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Exploring the Mechanism of Canmei Formula in Preventing and Treating Recurrence of Colorectal Adenoma Based on Data Mining and Algorithm Prediction

Xiaoling Fu[1,2*†], Yimin Xu[1,3†], Xinyue Han[1,2†], Xiangying Lin[1], Jingnan Wang[1], Guanhong Li[1], Xiaochen Fu[1] and Min Zhang[4*]

Abstract

Background The high incidence of recurrence and malignant transformation of colorectal adenoma (CRA) are current issues that need to be addressed in clinical practice. Canmei Formula (CMF) has shown promising results in the prevention and treatment, however, it lacks effective clinical data support and its mechanism of action is not fully elucidated.

Objective The aim of this study is to evaluate the clinical efficacy and safety of CMF in preventing and treating CRA, and to explore its effective chemical components and pharmacological mechanisms.

Method A randomized controlled clinical trial was conducted, with patients diagnosed with CRA within 6 months as the study subjects. After randomization, the patients were divided into a treatment group (receiving CMF granules) or a control group (receiving berberine hydrochloride tablets). The one-year recurrence rate of CRA was used as the key efficacy indicator to assess the effectiveness of CMF in preventing and treating CRA. The chemical components of CMF were identified using the UFLC-Q-TOF-MS/MS combined system. Data mining and the wSDTNBI algorithm were combined to construct a differential expression gene (DEG) - CMF prediction target interaction network for CRA. The core targets of CMF in CRA prevention and treatment were identified through topological analysis, and validated using molecular docking and in vitro experiments.

Result During the period from October 1 2021 to December 31 2023, a total of 228 participants were included in the study. After block randomization, 114 patients were assigned to each group. In the treatment group, 98 patients completed follow-up examinations, with 16 patients (14.0%) exhibiting shedding, Adenoma recurrence was identified in 24 (24.5%) patients through colonoscopy. In the control group, 99 cases completed the follow-up examination,

†Xiaoling Fu, Yimin Xu and Xinyue Han contributed equally to this work.

*Correspondence:
Xiaoling Fu
fuxiaoling111@163.com
Min Zhang
doctorzhangmin@163.com

Full list of author information is available at the end of the article

while 15 cases (13.2%) were lost to follow-up. There were 45 cases (45.5%) experienced recurrence of adenomas. During the follow-up period, no cases of colorectal cancer or severe adverse reactions were reported. UFLC-QTOF-MS/MS identification was combined with traditional Chinese medicine database mining to obtain 192 active chemical components of Canmei Formula. Using the wSDTNBI algorithm, 1044 prediction targets were predicted, and 3308 differentially expressed genes of CRA were extracted from the TCGA database. Network topology analysis and bioinformatics analysis were performed on 164 intersecting core targets. Molecular docking and qPCR analysis revealed that CMF downregulates angiotensin II type 1 receptor (AT1R) and regulated interleukin-8 (CXCL8) and matrix metalloproteinase 13 (MMP13) within the REN/Ang II/AT1R axis of the renin-angiotensin signaling pathway, thereby preventing and treating CRA.

Conclusion This small-scale randomized controlled clinical trial showed that CMF granules can safely and effectively reduce the risk of CRA recurrence. CMF prevents and treats colorectal adenomas by modulating the renin-angiotensin signaling pathway and the inflammatory response.

Keywords Canmei Formula, Colorectal adenoma recurrence, WSDTNBI algorithm, Renin-angiotensin signaling pathway

Introduction

Colorectal cancer (CRC) is a severe gastrocolorectal malignancy that poses a threat to human health and is the second leading cause of cancer-related deaths worldwide [1]. About 520,000 new cases of CRC are diagnosed in China each year [2]. Colorectal adenoma (CRA) represents an essential precancerous lesion for CRC, which often undergoing a series of oncogene alterations [3]. Over 85% of CRC develops from adenomatous polyps [4]. According to the pathological characteristics, CRA is mainly composed of tubular adenoma, villous adenoma, and mixed adenoma [5], collectively accounting for approximately 70% of colorectal polyps [6], and is a major threat to colorectal health. CRA are characterized by high recurrence rates: one-year recurrence exceeds 40%, and the five-year recurrence surpasses 60% for villous adenomas [7]. Furthermore, Villous adenoma also has the high carcinogenic characteristics, its single cancerous change rate is 20–30%, and multiple-lesion rates of 30–80% [8]. Colonoscopy combined with endoscopic polypectomy is the main treatment for CRA at present. However, studies reported recurrence rates of 36% ~ 61% within the first year post-procedure [9]. In recent years, aspirin, NSAIDs, vitamin D, calcium, folic acid, metformin and non-steroidal anti-inflammatory drug supplements have been used as primary or secondary chemoprevention agents for CRA [10]. However, aspirin and celecoxib can increase the risk of serious adverse reactions and cardiovascular comlications [11, 12], metformin is recommended for the prevention of CRA in patients with type 2 diabetes [13–15]. The role of vitamin D, calcium, folic acid and statins in chemoprevention is unclear [16–18], and not recommended for CRA prevention and treatment at this time. The high recurrence and high cancerous change rate of CRA are challenged, and therefore, exploring safe and effective CRA recurrence prevention and treatment drugs has become an urgent need in clinical practice.

Traditional Chinese medicine (TCM) has a long history of being used for treating diseases and has significant therapeutic effects. Within TCM, CRA is categorized under the "polyp" classification [19], attributed to pathological features like "phlegm stasis". In our clinical practice, we selected a modified formula from the classic Chinese medicine book "Hygienic Miscellaneous Vitality", the Canmei Formula (CMF). This formula is composed of three traditional Chinese medicines: black plum, stiff silkworm, and locust horn, which have the effect of "dissipating phlegm and resolving masses". Previous studies have found that CMF has preventive and therapeutic effects on AOM/DSS + HFD-induced colorectal adenoma in mice, potentially through the inhibition of inflammatory factors such as NF-κB, IL-17 C, and kEAP1/Nrf2/NQO1 signaling pathway [20]. Additionally, CMF also can regulate gut microbiota and changing them towards normal ones, upregulate beneficial bacteria like Bifidobacterium and Ruminococcaceae, while reducing harmful pathogens like Desulfovibrionaceae and Bacteroidaceae. These effects significantly reducing CRA incidence and cancerous change rate [21], In clinical applications, it has also been found to have significant therapeutic effects on the prevention and treatment of CRA. However, there is currently a lack of robust clinical data support for this effect and its mechanism of action has not been fully elucidated.

Chinese herbal medicine has the characteristics of multiple components, affecting multiple targets, and multiple pathways, but the complex formula of compounds in traditional Chinese medicine also poses difficulties in studying their molecular mechanisms of action. Network pharmacology or system pharmacology combined with multi-omics analysis and machine

learning can elucidate the potential mechanisms of multi component and multi target drugs by analyzing complex and multi-level interactions in various networks, demonstrating unique advantages in studying the pharmacological basis of traditional Chinese medicine and its therapeutic mechanisms of diseases [22–25]. Traditional network pharmacology methods, such as SwissTargetPrediction (http://SwissTargetPrediction.ch/), predict the chemical target based on molecular similarity. The principle is to predict targets of bioactive molecules based on a combination of 2D and 3D similarity measurements with known ligands [26], but the affinity of the molecule to the target was not considered. In recent years, machine learning researchers have shifted their focus to biological problems that are difficult to analyze with standard methods, helping to improve the accuracy of molecular target prediction [27]. The WSDTNBI algorithm employs a two-pronged approach based on weighted drug-target interactions and drug substructure association networks to calculate prediction scores, whose edge weights are correlated with binding affinity. It is a powerful tool for drug virtual screening based on network [28]. Based on the randomized controlled clinical trial that determined the effectiveness and safety of CMF in the prevention and treatment of CRA, this study adopts the method of combining WSDTNBI algorithm prediction, network analysis and experimental validation, to study the pharmacological mechanism of CMF on CRA prevention and treatment. As shown in Fig. 1, this study conducted a clinical efficacy trial and component analyses of CMF, and screened the core targets for its role based on data mining, WSDTNBI algorithm prediction, and network topology analysis. The reliability of these core target was verified by molecular docking and in vitro experiments, allowing for a discussion on the potential molecular mechanisms involved in the prevention and treatment.

Research Methods

Evaluation of Clinical Efficacy of Canmei Formula in Preventing and Treating Recurrence of Colorectal Adenoma after Endoscopic Resection

Case Screening

This randomized controlled single-center clinical trial was conducted at Yueyang Integrated Traditional Chinese and Western Medicine Hospital affiliated with Shanghai University of Traditional Chinese Medicine. The study was reviewed by the Yueyang Hospital Ethics Committee (Ethical Approval Number: 2021 – 124) and was registered in the Chinese Clinical Trial Registration Center (www.chictr.org.cn) (Registration No. ChiCTR210054824). All enrolled patients signed an informed consent form, which complies with the Helsinki Declaration and was designed and implemented in accordance with the clinical trial requirements of the CONSORT guidelines. Eligible subjects were determined by the staff during routine clinical diagnosis and treatment, and their clinical information was collected.

(1) Inclusion Criteria:

① Age ≥ 18 and ≤ 80 years old, regardless of gender;

② Within 6 months before enrollment, endoscopic or surgical polypectomy has been performed, and pathological confirmation indicates adenomatous polyps in the colon and rectum. The TCM syndrome belongs to "phlegm-blood stasis" type.

③ Patients understand and agree to participate in this study and sign an informed consent form.

(2) Exclusion Criteria:

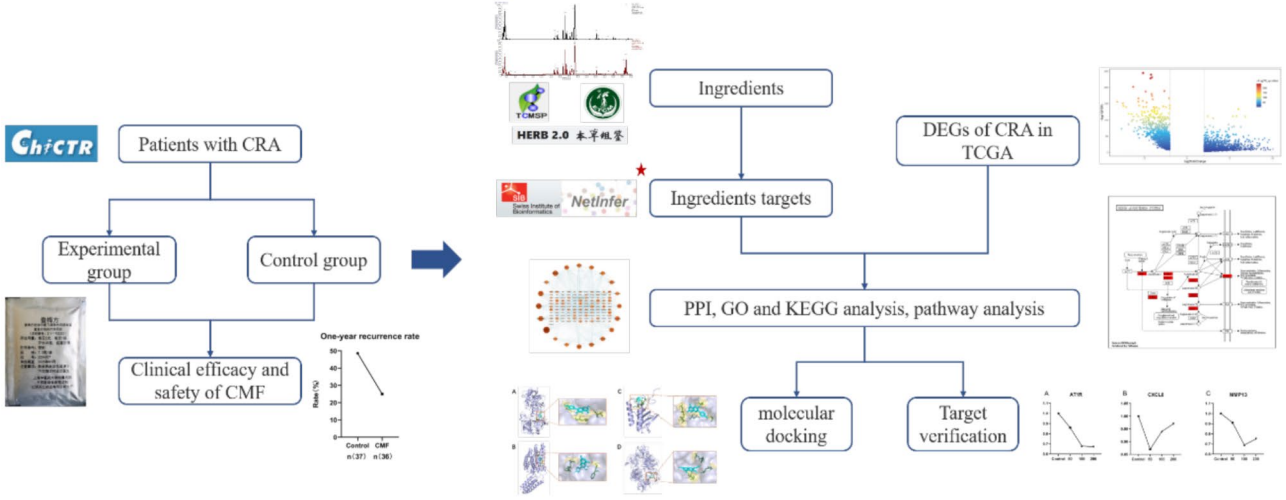

Fig. 1 Schema showing the strategy of exploring the molecular mechanism of CMF in the prevention and treatment of CRA using network analysis and experimental validation based on the clinical efficacy and safety of CMF

① During colonoscopy, the colorectal adenoma was not completely removed;
② Inability to undergo colonoscopy or poor bowel preparation (evaluated as "poor" and "insufficient" according to the Aronchik scale), with a short colonoscopy time and a stop time of less than 6 min;
③ Pregnant women or lactating women;
④ Having uncontrollable mental disorders and unable to cooperate;
⑤ Complicated with active tuberculosis or severe heart, liver or kidney diseases, or other serious infectious diseases;
⑥ Long-term use of drugs such as aspirin, NSAIDs, calcium, or vitamin D for at least three months (e.g., aspirin ≥ 100 mg/day or calcium ≥ 1200 mg/day);
⑦ Long-term (at least 6 months) use of Chinese herbal medicine or traditional Chinese patent medicines and simple preparations with the effect of clearing heat and detoxification, resolving phlegm and resolving stagnation, especially Chinese herbal prescriptions including fried Coptidis Rhizoma, Picrorhizae, and other drugs containing berberine;
⑧ Patients who cannot take oral medication or vomit frequently or suffer from severe constipation.

(3) Cases that have been included in the group but meet one of the following criteria for suspension or exclusion should be excluded:
① Misdiagnosis;
② Those who have not taken medicine, tested, or had poor compliance;
③ The patient was lost during follow-up or voluntarily gave up treatment;
④ During the experiment, there were serious cases of other concurrent diseases, deterioration of the condition, and the need to take emergency measures;
⑤ During the experiment, those who changed their medication or added non prescribed therapeutic medication (violating the protocol), which affected the effectiveness and safety judgment of treatment. For example, the use of certain prohibited drugs (such as aspirin, celecoxib, berberine, probiotics, etc.) makes it impossible to evaluate the efficacy.

Withdrawal cases must undergo corresponding clinical evaluation when discontinuing the clinical trial. For patients who request to withdraw from the clinical trial midway, the reasons should be clearly recorded. If the patient does not come to the hospital for follow-up on time, they should be inquired about the reasons through phone calls and letters, and be investigated about the subsequent process, and conduct a clinical evaluation of the patient's withdrawal from clinical trials midway.

Intervention Measures

A randomized controlled design was adopted in this study. SPSS 26.0 statistical software was used to realize random grouping and generate a group of random numbers ranging from 1 to 228. 228 subjects meeting the standard were divided into treatment group or control group according to random numbers.

The treatment group received intervention with CMF granules, while the control group received placebo granulesberberine hydrochloride tablets. The CMF granules and placebo were prepared by Jiangyin Tianjiang Pharmaceutical Co., Ltd. (Jiangsu, China, GMP number: JS20191115). CMF consists of 20 g black plum, 20 g stiff silkworm, and 10 g locust horn (ratio 2:2:1), which are equivalent to their traditional Chinese medicine decoction constituents. The granules have no additives or pigments, with one dose packaged into two small bags, taken once daily (one bag each in the morning and evening) with warm water.

Subjects assigned to the control group take a total of 0.6 g of berberine (0.2 g per dose, three times a day). The treatment period was 24 weeks, with 4 weeks as a course of treatment and 6 consecutive courses, which means the treatment period is half a year. During the experiment, all subjects received dietary and lifestyle adjustments, and maintained stable lifestyle and dietary habits throughout the entire experiment.

Therapeutic Indicator and Follow-up

After the intervention treatment, the subjects underwent a one-year follow-up colonoscopy to record the location, number, size, and histopathological type of adenomas. Baseline laboratory tests, including serum alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine, were conducted to evaluate the liver and kidney function.

The one-year recurrence rate of colorectal adenomas is the main indicator, polyp recurrence rate in each group = number of recurrence cases / number of cases in each group × 100%.

Follow-up was conducted through phone calls and clinical visits. Researchers made regular monthly phone calls to monitor compliance, side effects, and efficacy, and confirm that they have not used aspirin, non-steroidal anti-inflammatory drug (NSAID), or other prohibited drugs until the end of the trial.

Optimization Combination of Canmei Formula and Colorectal Adenoma Target Based on Data Mining and Algorithm Prediction

Identification of Chemical Constituents of Canmei Formula Granules

(1) Reagents.

CMF granules were provided by Jiangyin Tianjiang Pharmaceutical Co., Ltd. (Jiangsu, China, GMP number: JS20191115) and stored in the laboratory of Shanghai University of Traditional Chinese Medicine. HPLC grade acetonitrile was purchased from Merck Company (Darmstadt, Germany), HPLC grade methyl tert-butyl ether was purchased from CNW, AR grade acetic acid and GR grade ethanol were purchased from China National Pharmaceutical Group Chemical Reagents (Shanghai, China), and Cremophor EL (BR grade) was purchased from Shanghai Yuanye (Shanghai, China). The purified used throughout the entire study was prepared using a 611VP purified water system (Sartorius, Germany).

(2) UPLC-MS analysis.

The equipment used in the analysis included the CBM-20 A system controller, LC-20ADXRpump, SIL-20ACXRautosampler, CTO-10Avp column box and equipped with protective columns (C18, 4 mm × 3.0 mm, Phenomenex Co., Ltd., Torrance, CA, United States) and Shiseido C18 column (2.1 × 150 mm, 2.5 μm, Shiseido Co., Ltd., Tokyo, Japan). The CMF granules were analyzed using the Shimadzu HPLC coupled with mass spectrometry. The conditions of UHPLC were as follows: The LC system is thermo Dionex Ultimate 3000, Column is XBridge BEH C18 (2.1 × 150 mm, 2.5 μm), Column Temperature 40 °C, Flow Rate 0. 3mL/min, and a linear gradient procedure (Table 1), the mobile phase system consisted of solvent a (0.1% formic acid + H2O, V/V) and solvent B (0.1% formic acid + acetonitrile, V/V) .

The MS conditions are as follows: the MS system used was the Q Exactive, Ion mode was ESI− & ESI+, the Spray Voltage was 3500/3200V(+/-), the Vaporiser Temp was 350℃, with a sheath gas flow rate of 40 arb, and an auxiliary gas flow rate of 10 arb, the Capillary Temp was set to 320℃, the Scan Range was m/z 120–1500, Resolution was 70,000FWHM. The Top 3 ddms were selected for identification.

Approximately 500 mg of traditional CMF (which included stir fried silkworm 6.0 g, locust horn 6.0 g, black plum 6.0 g) was weighted. The sample was sonicated with 50% methanol for 20 min, then centrifuged at 12,000 r for 5 min. The supernatant was diluted and filtered through a 0.22 μ M microporous membrane. The filtrate sample 1 μ L was taken for analysis.

Table 1 Mobile Phase Gradient

Time/min	Flow rate ml/min	%A	%B
0	0.3	95	5
4	0.3	95	5
50	0.3	98	2
52	0.3	98	2
55	0.3	95	5
60	0.3	98	2

(3) Data processing.

The high-resolution information of quasimolecular ions and secondary fragments was obtained by HPLC-Q Exactive liquid-mass spectrometry. The data were analyzed by Compound Discoverer 2.1 software, Chromatogram peaks were inferred and identified by comparison of reference materials, MZ Vault, MZ clould, Chemspider and Pubchem.

Establishing a Database of Chemical Constituents of Canmei Formula

Literature searches were performed across three publicly available databases: TCMSP (https://old.tcmsp-e.com), ETCM (http://www.tcmip.cn/ETCM/) and HERB (http://herb.ac.cn/), to identify the chemical components of the three traditional Chinese medicines, black plum, stiff silkworm, and locust horn (https://pubchem.ncbi.nlm.nih.gov/). The standard SMILES format structures of compounds obtained from mass spectrometry and known compounds in the database were collected, and a total of 220 compounds with SMILES structures were obtained using SwissADME (http://www.swissadme.ch/). A network-based approach was used to filiter the ingredients based on the Lipinski "Rule of Five" [29], 28 compounds that did not meet the standards were removed, and ultimately 192 active ingredients were obtained. A CMF ingredient database was established, which includes basic information such as ingredient names, PubChem CID, SMILES structural formulas, and compliance with the Lipinski "Rule of Five".

wSDTNBI Algorithm for Predicting the Target Points of Canmei Formula Action

Using the wSDTNBI (http://lmmd.ecust.edu.cn/netinfer/) algorithm to predict the target of 192 active com pounds in CMF. For each compound, a target prediction set of 100 was generated, and prediction targets were screened based on a prediction score threshold of > 0.5. This approach identified ultimately 1044 potential for CMF. Compared with most current network-based methods, this prediction method can output prediction scores related to binding affinity, improving the accuracy of prediction results [28].

Data Mining from TCGA Database

The known CRA-related genes were retrieved by searching for "COAD" (Colon Adenocarcinoma) in the TCGA database, identified according to the standards of *P*-value < 0.05 and Fold Change (FC) > 2 or < 0.5. R programming language was used to screen

for differentially expressed genes (DEGs) between the CRA model group and the normal control group.

Drug Efficacy Evaluation Based on Network and Algorithm Prediction

Aspirin and berberine were used as positive drug controls. From the DrugBank database (https://go.drugbank.com/), 141 control drug targets were obtained from three databases: PubChem, ETCM, and HERB. Using two human genome protein-protein interaction (PPI) networks as the background network, one was the STRING network with a confidence level of ≥ 0.9 for human interactions, and the other was the BNet dataset, collected by Professor Barabasi's team. The Random Walk with Restart (RWR) algorithm was applied to the STRING network, and the correlation between drug targets and disease-related genes was evaluated using Z-score, and the proximity between drug targets and contrast agent targets was calculated.

Network Construction and Analysis

The overlapping targets of CMF prediction targets and CRA-related DEGs were obtained through STRING11.0 (https://string-db.org/). An interaction network of "CRA-related genes and CMF potential targets" was constructed by using the screening condition of "Homo sapiens". The results were saved, and the generated file was imported into Cytoscape v3.7.1 for visualization. This allowed for the identification of core targets within the protein-protein interaction (PPI) network.

Pathway Enrichment Analysis

The "clusterProfiler" package in R was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis on the interacting targets. This analysis aimed to elucidate the roles of core targets in gene function and associated signaling pathways.

Molecular Docking Analysis

We screen core targets based on the degree values of nodes in the protein-protein interaction (PPI) network, and perform molecular docking analysis with chemical components that comply with the five principles of Lipinski class drugs in mass spectrometry analysis. From Pubchem (https://pubchem.ncbi.nlm.nih.gov/), download the SDF file of the 2D structure of the core compound, utilize ChemBio3D software to optimize its mechanical structure and save it as a Mol2 file, import it into AutoDockTools1.5.6 software, and save it in pdbqt format. The 3D structure of key target proteins was downloaded from the PDB database (https://www.rcsb.org). The PyMOL software was used to remove water molecules and excess inactive ligands. The AutoDockTools 1.5.6 software was imported and saved in pdbqt format. Molecular docking simulations were conducted on potential targets and their corresponding components using AutoDock vina software. The global optimal binding conformation was obtained through DiscoveryStudio 4.5 software and saved as a PDB file. The docking results were visualized using the PyMOL software.

Table 2 Primer sequences

Name	Primer sequence(5′ to 3′)
AT1R/F	GATTGTCCCAAAGCTGGAAG
AT1R/R	ATCACCACCAAGCTGTTTCC
MMP13/F	GTTCTTCCCTTGATGGCCGATCATAT
MMP13/R	GTGATCCCTTGAGATATGGAAGGATGC
CXCL8/F	TGAAGGTGCAGTTTTGCCAAG
CXCL8/R	AACTTCTCCACAACCCTCTGC
REN/F	ACCTTGCTCTGTGAAGACGG
REN/R	ATACATAGTCCGCGCTGGTG

In vitro Experimental Verification

Cell Culture

The IH-CRA cells were obtained from the China Center for Type Culture Collection(CCTCC) with the cell line number C2019307. We filed a national invention patent for IH-CRA cells, patent number 201911261397.9 (Wuhan, China). Cells were cultured at 37 ° C and 5% CO_2 in a medium containing 10% fetal bovine serum (FBS) (F12 medium from Shanghai Beyonce Biotechnology Co., Ltd.). The cultured cells were washed twice in PBS and digested with trypsin. Cells were centrifuged at 800 rpm for 3 min, mixed in culture medium and inoculated into a culture dish. After 24 h of cell adhesion, the drug was administered, and the cells were collected for subsequent experiments.

qPCR

The total RNA was extracted using Trizol reagent, then treated with deoxyribonuclease without RNA enzyme, and reversely transcribed with oligomeric DT using MMLV reverse transcriptase according to the instructions of the reverse transcriptase kit. The conditions for reverse transcription reaction are: reaction at 25 °C for 5 min, reaction at 42 °C for 30 min, and reaction at 85 °C for 5 s. The reaction is carried out in a PCR machine. The primer sequence is shown in Table 2.

Statistic Analysis

All data were statistically processed using SPSS 26.0 software. Measurement data adopts mean plus minus

standard deviation $\left(\bar{x} \pm s\right)$. If the data follows a normal distribution and satisfies homogeneity of variance, paired sample t-tests are used for intra group comparisons before and after, and independent sample t-tests are used for inter group comparisons; For data with skewed distribution or uneven variance, non parametric testing is used; The counting data adopts chi square test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient registration started on October 1 2021, and ended on December 31 2023, with a total of 228 patients. These patients were randomly assigned to either the treatment group (114 cases) or the control group (114 cases). There were 16 patients (14.0%) in the treatment group and 15 patients (13.2%) in the control group failed the follow-up. In the end, a total of 197 patients in both groups completed colonoscopy reexamination.

The baseline characteristics of the two groups of patients were similar, and there were no significant differences between the groups in terms of age, gender, body mass index, and characteristics of adenomas. During the follow-up period, no patients reported taking drugs prohibited by the study protocol (such as aspirin and other non-steroidal anti-inflammatory drugs, calcium, or probiotics) for more than 2 weeks. In the treatment group, 98 patients completed follow-up examinations, with 16 patients (14.0%) shedding, Adenoma recurrence was found in 24 (24.5%) patients by colonoscopy. In the control group, 99 cases completed the follow-up examination, while 15 cases (13.2%) lost in the follow-up. There were 45 cases (45.5%) experienced recurrence of adenomas.

CMF Clinical Efficacy Evaluation Analysis

The colonoscopy results showed that 24 out of 98 subjects in the treatment group who completed follow-up and re examination had recurrence, with a recurrence rate of 24.5%; Among the 99 subjects in the control group who completed follow-up and re examination, there were 45 recurrence, with a recurrence rate of 45.5%. After conducting Pearson's chi square test, it was found that $\chi^2 = 4.063$, $P = 0.044 < 0.05$, with a statistically significant difference. These results demonstrated that there is a difference in the recurrence rate between the treatment group and the control group (Table 3), and the recurrence rate in the treatment group is significantly reduce.

Application of Canmei Formula in CRA Target Analysis

Isolation and Identification of Effective Constituents in Canmei Formula

The identification of chromatographic peaks for the chemical components of CMF granules, based on high-resolution quasi molecular ion and secondary fragment information analyzed by liquid chromatography-mass spectrometry, combined with relevant literature on the chemical components of Bombyx mori, Wumei, and Sophora japonica, a total of 33 chemical components were inferred. The total ion flow diagram of the granules is shown in Fig. 2. The retention time, molecular formula, compound name, high-resolution mass spectrometry parent ion and bias data, and fragment ion information of the granules are listed in Table 4.

Prediction of drug Efficacy

In order to predict the efficacy of CMF on CRA, the correlation between CMF targets and CRA related genes was analyzed in the algorithm network. The results showed a positive correlation between CMF and CRA related genes in the network, with significant correlation coefficients (Z-Score > 3), indicating that CMF is effective in affecting CRA at the molecular level (Table 5).

Construction of a Protein Protein Interaction Network (PPI) and Key Targets

Intersect of 1044 predicted targets that may be affected by Canmei Formula with 3308 DEGs mined from the TCGA database (Fig. 3) produced 164 intersecting targets. Their protein protein interaction network (PPI) was obtained through STRING, and imported into Cytoscape v3.8.2 software to calculate the interaction values and visualized (Fig. 4).

Bioinformatics Enrichment Analysis

The R language was used to perform GO and KEGG analysis on intersection targets, which explained the role of the core targets of CMF in gene function and signaling pathways (Fig. 5).

The results indicate that the Canmei Formula may act on core targets such as renin (REN), angiotensin II type 1 receptor (AT1R), interleukin-8 (CXCL8), and matrix metalloproteinase 13 (MMP13). By regulating

Table 3 Comparison of recurrence rates between two groups

Group	Recurrence *n*(%)	No recurrence *n*(%)	χ^2	*P*
Treatment group	24(24.5%)	74(75.5%)	4.063	0.044
Control group	45(45.5%)	54(54.5%)		

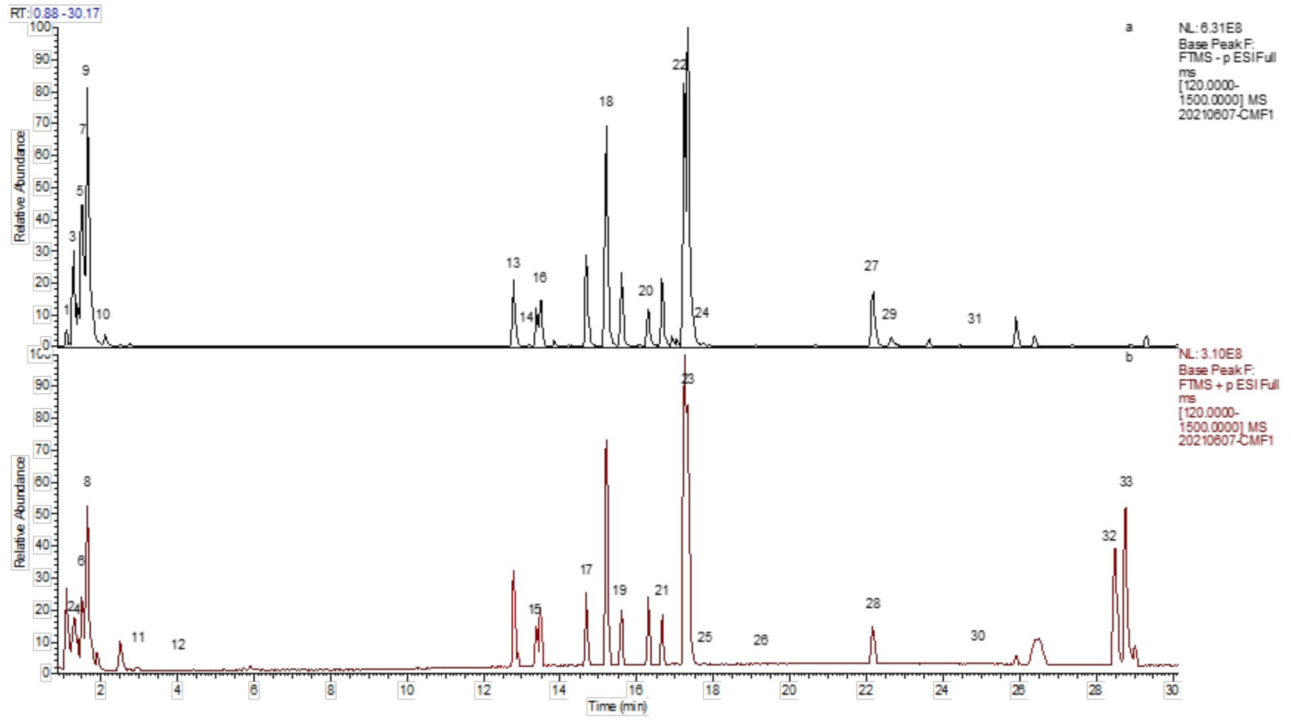

Fig. 2 Total ion flow diagram of 50% methanol extract of CMF granules. (**a**) negative ion scanning image, (**b**) positive ion scanning image

multiple biological processes such as transmembrane transport protein activity, it acts on the renin angiotensin signaling pathway, calcium signaling pathway, cAMP signaling pathway, and IL-17 signaling pathway, achieving a preventive and therapeutic effect on colorectal adenomas.

Molecular Docking

The active ingredients of CMF in mass spectrometry analysis comply with the five principles of Lipinski drugs with the core protein targets in the PPI network, namely REN, AT1R, CXCL8, and MMP13. In molecular docking analysis, the binding score is used to evaluate the binding degree between ligand molecules and receptor molecules. The lower the score, the lower the matching energy, indicating that the conformation of ligand receptor binding is more stable and the possibility of interaction is greater. The results showed that the common targeted binding energy of 15 active components of CMF with colorectal adenomas was negative, indicating that the compound had some binding activities with receptors, with a binding energy of less than − 1.2 kcal/mol, indicating strong binding activity (Fig. 6). The kaempferol and four core protein targets present in the mass spectrometry of Wumei, Bombyx mori, Sophora japonica were selected. The PyMol was used to display a three-dimensional view of their docking modes (Fig. 7).

In vitro Experimental Verification

To further evaluate the results of systematic pharmacological analysis, IH-CRA cells were treated with different concentrations of CMF and compared with the blank control group for the expression of REN, AT1R, CXCL8, and MMP13. The results showed that after CMF treatment, the expression of AT1R, CXCL8, and MMP13 genes decreased (Fig. 8), and the low expression of REN was not detected. This result indicated that CMF may regulate the renin angiotensin signaling pathway and inflammatory response by inhibiting the expression of AT1R, CXCL8, and matrix metalloproteinases, thereby preventing the recurrence of CRA. In vitro research provides supplementary information for screening components with potential effects, and demonstrates the rationality of molecular docking results and the reliability of screening strategies based on systemic pharmacology.

Discussion

Long term clinical practice has indicated that CMF granules are significantly effective in preventing and treating colorectal adenomas. This small sample, randomized controlled clinical trial supported that CMF granules is an effective drug for preventing recurrence after colorectal adenoma resection. Based on the complex functional characteristics of traditional Chinese medicine formulations, we validated the regulatory

Table 4 The chemical constituents in dispensing granules of traditional Chinese medicine

NO.	Molecular weight	Molecular formula	Compound name
1	174.1120	$C_6H_{14}N_4O_2$	DL-Arginine
2	182.0790	$C_6H_{14}O_6$	D-Galactitol
3	342.1162	$C_{12}H_{22}O_{11}$	Isomaltose
4	137.0478	$C_7H_7NO_2$	Anthranilic acid
5	192.0634	$C_7H_{12}O_6$	D-(-)-Quinic acid
6	151.0494	$C_5H_5N_5O$	Guanine
7	168.0280	$C_5H_4N_4O_3$	Uric acid
8	192.0270	$C_6H_8O_7$	Citric acid
9	181.0739	$C_9H_{11}NO_3$	DL-TYROSINE
10	170.0212	$C_7H_6O_5$	Gallic acid
11	165.0794	$C_9H_{11}NO_2$	L-Phenylalanine
12	154.0260	$C_7H_6O_4$	2,3-Dihydroxybenzoic acid
13	594.1585	$C_{27}H_{30}O_{15}$	Genistein 7,4′-di-O-beta-D-glucopyranoside
14	610.1534	$C_{27}H_{30}O_{16}$	Kaempferol-3-O-sophoroside
15	756.2113	$C_{33}H_{40}O_{20}$	Kaempferol 3-O-sophoroside 7-O-rhamnoside
16	740.2168	$C_{33}H_{40}O_{19}$	Apigenin 7-O-(2G-rhamnosyl)gentiobioside
17	756.2113	$C_{33}H_{40}O_{20}$	Kaempferol 3-O-sophoroside 7-O-rhamnoside
18	610.1534	$C_{27}H_{30}O_{16}$	Kaempferol-3-O-sophoroside
19	610.1534	$C_{27}H_{30}O_{16}$	Rutin
20	432.1057	$C_{21}H_{20}O_{10}$	Kaempferol-7-rhamnoside
21	594.1585	$C_{27}H_{30}O_{15}$	Genistein 7,4′-di-O-beta-D-glucopyranoside
22	432.1057	$C_{21}H_{20}O_{10}$	Kaempferol-7-rhamnoside
23	578.1636	$C_{27}H_{30}O_{14}$	Rhoifolin
24	462.1162	$C_{22}H_{22}O_{11}$	tectoridin
25	434.1213	$C_{21}H_{22}O_{10}$	Choerospondin
26	430.1264	$C_{22}H_{22}O_9$	Ononin
27	270.0528	$C_{15}H_{10}O_5$	Genistein
28	446.1213	$C_{22}H_{22}O_{10}$	Sissotrin
29	286.0477	$C_{15}H_{10}O_6$	Kaempferol
30	330.2406	$C_{18}H_{34}O_5$	(9E)-8,11,12-Trihydroxy-9-octadecenoic acid
31	268.0740	$C_{16}H_{12}O_4$	Formononetin
32	942.5204	$C_{48}H_{78}O_{18}$	Soyasaponin I
33	284.0688	$C_{16}H_{12}O_5$	Biochanin A

Table 5 Correlation between CMF targets, disease targets, and control drug targets

Relevance	STRING	HumanNet
Disease targets Z-Score	12.993335	10.80326577
Control drug targets Z-Score	19.74151933	27.54722259

effect of CMF granules on the REN/Ang II/AT1R axis through network analysis (Fig. 9).

The renin angiotensin system (RAS) is a complex peptide system, typically associated with the regulation of blood pressure and water electrolyte balance [30]. When blood pressure or sodium levels decrease, the kidneys release renin (REN), which breaks down angiotensinogen (AGT) into angiotensin I (Ang I), which is processed by angiotensin converting enzyme (ACE) to angiotensin II (Ang II) and promotes aldosterone production, raising blood pressure to normal levels [31]. In addition to its classic known functions, RAS was reported to be a complex system associated with several tissues, and its bioactive peptides locally regulate processes such as cell growth and proliferation. In recent years, many epidemiological, basic, and translational studies have shown that RAS imbalance is related to cancer development and progression [32]. A recent population-based cohort study suggested that patients who used RAS inhibitors had a lower cancer incidence rate than those who used other antihypertensive drugs [33]. Another regional study based on propensity score analysis found that ACE inhibitors/angiotensin receptor blockers (ARBs) are associated with a reduced risk of colorectal cancer [34]. There are also studies found that the use of RAS inhibitors may be related to an improvement in mortality after cancer surgery [35]. In addition, multiple experiments have shown that RAS inhibitors can interfere with cell proliferation, induce cell apoptosis, reduce tumor growth in colorectal cancer models, and improve fibrosis [36–39].

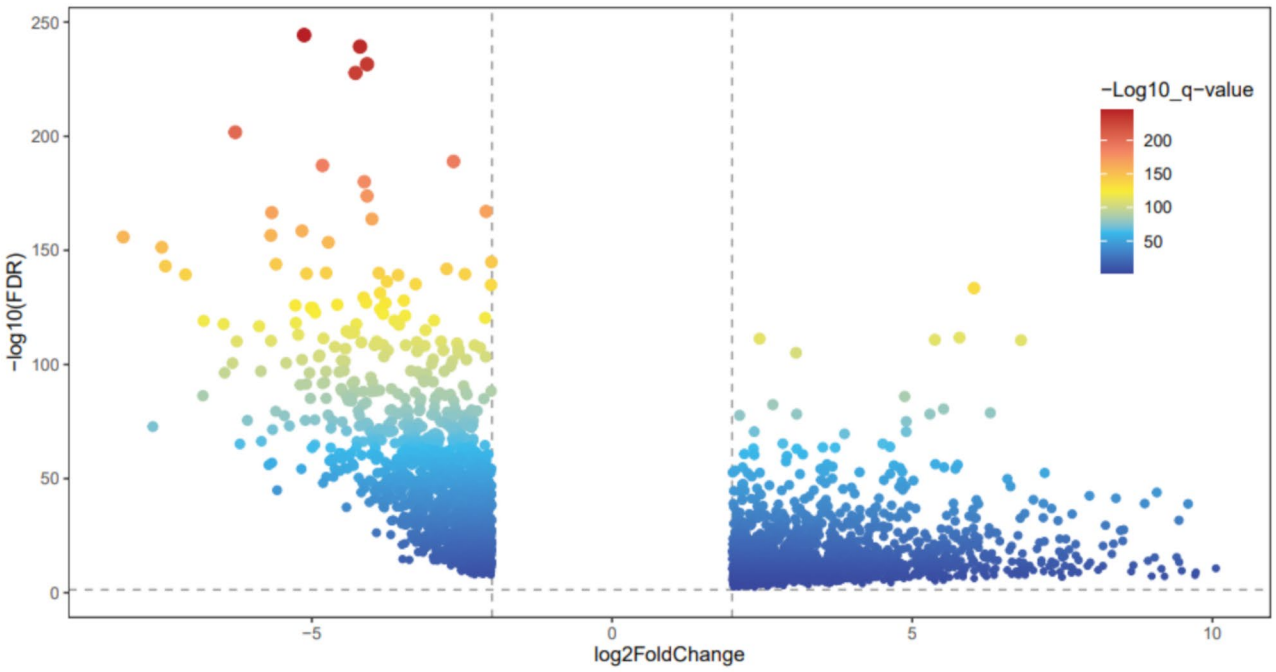

Fig. 3 CRA related differentially expressed genes in the TCGA database. The color bar ranges from blue to red, indicating that q-value ranges from high to low, that is, statistical significance ranges from low to high

Most Ang II functions involve the angiotensin II type 1 receptor (AT1R), a G protein coupled receptor encoded by a gene (AGTR1) in humans [40]. AT1R is present in various tissues, including vascular smooth muscle, endothelium, heart, brain, kidneys, adrenal gland, and adipose tissue [41]. The activation of AngII/AT1R promotes various complex signaling pathways. The binding of AngII to AT1R leads to the interaction between AT1R and heterotrimeric G proteins, followed by the second messenger signaling pathway including reactive oxygen species (ROS) [42]. The expression and catalytic activity of NADPH oxidase catalytic subunit Nox family proteins are increased, promoting ROS production and leading to oxidative stress [43]. AngII/AT1R signaling can increase the production and release of several pro-inflammatory cytokines, including TGF- β, IL-1a, IL-1 β, IL-6, IL-8, MCP-1 (monocyte chemoattractant peptide protein-1), M-CSF, COX-2 (cyclooxygenase-2), and CRP (C-reactive protein) in tumors and stromal cells [44]. Oxidative stress and infiltration of inflammatory cytokines promote the occurrence of cancer-related inflammation and enhance immune suppression in tumor microenvironment (TME) [45]. The activation of intermediate signals such as Ca2+, ROS, and metalloproteinases (ADAM) triggers transactivation of epidermal growth factor (EGF) receptor (EGFR), promoting cell migration [46]; Activation of Nox52 + and calmodulin by Ca2 + leads to the growth and inflammatory response of human microvascular endothelial cells [47]. Experimental studies have shown that AngII/AT1R signaling can promote the expression of VEGF in tumors and stromal cells to promote angiogenesis [48–50]. In addition, Ang II/AT1R signaling activates various intracellular protein kinases, including mitogen activated protein kinase (MAPK), PI3K, and Akt [42], activating IKK2/IKK- β, causing NF- κ B activation [51], while regulating cancer associated fibroblasts (CAFs) and pro fibrotic pathways such as TGF- β. Induction of epithelial mesenchymal transition (EMT) process [52]. EMT involves increasing production of matrix metalloproteinases (MMPs), where MMP-13 is elevated in inflammatory bowel disease [53] and is considered a biomarker for early cancer prediction [54, 55], which can be used to- α Cutting into biologically active 17-kD subunits leads to NF- κ Activation of the B signaling pathway [56]. NF- κB is a major inflammatory signaling pathway and a classic carcinogenic signaling pathway [57], NF- κ The activation of B can reverse induce EMT, promote tumor growth, and prevent cell apoptosis [58]. In summary, AngII/AT1R promotes the formation of TME through second messengers, the activation and remodeling of various kinases, and induction of inflammation [44, 59], and activates NF-κ B-class cancer related signaling pathways.

We used the wSDTNBI algorithm to predict and optimize the combination of components and targets within CMF granules. Then, molecular docking

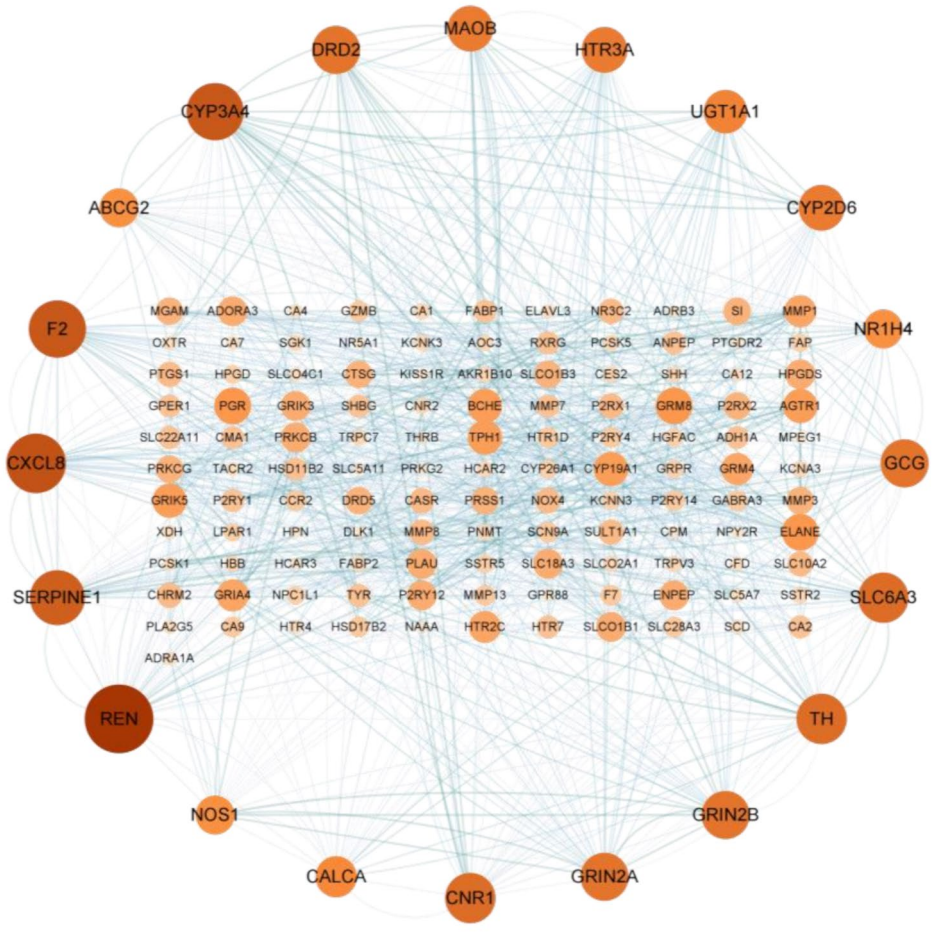

Fig. 4 Intersection Target PPI Network Diagram. The size of the circle represents the node degree of the target protein

and RT-qPCR were used to verify the CMF granules regulation of AT1R, CXCL8, and MMP13. This further confirmed that the CMF granules may achieve its preventive and therapeutic effects on CRA by regulating the REN/Ang II/AT1R axis, as well as inhibiting inflammatory reactions, EMT processes, and TME formation.

It is necessary to acknowledge the limitations of this work. The cases in this study all come from a single hospital. Whether the sample can fully represent the real status of patients after CRA surgery remains to be further studied, and this may affect the credibility and level of evidence-based evidence of this research. Moreover, the follow-up duration of this study may not be long enough to observe long-term effects or potential late-onset side effects. In our future work, we intend to conduct multicenter, large-sample, high-quality randomized controlled clinical studies on the prevention of CRA recurrence using the CMF. By extending the follow-up period and incorporating objective indicators, we will comprehensively assess the impact of CMF on patients after CRA surgery over a long period, from multiple perspectives and dimensions. Moreover, we have not yet conducted in vivo experimental validation, and cannot evaluate the plasma active ingredients and concentrations of CMF granules, as well as their relationship with adenoma recurrence. Therefore, further research is needed for in-depth mechanism exploration and comprehensive evaluation of clinical efficacy.

Conclusion

Based on the clinical trial, this study integrated chemical composition analysis, data mining, network prediction analysis, and experimental verification to prove that CMF can prevent the recurrence and progression of CRA by regulating the REN/Ang II/AT1R axis. The tool cell used in this in vitro experiment is supported by the national invention patent, which is a precancerous colorectal adenoma cell line similar to the type of adenoma in the enrolled patients, which provides an experimental basis for the clinical application of CMF for the prevention and treatment of colorectal adenoma patients. In the future, we intend to apply this

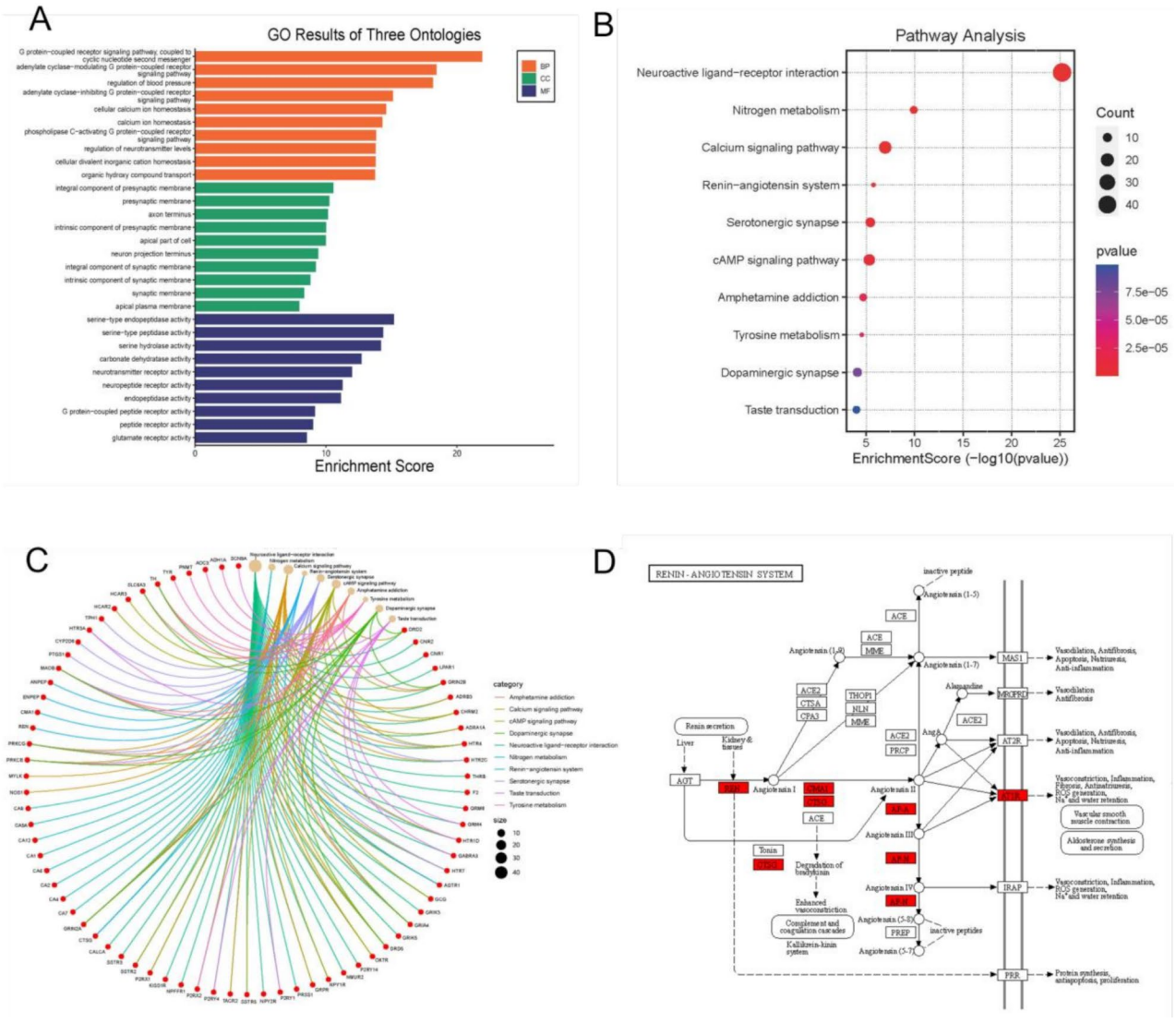

Fig. 5 (**A**) GO Enrichment Analysis Results, (**B**) results of KEGG enrichment analysi, (**C**) Core Target Related Signal Pathways, (**D**) Renin angiotensin signaling pathway

tool cell to more comprehensively explore the curative mechanism of the Canmei Formula to provide new insights into conditions such as colorectal precancerous lesions.

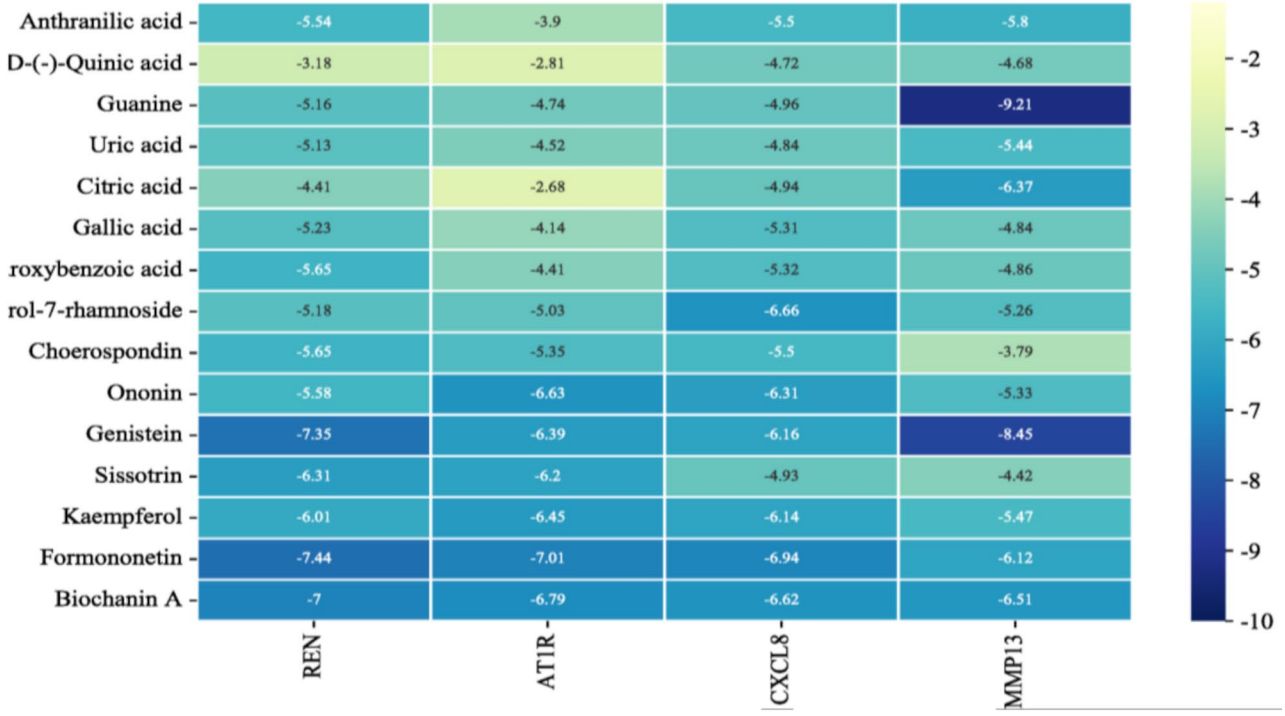

Fig. 6 The binding energy between active components of CMF and core targets. The depth of color indicates the magnitude of binding energy. The darker the color, the lower the binding energy, indicating stronger binding

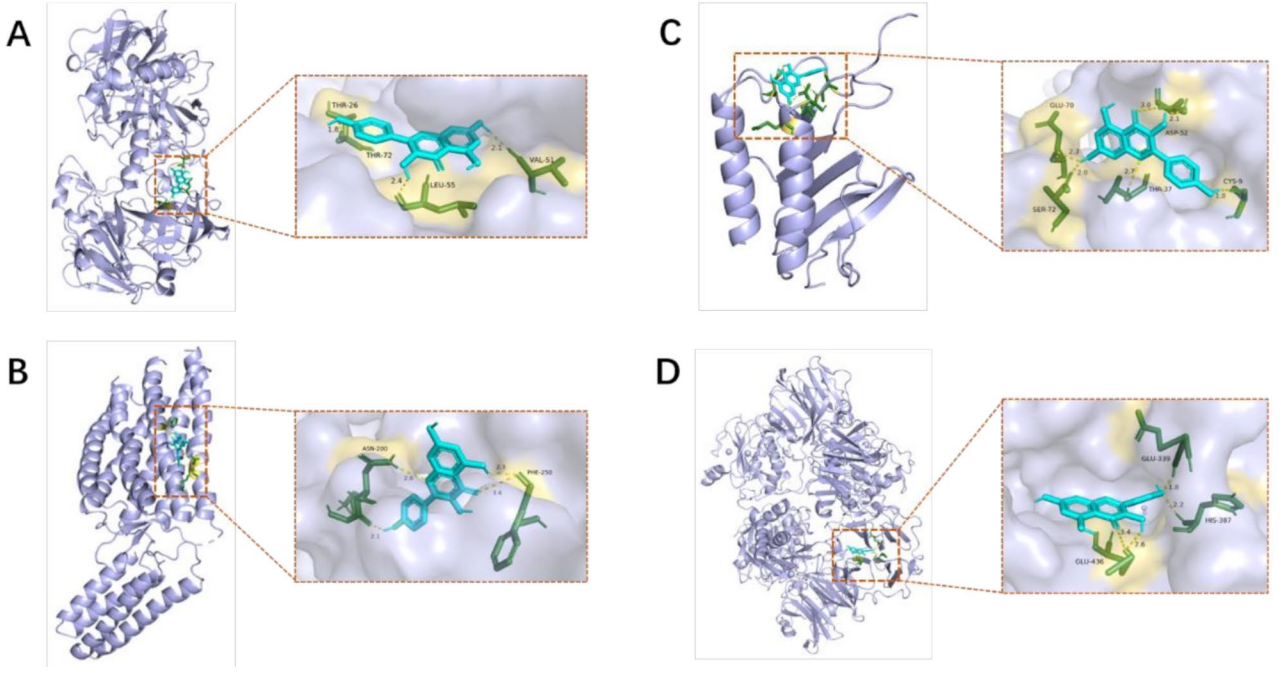

Fig. 7 Three dimensional view of molecular docking of kaempferol with REN (**A**), CXCL8 (**B**), AT1R (**C**), and MMP13 (**D**)

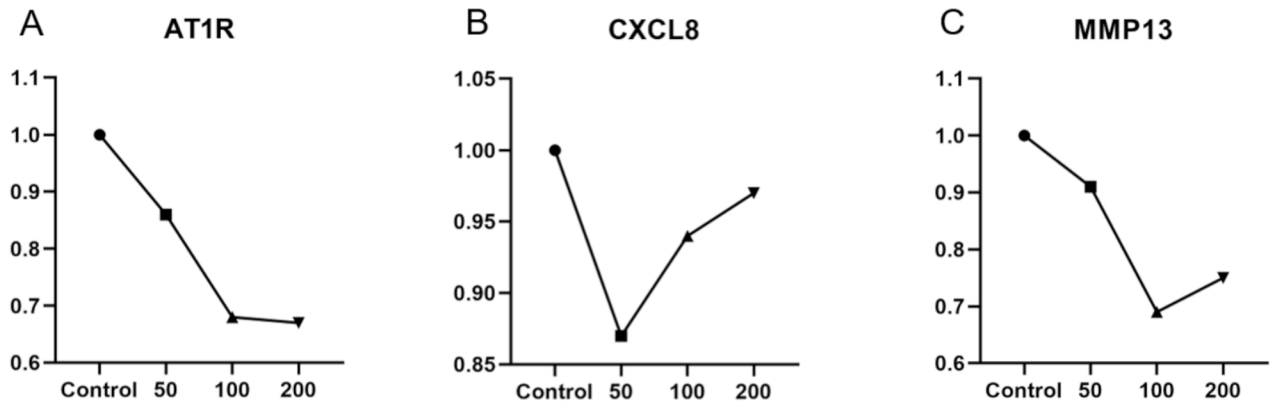

Fig. 8 The effect of different concentrations of CMF on AT1R (**A**), CXCL8 (**B**), and MMP13 (**C**) (the x-axis represents the administration concentration, and the y-axis represents the expression multiple)

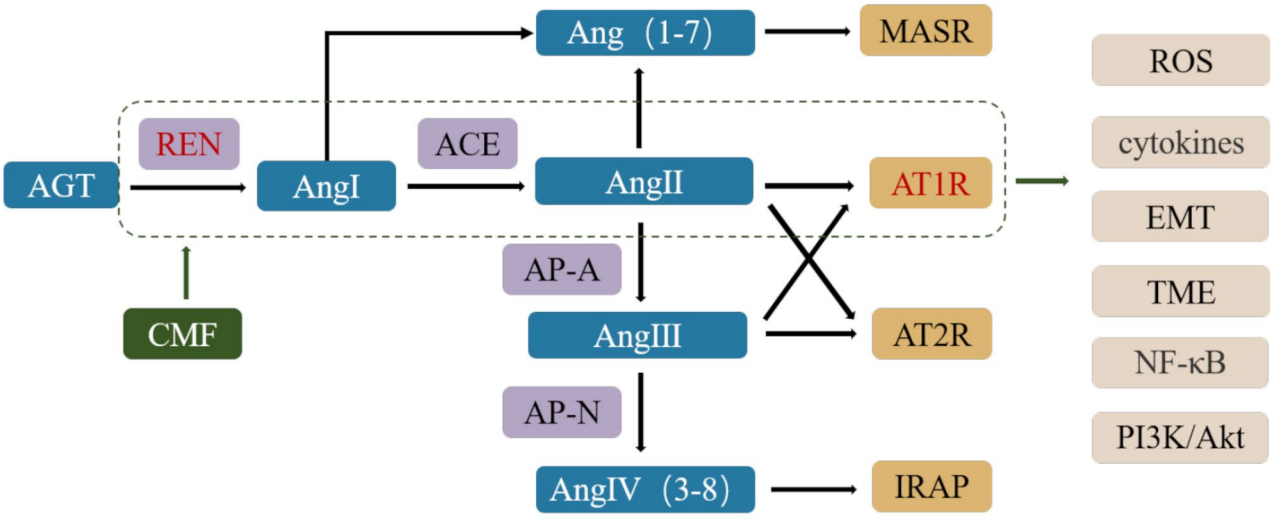

Fig. 9 Regulatory effect of CMF granules on REN/Ang II/AT1R axis

Author Contributions
Formal analysis, Guanhong Li, Xiaochen Fu and Jingnan Wang; Project administration, Xiaoling Fu; Validation, Min Zhang; Writing – original draft, Yimin Xu and Xinyue Han; Writing – review & editing, Xiangying Lin.All authors contributed to the article and approved the submitted version.

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Data Availability
The raw data supporting the conclusion of this article will be made available by the authors.

Declarations

Competing Interest
No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously. All the authors listed have approved the manuscript that is enclosed.

Ethics Approval and Consent to Participate
The study protocol was reviewed and approved by the Ethics Committee of Yueyang Hospital of Integrated Chinese and Western Medicine affiliated to Shanghai University of Traditional Chinese Medicine (Ethical Approval Number: 2021 – 124) and conformed to the ethical standards for medical research involving human subjects, as laid out in the 1964 Declaration of Helsinki and its later amendments. Participants provided written informed consent prior to taking part in the study.

Conflict of Interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author details
[1]Department of Oncology, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China
[2]Yiwu Traditional Chinese Medicine Hospital, Jinhua 322000, China
[3]Department of General Internal Medicine, Kunming Municipal Hospital of Traditional Chinese Medicine, Kunming 650000, China
[4]Department of Hospital Affairs, Yueyang Hospital of Integrative Medicine, Shanghai University of Traditional Chinese Medicine, Yueyang 200437, Shanghai, China

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