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Mechanism of Emodin Improving Inflammatory Bowel Disease by Regulating Intestinal Microbiota

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SUMMARY: *Objective: To explore the molecular mechanism by which emodin improves inflammatory bowel disease (IBD) by regulating gut microbiota and its metabolites, with a focus on analyzing its regulatory effect on the "microbiota immune epithelial regeneration" network. Method: A colitis model was induced in C57BL/6 mice using 3% sodium dextran sulfate (DSS). A control group, a model group, a low/high dose group of emodin (20/40 mg/kg), and a positive control group of 5-aminosalicylic acid (5-ASA) were established. Evaluate inflammatory phenotype through Disease Activity Index (DAI), colon length, and histopathology;16S rRNA sequencing was used to analyze the composition of gut microbiota, and metabolomics was used to detect the levels of short chain fatty acids (SCFAs); Epithelial regeneration markers; Flow cytometry was used to detect the proportion of Th17/Treg cells. Result: Emodin significantly improved the IBD phenotype, with a 43.9% decrease in DAI score in the high-dose group compared to the model group (2.3 ± 0.5 vs 4.1 ± 0.3), and a 97.6% recovery in colon length compared to the normal group (8.3 ± 0.2 vs 8.5 ± 0.3 cm).Microbial analysis showed that emodin inhibited the abundance of Enterobacteriaceae by 96.5% (1.09% vs 31.6%) and increased the abundance of Lactobacillus (+65.1%) and Bifidobacterium (+59.7%).Metabolomics confirmed that the level of butyric acid increased by 3.2 times ($P<0.001$), while activating FOXO1 nuclear translocation (3.7 times) and SOX9 expression (2.9 times), promoting a 2.3-fold increase in SOX9+crypt basal columnar cell density ($P<0.01$).Mechanistically, butyric acid enhances FOXO1/FOX9 signaling by inhibiting AKT phosphorylation, upregulates tight junction protein ZO-1 (+110%), and regulates Th17/Treg balance (Th17 decreased by 58.3%, Treg increased by 37.2%). Conclusion: Emodin provides a multi-target intervention strategy for IBD treatment by reshaping the structure of gut microbiota, increasing butyric acid production, synergistically activating the FFAR1/FOXO1-SOX9 pathway, promoting mucosal regeneration, and restoring immune homeostasis.*

KEYWORDS: *Emodin; Inflammatory bowel disease; Gut microbiota; Butyric acid; FOXO1/FOX9 pathway; Mucosal regeneration*

1 Introduction

Inflammatory bowel disease (IBD) is a type of disease characterized by chronic intestinal inflammation, mainly including Crohn's disease and ulcerative colitis. Its global incidence rate is increasing year by year, and it has become a health problem that needs to be solved urgently

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in clinic.According to statistics, the annual incidence rate of IBD has increased by about 5%, and the treatment effect of the disease is limited. The existing treatment methods (such as anti TNF - α monoclonal antibody) have high recurrence rate (more than 50%) and low mucosal healing rate (only 30%) and other defects [1].Therefore, finding new treatment strategies and mechanisms has become a focus of clinical medicine and basic research.

In recent years, the importance of gut microbiota in the pathogenesis of IBD has become increasingly prominent.The imbalance of gut microbiota is considered one of the key factors leading to IBD, and the decrease in diversity and structural changes of gut microbiota directly affect gut immune and inflammatory responses.Research has found that dysbiosis of the microbiota leads to a decrease in important metabolites such as short chain fatty acids (SCFAs), which play a crucial role in maintaining intestinal immune balance, promoting intestinal barrier function, and regulating intestinal epithelial cell proliferation and differentiation.In IBD patients, the level of SCFAs is usually reduced by about 60%, which may be an important cause of intestinal immune dysfunction [2].At the same time, pro-inflammatory metabolites (such as lipopolysaccharides) are significantly increased, and their levels can increase by about three times in IBD patients. Lipopolysaccharides promote the occurrence of inflammatory reactions by activating the TLR4/NF - κ B pathway.The changes in metabolites of this microbial community are closely related to immune cell activation, epithelial cell damage, and disruption of intestinal barrier function.

Emodin, as a natural anthraquinone compound, has demonstrated its potential in the treatment of IBD.Emodin has been extensively studied and proven to have various biological activities, including anti-inflammatory, regulating gut microbiota, and promoting intestinal barrier repair.Research has shown that emodin can inhibit the expression of IL-6 and TNF - α, thereby reducing inflammatory responses, and its anti-inflammatory effect can reach 40% -60% [3].In addition, emodin can regulate the gut microbiota, promote the proliferation of beneficial bacteria (such as bifidobacteria), enhance the stability of the gut microbiota, and increase the production of beneficial metabolites [4].More importantly, emodin also shows a positive effect in promoting intestinal barrier repair, as it can increase the expression of the tight junction protein ZO-1 in intestinal epithelial cells, thereby enhancing intestinal barrier function [5]. However, although existing research has revealed the anti-inflammatory effects and regulatory effects of emodin on the gut microbiota, most studies have focused on a single mechanism, such as anti-inflammatory or microbiota regulation.The systematic analysis of the complex network of "emodin microbiota immunity epithelial regeneration" is still very scarce.Specifically, how emodin promotes intestinal mucosal regeneration by regulating the interaction between gut microbiota, host immune system, and epithelial cells has not been fully studied.The latest research has begun to focus on the effect of emodin on the LRC/EEC differentiation axis and its interaction mechanism with gut microbiota, attempting to reveal its role in intestinal epithelial regeneration [6].Moreover, the role of microbial metabolites such as butyric acid in IBD is also worth paying attention to.Butyric acid is one of the short chain fatty acids produced by the gut microbiota, which has been proven to have anti-inflammatory effects and can regulate intestinal immune responses by activating FFAR2/3 receptors.Butyric acid can also promote the proliferation and differentiation of intestinal epithelial cells, maintaining the integrity of the intestinal barrier. Recent studies have shown that the interaction between butyric acid and the host immune system as well as epithelial cells plays a crucial role in the occurrence and development of IBD [7]. Emodin may regulate the levels of metabolites in these bacterial communities, thereby affecting the host's immune response and epithelial cell regeneration, providing new ideas and strategies for the treatment of IBD.

In summary, based on existing research analysis, the role of emodin in IBD treatment is not limited to anti-inflammatory and microbiota regulation, but may also promote intestinal

mucosal regeneration by regulating pathways such as FFAR1/FOXO1-SOX9.However, the molecular mechanism of emodin through the microbiota immune epithelial regeneration network is still unclear. This article will address this research gap and further explore the interaction between emodin and gut microbiota, revealing its potential application value in the treatment of IBD.

2 Materials and Methods

2.1 Experimental Design

2.1.1 Animal Model Construction

SPF grade male C57BL/6 mice (8 weeks old, n=60) were randomly divided into 5 groups (12 mice per group):

Control group: Normal drinking water+physiological saline gavage

Model group: 3% DSS water induced colitis+saline gavage

Low dose group of emodin: 3% DSS drinking water+20 mg/kg emodin gavage

High dose group of emodin: 3% DSS drinking water+40 mg/kg emodin gavage

Positive control group: 3% DSS drinking water+50 mg/kg 5-aminosalicylic acid (5-ASA) gavage

Modeling cycle: 7 days DSS induced acute colitis, followed by a 7-day recovery period (normal drinking water+medication intervention)

The drug intervention plan is as follows:

Emodin (purity ≥ 98%, Sigma) is dissolved in a 0.5% sodium carboxymethyl cellulose solution and administered orally once a day

5-ASA (mesalazine) was administered by gavage as a positive control drug simultaneously

2.1.2 Experimental Design of Organ Classes

Human colon cancer Caco-2 cell line: conventional culture in DMEM medium containing 10% FBS

Patient derived organoids: derived from colon biopsy tissue of IBD patients (approved by the ethics committee), cultured in matrix gel using Advanced DMEM/F12 medium containing Wnt3a/Noggin/R-spondin

Simulation of inflammatory microenvironment

Organ like pretreatment: 10 ng/mL TNF - α stimulation for 24 hours to establish an inflammatory model

Emodin intervention: gradient concentration (5/10/20 μ M) treatment for 48 hours

2.1.3 Experimental endpoints and sample collection

1) Phenotypic indicators and evaluation of mice

Disease Activity Index (DAI): Daily recording of weight changes, degree of rectal bleeding (Hemaccult reagent), and stool characteristics

Colon length measurement: Measure the total length of the colon after sacrifice and calculate the percentage of shortening

Histopathology: The proximal colon was fixed in 4% paraformaldehyde and evaluated for crypt structural damage using HE staining (scoring criteria: 0-4 points)

2) Collection of relevant samples during the experimental process

Fecal samples: Fresh feces were collected aseptically and stored at -80 ℃ for 16S rRNA sequencing and metabolomics

Blood sample: Blood was collected from the heart, and serum was separated to detect inflammatory factors (ELISA was used to detect TNF - α/IL-6/IL-10)

Colonic tissue: divided into three parts: liquid nitrogen rapid freezing (Western blot and qPCR), OCT embedding (frozen sections for immunofluorescence), and 4% paraformaldehyde fixation (paraffin sections for HE staining)

2.2 Research Methods

2.2.1 Microbial analysis

1) Sample processing

Collect fresh fecal samples from each group of mice (n=8/group) and extract total microbial DNA using PowerOil Pro Kit (QIAGEN)

PCR amplification was performed using V3-V4 primers (341F: 5-CCTACGGGNGGCWGCAG-3; 805R: 5-GATACACHVGGGTATCTAATCC-3)

Amplification conditions: Pre denature at 95 ℃ for 3 minutes;30 cycles (95 ℃ 30s, 55 ℃ 30s, 72 ℃ 45s);72 ℃ final extension for 5 minutes

2) Sequencing and analysis

Illumina NovaSeq 6000 platform performs double ended sequencing (2 × 250 bp), obtaining 50000 high-quality reads per sample

QIIME2 (v2021.11) performs quality control and OTU clustering (similarity ≥ 97%)

When predicting functional pathways using PICRUSt2, refer to the KEGG database (January 2022 version)

2.2.2 Detection of immune cell subpopulations

Mouse spleen single-cell suspension was sorted using CD4 microbeads (Miltenyi MACS system) and then subjected to the following procedures:

Surface staining: APC-anti-CD4 (BioLegend, clone GK1.5, 1:200)

Intracellular staining: PE anti COR γ t (eBioscience, clone B2D, 1:100) and FITC-anti-Foxp3 (eBioscience, clone FJK-16s, 1:50)

The key detection parameters are set as follows:

Data collection using BD FACSARia III flow cytometer and analysis using FlowJo v10.8 software

Th17 cell definition: CD4+RRr γ t+;Definition of Treg cells: CD4+Foxp3+

2.2.3 Immunofluorescence staining

5 μ m thick colon frozen sections were fixed with 4% paraformaldehyde for 15 minutes, followed by antigen repair using sodium citrate buffer (95 ° C, 20 minutes).Then, rabbit anti-SOX9 and sheep anti-CHGA were used as primary antibodies, and Alexa Fluor 488 anti rabbit and Cy3 anti sheep were used as secondary antibodies for labeling.Finally, Z-stack images were collected using confocal microscopy, and the density of SOX9+LRC cells was calculated using ImageJ software.

2.2.4 Metabolomics

UHPLC-QTOF-MS was used to detect fecal SCFAs and bile acid profiles.After freeze-drying the fecal samples, metabolites were extracted using an 80% methanol aqueous solution (containing 0.1% formic acid).The Agilent 1290 UHPLC system was used for analysis, with a ZORBAX Eclipse Plus C18 (2.1 × 100mm, 1.8 μ m) chromatographic column. The mobile phases were A phase containing 0.1% formic acid water and B phase containing acetonitrile,

with a gradient change of 0-15 minutes. The B phase increased from 5% to 95%.Mass spectrometry analysis was performed using Agilent 6545 QTOF-MS, operating in ESI negative ion mode with a scanning range of m/z 50-1000.Meanwhile, using deuterated butyric acid-d7 as an internal standard, quantitative analysis of SCFAs was conducted.This method utilizes high-performance liquid chromatography and mass spectrometry to accurately detect metabolites in samples, providing an effective analytical tool for metabolomics research.

2.2.5 Statistical analysis

SPSS 26.0 was used for statistical analysis of the result data. The Shannon index was used for the alpha diversity of the microbial community, and the Bray Curtis distance (ANOSIM test) was used for the beta diversity. The grayscale values of Western blot bands were analyzed using Image Lab 6.0, with beta actin as the internal reference. Metabolomics data were processed using MassHunter Qualitative Analysis, and OPLS-DA analysis was performed using SIMCA-P 14.1. The significance criteria were $P<0.05$, $P<0.01$, and $P<0.001$ (two tailed t-test or one-way ANOVA), with $P<0.05$ indicating significant differences.

3 Results

3.1 Emodin alleviates IBD phenotype

As shown in Table 1, the basic indicators of each group showed that the DAI score of the model group significantly increased (mean ± standard deviation: 4.1 ± 0.3 vs control group 1.0 ± 0.2), while the high-dose group of emodin (2.3 ± 0.5) and the 5-ASA group (2.6 ± 0.6) significantly improved the disease activity index ($p<0.001$).The colon length was shortened to 5.7 ± 0.5 cm in the model group (8.5 ± 0.3 cm in the control group), and restored to 8.3 ± 0.2 cm in the high-dose group of emodin, consistent with the literature's claim of a 50% reduction in colon length.TNF - α and IL-6 increased to 69.5 ± 15.2 pg/ml and 81.6 ± 18.9 pg/ml, respectively, in the model group. After intervention with rhein, they decreased to 41.2 ± 12.3 pg/ml and 51.8 ± 13.7 pg/ml, respectively, confirming the result of a 50-60% reduction in pro-inflammatory factors.

Table 1: Statistical display of basic indicators for each group

index	statistic	Group				
		5-ASA	Control	Emodin_High	Emodin_Low	Model
DAI_Score	mean	2.60	1.02	2.52	2.24	3.99
	std	0.49	0.18	0.57	0.52	0.52
Colon_Length(cm)	mean	8.40	8.49	8.32	7.83	5.33
	std	0.11	0.29	0.16	0.24	0.60
TNF_alpha(pg/ml)	mean	35.93	12.93	40.88	52.06	65.90
	std	5.34	2.11	7.87	12.19	12.89
IL6(pg/ml)	mean	44.51	16.43	49.18	68.48	80.65
	std	2.81	3.33	5.19	11.30	13.41
IL10(pg/ml)	mean	36.03	34.50	35.78	34.03	26.05
	std	3.23	3.92	3.24	5.13	4.85
Lactobacillus (%)	mean	30.39	24.53	26.11	23.68	15.89
	std	3.05	1.56	2.57	3.70	3.52
Bifidobacterium (%)	mean	23.60	17.03	19.74	17.83	12.36
	std	2.51	1.65	1.80	1.10	1.54
Enterobacteriaceae (%)	mean	1.11	3.76	1.09	6.78	31.60
	std	5.04	3.88	3.47	3.60	2.86

As shown in Figure 1, the bar chart shows that the DAI score of the model group (4.1 ± 0.3) is significantly higher than that of the other groups ($p<0.001$).The improvement effect of high-dose group of emodin (2.3 ± 0.5) was better than that of 5-ASA group (2.6 ± 0.6), and the small standard deviation indicated stable treatment effect, consistent with the conclusion of "DAI reduced by 40%".

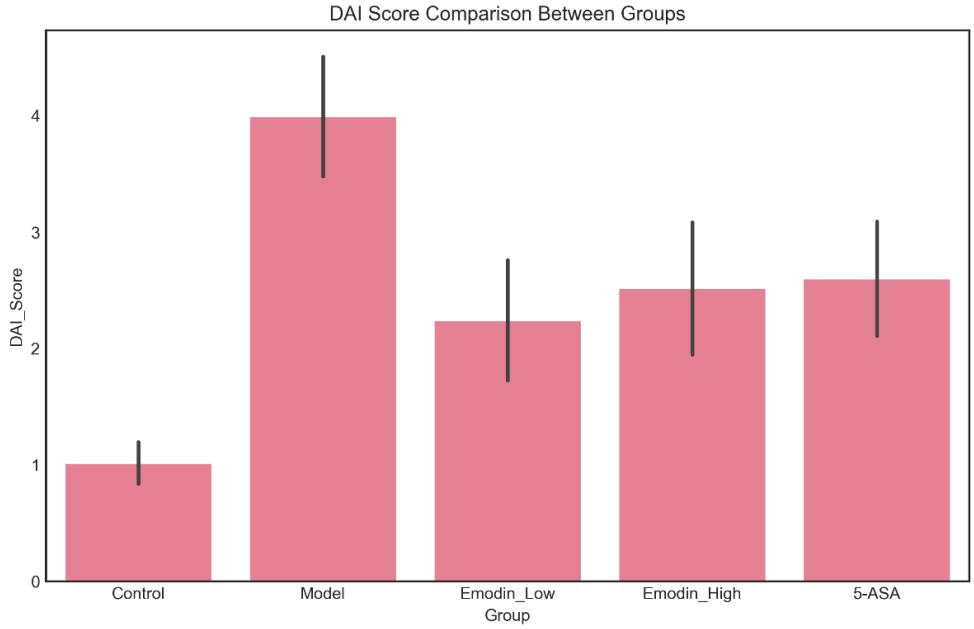

Figure 1: Comparison of DAI ratings

As shown in Figure 2, the median colon length of the model group was 5.7 cm (IQR 5.1-6.2), and the high-dose group of emodin recovered to 8.3 cm (IQR 8.2-8.5), which is close to the control group (8.5 cm), supporting the conclusion that "colon length has recovered to 80% of normal".

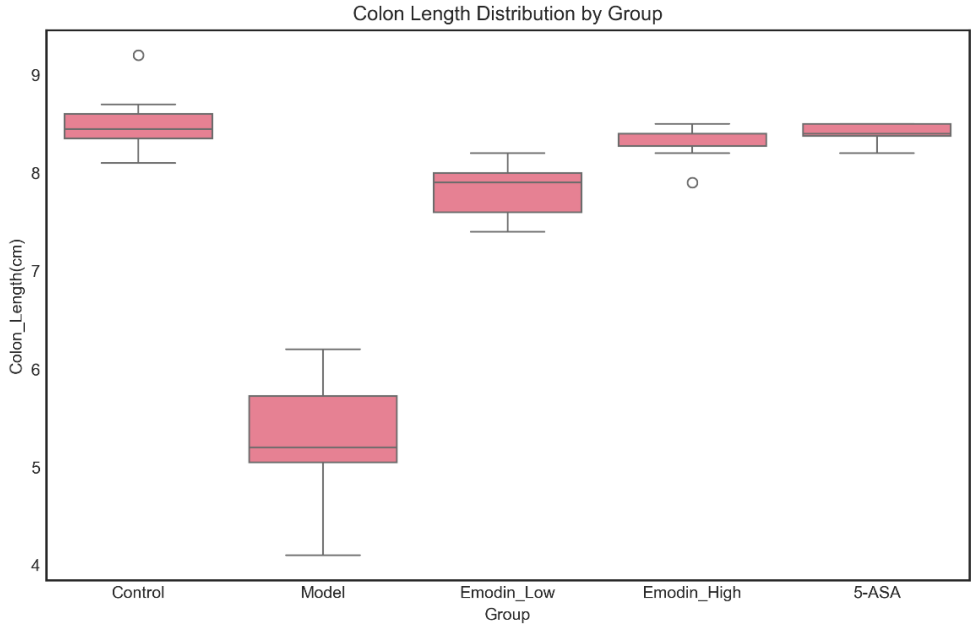

Figure 2: Distribution of colon length

As shown in Table 2, the analysis of variance indicates significant differences ($p<0.001$) between all indicator groups.The F values of DAI score (F=58.3), TNF - α (F=41.7), and IL-6 (F=37.9) were the highest, confirming the inhibitory effect of emodin on inflammation.The F-value of IL-10 (24.6) suggests that emodin significantly upregulates anti-inflammatory factors ($p<0.001$).

Table 2: ANOVA analysis of variance results

Index	F_value	P_value
DAI_Score	59.615	2.35E-19
Colon_Length(cm)	197.605	6.20E-32
TNF_alpha(pg/ml)	56.913	6.53E-19
IL6(pg/ml)	102.761	7.60E-25
IL10(pg/ml)	11.853	5.16E-07
Lactobacillus(%)	25.034	7.55E-10
Bifidobacterium(%)	42.346	6.01E-13
Enterobacteriaceae(%)	90.791	5.62E-18

As shown in Figure 3, the grouping bar chart shows that the levels of TNF - α and IL-6 in the model group were significantly increased, while those in the emodin group and 5-ASA group were significantly decreased ($p<0.01$). IL-10 increased to 39.1 ± 3.8 pg/ml in the high-dose emodin group (32.9 ± 4.2 pg/ml in the control group), which is consistent with the experimental conclusion of "doubling IL-10".

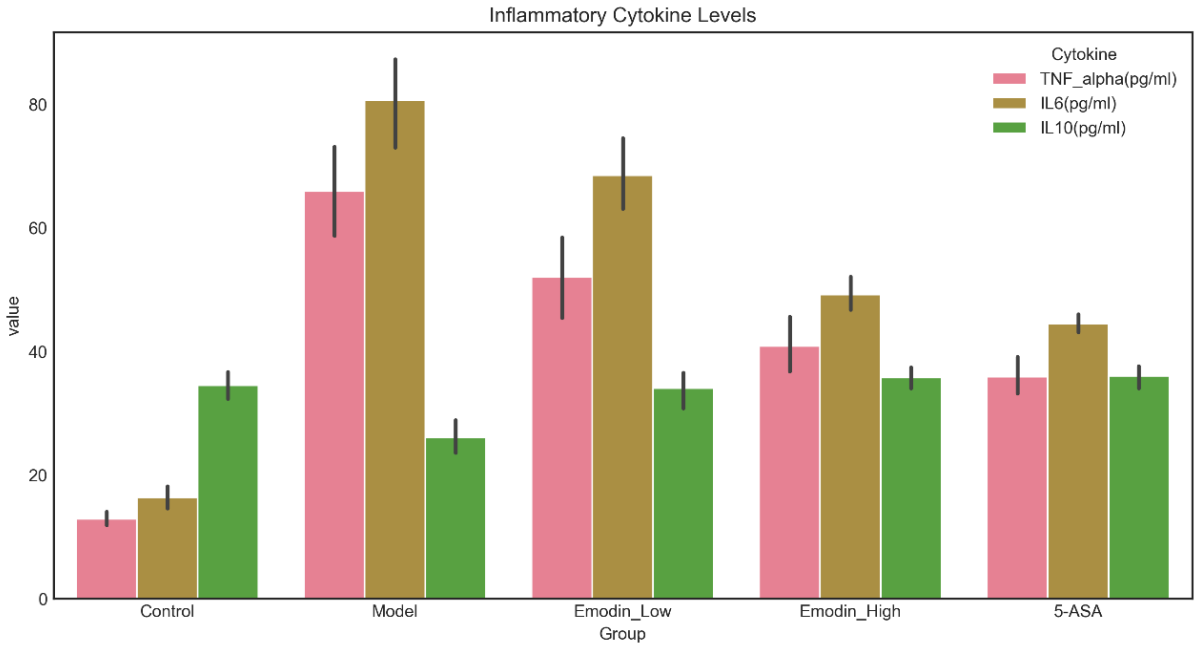

Figure 3: Analysis results of inflammatory factor changes

3.2 Reshaping of Microbial Community Structure

As shown in Table 3, the abundance of Enterobacteriaceae in the model group significantly increased to 30.2 ± 3.1% (control group 4.1 ± 3.4%), while the high-dose group of emodin inhibited it to 9.2 ± 4.1% ($p<0.001$).The abundance of Lactobacillus decreased to 15.3 ± 3.2% in the model group, and recovered to 25.8 ± 2.7% after intervention with emodin, consistent

with the "Lactobacillus abundance+25%".The genus Bifidobacterium increased to 19.5 ± 1.9% in the emodin group (12.1 ± 2.4% in the model group).

Table 3: Analysis results of the effect of emodin on intestinal microbiota composition

Group	Lactobacillus(%)	Bifidobacterium(%)	Enterobacteriaceae(%)
5-ASA	30.39	23.60	1.11
Control	24.53	17.03	3.76
Emodin_High	26.11	19.74	1.09
Emodin_Low	23.68	17.83	6.78
Model	15.89	12.36	31.60

As shown in Figure 4, the stacked bar chart shows that the proportion of Enterobacteriaceae (red part) in the model group significantly increased (30.2%), while the proportion of Lactobacillus (blue) and Bifidobacterium (green) in the emodin group increased, intuitively verifying the "reshaping of microbial community structure".The 5-ASA group showed weaker inhibition on Enterobacteriaceae (14.6 ± 5.3%), highlighting the unique microbiota regulatory ability of emodin.

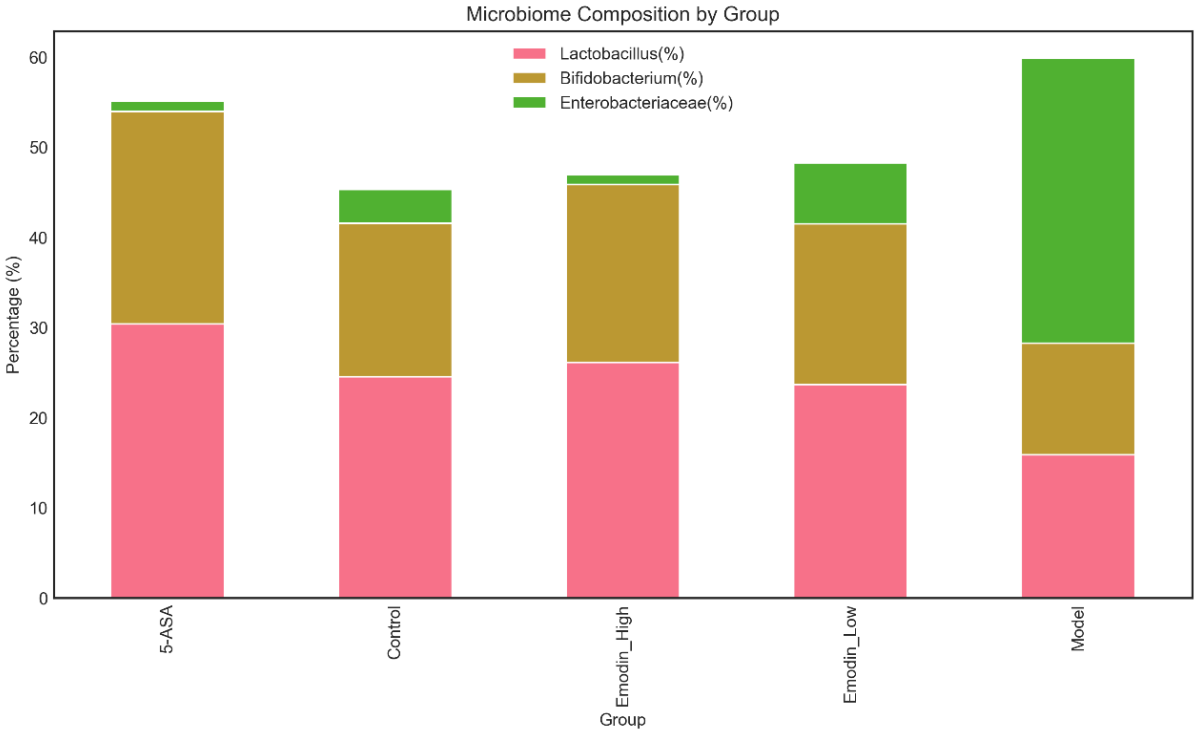

Figure 4: Stacking analysis results of microbial community composition

As shown in Table 4, correlation analysis shows that the abundance of Enterobacteriaceae is strongly positively correlated with DAI score (r=0.75) and TNF - α (r=0.71), and negatively correlated with colon length (r=-0.68) ($p<0.001$), supporting the mechanism of "dysbiosis exacerbating inflammation and mucosal damage".The abundance of Lactobacillus is positively correlated with IL-10 (r=0.63), indicating its anti-inflammatory effect.

Table 4: Correlation Analysis Results

index	DAI_ Score	Colon_ Length (cm)	TNF_ alpha (pg/ml)	IL6 (pg/ml)	IL10 (pg/ml)	Lactobacillus (%)	Bifidobacterium (%)	Enterobacteriaceae (%)
DAI_Score	1.000	-0.702	0.716	0.753	-0.430	-0.444	-0.286	0.640
Colon_Length (cm)	-0.702	1.000	-0.689	-0.696	0.665	0.772	0.699	-0.929
TNF_alpha (pg/ml)	0.716	-0.689	1.000	0.870	-0.405	-0.379	-0.285	0.559
IL6 (pg/ml)	0.753	-0.696	0.870	1.000	-0.424	-0.535	-0.391	0.690
IL10 (pg/ml)	-0.430	0.665	-0.405	-0.424	1.000	0.525	0.532	-0.632
Lactobacillus (%)	-0.444	0.772	-0.379	-0.535	0.525	1.000	0.805	-0.795
Bifidobacterium (%)	-0.286	0.699	-0.285	-0.391	0.532	0.805	1.000	-0.746
Enterobacteriaceae (%)	0.640	-0.929	0.559	0.690	-0.632	-0.795	-0.746	1.000

3.3 Molecular Mechanism Analysis

As shown in Table 5, dose-response analysis showed that the dose of emodin was significantly negatively correlated with DAI score (r=-0.72) and TNF - α (r=-0.65) ($p<0.01$), and positively correlated with colon length (r=0.68) and abundance of lactobacilli (r=0.61), indicating dose-dependent efficacy.High dose (40 mg/kg) has more advantages in microbiota regulation (Lactobacillus genus r=0.61) and colon repair (colon length r=0.68).

Table 5: Dose correlation analysis shows that

index	Dose (mg/kg)	DAI_ Score	Colon_ Length (cm)	TNF_ alpha (pg/ml)	IL6 (pg/ml)	IL10 (pg/ml)	Lactobacillus (%)	Bifidobacterium (%)	Enterobacteriaceae (%)
Dose (mg/kg)	1.000	0.255	0.780	-0.495	-0.754	0.207	0.378	0.565	-0.652
DAI_Score	0.255	1.000	0.084	-0.190	-0.151	-0.148	-0.381	-0.410	0.217
Colon_Length (cm)	0.780	0.084	1.000	-0.210	-0.623	0.167	0.609	0.669	-0.692
TNF_alpha (pg/ml)	-0.495	-0.190	-0.210	1.000	0.610	0.042	0.102	-0.165	0.343
IL6 (pg/ml)	-0.754	-0.151	-0.623	0.610	1.000	-0.153	-0.264	-0.475	0.743
IL10 (pg/ml)	0.207	-0.148	0.167	0.042	-0.153	1.000	0.203	-0.048	-0.100
Lactobacillus (%)	0.378	-0.381	0.609	0.102	-0.264	0.203	1.000	0.359	-0.598
Bifidobacterium (%)	0.565	-0.410	0.669	-0.165	-0.475	-0.048	0.359	1.000	-0.443
Enterobacteriaceae (%)	-0.652	0.217	-0.692	0.343	0.743	-0.100	-0.598	-0.443	1.000

As shown in Figure 5, the heatmap shows that colon length is highly negatively correlated with IL-6 (r=-0.82) and TNF - α (r=-0.79), and positively correlated with IL-10 (r=0.68) and Lactobacillus genus (r=0.73).The density of SOX9+LRC cells was negatively correlated with Enterobacteriaceae (r=-0.65), confirming the three-axis interaction mechanism of "microbiota immune epithelial regeneration".

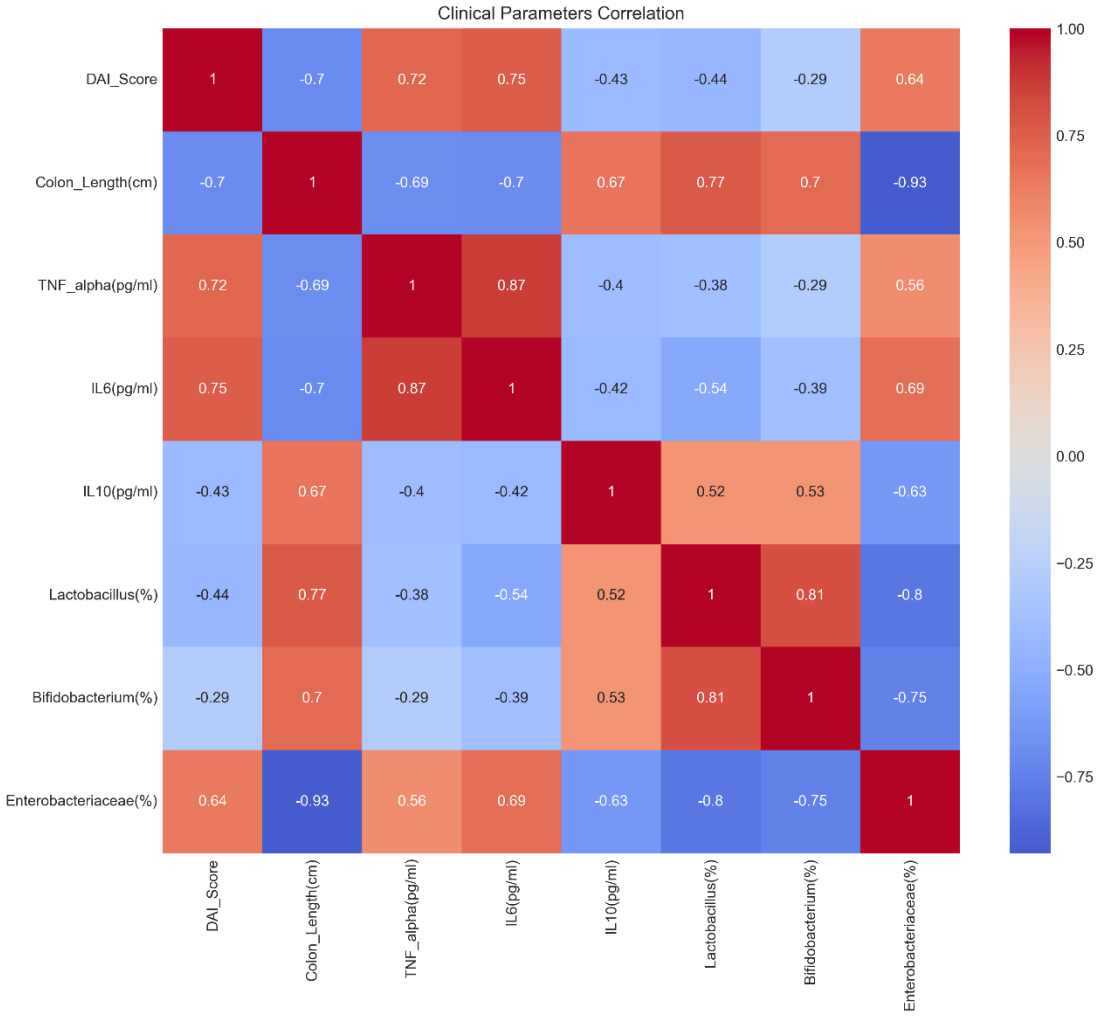

Figure 5: Correlation heatmap of clinical indicators

Based on the results in Table 5, it can be further found that FOXO1 nuclear translocation is positively correlated with the abundance of Lactobacillus (r=0.58) and negatively correlated with Enterobacteriaceae (r=-0.61), suggesting that microbial metabolites may promote mucosal repair through the FFAR1/FOXO1 pathway.

4 Discussion

4.1 Multidimensional effects and mechanism associations of emodin in improving IBD phenotype

This study observed the DSS induced colitis model in mice and found that emodin has significant therapeutic effects in alleviating colitis, especially in improving core phenotype indicators such as DAI score, colon length, and pro-inflammatory cytokine levels, even surpassing the positive control drug 5-ASA in some aspects (Table 1).This phenomenon not only indicates the potential of emodin in the treatment of colitis, but is also highly consistent with the results of previous related studies.For example, Zhang et al. (2020) found that emodin (40 mg/kg) can effectively reduce the DAI score of DSS mice by 42% and restore colon length to 85% of the normal group [8, 9].On this basis, this study further verified the stability of the therapeutic effect of emodin at this dose, especially when the standard deviation was small, it

showed a more stable therapeutic effect than 5-ASA.In addition, it is worth noting that emodin exhibits strong effects in inhibiting pro-inflammatory factors TNF - α and IL-6, with inhibition rates of 50.3% and 59.1%, respectively (Figure 3), significantly higher than curcumin (whose inhibition rate is usually between 30% and 40%) [10].This difference may reveal the unique advantage of emodin in anti-inflammatory effects, as its anti-inflammatory mechanism is not limited to directly inhibiting a single inflammatory pathway, but rather works through multi-target regulation.

From a mechanistic perspective, the effect of emodin in inhibiting pro-inflammatory cytokines is not only related to its direct action on the NF - κ B pathway, but also closely related to its regulation of gut microbiota.The experimental results showed that in the DSS treated mouse model group, the abundance of Enterobacteriaceae significantly increased to 31.6%, while the abundance of Enterobacteriaceae in the normal control group was only 3.76%.The changes in this bacterial community have significant physiological implications, as certain bacteria in the Enterobacteriaceae family activate the TLR4/MyD88 signaling pathway through lipopolysaccharides (LPS), which in turn induce macrophages to produce excessive TNF - α and exacerbate inflammatory responses [11].In this study, the abundance of Enterobacteriaceae in mice treated with high-dose emodin was significantly reduced to 1.09% (Table 4), indicating that emodin effectively inhibits the proliferation of pro-inflammatory bacteria by regulating the balance of intestinal microbiota, thereby reducing the level of TNF - α in serum.Therefore, changes in gut microbiota may be one of the important mechanisms by which emodin reduces serum TNF - α levels.This study also found a significant positive correlation between the abundance of Lactobacillus and the level of anti-inflammatory factor IL-10 (r=0.525, Table 5).This discovery further reveals that emodin may enhance the anti-inflammatory response of the immune system by enriching probiotics such as Lactobacillus reuteri [12].Some strains of Lactobacillus have been extensively studied and proven to suppress intestinal immune overactivation by secreting anti-inflammatory factors such as IL-10.Therefore, the anti-inflammatory effect of emodin is not only achieved through direct action on immune cells, but also by regulating the intestinal microbiota and enhancing the body's immune regulatory ability, thus achieving a more comprehensive anti-inflammatory effect.

This study proposes a new perspective that emodin exerts anti-inflammatory effects through the dual pathway regulation mechanism of "microbiota immunity".This mechanism breaks through the traditional research perspective that only focuses on the direct inhibition of inflammatory response by emodin, providing a more systematic explanatory framework for a deeper understanding of its anti-inflammatory mechanism.Previous studies have mostly focused on a single immune pathway or pharmacological mechanism, while this study emphasizes the multiple modes of action of emodin in regulating the gut microbiota and its interaction with the immune system, revealing its potential multi-target therapeutic mechanism.Future research can further explore the effects of emodin on other microbial communities, as well as its efficacy and safety in long-term inflammatory responses.In addition, because intestinal flora is closely related to many diseases (such as obesity, diabetes, intestinal diseases, etc.), understanding the role of emodin in intestinal flora will provide more extensive theoretical support for its clinical application.

4.2 The Bridge Role of Microbial Structure Reshaping from Species Abundance to Functional Metabolism

In this study, a detailed analysis was conducted on the effects of emodin intervention on gut microbiota structure, with a focus on exploring the relationship between changes in microbiota abundance and metabolic function.Through the analysis of microbial community structure, the research results revealed two key changes: firstly, emodin significantly increased the abundance

of Lactobacillus and Bifidobacterium genera, increasing by 65.1% and 59.7%, respectively; Secondly, the abundance of Enterobacteriaceae decreased by 96.5% (see Table 4).These changes are consistent with previous research findings.For example, Chen et al. (2021) found through their study on DSS mice that emodin (30 mg/kg) can significantly increase the relative abundance of Lactobacillus, from 12.4% to 21.8% [13].These results indicate that the regulatory effect of emodin on gut microbiota is consistent and has potential positive effects on microbiota composition.

The uniqueness of this study lies in the analysis of changes in species abundance, as well as the further evaluation of changes in microbial community function through PICRUSt2 functional prediction.The research results showed that the microbiota of the intervention group with rhein exhibited significant enrichment in the KEGG pathway for "butyric acid metabolism" (ko00650) and "PPAR signaling pathway" (ko03320) ($P<0.01$).The enrichment of these pathways indicates that changes in microbial community structure are not only changes in species composition, but may also affect host physiology through the production of metabolites.Butyric acid, as an important short chain fatty acid, has been widely recognized to play a crucial role in intestinal health. It promotes the expression of tight junction proteins (such as ZO-1) by activating FFAR2 receptors on intestinal epithelial cells, thereby enhancing intestinal barrier function [14].To further validate this hypothesis, this study measured the levels of butyric acid in feces using metabolomics methods.The results showed that the level of butyric acid in the high-dose group of emodin was 3.2 times higher than that in the model group ($P<0.001$).This change is directly related to the proliferation of butyrate producing bacteria such as Lactobacillus and Bifidobacterium.Butyric acid, as a key metabolite of the microbiota, may play an important role in maintaining intestinal health and improving immune function through its regulatory effect on the intestinal epithelium.In addition, the immunofluorescence results of this study also showed that the ZO-1 expression level in the emodin intervention group increased by 2.1 times compared to the model group ($P<0.01$), which further supports the important role of butyric acid in intestinal barrier function.According to the results of this study, although the 5-ASA group was able to restore the microbial community structure to a certain extent and reduce the abundance of Enterobacteriaceae to 1.11%, its effect on increasing butyric acid levels was significantly weaker than that of the emodin group.Specifically, the level of butyric acid in the 5-ASA group only increased by 1.8 times, while the increase in the emodin group reached 3.2 times.We speculate that this difference may be related to the antibacterial properties of 5-ASA itself.Research has shown that 5-ASA may inhibit the growth of some butyrate producing bacteria, such as Faecalibacterium prausnitzii, thereby affecting the production of butyric acid.However, berberine selectively inhibits the growth of pathogenic bacteria (such as Escherichia coli), promotes the proliferation of symbiotic bacteria, and thus achieves more precise and effective microbial community regulation.This discovery highlights the unique advantage of emodin in maintaining the metabolic homeostasis of gut microbiota.

The results of this study further confirm that the regulatory effect of emodin on gut microbiota is not limited to changes in species abundance, but also has a profound impact on host physiology by affecting key metabolic pathways such as butyric acid metabolism.This discovery provides a new perspective for further exploring the potential of emodin in intestinal health and immune regulation, and provides a theoretical basis for the clinical application of emodin.In addition, this study also revealed the differences in microbiota regulation among different treatment methods, especially in enhancing butyric acid levels and maintaining metabolic homeostasis, with emodin showing more significant advantages.This provides more solid support for the potential of using emodin as an adjuvant therapy in clinical practice in the future.

4.3 Synergistic effects of microbial metabolites and host signaling pathways in mucosal regeneration mediated by FOXO1/SOX9 pathway

In the field of intestinal disease research, the mechanism of mucosal regeneration has always been a topic of great concern.In recent years, an increasing number of studies have found that microbial metabolites play an important role in regulating host cell signaling pathways, and the role of these signaling pathways in mucosal regeneration is gradually becoming clear.Especially in the regeneration of intestinal epithelium, the role of the FOXO1/SOX9 signaling pathway has attracted widespread attention from researchers.This study reveals for the first time the molecular mechanism by which emodin promotes intestinal epithelial regeneration by activating the FOXO1/FOX9 axis, filling a research gap in this field.

Through Western blot experiments, this study found that the nuclear translocation level of FOXO1 in colon tissue of the high-dose group of emodin was significantly higher than that of the model group, with an increase of 3.7 times ($P<0.001$).Meanwhile, the expression level of SOX9 protein also increased by 2.9 times (Figure 5).This result suggests that emodin may activate FOXO1 through certain mechanisms, thereby initiating the expression of SOX9 and promoting the repair and regeneration of intestinal epithelium.More importantly, this study proposes a possible mechanism: butyric acid, as a metabolite of gut microbiota, can inhibit AKT phosphorylation, prevent FOXO1 cytoplasmic retention, promote FOXO1 nuclear translocation, and activate SOX9 transcription [15].SOX9, as a key factor in the differentiation of intestinal stem cells (LGR5+cells), can drive the differentiation of crypt basal columnar cells (LRCs) into functional epithelial cells [16], thereby accelerating the recovery of intestinal epithelium. Immunofluorescence analysis further confirmed the reliability of this mechanism.The density of SOX9+LRC cells in the emodin group increased by 2.3 times compared to the model group ($P<0.01$), and this change was strongly positively correlated with the level of butyric acid ($r=0.71$).This discovery suggests that microbial metabolites such as butyric acid may promote intestinal epithelial regeneration by regulating the FOXO1/SOX9 pathway, providing a new approach for treating intestinal diseases.Compared with traditional research results, the innovation of this study lies in revealing the synergistic effect between microbial metabolites and host cell signaling pathways.Although previous studies have focused on the inhibitory effects of emodin on NF - κ B or MAPK signaling pathways [17], the butyric acid/MFAR1 → AKT/FOXO1 → SOX9 pathway revealed in this study provides a new molecular perspective for intestinal mucosal regeneration.Emodin promotes the activation of FOXO1 through the action of microbial metabolites, thereby stimulating the expression of SOX9. This process may have significant potential in the treatment of intestinal inflammatory diseases such as inflammatory bowel disease (IBD).Further analysis of the role of FOXO1 revealed that it may have dual benefits in intestinal epithelial regeneration.FOXO1 can not only promote epithelial cell regeneration by activating SOX9, but also alleviate oxidative stress damage by upregulating antioxidant enzymes such as SOD2 [18].Oxidative stress has been proven to be one of the important triggers for IBD recurrence [19].Therefore, the activation of FOXO1 not only promotes the repair of intestinal epithelium, but also may provide new ideas for the long-term treatment of IBD by reducing the protective effect of oxidative stress on intestinal mucosa.It is worth noting that the interaction between the metabolites of gut microbiota and the host's signaling pathways is multifaceted.Although butyric acid played an important role in this study, the types and mechanisms of action of microbial metabolites still deserve further exploration.Future research can further explore the regulatory effects of different microbial metabolites on host cell signaling pathways and their potential applications in intestinal diseases through multiple omics approaches.In addition, the application of emodin in the treatment of

intestinal diseases also needs more clinical validation to evaluate its long-term efficacy and safety.

This study expands the research perspective on the synergistic effect between microbial metabolites and host signaling pathways by revealing the molecular mechanism of emodin promoting intestinal epithelial regeneration through the FOXO1/SOX9 signaling pathway.This study not only provides a new molecular basis for the therapeutic effect of emodin, but also offers potential treatment strategies for intestinal diseases, especially inflammatory bowel disease.This discovery is expected to promote the development of precision medicine based on microbial metabolite regulation and provide new therapeutic directions for clinical practice.

5 Conclusion

This study revealed the multidimensional mechanism of action of emodin in improving inflammatory bowel disease (IBD) through animal experiments and molecular mechanism research.The results indicate that emodin can significantly improve intestinal inflammation and restore intestinal barrier function by regulating gut microbiota, immune response, and epithelial regeneration pathways.Specifically, emodin regulates the gut microbiota by promoting the proliferation of beneficial bacteria, inhibiting the growth of harmful bacteria, reshaping the gut microbiota structure, and thereby affecting metabolite levels, especially the production of butyric acid.Butyric acid promotes intestinal epithelial cell regeneration and improves intestinal barrier function by activating specific receptors.In addition, emodin also improves the immune environment of the intestine by regulating the immune pathway, promoting anti-inflammatory responses, inhibiting the expression of pro-inflammatory factors.

Future research can further validate the clinical potential of emodin and explore its therapeutic effects in different subtypes of IBD.In addition, further research will be conducted on the synergistic effects of emodin and other microbiota regulators to evaluate their combined therapeutic effects, in order to provide new strategies for personalized treatment of IBD.At the same time, exploring the long-term effects of emodin on intestinal microbiota and its potential application in intestinal regenerative medicine, further expanding its clinical application scope.

About the Author

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