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Study of the Mechanism of Action of Fructus Meliae Toosendan in the Treatment of Colorectal Cancer Based on GEO Database, Network Pharmacology and Molecular Docking

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Abstract: This study aimed to elucidate the potential mechanism of action of Fructus Meliae Toosendan (FMT) in the treatment of Colorectal Cancer (CRC). We searched the Traditional Chinese Medicine System Pharmacology (TCMSP) database for the main active ingredients of FMT and their corresponding targets. Using the Gene Expression Omnibus (GEO) database, we organized the CRC therapeutic targets and utilized the Venny 2.1.0 platform to draw Venn diagrams. We constructed disease-component-target network diagrams and core genes using Cytoscape. Based on the co-acting targets, we performed Gene Ontology (GO) function enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Key targets and active ingredients were verified through molecular docking. We identified nine potentially active compounds corresponding to 78 potential drug targets. By mapping with 3,269 CRC disease targets, we obtained the top 10 core targets through topology analysis, including MYC, BCL2, CCND1, IL6, FOS, PLAU, CCNB1, IGFBP3, RUNX2, and CAV1. Enrichment analysis revealed that the primary therapeutic pathways for CRC include the AGE-RAGE signaling pathway in diabetic complications, the p53 signaling pathway, the HIF-1 signaling pathway, among others. Molecular docking results showed binding energies of Quercetin with BCL2, MYC, and CCND1, as well as Medioresil and Balanophonin with BCL2, were less than -7.0 kcal/mol, suggesting strong binding abilities between the receptors and ligands. Quercetin, Medioresil, and Balanophonin, active ingredients in FMT, primarily engage in mediating the p53 and HIF-1 signaling pathways to treat CRC by regulating core gene targets such as MYC, BCL2, CCND1, and IL6. This study provides insights into the potential mechanism of FMT for CRC treatment and offers guidance for subsequent pharmacological research and clinical applications.

CRC is a malignant tumor that affects the colon and rectum. It most commonly occurs in

individuals between the ages of 40 and 50, with a predilection for the junction of the rectum and sigmoid colon[1]. As society has developed, so too have people's dietary and living habits, which have undergone significant changes. These include a preference for foods with heavy tastes, high sugar and high fat diets, hunger and satiety disorders, unclean diets, and late-night activities. Collectively, these factors have contributed to an increase in the incidence of CRC among younger individuals[2]. CRC-specific symptoms are often subtle, developing insidiously over time and being challenging to detect. In severe cases, they may extend to invade peripheral or distant tissues[3]. Nevertheless, the cure rate for early-stage CRC is high, emphasizing the importance of early detection. Recent studies have demonstrated the efficacy of Chinese medicine in the treatment of CRC. These studies have shown that Chinese medicine can prolong survival, control pain, and improve quality of life. When used in conjunction with other treatments such as surgery, radiotherapy, and chemotherapy, Chinese medicine can increase efficacy and reduce toxicity. Furthermore, the efficacy of combined treatment with Chinese medicine is superior to that of Western medicine alone[4,5].

The anti-tumor effects of Chinese medicine have been gradually elucidated through the in-depth development and research in this field. Chinese medicine has been demonstrated to possess significant effects and unique advantages in the treatment of tumors[6,7]. The rapid development of network pharmacology analysis in recent years has established it as an accurate and efficient method for exploring pharmacology[8,9]. Accordingly, the objective of this paper is to conduct network pharmacology research for the treatment of CRC, with a particular emphasis on the key targets and signaling pathways that play a therapeutic role. This research endeavor is undertaken with the aim of providing a foundation for the advancement and clinical treatment of colon cancer.

1. Materials and Methods

1.1 Construction of active ingredient-target database

A search of the TCMSP database (https://old.tcmsp-e.com/tcmsp.php#) was conducted using the keyword "FMT". The search criteria included oral utilization (OB) ≥30% and drug-like properties (DL) ≥0.18, which were used to identify active ingredients with enhanced medicinal properties [10]. The nomenclature of each active ingredient was standardized using the PubChem database, and the structural formula of the corresponding compound SMILES was analyzed. A database of ingredient-target associations was constructed [11]. (Search deadline: August 1, 2024)

1.2 CRC disease therapeutic target acquisition

Access the GEO database (https://www.ncbi.nlm.nih.gov/geo/) of the National Center for Biotechnology Information (NCBI). Then, enter the key word "colorectal cancer" into the search field, with the screening criteria that (1) The dataset must originate from the same platform. (2) The samples must include CRC tissues and normal colorectal tissues from the same patient. (3) The total number of samples must be at least 20. (4) The samples must come from Homo sapiens. Three CRC datasets (GSE110224, GSE41328, GSE22598) were selected, all of which were based on the GPL570 platform and Affymetrix Human Genome U133 Plus 2.0 Array[12]. The data set GSE110224 comprises 17 normal tissue samples and 17 CRC samples, the data set GSE41328 contains 10 normal tissue samples and 10 CRC samples, and the data set GSE22598 consists of 17 normal tissue samples and 17 CRC samples. The differential genes of the CRC disease and control groups were analyzed using R software to create a differential gene volcano map. The top 20 differential genes were selected based on the criteria of $|\log FC| > 1$ and $P < 0.05$, which identified significantly differentially expressed genes. (Search as of August 1, 2024)

1.3 FMT-CRC intersection target screening and Protein-Protein Interaction (PPI) network construction

The aforementioned CRC disease targets and active ingredient targets were imported into the Venny 2.1.0 platform (https://bioinfogp.cnb.csic.es/tools/venny/index.html), and the FMT-CRC intersection target genes were obtained and plotted in the intersection Wayne diagram. The String platform (https://www.stringdb.org/) was utilized to import the key interaction targets, with the species set to "Homo sapiens," the minimum interaction score set to 0.9, and the Network Display Options set to hide nodes with no interactions. This was done to construct a PPI network diagram.

1.4 CRC-FMT active ingredient-Target Network map

The genes identified as intersecting with FMT compounds and CRC disease targets, as outlined in section 1.3, were imported into the Cytoscape 3.9.1 software to construct the Disease-Component-Target Network map. This graph represents the active ingredients and targets associated with FMT, and illustrates the relationships between them through nodes and edges.

1.5 Core target gene GO enrichment and KEGG pathway enrichment analysis

The obtained core intersecting targets were imported into the DAVID 6.8 platform (https://david.ncifcrf.gov/summary.jsp), with the species selected as human Homo sapiens. Subsequently, KEGG pathway enrichment analysis and GO enrichment analysis were performed, and GO functional enrichment analysis and KEGG pathway analysis were obtained.

1.6 Molecular docking

The active ingredients and key targets identified in section 1.3 were subjected to molecular docking analysis. The mode of action between the active ingredient and the key target was analyzed using AutoDock Vina 1.1.2 molecular docking software. The binding activity between the active ingredient and the target protein was evaluated based on the binding energy. The most stable conformation for binding was selected, and finally, PyMOL 2.5.1 software was used for visualization analysis.

2. Results

2.1 Active ingredient-target screening of FMT

Table 1: Key Active Ingredients of FMT

MOL ID	Molecule Name	OB (%)	DL
MOL001494	Mandenol	42.00	0.19
MOL001495	Ethyllinolenate	46.10	0.20
MOL002045	Stigmasterol	43.41	0.76
MOL002047	Melianone	40.73	0.81
MOL002048	Nimbolidin D	30.38	0.53
MOL002053	Nimbolin A	32.11	0.34
MOL002056	Balanophonin	54.74	0.40
MOL002058	Medioresil	57.20	0.62
MOL000098	Quercetin	46.43	0.28

A total of nine compounds were identified through a search of the TCMSP database using the following criteria: oral utilization OB ≥ 30% and drug-like properties DL ≥ 0.18. As illustrated in

Table 1, the identified compounds include Mandenol, Ethyllinolenate, Stigmasterol, Melianone, Nimbolidin D, Nimbolin A, Balanophonin, Medioresil, and Quercetin. After obtaining the corresponding targets of each component, the total number of targets of the active ingredient was 78.

2.2 CRC disease therapeutic target acquisition

The GSE110224, GSE41328, and GSE22598 datasets were standardized, and the results demonstrated that the expression of each sample in the three datasets was at an equivalent level, indicating that their standardization characteristics were effective. The three datasets were screened for differentially expressed genes between CRC and normal colorectal mucosa using the "limma" package, resulting in the identification of 3269 differentially expressed genes. The volcano and heat maps of these genes are presented in **Figure 1.**

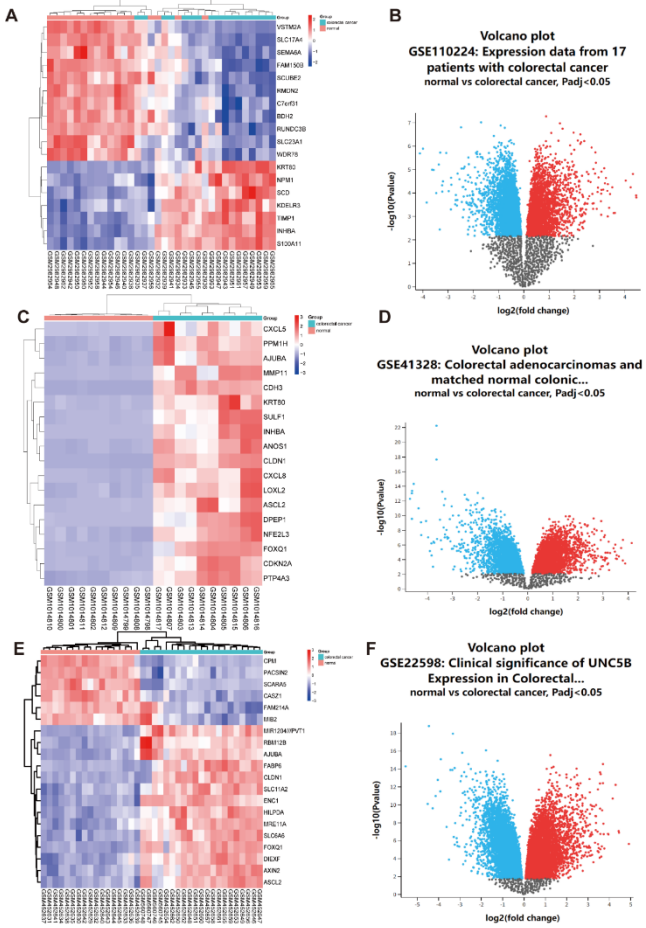

Figure 1: CRC therapeutic target data sets. (A), (B) demonstrate cohort GSE110224 differential gene heat map, volcano map; (C), (D) demonstrate cohort GSE41328 differential gene heat map, volcano map; (E), (F) demonstrate cohort GSE22598 differential gene heat map, volcano map.

2.3 FMT for CRC disease intersection target screening

The 78 FMT active ingredient action targets and 3269 CRC disease treatment targets were mapped through the Venny2.1.0 platform to create a cross-Wayne diagram (see **Figure 2A**), which identified 20 intersecting genes. The 20 genes were imported into the STRING website for protein-protein interaction (PPI) analysis, with a confidence threshold of ≥ 0.9, and the remaining genes were excluded to yield 19 core genes, as illustrated in **Figure 2B**. The MCC algorithm (topology analysis)

in Cytoscape software was employed to map Cytohubba, thereby obtaining the top 10 ranked core genes, which were MYC, BCL2, CCND1, IL6, FOS, PLAU, CCNB1, IGFBP3, RUNX2, and CAV1 (see **Figure 2C**).

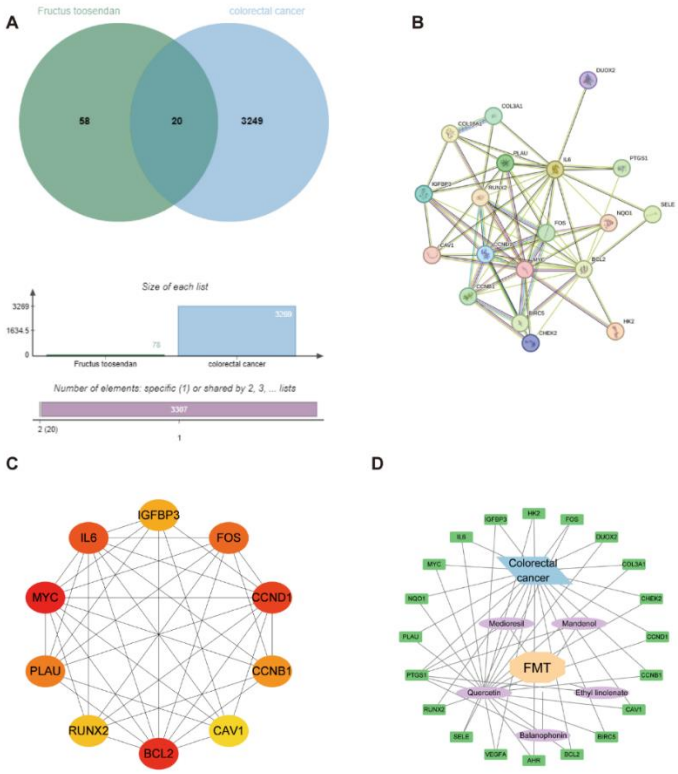

Figure 2: CRC-FMT target network diagram. (A) CRC and FMT shared genes; (B) PPI network diagram of intersecting genes; (C) HUB gene diagram; (D) CRC-FMT target network map.

2.4 CRC-FMT active ingredient-target network map

The network map of CRC disease and major active ingredient targets was constructed following the processing of data information from 20 shared target genes of diseases and drugs using Cytoscape software. This resulted in the identification of five active ingredient targets and CRC disease targets, for a total of 20 target genes, as illustrated in **Figure 2D**.

2.5 Analysis of shared drug-disease targets

The data were then processed further using Cytoscape software. The target genes that were simultaneously satisfied were identified according to the following criteria: Degree > 20, Betweenness > 15, and Closeness > 0.7 of the intersecting genes. This resulted in the identification of four protein-coding genes, which are presented in **Table 2**.

Table 2: Key targets of FMT in the treatment of colorectal cancer

Serial number	Gene symbol	Degree	Betweenness	Closeness
1	IL6	30	81.23	0.86
2	BCL2	30	56.44	0.86
3	MYC	28	39.75	0.82
4	CCND1	24	15.31	0.75

2.6 GO enrichment analysis of core targets

The Gene Ontology (GO) enrichment analysis encompassed three domains: biological process (BP), cellular component (CC), and molecular function (MF). The top 10 correlations were selected for visualization. The results of the GO-BP analysis indicated that FMT mainly exerted anti-CRC effects through biological processes, including peptidyl-serine phosphorylation, peptidyl-serine modification, and regulation of anoikis (see **Figure 3A**). The GO-CC analysis results indicated that FMT achieved its therapeutic effect on CRC through chromosomal region, condensed chromosome, kinetochore, and cellular components (see **Figure 3B**). The GO-MF analysis results indicated that FMT treated CRC through molecular functions such as antioxidant activity, channel inhibitor activity, and other molecular functions (see **Figure 3C**).

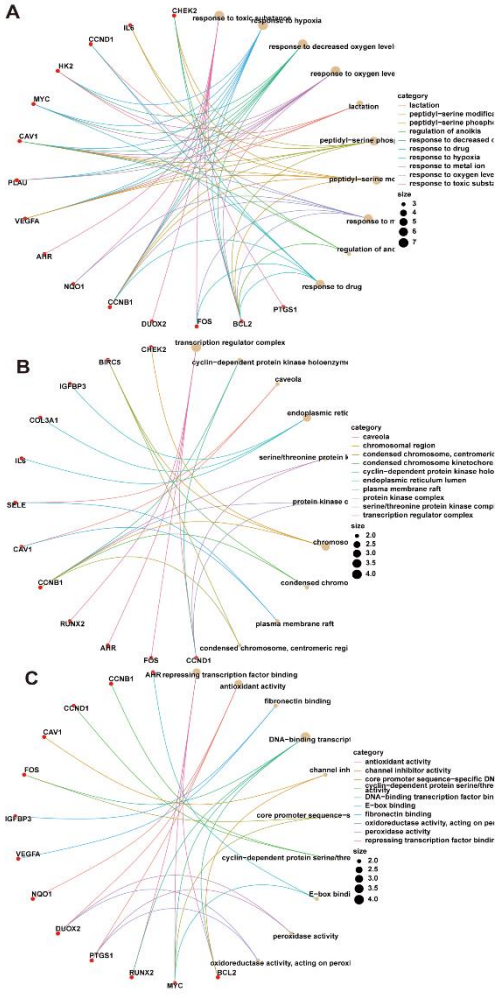

Figure 3: GO enrichment analysis. (A) represents biological process (BP); (B) represents cellular component (CC); (C) represents molecular function (MF).

2.7 KEGG pathway enrichment analysis of core targets

The results of the KEGG enrichment analysis indicated that a total of 121 KEGG signaling pathways were identified in this study. The pathways with higher target enrichment were predominantly concentrated in the AGE-RAGE signaling pathway in diabetic complications, the p53 signaling pathway, the HIF-1 signaling pathway, and other pathways. This suggests that the signaling pathways may be the primary mechanisms through which FMT exerts its therapeutic effects on CRC.

This is illustrated in **Figure 4**.

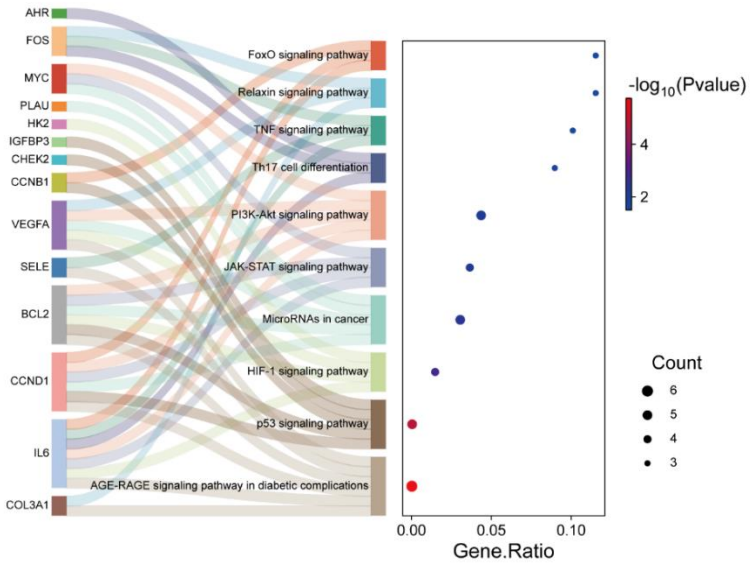

Figure 4: KEGG enrichment analysis results

2.8 Molecular docking validation of FMT for the treatment of colorectal cancer

The four core targets, IL6, BCL2, MYC, and CCND1, which were identified in Table 2, were subjected to molecular docking with five core compounds. The results demonstrated that the binding energies of quercetin with BCL2, MYC, and CCND1, as well as medioresil and balanophonin with BCL2, were less than -7.0 kcal/mol. This indicates that the receptor and ligand exhibit enhanced binding affinity, and that a lower binding energy value correlates with a more stable binding conformation. The core active ingredients with the lowest binding energies and their respective targets were selected and visualized using PYMOL software, as illustrated in **Figure 5**.

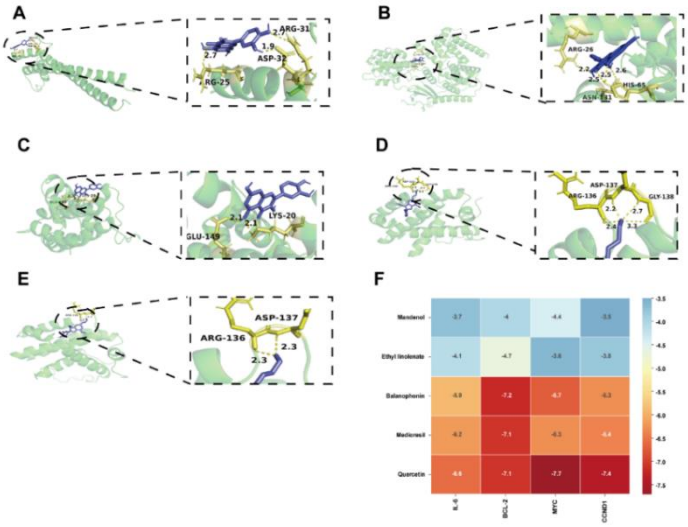

Figure 5: Results of molecular docking. (A) Quercetin docked with MYC molecule; (B) Quercetin docked with CCND1 molecule; (C) Quercetin docked with BCL2 molecule; (D) Balanophonin docked with BCL2 molecule; (E) Medioresil docked with BCL2 molecule; (F) Binding energy thermograms.

3. Discussion

CRC is one of the most prevalent malignant tumors of the digestive tract. It is treated with a range of modalities, including surgical resection, radiation therapy, chemotherapy, and targeted therapy[13]. While these conventional therapies have proven effective in treating colorectal cancer, their associated adverse effects often preclude their long-term use [14,15]. In recent years, the advancement of Chinese medicine has led to an increased recognition of its unique value in the treatment of colorectal cancer. Chinese medicine has demonstrated the ability to not only prolong the survival of patients but also to effectively reduce the risk of tumor recurrence and metastasis, indicating its potential as a valuable addition to the treatment of tumors[16,17]. In clinical practice, the combined application of Traditional Chinese Medicine (TCM) and radiotherapy has demonstrated remarkable therapeutic efficacy, and this comprehensive treatment strategy is increasingly emerging as a novel area of medical research[18]. In clinical practice, the combined application of TCM and radiotherapy has demonstrated remarkable therapeutic efficacy, and this comprehensive treatment strategy is increasingly emerging as a novel area of medical research. FMT is the dried mature fruit of Melia toosendan Sieb. et Zucc., which belongs to the Neem family. A chemical analysis revealed that FMT contains a variety of chemical constituents, including triterpenes, flavonoids, organic acids, and lignans. To date, these components have been demonstrated to possess a wide range of pharmacological effects, including antitumor, anti-inflammatory, analgesic, antiviral, and antioxidant properties[19]. The aqueous extract of FMT has been shown to inhibit the proliferation of A549 cells, which may be associated with its ability to activate the P53 gene and induce apoptosis through the modulation of the Bcl2 family of proteins[20].

The results of the study revealed the differential expression of 3269 CRC genes, the identification of nine active ingredients, and the mapping of 78 targets of action. By constructing a "CRC target-active ingredient" network pharmacology system, we identified the core ingredients for the treatment of CRC as Medioresil, Mandenol, Quercetin, Balanophonin, and Ethyl linolenate. Additionally, we determined the key targets to be IL-6, BCL2, MYC, and CCND1. In order to gain insight into the anti-CRC molecular mechanism of action, KEGG pathway analysis and GO function analysis were conducted in conjunction with the anti-CRC intersecting action targets obtained from the screening. The findings revealed that the treatment of colorectal cancer is predominantly associated with the AGE-RAGE signaling pathway in diabetic complications, the p53 signaling pathway, the HIF-1 signaling pathway, and other signaling pathways. Subsequently, molecular docking was conducted to confirm that the binding energies of quercetin with BCL2, MYC, and CCND1, as well as medioresinol and balanophonin with BCL2, exhibited a value less than -7.0 kcal/mol. It can be reasonably deduced that the active ingredients in neem, namely quercetin, medioresil, and balanophonin, may exert antitumor effects through several key targets, including BCL2, MYC, and CCND1. Additionally, these ingredients may affect the AGE-RAGE signaling pathway in diabetic complications, the p53 signaling pathway, the HIF-1 signaling pathway, and several other signaling pathways. Due to time constraints, the above-predicted results have not been verified at the in vivo and molecular levels. Further improvements will be made to this study, which explores the predicted results for the anti-colorectal cancer components and mechanisms of action. This study also provides a theoretical basis for anti-colorectal cancer pharmacodynamic substances.

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