncbi.nlm.nih.gov), gene targets for human adenoma were assessed. The target score of the results retrieved from the GeneCards database was screened using the median value to obtain more relevant targets.

2.3. Target prediction of TKD in the treatment of CRA

The screened drug target data and disease target data were imported into the Venny 2.1 software package, and a Venn diagram was drawn. The intersection of the drug-target and disease-target data was used to obtain common targets, which was then used to predict the drug target of CRC.

2.4. Protein–protein interaction (PPI) network construction and key targets screening

The common targets of TKD and CRA were inputted into the String database[13] (https://string-db.org/cgi/input.pl) and used for the construction of the Protein-Protein interaction network.The species was set as "*Homo sapiens*" and a confidence interval > 0.7 was used to generate the PPI network. The PPI network was then imported into Cytoscape 3.8.0.[14] Using the NetworkAnalyzer tool to perform topological analysis, targets were sorted by degree, and those with a score greater than the average score were determined as key targets. Key targets were then screened by molecular complex detection (MCODE) analysis.[15] The constructed PPI network was imported into Cytoscape 3.8.0, and the MCODE module was used for target cluster analysis and core target screening. To better understand the complex interaction between components, diseases, and corresponding targets, the component-disease-target network diagram was constructed.

2.5. Gene ontology (GO) enrichment analysis and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

The common targets of the drug-disease interaction as enriched by GO analysis included those involved in biological processes (BP), molecular functioning (MF), and cell components (CC). These targets were selected by referencing the String database and correcting the *P*-value < .05. KEGG enrichment analysis was utilized to assess signaling pathways enriched by the common targets of TKD and CRA. By referring to the String database, items with *P*-value < .05 were screened using R 4.0.3. All data were screened, using R 4.0.3 software. Specifically, the clusterProfiler, enrichplot, and ggplot2 packages were used to generate histograms and bubble charts.

2.6. Composition-disease-pathway-target network construction

The component-disease-pathway-target network file was imported into Cytoscape 3.8.0 and the network diagram was constructed. This strategy allowed for a more intuitive explanation of the characteristics of the multi-component-multi-target effect of TCM in the treatment of diseases. The specific design ideas of this study are shown in Figure 2.

3. Results

3.1. Screening and target prediction results of TKD

In this study, targets of the active ingredients of TKD were predicted using the TCMSP database. The 19 traditional Chinese medicines of TKD were characterized by OB ≥ 30% and DL ≥ 0.18, respectively. A total of 288 compounds were screened. Among them,"Tengligen 6," "Baihuasheshecao 7," "Banzhilian 29," "Chenpi 5," "Chishao 29," "Danshen 65," "Danggui 2," "Dangshen 21 ," "Fuling 15," "Fuzi 21," "Huangqi 20," "Jiezi 3," "Shanzha 6," "Shanzhuyu 20," "Shudi 2," "Tusizi 11," "Wumei 8," "Wuweizi 8," and "Zexie 10" were identified. The UniProt database was used to retrieve potential target information that corresponded to the active ingredients screened from the TCMSP database. Target gene names were standardized, and a total of 305 targets were predicted after they had been summarized and the [other repetitions genes] had been deleted.

3.2. Results of target screening for CRA

Using the keywords "colorectal adenoma" and "colorectal adenomas," the GeneCards[10] database (https://www.genecards.org/), OMIM database[11] (https://www.omim.org/), and the NCBI database[12] https://www.ncbi.nlm.nih.gov/) were searched for human adenoma targets. Using the target scores extracted from the GeneCards database, all potential hits were screened using the median value to obtain more relevant targets. After the removal of duplicates, a total of 1942 disease-related targets were obtained.

3.3. Prediction of therapeutic targets of TKD in patients with CRC

The screened TKD and CRA target data were imported into Venny 2.1. After the two datasets were intersected, 172 predicted targets for TKD were identified (Fig. 3).

3.4. PPI network construction and key target screening results

3.4.1. PPI network construction. To construct the PPI network, the shared targets of TKD and CRA were inputted into the String database. The species was set as Homo sapiens with a reliability score of >0.7 was used. One hundred seventy two nodes and 1673 edges were observed in the network, and the average degree value was 19.5. Within Figure 4, the PPI network diagram exported from the string website and the PPI network diagram drawn by the Cytoscape software is presented (Fig. 5A and B). The color and size of the nodes in Figure 4B were adjusted according to the degree value. The larger the shape and the intensity of the color indicate a large degree value. The thickness of the line (from thick to thin) indicates large to small

3.4.2. Screening of key targets based on PPI network. The PPI network was imported into Cytoscape 3.8.0 and topology analysis was conducted using the Network Analyzer tool. Targets with scores greater than average were selected as key targets by degree sorting. A total of 64 key targets were screened. The top 20 gene proteins were ranked by degree according to the target name, betweenness centrality, total centrality, clustering coefficient, degree, eccentricity, radiality, and topological coefficient (Table 1).

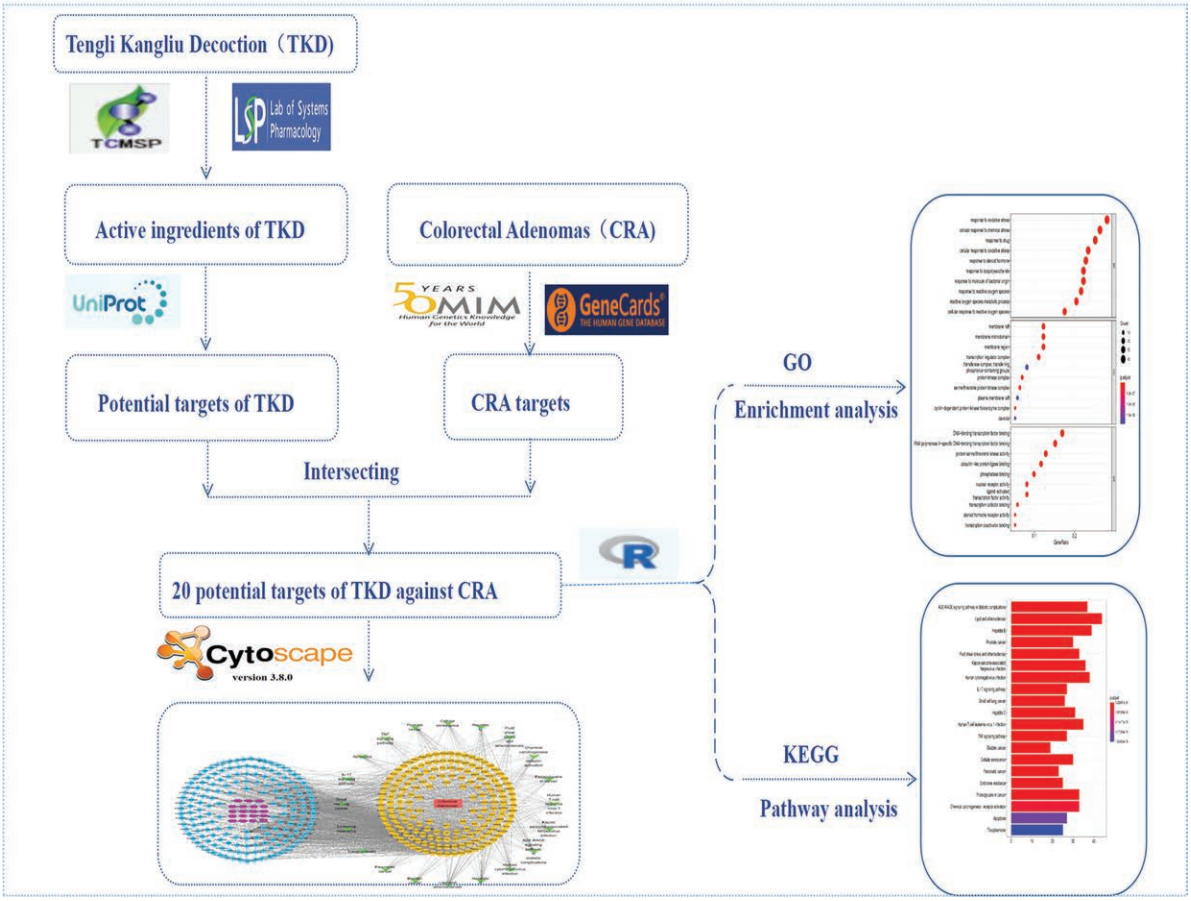

Figure 2. Research process.

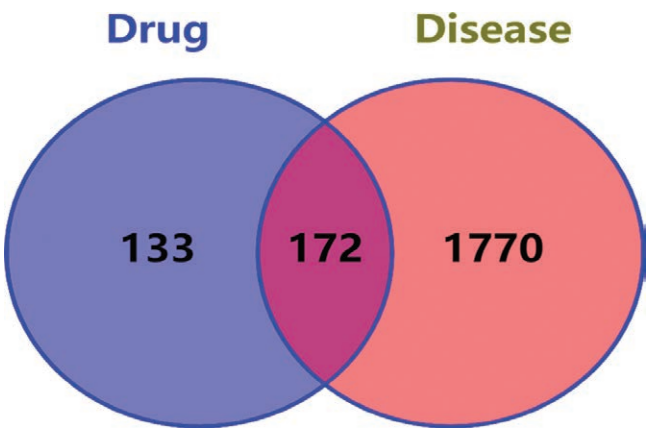

Figure 3. Intersection targets between Tengli Kangliu Decoction (TKD) and colorectal adenomas (CRA).

Additionally, the analysis of target clusters and re-screening of core targets was performed using the MCODE module. Using this method, a total of 8 target clusters and 7 core targets were obtained (Fig. 4). The core targets identified were SREBF1, ICAM1, AHR, FOS, SPP1, HIF1A, and PRKCD.

3.5. Component-disease target network construction

To better understand the complex interactions between the medicinal components, CRA, CRC, and the predicted drug targets, the component-disease-target network diagram was constructed (Fig. 6).

3.6. Key component screening

The composition-disease-target network diagram was imported into Cytoscape 3.8.0 for topology analysis and the components were ranked by degree. The higher the degree value was, the more important the components were determined to be (Table 2).

Using topological analysis, potential active components within TKD were predicted to be: quercetin, luteolin, kaempferol, wogonin, baicalein, baicalein, isorhamnetin, and sichuan chenpirin nobiletin, formononetin, sitosterol beta-sitosterol, naringenin or citrus peel naringenin, etc. The specific compound characteristics and structures are shown in Table 3. These active ingredients may play a relatively important role in the treatment of CRA, (Table 3).

3.7. GO enrichment analysis and KEGG pathway enrichment analysis

3.7.1. GO enrichment analysis. Using GO analysis, a total of 2550 BP were enriched, including 164 hits that were related to MF and 67 items related to cell composition (Fig. 7A and B).

3.7.2. KEGG pathway enrichment analysis. KEGG pathway enrichment analysis was performed on the common target genes of TKD and CRA. Results with a corrected *P*-value < .05 were screened using the String database and a total of 178 signal pathways were enriched (Table 4 and Fig. 8A and B).

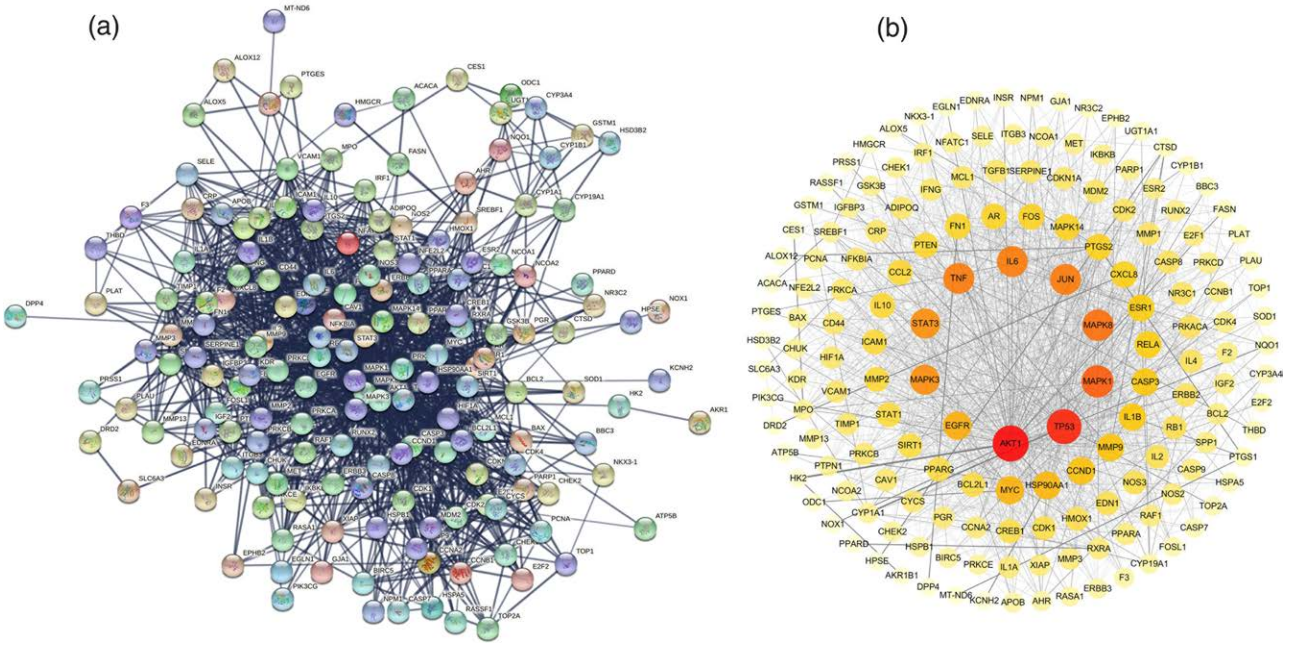

Figure 4. MCODE cluster analysis. MCODE = molecular complex detection.

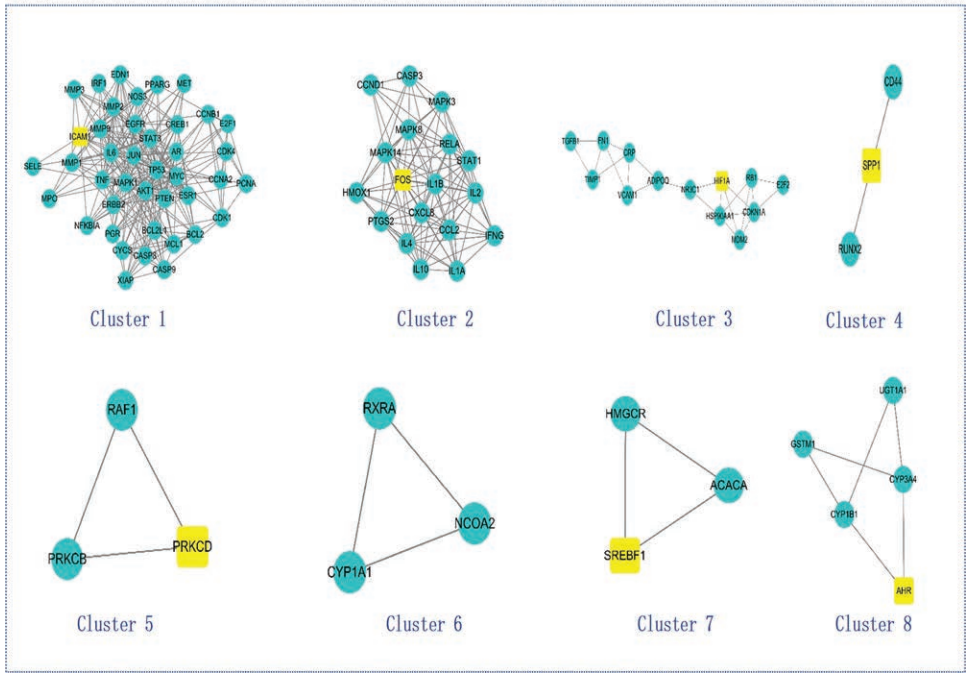

Figure 5. PPI network construction. PPI = protein–protein interaction.

3.8. Composition-disease-pathway-target network construction

The component-disease-pathway-target network file was imported into Cytoscape 3.8.0 and the path network diagram was constructed. This method allows for an intuitive demonstration of the multi-component, multi-pathway, and multi-target function characteristics of TKD active ingredients important in the treatment of CRA (Fig. 9).

4. Discussion

Globally, CRC is a significant contributor to cancer death. Further, due to the lack of effective therapeutic drugs, prevention remains the main strategy to reduce the incidence and mortality. CRC risk can be mitigated by avoiding known factors that increase the chance of developing CRC, or by targeting groups of subjects with a known risk of developing cancer. Early diagnosis of cancer or preneoplastic lesions and secondary

Table 1

Topological analysis of Tengli Kangliu Decoction (TKD) on colorectal adenomas (CRA) (Top 20).

Name	Betweenness centrality	Closeness centrality	Clustering coefficient	Degree	Eccentricity	Radiality	Topological coefficient
PTGS2	0.04155092	0.53594771	0.47948718	40	3	0.82682927	0.25376712
CXCL8	0.01873505	0.53074434	0.47804878	41	4	0.82317073	0.25077293
ESR1	0.02541646	0.55218855	0.38095238	43	3	0.83780488	0.22230722
RELA	0.00940484	0.5448505	0.42857143	43	3	0.83292683	0.24591452
IL1B	0.02022961	0.51735016	0.43333333	45	4	0.81341463	0.24006734
CASP3	0.01870111	0.55033557	0.36565657	45	3	0.83658537	0.22490679
MMP9	0.03429655	0.55218855	0.39323671	46	3	0.83780488	0.23266745
CCND1	0.01838555	0.54304636	0.386679	47	4	0.83170732	0.23345154
HSP90AA1	0.04440134	0.54849498	0.2542517	49	4	0.83536585	0.19723134
MYC	0.02667007	0.5559322	0.37714286	50	4	0.8402439	0.23238095
EGFR	0.0250921	0.56551724	0.31843137	51	4	0.84634146	0.21181631
STAT3	0.02120788	0.58156028	0.38717483	58	3	0.85609756	0.22765426
MAPK3	0.02411321	0.58571429	0.34180278	58	3	0.85853659	0.21473354
IL6	0.0309283	0.59636364	0.37652036	62	3	0.86463415	0.21789802
TNF	0.03628645	0.58992806	0.35854045	62	3	0.86097561	0.21402801
JUN	0.02411353	0.58781362	0.36805923	62	3	0.8597561	0.22377697
MAPK8	0.03726901	0.59205776	0.28701923	65	4	0.86219512	0.20050942
MAPK1	0.04045562	0.6119403	0.31457801	69	3	0.87317073	0.2059841
TP53	0.10578016	0.64313725	0.25308642	81	3	0.88902439	0.18067426
AKT1	0.14143163	0.65863454	0.20956963	87	3	0.89634146	0.17069712

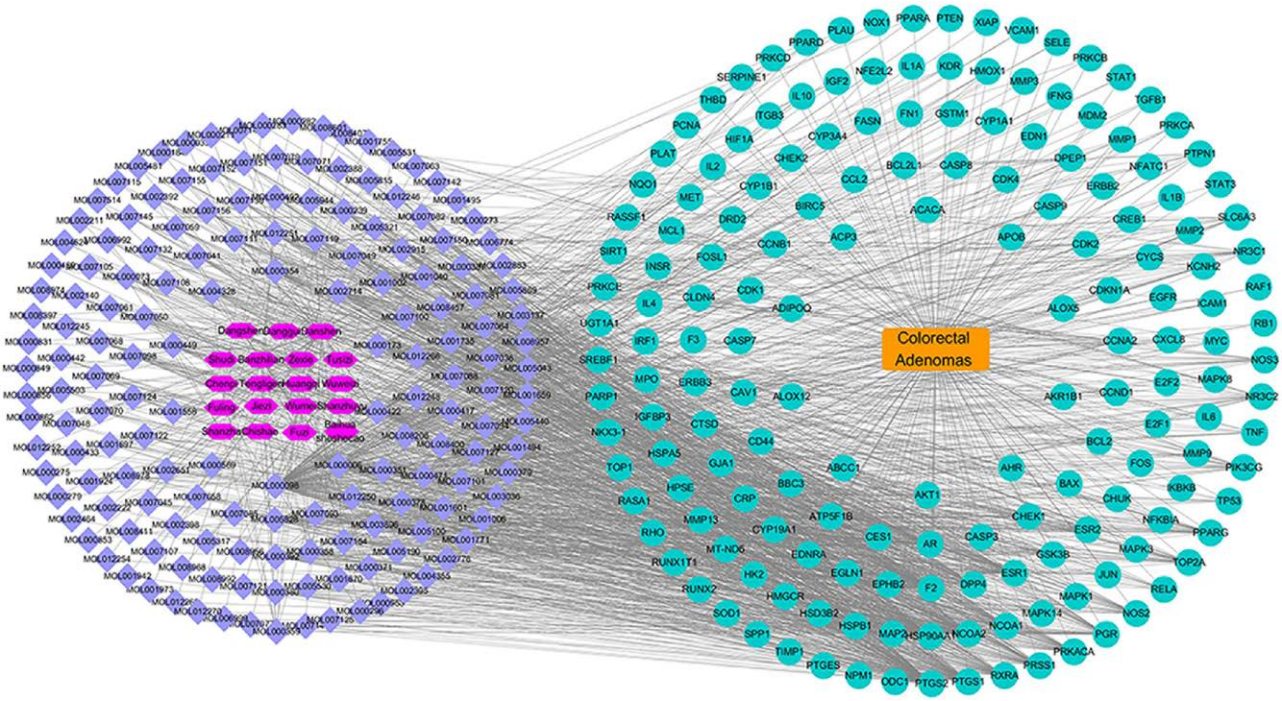

Figure 6. Component-disease target network construction. Note: In the network, the light blue is the active ingredient, the green is the target of the disease, the orange rectangle is the disease, and the purple is the drug.

prevention of intervention is essential to preventing the onset of disease.[16] The intervention study of TKD on CRA in this study belongs to the secondary prevention of CRC.

TKD is a prescription for personal experience summarized by Professor Renjie Shi, a famous traditional Chinese medicine doctor in Jiangsu Province. A CRA is a precancerous lesion of CRC with complex etiology. CRC pathogenesis is characterized by condensation or aggregation of phlegm, dampness, blood stasis, and poison due to a deficiency of positive qi. TKD is used for the treatment of several diseases and has heat-clearing, detoxification, and anti-tumor activity. TKD utilizes Teng ligen as the main medicinal component; however, the medicine is often supplemented with Huangqi, Danggui, Dangshen, Fuzi, Huangqi, Shanzhuyu, Shudi, Tusizi, Wuweizi, Yiqi yangxue, nourishing yin and tonifying yang, and fuzheng Guyuan. Additionally, "Baihuasheshecao," "Banzhilian, "Chishao," "Danshen"Jiezi," and "Wumei" are often used as adjuvants. The medicinal decoction is hypothesized to activate blood circulation, remove blood stasis, play a role in detoxication, activate collaterals, and eliminate stagnation. Further, "Chenpi ," "Fuling," "Zexie ," "Shanzha are believed to dissolve phlegm and dampness, eliminate lead stagnation, anti-greasy drugs and hinder gas. Together, these drugs strengthen solid yuan, resolving phlegm, dampness and stasis, detoxification, and sanjie. Thus, the same compounds may play a role in the prevention and treatment of CRA. Our previous studies have confirmed that TKD can reduce the recurrence of human CRAs after surgery and inhibit colorectal precancerous lesions in C57BL/6

Table 2

The active ingredients information of Tengli Kangliu Decoction (TKD) (Top 20).

Name	MOL ID	Average shortest path length	Betweenness centrality	Closeness centrality	Degree
Quercetin	MOL000098	1.985955	0.136921	0.503536	114
Luteolin	MOL000006	2.345506	0.035808	0.426347	51
Kaempferol	MOL000422	2.379213	0.025487	0.420307	42
Wogonin	MOL000173	2.530899	0.013568	0.395117	36
Baicalein	MOL002714	2.508427	0.014771	0.398656	30
Isorhamnetin	MOL000354	2.508427	0.008327	0.398656	28
Nobiletin	MOL005828	2.530899	0.011128	0.395117	28
Formononetin	MOL000392	2.620787	0.00823	0.381565	27
Beta-sitosterol	MOL000358	2.514045	0.015149	0.397765	25
Naringenin	MOL004328	2.69382	0.009073	0.37122	23
Tanshinone iia	MOL007154	2.66573	0.007404	0.375132	23
7-O-methylisomucronulatol	MOL000378	2.570225	0.003442	0.389071	22
7-Methoxy-2-methyl Isoflavone	MOL003896	2.575843	0.00397	0.388222	22
Stigmasterol	MOL000449	2.514045	0.017007	0.397765	20
Aloe-emodin	MOL000471	2.643258	0.006083	0.378321	20
Dan-shexinkum d	MOL007093	2.564607	0.00313	0.389923	20
7-Hydroxy-5,8-dimethoxy-2-phenyl-chromone	MOL012250	2.626404	0.002421	0.380749	19
Rhamnazin	MOL000351	2.564607	0.003272	0.389923	18
Calycosin	MOL000417	2.603933	0.002236	0.384035	18
Moslosooflavone	MOL008206	2.643258	0.002062	0.378321	18

Table 3

The active component information and chemical structure of Tengli Kangliu Decoction (TKD) (Top 10).

Compound name	Molecule ID	PubChem CID	Molecular formula	Chemical structural
Quercetin	MOL000098	5280343	$C_{15}H_{10}O_7$	
Luteolin	MOL000006	5280445	$C_{15}H_{10}O_6$	
Kaempferol	MOL000422	5280863	$C_{15}H_{10}O_6$	
Wogonin	MOL000173	5281703	$C_{16}H_{12}O_5$	
Baicalein	MOL002714	5281605	$C_{15}H_{10}O_5$	
Isorhamnetin	MOL000354	5281654	$C_{16}H_{12}O_7$	
Nobiletin	MOL005828	72344	C21H2208	
Formononetin	MOL000392	5280378	C16H1204	
Beta-sitosterol	MOL000358	222284	C29H500	
Naringenin	MOL004328	439246	C15H1205	

mice.[7] Together these lines of evidence suggest that TKD may be beneficial in CRC prevention.

In 1959, the use of Teng Ligen in TCM was first published the "Henan Chinese Herbal Medicine Handbook."In the handbook, Teng Ligen roots and root bark are described to be cold in nature and bitter in taste. Teng Ligen is a commonly used CM with anti-tumor activity. Additionally, it is used to clear away heat and for detoxification. It is endemic to and widely distributed throughout China. Modern pharmacological studies have found that that the roots of Teng Ligen mainly contain triterpenes, flavonoids, anthraquinones, steroids, and other active ingredients, which have anti-tumor, antioxidant, liver protection, and hypoglycemic effects.[17] Several studies have also shown that Teng Ligen has an inhibitory effect on a variety of tumors. For example, Zhao Chen Hui et al found that Teng Ligen ethanol extract can inhibit the proliferation and invasion of non-small cell lung cancer (NSCLC) A549 cells through the Mir-148B-3p/HSPA4L axis.[18] Additionally, Ye Huirong et alfound that an n-butanol Teng Ligen extract inhibited the phosphorylation in the PI3K/Akt-mTOR signaling pathway, and up-regulated autophagy and apoptosis in breast cancer cells (MDA-MB-231).[19] Further, this extract was also able to inhibit cell proliferation.[20] In their review on the active components and anti-tumor effects of Teng Ligen over 10 years, Yang Zihua et al proposed that Teng Ligen plays a key role in tumor proliferation, apoptosis, infiltration, and metastasis through a variety of mechanisms and pathways.[20] Furthermore, Teng Ligen has a synergistic or even reversal effect with western medicine.

In clinical settings, the multi-component, multi-channel, multi-target synergistic effect of traditional Chinese medicine is hypothesized to be better than single-target chemical drugs with large side effects and poor efficacy.[21] However, due to the paucity of sufficiently powered clinical trials as well as convincing mechanistic data, the modernization of Chinese medicine has been impaired. In recent years, the emergence of network pharmacology has provided new insights to clarify the mechanisms of action of traditional Chinese medicine. Network pharmacology is a research method based on "disease-gene-target-protein-drug" interaction networks. This strategy systematically and comprehensively interrogates the intervention and influence of drugs on disease networks, thereby revealing the effect of drugs and target proteins on the disease.[22] Furthermore, in biomedicine, network pharmacology reflects the trend of systematic research in the era of big data and adapts to the urgent need for systematic research methods in traditional Chinese medicine. Therefore, network pharmacology has become a cutting-edge analytical strategy and a hot spot in the field of medicine. This fact is especially true in the field of traditional Chinese medicine research as network pharmacology is expected to be a key tool used to bridge Chinese and Western medicine.[23] Traditional Chinese medicine has a multi-target therapeutic mechanism and has great potential for the treatment of human cancer in the future.

Using PPI network analysis to assess the 10 core targets for drug-diseases interactions, AKT1, TP53, MAPK1, MAPK8, JUN, IL6, TNF, STST3, MAPK3, EGFR, etc. AKT, also known

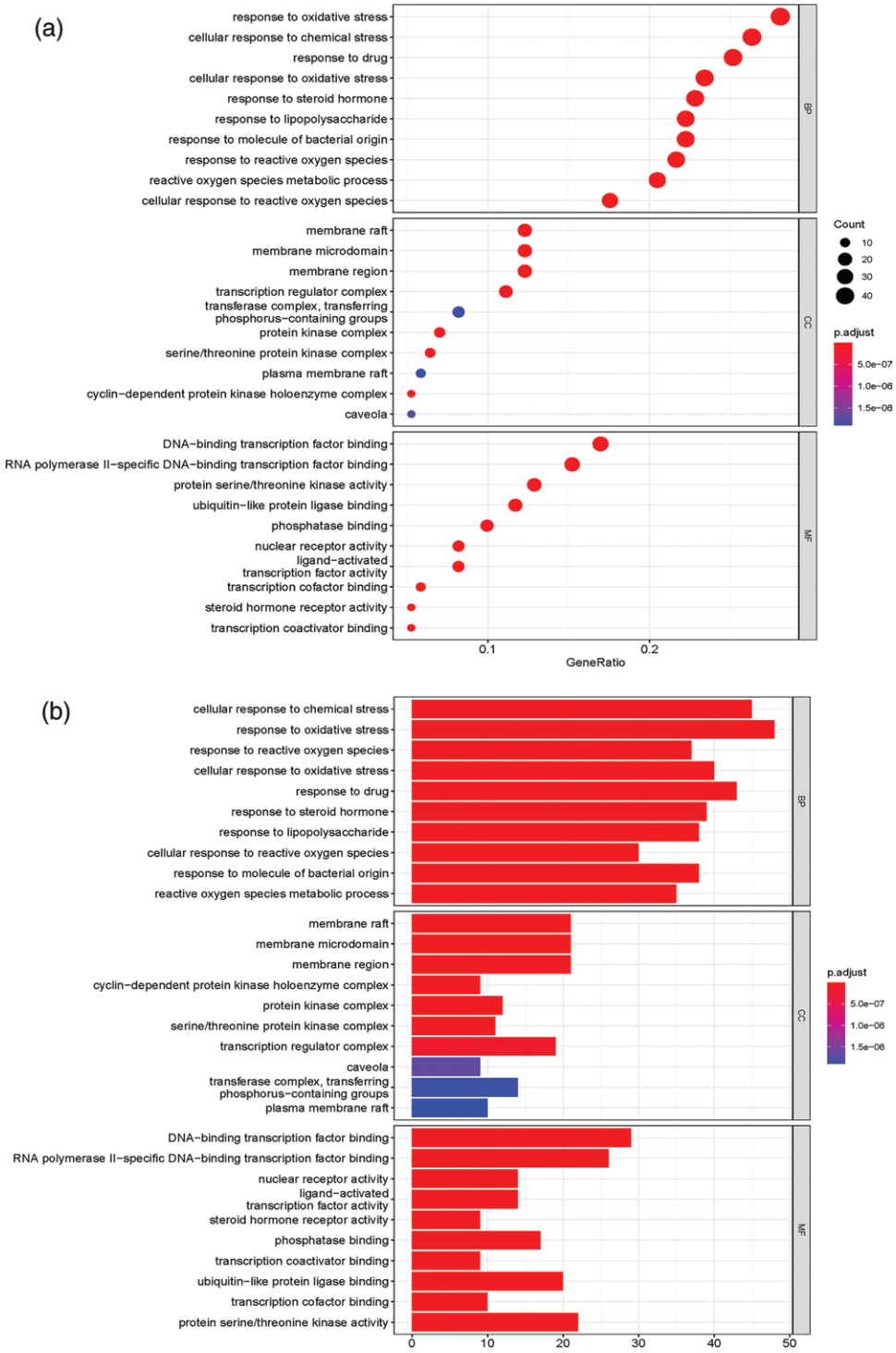

Figure 7. GO enrichment. GO = gene ontology.

as protein kinase B (PKB), is a serine/threonine kinase that participates in a variety of important cell signal transduction pathways, including cell survival, proliferation, invasion, apoptosis, and angiogenesis were observed.[24] Akt is a key effector in the PI3K/AKT1 mTOR signaling pathway, and its abnormal regulation plays an important role in the pathogenesis of many human tumors. Akt has three subtypes in mammals: Akt1, Akt2, and Akt3. Akt1 is one of the most frequently mutated subtypes of the AKT protein. Akt1 can inhibit apoptosis and promote cell growth, which not only affects the proliferation and apoptosis of tumor cells but also has an important impact on tumor invasion and metastasis.[25] The TP53 gene is located on the short arm of chromosome 17 (17p13). Previous studies have shown that exons 5-8 are tumor hotspot mutations. The DNA binding domain, encoding exons 5-8 of the TP53 gene, consists of 102-292 residues. In the DNA binding domain, the L2 loop (residues 163-195) and the L3 loop (residues 236-251) bind to the Zn atom and play a key role in DNA interaction. As one of the most common gene mutations[26] in most human tumors, the existence of TP53 gene mutation is often observed. Mitogen-activated protein kinase 1 (MAPK1) is an important protein in MAPK signal transduction. It causes MAPK inactivation by

Table 4

The top 20 significant enriched KEGG pathways for intersection target.

Description	P value	Count	GeneID
AGE-RAGE signaling pathway in diabetic complications	3.53E-38	37	AKT1/BAX/BCL2/CASP3/CCL2/CCND1/CDK4/CXCL8/EDN1/F3/FN1/ICAM1/IL1A/IL1B/IL6/JUN/MAPK1/MAPK14/MAPK3/MAPK8/MMP2/NFATC1/NOS3/NOX1/PRKCA/PRKCB/PRKCD/PRKCE/RELA/SELE/SERPINE1/STAT1/STAT3/TGFB1/THBD/TNF/VCAM1
Lipid and atherosclerosis	6.20E-33	44	AKT1/APOB/BAX/BCL2/BCL2L1/CASP3/CASP7/CASP8/CASP9/CCL2/CHUK/CXCL8/CYCS/CYP1A1/FOS/GSK3B/HSP90AA1/HSPA5/ICAM1/IKBKB/IL1B/IL6/JUN/MAPK1/MAPK14/MAPK3/MAPK8/MMP1/MMP3/MMP9/NFATC1/NFE2L2/NFKBIA/NOS3/NOX1/PPARG/PRKCA/RELA/RXRA/SELE/STAT3/TNF/TP53/VCAM1
Hepatitis B	5.07E-32	39	AKT1/BAX/BCL2/BIRC5/CASP3/CASP8/CASP9/CCNA2/CDK2/CDKN1A/CHUK/CREB1/CXCL8/CYCS/E2F1/E2F2/FOS/IKBKB/IL6/JUN/MAPK1/MAPK14/MAPK3/MAPK8/MMP9/MYC/NFATC1/NFKBIA/PCNA/PRKCA/PRKCB/RAF1/RB1/RELA/STAT1/STAT3/TGFB1/TNF/TP53
Prostate cancer	3.12E-28	30	AKT1/AR/BCL2/CASP9/CCND1/CDK2/CDKN1A/CHUK/CREB1/E2F1/E2F2/EGFR/ERBB2/GSK3B/HSP90AA1/IKBKB/MAPK1/MAPK3/MDM2/MMP3/MMP9/NFKBIA/NKX3-1/PLAT/PLAU/PTEN/RAF1/RB1/RELA/TP53
Fluid shear stress and atherosclerosis	7.38E-27	33	AKT1/BCL2/CAV1/CCL2/CHUK/EDN1/FOS/GSTM1/HMOX1/HSP90AA1/ICAM1/IFNG/IKBKB/IL1A/IL1B/ITGB3/JUN/KDR/MAPK14/MAPK8/MMP2/MMP9/NFE2L2/NOS3/NOX1/NQO1/PLAT/RELA/SELE/THBD/TNF/TP53/VCAM1
Kaposi sarcoma-associated herpesvirus infection	2.32E-25	36	AKT1/BAX/CASP3/CASP8/CASP9/CCND1/CDK4/CDKN1A/CHUK/CREB1/CXCL8/CYCS/E2F1/E2F2/FOS/GSK3B/HIF1A/ICAM1/IKBKB/IL6/JUN/MAPK1/MAPK14/MAPK3/MAPK8/MYC/NFATC1/NFKBIA/PIK3CG/PTGS2/RAF1/RB1/RELA/STAT1/STAT3/TP53
Human cytomegalovirus infection	4.00E-25	38	AKT1/BAX/CASP3/CASP8/CASP9/CCL2/CCND1/CDK4/CDKN1A/CHUK/CREB1/CXCL8/CYCS/E2F1/E2F2/EGFR/GSK3B/IKBKB/IL1B/IL6/ITGB3/MAPK1/MAPK14/MAPK3/MDM2/MYC/NFATC1/NFKBIA/PRKACA/PRKCA/PRKCB/PTGS2/RAF1/RB1/RELA/STAT3/TNF/TP53
IL-17 signaling pathway	1.77E-24	27	CASP3/CASP8/CCL2/CHUK/CXCL8/FOS/FOSL1/GSK3B/HSP90AA1/IFNG/IKBKB/IL1B/IL4/IL6/JUN/MAPK1/MAPK14/MAPK3/MAPK8/MMP1/MMP13/MMP3/MMP9/NFKBIA/PTGS2/RELA/TNF
Small cell lung cancer	2.16E-23	26	AKT1/BAX/BCL2/BCL2L1/CASP3/CASP9/CCND1/CDK2/CDK4/CDKN1A/CHUK/CYCS/E2F1/E2F2/FN1/IKBKB/MYC/NFKBIA/NOS2/PTEN/PTGS2/RB1/RELA/RXRA/TP53/XIAP
Hepatitis C	1.21E-22	31	AKT1/BAX/CASP3/CASP8/CASP9/CCND1/CDK2/CDK4/CDKN1A/CHUK/CLDN4/CYCS/E2F1/E2F2/EGFR/GSK3B/IFNG/IKBKB/MAPK1/MAPK3/MYC/NFKBIA/PPARA/RAF1/RB1/RELA/RXRA/STAT1/STAT3/TNF/TP53
Human T-cell leukemia virus 1 infection	2.66E-22	35	AKT1/BAX/BCL2L1/CCNA2/CCND1/CDK2/CDK4/CDKN1A/CHEK1/CHEK2/CHUK/CREB1/E2F1/E2F2/FOS/FOSL1/ICAM1/IKBKB/IL2/IL6/JUN/MAPK1/MAPK3/MAPK8/MYC/NFATC1/NFKBIA/PRKACA/PTEN/RB1/RELA/TGFB1/TNF/TP53/XIAP
TNF signaling pathway	3.08E-22	27	AKT1/CASP3/CASP7/CASP8/CCL2/CHUK/CREB1/EDN1/FOS/ICAM1/IKBKB/IL1B/IL6/IRF1/JUN/MAPK1/MAPK14/MAPK3/MAPK8/MMP3/MMP9/NFKBIA/PTGS2/RELA/SELE/TNF/VCAM1
Bladder cancer	3.73E-22	19	CCND1/CDK4/CDKN1A/CXCL8/E2F1/E2F2/EGFR/ERBB2/MAPK1/MAPK3/MDM2/MMP1/MMP2/MMP9/MYC/RAF1/RASSF1/RB1/TP53
Cellular senescence	1.42E-21	30	AKT1/CCNA2/CCNB1/CCND1/CDK1/CDK2/CDK4/CDKN1A/CHEK1/CHEK2/CXCL8/E2F1/E2F2/IGFBP3/IL1A/IL6/MAPK1/MAPK14/MAPK3/MDM2/MYC/NFATC1/PTEN/RAF1/RB1/RELA/SERPINE1/SIRT1/TGFB1/TP53
Pancreatic cancer	1.54E-21	23	AKT1/BAX/BCL2L1/CASP9/CCND1/CDK4/CDKN1A/CHUK/E2F1/E2F2/EGFR/ERBB2/IKBKB/MAPK1/MAPK3/MAPK8/RAF1/RB1/RELA/STAT1/STAT3/TGFB1/TP53
Endocrine resistance	2.67E-21	25	AKT1/BAX/BCL2/CCND1/CDK4/CDKN1A/E2F1/E2F2/EGFR/ERBB2/ESR1/ESR2/FOS/JUN/MAPK1/MAPK14/MAPK3/MAPK8/MDM2/MMP2/MMP9/PRKACA/RAF1/RB1/TP53
Proteoglycans in cancer	3.87E-21	33	AKT1/CASP3/CAV1/CCND1/CD44/CDKN1A/EGFR/ERBB2/ERBB3/ESR1/FN1/HIF1A/HPSE/IGF2/ITGB3/KDR/MAPK1/MAPK14/MAPK3/MDM2/MET/MMP2/MMP9/MYC/PLAU/PRKACA/PRKCA/PRKCB/RAF1/STAT3/TGFB1/TNF/TP53
Chemical carcinogenesis - receptor activation	1.15E-20	33	AHR/AKT1/AR/BCL2/BIRC5/CCND1/CREB1/CYP1A1/CYP1B1/CYP3A4/E2F1/EGFR/ESR1/ESR2/FOS/GSTM1/HSP90AA1/JUN/MAPK1/MAPK3/MYC/PGR/PPARA/PRKACA/PRKCA/PRKCB/RAF1/RB1/RELA/RXRA/STAT3/UGT1A1/XIAP
Apoptosis	7.47E-20	27	AKT1/BAX/BBC3/BCL2/BCL2L1/BIRC5/CASP3/CASP7/CASP8/CASP9/CHUK/CTSD/CYCS/FOS/IKBKB/JUN/MAPK1/MAPK3/MAPK8/MCL1/NFKBIA/PARP1/RAF1/RELA/TNF/TP53/XIAP
Toxoplasmosis	9.37E-20	25	AKT1/ALOX5/BCL2/BCL2L1/CASP3/CASP8/CASP9/CHUK/CYCS/IFNG/IKBKB/IL10/MAPK1/MAPK14/MAPK3/MAPK8/NFKBIA/NOS2/PIK3CG/RELA/STAT1/STAT3/TGFB1/TNF/XIAP

dephosphorylating serine, threonine, and tyrosine. As a negative regulator of MAPK signaling, MAPK1 plays an important role in regulating cell proliferation, growth, and differentiation.[27,28]

Through the construction of "active ingredient-target-pathway" networks and topology analysis, the main components of TKD that may affect CRA are quercetin, luteolin, kaempferol, wogonin, baicalein, isorhamnetin, nobiletin, formononetin, beta-sitosterol, and naringenin. These active ingredients may play an important role in the treatment of colon adenoma. Previous studies have found that quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a low molecular weight polyphenol phytochemical, usually in the form of quercetin glycoside compound that has chemopreventive and therapeutic effects on many diseases.[29] Its activity is attributed to anti-oxidation, anti-cancer, and immunity-enhancing effects.[30] Importantly, quercetin is cytotoxic to cancer cells but does not damage healthy cells. Further, quercetin can be used as a chemopreventive and therapeutic agent for a variety of cancers through its role in the inhibition of oncogene expression, cell cycle inhibition, induction of apoptosis. Additionally, it has been shown that the compound has anti-tumor effects by its ability to inhibit cancer cell invasion and metastasis.[31] Pang Linrong et al found that quercetin has an inhibitory effect on the proliferation, invasion, and migration of HSP-27 knocked-down colon cancer SW480 cells.[32] Together, their data indicated that the increase in apoptosis in HSP-27 knockdown SW480 cells and the regulation of

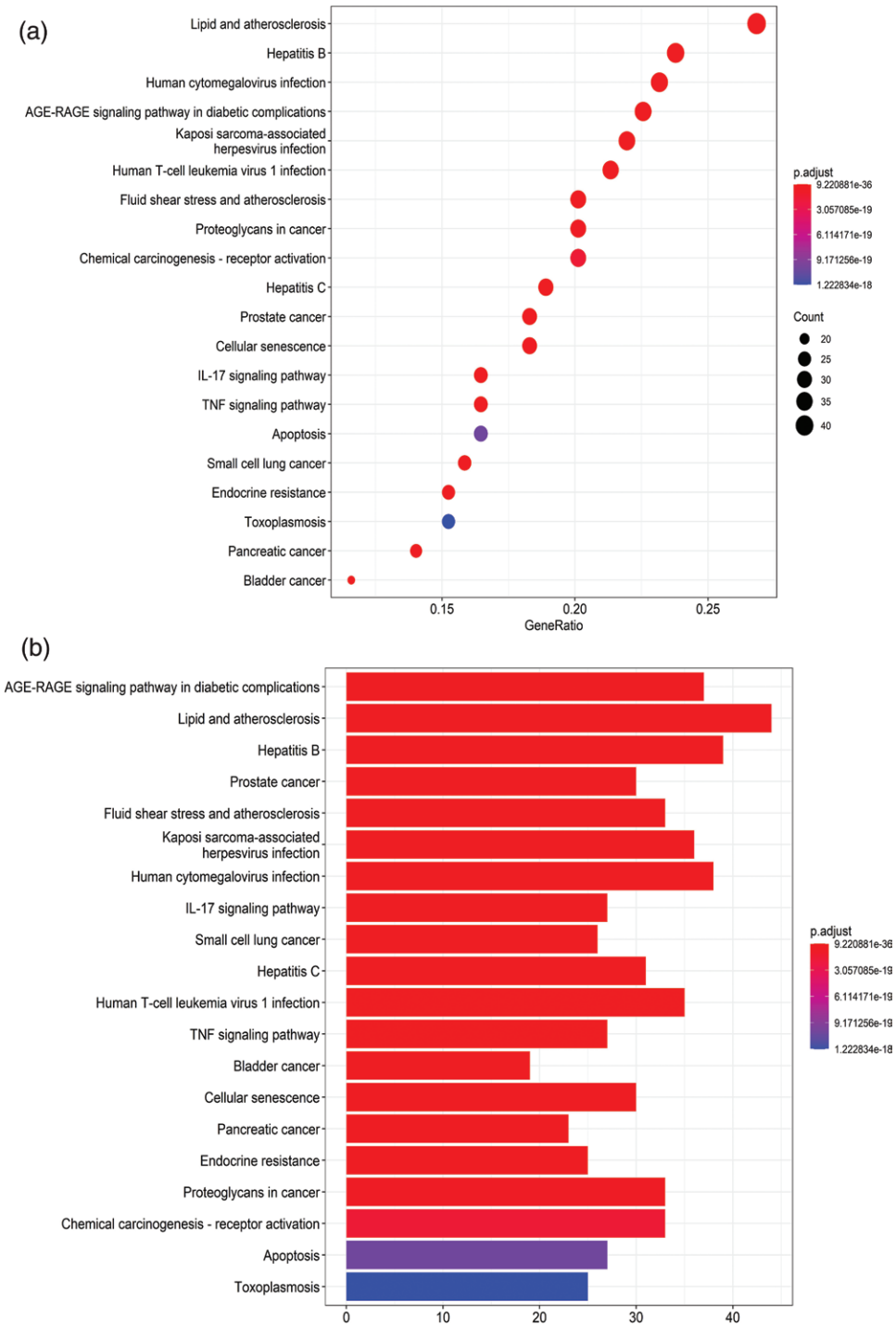

Figure 8. KEGG pathway enrichment. KEGG = Kyoto encyclopedia of genes and genomes.

apoptosis via Bcl-2 and Bax gene and protein expression are related. Luteolin is a flavonoid, that is mainly derived from Prunella vulgaris, nudiflora purple bead, dense chrysanthemum, wild chrysanthemum, and other plants.[33] Luteolin has anti-inflammatory, anti-tumor, anti-viral, and anti-oxidation activity. Further, it is hypothesized to have a role in liver protection and the enhancement of immune regulation and other biological activities. Due to its low side effects, this medicine has great clinical potential.[34,35] Kaempferol is a flavonoid derived from the rhizomes of the ginger herb, kaempferi. Its chemical structure is similar to quercetin and isorhamnetin.[36] Survey data has shown that the ingestion of foods high in kaempferol may reduce the risk of cancer and play a role in tumor prevention.[37] Furthermore, experimental studies have shown that kaempferol can inhibit the growth of malignant tumors through multiple pathways and multiple targets, including tumors related specific to the digestive system.[38,39] The anti-tumor mechanism of kaempferol involves PI3K/Akt, EGFR, MAPK, Wnt, and other signaling pathways. Specifically, the PI3K/Akt signaling pathway is hypothesized to be the main target of kaempferol. Interestingly, using network pharmacology, a similar finding was observed which indicated that the PI3K/Akt signaling pathway is an important pathway for the treatment of cancer by kaempferol.[40]

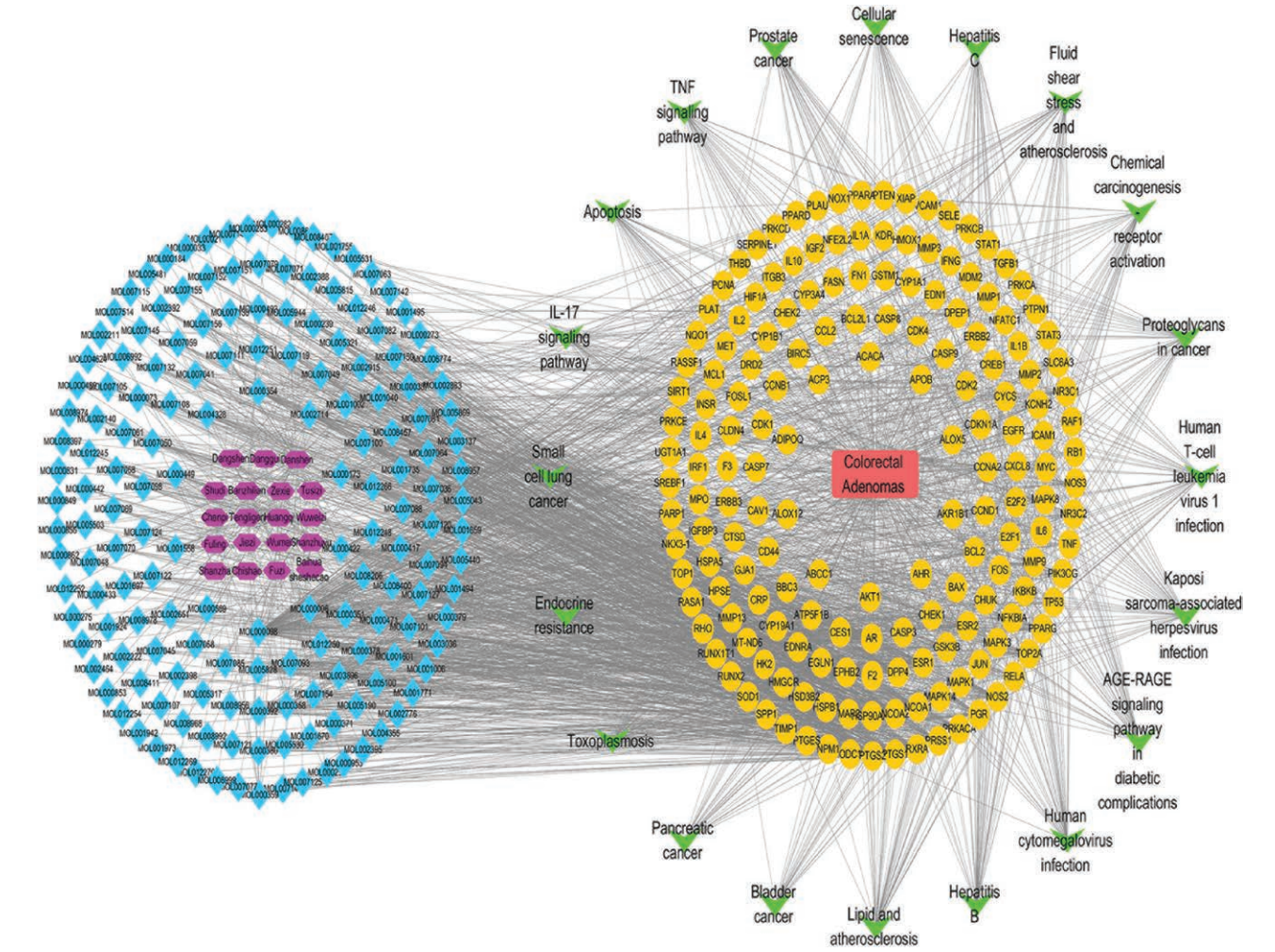

Figure 9. Component - Disease - Pathway - Target network. Note: Blue is the compound, yellow is the target of Chinese medicine on the disease, green is the first 20 most significant pathways, red is the disease, purple is the drug.

In the current study, GO enrichment analysis indicated that the TKD mechanism of action involves multiple BP and molecular functions. Among them, the main BP with the highest correlation include chemical stress responses, oxidative stress responses, responses to reactive oxygen species, drug responses, steroid hormone responses, etc. KEGG pathway enrichment analysis has shown that the TKD mechanism of action in the treatment of CRA is associated with the AGE-RAGE signaling pathway in diabetic complications, lipid metabolism, atherosclerosis, prostate cancer, IL-17 signaling, small cell lung cancer, hepatitis C, and human T-cell leukemia. Many pathways such as those involved in virus-1 infection and TNF signaling are closely related. From the results of both the GO and KEGG enrichment analysis, the effect of TKD on CRA highlights the advantages of traditional Chinese medicine.

5. Conclusion

In summary, this study demonstrated the potential mechanism of TKD in the treatment of preventing colorectal precancerous lesions based on network pharmacology. Our data indicate that quercetin, luteolin, kaempferol, wogonin, wogonin, baicalein, isorhamnetin, nobiletin, nobiletin, and formononetin, etc., affect multiple CRAs. The core targets (AKT1, TP53, MAPK1, MAPK8, JUN, IL6, TNF, STST3, MAPK3, and EGFR) play an important role in CRAs. In addition, the KEGG analysis conducted in this study shows that the intervention of TKD on CRA is related to multiple pathways, including the AGE-RAGE signaling pathway in diabetic complications, lipid metabolism, atherosclerosis, IL-17 signaling, small cell lung cancer, hepatitis-C, and TNF signaling. This study provides an important basis for the prevention of CRC using traditional Chinese medicine and highlights the importance of the further study of TKD.

Acknowledgments

We thank Prof Renjie-Shi, Department of Proctology, Jiangsu Hospital of Traditional Chinese Medicine, who provided guidance for the source of prescriptions and the basis of the previous study.

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Software: Xiang Zhu.

Supervision: Jiayi Jiang, Weixin Liu
Visualization: Han Jiang

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