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CASE REPORT

The study on the Impact of Fecal Microbiota Transplantation on Gut Microbiota Diversity in Mice With Colorectal Cancer

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ABSTRACT

This study aims to investigate the effects of fecal microbiota transplantation (FMT) on gut microbiota diversity in mice with colorectal cancer (CRC). A mouse model of colorectal cancer was induced using dextran sulfate sodium (DSS) combined with azoxymethane (AOM), followed by FMT intervention to assess its regulatory effects on the gut microbiota. The results showed that FMT significantly restored the imbalance in gut microbiota caused by cancer and its treatment, increasing gut microbiota diversity and the relative abundance of beneficial bacteria while inhibiting the overgrowth of harmful bacteria. This study suggests that FMT holds potential in regulating gut microbiota and improving the prognosis of colorectal cancer, providing new insights and references for future clinical treatments.

1. Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors of the digestive system worldwide. In recent years, its incidence has been rising steadily in China, making it the second leading cause of cancer-related deaths after lung cancer[1]. Although advances in diagnostic and therapeutic techniques have improved outcomes, surgery remains the primary treatment. However, the long treatment duration, high costs, and adverse side effects such as radiation enteritis and intestinal obstruction significantly impact patients' quality of life[2,3]. Therefore, exploring more effective treatment options to improve patient prognosis is of great clinical importance.

The gut microbiota plays a crucial role in the development and progression of CRC. Recent studies have shown that CRC patients often experience gut microbiota dysbiosis, characterized by a decrease in beneficial

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bacteria and an increase in harmful bacteria, which may promote tumor growth[4]. Fecal microbiota transplantation (FMT), a therapeutic method that transplants the gut microbiota from a healthy donor to a recipient, has shown great potential in restoring gut microbial balance and improving various diseases, such as inflammatory bowel disease and functional constipation[5]. Additionally, there is growing interest in the application of FMT for modulating gut microbiota to inhibit tumor growth[6,7].

This study aims to establish a mouse model of CRC using dextran sulfate sodium (DSS) combined with azoxymethane (AOM) and assess the effects of FMT intervention on gut microbiota diversity. Using 16S rDNA sequencing analysis, this research seeks to provide new theoretical support for the application of FMT in CRC treatment and lay the foundation for future clinical studies.

2. Materials and Methods

2.1. Experimental Animals

Thirty-six healthy female C57BL/6 mice, aged 7-8 weeks and weighing 20±5 g, were purchased from the Experimental Animal Center of Zhengzhou University. The animals were housed in the Medical College Animal Laboratory at Pingdingshan University under controlled conditions (temperature: 23±2°C, humidity: 55 ± 5% with free access to standard feed and water. All experimental procedures were approved by the Ethical Committee of Pingdingshan University.

2.2. Reagents and Materials

The major reagents used include dextran sulfate sodium (DSS), azoxymethane (AOM), sodium chloride, and the QIAamp PowerFecal Pro DNA Kit. Fresh fecal samples were collected from healthy donor mice. Reagents were prepared and handled following standard laboratory protocols.

2.3. Study Design and Grouping

The mice were randomly divided into three groups (n=12 per group):

Group A (Control): Mice received regular feed and a daily oral gavage of 15 mg/kg sterile saline.

Group B (CRC Model): Mice were administered AOM (10 mg/kg body weight) via intraperitoneal injection on the first day, followed by 2% DSS in drinking water for 7 days, with three cycles of treatment to establish the CRC model. They received a daily gavage of 15 mg/kg saline post-modeling.

Group C (FMT Treatment): After the CRC model was established as in Group B, mice were treated with a daily oral gavage of 15 mg/kg fecal microbiota suspension.

2.4. Fecal Microbiota Preparation

Fresh feces from healthy donor mice were collected and suspended in sterile saline (1:10 w/v). The suspension was filtered through a sterile mesh to remove large particles and centrifuged at 10,000 rpm for 2 minutes. The resulting pellet was resuspended in sterile saline, and the process was repeated three times. The final fecal microbiota solution was stored at 4°C and used for daily oral gavage.

2.5. Evaluation of CRC Development

Mice were monitored daily for general health, including activity, fur appearance, weight, and stool consistency. At the end of the experiment, mice were euthanized for necropsy. Tumors in the colon and rectum were counted, and the length of the colorectal region was measured. Histological samples were prepared for further analysis.

2.6. Fecal Collection and DNA Extraction

Fecal samples were collected from each group at baseline and at the end of the experiment. Samples were immediately frozen in liquid nitrogen and stored at -80°C. Microbial DNA was extracted using the QIAamp PowerFecal Pro DNA Kit, following the manufacturer's protocol.

2.7. 16S rDNA Sequencing and Microbial Analysis

The V3-V4 region of the 16S rDNA gene was amplified using specific primers. PCR products were purified and sequenced using the TruSeq DNA PCR-Free Sample Preparation Kit. Sequencing was performed on an Illumina platform, and data were processed to identify operational taxonomic units (OTUs). α-diversity indices, including Chao1, Ace, Shannon, and Simpson indices, were calculated to assess microbial richness and diversity.

2.8. Statistical Analysis

All statistical analyses were performed using SPSS 23.0 software. Group comparisons were made using one-way ANOVA for normally distributed data, and the Mann-Whitney U test for non-normally distributed data. Results with $P < 0.05$ were considered statistically significant.

3. Result

3.1 Establishment of the Mouse Colorectal Cancer Model Induced by DSS Combined with AOM

During the process of establishing the mouse colorectal cancer model induced by DSS combined with AOM, the mice in Group B (model group) gradually exhibited

typical symptoms of colorectal cancer, including lethargy, dull fur, constipation, and bloody stools. Dissection results showed that the colorectal length of the model group mice was significantly shorter than that of the blank control group (Group A) (8.2 cm vs. 8.5 cm), and multiple tumors of varying sizes appeared on the surface of the colon (Figure 1). These observations indicate that DSS combined with AOM successfully induced colorectal cancer in mice.

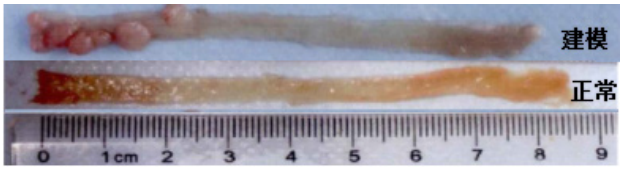


Figure 1. Comparison of the Colorectal of Normal Mice and Model Group Mice

3.2 Effect of Fecal Microbiota Transplantation on Mouse Weight Changes

During the modeling process, the mice in Group B gradually lost weight, with a significant weight reduction observed by day 7 ($P < 0.05$). In contrast, the mice in Group C (fecal microbiota transplantation group) showed a gradual increase in weight after receiving the transplantation, approaching the levels seen in Group A (Figure 2). The weight difference between Groups B and C at the end of the intervention was statistically significant ($P < 0.05$), indicating that fecal microbiota transplantation may help restore the normal physiological state of the mice.

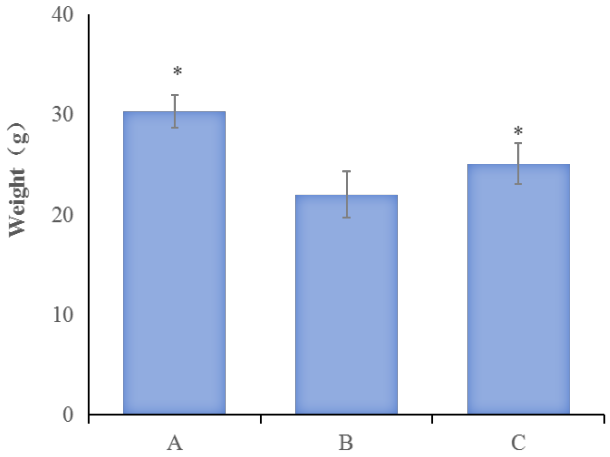


Figure 2. Weight of Mice in Each Group

Note: "*" indicates a statistically significant difference compared to Group B ($P < 0.05$).

3.3 Effect of Fecal Microbiota Transplantation on Mouse Gut Microbiota Diversity

3.3.1 OTU Analysis

The operational taxonomic unit (OTU) analysis based on sequence similarity revealed that the number of OTUs in Group A was 169, in Group B was 198, and in Group C was 183 (Figure 3). The higher OTU count in Group B compared to Group A may be associated with the dysbiosis caused by colorectal cancer, while the OTU count in Group C showed a decrease, trending toward healthy levels.

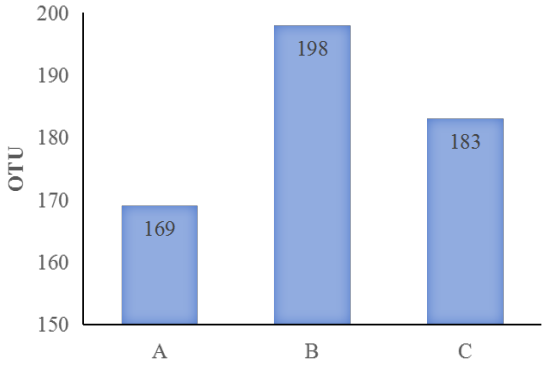


Figure 3. OTU Analysis of Mice in Each Group

3.3.2 α Diversity of Gut Microbiota in Each Group of Mice

The α diversity of the mice gut microbiota was analyzed using the Chao1, Ace, Shannon, and Simpson indices. The results showed that the Chao1 and Ace indices in Group B were significantly lower than those in Group A, indicating a decrease in microbiota richness in the model group ($P < 0.05$). However, after receiving fecal microbiota transplantation, the Chao1 and Ace indices in Group C significantly increased, approaching the levels observed in Group A, suggesting that fecal microbiota transplantation has a positive effect on restoring microbiota diversity (Table 3).

3.3.3 Effect of Fecal Microbiota Transplantation on the Relative Abundance of Gut Microbiota in Mice

At the phylum level, the abundance of *Firmicutes* in Group B significantly increased to 70.75%, while Group C showed a recovery to 48.64%, approaching the level of the control group (Group A was 55.56%). Additionally, mice in Group C exhibited a significant increase in the relative abundances of *Bacteroidetes* and *Actinobacteria*, indicating that fecal microbiota transplantation may restore gut health by increasing the proportion of these beneficial bacteria (Figure 4).

At the genus level, the relative abundances of *Akkermansia* and *Allobaculum* in Group C were significantly higher than those in Group B, indicating that fecal microbiota transplantation plays a crucial role in restoring these beneficial bacterial populations.

Additionally, the relative abundance of harmful bacteria, such as *Proteobacteria*, was significantly reduced in Group C (Figure 5). These results suggest that fecal microbiota transplantation effectively inhibits the overgrowth of pathogenic bacteria by modulating the relative abundances of gut microbiota.

Table 3. α Diversity of Gut Microbiota in Mice

Group	Chao1 Index (Mean ± SD)	Ace Index (Mean ± SD)	ShannonIIndex (Mean ± SD)	Simpson Index (Mean ± SD)
A	370.64±15.23*	354.21±17.56*	3.97±0.13*	0.65±0.09
B	305.89±7.62	302.23±8.41	3.87±0.16	0.58±0.06
C	357.24±14.34*	375.32±10.98*	3.93±0.11*	0.50±0.10

Note: "*" indicates a statistically significant difference compared to Group B ($P < 0.05$).

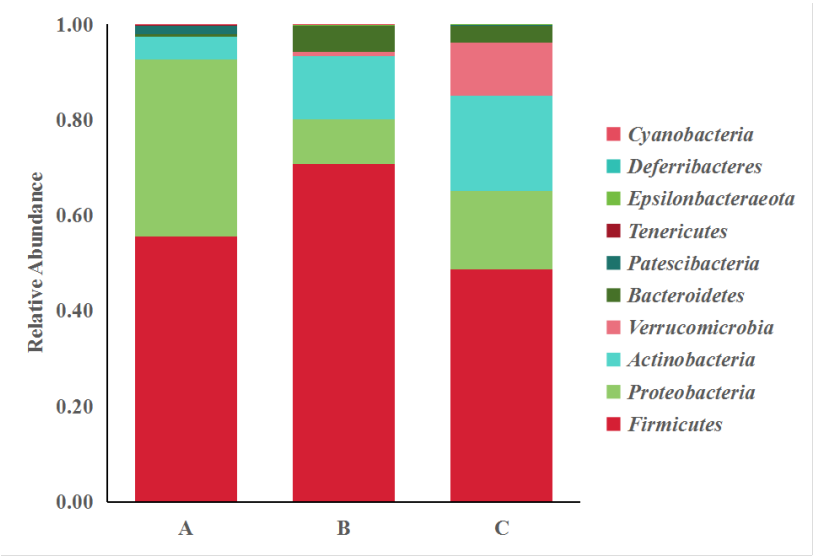
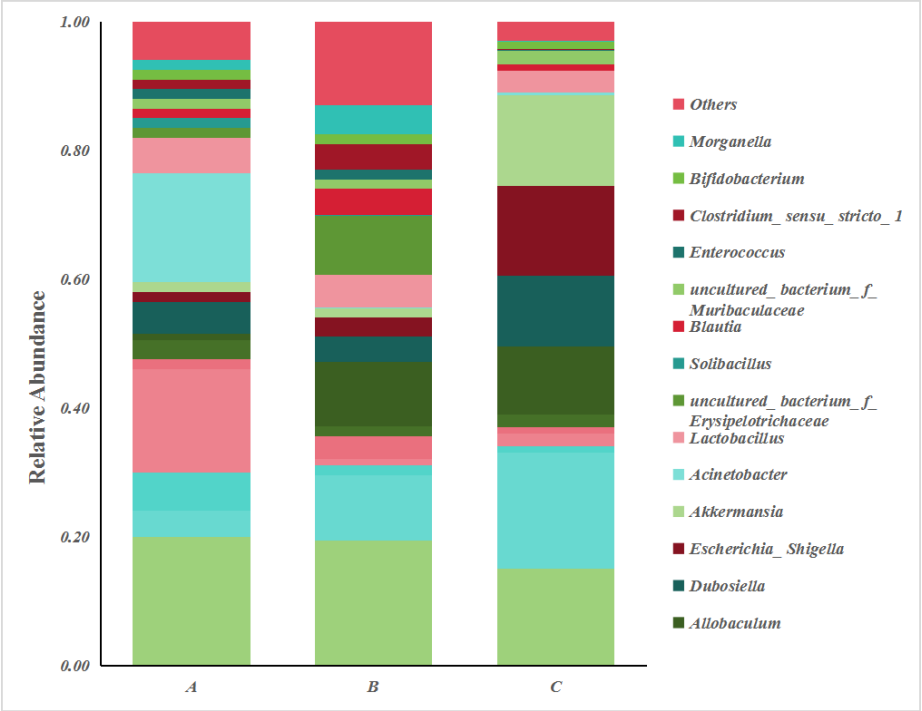


Figure 4. Diversity of Gut Microbiota at the Phylum Level in Each Mouse Group



4. Discussion

This study successfully established a mouse model of colorectal cancer through the use of the DSS combined with AOM induction method, aiming to explore the therapeutic effects of fecal microbiota transplantation (FMT) on the model mice and its impact on gut microbiota diversity. There are various methods for establishing colorectal cancer mouse models, among which the DSS combined with AOM induction method is widely used in related research due to its ability to induce colorectal cancer quickly and stably, along with its pathological characteristics resembling those of human colorectal cancer . In this study, this method was employed to successfully establish a tumor model, and clinical manifestations similar to those of human colorectal cancer were observed in the model mice, further validating the effectiveness of the model. After successful modeling, the mice received fecal microbiota transplantation treatment, resulting in a gradual increase in body weight and a significant reduction in tumor numbers. This suggests that fecal microbiota transplantation may delay tumor growth and improve survival rates by modulating the gut microbiota.

Our study results indicate that fecal microbiota transplantation has a positive therapeutic effect on colorectal cancer in mice. Research by Zackular et al. similarly found that fecal microbiota transplantation inhibits tumor occurrence and progression by reconstructing the intestinal microecology and reducing the proportion of pathogenic bacteria in the body[9]. Additionally, the study by Loo et al. pointed out that fecal microbiota transplantation can enhance the diversity of gut microbiota, restore a healthy environment, and subsequently slow tumor progression[10]. These studies support our findings, suggesting that fecal microbiota transplantation may have significant clinical implications in the regulation of gut microecology.

Through 16S rDNA sequencing analysis, we further observed significant differences in the OTU abundance, α diversity, and community structure of gut microbiota among the blank control group, model group, and fecal microbiota transplantation group. Specifically, the gut microbiota diversity in the model group was significantly reduced, with a decrease in beneficial bacteria abundance, whereas the gut microbiota diversity in the fecal microbiota transplantation group gradually restored, approaching the levels of the blank control group. This phenomenon indicates that fecal microbiota transplantation can effectively modulate and repair the gut microbiota imbalance caused by colorectal cancer and the modeling process. This result is consistent with the research of Zackular et al., who indicated that gut microbiota dysbiosis plays an important role in the occurrence of colorectal cancer, and that fecal microbiota transplantation can reduce inflammatory responses by restoring beneficial microbial communities, thereby inhibiting tumor occurrence[9]. Additionally, Seekatz et al. found that fecal microbiota transplantation can improve damaged gut microecology and restore microbial balance[11]. These studies further support our findings on the positive role of fecal microbiota transplantation in regulating microbiota diversity and highlight its potential value in disease treatment.

In the α diversity analysis, we found that, although there were differences in the microbiota structure among the three groups of mice, the diversity of the fecal microbiota transplantation group was relatively close to that of the blank control group. This indicates that fecal microbiota transplantation can effectively restore the gut microbiota imbalance caused by tumors and the modeling process, further supporting its significant role in regulating gut health. This result is consistent with the research by Wang et al., who demonstrated that fecal microbiota transplantation significantly increases gut microbiota diversity in diseased mice and restores a healthy microbial structure[11]. These findings further validate the critical role of fecal microbiota transplantation in restoring gut microecological balance.

At the phylum level, fecal microbiota transplantation significantly regulated the relative abundance of key microbial groups. In the model group of mice, the proportions of beneficial bacteria such as *Firmicutes* and *Bacteroidetes* were significantly reduced, while in the fecal microbiota transplantation group, these proportions gradually restored, indicating that fecal microbiota transplantation can effectively restore the balance of healthy microbial communities. At the same time, the proportion of certain potential pathogenic bacteria, such as *Proteobacteria*, increased in the model group, whereas in the fecal microbiota transplantation group, this proportion gradually approached that of the blank control group. This result suggests that fecal microbiota transplantation may restore microbial balance by inhibiting the excessive proliferation of harmful bacteria. The study by Zhou et al. similarly found that fecal microbiota transplantation could reduce the relative abundance of *Proteobacteria* and restore the proportions of healthy microbial communities[13]. Furthermore, Khan et al. noted that fecal microbiota transplantation effectively inhibits the excessive growth of harmful bacteria, promoting gut health[14].

In the analysis at the genus level, we observed that fecal microbiota transplantation had a significant impact on the relative abundance of several key genera. Specifically, *Acinetobacter* and *Psychrobacter* had the highest relative abundance in the blank control group, while the fecal microbiota transplantation group gradually approached these levels. Additionally, the relative abundance of *Akkermansia* significantly increased in the fecal microbiota transplantation group. Existing studies have indicated that *Akkermansia* is closely related to gut health, as it can restore gut function by maintaining intestinal barrier function and reducing inflammatory responses[15]. Li et al. also found that fecal microbiota transplantation significantly increased the abundance of *Akkermansia* in the intestines of colorectal cancer patients, reducing inflammation[16]. These findings further support the positive role of *Akkermansia* in regulating gut microbiota.

5. Conclusion

This study established a mouse model of colorectal cancer through the DSS combined with AOM method and implemented fecal microbiota transplantation interventions to explore the effects of fecal microbiota transplantation on gut microbiota diversity. The results indicate that, compared to the model group, mice in the fecal microbiota transplantation group showed a significant reduction in the number of intestinal tumors, and the diversity and species richness of gut microbiota were restored to near-normal levels. These findings suggest that fecal microbiota transplantation not only effectively inhibits the occurrence of tumors in colorectal cancer mice but also has the potential to regulate the structure of the intestinal microbial community. Therefore, the diversity of gut microbiota may play an important role in the development of colorectal cancer, supporting fecal microbiota transplantation as a potential therapeutic strategy to improve the prognosis and quality of life of colorectal cancer patients.

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