Journal of Cancer Research and Clinical Oncology
https://doi.org/10.1007/s00432-022-04553-6

RESEARCH

Influence of neo-adjuvant radiotherapy on the intestinal microbiota of rectal cancer patients

Siyang Xu[1] · Qimei Lv[1] · Ning Zou[2] · Yuling Zhang[1] · Jiucheng Zhang[2] · Qing Tang[1] · Shan-Ho Chou[1] · Li Lu[3] · Jin He[1]

Received: 11 November 2022 / Accepted: 21 December 2022

Abstract

Purpose Neo-adjuvant radiotherapy (NART) is a widely used pre-surgery radiotherapy for rectal cancer patients. Although NART is effective in reducing tumor burden before surgery, it may cause dysbiosis of intestinal microbiota. The intestinal microbiota shapes tumor inflammatory environment and influences cancer progression. However, how NART remodels the microbiota and how the microbiota affects therapeutic efficacy has been largely elusive. This study aimed to reveal the details of how NART affects the intestinal microbiota in patients with rectal cancer.

Methods Rectal cancer patients who received NART were recruited into the study, and their healthy family members on the same diet served as controls. Stool samples from five rectal cancer patients (28 in total) and five healthy individuals (16 in total) were collected for intestinal microbiota analysis by 16S rRNA gene amplicon sequencing. Samples from patients were divided into earlier- and later-NART according to the number of NART.

Results NART did not significantly affect the α diversity of intestinal microbiota. However, the abundance of bacterial genera associated with cancer progression tended to decrease in later-NART patients. More importantly, a variety of oral pathogenic bacteria were enriched in the intestine of later-NART patients. NART also affected functional pathways associated with the microbiota in DNA repair, metabolism, and bacterial infection.

Conclusion NART significantly altered the microbiota composition and function in rectal cancer patients, and some oral pathogens were found to translocate to the intestine. This is the first report to study the effect of NART on intestinal microbiota in patients with rectal cancer, exploring the importance of intestinal microbiota during the process of NART.

Keywords Rectum · Radiotherapy · Intestinal microbiota · 16S rRNA gene high-throughput sequencing · Therapeutic efficacy

Introduction

Colorectal cancer (CRC) is a common malignant tumor of the digestive tract, which usually occurs at the junction of the rectum and sigmoid colon. CRC ranks second in mortality and third in incidence of all cancers worldwide, with one-third of CRCs occurring in the rectum (Sung et al. 2021). Multiple factors, including individual genetic background, lifestyle, environmental factors (including diet and drugs), and intestinal microbiota, have been found to affect tumorigenesis of CRC (Arthur et al. 2014; O'Keefe 2016; Zhao et al. 2022; Zhiqin et al. 2014). Compared with healthy people, the composition and structure of intestinal microbiota in CRC patients may be changed, which further affects the tumorigenesis of CRC. In 1997, Dove et al. (1997) found that germ-free mice had fewer adenomas than specific pathogen-free mice, providing initial evidence for an association

Siyang Xu and Qimei Lv have contributed equally to this work.

✉ Li Lu
luli117@163.com

✉ Jin He
hejin@mail.hzau.edu.cn

[1] State Key Laboratory of Agricultural Microbiology & Hubei Hongshan Laboratory, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

[2] Department of Radiation Oncology, Hubei Cancer Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430071, People's Republic of China

[3] Department of Gastrointestinal Surgery, Hubei Cancer Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430071, People's Republic of China

Published online: 19 January 2023

between CRC and the intestinal microbiota. Scanlan et al. (2008) found that the temporal stability of intestinal microbiota was significantly reduced, whereas the diversity was increased in CRC patients compared to healthy individuals. To further confirm the association between CRC and intestinal microbiota, Wong et al. (2017) transplanted fecal microbiota from CRC patients and healthy individuals into the intestines of mice, and found that intestinal polyps and hyperplasia were significantly increased in mice carrying fecal microbiota from CRC patients. This result confirms that intestinal microbiota is tightly associated with tumorigenesis of CRC. With the development of next-generation sequencing technology, a large number of studies have been carried out on the differences in the composition and structure of intestinal microbiota between patients with CRC and healthy individuals. These findings also indicate that the structural dysbiosis of the intestinal microbiota plays a direct or indirect role in the tumorigenesis of CRC (Sheng et al. 2019; Zhang et al. 2019a, b, Zorron Cheng Tao Pu et al. 2020). Twelve species of intestinal bacteria, including *Bacteroides fragilis* (Thomas et al. 2016), *Campylobacter jejuni* (Pons et al. 2019), and *Fusobacterium nucleatum* (Rubinstein et al. 2013), were found to be enriched in tumor tissues of CRC patients (Xu et al. 2020), and play an important role in the CRC tumorigenesis. However, a range of issues such as whether changes in intestinal microbiota occur before or after CRC, whether and how changes in intestinal microbiota affect the outcomes of CRC patients, or whether these potential "carcinogens" play a role in the molecular mechanism of CRC tumorigenesis remain to be addressed urgently.

Among the most commonly used clinical therapies for rectal cancer, radiotherapy is a mature anti-tumor therapeutic method, and more than 50% of newly diagnosed cancer patients require radiotherapy in the clinic (Jaffray 2012). Radiotherapy can kill fast-dividing cancer cells by inducing DNA damage, high-dose radiotherapy also can induce immunogenic cell death through cross-sensitization triggered by tumor-associated antigen, thereby activating the body's adaptive anti-tumor immune response to assist therapy of tumors (Apetoh et al. 2007; Barker et al. 2015; Demaria et al. 2016; Herrera et al. 2017). However, radiotherapy also has certain side effects, such as ionizing radiation can cause intestinal epithelial ulceration by activating the coagulation system and induce intestinal inflammation by exposing the underlying tissue to the intestinal microbiota (Taghinezhad et al. 2021; Zhang et al. 2019a, b). Microbial metabolites detected in intestinal-associated lymphoid tissue and peripheral systems have been shown to control the initiation and progression of various diseases (Amoroso et al. 2020; Dzutsev, et al. 2015; Elinav et al. 2019; Viaud et al. 2013). Furthermore, the intestinal microbiota affects the function of T cells and other immune cell subsets (Fung et al. 2017; Ivanov et al. 2022). Therefore, intestinal microbiota dysbiosis is bound to affect the anti-tumor effect of radiotherapy, thereby affecting the therapeutic efficacy of rectal cancer.

Traditional radiotherapy is generally acted as adjuvant therapy after surgery, but patients still face the risk of cancer cell metastasis during surgical therapy. Therefore, the importance of neo-adjuvant radiotherapy (NART) is highlighted (Cedermark et al. 1997). NART is radiotherapy given before local therapy such as surgery to shrink tumor tissue and kill metastatic cancer cells, thereby facilitates subsequent surgery and other therapies. At present, how NART changes the intestinal microbiota of rectal cancer patients and how the altered intestinal microbiota affects the therapeutic efficacy of NART remain to be studied.

The study aimed to explore changes in intestinal microbiota in rectal cancer patients treated with NART, and to explore the potential role of these changes in subsequent therapeutic efficacy. Our results showed that NART did not significantly alter the α diversity of intestinal microbiota in rectal cancer patients. However, we found a downward trend in the abundance of bacterial genera known to be associated with cancer progression in patients receiving more NART. More importantly, we found that a variety of oral pathogens emerged in the intestine of later-NART patients, which may accelerate cancer progression. We speculated that these oral pathogens reach the intestine through the "oral-blood-intestine" pathway (Abed et al. 2020). With the progress of NART, 3 microbiota-associated functional pathways including non-homologous end-joining significantly increased in intestinal microbiota of patients with rectal cancer, while four function pathways including limonene and pinene degradation decreased significantly. This study is the first to report changes in the intestinal microbiota in rectal cancer patients receiving NART and discusses the potential impact of these changes on rectal cancer therapy. Our findings revealed the importance of the intestinal microbiota during NART and provided insights for improving therapeutic efficacy of rectal cancer patients.

Materials and Methods

16S rRNA gene sequencing sample collection

The objects of this study are rectal cancer patients admitted to Hubei Cancer Hospital who needed conventional NART. The therapy cycle is generally 5–6 weeks, the radiotherapy dose is 50 Gy, divided into 25 fractions (2.0 Gy per time), and NART was performed five times per week. We used a fecal sampling tube to collect feces from rectal cancer patients receiving NART. Three technique replicates with

2.0–5.0 g for each tube were collected at a time (one tube for follow-up sequencing and two for archiving), which were immediately frozen and stored at − 80 °C for later use. Samples from healthy family members (children or spouses) of the corresponding rectal cancer patients were collected using the same sampling method. A total of 44 samples were collected for 16S rRNA gene sequencing (including 28 samples from 5 rectal cancer patients and 16 samples from 5 healthy individuals). See Supplemental Table 1 for detailed sample descriptions. Samples from rectal cancer patients were divided into earlier- and later-NART according to the number of NART that patient received (for earlier-NART, the number of NARTs is less than 15, whereas for later-NART, the number of NARTs is more than 15).

High-throughput sequencing of 16S rRNA gene of intestinal microbiota

For high-throughput 16S rRNA gene sequencing of intestinal microbiota, stool samples were thawed and centrifuged at 13,000 × *g* for 2 min, and then, the supernatant was removed. Total bacterial DNA in stool samples was extracted using the QIAamp DNA Mini Kit (QIAGEN, Stockach, Germany), and the extracted genomic DNA was detected using 1% agarose gel electrophoresis. Specific primers with barcode (338F: 5′-ACTCCTACGGGAGGCAGCAG-3′; 806R: 5′-GGACTACHVGGGTWTCTAAT-3′) were synthesized according to the variable region of bacterial 16S rRNA gene V3-V4; and the target fragment was amplified from each sample using TransStart Fastpfu DNA Polymerase (TransGen Biotech, Beijing, China). The correctness of the amplified product was confirmed by 2% agarose gel electrophoresis. The PCR products was purified with the AxyPrep-DNA Gel Recovery Kit (Axygen, Union City, Californian, USA), and quantified with QuantiFluor™-ST Kit (Promega, Madison, Wisconsin, USA). Purified amplicons were pooled in equimolar amounts and sequenced in paired-end mode on an Illumina MiSeq PE300 platform (Illumina, San Diego, Californian, USA).

Bioinformatics analysis of sequencing data

Raw fastq files were de-multiplexed using an in-house Perl script, and then quality-filtered by fastp version 0.19.6 (Chen et al. 2018) and merged by FLASH version 1.2.7 (Magoč and Salzberg 2011) using the following procedures. (i) 300 bp reads were truncated at any site receiving an average quality score of < 20 over a sliding window of 50 bp, and truncated reads shorter than 50 bp were discarded, as were reads containing ambiguous characters. (ii) Reads were assembled according to overlapping sequences (overlapping sequence > 10 bp, mismatch ratio < 0.2), while unassembled reads were discarded. (iii) Different samples were distinguished according to barcode. Sequences were clustered into operational taxonomic units (OTUs) using UPARSE 7.1 (Edgar 2013) with a sequence similarity of 97%. The number of sequences per sample was rarefied according to the minimum number of sample sequences (33,037 reads).

The taxonomy of each OTU representative sequence was analyzed by the RDP Classifier version 2.2 (Wang et al. 2007) against the Silva v138 database using a confidence

Table 1 Bacterial genera with significant differences in the intestinal microbiota between the earlier- and later-NART patients

Phylum/class	Order	Family	Genus
Actinobacteria			
Actinobacteria	*Bifidobacteriales*↓	*Bifidobacteriaceae*↓	*Bifidobacterium*↓
			Parascardovia↑
Proteobacteria			
Gammaproteobacteria	*Enterobacteriales*	*Enterobacteriaceae*	*Enterobacter*↓
			Citrobacter↓
Firmicutes			
Tissierellia	*Tissierellales*	*Peptoniphilaceae*	*Peptoniphilus*↓
Negativicutes	*Veillonellales*	*Veillonellaceae*	*Dialister*↓
Clostridia	*Eubacteriales*	*Peptostreptococcaceae*	*Intestinibacter*↓
		Lachnospiraceae	*Shuttleworthia*↑
		Lachnospiraceae	*Lachnoanaerobaculum*↑
		Oscillospiraceae	*Anaerotruncus*↑
Bacilli	*Bacillales*↑		
	Lactobacillales	*Streptococcaceae*	*Streptococcus*↑
		Enterococcaceae↓	*Enterococcus*↓

*: ↑ indicates increased abundance in the intestinal microbiota of the later-NART patients relative to the earlier-NART patients; ↓indicates reduced abundance in the intestinal microbiota of the later-NART relative to the earlier-NART patients

threshold of 0.7. Metagenomic functions were predicted based on OTUs by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt).

Bioinformatic analysis of intestinal microbiota was carried out using the Majorbio Cloud platform (https://cloud.majorbio.com). α diversity indices (Sob, Shannon, Simpson, ACE, Coverage) were calculated with Mothur v1.30.1 (Schloss et al. 2009) and β diversity indices calculated by principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) based on unweighted unifrac using the Vegan v2.5–3 package. Linear discriminant analysis (LDA) effect sizes (LEfSe) (Segata, et al. 2011) were used to identify the significantly enriched taxa (phylum to genera) of bacteria in different groups (LDA score > 3.5). Data analysis was performed using IBM SPSS software, and Kruskal–Wallis test was used to determine the difference in three or more groups, and the Student's t test for two groups.

Results

Effects of NART on the diversity of intestinal microbiota in rectal cancer patients

To reveal if NART affects the intestinal microbiota diversity of rectal cancer patients, the richness (Sobs index, Chao index, and ACE indices), diversity (Shannon, and Simpson indices), and coverage (Coverage index) of microbiota in different samples were evaluated by diversity to explore the effect of NART on the diversity of intestinal microbiota in rectal cancer patients. The results showed that there was no significant difference between the different groups in diversity (Fig. 1), indicating that NART does not change intestinal microbiota to a large extent.

Given that the diversity of the human intestinal microbiota can be affected by human genetics, intestinal microenvironment, and host immunity (Cogdill et al. 2018), we therefore hypothesize that NART affects the composition and structure of intestinal microbiota. By performing community composition analysis, we found that the most abundant bacterial phyla in all human stool samples were *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinomycetes*, and *Verrucomicrobia*, while the abundances of other bacterial phyla were all below 1% (Fig. 2A). In addition, we found that at the genus level, the highest abundance in all samples was *Escherichia/Shigella*, *Bacteroides*, *Blautia*, *Lactobacillus, Enterobacter*, *Lysinibacillus*, and *Agathobacter* (Fig. 2B). We further used PCoA and NMDS to explore the differences in intestinal microbiota in different groups (β diversity). Both analyses showed that the intestinal microbiota was relatively consistent between the earlier- and later-NART patients, but was significantly different from those of healthy individuals (Fig. 2C and D).

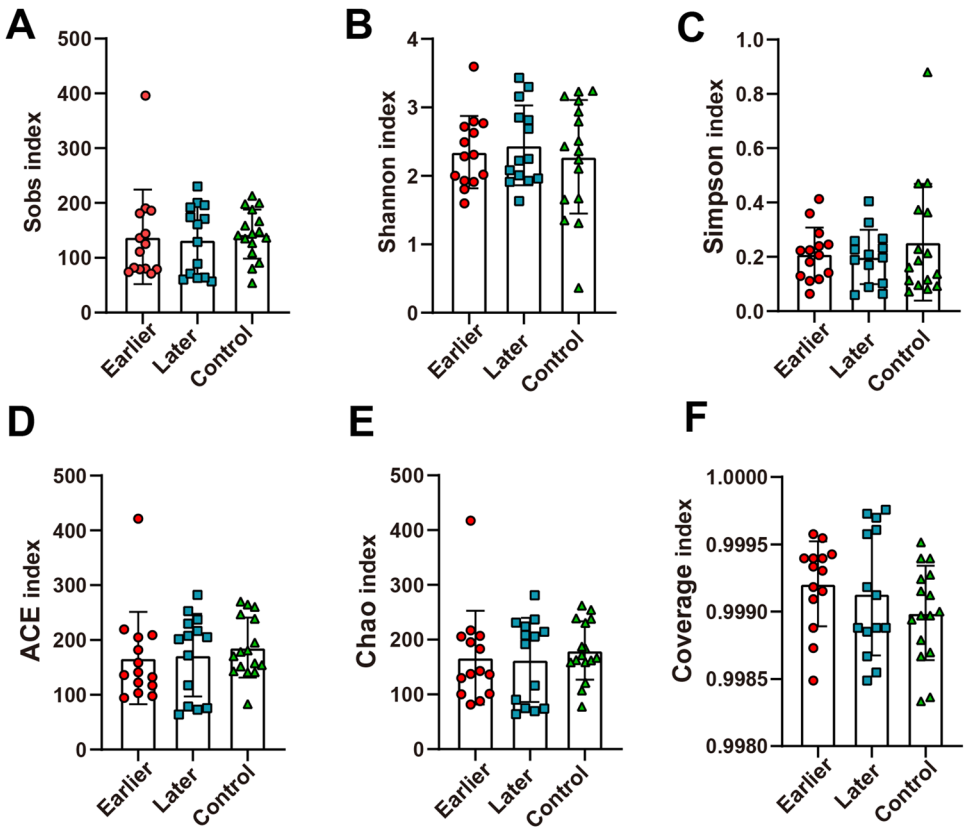

Fig. 1 Effects of NART on the α diversity of intestinal microbiota in rectal cancer patients. All samples were tested for α diversity at the OTU level. **A** Sob index. **B** Shannon index. **C** Simpson index. **D** ACE index. **E** Chao index. **F** Coverage index. The "Earlier" ($n = 14$) and "Later" ($n = 14$) represent the samples from earlier- and later-NART patients, respectively. The "Control" ($n = 16$) represents the samples from healthy family members of corresponding rectal cancer patients. The values represent the mean ± standard error of mean. Statistical analysis was performed using Kruskal–Wallis test. None of the above diversity indices had any significant difference ($p > 0.05$)

Differences in intestinal microbiota between the earlier- and later-NART patients

We used LEfSe analysis to explore the differences in the intestinal microbiota of the earlier- and later-NART patients. In general, the bacterial genera with significant changes in abundance after receiving NART were mainly distributed in the phyla of *Actinobacteria*, *Proteobacteria*, and *Firmicutes* (Table 1, Fig. 3A).

We found that the genera *Enterobacter*, *Bifidobacterium*, *Enterococcus*, *Citrobacter*, *Peptoniphilus*, *Dialister*, and *Intestinibacter* were reduced in the intestinal microbiota of the later-NART patients (Fig. 3A–G). Specifically, the abundance of the pro-inflammatory genera *Enterococcus*, *Citrobacter*, and *Dialister* in the intestinal microbiota of rectal cancer patients declined after NART, exhibiting comparable abundance with healthy individuals (Fig. 3D, E and H). Furthermore, the abundance of *Bifidobacterium* in earlier-NART patients was higher than in both the later-NART and healthy individuals (Fig. 3C). *Bifidobacterium* was known as a probiotic; however, increasing evidence has shown that *Bifidobacterium* is enriched in tumor tissue of CRC patients (Hasan et al. 2022). These results indicate that NART is likely beneficial in limiting intestinal inflammation

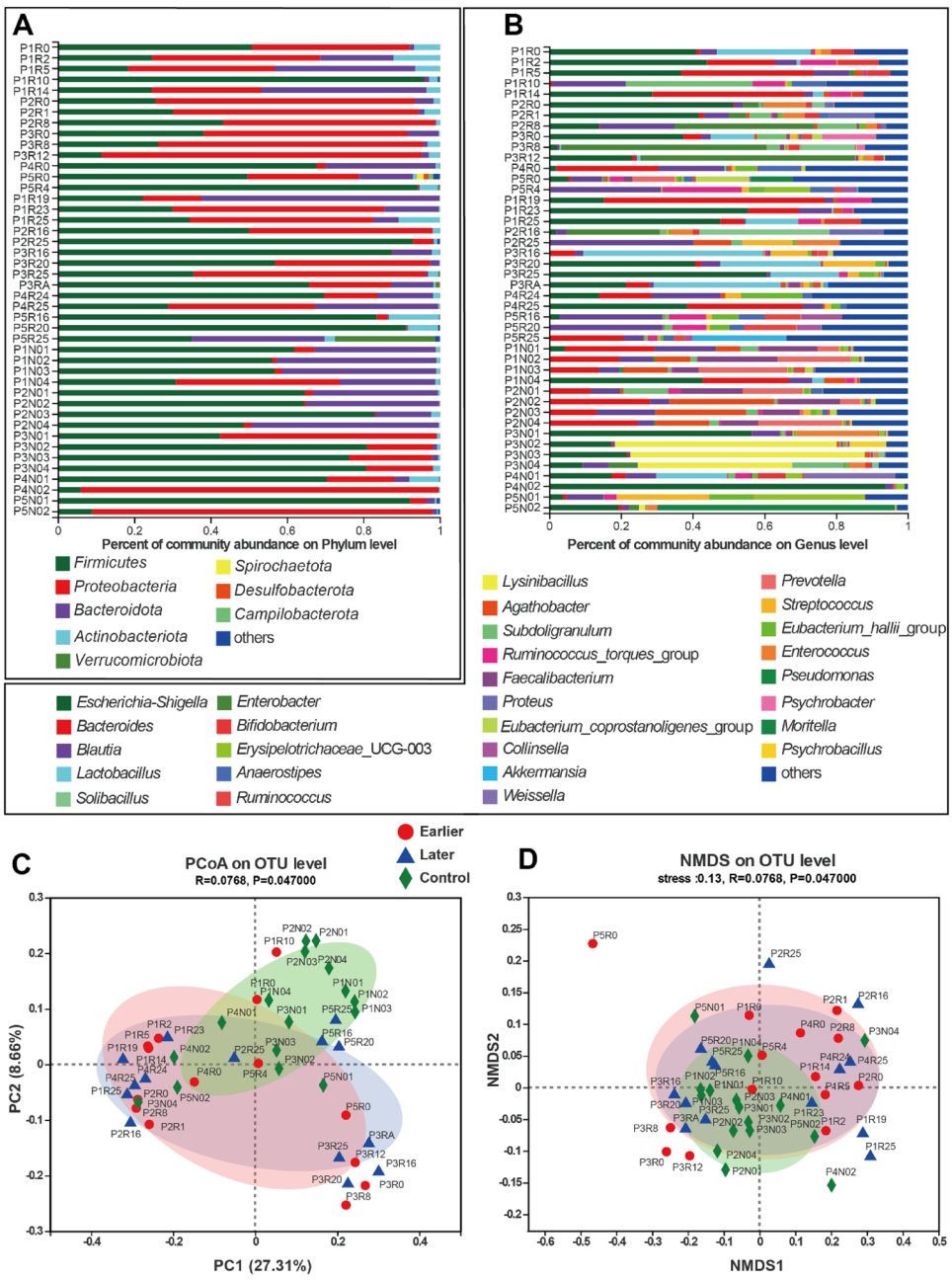

Fig. 2 Analysis of community composition and β diversity of intestinal microbiota in rectal cancer patients received NART. **A** Analysis of the phylum-level community composition. **B** Analysis of the genus-level community composition. **C** PCoA analysis of intestinal microbiota in different groups based on unweighted uniFrac distance (on OTU). **D** NMDS analysis of intestinal microbiota in different groups based on unweighted uniFrac distance (on OTU). The "Earlier" (red solid circle) and "Later" (blue solid triangle) represent the samples from earlier- and later-NART patients, and the "Control" (green solid diamond) represents the samples from healthy family members of corresponding rectal cancer patients. *Sample description: samples from patients are labeled in the format of "PnRm", "Pn": patient ID; "Rm": number of NART, "RA": 2 months after the patient completes the course of NART. Samples from healthy individuals are labeled in the format of "PnNm", "Nm": sample number. Please refer to Supplemental Table 1 for detailed sample description

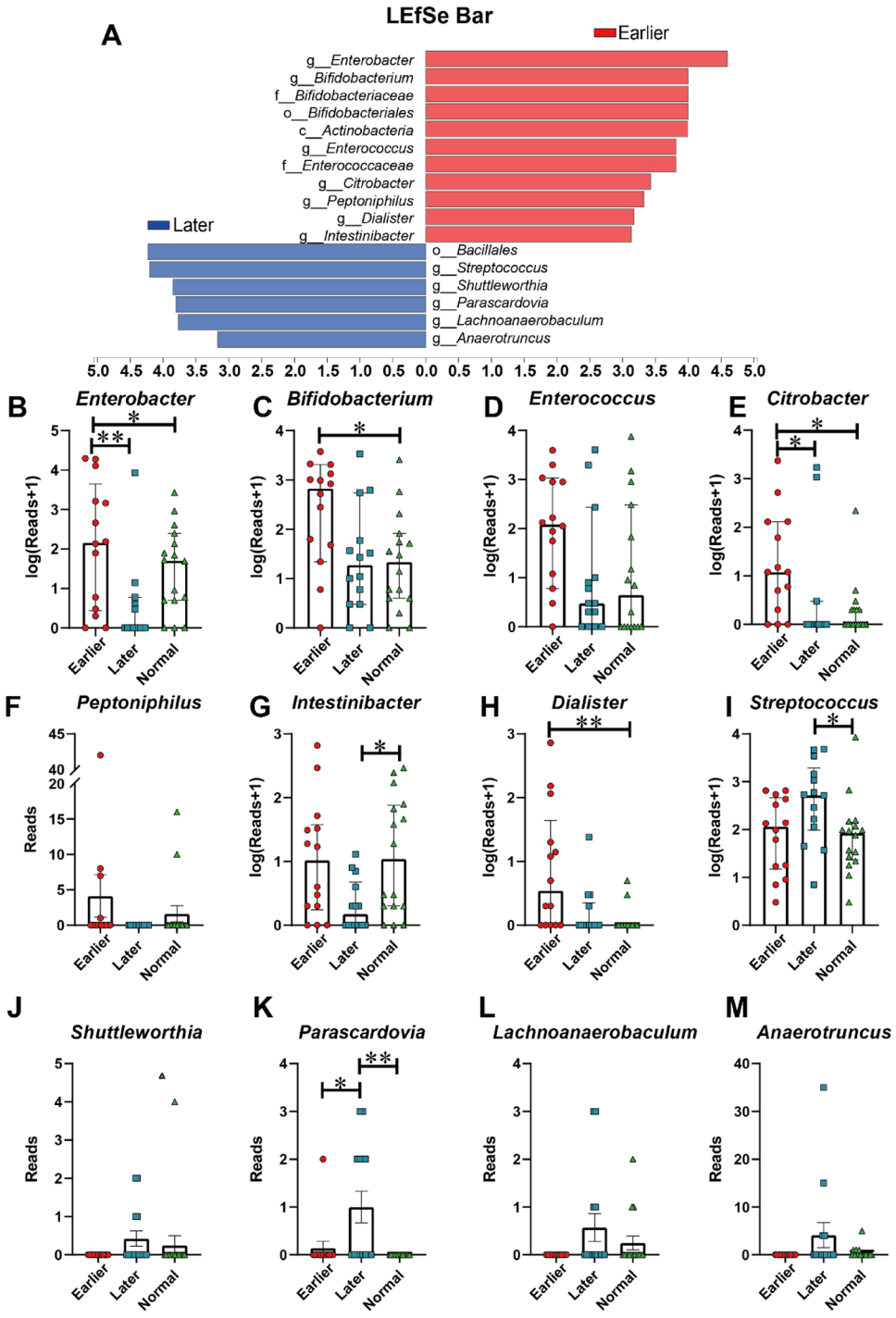

Fig. 3 Bacterial genera with significant differences in intestinal microbiota between the earlier- and later-NART patients. **A** LEfSe analysis of differential abundance of intestinal microbiota. Significant differences were found in intestinal microbiota between the earlier- and later-NART patients**.** All-against-all strategy was used for LEfSe. The "Earlier" and "Later" represent the samples from earlier- and later-NART patients. **B**–**M** Bar chart of bacterial genera with significant differences between the earlier- and later-NART patients by LEfSe analysis. The "Control" represents the samples from healthy family members of corresponding rectal cancer patients. The values of **B, C, D, E, G, H, I** represent the median with interquartile range. The values of **F, J, K, L, M** represent the mean ± standard error of mean. Statistical analysis was performed using Kruskal–Wallis test, *: $p \leq 0.05$, **: $p \leq 0.01$

caused by resident intestinal pro-inflammatory bacteria. Furthermore, the commensal bacteria *Enterobacter*, *Peptoniphilus*, and *Intestinibacter* also exhibited a dramatic decrease in later-NART patients when compared with the earlier-NART patients and healthy individuals (Fig. 3B, F and G). Given that genera *Enterobacter*, *Peptoniphilus*, and *Intestinibacter* are naturally abundant in healthy individuals, abolishing intestinal *Peptoniphilus* or *Intestinibacter* may lead to an unbalanced intestinal microbiota; however, there is currently no report on the association between *Peptoniphilus* or *Intestinibacter* with rectal cancer pathology.

On the other hand, we found that the abundances of genera *Streptococcus*, *Shuttleworthia*, *Parascardovia*, *Lachnoanaerobaculum*, and *Anaerotruncus* in the intestinal microbiota of the later-NART patients were increased compared with those of the earlier-NART patients (Table 1,

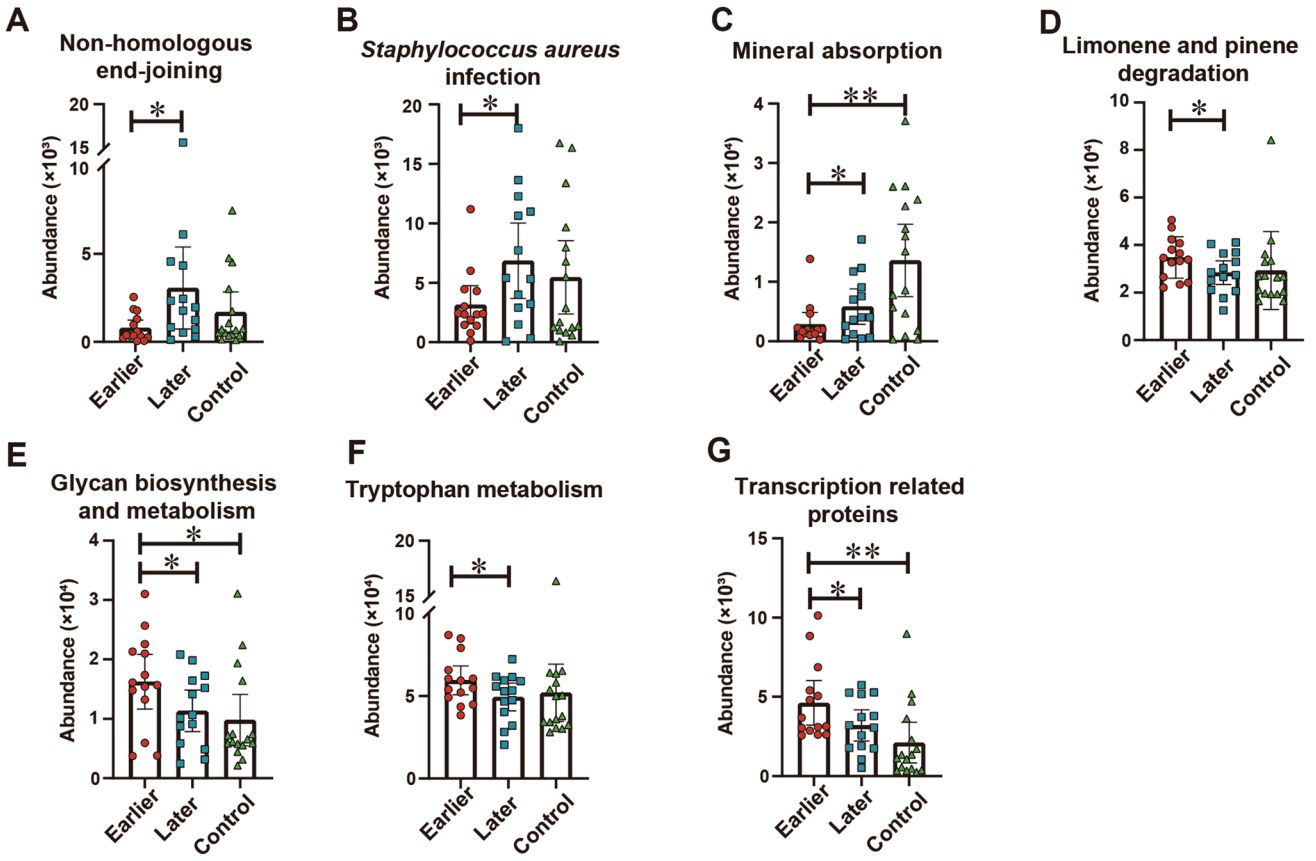

Fig. 4 Differences in intestinal microbial functions between the earlier- and later-NART patients based on PICRUSt analysis. **A–G** represent bacterial phycological functions that changed significantly after NART. The "Earlier" and "Later" represent the samples from earlier- and later-NART patients. The values represent the median with interquartile range. Statistical analysis was performed using a Student's t test. *: $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$

Fig. 3A and I–M). *Streptococcus* and *Anaerotruncus*, which display a strong association with CRC, exhibited a higher abundance in later-NART patients compared with both the earlier-NART patients and healthy individuals (Fig. 3I). In addition, *Shuttleworthia*, *Parascardovia*, and *Lachnoanaerobaculum*, which have been widely known as oral pathogens (Chen et al. 2021; Downes et al. 2002; Ida et al. 2022; Lim et al. 2019; Liu et al. 2021), showed a low abundance in the intestinal microbiota of healthy individuals and most earlier-NART patients, whereas showed a robust increase in the intestine of the later-NART patients (Fig. 3J–L). These data indicate that NART might lead to the translocation of oral microbes into the intestine.

Taken together, NART is associated with the elevated abundance of pathogens in the intestinal microbiota, which may exacerbate intestinal inflammation and dampen the therapeutic efficacy of CRC.

Functional properties predicted by PICRUSt

To further investigate whether NART leads to changes in microbial function in rectal cancer patients, a PICRUSt analysis was performed (Fig. 4). We found that non-homologous end-joining, *Staphylococcus aureus* infection, and mineral absorption in the intestinal microbiota of the later-NART patients were significantly higher than those of the earlier-NART patients ($p < 0.05$). We speculated that NART damages intestinal bacterial DNA and thereby activates non-homologous end-joining DNA repair (Fig. 4A), which concurrently promotes intestinal infection and elevates bacterial absorption of minerals (Fig. 4B and C). Meanwhile, limonene and pinene degradation (Fig. 4D), glycan biosynthesis and metabolism (Fig. 4E), tryptophan metabolism (Fig. 4F), and transcription-related proteins (Fig. 4G) were also significantly decreased ($p < 0.05$). These results suggested that NART reduced the viability and metabolism of intestinal microbiota in rectal cancer patients.

Furthermore, to reveal the association between bacterial abundance with microbial functional differences (Fig. 5),

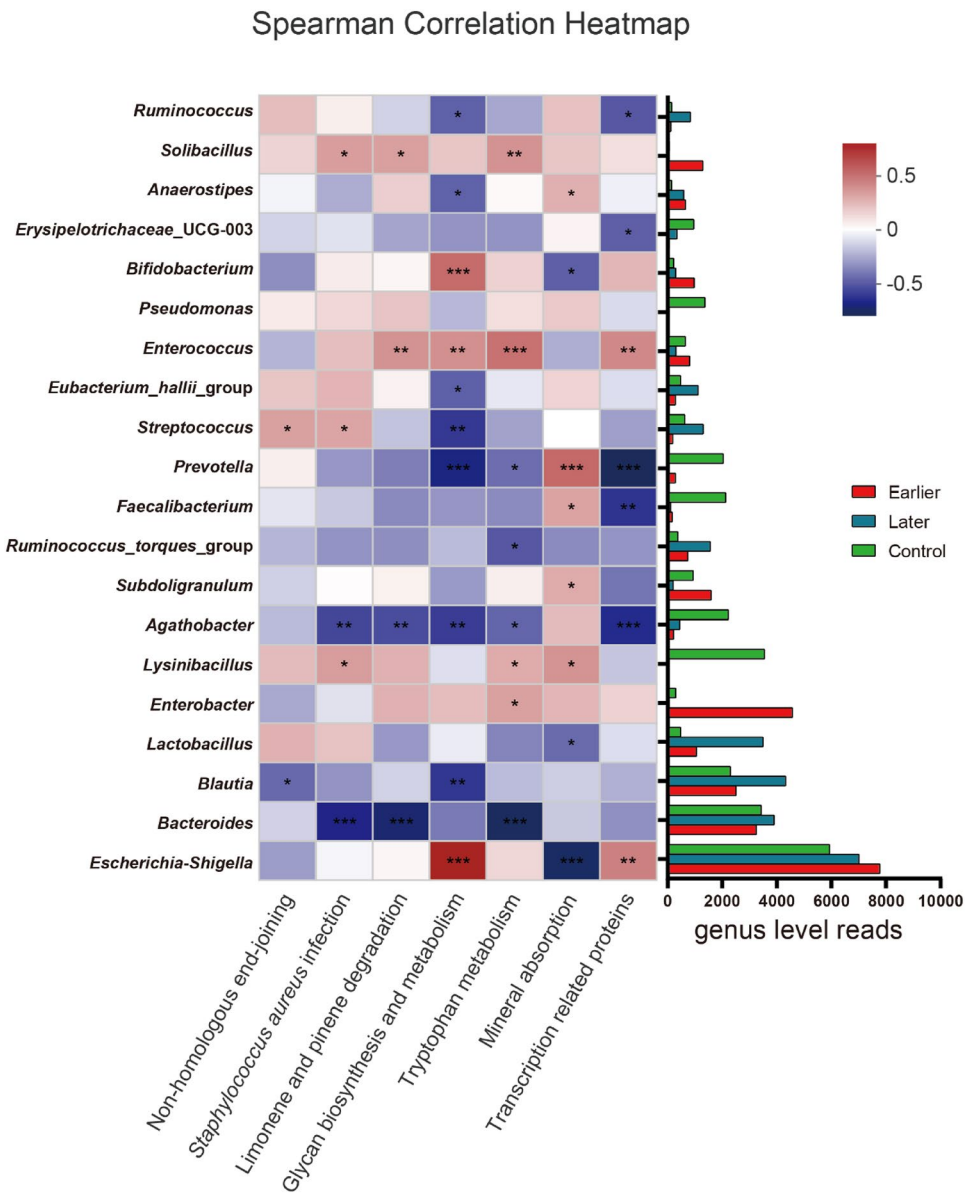

Fig. 5 Association analysis of genus-level bacterial abundance with microbial function. Using Spearman to analyze the association of the top 20 bacterial genera with the function of the intestinal microbiota; the right panel of the image shows the reads of the top 20 bacterial genera in different groups. The "Earlier" and "Later" represent the samples from earlier- and later-NART patients, and the "Control" represents the samples from healthy family members of corresponding rectal cancer patients. *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$

the PICRUSt analysis data were combined with microbiota abundance. Our results showed that the most abundant bacteria *Escherichia-Shigella* were positively correlated with glycan biosynthesis and metabolism, and transcription-related proteins, while negatively correlated with mineral absorption (Fig. 5).

Discussion

NART affects the abundance of pathogenic bacteria in the intestines

The intestinal microbiota is closely related to host health. Understanding the function of intestinal microbiota in host disease and the mechanisms of host–microbiota interactions are the basis for future human health management and therapeutic drug development. Specifically, understanding how NART shapes microbiota is critical for the radiotherapy efficacy of CRC. In this study, we compared the composition of intestinal microbiota in rectal cancer patients at different NART stages. NART is likely to have both beneficial and detrimental effects on the host. We found that the abundance of resident intestinal bacteria *Citrobacter* and *Dialister* was higher in the intestinal microbiota of CRC patients than those of the healthy individuals, whereas NART decreased the abundance of both genera. *Citrobacter* and *Dialister* are known as potential biomarkers for the diagnosis of CRC (Kharrat et al. 2019), indicating that NART is potentially beneficial to the therapeutic efficacy. On the other hand,

NART increased the abundance of several bacterial pathogens, including *Streptococcus* and *Anaerotruncus*, in the CRC intestines. Studies have found that many *Streptococci* are present in CRC patients, including *S. gallolyticus* subsp. *Gallolyticus* (Butt et al. 2016; Périchon et al. 2022), *S. gallolyticus* subsp. *Pasteuranus* (Agnes et al. 2021), and *S. bovis* (Deng et al. 2020). Among these species, *S. bovis* is positively associated with colorectal cancer and adenoma (Abdulamir et al. 2011). *Anaerotruncus* is a potential biomarker for predicting CRC recurrence as it is associated with CRC recurrence (Huo et al. 2022). As a result, the elevation of *Streptococcus* and *Anaerotruncus* in the intestinal microbiota is likely unfavorable for later-NART patients.

In addition, we also observed decreased abundance of *Bifidobacterium* and *Enterococcus*. However, whether the decreased abundance of both bacteria is beneficial or detrimental to the host requires further study. *Bifidobacterium* is known as a probiotic and has anti-cancer effects (Asadollahi et al. 2020; Fahmy et al. 2019; Wang et al.2020); however, *B. pseudocatenulatum* is enriched in tumor tissue of CRC patients (Hasan et al. 2022). Therefore, specific species need to be identified to reveal the function of *Bifidobacterium* in CRC. The effect of *Enterococcus* on cancer progression is also controversial. The human intestine is dominated by *E. faecalis* and *E. faecium* (de Almeida et al. 2018). *E. faecalis* has anti-inflammatory activity and is a potential probiotic (Are et al. 2008; Wang et al. 2014), but other studies have shown that *E. faecalis* produces metalloproteinases that disrupt the intestinal epithelial barrier and induce an inflammatory response (Steck et al. 2011).

Our study also revealed an unappreciated link between oral pathogens with intestinal microbiota in CRC. We observed that the abundance of *Shuttleworthia*, *Parascardovia*, and *Lachnoanaerobaculum* dramatically increased after NART. In fact, *Shuttleworthia*, *Parascardovia*, and *Lachnoanaerobaculum* have been extensively known as oral bacteria (Chen et al. 2021; Downes et al. 2002; Ida et al. 2022; Lim et al. 2019; Liu et al. 2021) and are associated with diseases such as systemic lupus erythematosus (Lim et al. 2019), type 2 diabetes (Liu et al. 2021), dental caries (Chalmers et al. 2015), and gingivitis (Lim et al. 2019). Although these bacteria were absent in the healthy intestine, our results suggested that long-term NART might lead to the translocation of oral microbes into the intestine, which might affect therapeutic efficacy of NART. Previous studies have revealed that the oral bacterium *F. nucleatum* is frequently detected in the intestine of CRC patients with periodontal disease (Komiya et al. 2019). Subsequent studies have revealed that periodontal *F. nucleatum* can enter the bloodstream and colonize intestines when tumor disrupts the intestinal barrier, therefore accelerating tumorigenesis (Abed et al. 2020; Dong et al. 2021). Our hypothesis is also supported by the study from Dong et al. (2021), which found that oral microbiota affects the therapeutic efficacy of NART in CRC mouse model. Previous studies and our study all suggest that rectal cancer patients need to control their oral microbiota when receiving NART, so as to prevent the translocation of oral pathogens to the intestine to affect the therapeutic effect.

The effects of microbiota on the radiotherapy therapeutic efficacy of CRC

The intestinal microbiota regulates the tumorigenesis of cancer through various pathways. However, how radiotherapy affects intestinal microbiota and subsequent tumorigenesis remains unclear. In our study, we observed that NART affects the metabolism of microbiota, which could potentially affect the tumor microenvironment and therapeutic efficacy. Specifically, a possible relationship between intestinal microbiota, radiotherapy, and cancer could be proposed by the fact that intestinal microbiota might metabolize polysaccharides that are difficult to use for the human body into a variety of short-chain fatty acids, among which valerianic acid (VA) has been shown to significantly prevent radiation damage caused by radiation (Li et al. 2020). In studies using the mouse models, VA supplementation improves survival in radiated mice, protects hematopoietic organs and intestinal epithelial integrity, and improves gastrointestinal function. Mechanistically, VA protects against radiation damage by acting on keratin 1 (KRT1), attenuates radiation enteritis, and prevents dextran sulfate sodium (DSS)-induced colitis in mice (Li et al. 2020). Since NART reduces the metabolic activity of the intestinal microbiota, we also suggest that these patients supplement probiotics and prebiotics to improve the metabolic activity of intestinal microbiota.

Future directions of microbiota study with NART

In the future, multiple omics approaches should be used to explore the function of intestinal microbiota in patients who receive NART, and systematically analyze the influence of patients' intestinal microbiota on the therapeutic efficacy of NART. Radiation therapy can lead to changes in the intestinal microbiota of patients, but whether these changes are beneficial or detrimental still needs further study. By analyzing altered bacteria and using in vitro or in vivo experiments to explore their ability to affect cancer, we will be able to draw corresponding conclusions and explore the potential application value of intestinal bacteria in the therapy of cancer. For example, our results indicated that some oral pathogenic bacteria could translocate into the intestines of rectal cancer patients after NART. We could then purposefully modify the oral microbiota of rectal cancer patients and prevent the impact of oral pathogens on rectal cancer. According to the results of microbiota changes after radiotherapy,

we could purposefully repress the adverse effects of these "harmful bacteria" and avoid the occurrence of side effects.

Conclusions

Although NART has little impact on the α diversity of the intestinal microbiota in rectal cancer patients, it can significantly change the abundance of some bacteria and affect the function of intestinal microbiota simultaneously. Intestinal tissue injury caused by NART would also increase the possibility that oral pathogenic bacteria translocated to the intestines, which may accelerate the development of rectal cancer, and we recommend that patients who after NART need to pay attention to their oral hygiene to eliminate oral pathogenic bacteria. Simultaneously, we found that some traits, such as non-homologous end-joining, *S. aureus* infection, and mineral absorption, were significantly increased, while limonene and pinene degradation, glycan biosynthesis and metabolism, tryptophan metabolism, and transcription-related proteins were significantly reduced in the later-NART patients. We suggest that these patients regulate the intestinal microbiota and improve their beneficial metabolic activities by probiotics.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00432-022-04553-6.

Acknowledgements This research was funded by the Health Commission of Hubei Province (WJ2021M193), the Biomedical Center of Hubei Cancer Hospital (2022SWZX13), Natural Science Foundation of Hubei Province (2016CFB405), Health and Family Planning Commission of Hubei Province (WJ2017M144), and the Fundamental Research Funds for the Central Universities (2662017PY112).

Author contributions SX: data curation; formal analysis; investigation; methodology; visualization; roles/writing—original draft. QL: data curation; formal analysis; investigation; methodology; resources; writing—review and editing. NZ: conceptualization; formal analysis; funding acquisition; resources; writing—review and editing. YZ: investigation; methodology; writing—review and editing. JZ: conceptualization; funding acquisition; project administration; resources; writing—review and editing. QT: conceptualization; data curation; formal analysis; project administration; writing—review and editing. S-HC: writing—review and editing. LL: funding acquisition; formal analysis; project administration; writing—review and editing. JH: conceptualization; formal analysis; funding acquisition; project administration; writing—review & editing. All authors read and approved the final manuscript.

Funding This research was funded by the Health Commission of Hubei Province (WJ2021M193), the Biomedical Center of Hubei Cancer Hospital (2022SWZX13), Natural Science Foundation of Hubei Province (2016CFB405), Health and Family Planning Commission of Hubei Province (WJ2017M144), and the Fundamental Research Funds for the Central Universities (2662017PY112).

Data availability Contact the corresponding author for the raw sequencing reads.

Declarations

Conflict of interest The authors declare no conflict of interest.

Institutional review board statement This is certify that the design and methods of the research are in accordance with the requirements of related regulations and procedures as well as the ethical principles, and approved by the Institutional Review Board of Hubei Cancer Hospital, project associated code LLHBCH2022YN-044.

Informed consent Informed consent was obtained from all subjects involved in the study.

References

Abdulamir AS, Hafidh RR, Abu BF (2011) The association of *Streptococcus bovis*/*gallolyticus* with colorectal tumors: the nature and the underlying mechanisms of its etiological role. J Exp Clin Cancer Res 30:11

Abed J, Maalouf N, Manson AL, Earl AM, Parhi L, Emgård JEM et al (2020) Colon cancer-associated *Fusobacterium nucleatum* may originate from the oral cavity and reach colon tumors via the circulatory system. Front Cell Infect Microbiol 10:400

Agnes A, Biondi A, Belia F, Di Giambenedetto S, Addolorato G, Antonelli M et al (2021) Association between colorectal cancer and *Streptococcus gallolyticus* subsp. *pasteuranus* (former *S. bovis*) endocarditis: clinical relevance and cues for microbiota science. Case report and review of the literature. Eur Rev Med Pharmacol Sci 25:480–486

Amoroso C, Perillo F, Strati F, Fantini MC, Caprioli F, Facciotti F (2020) The role of gut microbiota biomodulators on mucosal immunity and intestinal inflammation. Cells 9:1234

Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A et al (2007) Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 13:1050–1059

Are A, Aronsson L, Wang S, Greicius G, Lee YK, Gustafsson JA et al (2008) *Enterococcus faecalis* from newborn babies regulate endogenous PPARgamma activity and IL-10 levels in colonic epithelial cells. Proc Natl Acad Sci USA 105:1943–1948

Arthur JC, Gharaibeh RZ, Mühlbauer M, Perez-Chanona E, Uronis JM, McCafferty J et al (2014) Microbial genomic analysis reveals the essential role of inflammation in bacteria-induced colorectal cancer. Nat Commun 5:4724

Asadollahi P, Ghanavati R, Rohani M, Razavi S, Esghaei M, Talebi M (2020) Anti-cancer effects of *Bifidobacterium* species in colon cancer cells and a mouse model of carcinogenesis. PLoS One 15:e0232930

Barker HE, Paget JT, Khan AA, Harrington KJ (2015) The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence. Nat Rev Cancer 15:409–425

Butt J, Romero-Hernández B, Pérez-Gómez B, Willhauck-Fleckenstein M, Holzinger D, Martin V et al (2016) Association of *Streptococcus gallolyticus* subspecies *gallolyticus* with colorectal cancer:serological evidence. Int J Cancer 138:1670–1679

Cedermark B, Dahlberg M, Glimelius B, Påhlman L, Rutqvist LE, Wilking N (1997) Improved survival with preoperative radiotherapy in resectable rectal cancer. N Engl J Med 336:980–987

Chalmers NI, Oh K, Hughes CV, Pradhan N, Kanasi E, Ehrlich Y et al (2015) Pulp and plaque microbiotas of children with severe early childhood caries. J Oral Microbiol 7:25951

Chen S, Zhou Y, Chen Y, Gu J (2018) Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890

Chen BD, Jia XM, Xu JY, Zhao LD, Ji JY, Wu BX et al (2021) An autoimmunogenic and proinflammatory profile defined by the gut microbiota of patients with untreated systemic lupus erythematosus. Arthritis Rheumatol 73:232–243

Cogdill AP, Gaudreau PO, Arora R, Gopalakrishnan V, Wargo JA (2018) The impact of intratumoral and gastrointestinal microbiota on systemic cancer therapy. Trends Immunol 39:900–920

de Almeida CV, Taddei A, Amedei A (2018) The controversial role of *Enterococcus faecalis* in colorectal cancer. Therap Adv Gastroenterol 11:1756284818783606

Demaria S, Coleman CN, Formenti SC (2016) Radiotherapy: changing the game in immunotherapy. Trends Cancer 2:286–294

Deng Q, Wang C, Yu K, Wang Y, Yang Q, Zhang J et al (2020) *Streptococcus bovis* contributes to the development of colorectal cancer via recruiting $CD11b^{+}TLR\text{-}4^{+}$ cells. Med Sci Monit 26:e921886

Dong J, Li Y, Xiao H, Zhang S, Wang B, Wang H et al (2021) Oral microbiota affects the efficacy and prognosis of radiotherapy for colorectal cancer in mouse models. Cell Rep 37:109886

Dove WF, Clipson L, Gould KA, Luongo C, Marshall DJ, Moser AR et al (1997) Intestinal neoplasia in the ApcMin mouse: independence from the microbial and natural killer (beige locus) status. Cancer Res 57:812–814

Downes J, Munson MA, Radford DR, Spratt DA, Wade WG (2002) *Shuttleworthia satelles* gen. nov., sp. nov., isolated from the human oral cavity. Int J Syst Evol Microbiol 52:1469–1475

Dzutsev A, Goldszmid RS, Viaud S, Zitvogel L, Trinchieri G (2015) The role of the microbiota in inflammation, carcinogenesis, and cancer therapy. Eur J Immunol 45:17–31

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996–998

Elinav E, Garrett WS, Trinchieri G, Wargo J (2019) The cancer microbiome. Nat Rev Cancer 19:371–376

Fahmy CA, Gamal-Eldeen AM, El-Hussieny EA, Raafat BM, Mehanna NS, Talaat RM et al (2019) *Bifidobacterium longum* suppresses murine colorectal cancer through the modulation of oncomiRs and tumor suppressor miRNAs. Nutr Cancer 71:688–700

Fung TC, Olson CA, Hsiao EY (2017) Interactions between the microbiota, immune and nervous systems in health and disease. Nat Neurosci 20:145–155

Hasan R, Bose S, Roy R, Paul D, Rawat S, Nilwe P et al (2022) Tumor tissue-specific bacterial biomarker panel for colorectal cancer: *Bacteroides massiliensis*, *Alistipes species*, *Alistipes onderdonkii*, *Bifidobacterium pseudocatenulatum*, *Corynebacterium appendicis.* Arch Microbiol 204:348

Herrera FG, Bourhis J, Coukos G (2017) Radiotherapy combination opportunities leveraging immunity for the next oncology practice. CA Cancer J Clin 67:65–85

Huo RX, Wang YJ, Hou SB, Wang W, Zhang CZ, Wan XH (2022) Gut mucosal microbiota profiles linked to colorectal cancer recurrence. World J Gastroenterol 28:1946–1964

Ida Y, Okuyama T, Araki K, Sekiguchi K, Watanabe T, Ohnishi H (2022) First description of *Lachnoanaerobaculum orale* as a possible cause of human bacteremia. Anaerobe 73:102506

Ivanov II, Tuganbaev T, Skelly AN, Honda K (2022) T cell responses to the microbiota. Annu Rev Immunol 40:559–587

Jaffray DA (2012) Image-guided radiotherapy: from current concept to future perspectives. Nat Rev Clin Oncol 9:688–699

Kharrat N, Assidi M, Abu-Elmagd M, Pushparaj PN, Alkhaldy A, Arfaoui L et al (2019) Data mining analysis of human gut microbiota links *Fusobacterium* spp. with colorectal cancer onset. Bioinformation 15:372–379

Komiya Y, Shimomura Y, Higurashi T, Sugi Y, Arimoto J, Umezawa S et al (2019) Patients with colorectal cancer have identical strains of *Fusobacterium nucleatum* in their colorectal cancer and oral cavity. Gut 68:1335–1337

Li Y, Dong J, Xiao H, Zhang S, Wang B, Cui M et al (2020) Gut commensal derived-valeric acid protects against radiation injuries. Gut Microbes 11:789–806

Lim YK, Park SN, Jo E, Shin JH, Chang YH, Shin Y et al (2019) *Lachnoanaerobaculum gingivalis* sp. nov., isolated from human subgingival dental plaque of a gingivitis lesion. Curr Microbiol 76:1147–1151

Liu YK, Chen V, He JZ, Zheng X, Xu X, Zhou XD (2021) A salivary microbiome-based auxiliary diagnostic model for type 2 diabetes mellitus. Arch Oral Biol 126:105118

Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963

O'Keefe SJ (2016) Diet, microorganisms and their metabolites, and colon cancer. Nat Rev Gastroenterol Hepatol 13:691–706

Périchon B, Lichtl-Häfele J, Bergsten E, Delage V, Trieu-Cuot P, Sansonetti P et al (2022) Detection of *Streptococcus gallolyticus* and four other CRC-associated bacteria in patient stools reveals a potential "driver" role for enterotoxigenic *Bacteroides fragilis*. Front Cell Infect Microbiol 12:794391

Pons BJ, Vignard J, Mirey G (2019) Cytolethal distending toxin subunit b: a review of structure-function relationship. Toxins (Basel) 11:595

Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW (2013) *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. Cell Host Microbe 14:195–206

Scanlan PD, Shanahan F, Clune Y, Collins JK, O'Sullivan GC, O'Riordan M et al (2008) Culture-independent analysis of the gut microbiota in colorectal cancer and polyposis. Environ Microbiol 10:789–798

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS et al (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12:R60

Sheng Q, Du H, Cheng X, Cheng X, Tang Y, Pan L et al (2019) Characteristics of fecal gut microbiota in patients with colorectal cancer at different stages and different sites. Oncol Lett 18:4834–4844

Steck N, Hoffmann M, Sava IG, Kim SC, Hahne H, Tonkonogy SL et al (2011) *Enterococcus faecalis* metalloprotease compromises epithelial barrier and contributes to intestinal inflammation. Gastroenterology 141:959–971

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71:209–249

Taghinezhad SS, Mohseni AH, Fu X (2021) Intervention on gut microbiota may change the strategy for management of colorectal cancer. J Gastroenterol Hepatol 36:1508–1517

Thomas AM, Jesus EC, Lopes A, Aguiar S, Begnami MD, Rocha RM et al (2016) Tissue-associated bacterial alterations in rectal carcinoma patients revealed by 16S rRNA community profiling. Front Cell Infect Microbiol 6:179

Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillère R, Hannani D et al (2013) The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 342:971–976

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267

Wang S, Hibberd ML, Pettersson S, Lee YK (2014) *Enterococcus faecalis* from healthy infants modulates inflammation through MAPK signaling pathways. PLoS One 9:e97523

Wang Q, Wang K, Wu W, Lv L, Bian X, Yang L et al (2020) Administration of *Bifidobacterium bifidum* CGMCC 15068 modulates gut microbiota and metabolome in azoxymethane (AOM)/dextran sulphate sodium (DSS)-induced colitis-associated colon cancer (CAC) in mice. Appl Microbiol Biotechnol 104:5915–5928

Wong SH, Zhao L, Zhang X, Nakatsu G, Han J, Xu W et al (2017) Gavage of fecal samples from patients with colorectal cancer promotes intestinal carcinogenesis in germ-free and conventional mice. Gastroenterology 153:1621-1633.e1626

Xu S, Yin W, Zhang Y, Lv Q, Yang Y, He J (2020) Foes or friends? bacteria enriched in the tumor microenvironment of colorectal cancer. Cancers (Basel) 12:372

Zhang H, Chang Y, Zheng Q, Zhang R, Hu C, Jia W (2019a) Altered intestinal microbiota associated with colorectal cancer. Front Med 13:461–470

Zhang S, Wang Q, Zhou C, Chen K, Chang H, Xiao W et al (2019b) Colorectal cancer, radiotherapy and gut microbiota. Chin J Cancer Res 31:212–222

Zhao Z, Wang H, Zhang D, Guan Y, Siddiqui SA, Feng-Shan X et al (2022) Oral vaccination with recombinant *Lactobacillus casei* expressing *Aeromonas hydrophila* Aha1 against *A. hydrophila* infections in common carps. Virulence 13:794–807

Zhiqin W, Palaniappan S, Raja Ali RA (2014) Inflammatory bowel disease-related colorectal cancer in the Asia-Pacific region: past, present, and future. Intest Res 12:194–204

Zorron Cheng Tao PuL, Yamamoto K, Honda T, Nakamura M, Yamamura T, Hattori S et al (2020) Microbiota profile is different for early and invasive colorectal cancer and is consistent throughout the colon. J Gastroenterol Hepatol 35:433–437