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Fecal microbiota transplantation enhanced the effect of chemoimmunotherapy by restoring intestinal microbiota in LLC tumor-bearing mice

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Abstract

Objective To assess the effect of half-dose chemotherapy (HDC) and standard-dose chemotherapy (SDC) on the intestinal microbiota and to investigate whether fecal microbiota transplantation (FMT) can restore the intestinal microecology to enhance the efficacy of chemoimmunotherapy containing an anti-PD- 1 antibody (PD1).

Methods Lewis lung cancer (LLC) tumor-bearing mice were divided into six groups, including Control, HDC, SDC, SDC + FMT, SDC + PD1, and SDC + PD1 + FMT. After the treatment, analyses were conducted on intestinal microbiota using 16S rRNA sequencing, immune cells through flow cytometry, cytokines and chemokines via polymerase chain reaction (PCR), and programmed death-ligand 1 (PD-L1) expression in tumor tissues by immunohistochemistry.

Results Alpha and beta diversity of intestinal flora were not significantly different between HDC and SDC groups, nor was there a significant difference in the abundance of the top 10 species at the phylum, class, order, family, genus, or species levels. FMT increased both alpha and beta diversity and led to an increase in the abundance of *Ruminococcus_callidus* and *Alistipes_finegoldii* at the species level in mice receiving SDC + FMT. Besides, tumor growth was significantly slowed in SDC + PD1 + FMT compared to SDC + PD1 group, accompanied by an up-regulated *Bacteroidetes/Firmicutes* ratio, down-regulated abundance of *Proteobacteria* species (including *Pseudolabrys*, *Comamonas*, *Alcaligenaceae*, *Xanthobacteraceae* and *Comamonadaceae*), as well as *Faecalicoccus* of *Firmicutes*, the increased number of cDC1 cells, cDC2 cells, $CD4^+$ T cells and $CD8^+$ T cells in the peripheral blood, and IFN-γ^+CD8^+ T cells, IFN-γ, granzyme B, TNF-α, CXCL9 and CXCL10 in intestinal tissues.

Conclusions There were no significant differences between HDC and SDC in their effects on the intestinal microbiota. FMT exhibited a beneficial impact on gut microbiota and improved the efficacy of chemoimmunotherapy, possibly associated with the increase of immune cells and the modulation of related cytokines and chemokines.

Keywords Fecal microbiota transplantation, Chemoimmunotherapy, Intestinal microecology, Half-dose chemotherapy, Immune checkpoint inhibitors

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Introduction

Chemotherapy serves as the cornerstone of anti-tumor treatment, and its combination with immune checkpoint inhibitors (ICIs) is the first-line standard treatment regimen for many advanced tumors, which holds promising clinical application prospects [1, 2]. However, there are still certain challenges that need to be addressed. On one hand, the gut microbiota plays a critical role in the development of malignant tumors. Certain gut microorganisms, such as *Fusobacterium nucleatum* can produce FadA adhesin to promote the occurrence of colorectal cancer [3]. In patients with advanced tumors, repeated chemotherapy may damage the mucosal epithelium and lead to intestinal bacterial dysbiosis [4]. The dysregulated gut microbiota and its metabolites can trigger systemic immune disorders by inducing inflammation, inactivating and activating tumor suppressor genes, and inducing immunosuppressive tumor microenvironment in multiple ways to directly participate in the development of tumors [5–7]. To solve this, appropriate interventions aimed at regulating intestinal bacteria have been explored [8, 9]. Among them, fecal microbiota transplantation (FMT) has emerged as a highly effective approach which can modulate the intestinal microbiota [9, 10] by introducing a variety of metabolically active bacteria to enhance the effectiveness of chemotherapy and alleviate treatment associated side effects [8]. For instance, FMT from overweight or obese donors has been shown to improve chemotherapy response and prolong survival in patients with metastatic gastroesophageal cancer [11]. Additionally, the transplantation of *Bifidobacterium* or microbiota from healthy mice reduced the incidence of weight loss and colon shortening in mice undergoing 5-fluorouracil treatment [12, 13]. Several small-sample clinical trials have suggested that FMT may alleviate chemotherapy-induced diarrhea, although these findings were not yet confirmed by large-scale clinical studies.

On the other hand, some patients exhibit either primary unresponsiveness or rapid acquired resistance to ICIs [14, 15]. This phenomenon may be related to the aberrant composition of the intestinal microbiota as well [16]. By modulating the intestinal microbiota and promoting immune cell proliferation, FMT has the potential to mitigate both primary and secondary resistance to ICIs. For example, co-administration of *Bacteroides fragilis*, *Bifidobacterium*, and *Akkermansia muciniphila* with ICIs has been shown to enhance the anti-tumor efficacy in preclinical tumor models as well as in cancer patients [16–19]. The 11 strains isolated from the feces of patients who responded well to ICIs could effectively inhibit tumor growth in mouse models when combined with ICIs [20]. Similarly, FMT has improved the efficacy of ICIs in refractory melanoma patients in two independent clinical studies [15, 21]. Consequently, the combination of FMT with ICIs is currently undergoing extensive investigation [17, 22].

However, current research predominantly focuses on the effects of FMT on chemotherapy or immunotherapy alone, with limited attention to its impact when combined with chemoimmunotherapy. Moreover, whether adjusting chemotherapy dosage can minimize its adverse effects on the intestinal microbiota remains unresolved. Therefore, our study aimed to investigate how different dose-intensity chemotherapy affects the intestinal bacteria in tumor-bearing mice, and to determine whether FMT can restore the intestinal microecology, thereby enhancing efficacy of chemoimmunotherapy.

Materials and methods

FMT experiments

Fecal samples obtained from healthy human donors were mixed thoroughly in sterile saline (mass: volume = 1: 5) under an anaerobic environment. The fecal solids and impurities were removed, and the sediment was collected through filtration and centrifugation. Bacterial viability was assessed using flow cytometry and anaerobic plate counts. The bacterial solution was adjusted to a concentration of 5×10^9 colony-forming units (CFU/mL), followed by the addition of high-pressure glycerol. Then FMT was performed by gavage [16, 18, 20, 23].

Mouse experiments

C57BL/6 (7–8 weeks old) mice were purchased from Zhejiang Baiyue Biological Technology Company Limited (Zhejiang, China). 250 µL of Lewis lung cancer (LLC) cells (8×10^6 cells/mL) were injected subcutaneously into each mouse. Tumor volume was measured every other day using the formula: length × width^2 × 0.5. Mice were divided into six groups including Control, HDC, SDC, SDC + FMT, SDC + PD1, SDC + PD1 + FMT groups, and 5 mice were assigned to each group. The treatment was initiated when tumor volume reached a minimum of 80 mm^3 (approximately on day 7). On days 7, 10, and 13, mice received intraperitoneal injections of either PBS at a dose of 20 mg/kg or Nanoparticle Albumin-Bound Paclitaxel (Nab-PTX) at a dose of 10 mg/kg or Nab-PTX at a dose of 20 mg/kg. On days 14, 16, and 18, mice were administered either PBS at a dose of 10 mL/kg or FMT by gavage at the same dosage. Finally, on days 21, 24, and 27, mice received tail vein injections with either PBS at a dose of 10 mg/kg or 200 µg of murine Anti-PD- 1 antibody (αPD- 1) (Fig. 1). The experiment concluded on day 30 and then mice were sacrificed by ether inhaled anesthesia followed by cervical dislocation. All animal procedures were carried out in strict adherence to the guidelines set by the Animal Care Committee of Zhejiang Baiyue Biological Technology Company Limited (No. ZJBYLA-IACUC- 20230209).

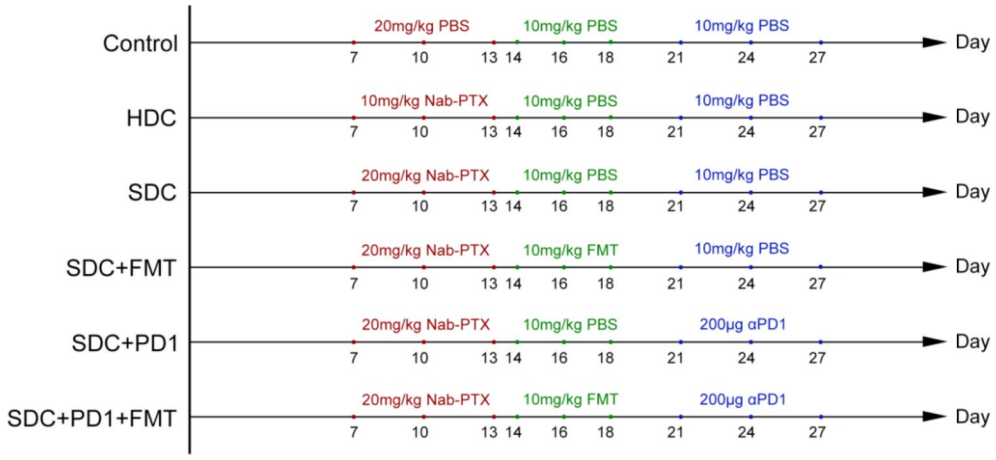

Fig. 1 Animal experiment flowchart. HDC: Half-dose chemotherapy, SDC: Standard-dose chemotherapy, FMT: Fecal microbiota transplantation, PD1: Anti-PD- 1 antibody (αPD- 1), Nab-PTX: Nanoparticle Albumin-Bound Paclitaxel. Nab-PTX, intraperitoneal injection; FMT, via gavage; αPD- 1, via tail vein injection

Table 1 The primers used in the PCR analysis

Primer name	(5′ to 3′)	
Mus-GAPDH	F	GAGAAACCTGCCAAGTATGATGAC
	R	AGAGTGGGAGTTGCTGTTGAAG
CXCL9	F	TGAAGTCCGCTGTTCTTTTCCT
	R	ATTCCTTATCACTAGGGTTCCTCG
CXCL10	F	CGTGTTGAGATCATTGCCACGAT
	R	AGACCTTTTTTGGCTAAACGCTTTC
IFN-γ	F	TGGCTGTTTCTGGCTGTTACT
	R	GATTTTCATGTCACCATCCTTTTGC
Granzyme B	F	GGAGAAGACCCAGCAAGTCA
	R	GCCTTACTCTTCAGCTTTAGCA
TNF-α	F	GATCGGTCCCCAAAGGGATG
	R	TGTGAGGGTCTGGGCCATAG

16S rRNA gene sequence analysis

With 30 ng of high-quality genomic DNA, we designed polymerase chain reaction (PCR) primers to establish a PCR system. For PCR amplification, PCR reaction parameters were established. To complete the library assembly, PCR amplification products were purified. The Agilent 2100 Bioanalyzer was utilized to analyze the library and sequencing. Clean data were collected and subjected to further analysis. Pairs of short sequences were assembled into contiguous sequence to generate Tags, and Operational Taxonomic Units (OTUs) were formed by clustering the Tags. These OTUs were checked against a reference database and annotated with species information. Subsequently, the species complexity of the samples, differences in species between groups, and associations were analyzed.

Quantitative real-time PCR

We isolated total RNA from tumor or intestinal tissues of mice in each group, followed by reverse transcription using conventional methods to generate cDNA. Subsequently, mRNA levels of CXCL9, CXCL10, TNF-α, IFN-γ, and granzyme B were detected. The PCR amplification system was prepared based on the instructions provided with the PCR kit (Genepharma, China) with a total reaction volume of 50 μL. Primer sequences for the target genes were synthesized to standardize the mRNA transcript levels of GAPDH (Table 1).

Flow cytometry analysis

Blood and intestinal tissues were collected to obtain resuspended cells. The cells were subsequently washed and centrifuged at 1000 r/min for 5 min in PBS, and labeled with anti-mouse antibodies at a final concentration of 10 μg/mL (Anti-CDC123 antibody [ab129101, Abcam], Anti-IFN gamma Receptor beta/AF- 1 antibody [ab224197, Abcam], Anti-CD8 alpha antibody [ab217344, Abcam], Anti-CDK1 antibody [ab18, Abcam], Anti-CD4 antibody [ab207755, Abcam]). Afterward, the cells were washed again and incubated in the dark at 4 °C for 30 min. Analysis was performed using FACSCanto II flow cytometry.

Immunohistochemical staining

The tissue sections were deparaffinized, repaired by heating with sodium citrate, treated with 1% hydrogen peroxide to eliminate endogenous peroxidase activity, and blocked by 5% goat serum. The sections were shaken to remove the blocking solution, and then 50 μL of the primary antibody (PD-L1) was added dropwise to each section, followed by overnight incubation at 4 °C. The next day, the samples were rewarmed to room temperature and incubated for 2 h with a gradual addition of a secondary antibody. Diaminobenzidine (DAB) was developed, and hematoxylin was restained. Using light microscope, the proportion of PD-L1-positive cells relative to all tumor cells was measured. The results were interpreted

by two pathologists using a double-blind method. The PD-L1 antibody [Proteintech, 66248 - 1-Ig] was diluted at a ratio of 1 : 2000.

Statistical analysis

All measurements were reported as mean ± standard deviation (SD). One-way ANOVA was used for comparisons between multiple groups. Diversity, downscaling, and species analyses were performed using the GBI platform (http://meta.bgi.com). Statistical tests were performed with GraphPad Prism 9.0 software. Statistical significance was considered when $p < 0.05$. The p-values were adjusted by FDR when making multiple comparisons. ns, not significant; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Results

Effects of HDC and SDC on intestinal microbiota

To investigate the impact of different chemotherapy doses on intestinal microbiota, 16S rRNA sequencing was performed after HDC or SDC treatment. Alpha diversity was measured by abundance-based coverage estimator (ACE) and Shannon index analysis. beta diversity was measured through unweighted UniFrac analysis. As the results showed, there was no significant difference in alpha and beta diversity between HDC and SDC (Fig. 2A-C). Although *Ruminococcus_callidus* increased, and *Alistipes_finegoldii* decreased in HDC among the top 10 species at the species level (Fig. 2D), these changes did not reach statistical significance. At the same time, no significant difference was observed in the relative abundance of the top 10 species at the phylum, class, order, family, and genus levels (Figure S1). As a result, there was no significant difference in the effects of intestinal microbiota for mice receiving HDC and SDC treatment.

FMT increased the abundance of "beneficial bacteria" in the gut of mice receiving chemotherapy

Alpha and beta diversity were slightly elevated in SDC + FMT compared with SDC, though the differences were not statistically significant (Fig. 2A-C). At the species level, *Ruminococcus_callidus* and *Alistipes_finegoldii* were significantly enriched in SDC + FMT (Fig. 2D), both of which are considered "beneficial bacteria" due to their ability to enhance the anti-tumor efficacy by producing short-chain fatty acids [12, 24]. Similarly, no significant differences were observed among the top 10 species abundance at the phylum, class, order, family, and genus levels (Figure S1). The findings suggested FMT may contribute to an increase in the abundance of "beneficial bacteria" in the gut.

FMT enhanced the efficacy of chemoimmunotherapy in tumor-bearing mice

Next, we analyzed the impact of FMT on the efficacy of the combination of chemoimmunotherapy. Notably, tumor growth was significantly inhibited in SDC + PD1 + FMT compared to SDC + PD1, and a similar trend was observed in SDC + FMT versus SDC (Fig. 3A). What's more, it is noteworthy that the tumor suppression effect of SDC + FMT was even slightly better than that of SDC + PD1 (Fig. 3B-D). Hence, FMT demonstrated its potential to enhance the effectiveness of both chemotherapy and chemoimmunotherapy, even achieving comparable efficacy to αPD- 1 treatment.

FMT exhibited a beneficial impact on gut microbiota when combined with chemoimmunotherapy

To investigate how FMT enhanced the effects of chemoimmunotherapy, 16S rRNA sequencing of intestinal microbiota was conducted. Any disruption in the balance between *Bacteroidetes* and *Firmicutes* may disturb gut microbial homeostasis [25] and a reduced *Bacteroidetes/Firmicutes* (B/F) ratio is associated with poor treatment prognosis [23, 26]. In our study, analysis at the phylum level revealed a significant decrease in the relative abundance of *Firmicutes* (41.35%) and a notable increase in *Bacteroidetes* (51.13%) within the SDC + PD1 + FMT group, which resulted in a significant improvement in the B/F ratio (Fig. 3E). Subsequently, linear discriminant analysis effect size (LEfSe) analysis was employed, revealing 9 species differed in abundance between SDC + PD1 + FMT and SDC + PD1. To be specific, *Proteobacteria* species (including *Pseudolabrys*, *Comamonas*, *Alcaligenaceae*, *Xanthobacteraceae* and *Comamonadaceae*), as well as *Faecalicoccus* of *Firmicutes*, were reduced in SDC + PD1 + FMT compared to SDC + PD1 (Fig. 3F). Therefore, FMT modulated the composition of intestinal microbiota in a manner favorable to the efficacy of chemoimmunotherapy.

FMT increased the number of immune cells in peripheral blood

PD-L1 expression in tumor tissues, as assessed by immunohistochemical staining, did not significantly differ among the groups, indicating that the enhanced efficacy in the SDC + PD1 + FMT was not attributable to changes in PD-L1 expression (Fig. 4A-B). Subsequently, an analysis of immune cells in peripheral blood from each group was conducted. In SDC + PD1 + FMT, there was a significant increase in the numbers of cDC1 cells, cDC2 cells, $CD4^{+}$ T cells and $CD8^{+}$ T cells compared to SDC + PD1. A similar difference was also observed in SDC + FMT compared to SDC (Fig. 4C-F). Therefore, the enhanced anti-tumor efficacy of FMT may be associated with the increase of immune cells in peripheral blood.

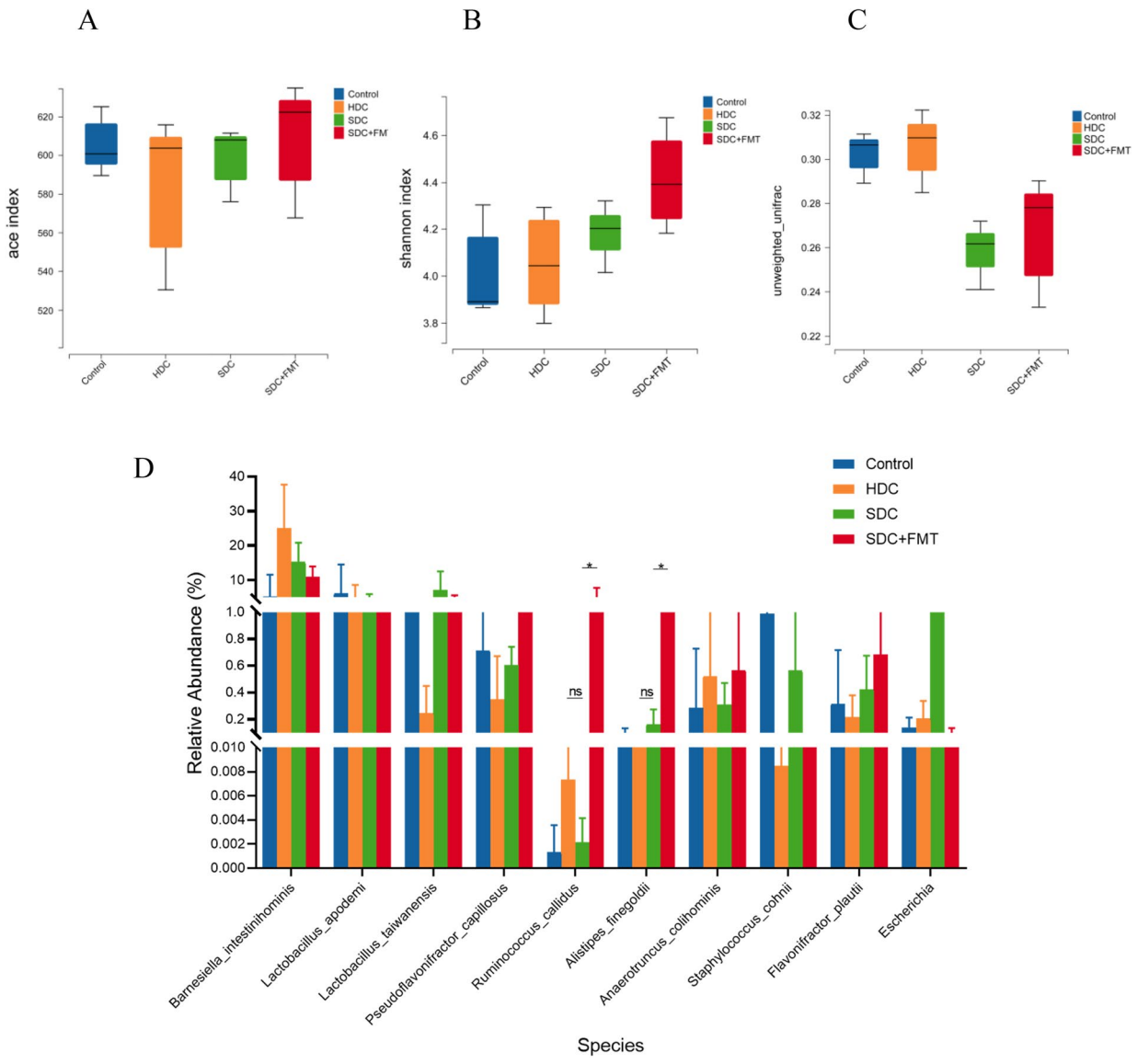

Fig. 2 Alpha, beta diversity, and the abundance of different species diversity of gut microbiota in different treatment groups. **A** Ace index analysis for alpha diversity ($p = 0.8261$). **B** Shannon index analysis for alpha diversity ($p = 0.5566$). **C** Unweighted analysis of beta diversity ($p = 0.0752$). **D** Differences among the top 10 species in relative abundance at the species level (*Ruminococcus_callidus*, $p = 0.0497$; *Alistipes_finegoldii*, $p = 0.0434$). Data are represented as means ± SDs, $n = 3$. One-way ANOVA was used for comparisons between multiple groups. ns, not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

FMT increased the killing efficacy of CD8$^+$ T cells in intestinal tissues by regulating cytokines and chemokines

Finally, we utilized flow cytometry to analyze the population of IFN-γ$^+$CD8$^+$ T cells in intestinal tissues. Similarly, SDC + PD1 + FMT exhibited a significantly higher proportion of IFN-γ$^+$CD8$^+$ T compared to SDC + PD1, and SDC + FMT had a significantly higher proportion than SDC (Fig. 5A). We then further detected the mRNA expression levels of granzyme B, TNF-α, IFN-γ, CXCL9 and CXCL10 in intestinal tissues by qPCR. The expression of IFN-γ was significantly higher in SDC + PD1 + FMT compared to SDC + PD1, and SDC + FMT showed a higher level than SDC. Although the expression levels of granzyme B and TNF-α all elevated, the difference was not significant (Fig. 5B-D). Coincidentally, we also noted significantly elevated expression levels of CXCL9 and CXCL10, particularly CXCL9, in the SDC + PD1 + FMT relative to the SDC + PD1 (Fig. 5E-F). These findings suggested that FMT may enhance the killing efficacy of CD8$^+$ T cells in intestinal tissues by modulating associated cytokines and chemokines.

Discussion

To date, many reports have documented strategies for optimizing chemotherapy in conjunction with immunotherapy. One such approach involves the combination of

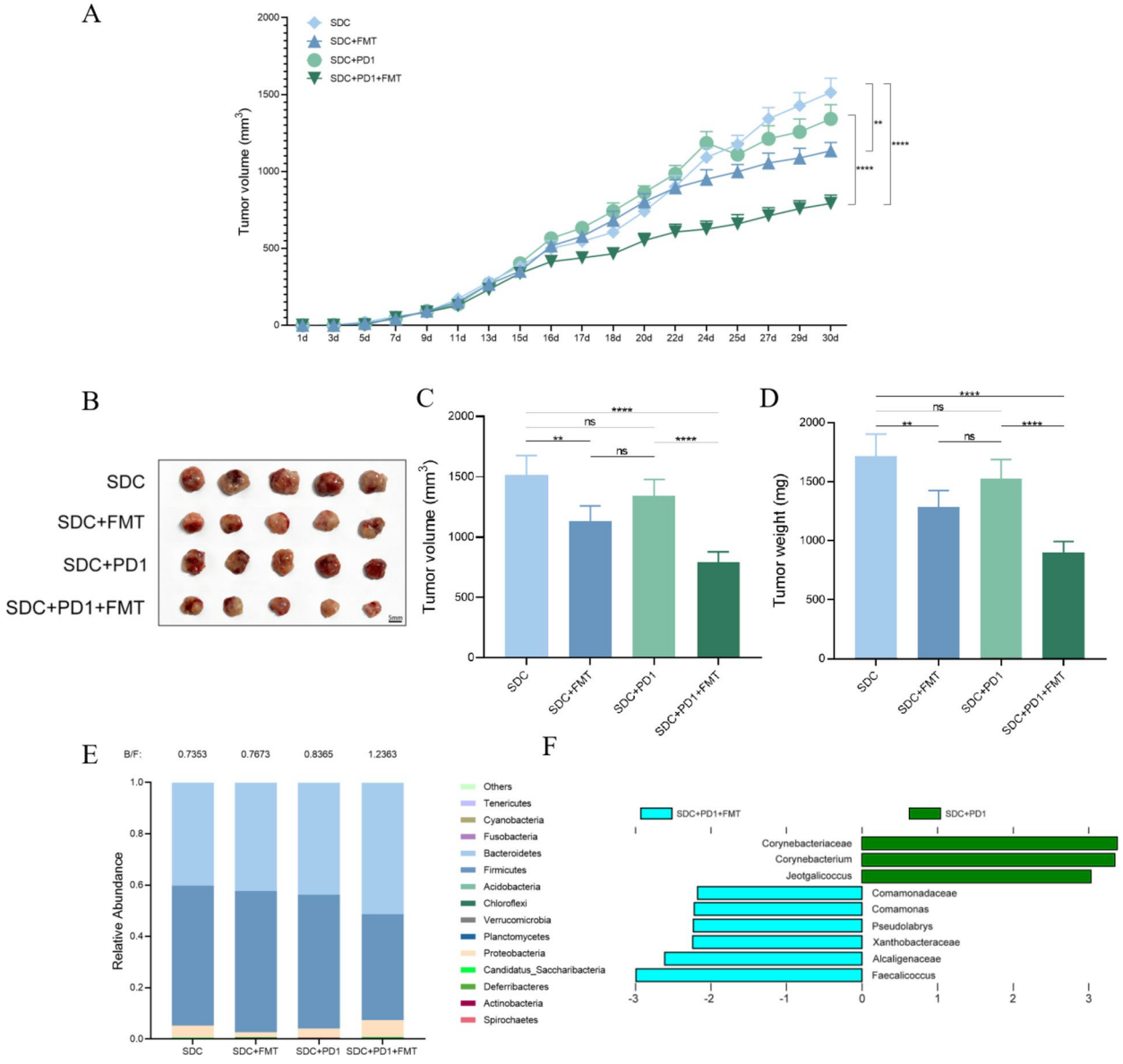

Fig. 3 FMT and chemoimmunotherapy exerted synergistic anti-tumor efficacy. **A** Tumor growth curves in each group. **B** Tumor macroscopic views in each group. Differences in tumor volume (**C**) and tumor weight (**D**) among the mice. **E** The species composition of the intestinal microbiota at the phylum level in each group. **F** LEfSe analysis showed differentially abundant bacterial species between SDC + PD1 and SDC + PD1 + FMT. Data are represented as means ± SDs, $n = 5$. Tumor volume and weight were analyzed with a one-way ANOVA test. ns, not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

low-dose chemotherapy represented by half-dose chemotherapy [16, 17]. Studies have shown that the combination of ICIs with a reduced dosage of chemotherapy can mitigate adverse effects [27] without affecting the efficacy of treatment [28–31], especially in elderly patients [32]. However, studies related to the effects of different dose-intensity chemotherapy on intestinal microbiota are less common. As the first study to explore the effect of different doses of chemotherapy on gut microbiota, we found that HDC and SDC had nearly no difference in effect on intestinal microflora. Although the number of *Ruminococcus_callidus* showed an increasing trend and the number of *Alistipes_finegoldii* showed a decreasing trend in HDC at the species level, the difference was not statistically significant. We thought that the insignificant changes in the overall diversity were possibly due to the infrequent chemotherapy and short treatment cycles as well as the small sample size. Future research should explore the long-term impacts of repeated cycles of chemotherapy with different dosage on the intestinal microbiota.

The effects of FMT on intestinal bacteria can counteract the impact of chemotherapy and chemoimmunotherapy by enhancing immunity and improving

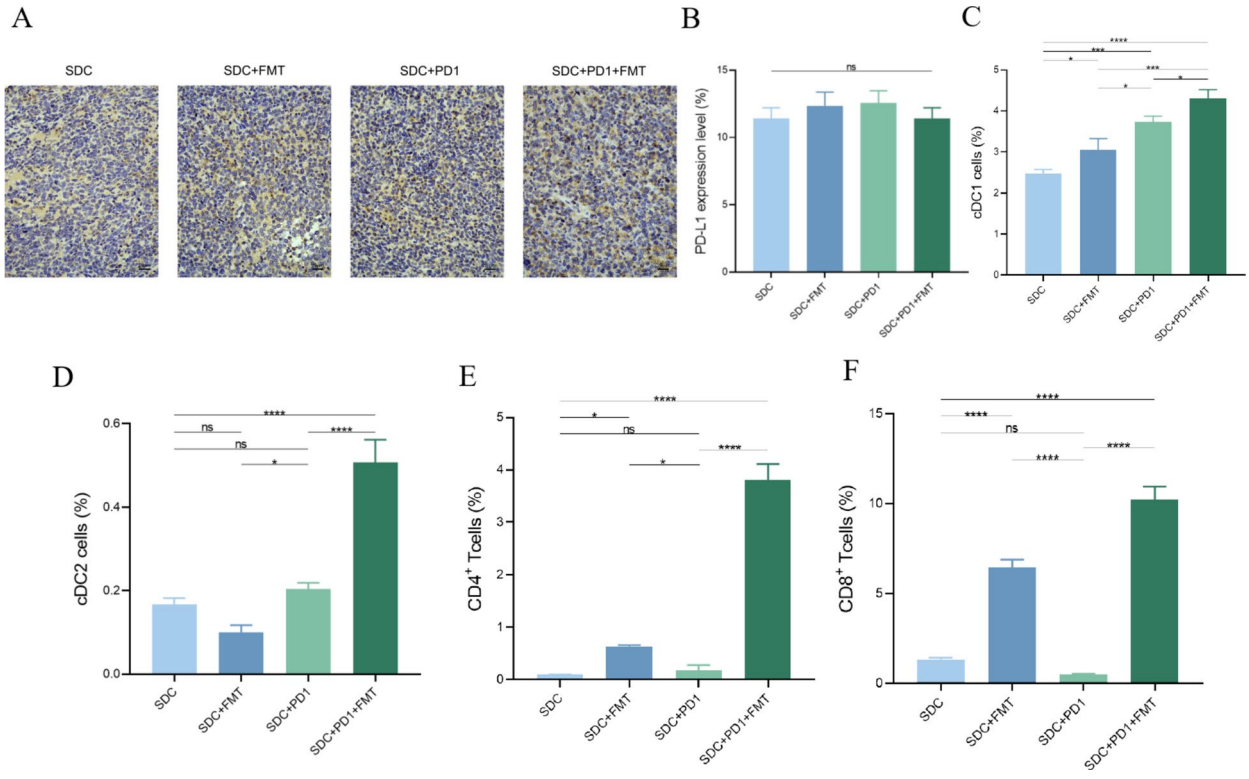

Fig. 4 FMT could increase the number of immune cells in peripheral blood of tumor-bearing mice. **A** The immunohistochemical staining of PD-L1 expression (400× magnification; scale bar = 20 μm). **B** PD-L1 levels were detected by immunohistochemical staining. Differences in cDC1 cells (**C**), cDC2 cells (**D**), $CD4^+$ T cells (**E**) and $CD8^+$ T cells (**F**) in peripheral blood detected by flow cytometry. Data are represented as means ± SDs, $n = 5$. One-way ANOVA was used for comparisons between multiple groups. ns, not significant, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$

colonization of the intestinal microbiota [14, 15]. Alpha and beta diversity of the gut microbiota can stratify cancer patients into responders and non-responders in terms of anti-tumor efficacy [33], and can even serve as potential biomarkers for predicting the efficacy of immunotherapy [34]. Increased alpha and beta diversity was often associated with better treatment outcomes. Zhang et al. found that the enriched genera like *Ruminococcus gnavus (Rg.)* can promote the activation and the immune surveillance function of $CD8^+$ T cells, thereby inhibiting the growth of colon tumors [35]. In addition, both animal-based study and clinical trials have demonstrated that *Ruminococcaceae* and *Alistipes* could work together to induce IFN-γ^+CD8^+ T cells and could effectively inhibit tumor growth when combined with ICIs [15, 24, 28]. These are exactly in line with our study. As a result, the increased alpha and beta diversity, along with the enriched abundance of certain "beneficial bacteria" in the gut microbiota might be related with the enhanced efficacy observed in SDC + FMT compared to SDC alone. Moreover, the B/F ratio, which was positively correlated with the efficacy of ICIs [23, 26], was also elevated after the addition of FMT. The levels of 4 'harmful bacteria' (including *Comamonas*, *Alcaligenaceae*, *Xanthobacteraceae*, and *Faecalicoccus* of *Firmicutes*) within the intestinal bacteria were decreased in SDC + PD1 + FMT, which also related to better treatment outcomes [36–39]. As a result, FMT appears to help improve the structure of the gut flora in a manner favorable to the effectiveness of chemoimmunotherapy.

As we all know, the efficacy of αPD- 1 relies on effective communication between DC and T cells (DC-T crosstalk) [40], a process integral to the cancer-immunity cycle (CIC). Activation and recruitment of immune cells are crucial components of this cycle [41]. Prior studies have demonstrated that FMT can increase the number of DC cells, which play a crucial role in modulating immune responses by initiating innate immunity and regulating adaptive immunity [42]. Furthermore, effective interactions between DC cells and T cells could potentiate the anti-tumor efficacy by increasing the secretion of IFN-γ, TNF-α and granzyme B effectively [43]. Some chemokines, such as CXCL9 and CXCL10, could facilitate the recruitment of more immune cells into the tumor tissues, thereby enhancing the effectiveness of αPD- 1 [18, 23]. In our study, it was observed that subsequent to the addition of FMT, the proportion of cDC1 and cDC2 cells exhibited an increment, accompanied by a synchronous elevation in the proportions of both $CD4^+$ and $CD8^+$ T cells in peripheral blood. Apart from this, more

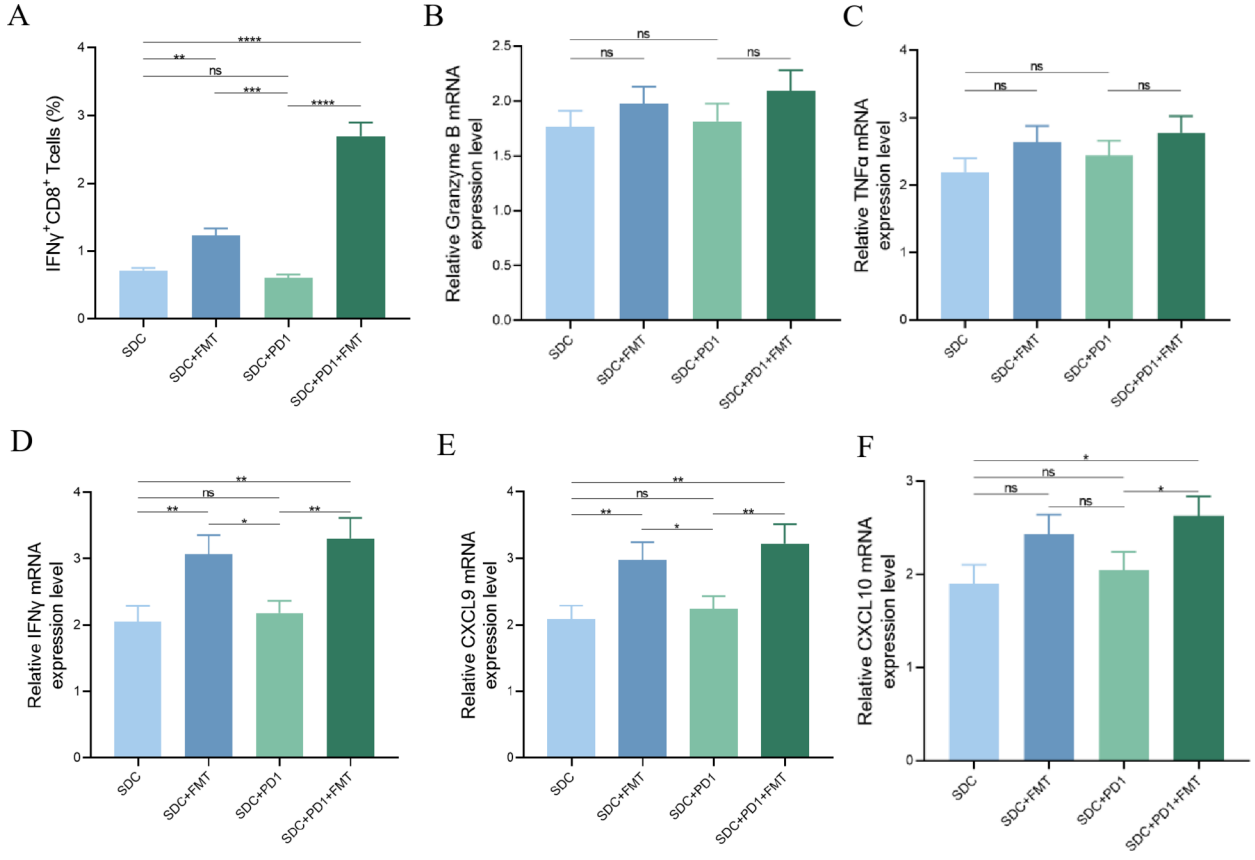

Fig. 5 FMT could increase the killing efficacy of $CD8^+$ T cells in intestinal tissues by regulating cytokines and chemokines. Differences in IFN-γ^+CD8^+ T cells (**A**) in intestinal tissues determined by flow cytometry. PCR assay detected differential mRNA expression of granzyme B (**B**), TNF-α (**C**), IFN-γ (**D**), CXCL9 (**E**) and CXCL10 (**F**) in mouse intestinal tissues. Data are represented as means ± SDs, $n = 5$. One-way ANOVA was used for comparisons between multiple groups. ns, not significant, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$

IFN-γ^+CD8^+ T cells and higher levels of IFN-γ were detected in intestinal tissues, indicating that FMT might augment the DC-T crosstalk, thereby causing $CD8^+$ T cells to secrete a greater quantity of IFN-γ [42, 44, 45]. Although the expression levels of granzyme B and TNF-α all elevated, the difference was not significant, which may not be the predominant way for T cells to kill tumor cells. This may also be related to our smaller sample size. Besides, we also discerned that CXCL9 and CXCL10 within the intestinal tissue manifested a pronounced elevation following FMT treatment, with CXCL9 being particularly prominent, which promoted the recruitment of T cells to migrate towards the tumor. The above results are consistent with those of previous studies [42, 44]. In conclusion, FMT not only recruited more immune cells into intestinal tissues by increasing levels of chemokines, but also activated more IFN-γ^+CD8^+ cells to secrete more cytokines, IFN-γ especially, for tumor killing by increasing the number of DC and T cells, and facilitating the DC-T crosstalk.

Most current studies focus on the effects of probiotics solely on intestinal bacteria, while our study delved deeper into the impact of FMT on the entire intestinal microbiota. Our findings also shed light on whether different chemotherapy doses affect the intestinal microbiota and offered new insights for optimizing FMT and chemoimmunotherapy. The primary limitations of our study were that we did not analyze the microbiota between normal and tumor-bearing mice, and we didn't examine the structure of the FMT solution before the experiment, which were very important when clarifying the mechanism of FMT. Further metabolite analyses were also needed to identify specific targeted metabolites of FMT. Besides, we did not monitor weight changes or adverse effects in mice undergoing chemotherapy and chemoimmunotherapy. Currently, we are conducting clinical studies (Trial registration NCT06405113, Registered 8 May 2024. Trial registration NCT06403111, Registered 7 May 2024.) to investigate whether the addition of FMT could enhance the efficacy of chemoimmunotherapy as the first-line treatment in gastric cancer and non-small cell lung cancer.

In all, no significant differences were observed in the effects of HDC and SDC on the intestinal microbiota.

FMT exhibited a beneficial impact on the intestinal microbiota and improved the efficacy of chemoimmunotherapy, possibly associated with the increase of immune cells and the modulation of related cytokines and chemokines.

Abbreviations

FMT	Fecal microbiota transplantation
LLC	Lewis lung cancer
ICIs	Immune checkpoint inhibitors
PD-L1	Programmed death-ligand 1
αPD- 1	Anti-PD- 1 antibody
Nab-PTX	Nanoparticle albumin-bound paclitaxel
HDC	Half-dose chemotherapy
SDC	Standard-dose chemotherapy
SDC + FMT	Standard-dose chemotherapy + FMT
SDC + PD1	Standard-dose chemotherapy + Anti-PD- 1 antibody
SDC + PD1 + FMT	Standard-dose chemotherapy +Anti-PD-1 antibody + FMT
PCR	Polymerase chain reaction
IHC	Immunohistochemistry
LEfSe	Linear discriminant analysis effect size
DAB	Diaminobenzidine
SD	Standard deviation
CFU	Colony-forming units
OTUs	Operational Taxonomic Units
IFN	Interferon
TNF	Tumor necrosis factor
CXCL9	Chemokine ligand 9
CXCL10	Chemokine ligand 10
SCFAs	Short chain fatty acids
CIC	Cancer-immunity cycle

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12865-025-00710-x.

Supplementary Material 1.

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Authors' contributions

HJ and LQ conceived of the presented idea. XM and LQ were mainly in charge of writing the manuscript. QG, JY, and CQ participated in the whole experiment as an assistant. HJ and QG supervised the findings of this work. All authors contributed to the article and approved the submitted version.

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Data availability

The 16S rRNA sequencing data generated during the current study are available in the BioProject database repository (BioProject ID: PRJNA1177666) in GenBank section, https://submit.ncbi.nlm.nih.gov/.

Declarations

Ethics approval and consent to participate

The collection procedure of fecal samples was developed in accordance with the guidelines that have been approved by the Ethics Committee of Changzhou No.2 People's Hospital, the Third Affiliated Hospital of Nanjing Medical University. All donors gave informed consent for their samples to be used in scientific studies. All animal procedures were carried out in strict adherence to the guidelines set by the Animal Care Committee of Zhejiang Baiyue Biological Technology Company Limited (No. ZJBYLA-IACUC-20230209).

Competing interests

The authors declare no competing interests.

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