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Qu-Yu-Jie-Du Decoction Inhibits the Recurrence and Metastasis of Stage III Colorectal Cancer by Targeting Inflammatory Pathways: A Retrospective Clinical Study

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Introduction

Background: Colorectal Cancer (CRC) is among the leading causes of cancer-related mortality and morbidity worldwide. Qu-Yu-Jie-Du (QYJD) decoction 10 prevents enteritis and prolongs CRC survival time; however, large11 scale clinical research and mechanism analysis are needed. Hence, our study aimed to confirm the effectiveness of QYJD decoction in 13 stage III CRC patients and its potential anticancer mechanisms.

Methods: We conducted a retrospective clinical study among 177 patients with stage III CRC. An ancient and modern medical record cloud platform.

Software was used to analyse the high-frequency traditional Chinese medicines of stasis and toxin syndrome. Additionally, we confirmed the antitumor effect of QYJD decoction in a mouse model of inflammatorycoecal xenografts and analysed the mechanism of QYJD 21 decoction using systems pharmacology and bioinformatics.

Results: Multivariate-adjusted Chinese Herbal Medicine (CHM) intake was related to better disease-free survival (hazard ratio [HR] =0.48, P=0.0147), especially in the regular CHM intake group, which had a 73% lower risk of recurrence (HR=0.27, P=0.0019). A total of 166 traditional Chinese medicines were among the 416 prescription medicines analysed. We used cluster analysis to identify 18 traditional Chinese medicines, which coincide with QYJD decoction. Animal experiments suggest that QYJD decoction can inhibit intestinal inflammation and tumour growth. The network pharmacology study predicted 518 QYJD anti-CRC targets. QYJD decoction improves 33 disease-free survival in stage III CRC patients.

Conclusion: Our results show that QYJD decoction inhibits tumour growth by anti36 inflammatory activity, making it a promising anti-CRC herbal compound.

Keywords: Quyujiedu decoction; Colorectal cancer; Stage III; Disease-free survival; Cohort study; Anti-inflammatory; Tumour inhibition.

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Introduction

Colorectal Cancer (CRC) is one of the most common gastrointestinal malignancies worldwide, with the third-highest rate of occurrence and the second-highest rate of mortality of all cancers [1,2]. Recurrence and metastasis are the principal reasons for CRC treatment failure; the 5-year survival rate of patients with metastasis is only 10% [3]. An international study found that more than 25% of patients with stage III colon cancer experienced recurrence or metastasis within 3 years of surgery [4]. The median Disease-Free Survival (DFS) of patients with stage III CRC is 19 months [5]. Therefore, the prevention of postoperative recurrence or metastasis of stage III 61 CRC remains an urgent clinical problem. According to the National Comprehensive Cancer Network® guidelines, stage III CRC treatment is based on surgical procedures, with radiotherapy and chemotherapy as postoperative adjuvant treatment [6]. Adjuvant chemotherapy has become a routine treatment for stage III colorectal cancer in China and worldwide [7]; however, the curative effect of these treatments remains unfavorable, and the efficacy of 5-flurouracil-based therapy is frequently compromised by the development of chemotherapy resistance [8]. Recently, there has been an increase in clinical research on Chinese Herbal Medicine (CHM) for CRC. Recent studies found that CHM can be administered as an effective auxiliary therapy to significantly improve the quality of life and reduce the adverse reactions to chemotherapy in patients with CRC [9-11]. In addition to adjuvant chemotherapy, many Chinese patients also use CHM to delay CRC recurrence [12-14]. A prospective multicentre study confirmed that a longer duration of CHM use significantly improved the survival outcomes of patients with stage II–III CRC in Beijing, China [12]. Additionally, a randomised, double-blind, placebo-controlled clinical trial in Shanghai, Nanjing, and the Central Plains of China reported that PRM1201 improved the adjuvant treatment of stage III colon cancer [14]. Therefore, concomitant with the ever-increasing categorical evidence-based pharmacological therapies, the clinical utilisation of CHM has proven to be a valuable approach for treating CRC.CHM is deeply rooted in Chinese culture, especially in the Guangdong province, where it has been used for thousands of years as the preferred method of treating diseases [15]. However, CHM use varies across regions. For example, the climate in southern China is humid, which is significantly different from that of the northern region, and, thus, treatment concepts and curative effects are specific and based on the characteristics of Lingnan CHM. According to pre-clinical data in one report, in the classification of patients for stage III CRC admission, colonic damp-heat and stasis-toxin syndrome was observed in 60% of patients, for whom the indicated Traditional Chinese Medicine (TCM) treatment is blood stasis removal, detoxification, and clearing of heat [15,16]. A previous study showed that the Qu-Yu-Jie-Du (QYJD) CHM therapy was related to improved survival outcomes in CRC patients with liver-limited metastases [17], preliminarily confirming that QYJD decoction may inhibit intestinal cancer cells by activating the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway through the epigenetic regulation of demethylation [18]. In Guangdong province, the effects of QYJD decoction on the survival outcomes of patients with stage III CRC remain unknown. Hence, we conducted this study to clarify whether QYJD decoction is associated with a reduced risk of recurrence in patients with stage III CRC and clarified the mechanism by which QYJD decoction delays tumour occurrence and development through network pharmacology and animal experiments.

Materials and methods

Study participants: The retrospective cohort study was conducted at our hospital in China.

Inclusion and exclusion criteria: Patients diagnosed with stage III CRC pathologically or clinically, who had undergone radical surgery and were admitted to our hospital between 2016 and 2018 were included in our study. Patients were excluded if they had been diagnosed with secondary CRC; had additional organ malignancies; had received neoadjuvantchemotherapy, radiotherapy, or targeted therapy; had pathological tumour classification or clinical characteristics that were severely fragmented, or presented with severe primary diseases, such as heart, kidney, liver diseases; or had a DFS period of less than 3 months.

Ethics: This study was approved by the ethics committee of our hospital, and the need for informed consent was waived. The research was conducted in accordance with the World Medical Association Declaration of Helsinki.

Exposure: CHM therapy use Patients were assigned to exposure groups according to the literature; those who had taken CHM for more than 3 months during the study period were allocated to the Chinese medicine treatment group (CHM intake group), and other patients were assigned to the group that did not take CHM (non-CHM intake group). Based on prognostic follow-up data, we also calculated the duration of CHM therapy received by classifying TCM users into two groups as follows: a regular Chinese medicine group comprising patients who took Chinese medicine regularly (at least 14 doses per month) for at least 12 months and an intermittent CHM group comprising those who took CHM irregularly (less than 14 doses per month) for between 3 months and 1 year.

Covariates: In the retrospective cohort study, we recorded information, including sex, age, pathological tumour type, clinical TNM staging, tumour differentiation, anatomical location of the tumour, adjuvant radiotherapy, and adjuvant chemotherapy. According to previous studies, we collected variables that have a known impact on the prognosis of stage III CRC, including Right-Sided Colon Cancer (RCC) and Left-Sided Colon Cancer (LCRC); intravascular tumour thrombus; nerve bundle infiltration; and unstable microsatellites. The chemotherapy regimens used for the enrolled patients were as follows: oxaliplatin + leucovorin + fluorouracil, oxaliplatin + capecitabine, and other combinations. The ancient and modern medical record cloud platform software Preclinical data statistics show that blood stasis removal and detoxification is the basic treatment method. We collected the available TCM prescriptions and input the data into a cloud platform software of ancient and modern medical records. A database of patients with stage III CRC was established; the data were standardised and added to the analysis pool for data mining analysis. Moreover, we used binomial association rule and cluster analyses to conduct data mining on the frequency of use of high-frequency drugs with the accumulation of stasis and toxins.

Therapeutic efficacy evaluation

DFS time, defined as the time from the start of the treatment to

the observation of metastasis/recurrence or death, was the main endpoint indicator for evaluating the survival outcome of patients with CRC [5]. Data were acquired by the hospital medical record system or telephone follow-up. DFS time was calculated in months, and the assessors were blinded to patient information to remove assessment bias. All data were updated on August 30, 2021.

Cell culture

Human LoVo CRC cells were purchased from the Cell Bank of the Chinese Academy of Sciences in Shanghai, and cells were cultured in RPMI-1640 complete medium containing 10% fetal bovine serum and incubated at 37°C and 5% CO_2. LoVo cells were seeded into mouse 184 coecal sub mucosal tissue at a concentration of 5×10^4/mL in a volume of 10–15 μL.

Animal source

BALB/c-nu nude female mice, SPF grade, weighing 18~22 g, were purchased from Nanjing University-Nanjing Institute of Biomedicine (laboratory animal certificate number: NO. 32002100007118). The experimental animals were kept in the SPF animal room of Guangdong Pharmaceutical University (experimental unit license number: SYXK(Guangdong 2014-0125) and randomly grouped in individual experiments (n = 6 per group).

Experimental drug

(1) QYJD decoction: The prescription for removing blood stasis and detoxification comprises Coix seed, Burnet, Sophora japonica, peach kernel, and turtle bug, among others. All CHM in the recipe were purchased from the Chinese Pharmacy of the First Affiliated Hospital of Guangzhou University of Chinese Medicine. Using the body surface area (BSA) method, we calculated the daily dose of 21.6 g/kg for the 203 mice (equivalent to the adult daily dose).

(2) Acetylsalicylic acid (ASA, Aspirin): Purchased from the First Affiliated Hospital of Guangzhou University of Chinese Medicine, was dissolved in water (2 mg/mL) at a dose of 20 mg/kg/day for the experimental mice (corresponding to a daily dose of 150 mg for an adult according to the BSA method).

Coecal xenograft mouse models in an enteritis-simulated environment. Acute enteritis was induced by administering 1.5% dextran sulfate sodium salt (DSS) for 1 week, simultaneous with the initiation of the experiment of the drug treatment group. During the period, the disease index was scored according to the weight of mice, stool characteristics, and presence of blood in stool; 1–3 days after the end of DSS administration, LoVo cells were planted in the submucosa of the coecum, and the drug was continued for 2 weeks. After the treatment, mice in each group were sacrificed after sodium pentobarbital-induced anaesthesia; the entire colon was dissected, the contents were taken out, the colon length was measured, and the tumour volume was calculated.

Immunohistochemistry assay

The tumour tissues were sliced into 4–6 μm sections, and antigens were retrieved after deparaffinisation with graded alcohol. Tissue sections were subsequently blocked with 5% bovine serum albumin for 60 min. Tissue sections were incubated with anti-ki67 (1:100) antibodies at 4°C overnight. The secondary antibody was added, and the resulting mixture was incubated at room temperature for 1 h after washing three times. Subsequently, diaminobenzidine solution was used for staining; the sections were counterstained with hematoxylin, dehydrated, and mounted on slides. Sections showing brownish yellow under the microscope indicated positive results.

Network pharmacology analysis

Based on the network pharmacology 'disease-gene-target-drug' interaction network, the pharmacokinetic parameters oral availability ≥30% and drug similarity ≥0.18 in the Traditional Chinese Medicine Systems Pharmacology Technology Platform (TCMSP) and a Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine (BATMAN-TCM) were used to screen the potential 241 active ingredients of QYJD decoction. A score cutoff ≥20 (P≤0.05) was used to predict and screen the corresponding targets. With a score ≥5 as the screening criterion, the target genes related to CRC were retrieved from the Gene cards database, and the common targets were screened by intersecting with the active ingredient target genes in QYJD decoction. The String database and Cytoscape were used to build a 'component-target' network. Using the database for annotation, visualisation, and integrated discovery (DAVID) database, Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway analysis were performed on the common targets of the QYJD decoction and CRC.

Statistical analysis

Baseline differences between the CHM and non-CHM intake groups were compared. Data were presented as means ± standard deviation for continuous variables and as frequencies (percentages) for categorical variables according to CHM intake. Three variables, including intravascular cancer thrombus, bundle invasion, and microsatellite state showed a high missing rate. We used the multiple imputation function of missing data to improve the missing data of the three sets of variables. One-way analysis of variance (ANOVA), Kruskal-Wallis H tests, and chi-squared tests were employed to evaluate any statistical differences between groups. The association between CHM therapy and DFS in stage III CRC patients was determined using a univariate linear regression model and multivariate-adjusted models. Considering the unbalanced baseline between the CHM and non-CHM groups, we conducted exploratory subgroup analyses and interaction detection to test the stability of the relationship. The mean ± standard error (x ± s) of the experimental data is expressed. For normally distributed continuous data, one-way ANOVA was used for comparison among groups. Otherwise, the Kruskal-Wallis H test was used for comparison among groups. If the difference between groups was statistically significant, the Student-Newman-Keuls method, Bonferroni method, or the Dwas, Steel, Critchlow Flinger method was used for multiple comparisons. For repeated data at different time points in three or more groups, such as the body weight of mice and disease activity index (DAI) indicators, repeated measures analysis of variance was used. Analyses were performed using the statistical software packages R and Empower States. All tests were two-sided, and the statistical significance level was set at 0.05.

Results

In our study, the high-frequency medicines for removing blood stasis and detoxification were extracted from all Chinese medi-

cine prescriptions in the retrospective cohort study, which almost coincides with QYJD decoction. Animal experiments were used to confirm the anti-inflammatory and tumour-inhibiting effects of QYJD decoction. The main active components and action pathways of QYJD 291 decoction were speculated based on network pharmacology. The flow diagram of the present study is shown in Figure 1.

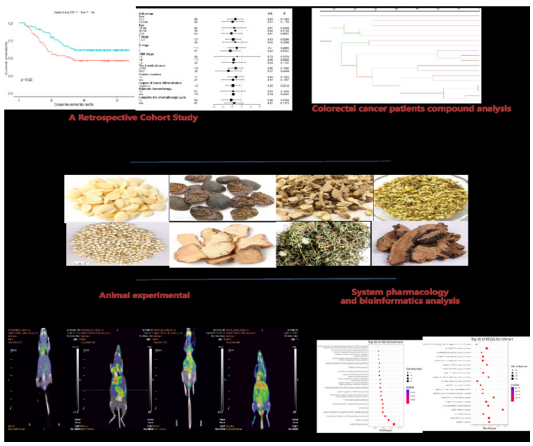

Figure 1: Flow diagram.

Patients

By July 30, 2018, 206 medical records were collected; 177 patients met the inclusion criteria, whereas 40 were excluded (Figure 2A). Of these, 121 (69.94%) patients received TCM therapy, 64 were taking Chinese medicine intermittently, and 57 were taking Chinese medicine regularly. After August 2020, those whose information had not been reviewed through telephone follow-up or the electronic medical record system were considered lost to follow-up. In this study, 13 patients were lost to follow-up, with a loss follow-up ratio of 7.34%. Information on the DFS of 111 (62.71%) and 53 (29.94%) patients was collected via the hospital medical record system and telephone follow-up, respectively.

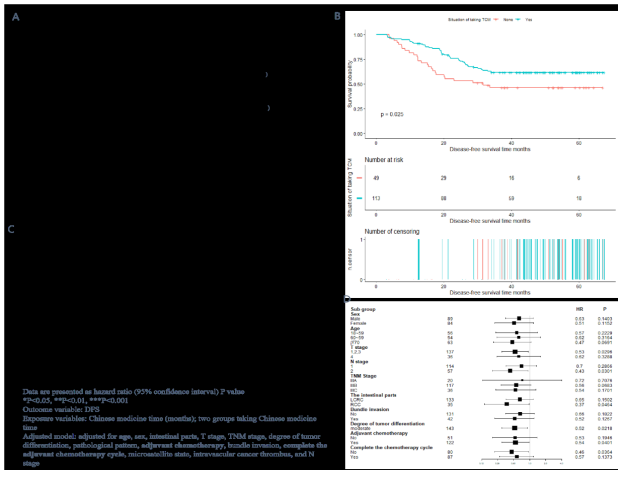

Figure 2: (A) Flow chart of patient selection for the cohort study **(B)** Kaplan-Meier curve of CHM therapy to DFS showing fewer events of cancer recurrence/metastasis in the CHM intake group than the no CHM intake group. **(C)** Univariate and multivariable Cox regression model between the time of CHM intake and disease-free survival. **(D)** Forest plot of HR and 95% confidence interval between the CHM group and the no CHM group.CHM, Chinese herbal medicine; DFS, disease-free survival; TCM, traditional Chinese medicine; HR, hazard ratio; LCRC, left-sided colon cancer; RCC, right-sided colon cancer.

Participant characteristics

The study participants were enrolled from January 5, 2016 to June 25, 2018. The median follow-up time was 51.07 months; however, the median DFS could not be determined; the average DFS time was 34.87 ± 19.59 months. Of the 173 patients, 69 (42.07%) experienced recurrence or metastasis. The demographic and clinical characteristics of the study patients classified by CHM use are presented in Table 1. As shown in Table 1, the two groups were unevenly distributed with regard to factors such as age, bundle invasion, adjuvant chemotherapy, and complete adjuvant chemotherapy cycle. Moreover, a higher proportion of patients received chemotherapy (75.21% vs. 59.62%) and completed chemotherapy (57.26% vs. 40.00%) in the CHM intake group than in the CHM intake group. Baseline characteristics and clinical features classified by CHM intake (none, intermittent, or regular CHM intake) showed that age (P=0.075), T stage (P=0.088), bundle invasion (P=0.099), and adjuvant chemotherapy (P=0.036) were unevenly distributed.

CHM therapy and DFS

The median survival and hazard ratio (HR) of mortality for each subgroup are shown in Figure 2. The predefined primary endpoint in our analysis was the DFS. The median DFS for the CHM intake group 333 could not be determined; however, it was 31.67 months in the non-CHM intake group. The 1-, 2-, and 3-year recurrence and metastasis rates in the CHM intake group were 7.96%, 24.29%, and 38.21%, respectively, while those in the non-CHM intake group were 20.41%, 337 46.94%, and 53.52%, respectively (Figure 2B). Univariate Cox proportional-hazards regression model analysis revealed that the CHM intake group showed a notable 43% decrease in recurrence/metastasis rate (HR = 0.570; 95% confidence interval [CI], 0.35–0.94, P=0.0266). In particular, on applying the Cox proportional-hazards regression model to adjust covariates such as age, bundle invasion, adjuvant chemotherapy, completion of the chemotherapy cycle, and so on, the HR was 0.48 (95% CI, 0.27–0.87; 345 P=0.0147). Moreover, relative to the non-CHM intake group, the multivariate Cox proportional-hazard regression model analysis result showed that the risk of recurrence/metastasis in the intermittent CHM intake group was significantly reduced by 31% (HR=0.69, P=0.2416) and it was decreased by 73% (HR=0.27, P=0.0019) in the regular 350 CHM intake group. Considering the baseline differences between the two groups, we further conducted stratified analysis and statistical interaction methods to confirm the influence of CHM on the prognosis of stage III 354 CRC patients. We examined the effect of CHM on DFS in patients in different variable stratifications (Figure 2C). The association between CHM intake and DFS in patient outcomes seemed consistently stratified by 358 age, sex, tumour characteristics, and different treatments. The relationship between enteritis and DFS in stage III CRC patients. The univariate Cox regression model showed that stage III CRC patients with a history of colitis had a 1.45-fold increased risk of recurrence and metastasis (HR=2.45; 95% CI, 1.14–5.25; P=0.0218). The multivariate Cox regression models suggested a 2.08-fold increased risk of recurrence and metastasis (HR=3.08; 95% CI, 0.95–10.02; P=0.0613).

QYJD decoction and stage III CRC patients

Even more, in the univariate analysis, we found that the syn-

drome of heat-toxin and blood stasis can increase the risk of recurrence and metastasis in patients with stage III CRC by 1.37 times (HR=2.37; 95% 372 CI, 1.07–5.23; P=0.0332). The syndrome of heat-toxin and blood stasis is one of the main syndrome types of colorectal cancer, which is generally treated with the QYJD decoction. In order to further confirm the high-frequency TCM combination for stage III CRC stasis and toxin accumulation syndrome, a total of 416 CHM prescriptions for the large intestine stasis-toxin syndrome (stasis-toxin accumulation) were analysed. Among them, there were 324 prescriptions for simple TCM maintenance treatment and 92 prescriptions for adjuvant chemotherapy. The statistics of the 92 prescriptions for the stasis-toxin accumulation syndrome type involved a total of 166 TCMs. The frequency of all medicines was counted. Furthermore, 10 medicines with a frequency of use of ≥50% are listed in descending order of frequency as follows: Semen persicae, Ground beetle, Radix sophoraefalvescentis, Radix Sanguisorbae, Solanum nigrum, Herba Sarcandrae, Shancigu, Pachymacocos, Atractylodes, and Taxus chinensis.We used cluster analysis to screen out 18 TCMs that were used more than 100 times, which almost coincides with QYJD decoction.The ranking results are shown in Figure 3A.

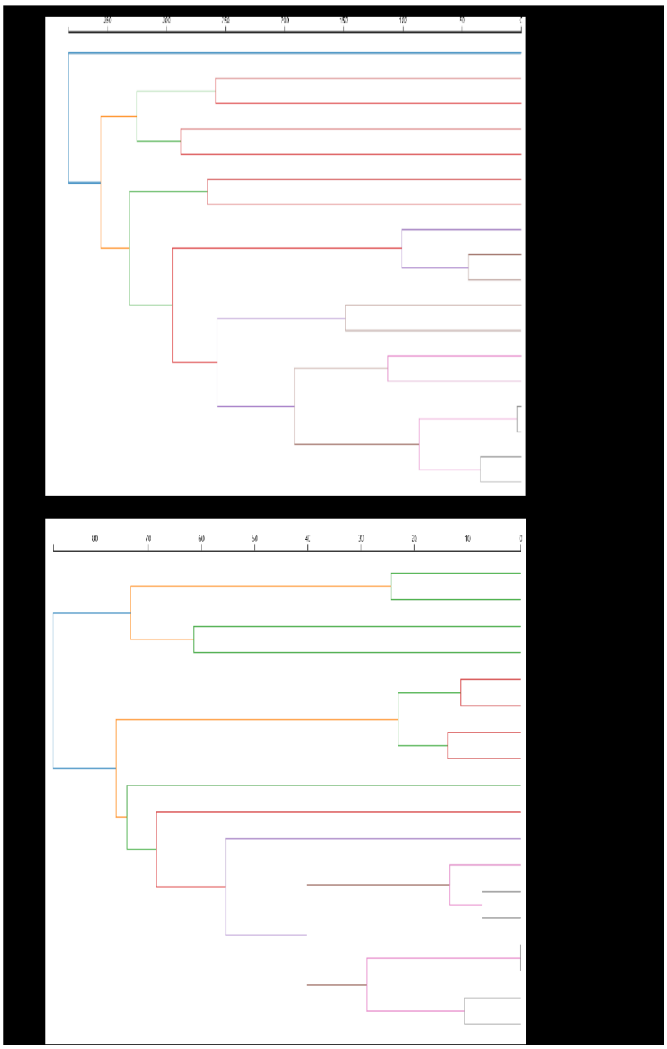

Figure 3: (A) Cluster analysis diagram of high-frequency CHM forheat-toxin syndrome and blood stasis in stage III CRC; **(B)** Clusteranalysis diagram of high-frequency CHM during adjuvant chemotherapy. CRC, colorectal cancer; GGEC, Galli Gigeriae Endothelium Corneum.

Clinically, apart from the method of removing blood stasis and detoxification being useful in attacking cancer pathogens, it also has great importance in strengthening the spleen, nourishing qi, and protecting stomach qi. Especially during adjuvant chemotherapy, the frequency of use of ChenxiaSijunzi decoction was significantly higher than that of TCM maintenance therapy (Figure 3B). QYJD decoction inhibits enteritis symptoms in mice with coecaltumours in an enteritis-simulated environment Repeated measures ANOVA indicated that there was a significant difference in body weight between different groups (P=0.001), and there was a significant interaction between body weight and time (Greenhouse-Geisser test, P=0.017) (Figure 4B). There were differences in the body weight of mice between groups at different experimental times. From the 10th day of the experiment, the model group mice body weight significantly decreased (P<0.1), whereas that of mice treated with QYJD decoction and ASA significantly increased (P<0.1).

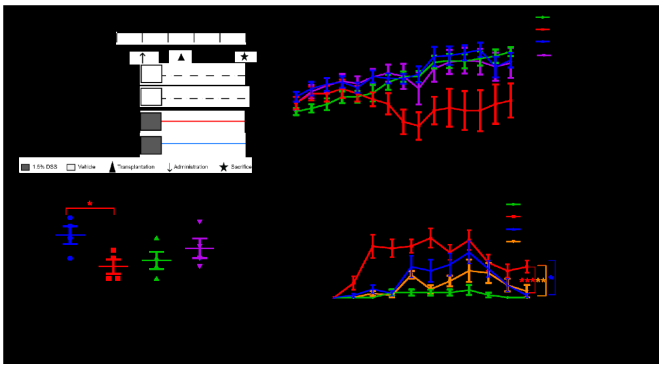

Figure 4: (A) Animal experiment flow chart. **(B)** QYJD decoction canimprove the weight loss of model mice; **(C)** QYJD decoction and ASA may prolong colon length in model mice; **(D)** QYJD decoction and ASAcan reduce the DAI score of model mice. DSS, dextran sulfate sodium salt; OCMI, ; ASA, acetylsalicylic acid; QYJD, Qu-Yu-Jie-Du; Con, control.

QYJD decoction inhibits inflammatory symptoms in mice with intestinal cancer in an enteritis-simulated environment. We measured the colon length (the length from the coecum to the anus) of mice in each group: compared with the control group, the colon length of mice in the model group was significantly shorter (P=0.024). Compared with the model group, colon length of the QYJD group was longer but the difference was not significant (P=0.16) (Figure 4C). After the mice in each group were orally administered DSS, the body weight, stool quality, and presence of blood in stool were monitored daily. DAI was assessed in all groups. Repeated-measures ANOVA showed that there was a significant difference in DAI value between different groups (P=0.02), and there was a significant interaction between DAI value and time (Greenhouse-Geisser test, P=0.000). Compared with mice in the blank group, the DAI of mice in the model group changed significantly (P=0.000), and this change became apparent from the 4th day, reached the maximum on the 7th day, and lasted for 21 days. Compared with mice in the model group, the DAI values of the mice in the QYJD decoction group (P=0.002) and ASA (P=0.022) group were significantly lower (Figure 4D).

QYJD decoction inhibits tumour proliferation in mice with coecaltumours in an enteritis-simulated environment. The influence of QJYD decoction on the inhibition of tumours in mice: QYJD de-

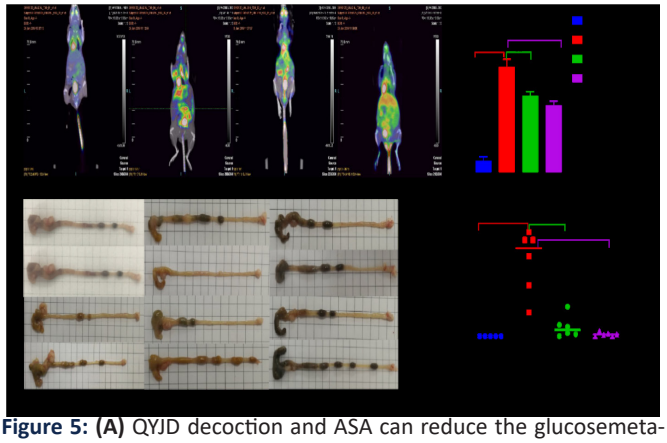

Figure 5: (A) QYJD decoction and ASA can reduce the glucosemetabolism by a tumour in mice; **(B)** Fresh colon tissue and tumour after dissection of different groups of mice; **(C)** The tumour volume ofdifferent groups of mice. The tumour volume in the QYJD group wassignificantly reduced.DSS, dextran sulfate sodium salt; OCMI, ; ASA, acetylsalicylic acid; QYJD, Qu-Yu-Jie-Du; Con, control.

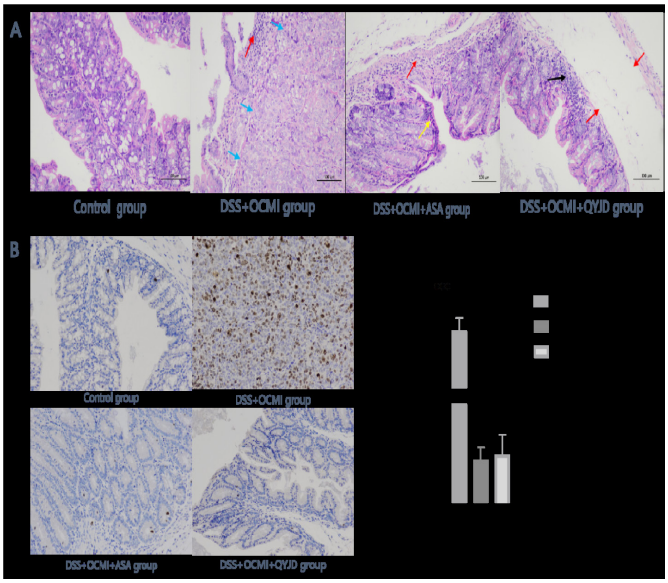

Figure 6: (A) HE staining results of different groups of mice (×100); **(B)** Immunohistochemistry showing Ki4 expression levels in different groups (×200). **(A)** shows the results of colonic HE staining inthe model mice treated with QYJD decoction and aspirin: In the normal group, the colon tissue structure of mice is complete, thenumber of intestinal glands is abundant, the cell morphology is normal, and the staining is uniform; In the model group, the sub mucosa of the mouse model group shows clustered tumour metastases (indicated by blue arrows), the mucosal epithelium and intestinal glands are lost, the damage invades the submucosa, and many inflammatory cells have infiltrated in the mucosa and sub mucosa (indicated by the red arrow). The local intestinal gland structure has disappeared in the colon tissue of mice in the ASA group, and a small amount of inflammatory cell infiltration is seen (red arrow); pyknosis is hyper chromatic, and cytoplasmic eosinophilia is enhanced (yellow arrow). The colon tissue structure of the mice in the QYJD group is complete, the local intestinal gland structure has disappeared, a small amount of inflammatory cells have infiltrated (black arrows), and the sub mucosal space is widened (red arrows).HE, hematoxylin and eosin; DSS, dextran sulfate sodium salt; OCMI,; ASA, acetylsalicylic acid; QYJD, Qu-Yu-Jie-Du.

coction can reduce the glucose metabolism in the tumour. After 21 days of administration, 3 mice in each group were randomly selected for positron emission tomography/computed tomography. The level of SUVmax could reflect the uptake of glucose metabolism by the lesions; the SUVmax value of the model group was significantly higher than that of the control group (P=0.000); compared with the model group, the SUVmax value of the QYJD decoction group P=0.001) and the ASA group (P=0.005) decreased significantly. The effect of QYJD decoction on the volume of tumours in the intestinal cancer mouse model: The colon tumour volume of mice in the model group was significantly larger than that of normal mice (P=0.012). Compared with the model group, the tumour volumes of mice in the QYJD group (P=0.013) and ASA group (P=0.015) were significantly reduced.

The expression of Ki67 in the intestinal tract of mice in the model group and the QYJD decoction group Ki-67 is a nuclear antigen related to cell proliferation, which only exists in cells in the proliferation cycle. The Ki67 value of the model group was significantly increased compared with that of the normal group; additionally, the QYJD decoction and aspirin groups had significantly reduced expression of tumour tissue growth-related protein Ki67 compared with the model group (Figure 6B).

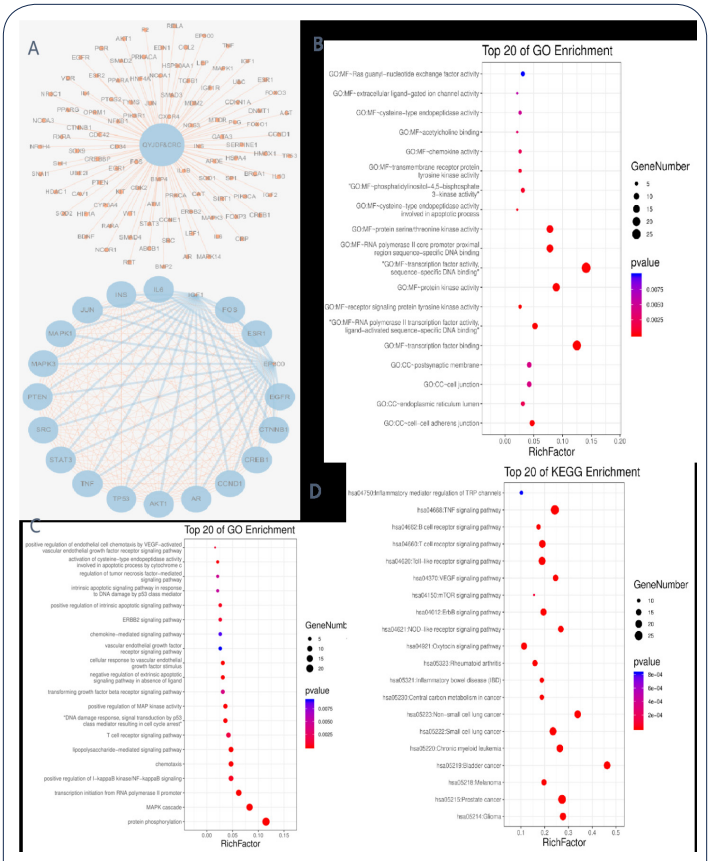

Figure 7: (A) Selected 105 target genes that meet the card value; all target genes and the top 20 target genes are plotted separately for visualisation. (B) Enrichment analysis of the GO-MF/CC pathway of potential targets of active ingredients of QYJD decoction and colorectal cancer. (C) Enrichment analysis of GO-MF/CC, GO-BP pathway of potential targets of active ingredients of QYJD decoction and colorectal cancer. (D) Enrichment analysis of KEGG pathway of potential targets of active ingredients in QYJD decoction and colorectal cancer. GO, Gene Ontology; MF/CC, molecular function/cell composition; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes.

QYJD decoction & CRC network pharmacological analysis. With the limited conditions, there were 23 active components and 95 target proteins in peach kernel; 1 active component and 32 target proteins in P. chinensis; 8 active components and 122 target proteins in dandelion; 42 active components and 971 target proteins in Sophoraflavescens; 15 in Tuckahoe 1 active ingredients and 222 target proteins, 9 active ingredients and 48 target proteins of Coix seed, 9 active ingredients and 496 targets of Burnet, 6 active ingredients and 366 targets of Sophora japonica. After removing the common targets, we finally got a total of 1,411 component targets. A total of 8,389 CRC-related targets were collected from the Genecards database, and a total of 2,740 CRC target genes were collected with a score ≥5 as the screening condition. This intersected with the 1,411 targets corresponding to the active ingredients of the QYJD decoction, and we obtained 518 common targets, which were the predicted targets for the anti-colorectal cancer effect of the QYJD decoction. We imported the target interaction relationship obtained from the STRING database into the Cytoscape 3.6.0 software and selected all 105 target genes and the top 20 target genes that meet the card value for visualisation, as shown in Figure 7A. TP53, AKT1, INS, IL-6, EGFR, ESR1, MAPK3, MAPK1, SRC, STAT3, JUN, TNF, PTEN, CTNNB1, CCND1, IGF1, EP300, FOS, AR, CREB1, IL1B, MTOR, CXCR4, and PIK3R1 are all potential targets for the treatment of CRC with QYJD decoction.

GO enrichment and KEGG pathway enrichment analyses were performed on the targets of QYJD decoction against CRC by the DAVID database. The GO enrichment analysis results showed that the enriched genes satisfying molecular function analysis P<0.01 were included in cadherin binding involved in cell adhesion, chemokine activity, apoptosis-related cysteine-type endopeptidase activity, protein serine/threonine kinase activity, and transcription factor binding. A large number of enriched genes satisfying cell composition analysis P<0.01 were associated with the postsynaptic membrane, cell junction, endoplasmic reticulum, and cell-cell adherens junction, which are closely related to the mechanism of CRC occurrence and development (Figure 7B). In the biological process analysis of cell components, the genes that satisfy P<0.01 and have a large number of enriched genes were involved in apoptosis, chemokines, protein phosphorylation, vascular endothelial growth factor receptor signaling pathway, and I-kappa B positive regulation of kinase/NF-kappa B signaling, cell cycle arrest, positive regulation of MAP kinase activity, transforming growth factor beta-receptor signaling, P53-mediated cell cycle arrest signaling, extra cellularity in the absence of ligands, and negative regulation of 515 the apoptotic signaling pathway (Figure 7C). Based on the KEGG pathway enrichment analysis, we speculated that tumour choline metabolism, regulation of Transient receptor potential channels by inflammatory mediators, tumour necrosis factor signaling pathway, B cell receptor signaling pathway, T cell receptor signaling pathway, toll-like receptor signaling pathway, VEGF signaling pathway, mTOR signaling pathway, ErbB signaling pathway, and NOD-like receptor signaling pathway, among other pathways, lay a primary role in the treatment of CRC (Figure 7D).

Discussion

This is a study on the clinical application and formation of empirical prescriptions. From basic experiments to clinical research, we have repeatedly demonstrated that QYJD decoction may inhibit the occurrence and development of tumours by targeting anti-inflammatory pathways. The data in this study were collected from the CRC database of the Cancer Center in our hospital. Patients in this database were followed up regularly. The data covariates of this study were comprehensively collected; the data were true and reliable, the loss to follow-up rate was low, and the data quality was high. To avoid confounding factors affecting the core outcome, we collected literature reports on confounding factors related to the prognosis of CRC, such as the left and right hemiguts [19], stability of MSI [20], and the presence of vascular invasion. In this study, we found a notable decrease in recurrence and metastasis risk in patients who received CHM therapy for over 3 months, indicating that longer-lasting CHM intake correlated with better DFS. In addition, the results of univariate and multivariate analyses suggest that inflammatory bowel disease is an independent risk factor for recurrence and metastasis in patients with stage III CRC after radical resection. Further, the stasis-toxin accumulation syndrome type is a risk factor for stage III CRC. The top 18 Chinese medicines in the prescriptions of stasis and poison accumulation syndrome include QYJD decoction except for CHM Tufuling (Smilax 550 glabraRoxb.). Previous research results have confirmed that QYJD decoction has an evident anti-inflammatory effect, 18 Inflammation-cancer transformation is one of the classic pathways of CRC [21-23], and inflammation-related biomarkers may be used to predict the prognosis of colorectal cancer [24]. Inflammation can promote cell proliferation, inhibit apoptosis, recruit immunocytes or enhance cytoskeletal remodeling by microRNA signaling, affect genome stability, and lead to tumourformation [25,26]. Gut microbiota dysbios is alters mitochondrial metabolism in mucosal cells, induces activation of inflammasome signaling pathways, and alters epithelial barrier function [27]. In addition, there is increasing evidence that inflammation interacts with epithelial-mesenchymal transition and cancer stem cells. With the malignant initiation of epithelial cells, chronic inflammation further maintains a pro-inflammatory and tumour565 promoting microenvironment, mediating tumour progression and spread [28]. Studies have found that the NF-kappa B (NF-κB) signaling pathway plays an extremely important role in the maintenance of chronic inflammation and is an important mediator of epithelial cell growth and deterioration in a pro-inflammatory environment [29,30]. The TLR4/NF-κB signaling pathway or the PI3K/AKT/NF-κB signaling pathway is activated in colon cancer, causing the production of IL-6 and TNF-α, as well as tumour growth and metastasis [31,32]. Therefore, we speculate that QYJD decoction could inhibit the NF-kB inflammatory pathway in CRC, as demonstrated by network pharmacology. The prevention of recurrence after radical resection for CRC conforms to the characteristic of 'healing and preventing recovery,' which is an aspect of TCM treatment. It is also the entry point for the use of CHM to treat tumours. The core study results preliminarily confirmed the clinical application and mechanism of action of the method of removing blood stasis and detoxification in the Lingnan region of China after surgery for stage III CRC. This provides a new possibility for clinical adjuvant therapy after CRC surgery. However, as a single-centre retrospective cohort study, our study also had certain limitations. The level of evidence is not sufficiently high, and the mechanism of action of the inflammatory pathway is not proven. Therefore, future multi-centre prospective cohort studies and basic experimental verification studies in the Lingnan Regional TCM Oncology Center are required.

Conclusions

This study confirms that removing blood stasis and detoxification can reduce postoperative recurrence or metastasis of stage III CRC, and its mechanism may involve the inhibition of the inflammatory pathway.

Abbreviations

ANOVA: Analysis Of Variance; **BSA:** Body Surface Area; **CHM:** Chinese Herbal Medicine; **CRC:** Colorectal Cancer; DAI: Disease Activity Index; **DAVID:** Database For Annotation, Visualization, and Integrated Discovery; **DFS:** Disease-Free Survival; **DSS:** Dextran Sulfate Sodium salt; **GO**: Gene Ontology; **HR**: Hazard Ratio; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **LCRC**: left-sided colon cancer; **QYJD:** Qu-Yu-Jie-Du; **RCC:** Right-Sided Colon Cancer.

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