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Roles of gut microbiota in cancers of the gastrointestinal tract

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Roles of gut microbiota in cancers of the gastrointestinal tract

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Editorial: Roles of gut microbiota in cancers of the gastrointestinal tract

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Editorial on the Research Topic
Roles of gut microbiota in cancers of the gastrointestinal tract

The gut microbiota is a dynamic ecosystem residing within the human gastrointestinal tract, and is progressively recognized as a crucial factor influencing cancer initiation, progression, and treatment response. Mounting evidence underscores the microbiota's role as a key regulator of host immunity, metabolism, and inflammation, which are pathways linked to carcinogenesis. Of particular interest is the microbiota's involvement in digestive cancers, including colorectal cancer (CRC), gastric cancer, and esophageal cancer, where microbial dysbiosis has been consistently reported (Garrett, 2019; Wong and Yu, 2019). Despite extensive research into CRC microbiota, the gastric and esophageal microbiotas were relatively less studied until recently; however, these areas are now garnering increased research attention as potential sources of novel biomarkers and therapeutic targets.

Research trend and microbial pathogenesis in digestive cancers

A recent bibliometric analysis by Ke et al. identifies a clear and accelerating trend toward investigating the gastric microbiota in gastric cancer research. This comprehensive study reveals a notable shift from the previously dominant focus on *Helicobacter pylori* alone toward broader exploration of the gastric microbiota, including emerging non-*Helicobacter* bacteria (e.g., *Fusobacterium nucleatum*, *Streptococcus anginosus*). In this connection, *F. nucleatum* could promote immune evasion in gastric cancer via recruiting tumor-associated neutrophils while *S. anginosus* could promote gastric tumorigenesis through the Annexin A2-mitogen-activated protein kinase axis (Zhang et al., 2025; Fu et al., 2024). The study by Ke et al. highlights the increasing emphasis on elucidating the microbial mechanisms underlying gastric carcinogenesis. Similarly, a systematic review of case-control studies by Zhang R. et al. provides evidence that gastric cancer patients harbor distinct microbial signatures, such as increased *Lactobacillus spp.* and *Streptococcus spp.* and decreased *Porphyromonas spp.* and *Rothia spp.*. Such findings

underscore the necessity for consensus microbial signatures across diverse populations and methodologies, reflecting the complexity and specificity of microbiota alterations in cancer.

To move beyond observational associations toward establishing causality, increased efforts are focusing on methodologies such as Mendelian randomization. Recent analyses by Ma et al. for CRC and Zhang Z. et al. for esophageal cancer have pinpointed potentially causal microbial taxa in carcinogenesis. Ma et al. demonstrated the positive associations of *Porphyromonadaceae spp., Lachnospiraceae UCG010, Lachnospira,* and *Sellimonas* with CRC. Notably, although *Lachnospiraceae UCG010* exhibited a negative correlation with interleukin-10, the analysis suggested that the CRC-promoting effect of *Lachnospiraceae UCG010* was independent of this cytokine. Likewise, Zhang Z. et al. identified the negative associations of *Romboutsia*, *Lachnospira*, and *Eubacterium* with esophageal cancer. These protective microbes might protect against esophageal cancer formation through enhancing cellular resistance to endoplasmic reticulum stress, inhibiting inflammatory responses, and scavenging free radicals. The authors also identified the potential pathogenic role for *Veillonella* in esophageal cancer. To this end, this bacterium has been shown to promote inflammatory responses via activating the Toll-like receptor 4 pathway in macrophages. These studies reinforce the importance of distinguishing correlation from causation, guiding the identification of microbial culprits, and refining targets for microbiota-based interventions.

While colorectal microbiota research remains robust, the previously less-explored gastric and esophageal microbiotas are now emerging as some areas of research focus. Future efforts must aim for cross-population validation, interdisciplinary collaboration and biological insight to fully harness the microbiota's potential for translation.

Microbiota-based biomarkers for digestive cancers

Over the last decade, the scientific community has witnessed a rapid expansion in the number of studies utilizing gut microbes as biomarkers for the detection of neoplastic lesions, especially those of the digestive organs. In this Research Topic, Cui et al. introduces a deep learning model named multi-view convolutional variational information bottleneck (MV-CVIB) for predicting metastatic colorectal cancer (mCRC) using 16S rDNA sequencing-based gut microbiota data. The model integrates microbial abundance data with nearest neighbor information, achieving an area under the receiver operating characteristic curve (AUROC) of >0.9 on the mCRC dataset and demonstrating good performance for distinguishing CRC patients from healthy subjects on two additional CRC datasets (AUROC = 0.82 and 0.83, respectively). The study also identified significant microbial differences between mCRC and non-mCRC patients, particularly the enrichment of *Propionibacterium acnes* in the former. MV-CVIB thus represents a new deep learning tool for microbiota-based disease classification. Similarly, Zhou et al. compared the microbial communities in mCRC and non-mCRC, but they focused on tissue-associated instead of luminal bacteria. The researchers found that mCRC was characterized by an increase in *Bacteroides*, particularly *B. fragilis* and *B. uniformis*, and a decrease in *Streptococcus*. Interestingly, microbial differences in tumor-adjacent tissues from mCRC and non-mCRC persisted, indicating that a microbial "field defect" might contribute to CRC metastasis. In terms of the classification performance, these bacteria only exhibited a modest accuracy (AUROC = 0.64 for *Bacteroides* or *Streptococcus*) but their combination with carcinoembryonic antigen (CEA) improved the prediction (AUROC = 0.71 for CEA + *Streptococcus*). This study again suggests a potential role of the gut microbiota in CRC metastasis. Unlike the role in CRC, authors of another article in this Research Topic identified the enrichment of *Streptococcus* in the gut microbiota among patients with pancreatic cancer (PC), especially those with liver metastasis (PCLM) (Yang et al.). In this respect, *Streptococcus* could discriminate PC patients and PCLM patients from healthy subjects and non-metastatic PC patients, respectively, with high accuracy (AUROC = 0.93 for PC; AUROC = 0.80 for PCLM).

Different from the bacteriome, the use of plasmids (small circular, non-chromosomal DNA molecules found in bacteria) as biomarkers has been understudied. In this connection, Cai et al. examined the potential of using gut plasmids as novel diagnostic biomarkers for CRC. By analyzing metagenomic data from over 1,200 samples across eight cohorts, the researchers identified 198 plasmid sequences differentially abundant in CRC patients. A diagnostic model using 21 plasmid markers achieved a moderate accuracy with an AUROC of 0.70. Combining the plasmid markers with the bacterial markers further improved the accuracy (mean AUROC = 0.80). These findings underscore the utility of plasmids in enhancing diagnostic models despite the current challenges in their detection from short-read sequencing data.

Microbiota-based therapeutics for digestive cancers

Inflammatory bowel disease, including ulcerative colitis, is associated with an increased risk of CRC whereas the traditional Chinese medicine maggot has demonstrated anti-inflammatory properties in other disease contexts. In the work by Tang et al., maggot extract was shown to reverse the alterations of the gut microbiota and the associated metabolome in a murine model of colitis-associated CRC. Importantly, the reversal of dysbiosis was accompanied by the improvement of gut barrier function and the alleviation of inflammatory signals, hinting at the therapeutic potential of the restoration of a healthy gut microbiota in preventing colitis-associated CRC. Feng et al. also elegantly summarize how the gut microbiota might interact with the tumor microenvironment that has a direct impact on the therapeutic response, especially in the context of cancer immunotherapy. For instance, *Bifidobacterium pseudolongum* could drive T helper 1 cell differentiation and its high abundance is associated with response to immune checkpoint inhibitors. Short-chain fatty acids produced by commensals can also promote the memory potential of activated

$CD8^+$ T cells, contributing to the responsiveness to immune checkpoint inhibitors.

Concluding remarks

This Research Topic represents a notable collection of articles highlighting the current research trends in the field of gut microbiota in cancers of the gastrointestinal tract. Pathogenic roles of specific gut microbes have been scrutinized by systematic reviews and Mendelian randomization. With clinical relevance, several articles highlight the potential of using gut microbial biomarkers for non-invasive screening of gastrointestinal cancers and predicting their metastasis. However, the small sample size of some of these studies may raise concerns about overfitting. Their retrospective design also limits the generalizability. Larger-cohort validation, particularly across geographic regions and with subjects recruited in a prospective manner, is thus needed to confirm the clinical utility. Therapeutically, manipulating the gut microbiota might hold promise for preventing gastrointestinal cancers and improving response to systemic therapies.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Fu, K., Cheung, A. H. K., Wong, C. C., Liu, W., Zhou, Y., Wang, F., et al. (2024). *Streptococcus anginosus* promotes gastric inflammation, atrophy, and tumorigenesis in mice. *Cell* 187, 882–896. doi: 10.1016/j.cell.2024.01.004

Garrett, W. S. (2019). The gut microbiota and colon cancer. *Science* 364, 1133–1135. doi: 10.1126/science.aaw2367

Wong, S. H., and Yu, J. (2019). Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat. Rev. Gastroenterol. Hepatol.* 16, 690–704. doi: 10.1038/s41575-019-0209-8

Zhang, T., Li, Y., Zhai, E., Zhao, R., Qian, Y., Huang, Z., et al. (2025). Intratumoral *Fusobacterium nucleatum* recruits tumor-associated neutrophils to promote gastric cancer progression and immune evasion. *Cancer Res.* doi: 10.1158/0008-5472.CAN-24-2580

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Maggot extracts chemo-prevent inflammation and tumorigenesis accompanied by changes in the intestinal microbiome and metabolome in AOM/DSS-induced mice

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Inflammatory responses and intestinal microbiome play a crucial role in the progression of colitis-associated carcinoma (CAC). The traditional Chinese medicine maggot has been widely known owing to its clinical application and anti-inflammatory function. In this study, we investigated the preventive effects of maggot extract (ME) by intragastric administration prior to azoxymethane (AOM) and dextran sulfate sodium (DSS)-induced CAC in mice. The results showed that ME had superior advantages in ameliorating disease activity index score and inflammatory phenotype, in comparison with the AOM/DSS group. The number and size of polypoid colonic tumors were decreased after pre-administration of ME. In addition, ME was found to reverse the downregulation of tight junction proteins (zonula occluden-1 and occluding) while suppressing the levels of inflammatory factors (IL-1β and IL-6) in models. Moreover, Toll-like receptor 4 (TLR4) mediated intracellular nuclear factor-κB (NF-κB)-containing signaling cascades, including inducible nitric oxide synthase and cyclooxygenase-2, and exhibited decreasing expression in the mice model after ME pre-administration. 16s rRNA analysis and untargeted-metabolomics profiling of fecal samples inferred that ME revealed ideal prevention of intestinal dysbiosis in CAC mice, accompanied by and correlated with alterations in the composition of metabolites. Overall, ME pre-administration might be a chemo-preventive candidate in the initiation and development of CAC.

KEYWORDS

maggot extract (ME), inflammation, colitis-associated colon cancer (CAC), intestinal microbiota, metabolome

1. Introduction

Colorectal cancer (CRC) is the third most common malignancy and the second mortality of cancer death globally (Siegel et al., 2020). Inflammatory factors, such as bowel disease (IBD), play an etiologic part in CRC, predisposing patients to a high risk of morbidity and cancerization (Blackman et al., 2021). The epidemiological investigation has indicated that IBD cancer accounts for only 1–2% of CRC, but it is the main cause of death for IBD patients that is often recurrent (Li et al., 2019). Indeed, such a causal link between chronic inflammation and colitis-associated carcinoma (CAC) has served to be confirmed. A tumor microenvironment containing the immune cells that secrete proinflammatory and anti-inflammatory factors and release reactive oxygen and nitrogen species have been suspected to promote tumor initiation and progression (Chen et al., 2021; Overacre-Delgoffe et al., 2021). Another direct example of the transformation of inflammatory cancer was supplied by mucosa-associated lymphoid tissue (MALT) lymphoma, which was caused by chronic infection from persistent activation of B cells to genetic arrangement leading to carcinogenesis eventually (Thieblemont et al., 2014). Anti-inflammatory treatment is very important and effective, so chemo-prevention strategies are necessary. Nowadays, food-origin and herb-origin products with diverse functions are emerged as novel chemo-prevention agents and are used in clinical medicine owing to their anti-inflammatory effects and safe benefits (Chung et al., 2018; Fong et al., 2020; Chen et al., 2021; Iqbal et al., 2021; Sameni et al., 2021). In addition, newer precision medicine, such as aspirin (acetylsalicylic acid), is placed with great hopes to get better control of the potential risks of cancerization (Gilligan et al., 2019; Hua et al., 2019).

The gut microbiome (GMB) is very large, and its interaction with the human body is highly complex. Studies had shown that the balance of GMB played a crucial role in intestinal immunity and host health, and changes in GMB might lead to a variety of metabolomics through their metabolites (Stutz et al., 2022). On the other hand, dysbiosis of the microbial population may promote mucosal injury by means of driving gut inflammation (Bajic et al., 2020; Dooyema et al., 2022). Accumulating evidence suggests that intestinal microorganism disorder in CAC patients induces an abnormal immune response, destroys intestinal homeostasis, and eventually leads to the loss of intestinal mucosal barrier integrity (Tilg et al., 2020). Pathogenic or probiotic bacterial infection is a key part of the triggering of IBD cancerization. For instance, *Akkermansia muciniphila*, a type of probiotic, can repair the gut barrier and blunt CAC by modulation of immune cells (Wang et al., 2022a). A link between dysbacteriosis and CAC was validated. It is, thus, urgent to find approaches to reverse intestinal flora and barrier function as a notable strategy in the prevention of CAC (Wang et al., 2020a; Chang et al., 2022).

The Chinese medicine maggot is the larva of *Chrysomya megacephala* (Fabricius, named "larvae of *Lucilia sericata*" in Latin) and its relatives, belonging to the Calliphoridae family. Maggot was widely used in traditional prescriptions and such classic works as "Compendium of Materia Medica" originated from the 16th century in the Ming dynasty and listed thousands of natural herb medicines described in detail. Maggot therapy can accelerate the removal of necrotic tissue and recovery of wounds, which shortens the treatment process of patients with diabetic foot (Bazalinski et al., 2022). Recent research studies have shown that the chemical composition of a maggot is made up of protein, fatty acid, chitin, etc (Taowen et al., 2022). The clinical application of the maggot is still widely concerned, although maggot standards continue to be improved. Pharmacological effects were also confirmed such as antimicrobial acerating, wound healing, blood glucose and lipid lowering, anti-inflammatory, immune regulation, and tissue reconstruction (Wang et al., 2020b, 2021; Lema et al., 2022; Shi et al., 2022). At present, the function of maggot extract (ME) on CAC still remains unknown, and whether ME pre-administration plays a chemo-preventive role in the initiation and development of CAC has not been reported.

Here, we focused on the preventive effects of ME by intragastric treatment prior to azoxymethane and dextran sulfate sodium (AOM/DSS)-induced CAC. In addition, the possible mechanism was investigated from the aspects of intestinal barrier repairing, inflammatory factor decreasing, and fecal microbial composition changing in the CAC model. These alterations were coupled and related to fecal non-targeted metabolic substance variation. Integrative analysis was used to clarify the relationship between gut microbiota and fecal metabolites in the divided groups.

2. Materials and methods

2.1. Animals and ethical considerations

The experiment was performed on male C57BL/6 mice approximately 6 weeks old (Model Animal Research Center of Nanjing University), housing in a specific pathogen-free (SPF) environment (temperature 22±2°C; controlled humidity 50%;12/12 h day/night cycles). Ethical approval was listed by the animal ethics committee with a license. Animals were randomly assigned into four experimental groups ($n = 6$ in each group), as shown in Figure 1A: (a) normal control: sterile water daily, (b) the CAC model: azoxymethane (AOM) and dextran sulfate sodium salt (DSS), (c) ME pre-administration in model mice AOM/DSS plus ME administration consecutively for 21 days, and (d) only ME administration consecutively for 21 days.

Animals were cohoused for 1 week before the experiment. The control group drank sterile water every day. For the ME administrated group, the individuals were given (i.g.) 1 g/kg every day for 21 days from the first day of the experiment (day 1). The model mice were also injected intraperitoneally (i.p.) with 12.5 mg/kg of AOM (Sigma–Aldrich) per mouse on the first day (day 1). After a week, 2.5% DSS (MW36-50 kDa, MP biomedicals, United States) was given in drinking water for 5 days in the first cycle, followed by regular sterile water for the next 2 weeks. If mice lost 35% of their body weight, DSS can be replaced by drinking water, and then, DSS was allowed to continue for extra 2–5 days after mice were recovered from the loss. The cycle was repeated two times (5 days of 2%DSS), and all mice were then sacrificed 2 weeks later since the last cycle.

The colon was separated longitudinally, washed with phosphate buffer saline (PBS), and fixed as a Swiss roll in 4% paraformaldehyde after counting the number and size of polys in a blind trial. The specimens were embedded in paraffin, and serial

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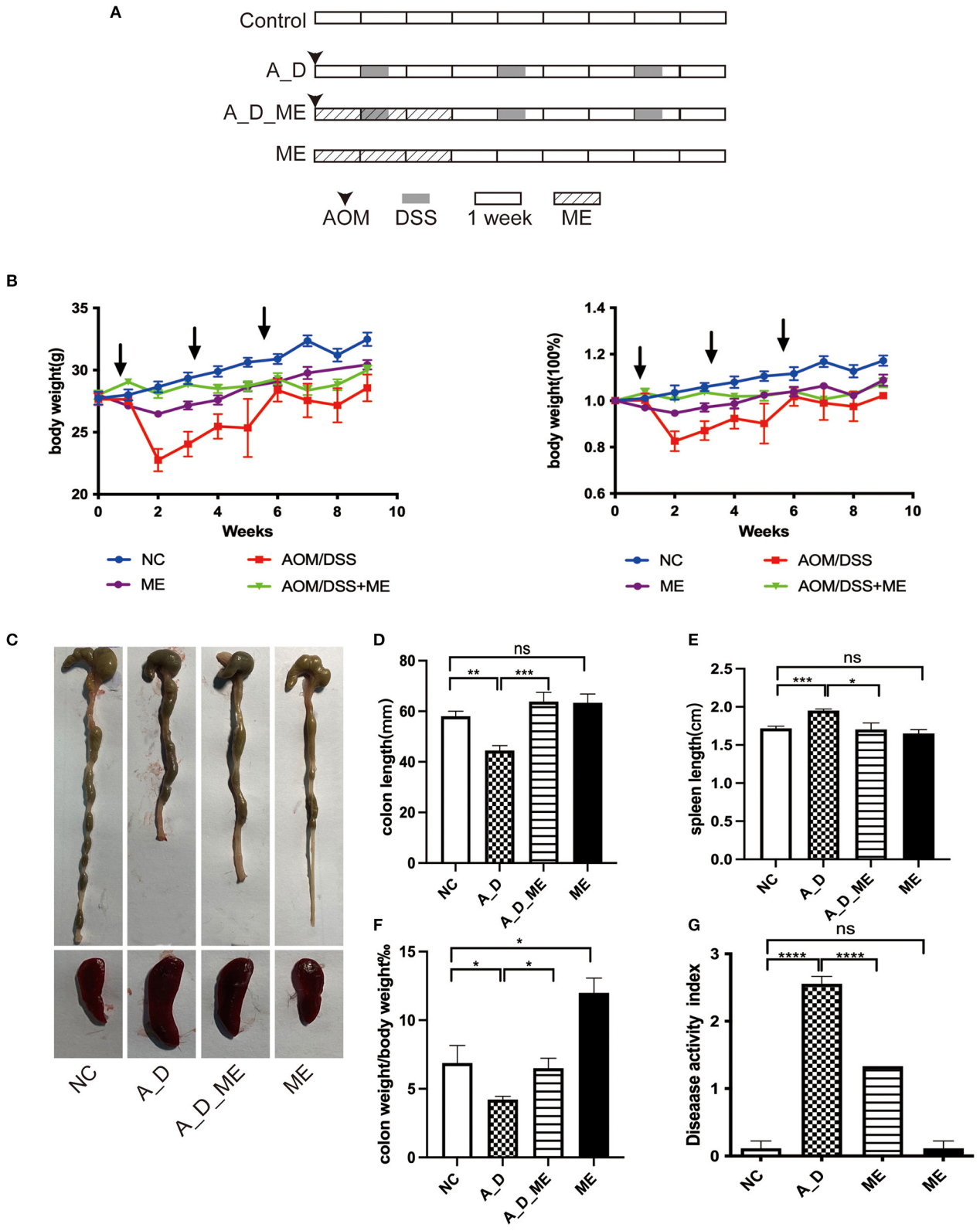

FIGURE 1
Effects of ME administration on the development of AOM/DSS-induced tumorigenesis in C57BL/6 mice ($n = 6$). **(A)** protocol, **(B)** body weight changes and the ratio of weight changes to the starting weight, **(C)** macroscopic morphologies of colon and spleen, **(D)** colon length, **(E)** spleen length, **(F)** the radio of colon weight to body weight, **(G)** DAI.

sections were stained by HE (Hematoxylin–Eosin staining), IHC (immunohistochemistry), or IF (immunofluorescence). Blood, colon, liver, spleen, lung, and kidney tissues were collected for further experiments. During the experiment, the weight of mice was measured weekly, and stool was collected two times a week. The damage of disease was scored by the disease activity index (DAI), including weight loss, stool consistency, and bleeding. The scores were recorded after dividing the sum of subfractions by 3 and ranged from 0 to 4 individually.

2.2. ME preparation

The preparation and dose of ME were performed based on previous articles (Wang et al., 2019, 2021). In brief, blowflies were fed from larvae to maggots on wheat seeds, powdered milk, and yeast extracts. Large amounts of fresh maggot were flushed three times with water. After freeze-drying, maggots were ground into powder. Then, water solutions were obtained from PBS addition (twice the volume of the power), and the supernatant was collected by centrifugation at 15,000 r/min for 10 min after water-bath processing. Finally, the solutions were filtered through a 0.22μm membrane. The ME stock solution (500 mg/ml) was obtained.

2.3. Histological analysis (HE, IHC, and IF)

Tissues were prepared for sections (5μm), stepwise (200μm) through the paraffin block. The slides were dehydrated by gradient alcohol and stained with HE. The colon tissue of epithelial injury, inflammatory infiltration, and dysplastic hyperplasia was evaluated by pathologists separately.

As described above for IHC, the slides were blocked with 5% BSA for 1 h and incubated with primary antibody against ZO-1 (GB111402, Servicebio, China) and occluding (27260-1-ap, Proteintech, China) overnight at 4°C followed by incubation with secondary antibody for 1 h at room temperature. The sections were stained with DAB and then counterstained with hematoxylin.

To stain immunofluorescence, the experiment was carried out as mentioned above until the antigen is retrieved by citric acid buffer (PH6.0) through the microwave. Then, the slides were incubated with primary antibody overnight at 4°C after blocking. The secondary antibody conjugated with Alexa Flour 488 or 594 was used to incubate with the slides for immunofluorescence and DAPI for nuclei. The stained specimens were scanned by the laser scanning confocal microscopy (Leica DMIRE2, Germany).

2.4. Enzyme-linked immunosorbent assay (ELISA)

IL-1β and IL-6 in serum obtained on the last day were measured by mouse ELISA kit (Solarbio, China). The assays were executed according to the manufacturers' instructions. The absorbance of the specimen was detected at 450 nm by a microplate reader.

2.5. Western blot analysis

Colon tissue was cut and stored at −80°C in RIPA buffer (Beyotime, China) mixed with phosphatase inhibitor (Thermos Scientific, CA, United States) and protease inhibitor cocktail (Thermos Scientific, CA, United States). The lysates were centrifuged at 4°C (12,000 rpm, 20 min), and the supernatant was obtained. Protein quantification was performed by Enhanced BCA Protein Assay Kit (Beyotime, China). Proteins (30μg) per sample were used for Western blot analysis.

2.6. Quantitative Real-Time PCR assay

Tissue Total RNA Isolation Kit (Vayzme, China) was used to extract total RNA according to the protocol. Reverse transcription was performed to synthesize cDNA using PrimeScriptTM RT Master Mix (Takara, China), and then, cDNA was used. The primer sequences were shown in our previous studies (Wang et al., 2019). The relative expression of target mRNA was normalized by GAPDH and calculated a 2-ΔCt after obtaining a mean ΔC value. All results in triplicate were repeated three times.

2.7. 16s DNA sequencing

DNA was extracted from 200 mg stool of each sample. Specific primers with barcodes were used to amplify the conserved regions (V3-V4 region) (Guo et al., 2017) of ribosome RNA (rRNA). The primers were listed as follows: forward 5′-CCTACGGGNGGCWGCAG-3′ and reverse 5′-GGACTACHVGGGTATCTAAT-3′. The true PCR amplification products, with an average length of 466 base pairs, were recovered from the gel and quantified by the QuantiFluorTM fluorometer. The purified products were mixed in equal volumes and connected with sequencing adaptors to construct a sequencing library on the Illumina PE250 platform by Gene Denovo Biotechnology Co., Ltd (Guangzhou, China).

2.8. Bioinformatics processing

Sequencing reads were filtered to remove low-quality reads by FASTP (Chen et al., 2018). The rest of the reads was spliced paired-end with FLASH (version 1.2.11) and concatenated to create raw tags (Magoc and Salzberg, 2011). Then, raw tags were assembled, and clean tags were extracted (Bokulich et al., 2013). After strict quality checks, clean tags were clustered, and the chimeric tag (Edgar et al., 2011) was possibly removed by the UCHIM algorithm in USEARCH version 9.2.64 software (Supplementary Table S1). The Greengene database (version gg_13_5) was used as standard reference data (DeSantis et al., 2006). Finally, effective tags were obtained, and the abundance of operational taxonomic units (OTUs) was analyzed based on the effective tags by using USEARCH software (Edgar, 2013). Qiime (version 1.9.1) was used to estimate alpha and beta diversity indices (Caporaso et al., 2010) on a thin table of OTUs. The Shannon and Simpson metrics

and ACE and Chao1 estimators were analyzed. The information of structural differences among samples was summarized from weighted-unifrac distances by an unweighted pair-group method with arithmetic means (UPGMA) tree. The linear discriminant analysis effect size (LEfSe) was employed to analyze differences between the groups shown by linear discriminant scores. Maps visualizing the principal coordinates analysis (PCoA) plots from the weighted and unweighted unifrac distances were drawn in R with the ggplot2, labdsv, and vegan packages (Lozupone and Knight, 2005; Hoegh and Roberts, 2020; Gao et al., 2021a; Liu et al., 2021). The characteristics of the microbiome were displayed at the taxonomic levels of phylum and family. A combination of PICRUSt2 (phylogenetic investigation of communities by reconstruction of unobserved states) and the Integrated Microbial Genomes database was used to construct phylogenetic trees, predicting bacterial genomics. Functions were, then, predicted based on the gene families and abundances using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. For the multivariate patterns represented numerically, outlier data were eliminated to prevent interference with the analysis. Items of information from 20 samples ($n = 5$ per group) were incorporated into the research.

2.9. Metabolomics profiling: ultra high-performance liquid chromatography-mass spectrometry (UHPLC-MS)

The stool samples were frozen at −80°C before a UHPLC-MS analysis. Each sample (50 mg) was added to a precooled solution of methanol/acetonitrile/water (2:2:1, v/v/v), which was mixed by ultrasound at a low temperature for 30 min. After standing for 10min at −20°C, the mixture was centrifuged at 14,000 g, at 4°C for 20 min, and the supernatant was dried under vacuum. During mass spectrometry, 100 μl of aqueous acetonitrile solution (acetonitrile: water =1:1, v/v) per sample was added to redissolve thoroughly and centrifuged at 14,000 g,at 4°C for 15 min. Samples of quality control were performed by blending 10 μl of every sample and then profiling with the whole samples meanwhile. QC got involved termly and studied at intervals of five samples to check the repeatability of the whole analysis.

The derivative was injected into a UHPLC (1290 Infinity LC, Agilent Technologies) coupled to a quadrupole time-of-flight (AB Sciex TripleTOF 6600) for analyzing untargeted metabolomics profiling of 20 fecal samples. A 2.1 mm × 100 mm ACQUIY UPLC BEH 1.7 μm column (waters, Ireland) was employed for RPLC separation.

2.10. Metabolomics data mining

MS raw (.raw) documents were transformed into the mzML format by proteowizard and were analyzed in R with the XCMS package, consisting of retention time alignment, peak identification, and peaks matching. After the preprocessing of the data matrix, it was formed including mass-to-charge ratio, retention time, and peak area. Precursor molecules in positive and negative ion modes were accessed, and the molecules were normalized to obtain quantitative results. Identified metabolites were projected to KEGG pathways. The detailed descriptions of data mining and statistical analysis are presented in Supplementary material.

2.11. Statistical analysis

The Kruskal–Wallis test was accomplished by LEFse to value the differences among the microbiota compositions of the four compartments. Moreover, the selected differences were compared between any two groups by the Wilcoxon rank sum test. The final differences were ranked using the results of a linear discriminant analysis (LDA). The VIP value of multivariate statistical analysis of OPLS-DA was combined with the *P*-value of univariate statistical analysis in a T-test, screening the differential metabolites between different groups. The threshold of differences was as follows: VIP≥ 1 in the OPLS-DA as well as p<0.05 in the *t*-test. The correlation between gut microbiota communities and fecal metabolites was analyzed by Pearson's correlation coefficients. The *p*-value was calculated based on Fisher's Z-transformation. Differences were statistically significant at $p < 0.05$.

3. Results

3.1. Effects of ME administration on the development of AOM/DSS-induced tumorigenesis in C57BL/6 mice

During a 15-week period in an AOM/DSS-induced tumorigenesis, weight loss was observed compared with the NC group, particularly DSS water drinking in the 2nd, 5th, and 8th weeks. Upon changing from DSS to sterile water, the body weight was recovered. There were significant differences in the ME-treated group throughout the period of 21 days, but no differences were observed on the day of sacrifice (Figure 1B). ME treated for 21 days in mice proved no toxicity in the aspects of gross abnormality and serological indicators (Supplementary Figure S1). Except for the NC and ME groups, the mice in the rest of the two groups caused bloody stool and ulcers (not shown). Morphology was visibly altered in the terms of the colon and spleen (Figures 1C–E). As shown in Figures 1D, F, AOM/DSS caused length shortening and weight reduction in the colon tissue. However, the length and weight of the large bowel in ME-treated CAC mice were improved compared with those in the AOM/DSS group ($p < 0.05$). Obvious splenomegaly led by AOM/DSS was also visible while an effective reversal on the enlargement of the spleen was shown compared with the supplement of ME in the models. Compared with the control group, the DAI was scored on the last day, increasing in AOM/DSS-exposed mice, and pre-administration of ME showed improvement clearly (Figure 1G).

3.2. Decreasing severity of inflammation and carcinogenesis in AOM/DSS-treated mice after ME administration

To investigate the function of ME in CAC, the model we used was administrated by a dose of AOM and three cycles of DSS (Figure 1A). The repeated DSS-induced IBD, as a result of chronic inflammation, slightly increases the incidence of AOM-caused tumors. We noticed a dramatic reduction of approximately 67% in polypoid colonic tumor incidence in the ME pre-administration group (Figure 2B). These tumors were macroscopically located in the middle and distal colon (Figure 2A), where inflammation induced by DSS occurs severely, indicating that the severity of colitis was identified with the incidence of a tumor. We divided the tumors into three types: 0–2, 2–4, and >4 mm; a larger diameter means the cancer is more serious. Significant differences in size between tumors in model mice and ME-treated models could be detected, indicating that ME pre-administration alleviates inflammation-associated carcinogenesis in the model colon (Figure 2C). The tumors were largely adenomas with low-grade or high-grade differentiation in intraepithelial neoplasia and different degrees of inflammatory cell invasion (Figures 2D–I, Supplementary Figures S2A, B). A decreasing expression of β-catenin and ki67, very representative markers in colorectal carcinogenesis, was observed in a colonic crypt in the ME-treated group (Figures 2J–L).

3.3. Effects of ME administration on the regulation of tight junction proteins and inflammatory responses in AOM/DSS-treated mice

Pre-administration of ME was found to reverse the downregulation of zonula occluden-1 (ZO-1) and occluding, which were significantly reduced in the AOM/DSS group (Figures 3A, B). As a result, we suggested that the effects of ME on the CAC model mice were in connection with the regulation of ZO-1 and occluding, functioning in the aspects of the intestinal mucosal barrier homeostasis. The reports showed that CAC had a sign with the production of a variety of inflammatory factors such as IL-1β and IL-6. Our ELISA results demonstrated that the serum levels of IL-1β and IL-6 expanding in mice treated with AOM/DSS were decreased by ME (Figures 3C, D). Meanwhile, it was also shown that after ME administration, the impression of AOM/DSS on CAC was partially offset in the mRNA expression of IL-1β and IL-6 in colonic tissues (Figure 3E).

Colitis-associated carcinoma has proven to be a complicated process. In AOM/DSS-induced mice, Toll-like receptor 4 (TLR4) mediated intracellular nuclear factor-κB (NF-κB)-containing signaling cascades, encouraging the progression of cancer. After NF-κB was activated, it leads to the release of pro-inflammatory mediators including interleukin-1β (IL-1β), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). Our results of immunofluorescence staining exhibited that the positive cells of TLR4, NF-κB, iNOS, and COX2 showed the highest expression in the colon tissue of the AOM/DSS group. However, we observed that the levels of TLR4, NF-κB, iNOS, and COX2 in the ME pre-administrated group showed lower expression than that in the AOM/DSS group. Data revealed that the increasing expression of TLR4, NF-κB, iNOS, and COX2 in the AOM/DSS group was inhibited by ME treatment (Figure 4).

3.4. Mice treated by ME and fecal microbiome

To investigate the association between ME's impact and intestinal microbiome, we focus on the composition of the fecal bacterium by 16s rRNA sequencing. After extracting clean reads and producing effective tags, high-quality sequencing and quality control were used for subsequent taxonomy analysis (Supplementary Table S1). The multi-sample rarefaction curves of Shannon and Simpson indices tended to be smooth when the sample tags added up to approximately 2,000, indicating an extensive sequencing depth and the most captured diversity for fecal microbiome analysis (Figure 5A). Moreover, Simpson's results were similar to Shannon's. Microbial community alpha diversity metrics (Shannon, Simpson, Chao, Goods' coverage, Pielou, and pd shown in Supplementary Table S2) and beta diversity indices (NMDS and PCA, shown in Figures 5B, C) were significantly different between the groups with and without ME (NC vs. ME, A_D vs. A_D_ME). The NMDS and PCA plots of weighted unifrac_distances were clearly separated observing by ME status. The obvious shift of the ME group was narrowed compared to the control (A_D vs. A_D_ME compared to NC vs. A_D). Moreover, ANOSIM analysis showed that the effects among the three groups were also significantly different (Figure 5D). The unweighted pair-group method with arithmetic means (UPGMA) clustering was likewise employed to access the beta diversity of gut microbiome among groups (Figure 5E). The UPGMA method divided the individuals into the A_D group and the other groups, suggesting that the microbial profile was definitely diverse between the model mice and ME-treated model mice. There was a certain degree of similarity in the NC group and A_D_ME group, which reveals that ME administration reversed the microbial profile of the AOM/DSS model mice. Together, ME had obvious effects on the alpha and beta diversities of the gut microbiome.

According to operational taxonomic units (OTUs) identified from sequenced samples, the most relative abundances in the level of phylum were *Firmicutes*, *Bacteroidetes, and Proteobacteria* (Supplementary Figure S3). At the family level, the microbial profile of the NC group, A_D group, A_D_ME group, and ME group belonged to the most 10 common families as follows: *Muribaculaceae, Erysipelotrichaceae, Lactobacillaceae, Moraxellaceae, Enterobacteriaceae, Ruminococcaceae, Bifidobacteriaceae,* and other three families (Figure 6A). LEfSe analysis showed that CAC mice treated by ME had a correlation with rich abundances of *Lactobacillaceae and Bacilli* and poor abundances of *Erysipelotrichaceae* and Coriobacteriales_Incertae_Sedis (Figure 6B). Welch's t-test was used to analyze the top biomarkers in the taxa that could identify the NC group, A_D group, and A_D_ME group (Figures 6C, D). A total of three probiotics (*Lactobacillaceae,*

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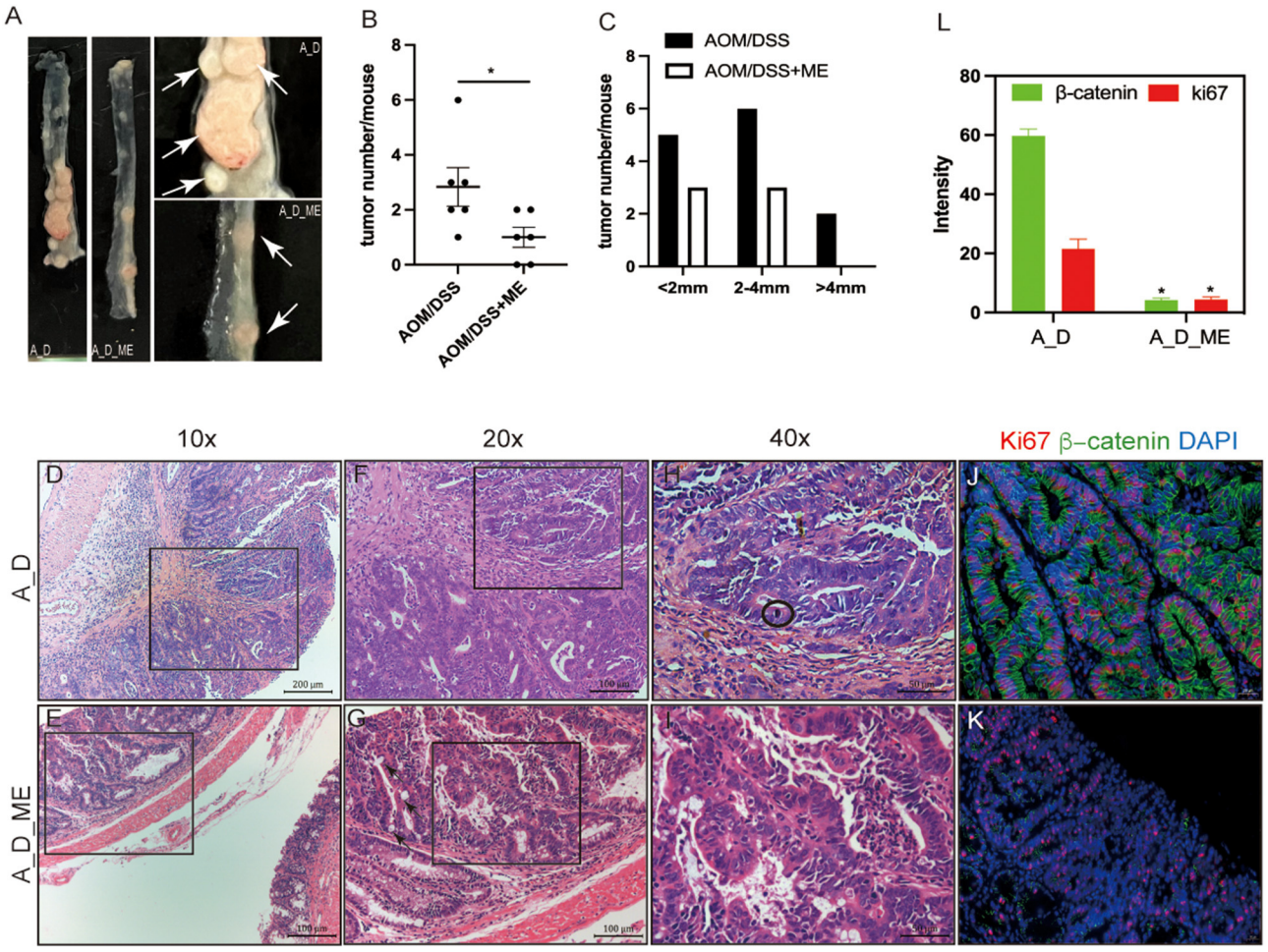

FIGURE 2
Decreasing severity of inflammation and carcinogenesis in AOM/DSS-treated mice after ME administration. **(A)** tumors formed in the large intestinal tracts. The white arrow represents tumors. **(B, C)** numbers and distributions of tumors in the AOM/DSS mice ($n = 6$ per group). **(D–I)** representative micrographs of H&E staining, **(D, E)** representative adenomas, infiltrating inflammatory cells in the surface of the rectum, scale bar=200μm, 10×magnification, **(F, G)** pathology damaged in the colonic mucosa, scale bar = 100 μm, 20×magnification, the black arrow represents intestinal metaplasia, **(H, I)** degree and type of cell differentiation, evaluation of mitotic figure can be hard(encircled). scale bar=50μm, 40×magnification. **(J–L)** immunofluorescent staining of ki67 (red) and β-catenin(green) in the adenocarcinoma of AOM/DSS model treated with ME or not, scale bar=20μm, fluorescence intensity is analyzed by image J. Wilcoxon rank sum test was used, *$p < 0.05$.

Bifidobacteriaceae, and *Eggerthellaceae*) decreased in A_D but increased in the NC and A_D_ME groups, while another pathogen (*Erysipelotrichaceae*) increased in A_D but decreased in the NC and A_D_ME groups (Figures 6E–H). Therefore, ME-associated families including *Lactobacillaceae*, *Eggerthellaceae*, *Erysipelotrichaceae*, and *Bifidobacteriaceae* were incorporated into the following analysis. The *Lactobacillaceae* and *Erysipelotrichaceae* families were classified into the same and most phylum Firmicutes, while *Eggerthellaceae* and *Bifidobacteriaceae* were classified into the phylum Actinobacteria.

3.5. Mice treated by ME and fecal metabolomic profile

Metabolite differentiation was presented among the treated groups by the Partial least squares-discriminant analysis (PLS-DA), and all four groups were almost separated (Figure 7A). An orthogonal projection to latent structures-discriminant analysis (OPLS-DA) also revealed an obvious distinction between NC and A_D mice and between the A_D group and A_D_ME group (Figures 7B, C). As shown in Figure 7D, a total of 48 fecal metabolites in the relevant three groups were listed. The results demonstrated that the relative abundances of presented fecal metabolites in CAC mice were quite different from those in the control group, implying that AOM/DSS treatment has a profound and lasting influence on fecal metabolic profiles. ME administration in CAC mice also showed significant differences in the abundance of fecal metabolites. The changes in the listed 25 metabolites caused by AOM/DSS treatment were attenuated by ME administration, including G-quanidinobutyrate, Triameinalana dissatsta, Stachydrine, Isoevernic acid, Prostaglandin i2, and I-alaninamide. In addition to that, the abundances of 12-ketodeoxycholic acid, Tetradecanedioic acid, 3-aminobutanoic acid, 5alpha-Androstane-3,17-dione, 3-aminopyrazine-2-carboxylic acid,

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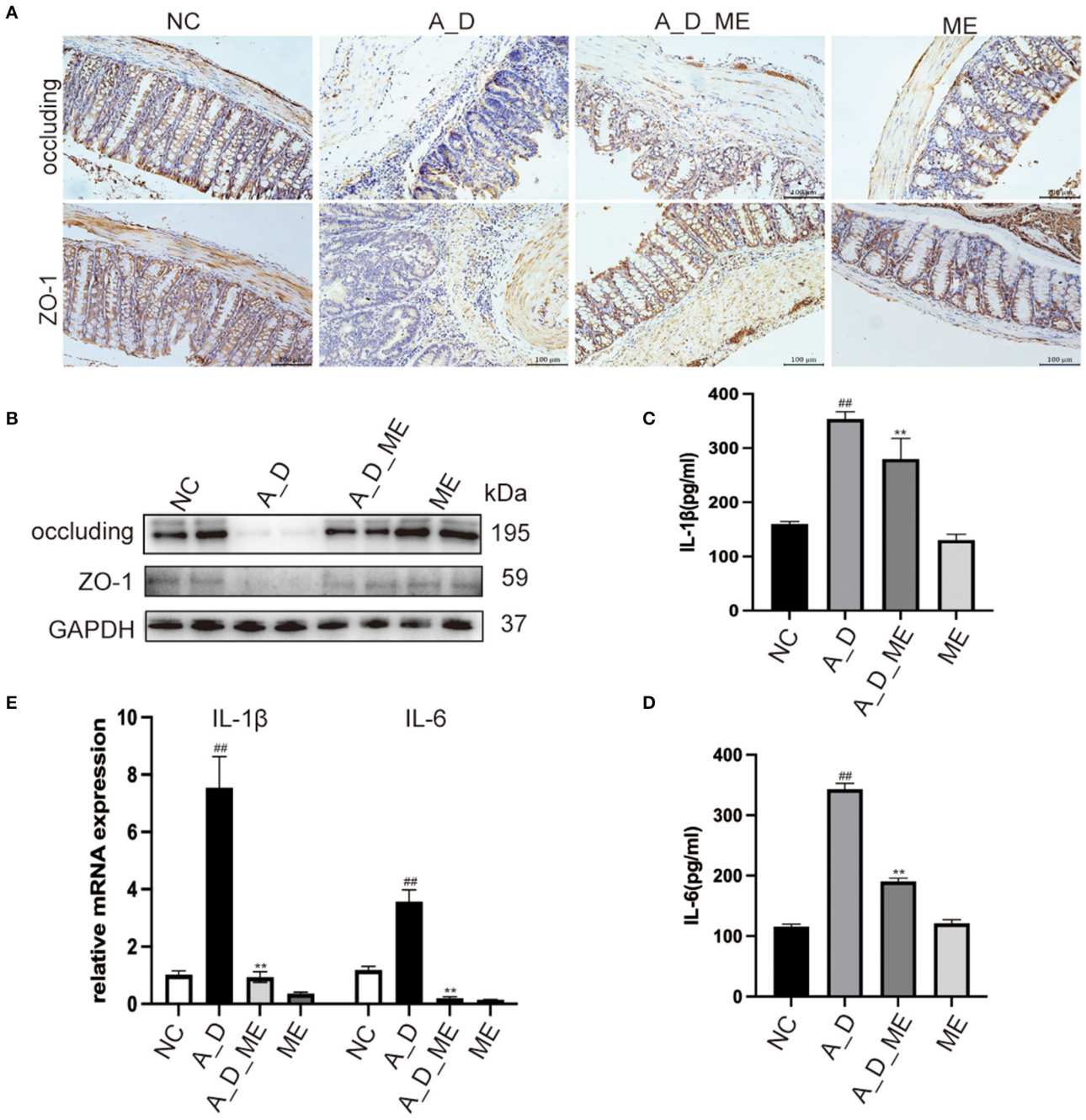

FIGURE 3
Effects of ME on the regulation of tight junction proteins and inflammatory responses in AOM/DSS-treated mice. **(A)** representative IHC staining of occluding and ZO-1 in treated mice as expressed. Scale bar = 100 m. **(B)** the expression of occluding and ZO-1 in colon tissues was detected by Western blotting. Graphs were quantified using GAPDH as the internal reference. **(C, D)** the serum levels of IL-1β as well as IL-6 in four groups. **(E)** the relative mRNA levels of IL-1β as well as IL-6 in the colon tissues. Data represent the mean ± SD, $^{\#\#}p < 0.01$ compared to normal control, $^{**}p < 0.01$ compared with the model group. $N = 6$/group.

Arg-Gln, and Palythine were decreased in the model mice, whereas the alterations were diminished after ME administration.

To evaluate the importance of 48 chemical compounds, metabolite pathways were analyzed, involved in the regulation of lipolysis in adipocytes, prion disease, the CGMP-PKG signaling pathway, renin secretion, vascular smooth muscle contraction, morphine addiction, alcoholism, and aldosterone synthesis and secretion (Figure 7E).

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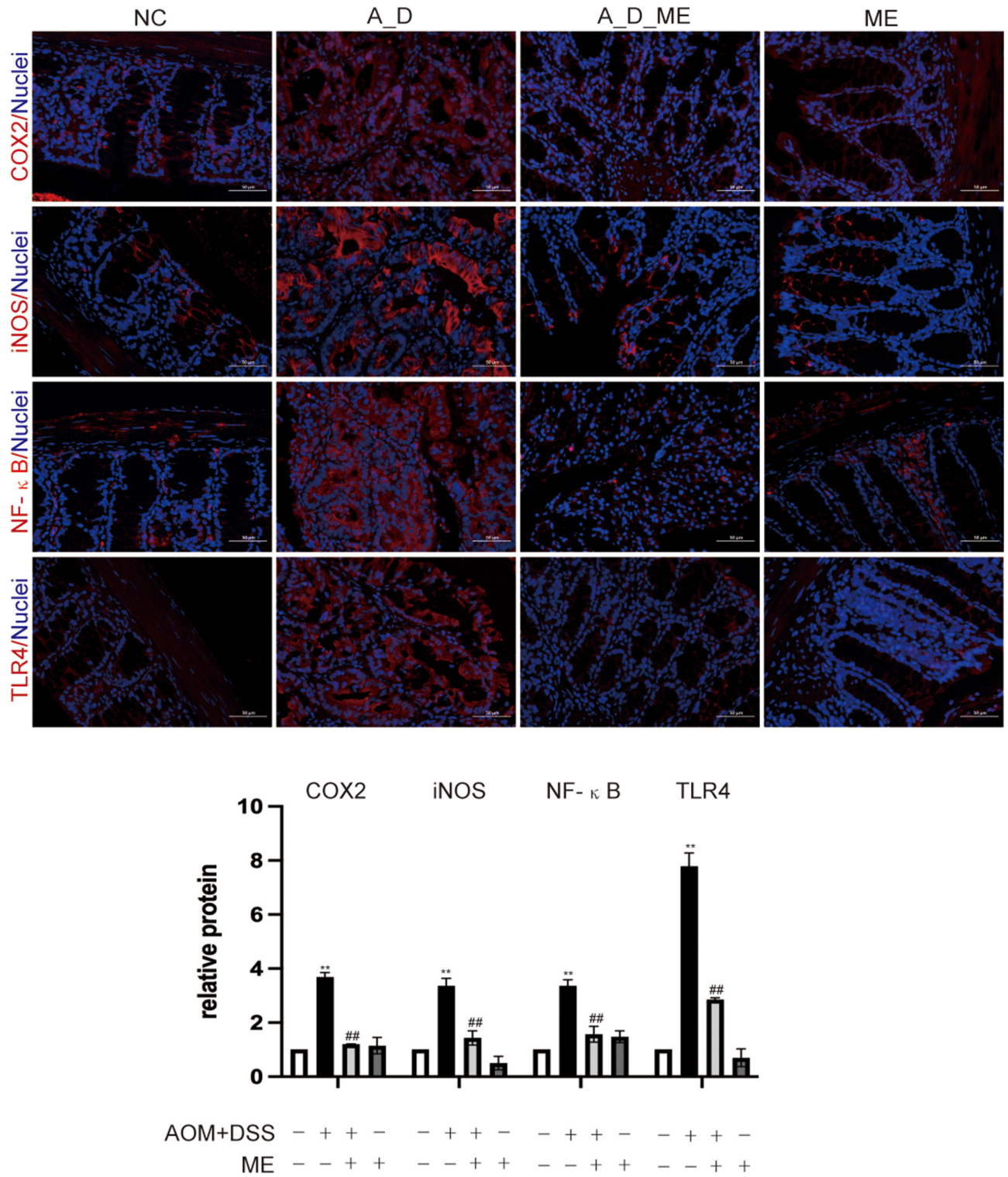

FIGURE 4
Aberrant regulation of TLR4 and NF-κB signaling pathway in the large intestinal tissues. COX2, iNOS, NF-κB, and TLR4 were assessed by immunofluorescence staining of the mice colonic slides in four groups (400× magnification). Histogram showing the percent of positive staining in the tissue. Data represent the mean ± SD, $^{\#\#}p < 0.01$ compared with normal control, $^{**}p < 0.01$ compared with the model group. $N = 6$/group.

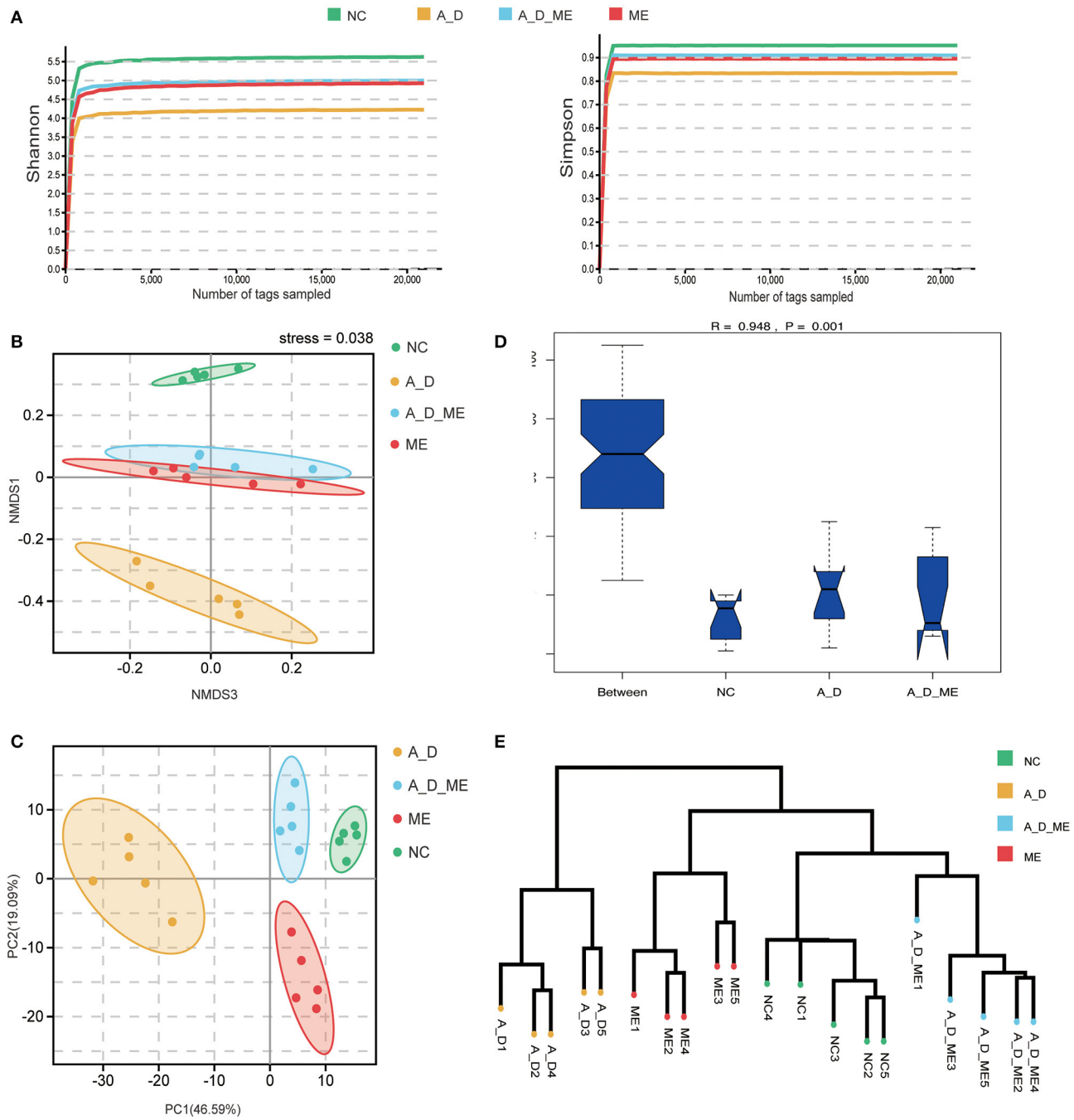

FIGURE 5
Effects of ME intragastric administration on microbial diversity in intestinal fetal, decided by 16s rRNA sequencing. **(A)** rarefaction curves of Shannon and Simpson index, **(B, C)** plots of nonmetric multidimensional scaling (NMDS) analysis, and Principal Component Analysis (PCA) based on weighted unifrac distance. The more similar the sample had, the closer the distance reflected in the plot. **(D)** boxplot of analysis of similarity (ANOSIM) based on weighted_unifrac among the three groups, NC vs. A_D R = 0.996 $p < 0.05$*, A_D vs. A_D_ME R=0.776, **$p < 0.01$, **(E)** UPGMA clustering method of all samples was classified on the basis of the unifrac-distance matrix, R=0.948, **$p < 0.01$. The more similar samples had shorter common branches.

3.6. Correlations between host fecal microbiota and metabolites

As shown in Figure 8, correlations between 4 ME-associated bacterial families and the top 20 of all the altered metabolites in the fecal were analyzed. For example, fecal Tetrandrine, Adrenosterone, and 2-heptyl-4-hydroxyquinoline n-oxide had positive relations with three ME-increased bacterial families, notably *Lactobacillaceae*, *Bifidobacteriaceae*, and *Eggerthellaceae*, but negative relations with ME-decreased bacterial family, *Erysipelotrichaceae*. In addition, *Erysipelotrichaceae* was not related to Gln-Gln-Arg, 4-Hexen-1-ol, 3beta-hydroxydeoxodihydrodeoxygedunin, Salvinonin a, Gly-pro-arg-pro-amide, Glycerol 3-phosphate, Salidroside, or

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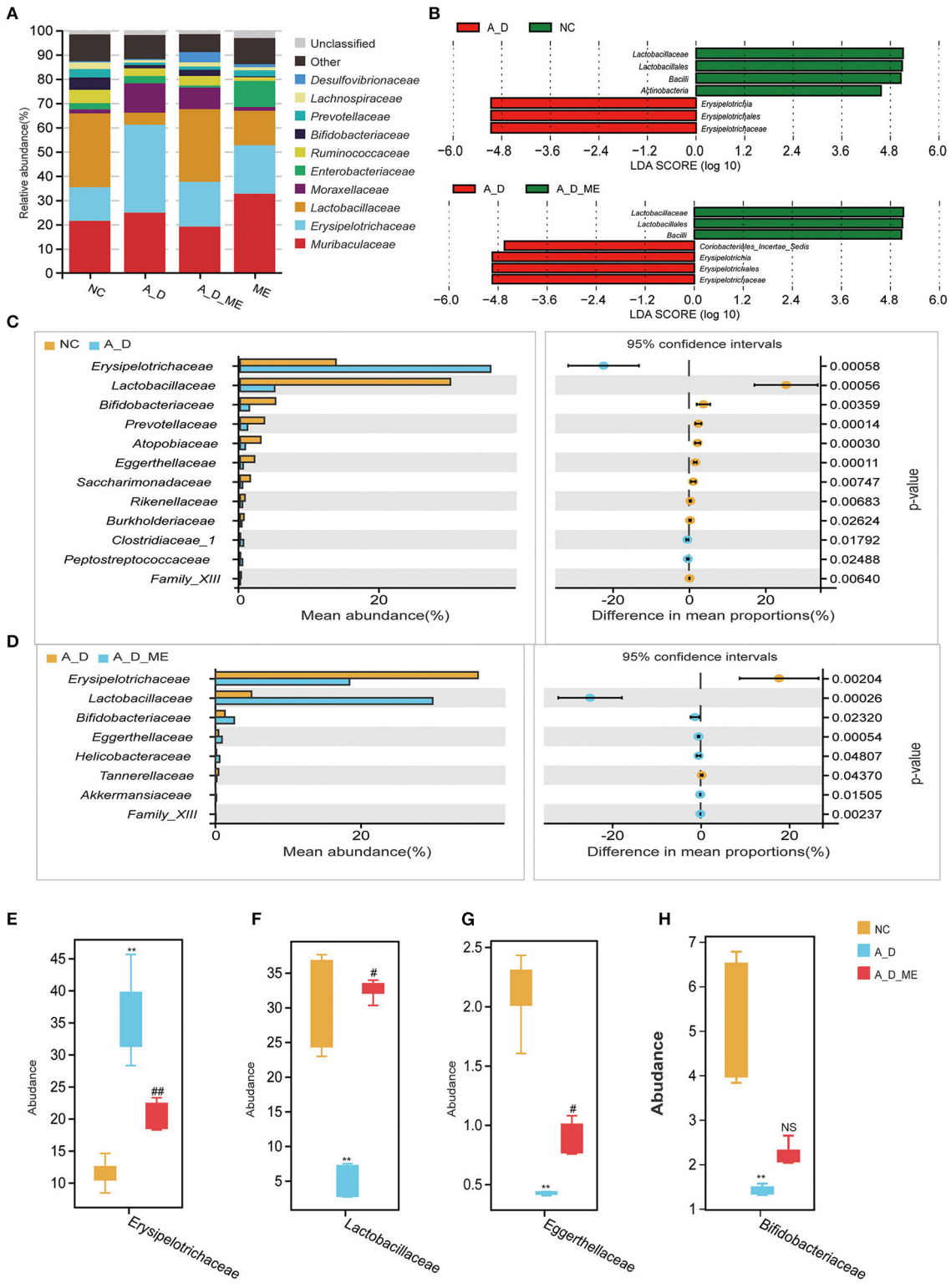

FIGURE 6
Effects of ME intragastric administration on the proportion of intestinal microbiota at the family level. **(A)** Each column in the histogram shows the relative abundance for one group and detailing the top 10 species among the mean abundance of each sample. The remaining unknown species and unclassified sequencing data were separately marked as "Other" and "Unclassified". **(B)** differences in biomarkers by means of taxonomic line discriminant analysis (LDA) effect size (LEfSe) method among the NC, A_D, A_D_ME groups. LDA scores indicated by the bar graph represent the effect of the different species at the family level. **(C, D)** differences of abundance at the family level in the NC group compared with the A_D group **(C)** and the A_D group compared with the A_D_ME group **(D)**, Welch's t-test is used to identify the difference between the two groups. Differentially abundant family according to ME administration. **(E–H)** Box plots are shown by mean data (SD) of the abundance ratio of four families (*Erysipelotrichaceae, Lactobacillaceae, Eggerthellaceae, and Bifidobacteriaceae* among five individuals in every three groups. Tukey's HSD test is used to analyze the differences. $N = 5$/group, * $p < 0.05$, *vs.* NC, ** $p < 0.01$, *vs.* NC; # $p < 0.05$, *vs.* A_D, ## $p < 0.01$, *vs.* A_D, ns means no significance.

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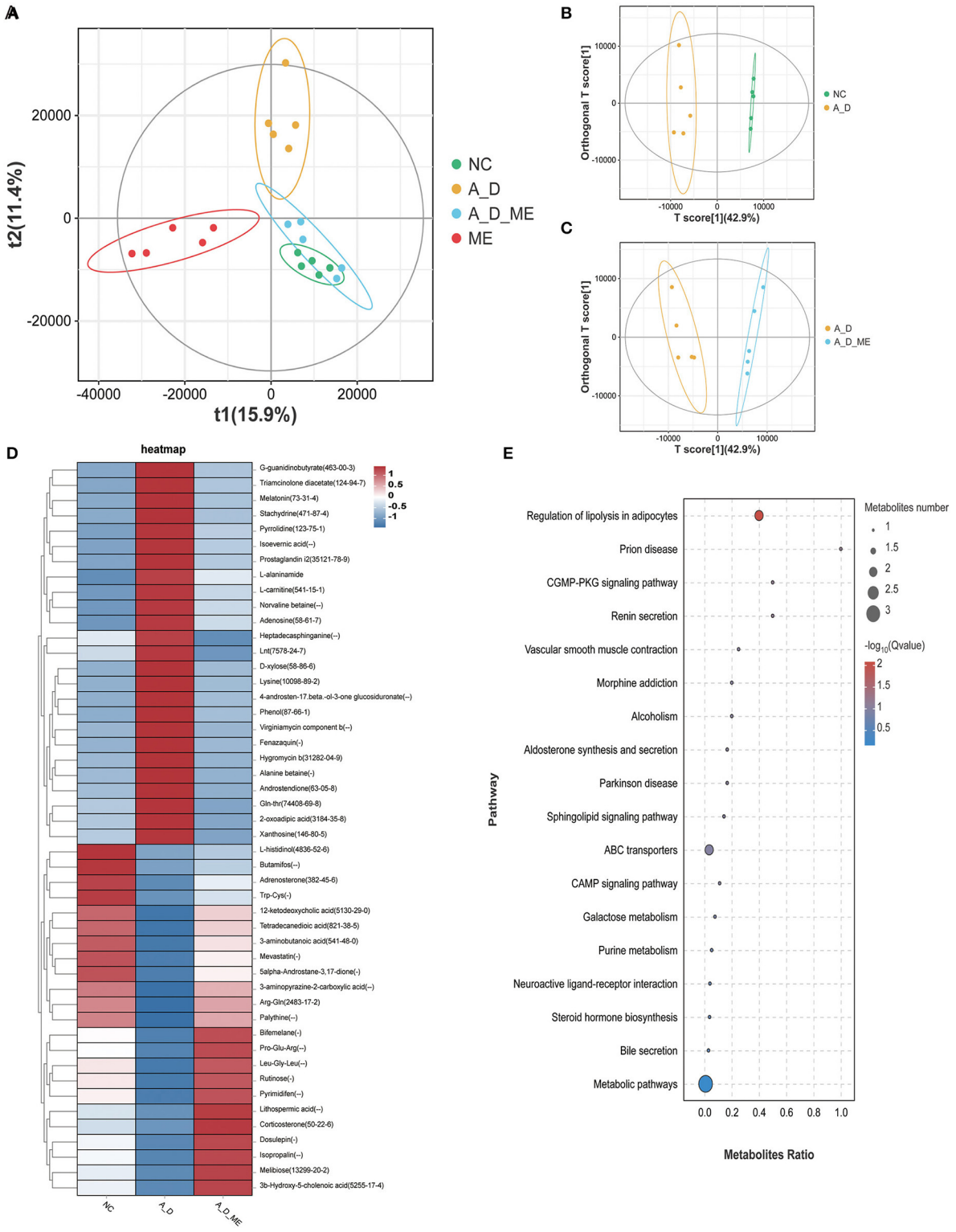

FIGURE 7
Alteration of ME administration on metabolomics in the fecal. score scatter plots for (PLS-DA) the model showed that almost all of the data is in 95% hotelling's T-squared ellipse. The X and Y axes are the scores of the first and second principal components **(A)**. Combining orthogonal signal correction (OSC) and PLS-DA, the X matrix information can be decomposed into two types of information related to Y and irrelevant. By removing the irrelevant differences, the relevant information is concentrated in the first predictive component (the predicted score of the X-axis). The Y axis represents the score of the main orthogonal component. An orthogonal projection to latent structures-discriminant analysis (OPLS-DA) model was employed to analyze and screen differential metabolites. NC vs. A_D **(B)**, A_D vs. A_D_ME **(C)**. **(D)** heatmap of 48 differential metabolites enriched in the three groups, **(E)** bubble map of KEGG enrichment pathway. The top 20 pathways with the lowest Q value are used to draw the map. The X axis is the pathway and the Y axis is the ratio of the metabolites (the number of differential metabolites in the pathway divided by all the numbers in the pathway). The size stands for the number and the color stands for the Q value. $N = 5$ mice per group.

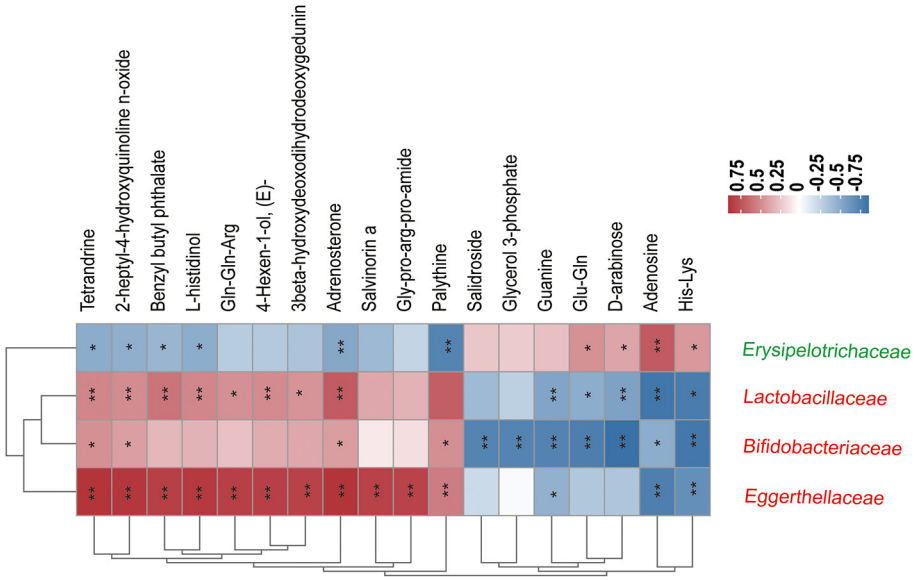

FIGURE 8
Correlation coefficients between the relative abundance of four ME-associated bacterial families and the top 20 fecal metabolites in the treated groups. The color depth of the grids depicted the strength of the correlation (red square= positive correlation, blue square = negative correlation. *$p < 0.05$, **$p < 0.01$). Increased and decreased relative abundance in AOM/DSS mice treated by ME were denoted by red and green tags, respectively.

Guanine, except for a positive relation with Gln-Gln, D-arabinose, Adenosine, and His-Lys. The data showed that host gut microbiota composition was identified to work on fecal metabolites.

4. Discussion

CRC fatality keeps rising worldwide, sometimes CRC is caused by genetic or familial basis (Siegel et al., 2020), but seldom the results from exclusively intestinal inflammation which was a double-edged sword in tumorigenesis (Schmitt and Greten, 2021). Though approximately 20% of IBD will be with CAC, more than 50% of them die of CAC, and it is one of the most worrisome complications of IBD (Nadeem et al., 2020). Data obviously showed that anti-inflammatory treatment with drugs was practical in preventing or alleviating colon cancer stages (Malik et al., 2018). As a result, it is urgent to develop precision medicine that delay or even stop the conversion from inflammation to cancer, as well as improve living quality with low toxicities. According to this strategy, ME was chosen as a new bioactive insect, owing to its multiple biological activities, especially its anti-inflammatory effects (Wang et al., 2021; Lema et al., 2022). Bioassay-guided fractionation was used to isolate antibacterial substances from the secretions of the living maggot body (Gao et al., 2015). The peptide compounds of maggot had been confirmed to influence the treatment of diabetic foot (Taowen et al., 2022). Medical maggots were also undertaken by therapeutic nurses at the ocular surface, with the attribution to the validation of peptide compounds (Bazalinski et al., 2022; Lema et al., 2022). Polysaccharide substances were extracted from the maggot body and induced the composition of GMB in high-fat diet mice (Wang et al., 2020b; Shi et al., 2022). Additionally, ME ameliorated intestinal fibrosis in DSS-induced chronic colitis (Wang et al., 2021). Our previous study reported the anti-cancer effects of ME in human ovarian cancer cells (Wang et al., 2022a). Moreover, its powder was investigated for therapeutic function by interrupting bacterial biofilm (Becerikli et al., 2022). The toxicity of altered intestinal and other major organs after ME administration was unlikely to appear because ME had no significant influences on clinic indicators and morphological features (Supplementary Figure S1).

In the present research, one of the key findings was that ME had superior positions in ameliorating AOM-induced and DSS-induced splenomegaly, colon length reduction, intestinal barrier damage, as well as inflammation of colon cells in mice. The results also inferred for the first time that ME revealed ideal prevention of intestinal dysbiosis in CAC mice, accompanied by and correlated with alterations in the composition of metabolites. Although specific ingredients of ME were not evaluated in this research, it still deserves further study.

The observed mucosal barrier changes were similar to previous reports on AOM/DSS-induced enteropathy (Li et al., 2019; Oh et al., 2020; Luan et al., 2022), mainly featured by losing the expression of ZO-1 and occluding and blooming release of inflammatory factors, IL-1β and -IL6 (Figure 3). In previous studies, DSS activated the TLR4-mediated signal pathways, afterward NF-κB phosphorylation cascade, to regulate the feedback of inflammatory factors, which was correlated with the tight junction protein (Sinha et al., 2020; Jin et al., 2021). This explained the increased levels of ZO-1 and occluding in a way, resulting from the participation of factors in promoting intestinal epithelial permeability. Since the activation of NF-κB leads to increasing transcription of abundant genes, that functioning in the aspects of immune responses, proinflammatory effects and cell apoptosis. (Jayandharan et al., 2011; Bessa-Goncalves et al., 2020). Indeed, one can predict that in some types of cells, the activated NF-κB may enhance tumor development but inhibit tumor incidences in

other types (Gao et al., 2021b; Mirzaei et al., 2021). Interestingly, our results confirmed that the enterocyte proliferation (ki67 and β-catenin) was reduced after the administration of ME, associated with less tumor burden (Figure 2). ME reversed the expressions of intestinal mucosal barrier markers occluding and ZO-1 in the CAC model by repairing the TLR4 cascade and decreasing inflammatory genes, therefore slowing tumor progression (Figure 4).

It was worth noting that the enterocytes had diametrically opposed influences on LPS from gram-negative bacteria for a long time. Since LPS can stimulate TLR4, which leads to initial immune responses in enterocytes, the role of TLR4 mediating the development of colitis-associated tumorigenesis has been established in the aspects of enhancing direct recruitment of NF-κB and the large increase in cytokines (Park et al., 2009; Olona et al., 2021). One way to activate the interaction between TLR4 and NF-κB and thereby drive LPS-dependent proinflammatory progression may be important for the severity of intestinal inflammatory response. The reports have provided a few examples that TLRs, interacting with endogenous ligands from the host, especially TLR4, are engaged in the process of infectious and non-infectious diseases (Tan et al., 2015). The present study revealed that ME had a repressive response to TLR4 using a chemical carcinogenesis model, and its inhibition suggested that TLR4-mediated NF-κB exerted an effect on tumor burden reduction. Consequently, downregulated expression of NF-κB contributed to the inhibition of COX-2 and iNOS (Figure 4). Wang et al. reported that ME repressed the Nrf2/NF-κB signaling pathway, including the production of downstream kinases in DSS-induced colitis (Wang et al., 2019, 2021). Furthermore, iNOS is an important enzyme and produces some compounds, involved in oxidative stress and inflammatory response (Cinelli et al., 2020). As far as we know,COX-2 catalyzes arachidonic acid into prostaglandin, acting as a mediator that cause pain or inflammation. The inhibitor of COX-2, such as 5-ASA and aspirin both successfully supported in chemoprevention, has been proven to be a key point to alleviate colonic inflammation even with CRC occurrence (Burn et al., 2011; Kaur et al., 2020). In this study, despite increasing productions of iNOS and COX-2 induced by AOM/DSS, ME pre-administration suppressed their proteins in the colon tissue of model mice, suggesting that ME could reduce chronic inflammation-associated tumor initiation by the inhibition of TLR4, NF-κB, iNOS, and COX-2. The relationships of TLR4-mediated NF-κB signal pathway in intestinal cells have been supported in many research studies; thus, various mechanisms have been established (Jin et al., 2021). One question still remains unclear that which types of cells act for ME-reversed inflammatory hallmarks in CAC.

Gastrointestinal cancer has proven to have connections with intestinal flora (Janney et al., 2020; DeDecker et al., 2021). Moreover, chronic inflammatory response and intestinal barrier damage might be related to dysbiosis in the gut flora as described above. Consistently, the enteropathy will tend to be recovered to normal condition via administration with probiotic bacteria directly (Suez et al., 2019; Samara et al., 2022). This study focused on the changing microbiome caused by ME pre-administration for 21 consecutive days in the initial phase of the model. At the end of the period, 16S rRNA analysis showed the improving relative abundance of the intestinal bacterium in the model treated with ME, and then, we identified four microbial families, namely *Erysipelotrichaceae, Lactobacillaceae, Bifidobacteriaceae,* and *Eggerthellaceae*, the last three probiotics of which were dramatically enriched (Figures 5, 6). The results also showed the inhibition of pro-inflammatory *Erysipelotrichaceae* and the acceleration of anti-inflammatory probiotics after ME administration. The family *Lactobacillaceae* has been reported to regulate the immune system, including the management of initial immune response, improvement of cellular and humoral immunity, and inhibition of pathogenic microorganisms (Lin et al., 2020). It has been reported that probiotics weaken the capability of proliferation in colon cells and prevented tumor migration or angiogenesis. Previous studies reported that pro-inflammatory cytokines, such as IL-1β, IL-6, IL-17, and IL-22, in the blood reduced significantly and were observed in a CRC patient trial with consumption of 6-month probiotics (IL-1 is required for tumor invasiveness and angiogenesis) (Samara et al., 2022).

The score plots of both PLS-DA and OPLS-DA revealed the changing metabolic profiles in fecal samples. Our study confirmed the disordered host microbiota and compositional-metabolomic fluctuations (48 chemical compounds included) after ME treatment, as a result of the prevention of CAC occurrence (Figures 7, 8). Metabolite pathways were also analyzed by topology programs, greatly differing between the A_D and A_D_ME groups. We hypothesized that pre-administration of ME could change the levels of metabolomics, contributing to the homeostasis of the gut microbiota. ME administration significantly increased products of fat metabolism, including 12-ketodeoxycholic acid, tetradecanedioic acid, 3-aminobutanoic acid, and 3-aminopyrazine-2-carboxylic acid in the fecal of AOM/DSS mice. Fecal secondary bile acids, 12-ketodeoxycholic acid included, were significantly higher during the ME supplementation periods. Data showed that secondary bile acids functioned in the regulation of cholesterol and lipid and the production of active oxygen and nitrogen (Reinicke et al., 2018; di Gregorio et al., 2021), as well as reducing the levels of cytokines engaged in inflammation (Sinha et al., 2020; Feng et al., 2022). Thus, we speculate that ME exacerbates bile acid metabolism and has an impact on gut homeostasis. Despite the role of Prostaglandin in CAC being controversial (Hirano et al., 2020), our analysis demonstrated that Prostaglandin I2 was significantly amplified in the model mice. In accordance with studies reported (Iwanaga et al., 2014; Wang et al., 2022b), Prostaglandin I2, as the product of Prostaglandin I synthase, was a chemo-preventive or antimitogenic agent in tumor angiogenesis or growth (Cathcart et al., 2010; Minami et al., 2015). Meanwhile, major urinary metabolic products of prostaglandin had been verified for its clinical benefits monitoring as a non-invasive biomarker in ulcerative colitis (Gao et al., 2021c). Notably, some metabolites (e.g., phenol in fecal samples) are most possibly toxic to bodies (Van Hecke et al., 2021). A significant loss of fecal phenol shown in the ME-treated and NC groups compared with model mice may be related to some bacterium shift in intestinal microbial composition. Adenosine, acting in many pathophysiological

processes, potentially mediates the proliferation through mutual effects with receptors. Interestingly, we found the reversed levels in ME-treated mice. The proliferative functions of adenosine may be active in our model.

In brief, ME protected against AOM/DSS-induced carcinoma by reducing intestinal inflammation, repairing intestinal barrier damage, restoring gut homeostasis, and linking the microbiota and metabolites. The data above suggested that ME administration might be a possible therapeutic strategy for CAC.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI - PRJNA924979.

Ethics statement

The animal study was reviewed and approved by the Institutional Animal Ethics Committee of Nanjing University (IACUC-2003031, 18 March 2020).

Author contributions

XT, LW, and GT: conceptualization and methodology. LT, DW, and YZ: validation and performing. XT and TW: analysis and writing. QW, ZZ, and YWe: supervision and review. FY and YWa: project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

Bajic, D., Niemann, A., Hillmer, A. K., Mejias-Luque, R., Bluemel, S., Docampo, M., et al. (2020). Gut microbiota-derived propionate regulates the expression of reg3 mucosal lectins and ameliorates experimental colitis in mice. *J. Crohns Colitis.* 14, 1462–1472.10.1093/ecco-jcc/jjaa065

Bazalinski, D., Przybek Mita, J., Scislo, L., and Wiech, P. (2022). Perception and readiness to undertake maggot debridement therapy with the use of lucilia sericata larvae in the group of nurses. *Int. J. Environ. Res. Public Health.* 19, 2895. doi: 10.3390/ijerph19052895

Becerikli, M., Wallner, C., Dadras, M., Wagner, J. M., Dittfeld, S., Jettkant, B., et al. (2022). Maggot extract interrupts bacterial biofilm formation and maturation in combination with antibiotics by reducing the expression of virulence genes. *Life (Basel).* 12, 27. doi: 10.3390/life12020237

Bessa-Goncalves, M., Silva, A. M., Bras, J. P., Helmholz, H., Luthringer-Feyerabend, B. J. C., Willumeit-Romer, R., et al. (2020). Fibrinogen and magnesium combination biomaterials modulate macrophage phenotype, NF-kB signaling and crosstalk with mesenchymal stem/stromal cells. *Acta Biomater.* 114, 471–484. doi: 10.1016/j.actbio.2020.07.028

Blackman, E. L., Ragin, C., and Jones, R. M. (2021). Colorectal cancer screening prevalence and adherence for the cancer prevention project of Philadelphia (CAP3) participants who self-identify as black. *Front. Oncol.* 11, 690718. doi: 10.3389/fonc.2021.690718

Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., et al. (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat Methods.* 10, 57–59. doi: 10.1038/nmeth.2276

Burn, J., Gerdes, A. M., Macrae, F., Mecklin, J. P., Moeslein, G., Olschwang, S., et al. (2011). Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. *Lancet.* 378, 2081–2087.10.1016/S0140-6736(11)61049-0

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat Methods.* 7, 335-6.10.1038/nmeth.f.303

Cathcart, M. C., Reynolds, J. V., O'Byrne, K. J., and Pidgeon, G. P. (2010). The role of prostacyclin synthase and thromboxane synthase signaling in the development and progression of cancer. *Biochim Biophys Acta.* 1805, 153-66.10.1016/j.bbcan.2010.01.006

Chang, Z. Y., Liu, H. M., Leu, Y. L., Hsu, C. H., and Lee, T. Y. (2022). Modulation of gut microbiota combined with upregulation of intestinal tight junction explains anti-inflammatory effect of corylin on colitis-associated cancer in mice. *Int. J. Mol Sci.* 23, 2667. doi: 10.3390/ijms23052667

Chen, B., Scurrah, C. R., McKinley, E. T., Simmons, A. J., Ramirez-Solano, M. A., Zhu, X., et al. (2021). Differential pre-malignant programs and microenvironment chart distinct paths to malignancy in human colorectal polyps. *Cell.* 184, 6262–6280 e26. doi: 10.1016/j.cell.2021.11.031

Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics.* 34, i884–i890. doi: 10.1093/bioinformatics/bty560

Chen, Y., Wang, B., Yuan, X., Lu, Y., Hu, J., Gao, J., et al. (2021). Vitexin prevents colitis-associated carcinogenesis in mice through regulating macrophage polarization. *Phytomedicine.* 83,153489. 10.1016/j.phymed.2021.153489

Chung, K. S., Cheon, S. Y., Roh, S. S., Lee, M., and An, H. J. (2018). Chemopreventive effect of aster glehni on inflammation-induced colorectal carcinogenesis in Mice. *Nutrients.* 10, 202. doi: 10.3390/nu10020202

Cinelli, M. A., Do, H. T., Miley, G. P., and Silverman, R. B. (2020). Inducible nitric oxide synthase: Regulation, structure, and inhibition. *Med. Res. Rev.* 40, 158–189. doi: 10.1002/med.21599

DeDecker, L., Coppedge, B., Avelar-Barragan, J., Karnes, W., and Whiteson, K. (2021). Microbiome distinctions between the CRC carcinogenic pathways. *Gut Microbes.* 13, 1854641. 10.1080/19490976.2020.1854641

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., et al. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol.* 72, 5069-72. doi: 10.1128/AEM.03006-05

di Gregorio, M. C., Cautela, J., and Galantini, L. (2021). Physiology and Physical Chemistry of Bile Acids. *Int J Mol Sci.* 22. doi: 10.3390/ijms22041780

Dooyema, S. D. R., Noto, J. M., Wroblewski, L. E., Piazuelo, M. B., Krishna, U., Suarez, G., et al. (2022). Helicobacter pylori actively suppresses innate immune nucleic acid receptors. *Gut Microbes.* 14, 2105102. doi: 10.1080/19490976.2022.2105102

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods.* 10, 996–998. doi: 10.1038/nmeth.2604

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., and Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics.* 27, 2194–200. doi: 10.1093/bioinformatics/btr381

Feng, P., Li, Q., Liu, L., Wang, S., Wu, Z., Tao, Y., et al. (2022). Crocetin prolongs recovery period of DSS-induced colitis via altering intestinal microbiome and increasing intestinal permeability. *Int J Mol Sci.* 23, 3832. doi: 10.3390/ijms23073832

Fong, W., Li, Q., and Yu, J. (2020). Gut microbiota modulation: a novel strategy for prevention and treatment of colorectal cancer. *Oncogene.* 39.4925-4943. 10.1038/s41388-020-1341-1

Gao, C. H., Yu, G., and Cai, P. (2021a). ggVennDiagram: an intuitive, easy-to-use, and highly customizable r package to generate venn diagram. *Front Genet.* 12, 706907. doi: 10.3389/fgene.2021.706907

Gao, J., Li, W., Niu, L., Cao, R., and Yin, W. (2015). Isolation and structural elucidation of novel antimicrobial compounds from maggots of Chrysomyis megacephala Fabricius. *Nat. Prod. Res.* 29, 239–246. 10.1080/14786419.2014.948875

Gao, J., Luo, T., and Wang, J. (2021b). Gene interfered-ferroptosis therapy for cancers. *Nat Commun.* 12, 5311. doi: 10.1038/s41467-021-25632-1

Gao, L., Yu, Q., Zhang, H., Wang, Z., Zhang, T., Xiang, J., et al. (2021c). A resident stromal cell population actively restrains innate immune response in the propagation phase of colitis pathogenesis in mice. *Sci Transl Med.* 13, eabb5071. doi: 10.1126/scitranslmed.abb5071

Gilligan, M. M., Gartung, A., Sulciner, M. L., Norris, P. C., Sukhatme, V. P., Bielenberg, D. R., et al. (2019). Aspirin-triggered proresolving mediators stimulate resolution in cancer. *Proc. Natl. Acad. Sci. U S A.* 116, 6292–6297. doi: 10.1073/pnas.1804000116

Guo, M., Wu, F., Hao, G., Qi, Q., Li, R., Li, N., et al. (2017). Bacillus subtilis improves immunity and disease resistance in rabbits. *Front Immunol.* 8, 354. doi: 10.3389/fimmu.2017.00354

Hirano, T., Hirayama, D., Wagatsuma, K., Yamakawa, T., Yokoyama, Y., and Nakase, H. (2020). Immunological mechanisms in inflammation-associated colon carcinogenesis. *Int J Mol Sci.* 21, 3062. doi: 10.3390/ijms21093062

Hoegh, A., and Roberts, D. W. (2020). Evaluating and presenting uncertainty in model-based unconstrained ordination. *Ecol. Evol.* 10, 59–69. doi: 10.1002/ece3.5752

Hua, H., Zhang, H., Kong, Q., Wang, J., and Jiang, Y. (2019). Complex roles of the old drug aspirin in cancer chemoprevention and therapy. *Med. Res. Rev.* 39, 114–145. doi: 10.1002/med.21514

Iqbal, M., Shah, M. D., Vun-Sang, S., Okazaki, Y., Okada, S., et al. (2021). The therapeutic potential of curcumin in alleviating N-diethylnitrosamine and iron nitrilotriacetate induced renal cell tumours in mice via inhibition of oxidative stress: implications for cancer chemoprevention. *Biomed Pharmacother.* 139, 111636. 10.1016/j.biopha.2021.111636

Iwanaga, K., Nakamura, T., Maeda, S., Aritake, K., Hori, M., Urade, Y., et al. (2014). Mast cell-derived prostaglandin D2 inhibits colitis and colitis-associated colon cancer in mice. *Cancer Res.* 74, 3011–3019. doi: 10.1158/0008-5472.CAN-13-2792

Janney, A., Powrie, F., and Mann, E. H. (2020). Host-microbiota maladaptation in colorectal cancer. *Nature.* 585, 509–517. doi: 10.1038/s41586-020-2729-3

Jayandharan, G. R., Aslanidi, G., Martino, A. T., Jahn, S. C., Perrin, G. Q., Herzog, R. W., et al. (2011). Activation of the NF-kappaB pathway by adeno-associated virus (AAV) vectors and its implications in immune response and gene therapy. *Proc Natl Acad Sci U S A.* 108, 3743–3748. doi: 10.1073/pnas.1012753108

Jin, B. R., Chung, K. S., Hwang, S., Hwang, S. N., Rhee, K. J., Lee, M., et al. (2021). Rosmarinic acid represses colitis-associated colon cancer: a pivotal involvement of the TLR4-mediated NF-kappaB-STAT3 axis. *Neoplasia.* 23, 561–573. doi: 10.1016/j.neo.2021.05.002

Kaur, L., Gordon, M., Baines, P. A., Iheozor-Ejiofor, Z., Sinopoulou, V., and Akobeng, A. K. (2020). Probiotics for induction of remission in ulcerative colitis. *Cochrane Database Syst. Rev.* 3, CD005573. doi: 10.1002/14651858.CD005573.pub3

Lema, C., Baidouri, H., Sun, M., Pohl, S., Cookson, S., Redfern, R., et al. (2022). Anti-inflammatory and wound healing potential of medicinal maggot excretions/secretions at the ocular surface. *Ocul. Surf.* 26, 244-254. doi: 10.1016/j.jtos.2022.09.003

Li, Z. W., Sun, B., Gong, T., Guo, S., Zhang, J., Wang, J., et al. (2019). GNAI1 and GNAI3 reduce colitis-associated tumorigenesis in mice by blocking il6 signaling and down-regulating expression of GNAI2. *Gastroenterology.* 156, 2297–2312. doi: 10.1053/j.gastro.2019.02.040

Lin, C. C., Huang, W. C., Su, C. H., Lin, W. D., Wu, W. T., Yu, B., et al. (2020). Effects of multi-strain probiotics on immune responses and metabolic balance in helicobacter pylori-infected mice. *Nutrients.* 12, 2476. doi: 10.3390/nu12082476

Liu, C., Cui, Y., Li, X., and Yao, M. (2021). Microeco: An R package for data mining in microbial community ecology. *FEMS Microbiol. Ecol.* 97, fiaa255. doi: 10.1093/femsec/fiaa255

Lozupone, C., and Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* 71, 8228–8235. doi: 10.1128/AEM.71.12.8228-8235.2005

Luan, Y., Zhang, Y., Yu, S. Y., You, M., Xu, P. C., Chung, S., et al. (2022). Development of ovarian tumour causes significant loss of muscle and adipose tissue: a novel mouse model for cancer cachexia study. *J. Cachexia Sarcopenia Muscle.* 13, 1289–1301. doi: 10.1002/jcsm.12864

Magoc, T., and Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics.* 27, 2957–2963. doi: 10.1093/bioinformatics/btr507

Malik, A., Sharma, D., Malireddi, R. K. S., Guy, C. S., Chang, T. C., Olsen, S. R., et al. (2018). SYK-CARD9 signaling axis promotes gut fungi-mediated inflammasome activation to restrict colitis and colon cancer. *Immunity.* 49, 515–530 e5. doi: 10.1016/j.immuni.2018.08.024

Minami, Y., Sasaki, T., Bochimoto, H., Kawabe, J., Endo, S., Hira, Y., et al. (2015). Prostaglandin I2 analog suppresses lung metastasis by recruiting pericytes in tumor angiogenesis. *Int. J. Oncol.* 46, 548–554. doi: 10.3892/ijo.2014.2783

Mirzaei, S., Zarrabi, A., Hashemi, F., Zabolian, A., Saleki, H., Ranjbar, A., et al. (2021). Regulation of nuclear factor-kappaB (NF-kappaB) signaling pathway by non-coding RNAs in cancer: Inhibiting or promoting carcinogenesis? *Cancer Lett.* 509, 63–80. doi: 10.1016/j.canlet.2021.03.025

Nadeem, M. S., Kumar, V., Al-Abbasi, F. A., Kamal, M. A., and Anwar, F. (2020). Risk of colorectal cancer in inflammatory bowel diseases. *Semin. Cancer Biol.* 64, 51–60. doi: 10.1016/j.semcancer.2019.05.001

Oh, N. S., Joung, J. Y., Lee, J. Y., Kim, Y. J., Kim, Y., and Kim, S. H. (2020). A synbiotic combination of Lactobacillus gasseri 505 and Cudrania tricuspidata leaf extract prevents hepatic toxicity induced by colorectal cancer in mice. *J. Dairy Sci.* 103, 2947–2955. doi: 10.3168/jds.2019-17411

Olona, A., Hateley, C., Muralidharan, S., Wenk, M. R., Torta, F., and Behmoaras, J. (2021). Sphingolipid metabolism during Toll-like receptor 4 (TLR4)-mediated macrophage activation. *Br. J. Pharmacol.* 178, 4575–4587. doi: 10.1111/bph.15642

Overacre-Delgoffe, A. E., Bumgarner, H. J., Cillo, A. R., Burr, A. H. P., Tometich, J. T., Bhattacharjee, A., et al. (2021). Microbiota-specific T follicular helper cells drive tertiary lymphoid structures and anti-tumor immunity against colorectal cancer. *Immunity.* 54, 2812–2824 e4. doi: 10.1016/j.immuni.2021.11.003

Park, B. S., Song, D. H., Kim, H. M., Choi, B. S., Lee, H., and Lee, J. O. (2009). The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature.* 458, 1191–1195. doi: 10.1038/nature07830

Reinicke, M., Schroter, J., Muller-Klieser, D., Helmschrodt, C., and Ceglarek, U. (2018). Free oxysterols and bile acids including conjugates - Simultaneous quantification in human plasma and cerebrospinal fluid by liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta.* 1037, 245–255. doi: 10.1016/j.aca.2018.02.049

Samara, J., Moossavi, S., Alshaikh, B., Ortega, V. A., Pettersen, V. K., Ferdous, T., et al. (2022). Supplementation with a probiotic mixture accelerates gut microbiome maturation and reduces intestinal inflammation in extremely preterm infants. *Cell Host. Microbe.* 30, 696–711 e5. doi: 10.1016/j.chom.2022.04.005

Sameni, H. R., Yosefi, S., Alipour, M., Pakdel, A., Torabizadeh, N., Semnani, V., et al. (2021). Co-administration of 5FU and propolis on AOM/DSS induced colorectal cancer in BALB-c mice. *Life Sci.* 276, 119390. 10.1016/j.lfs.2021.119390

Schmitt, M., and Greten, F. R. (2021). The inflammatory pathogenesis of colorectal cancer. *Nat. Rev. Immunol.* 21, 653–667. doi: 10.1038/s41577-021-00534-x

Shi, P., Zhao, T., Wang, W., Peng, F., Wang, T., Jia, Y., et al. (2022). Protective effect of homogeneous polysaccharides of Wuguchong (HPW) on intestinal mucositis induced by 5-fluorouracil in mice. *Nutr. Metab (Lond).* 19, 36. doi: 10.1186/s12986-022-00669-1

Siegel, R. L., Miller, K. D., Goding Sauer, A., Fedewa, S. A., Butterly, L. F., Anderson, J. C., et al. (2020). Colorectal cancer statistics, 2020. *CA Cancer J. Clin.* 70, 145–164. doi: 10.3322/caac.21601

Sinha, S. R., Haileselassie, Y., Nguyen, L. P., Tropini, C., Wang, M., Becker, L. S., et al. (2020). Dysbiosis-induced secondary bile acid deficiency promotes intestinal inflammation. *Cell Host Microbe.* 27, 659-670 e5. doi: 10.1016/j.chom.2020.01.021

Stutz, M. R., Dylla, N. P., Pearson, S. D., Lecompte-Osorio, P., Nayak, R., Khalid, M., et al. (2022). Immunomodulatory fecal metabolites are associated with mortality in COVID-19 patients with respiratory failure. *Nat. Commun*. 13, 6615. doi: 10.1038/s41467-022-34260-2

Suez, J., Zmora, N., Segal, E., and Elinav, E. (2019). The pros, cons, and many unknowns of probiotics. *Nat. Med.* 25, 716–729. doi: 10.1038/s41591-019-0439-x

Tan, Y., Zanoni, I., Cullen, T. W., Goodman, A. L., and Kagan, J. C. (2015). Mechanisms of toll-like receptor 4 endocytosis reveal a common immune-evasion strategy used by pathogenic and commensal bacteria. *Immunity*. 43, 909–922. doi: 10.1016/j.immuni.2015.10.008

Taowen, P., Shuyuan, F., Xiaoli, S., Annan, W., Feng, Q., Yizhong, Z., et al. (2022). Study on the action mechanism of the peptide compounds of Wuguchong on diabetic ulcers, based on UHPLC-Q-TOF-MS, network pharmacology and experimental validation. *J. Ethnopharmacol.* 288, 114974. doi: 10.1016/j.jep.2022.114974

Thieblemont, C., Bertoni, F., Copie-Bergman, C., Ferreri, A. J., and Ponzoni, M. (2014). Chronic inflammation and extra-nodal marginal-zone lymphomas of MALT-type. *Semin. Cancer Biol.* 24, 33–42. doi: 10.1016/j.semcancer.2013.11.005

Tilg, H., Zmora, N., Adolph, T. E., and Elinav, E. (2020). The intestinal microbiota fuelling metabolic inflammation. *Nat Rev Immunol.* 20, 40-54. doi: 10.1038/s41577-019-0198-4

Van Hecke, T., Vossen, E., Goethals, S., Boon, N., De Vrieze, J., and De Smet, S. (2021). In vitro and in vivo digestion of red cured cooked meat: oxidation, intestinal microbiota and fecal metabolites. *Food Res. Int.* 142, 110203. doi: 10.1016/j.foodres.2021.110203

Wang, D., Wang, R., Cai, M., Zhang, Y., Zhu, Z., Weng, Y., et al. (2022a). Maggot extract inhibits cell migration and tumor growth by targeting HSP90AB1 in ovarian cancer. *J. Clin. Med.* 11. doi: 10.3390/jcm11216271

Wang, L., Tang, L., Feng, Y., Zhao, S., Han, M., Zhang, C., et al. (2020a). A purified membrane protein from Akkermansia muciniphila or the pasteurised bacterium blunts colitis associated tumourigenesis by modulation of CD8(+) T cells in mice. *Gut.* 69, 1988–1997. doi: 10.1136/gutjnl-2019-320105

Wang, Q., Morris, R. J., Bode, A. M., and Zhang, T. (2022b). Prostaglandin pathways: opportunities for cancer prevention and therapy. *Cancer Res.* 82, 949–965. doi: 10.1158/0008-5472.CAN-21-2297

Wang, R., Luo, Y., Lu, Y., Wang, D., Wang, T., Pu, W., et al. (2019). Maggot extracts alleviate inflammation and oxidative stress in acute experimental colitis via the activation of Nrf2. *Oxid Med. Cell Longev.* 2019, 4703253. doi: 10.1155/2019/4703253

Wang, R., Wang, D., Wang, H., Wang, T., Weng, Y., Zhang, Y., et al. (2021). Therapeutic targeting of Nrf2 signaling by maggot extracts ameliorates inflammation-associated intestinal fibrosis in chronic DSS-induced colitis. *Front. Immunol.* 12, 670159. doi: 10.3389/fimmu.2021.670159

Wang, W., Zhong, M., Yu, T., Chen, L., Shi, L., Zong, J., et al. (2020b). Polysaccharide extracted from WuGuChong reduces high-fat diet-induced obesity in mice by regulating the composition of intestinal microbiota. *Nutr. Metab. (Lond).* 17, 27. doi: 10.1186/s12986-020-00442-2

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Metagenomic analysis reveals gut plasmids as diagnosis markers for colorectal cancer

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Background: Colorectal cancer (CRC) is linked to distinct gut microbiome patterns. The efficacy of gut bacteria as diagnostic biomarkers for CRC has been confirmed. Despite the potential to influence microbiome physiology and evolution, the set of plasmids in the gut microbiome remains understudied.

Methods: We investigated the essential features of gut plasmid using metagenomic data of 1,242 samples from eight distinct geographic cohorts. We identified 198 plasmid-related sequences that differed in abundance between CRC patients and controls and screened 21 markers for the CRC diagnosis model. We utilize these plasmid markers combined with bacteria to construct a random forest classifier model to diagnose CRC.

Results: The plasmid markers were able to distinguish between the CRC patients and controls [mean area under the receiver operating characteristic curve (AUC=0.70)] and maintained accuracy in two independent cohorts. In comparison to the bacteria-only model, the performance of the composite panel created by combining plasmid and bacteria features was significantly improved in all training cohorts (mean $AUC_{composite}$=0.804 and mean $AUC_{bacteria}$=0.787) and maintained high accuracy in all independent cohorts (mean $AUC_{composite}$=0.839 and mean $AUC_{bacteria}$=0.821). In comparison to controls, we found that the bacteria-plasmid correlation strength was weaker in CRC patients. Additionally, the KEGG orthology (KO) genes in plasmids that are independent of bacteria or plasmids significantly correlated with CRC.

Conclusion: We identified plasmid features associated with CRC and showed how plasmid and bacterial markers could be combined to further enhance CRC diagnosis accuracy.

KEYWORDS

metagenome, colorectal cancer, plasmid, biomarkers, diagnosis, gut microbiome

1. Introduction

Colorectal cancer (CRC) is the most common clinical malignant tumor of the digestive system and poses a huge threat to human health and society (Bray et al., 2018). Most CRC patients are diagnosed at an advanced stage and lose the opportunity for radical surgery (Di Nicolantonio et al., 2021). Prompt diagnosis of CRC is essential for effective treatment and favorable prognosis (Tomizawa et al., 2017). Colonoscopy and biopsy are currently considered the gold standard for the screening of CRC (Rex et al., 2006). Fecal occult blood test (FOBT) is non-invasive and the most commonly used method for colorectal cancer screening currently (Faivre et al., 2004; Lee et al., 2020). The specificity of FOBT for CRC detection was 92.4%, but the sensitivity was only 30.8% (Allison et al., 1996). Due to its dependence on tumor tissue

bleeding, FOBT has limited sensitivity and accuracy for CRC (Hardcastle et al., 1996). Therefore, there is an urgent need for reliable and efficient biomarkers for the diagnosis of colorectal cancer.

With the development of metagenomic technology, an increasing number of recent studies have highlighted the vital role of the gut microbiome in regulating human health and disease (Ghaisas et al., 2016; Schmidt et al., 2018; Gurung et al., 2020). The gut microbiome may have an impact on the onset and development of CRC (Zamani et al., 2019), while some intestinal bacteria may slow the disease's progression (Chan et al., 2019). The efficacy of gut bacteria as diagnostic biomarkers for CRC has been confirmed (Dai et al., 2018; Liu et al., 2022).

Plasmids play important roles in the evolutionary events of microbial communities, and many plasmid genes are involved in bacterial survival and adaptation to environmental changes (Fondi et al., 2010; Dib et al., 2015). Many bacteria can exchange genetic material through horizontal gene transfer, which is facilitated by plasmids and transposable elements carried by plasmids (Smalla and Sobecky, 2002). It indicates that plasmids should not be disregarded in research. Plasmidomics refers to the whole plasmid DNA of the samples (Brown Kav et al., 2012; Bleicher et al., 2013). With the advancement of next-generation sequencing technology and the development of bioinformatics tools, numerous methods were developed for identifying plasmid sequences in metagenomic data, such as Plasflow (Krawczyk et al., 2018), Plasmidseeker (Roosaare et al., 2018), PlasmidFinder (Carattoli et al., 2014), SCAPP (Pellow et al., 2021), and cBar (Zhou and Xu, 2010). For short-reads metagenomic sequencing, PlasFlow software based on deep neural networks is the way of maximizing plasmid coverage and minimizing false positives currently (Hilpert et al., 2021). With the help of these techniques, we can examine how intestinal plasmids and plasmid genes change during diseases.

Many human diseases are closely associated with plasmids, particularly those involving antibiotic resistance genes and virulence genes (Cheung et al., 2004; Dolejska and Papagiannitsis, 2018). Enterotoxigenic *Escherichia coli* (ETEC) causes numerous cases of diarrheal disease worldwide, which is linked to the virulence plasmid pEntYN10 within ETEC (Ban et al., 2015). Emerging research points to the significance of other microbial kingdoms in gastrointestinal disease in addition to gut bacteria (Liu et al., 2022), but no studies on intestinal plasmids in CRC patients have been explored. The primary goal of this study is to examine the key characteristics of the plasmids in the gut microbiomes of CRC patients from eight cohorts worldwide. We seek to expand existing CRC diagnosis biomarkers and develop a more precise diagnosis model using newly discovered plasmid biomarkers.

2. Methods

2.1. Public data collection

We used the terms "Colorectal cancer" and "Human gut metagenomics" to search the NCBI database,[1] and we found a total of nine CRC gut metagenomic cohorts. We excluded the Italian cohort (PRJNA447983) since we were unable to determine the case–control status that matched the sequencing data in that dataset. We selected an Asian cohort from China and a European cohort from Germany as independent validation datasets, and the other six cohorts as training datasets, to ensure the reliability and generalizability of the prediction model. We downloaded fecal metagenomic sequencing data of the eight cohorts in NCBI on CRC patients and healthy controls (Supplementary Table 1). For discovery cohorts ($n = 1{,}123$), Accession of China Cohort1 (CHN1) is PRJNA763023 (Yang et al., 2021), CRC, $n = 100$; and Control, $n = 100$. Accession of China Cohort2 (CHN2) is PRJNA731589 (Liu et al., 2022), CRC, $n = 80$; and Control, $n = 86$. Accession of Japan (JPN) is PRJDB4176 (Yachida et al., 2019), CRC, $n = 218$; and Control, $n = 212$. Accession of Austria (AUS) is PRJEB7774 (Feng et al., 2015), CRC, $n = 46$; and Control, $n = 63$. Accession of France (FRA) is PRJEB6070 (Zeller et al., 2014), CRC, $n = 53$; and Control, $n = 61$. Accession of the United States of America (USA) is PRJEB12449 (Vogtmann et al., 2016), CRC, $n = 52$; and Control, $n = 52$. For validation cohorts ($n = 119$), Accession of China Cohort3 (CHN3) is PRJNA514108 (Gao et al., 2022), CRC, $n = 32$; and Control, $n = 44$. Accession of Germany (GER) is PRJEB6070 (Zeller et al., 2014), CRC, $n = 38$; and Control, $n = 5$. The cohorts' characteristics are listed in Supplementary Table 1.

2.2. Sequencing data processing

KneadData[2] v0.7.4 was used to obtain high-quality microbial reads. The metagenomic shotgun sequencing data were trimmed using Trimmomatic (Bolger et al., 2014; v0.39) with the following parameters: SLIDINGWINDOW:4:20 MINLEN:50. Then, human reads were mapped to hg37 human reference genome and discarded by bowtie2 (v.2.4.3; —very-sensitive --dovetail; Langmead and Salzberg, 2012). High-quality reads were used to conduct species-level community profiling with relative abundance by MetaPhlAn2 (v2.8.1) using the setting "-a" to determine all taxonomic level (Truong et al., 2015). Quality-controlled reads were assembled into contigs with Megahit (v.1.2.9) using the default parameters: "--min-contig-len 200, —disconnect-ratio 0.1" (Li et al., 2015). PlasFlow was run with a minimum posterior probability of 0.7 to filter plasmid contigs longer than 1,000 bp (Hilpert et al., 2021). We compared the plasmid contigs to the NCBI plasmid reference sequence database (accessed on 2021-06-28) by using BLAST (Altschul et al., 1990; v 2.11) with an *E*-value of 10^{-5} and coverage of 50% as the cut-off. The plasmid genes were predicted by Prodigal (Hyatt et al., 2010) via the metagenome mode. CD-HIT (Fu et al., 2012; v4.8.1) was used to create a non-redundant plasmid gene catalog, with an identity cut-off of 0.95 and a coverage cut-off of 90%. The plasmid gene catalog was annotated with EggNOG mapper (Cantalapiedra et al., 2021; v.2.1.5) based on EggNOG DB (Huerta-Cepas et al., 2019; v5.02). The carbohydrate-active enzymes (CAZy) genes were identified using run_dbcan (v2.0.11; Zhang et al., 2018). Moreover, the relative abundance of plasmid and plasmid genes was determined using salmon (Patro et al., 2017; v.1.5.2) with settings "--meta."

1 https://www.ncbi.nlm.nih.gov/

2 http://huttenhower.sph.harvard.edu/kneaddata

2.3. Annotation of plasmid

Host taxa information for plasmids was obtained from the NCBI plasmid reference. Antibiotic resistance genes were annotated through the ResFinder database (Bortolaia et al., 2020; https://cge.cbs.dtu.dk/services/ResFinder/) by BLAST (*E* value, $<10^{-5}$; identity, >80%). The oriT regions and relaxase genes were identified based on the oriTDB database (Li et al., 2018; https://bioinfo-mml.sjtu.edu.cn/oriTDB/) by BLAST (*E* value, $<10^{-5}$; identity, >80%). It was determined that plasmids containing both the oriT region and relaxase gene are conjugative plasmids (Smillie et al., 2010).

2.4. Microbial ecological analysis

For each sample, Shannon metrics of plasmids were used to calculate alpha diversity. The Bray-Curtis distance was used to calculate the beta diversity. Using the "Vegan" R package (v 2.6–2) in R software (Jari Oksanen et al., 2022), Shannon's index for each sample and the Bray-Curtis distance between samples was both evaluated. Using principal coordinates analysis (PCoA), the Bray–Curtis dissimilarity index was used to visualize the microbial community structures. Permutational multivariate ANOVA (PERMANOVA) was performed to reveal the plasmid community differences between groups or cohorts with 999 permutations (Anderson, 2001).

2.5. Feature selection

Plasmid community batch effects among cohorts were corrected using the "adjust_batch" function of the MMUPHin R package (v 2.6-2; Ma et al., 2022). We identified differential plasmids as candidate features for the CRC diagnosis models with the "lm_meta" function of MMUPHin. Subsequently, feature selection was performed using the package Boruta (Miron and Kursa, 2010; v7.0.0) with default settings (pValue = 0.01, mcAdj = T, maxRuns = 100). Differential EggNOG gene KOs, CAZY, and bacteria species were selected with the same pipeline.

2.6. Prediction model construction and validation

Random forest prediction model was constructed using "random forest" R package with 500 trees (Breiman, 2001). Based on differential plasmids and bacteria signatures, the random forest prediction model for CRC was trained with 10-fold cross-validation on the discovery cohorts. Model evaluation was performed with cohort-to-cohort transfer validation, leave-one-cohort-out (LOCO) evaluation, and independent validation. In cohort-to-cohort validation, the models were trained on a single cohort and their performances were evaluated in the other cohorts. *In LOCO* evaluation, the models were trained on five of the six cohorts in the discovery dataset and their performances were evaluated on the sixth cohort. Furthermore, an independent validation analysis was conducted in order to assess the reliability of microbial features as CRC diagnostic markers, and two additional datasets from CHN3 and GER were used in the process.

2.7. Associations between species and function

Associations between bacteria, plasmids, and their KO genes were performed by Spearman correlation using the "corAndPvalue" function of the "WGCNA" R package (Langfelder and Horvath, 2008).

2.8. Statistical analysis

All statistical analyses were conducted by R software (v 4.1.2, the R Project for Statistical Computing). In order to compare the two groups, Wilcoxon rank-sum test was used. Correlations were calculated using Spearman's rank correlation. The Benjamini-Hochberg method was used to adjust *p* values for multiple testing to account for the false discovery rate (FDR). *p* value <0.05 is considered statistically significant.

3. Results

3.1. Characterization of CRC cohorts

We gathered metagenomic data from 1,242 samples across eight publicly available CRC cohorts worldwide (Supplementary Table 2). We included six of these cohorts as discovery cohorts to identify gut plasmids as biomarkers for CRC diagnosis, consisting of 549 CRC patients and 574 tumor-free controls from five countries (China, CHN1 and CHN2; Japan, JPN; Austria, AUS; France, FRA; and the United States, USA). As a result, the independent validation dataset, which comprised 70 CRC patients and 49 tumor-free controls from two countries, was created (China, CHN3 and Germany, GER). The bioinformatics analysis of all raw shotgun sequencing data was conducted consistently to reduce technical bias.

3.2. Alteration of the intestinal plasmids in CRC patients

In the discovery cohorts, we identified a total of 12,515 plasmids using metagenomic approaches. Only 628 plasmids were present in all six cohorts, with more cohort-specific plasmids being found in CHN1, CHN2, and JPN cohorts (Figure 1A). We found that Proteobacteria and Firmicutes phylas made up the majority of the host taxa for each cohort of plasmids, and that there were no differences in these proportions between CRC patients and healthy controls. However, compared to other cohorts, a greater percentage of plasmids in the US cohort had Bacteroidetes phyla as their host (Figure 1B). We found no discernible differences in the proportion of plasmids between CRC patients and controls, although a smaller portion of the identified plasmids were conjugative or carried antibiotic-resistance genes (Supplementary Figure 1).

We then assessed differences in intestinal plasmid alpha diversity between CRC patients and controls. According to the Shannon index in the discovery cohorts, we observed increased plasmid alpha diversity in CRC patients ($p = 0.015$; Figure 1C). Meanwhile, geographic differences are visible in intestinal plasmid alpha diversity (Supplementary Figure 2). The difference in intestinal plasmid alpha

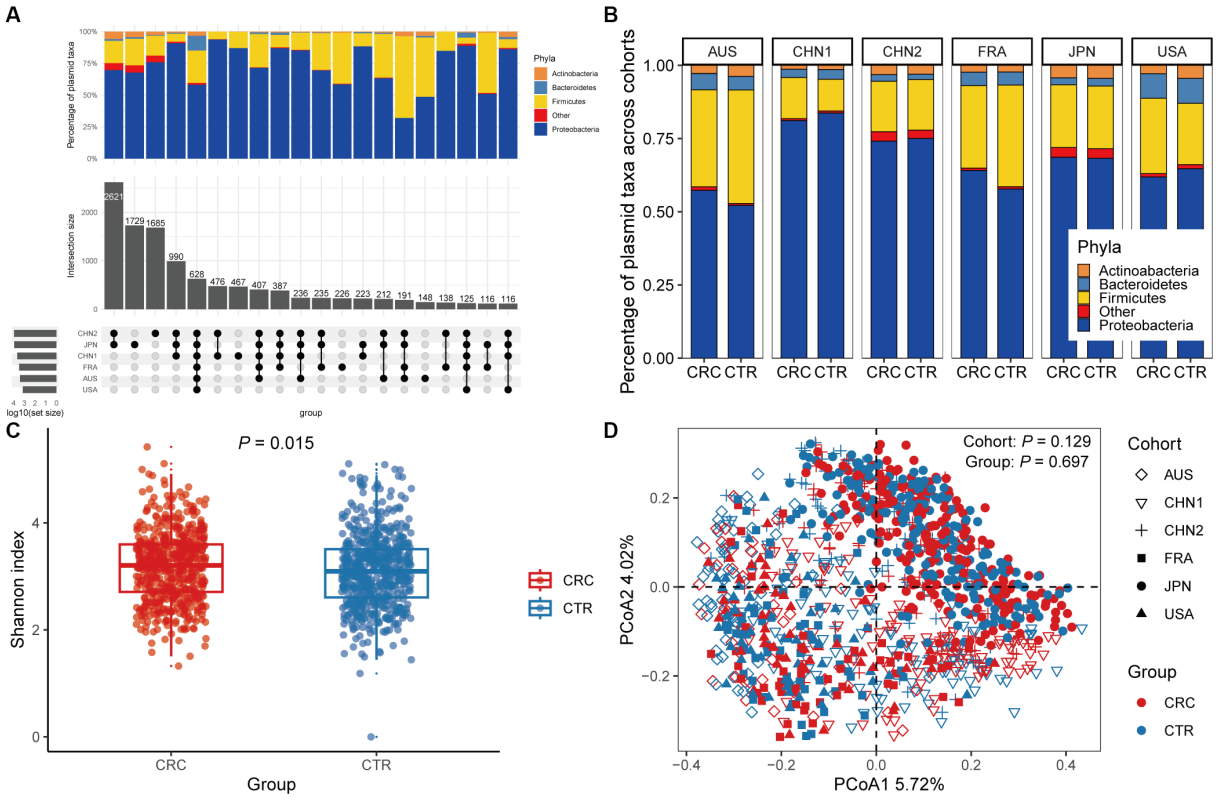

FIGURE 1
The gut plasmid comparison of patients with colorectal cancer (CRC) and controls. **(A)** Upset plot for host taxa of plasmids per cohort. There are a total of 12,515 plasmids observed across six discovery cohorts. **(B)** Stacked column chart showing the proportion of host taxa of plasmids per cohort. **(C)** Alpha diversity measured by the Shannon index of the gut plasmid of patients with CRC (red, n=549) and control individuals (blue, n=574; Wilcoxon rank-sum test, p=0.015). Boxplots indicate medians (horizontal line in the box), interquartile (boxes), and ranges (whiskers). **(D)** Principal coordinate analysis (PCoA) of samples from all six cohorts based on Bray–Curtis distance, which shows that microbial composition was not different between groups (p=0.697) and cohorts (p=0.129). p values of beta diversity based on Bray–Curtis distance corresponds to Adonis PERMANOVA tests by 999 permutations (two-sided test). The cohort is shape-coded while the group is color-coded.

diversity between CRC patients and healthy controls was only found in the CHN1 cohort (p = 0.03). In other cohorts, the intestinal plasmid alpha diversity between CRC patients and healthy controls was not significantly different (Supplementary Figure 2). Based on the analysis of beta diversity, the beta diversity of intestinal plasmids was not associated with CRC (p = 0.129, Figure 1D), nor was there a significant difference between cohorts (p = 0.697; Figure 1D).

3.3. Plasmid biomarkers for CRC diagnosis

We conducted a meta-analysis of six datasets from the discovery cohort in order to find plasmids that could be used as diagnostic markers for CRC. After that, we discovered 198 plasmids that had different abundances in patients with CRC and controls (Supplementary Table 3), 108 of which were highly abundant in the guts of CRC patients (p < 0.05), and 90 of which were decreased in the guts of CRC patients (p < 0.05). To screen out plasmid signatures for diagnosing CRC, we performed further signature selection on these 198 plasmids using Boruta. We screened 21 plasmids, of which 13 (including NZ_CP036554.1) were more prevalent in CRC patients and eight (including NZ_AP023416.1) were less prevalent in CRC patients (Figure 2A). We first trained the random forest classifier with the 21 plasmid features in each dataset used 20 times repeated 10-fold cross-validation to assess the diagnostic accuracy of the plasmid features for diagnosing CRC. Depending on the region, the plasmid random forest classifier performed differently. The plasmid random forest classifier demonstrated strong predictive power in the CHN1, CHN2, and FRA cohorts, with mean AUC ranging from 0.75 to 0.80 across cohorts that were 20 times repeated using 10-fold cross-validation. In contrast, the plasmid random forest classifier performs worse in JPN (AUC, 0.58), AUS (AUC, 0.67), and USA (AUC, 0.62) datasets (Figure 2B).

We conducted cohort-to-cohort validation and leave-one-cohort-out (LOCO) validation on the training cohorts to evaluate the geographical robustness of plasmid signatures as a universal biomarker. In cohort-to-cohort validation, the mean AUC of the plasmid random forest model ranged from 0.51 to 0.75 (Figure 2C). The LOCO performance of the plasmid model ranged from 0.59 to 0.71 (Figure 2D). To further test predictive performance, the plasmid classifiers trained within study cross-validation were applied to two independent validation sets. In the CHN3 and GER cohorts, the model's average AUC was 0.79 and 0.66, respectively (Figure 2E).

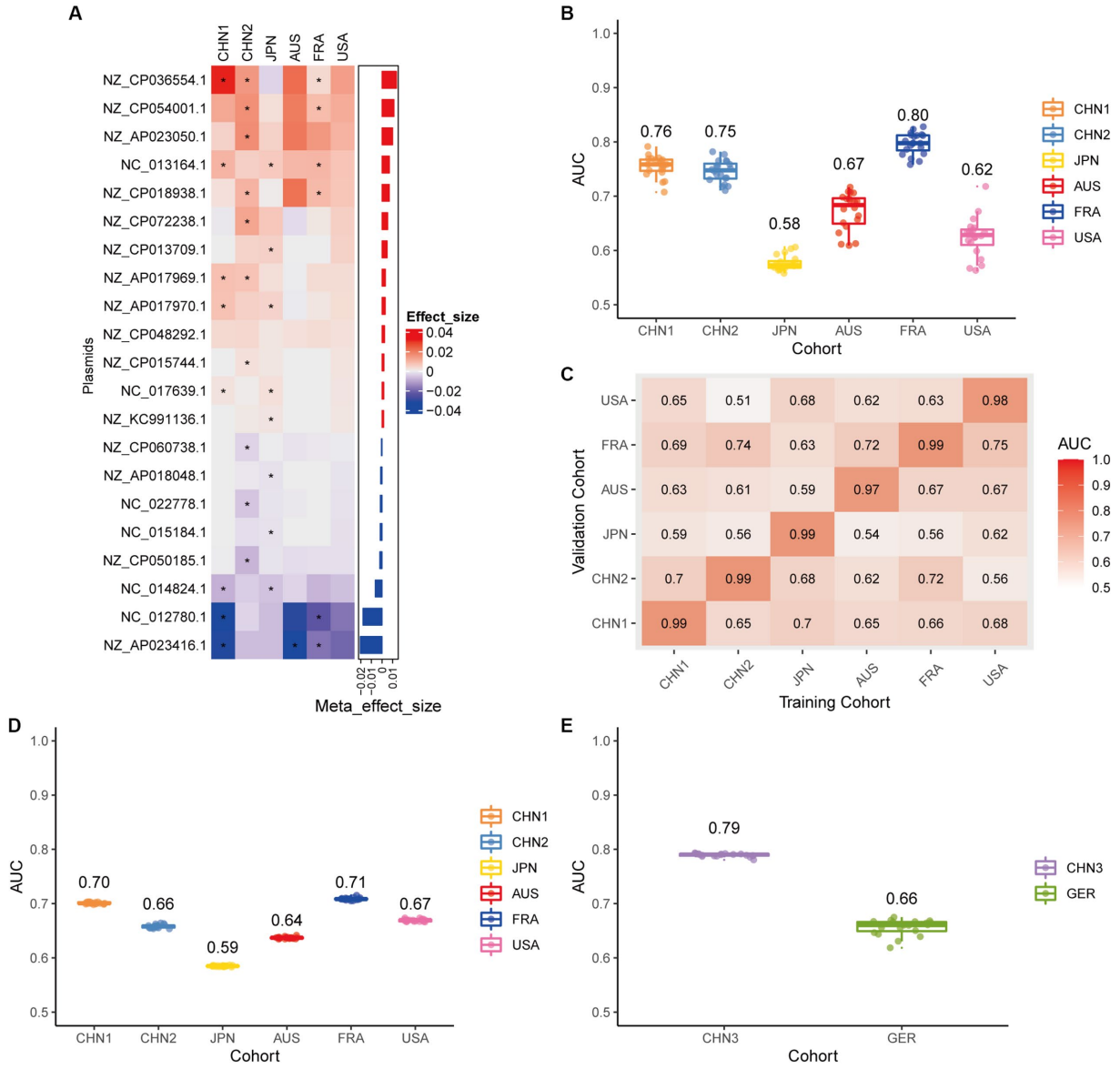

FIGURE 2
Plasmid metagenomic classification models generalize across different cohorts. **(A)** Bar plot of the 21 plasmid features' effect sizes for the prediction of CRC diagnosis, as determined by MMUPHin and Boruta. The significance of the difference between patients with CRC and controls was determined via Wilcoxon rank-sum test: *p<0.05. **(B)** CRC classification performances (AUC) calculated through the cohort-to-cohort model transfer for the random forest classifier trained on relative abundance profiles of plasmids. The values refer to an average value of 20 times repeated 10-fold cross-validation. **(C)** CRC classification performances (AUC) calculated through 20 times repeated 10-fold cross-validation within each study for the random forest classifier trained on relative abundance profiles of plasmids. **(D)** CRC classification performances (AUC) calculated through leave-one-cohort-out validation (LOCO, Model was trained using five of six cohorts and validated by the other one) for random forest classifier trained on relative abundance profiles of plasmids. **(E)** Validation of the plasmid random forest classifier in two independent cohorts (CHN3 and GER). The CRC classification performances (AUC) of the plasmid random forest classifier trained with all the training cohorts were obtained in the CHN3 and GER cohorts.

3.4. Improved predictability based on a combination of plasmid and bacterial features

Using the same pipeline as plasmids, 91 differential bacteria species were identified (p < 0.05), and 39 of them were extracted as biomarkers for the diagnosis of CRC (Supplementary Figure 3A; Supplementary Table 4). Previous studies have demonstrated a strong link between gut bacteria and the occurrence and progression of CRC (Sang et al., 2020; Yinhang et al., 2022). Bacterial classifiers are effective at detecting CRC (Wirbel et al., 2019). The bacterial random forest classifier performed admirably in diagnosing CRC in our study. The bacteria random forest classifier showed strong predictive power within cohorts, with a mean AUC ranging from 0.81 to 0.93 except for the JPN (0.68) and USA (0.63) cohorts due to the distinct food culture of Japanese and the prolonged cryopreservation of fecal specimens in USA cohort, respectively (Supplementary Figure 3B). The cohort-to-cohort validation (Supplementary Figure 3C) and LOCO validation had similar outcomes (Supplementary Figure 3D). In independent validation, the average AUC of the model obtained in the CHN3 and

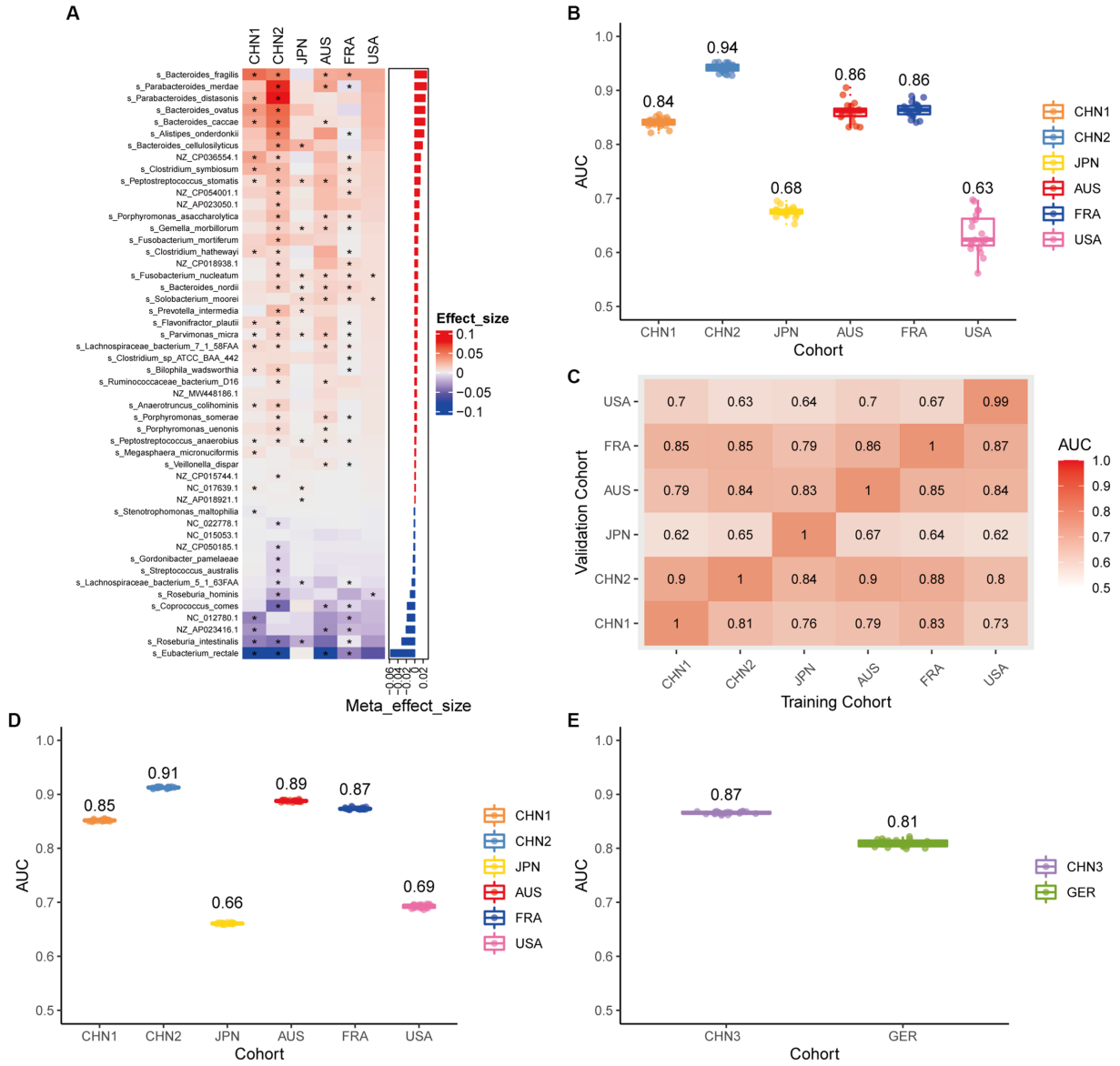

FIGURE 3
Bacterial metagenomic classification models generalize across different cohorts. **(A)** Bar plot of the 50 plasmid and bacterial features' importance for the prediction of CRC diagnosis, as determined by MMUPHin and Boruta. The significance of the difference between patients with CRC and controls was determined via Wilcoxon rank-sum test: *p<0.05. **(B)** CRC classification performances (AUC) calculated through the cohort-to-cohort model transfer for the random forest classifier trained on relative abundance profiles of plasmid and bacterial species. The values refer to an average value of 20 times repeated 10-fold cross-validation. **(C)** CRC classification performances (AUC) calculated through 20 times repeated 10-fold cross-validation within each study for the random forest classifier trained on relative abundance profiles of plasmid and bacterial species. **(D)** CRC classification performances (AUC) calculated through leave-one-cohort-out validation (LOCO, Model was trained using two of three cohorts and validated by the other one) for random forest classifier trained on relative abundance profiles of plasmid and bacterial species. **(E)** Validation of the plasmid and bacterial random forest classifier in two independent cohorts (CHN3 and GER). The CRC classification performances (AUC) of the plasmid and bacterial random forest classifier trained with all the training cohorts were obtained in the CHN3 and GER cohorts.

GER cohorts were 0.84 and 0.86, respectively (Supplementary Figure 3E). We investigated whether creating a diagnostic panel with plasmids and bacterial species would result in better performance. 13 plasmids and 37 bacteria made up the panel after feature screening (Figure 3A). 10 of the 37 bacteria have also been linked to CRC in previous studies, including *Parvimonas micra*, *Peptostreptococcus stomatis*, *Prevotella intermedia*, *Porphyromonas asaccharolytica*, *Porphyromonas somerae*, *Porphyromonas uenonis*, *Gemella morbillorum*, *Fusobacterium nucleatum*, *Roseburia hominis*, and *Roseburia intestinalis* (Wirbel et al., 2019; Liu et al., 2022). The 10-fold cross-validation AUC scores for the various cohorts were 0.84 for CHN1, 0.94 for CHN2, 0.68 for JPN, 0.86 for AUS, 0.86 for FRA, and 0.63 for USA (Figure 3B). The model showed valuable prediction performance in cohort-to-cohort validation (Figure 3C) and LOCO validation (Figure 3D). The average AUC of the model obtained in the CHN3 and GER cohorts during independent validation was 0.87 and 0.81, respectively (Figure 3E). In all training cohorts (Composite model, AUC = 0.804; Bacterial model, AUC = 0.787) and all independent cohorts (Composite model, AUC = 0.839; Bacterial

model, AUC = 0.821), the prediction performance of the composite panel by combining the plasmid and bacterial features was significantly better than the bacteria-only model was significantly improved (Figure 4). In comparison to the bacteria-only model, the average AUROC of the cross-validation models with the combined panel for all independent cohorts was 0.88 (Supplementary Figure 4).

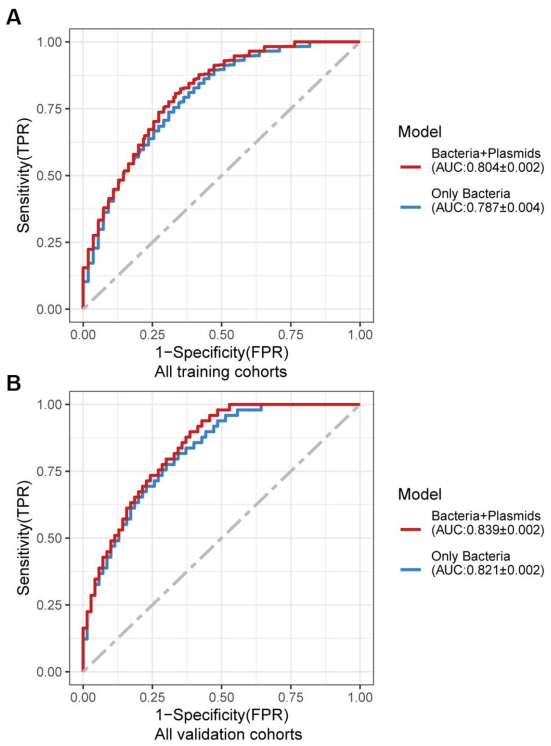

FIGURE 4
Average ROC curve obtained through 20 times repeated 10-fold cross-validation. **(A)** Average ROC curve obtained through 20 times repeated 10-fold cross-validation on all the training cohorts. **(B)** Average ROC curve obtained through independent validation on all the independent cohorts using the random forest classifier trained with 20 times repeated 10-fold cross-validation of all the training cohorts. AUC data are shown as (average of AUC)±SD.

3.5. Correlations between gut bacterial features and plasmids

We further investigated the correlations between the bacteria and plasmids based on the Spearman correlation analysis in the controls and patients with CRC, respectively, to gain insights into the bacteria-plasmid interactions from an ecological perspective. In comparison to CRC cases, we found that the bacteria-plasmid correlation strength was stronger in controls. NZ_CP041417.1 (*Escherichia coli* strain STEC711 plasmid pSTEC711_1) in the gut of CRC patients served as the hub of the correlation network. And the relevant network in the control group's NZ_CP059935.1 (*Escherichia coli* strain 28.1 plasmid p4) was at its hub. *Escherichia coli* and plasmids were strongly associated in both CRC patients and controls. In addition, we found other bacteria that were closely related to the plasmids only in controls, particularly *Enterobacter cloacae* and *Atopobium parvulum* (Figure 5).

3.6. Plasmid functional alterations in CRC

We looked at the plasmid functional alterations at the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KO) genes and carbohydrate-active enzymes (CAZy) genes in order to investigate the plasmid metagenomic functions of pathogenesis in CRC. From 9,514 plasmids KO genes, we first identified 613 differential KO genes ($p < 0.05$), including 333 KO genes with increased abundance and 280 KO genes with decreased abundance in CRC patients compared to controls (Supplementary Table 5).

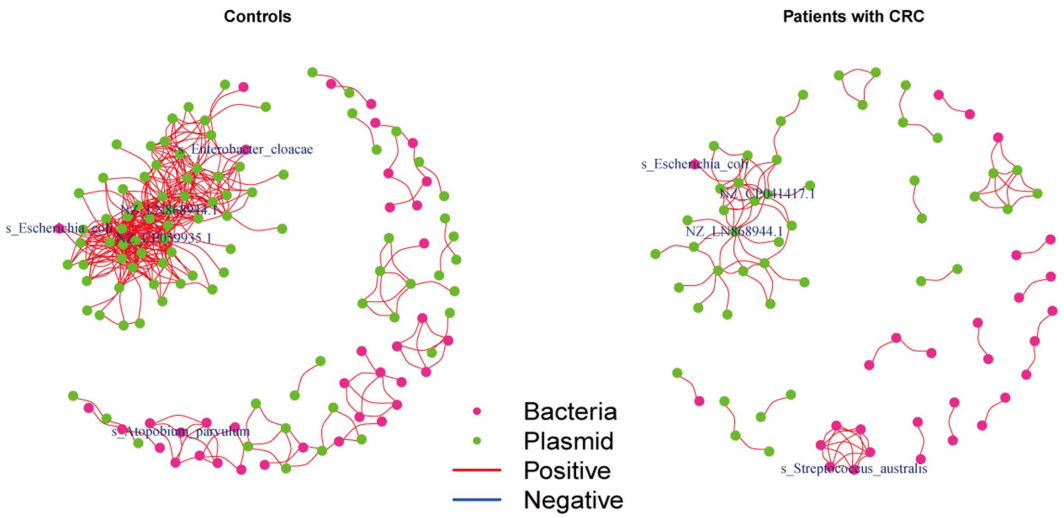

FIGURE 5
Coabundance correlations between plasmids and bacterial species in patients with CRC and controls. Coabundance networks involving plasmids and bacterial species in the CRC and control samples, with absolute correlations above 0.7 and with a significance cut-off of FDR<0.05. The colors of nodes indicate plasmids (green) and bacterial species (deep pink).

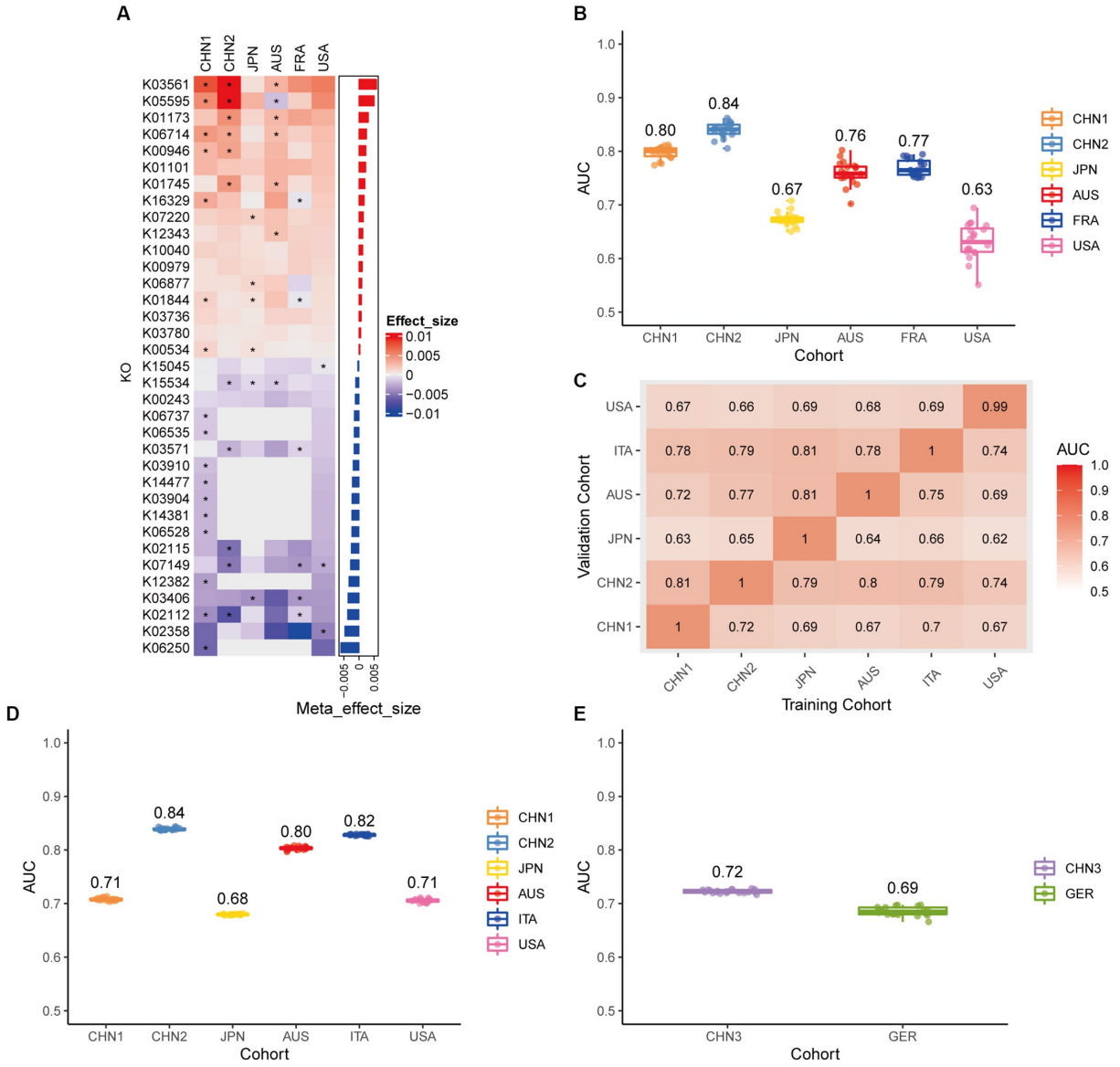

FIGURE 6
Plasmid functional classification models generalize across different cohorts. **(A)** Bar plot of the 34 plasmid gene KO features' importance for the prediction of CRC diagnosis, as determined by MMUPHin and Boruta. The significance of the difference between patients with CRC and controls was determined via Wilcoxon rank-sum test: *p<0.05. **(B)** CRC classification performances (AUC) calculated through the cohort-to-cohort model transfer for the random forest classifier trained on relative abundance profiles of plasmid KO genes. The values refer to an average value of 20 times repeated 10-fold cross-validation. **(C)** CRC classification performances (AUC) calculated through 20 times repeated 10-fold cross-validation within each study for the random forest classifier trained on relative abundance profiles of plasmid KO genes. **(D)** CRC classification performances (AUC) calculated through leave-one-cohort-out validation (LOCO, Model was trained using two of three cohorts and validated by the other one) for random forest classifier trained on relative abundance profiles of plasmid KO genes. **(E)** Validation of the plasmid KO gene random forest classifier in two independent cohorts (CHN3 and GER). The CRC classification performances (AUC) of the plasmid KO gene random forest classifier were obtained by using 20× repeated 10-fold cross-validation in the CHN3 and GER cohort.

Following feature screening, 35 KO genes (including K03561, K05595, and K06250), mainly related to metabolism, were found to be potential biomarkers for CRC prediction (Figure 6A). The plasmid KO random forest classifier showed strong predictive power within cohorts 20 times repeated 10-fold cross-validation, with mean AUC ranging from 0.63 to 0.84 (Figure 6B). The mean AUC of the plasmid KO random forest model ranged from 0.63 to 0.81 in cohort-to-cohort validation (Figure 6C). The LOCO performance of the plasmid KO model ranged from 0.68 and 0.84 (Figure 6D). In independent validation sets, the average AUC was 0.72 and 0.69, respectively, in the CHN3 and GER cohorts (Figure 6E). We carried out the Spearman correlation analysis of differential plasmid KO genes with differential plasmids or bacteria to comprehend the relationship between differential KO and differential bacteria or plasmids, Differential plasmid KO genes had no significant correlation with differential plasmids or bacteria (Supplementary Figure 5). Plasmid KO genes might serve as biomarkers for diagnosing CRC, which is independent of bacteria and plasmids. From 414 plasmids CAZy

genes, we first identified 43 differential CAZy genes ($p < 0.05$), including 16 CAZy genes with increased abundance and 27 CAZy genes with decreased abundance in CRC patients compared to controls (Supplementary Figure 6A; Supplementary Table 6). The plasmid CAZy random forest classifier showed strong predictive power with mean AUC ranging from 0.61 to 0.71 in cross-validation (Supplementary Figure 6B). The mean AUC of the plasmid CAZy random forest model ranged from 0.63 to 0.61 in cohort-to-cohort validation (Supplementary Figure 6C). The plasmid CAZy model's LOCO performance ranged from 0.62 and 0.72 (Supplementary Figure 6D). In independent validation sets, while the average AUC of the model obtained in the GER cohort was 0.51, it was 0.76 on average for the CHN3 (Supplementary Figure 6E). Plasmid CAZy genes were less effective as diagnostic indicators for CRC than plasmid KO genes.

4. Discussion

Plasmid-mediated horizontal gene transfer is regarded as a major driver of bacterial adaptation and diversification, as demonstrated by several studies (Smalla et al., 2015; Wein et al., 2020; Rodríguez-Beltrán et al., 2021). Plasmids can provide ecological benefits to their host bacteria (Di Venanzio et al., 2019). These plasmids may change the biological characteristics of their bacterial hosts, which may have an impact on human health (Rozwandowicz et al., 2018). However, little is known about the function of gut plasmids, which are carried by bacteria that cause disease. We thoroughly analyzed the plasmidome in this study across eight different CRC cohorts. This study provides the most comprehensive metagenomic sequencing-based gut plasmidomic study to date in the largest sample of CRC patients. The bioinformatics pipeline allowed us to locate 12,515 intestinal plasmids in total. We observed that compared to healthy controls, intestinal plasmid diversity was higher in CRC patients. It might imply that CRC patients' intestinal environments were more stressful than those of controls, where bacteria required more plasmids to adjust to changes. To the best of our knowledge, our study is the first to pinpoint differential intestinal plasmids in patients with colorectal cancer. Some of the 198 differential plasmids, including NC_012780.1 (*Eubacterium eligens* ATCC 27750 plasmid unnamed, complete), corresponding bacteria that were equally abundant in CRC patients and controls. Such bacteria may increase the abundance of their associated plasmids to increase their tolerance rather than changing their own abundance in order to adapt to changes in the gut environment of colorectal cancer patients. The bacteria corresponding to other differential plasmids, like NZ_CP036554.1 (*Bacteroides fragilis* strain DCMOUH0067B plasmid pBFO67_1, complete), are also differential in abundance between CRC patients and controls. Although these bacteria also affected the plasmids they were associated with, changes in the colorectal cancer patients' intestinal environment could also affect the abundance of these bacteria. In contrast to controls, the abundance of intestinal plasmids in CRC patients was more independent of their gut microbiota's abundance. According to this, the relationships between bacteria and plasmids may be relevant in the microbiome-mediated tumorigenesis of CRC. An additional layer of information about the contribution of plasmid genes to host health independent of changes in bacterial abundance was revealed by the intriguing fact that the differential plasmid genes in our study were not associated with differential gut bacteria or differential gut plasmids.

The prognosis of CRC is closely related to the stage of the patient at the time of diagnosis (Bruni et al., 2020). Host gene variation (Schmit et al., 2019), RNAs (Wu et al., 2021), proteins (Li et al., 2020), metabolites (Chen et al., 2022), and gut microbes (Liu et al., 2022) are some of the currently validated colorectal cancer markers; however, more work needs to be done to increase their predictive power. A non-invasive, effective, and efficient diagnostic method is urgently needed for colorectal cancer patients who are asymptomatic in order to lower CRC morbidity and mortality, and thereby lower the economic costs of CRC. We screened 21 plasmids, including NZ_CP036554.1 and NZ_AP023416.1, and created a colorectal cancer prediction model based on these intestinal plasmids for the first time, applying various validation techniques to demonstrate the robustness and accuracy of the model. Additionally, we observed that the combination of plasmids and bacteria markers could further improve the predictive power of CRC. In the external validation, the mean specificity and sensitivity of the plasmid and bacterial marker combo for CRC detection were 65.2 and 88.5%, respectively. Our plasmid and bacterial marker combo predict CRC with high accuracy and is as non-invasive as FOBT. Our model has a relatively low predictive effect for the Japan cohort. We suspect that this may be related to the regional heterogeneity of the gut microbiome. It has been shown that glycoceramides contained in the Japanese diet increase the abundance of *Blautia coccoides* in the intestine, which affects the composition of the intestinal flora (Hamajima et al., 2016). Meanwhile, glycoceramides inhibited the development of colorectal cancer in multiple intestinal neoplasia (min) mice (Symolon et al., 2004). The regional heterogeneity of intestinal bacteria in the Japanese cohort is likely due to Japanese diet. Further experimental verification of the specific mechanism is needed.

Several limitations of this study are noted. Identification of plasmids from short-read metagenomic sequencing data remains challenging. It can be difficult to detect and extract a complete plasmid since plasmids can vary greatly in size, have high homology with other plasmids or with the host genome, often contain repetitive regions, or may be incomplete or missing key regions. We have used filtering techniques to exclude less accurate plasmid contigs in light of these difficulties, but we cannot completely rule out the possibility of false positives. As a result, long-read sequencing technology (Pacific Biosciences and Oxford Nanopore Technology) and future tool development may enable us to fully understand the structure of human gut plasmids (Suzuki et al., 2019). The staging of tumors, gender, age, and other factors affecting the incidence of CRC were not taken into consideration. The controls in the majority of cohorts were determined by colonoscopy without detecting CRC, yet the controls in the CHN2 cohort were selected from Taizhou Imaging Study who did not undergo colonoscopy, which could potentially introduce detection bias. A fourth limitation is the cohort effect due to variations in the distribution of gut flora across regions and the use of different sequencing platforms, even though we eliminated the batch effect through MMUPHin. We were unable to determine the actual host of the plasmids because of the phenomenon of the horizontal transfer of plasmids. A high-throughput technique called Microbe-seq was created by Zheng et al. to examine individual bacterial cells in the microbiota. This approach enables further exploration of plasmid horizontal transfer and the host profile of plasmids (Zheng et al., 2022). Future prospective studies with

large patient cohorts are needed to validate the results. We cannot establish a causal relationship between CRC and plasmids in the current data collection. We anticipate that long-read metagenomic sequencing and upcoming experimental research will clarify the causal relationship between CRC and plasmids.

In conclusion, we used plasmid-related sequences to identify the corresponding plasmids and found that they were able to distinguish between CRC patients and controls. We constructed a combined plasmid and bacteria panel, which performed superior at predicting CRC than bacteria alone. Our study expands the knowledge of the function of plasmids in CRC patients may lead to further research into potential CRC diagnosis applications. Plasmids should be taken into account when studying the gut microbiota.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://www.ncbi.nlm.nih.gov/sra.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

ML and ZC designed the research. ZC, PL, WZ, and JW collected the data. ZC, JL, XS, KL, and SL performed the statistical analysis. ML and ZC wrote the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1130446/full#supplementary-material

References

Allison, J. E., Tekawa, I. S., Ransom, L. J., and Adrain, A. L. (1996). A comparison of fecal occult-blood tests for colorectal-cancer screening. *N. Engl. J. Med.* 334, 155–160. doi: 10.1056/NEJM199601183340304

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. doi: 10.1016/S0022-2836(05)80360-2

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral. Ecol.* 26, 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x

Ban, E., Yoshida, Y., Wakushima, M., Wajima, T., Hamabata, T., Ichikawa, N., et al. (2015). Characterization of unstable pEntYN10 from enterotoxigenic *Escherichia coli* (ETEC) O169:H41. *Virulence* 6, 735–744. doi: 10.1080/21505594.2015.1094606

Bleicher, A., Schöfl, G., Rodicio, M. E. R., and Saluz, H. P. (2013). The plasmidome of a *Salmonella enterica* serovar Derby isolated from pork meat. *Plasmid* 69, 202–210. doi: 10.1016/j.plasmid.2013.01.001

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi: 10.1093/bioinformatics/btu170

Bortolaia, V., Kaas, R. S., Ruppe, E., Roberts, M. C., Schwarz, S., Cattoir, V., et al. (2020). ResFinder 4.0 for predictions of phenotypes from genotypes. *J. Antimicrob. Chemother.* 75, 3491–3500. doi: 10.1093/jac/dkaa345

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 68, 394–424. doi: 10.3322/caac.21492

Breiman, L. (2001). Random forests. *Mach. Learn.* 45, 5–32. doi: 10.1023/A:1010933404324

Brown Kav, A., Sasson, G., Jami, E., Doron-Faigenboim, A., Benhar, I., and Mizrahi, I. (2012). Insights into the bovine rumen plasmidome. *Proc. Natl. Acad. Sci. U. S. A.* 109, 5452–5457. doi: 10.1073/pnas.1116410109

Bruni, D., Angell, H. K., and Galon, J. (2020). The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. *Nat. Rev. Cancer* 20, 662–680. doi: 10.1038/s41568-020-0285-7

Cantalapiedra, C. P., Hernandez-Plaza, A., Letunic, I., Bork, P., and Huerta-Cepas, J. (2021). eggNOG-mapper v2: functional annotation, Orthology assignments, and domain prediction at the metagenomic scale. *Mol. Biol. Evol.* 38, 5825–5829. doi: 10.1093/molbev/msab293

Carattoli, A., Zankari, E., García-Fernández, A., Voldby Larsen, M., Lund, O., Villa, L., et al. (2014). In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob. Agents Chemother.* 58, 3895–3903. doi: 10.1128/aac.02412-14

Chan, J. L., Wu, S., Geis, A. L., Chan, G. V., Gomes, T. A. M., Beck, S. E., et al. (2019). Non-toxigenic *Bacteroides fragilis* (NTBF) administration reduces bacteria-driven chronic colitis and tumor development independent of polysaccharide A. *Mucosal Immunol.* 12, 164–177. doi: 10.1038/s41385-018-0085-5

Chen, F., Dai, X., Zhou, C. C., Li, K. X., Zhang, Y. J., Lou, X. Y., et al. (2022). Integrated analysis of the faecal metagenome and serum metabolome reveals the role of gut microbiome-associated metabolites in the detection of colorectal cancer and adenoma. *Gut* 71, 1315–1325. doi: 10.1136/gutjnl-2020-323476

Cheung, A. M., Farizo, K. M., and Burns, D. L. (2004). Analysis of relative levels of production of pertussis toxin subunits and Ptl proteins in *Bordetella pertussis*. *Infect. Immun.* 72, 2057–2066. doi: 10.1128/IAI.72.4.2057-2066.2004

Dai, Z., Coker, O. O., Nakatsu, G., Wu, W. K. K., Zhao, L., Chen, Z., et al. (2018). Multi-cohort analysis of colorectal cancer metagenome identified altered bacteria across populations and universal bacterial markers. *Microbiome* 6:70. doi: 10.1186/s40168-018-0451-2

di Nicolantonio, F., Vitiello, P. P., Marsoni, S., Siena, S., Tabernero, J., Trusolino, L., et al. (2021). Precision oncology in metastatic colorectal cancer - from biology to medicine. *Nat. Rev. Clin. Oncol.* 18, 506–525. doi: 10.1038/s41571-021-00495-z

di Venanzio, G., Flores-Mireles, A. L., Calix, J. J., Haurat, M. F., Scott, N. E., Palmer, L. D., et al. (2019). Urinary tract colonization is enhanced by a plasmid that regulates uropathogenic *Acinetobacter baumannii* chromosomal genes. *Nat. Commun.* 10:2763. doi: 10.1038/s41467-019-10706-y

Dib, J. R., Wagenknecht, M., Farías, M. E., and Meinhardt, F. (2015). Strategies and approaches in plasmidome studies-uncovering plasmid diversity disregarding of linear elements? *Front. Microbiol.* 6:463. doi: 10.3389/fmicb.2015.00463

Dolejska, M., and Papagiannitsis, C. C. (2018). Plasmid-mediated resistance is going wild. *Plasmid* 99, 99–111. doi: 10.1016/j.plasmid.2018.09.010

Faivre, J., Dancourt, V., Lejeune, C., Tazi, M. A., Lamour, J., Gerard, D., et al. (2004). Reduction in colorectal cancer mortality by fecal occult blood screening in a French controlled study. *Gastroenterology* 126, 1674–1680. doi: 10.1053/j.gastro.2004.02.018

Feng, Q., Liang, S., Jia, H., Stadlmayr, A., Tang, L., Lan, Z., et al. (2015). Gut microbiome development along the colorectal adenoma-carcinoma sequence. *Nat. Commun.* 6:6528. doi: 10.1038/ncomms7528

Fondi, M., Bacci, G., Brilli, M., Papaleo, M. C., Mengoni, A., Vaneechoutte, M., et al. (2010). Exploring the evolutionary dynamics of plasmids: the Acinetobacter pan-plasmidome. *BMC Evol. Biol.* 10:59. doi: 10.1186/1471-2148-10-59

Fu, L., Niu, B., Zhu, Z., Wu, S., and Li, W. (2012). CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics* 28, 3150–3152. doi: 10.1093/bioinformatics/bts565

Gao, R., Wu, C., Zhu, Y., Kong, C., Zhu, Y., Gao, Y., et al. (2022). Integrated analysis of colorectal Cancer reveals cross-cohort gut microbial signatures and associated serum metabolites. *Gastroenterology* 163, 1024–1037.e9. doi: 10.1053/j.gastro.2022.06.069

Ghaisas, S., Maher, J., and Kanthasamy, A. (2016). Gut microbiome in health and disease: linking the microbiome-gut-brain axis and environmental factors in the pathogenesis of systemic and neurodegenerative diseases. *Pharmacol. Ther.* 158, 52–62. doi: 10.1016/j.pharmthera.2015.11.012

Gurung, M., Li, Z., You, H., Rodrigues, R., Jump, D. B., Morgun, A., et al. (2020). Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine* 51:102590. doi: 10.1016/j.ebiom.2019.11.051

Hamajima, H., Matsunaga, H., Fujikawa, A., Sato, T., Mitsutake, S., Yanagita, T., et al. (2016). Japanese traditional dietary fungus koji aspergillus oryzae functions as a prebiotic for *Blautia coccoides* through glycosylceramide: Japanese dietary fungus koji is a new prebiotic. *Springerplus* 5:1321. doi: 10.1186/s40064-016-2950-6

Hardcastle, J. D., Chamberlain, J. O., Robinson, M. H., Moss, S. M., Amar, S. S., Balfour, T. W., et al. (1996). Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* 348, 1472–1477. doi: 10.1016/S0140-6736(96)03386-7

Hilpert, C., Bricheux, G., and Debroas, D. (2021). Reconstruction of plasmids by shotgun sequencing from environmental DNA: which bioinformatic workflow? *Brief. Bioinform.* 22. doi: 10.1093/bib/bbaa059

Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., et al. (2019). eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res.* 47, D309–D314. doi: 10.1093/nar/gky1085

Hyatt, D., Chen, G. L., Locascio, P. F., Land, M. L., Larimer, F. W., and Hauser, L. J. (2010). Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. doi: 10.1186/1471-2105-11-119

Jari Oksanen, G.L.S., Guillaume Blanchet, F., Kindt, R, Legendre, P, Minchin, P.R., O'Hara, R.B., et al. (2022). Vegan: community ecology package. Ter Braak and James Weedon R package version 2.6-2. Available at: https://CRAN.R-project.org/package=vegan

Krawczyk, P. S., Lipinski, L., and Dziembowski, A. (2018). PlasFlow: predicting plasmid sequences in metagenomic data using genome signatures. *Nucleic Acids Res.* 46:e35. doi: 10.1093/nar/gkx1321

Langfelder, P., and Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 9:559. doi: 10.1186/1471-2105-9-559

Langmead, B., and Salzberg, S. L. (2012). Fast gapped-read alignment with bowtie 2. *Nat. Methods* 9, 357–359. doi: 10.1038/nmeth.1923

Lee, M. W., Pourmorady, J. S., and Laine, L. (2020). Use of fecal occult blood testing as a diagnostic tool for clinical indications: A systematic review and Meta-analysis. *Am. J. Gastroenterol.* 115, 662–670. doi: 10.14309/ajg.0000000000000495

Li, D., Liu, C. M., Luo, R., Sadakane, K., and Lam, T. W. (2015). MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* 31, 1674–1676. doi: 10.1093/bioinformatics/btv033

Li, C., Sun, Y. D., Yu, G. Y., Cui, J. R., Lou, Z., Zhang, H., et al. (2020). Integrated omics of metastatic colorectal Cancer. *Cancer Cell* 38, 734–747.e9. doi: 10.1016/j.ccell.2020.08.002

Li, X., Xie, Y., Liu, M., Tai, C., Sun, J., Deng, Z., et al. (2018). oriTfinder: a web-based tool for the identification of origin of transfers in DNA sequences of bacterial mobile genetic elements. *Nucleic Acids Res.* 46, W229–W234. doi: 10.1093/nar/gky352

Liu, N. N., Jiao, N., Tan, J. C., Wang, Z., Wu, D., Wang, A. J., et al. (2022). Multi-kingdom microbiota analyses identify bacterial-fungal interactions and biomarkers of colorectal cancer across cohorts. *Nat. Microbiol.* 7, 238–250. doi: 10.1038/s41564-021-01030-7

Ma, S., Shungin, D., Mallick, H., Schirmer, M., Nguyen, L. H., Kolde, R., et al. (2022). Population structure discovery in meta-analyzed microbial communities and inflammatory bowel disease using MMUPHin. *Genome Biol.* 23:208. doi: 10.1186/s13059-022-02753-4

Miron, B., and Kursa, W. R. R. (2010). Feature selection with the Boruta package. *J. Stat. Softw.* 36, 1–13. doi: 10.18637/jss.v036.i11

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., and Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nat. Methods* 14, 417–419. doi: 10.1038/nmeth.4197

Pellow, D., Zorea, A., Probst, M., Furman, O., Segal, A., Mizrahi, I., et al. (2021). SCAPP: an algorithm for improved plasmid assembly in metagenomes. *Microbiome* 9:144. doi: 10.1186/s40168-021-01068-z

Rex, D. K., Petrini, J. L., Baron, T. H., Chak, A., Cohen, J., Deal, S. E., et al. (2006). Quality indicators for colonoscopy. *Gastrointest. Endosc.* 63, S16–S28. doi: 10.1016/j.gie.2006.02.021

Rodríguez-Beltrán, J., DelaFuente, J., León-Sampedro, R., MacLean, R. C., and San Millán, Á. (2021). Beyond horizontal gene transfer: the role of plasmids in bacterial evolution. *Nat. Rev. Microbiol.* 19, 347–359. doi: 10.1038/s41579-020-00497-1

Roosaare, M., Puustusmaa, M., Möls, M., Vaher, M., and Remm, M. (2018). PlasmidSeeker: identification of known plasmids from bacterial whole genome sequencing reads. *PeerJ* 6:e4588. doi: 10.7717/peerj.4588

Rozwandowicz, M., Brouwer, M. S. M., Fischer, J., Wagenaar, J. A., Gonzalez-Zorn, B., Guerra, B., et al. (2018). Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. *J. Antimicrob. Chemother.* 73, 1121–1137. doi: 10.1093/jac/dkx488

Sang, T., Qiu, W., Li, W., Zhou, H., Chen, H., and Zhou, H. (2020). The relationship between prevention and treatment of colorectal Cancer and cancerous toxin pathogenesis theory basing on gut microbiota. *Evid. Based Complement. Alternat. Med.* 2020, 7162545–7162549. doi: 10.1155/2020/7162545

Schmidt, T. S. B., Raes, J., and Bork, P. (2018). The human gut microbiome: from association to modulation. *Cells* 172, 1198–1215. doi: 10.1016/j.cell.2018.02.044

Schmit, S. L., Edlund, C. K., Schumacher, F. R., Gong, J., Harrison, T. A., Huyghe, J. R., et al. (2019). Novel common genetic susceptibility loci for colorectal Cancer. *J. Natl. Cancer Inst.* 111, 146–157. doi: 10.1093/jnci/djy099

Smalla, K., Jechalke, S., and Top, E. M. (2015). Plasmid detection, characterization, and ecology. *Microbiol. Spectr.* 3:PLAS-0038-2014. doi: 10.1128/microbiolspec.PLAS-0038-2014

Smalla, K., and Sobecky, P. A. (2002). The prevalence and diversity of mobile genetic elements in bacterial communities of different environmental habitats: insights gained from different methodological approaches. *FEMS Microbiol. Ecol.* 42, 165–175. doi: 10.1111/j.1574-6941.2002.tb01006.x

Smillie, C., Garcillan-Barcia, M. P., Francia, M. V., Rocha, E. P., and de la Cruz, F. (2010). Mobility of plasmids. *Microbiol. Mol. Biol. Rev.* 74, 434–452. doi: 10.1128/MMBR.00020-10

Suzuki, Y., Nishijima, S., Furuta, Y., Yoshimura, J., Suda, W., Oshima, K., et al. (2019). Long-read metagenomic exploration of extrachromosomal mobile genetic elements in the human gut. *Microbiome* 7:119. doi: 10.1186/s40168-019-0737-z

Symolon, H., Schmelz, E. M., Dillehay, D. L., and Merrill, A. H. Jr. (2004). Dietary soy sphingolipids suppress tumorigenesis and gene expression in 1,2-dimethylhydrazine-treated CF1 mice and ApcMin/+ mice. *J. Nutr.* 134, 1157–1161. doi: 10.1093/jn/134.5.1157

Tomizawa, M., Shinozaki, F., Uchida, Y., Uchiyama, K., Fugo, K., Sunaoshi, T., et al. (2017). Diffusion-weighted whole-body imaging with background body signal suppression/T2 image fusion for the diagnosis of colorectal polyp and cancer. *Exp. Ther. Med.* 13, 639–644. doi: 10.3892/etm.2016.3981

Truong, D. T., Franzosa, E. A., Tickle, T. L., Scholz, M., Weingart, G., Pasolli, E., et al. (2015). MetaPhlAn2 for enhanced metagenomic taxonomic profiling. *Nat. Methods* 12, 902–903. doi: 10.1038/nmeth.3589

Vogtmann, E., Hua, X., Zeller, G., Sunagawa, S., Voigt, A. Y., Hercog, R., et al. (2016). Colorectal Cancer and the human gut microbiome: reproducibility with

whole-genome shotgun sequencing. *PLoS One* 11:e0155362. doi: 10.1371/journal.pone.0155362

Wein, T., Wang, Y., Hülter, N. F., Hammerschmidt, K., and Dagan, T. (2020). Antibiotics interfere with the evolution of plasmid stability. *Curr. Biol.* 30, 3841–3847.e4. doi: 10.1016/j.cub.2020.07.019

Wirbel, J., Pyl, P. T., Kartal, E., Zych, K., Kashani, A., Milanese, A., et al. (2019). Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. *Nat. Med.* 25, 679–689. doi: 10.1038/s41591-019-0406-6

Wu, Y., Yang, X., Jiang, G., Zhang, H., Ge, L., Chen, F., et al. (2021). 5'-tRF-GlyGCC: a tRNA-derived small RNA as a novel biomarker for colorectal cancer diagnosis. *Genome Med.* 13:20. doi: 10.1186/s13073-021-00833-x

Yachida, S., Mizutani, S., Shiroma, H., Shiba, S., Nakajima, T., Sakamoto, T., et al. (2019). Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat. Med.* 25, 968–976. doi: 10.1038/s41591-019-0458-7

Yang, Y., du, L., Shi, D., Kong, C., Liu, J., Liu, G., et al. (2021). Dysbiosis of human gut microbiome in young-onset colorectal cancer. *Nat. Commun.* 12:6757. doi: 10.1038/s41467-021-27112-y

Yinhang, W., Wei, W., Jing, Z., Qing, Z., Yani, Z., Yangyanqiu, W., et al. (2022). Biological roles of toll-like receptors and gut microbiota in colorectal cancer. *Future Microbiol.* 17, 1071–1089. doi: 10.2217/fmb-2021-0072

Zamani, S., Taslimi, R., Sarabi, A., Jasemi, S., Sechi, L. A., and Feizabadi, M. M. (2019). Enterotoxigenic *Bacteroides fragilis*: A possible etiological candidate for bacterially-induced colorectal precancerous and cancerous lesions. *Front. Cell. Infect. Microbiol.* 9:449. doi: 10.3389/fcimb.2019.00449

Zeller, G., Tap, J., Voigt, A. Y., Sunagawa, S., Kultima, J. R., Costea, P. I., et al. (2014). Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol. Syst. Biol.* 10:766. doi: 10.15252/msb.20145645

Zhang, H., Yohe, T., Huang, L., Entwistle, S., Wu, P., Yang, Z., et al. (2018). dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. *Nucleic Acids Res.* 46, W95–W101. doi: 10.1093/nar/gky418

Zheng, W., Zhao, S., Yin, Y., Zhang, H., Needham, D. M., Evans, E. D., et al. (2022). High-throughput, single-microbe genomics with strain resolution, applied to a human gut microbiome. *Science* 376:eabm1483. doi: 10.1126/science.abm1483

Zhou, F., and Xu, Y. (2010). cBar: a computer program to distinguish plasmid-derived from chromosome-derived sequence fragments in metagenomics data. *Bioinformatics* 26, 2051–2052. doi: 10.1093/bioinformatics/btq299

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Differences in tissue-associated bacteria between metastatic and non-metastatic colorectal cancer

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Background and aims: Accumulated evidence indicates that the intestinal microbiota plays crucial roles in the initiation and progression of colorectal cancer (CRC). However, the effects of the tissue-associated microbiota on CRC metastasis are poorly defined. The aim of this study was to explore the differences in bacteria between metastatic and non-metastatic CRC tissues and identify potential bacterial species that associate with CRC metastasis.

Methods: 16S rDNA amplicon high-throughput sequencing was used to test the intestinal tissue-associated microbiota in patients with metastatic CRC (n = 48) and non-metastatic CRC (n = 44). The microbial diversity and differential species were analysed by standard microbiological methods, and then the differential bacteria were confirmed by qPCR. Receiver operating characteristic (ROC) curves were plotted to evaluate the ability of the differential bacteria in predicting the metastasis of CRC. In addition, the microbial compositions of tumor-adjacent tissues from the metastatic and non-metastatic CRC groups were analysed.

Results: The α- or β-diversity of microbial community between the metastatic and non-metastatic CRC groups did not exhibit significant differences. However, some bacterial abundances between two groups showed significant differences. At the phylum level, Bacteroidota and Desulfobacterota were significantly higher in the metastatic group than in the non-metastatic group, while Proteobacteria was significantly decreased in the metastatic group. At the genus level, *Bacteroides* (mainly composed of *Bacteroides fragilis* and *Bacteroides uniformis*) was significantly higher in the metastatic group than in the non-metastatic group, while *Streptococcus* and *Escherichia-Shigella* were significantly decreased. The ROC curves of the selected bacteria showed area under the curve (AUC) values ranging from 0.598 to 0.69; when CEA and the selected bacteria were combined, the AUC values increased from 0.678 to 0.705. In addition, the bacterial composition of tumor-adjacent tissues from the metastatic and non-metastatic CRC groups were also different, and the differential bacteria were consistent with those between metastatic and non-metastatic CRC tumor tissues.

Conclusion: The bacterial composition of tumor and tumor adjacent tissue from the metastatic CRC group was different from that of the non-metastatic CRC

group; in particular, *Bacteroides* was increased, and *Streptococcus* was decreased. These findings are helpful to further reveal the mechanism of CRC metastasis and provide new ideas for the clinical diagnosis and treatment of CRC metastasis.

KEYWORDS

colorectal cancer (CRC), metastasis, tissue-associated bacteria, *Bacteroides*, *Streptococcus*

Introduction

Colorectal cancer (CRC) is one of the most common gastroenterological tumors. According to 2020 epidemiological data, CRC is the third most common diagnosed and second most deadly cancer worldwide (Sung et al., 2021). The process of intestinal cancer development usually takes 10–15 years, including the initiation, promotion, progression, and metastasis stages (Dekker et al., 2019). Metastasis is known as the main cause of death in CRC patients, with a 5 years survival rate of less than 20% (Pretzsch et al., 2019; Biller and Schrag, 2021). Therefore, it is of great importance and necessity to understand the potential risk factors that promote CRC metastasis, which can serve as targets to block CRC metastasis (Fong et al., 2020).

The pathogenesis of CRC is highly complex and involves both genetic and environmental factors (Biller and Schrag, 2021). In recent years, numerous studies have supported the notion that the intestinal microbiota plays a crucial role in the initiation and progression of CRC (Wong and Yu, 2019; Cheng et al., 2020; Rebersek, 2021). Usually, the microbiota significantly changed in CRC patients, with tumor-promoting bacteria enriched and tumor-inhibiting bacteria depleted. Some bacteria, such as colibactin-producing *Escherichia coli,* enterotoxigenic *Bacteroides fragilis*, and *Fusobacterium nucleatum*, are enriched in the intestinal microbiota (Cheng et al., 2020; Tabowei et al., 2022). They can promote CRC initiation and progression by inducing host DNA damage, stimulating oncogenic pathways related to cell growth and proliferation, or creating a proinflammatory environment (Clay et al., 2022). However, studies focusing on specific bacteria that can promote CRC metastasis are limited.

It has been reported that the bacterial burden in CRC mucosal tissue is higher than that in healthy controls. Interestingly, the composition of the intestinal microbiota and tissue-associated bacteria are significantly different (Keku et al., 2015; Flemer et al., 2017), suggesting that they may play different roles in CRC progression. To date, many studies have revealed the functions of the intestinal microbiota (fecal sample) in CRC, but only a few have focused on tissue-associated bacteria (CRC tissue samples) (Costa et al., 2022). Thus, the features and functions of tissue-associated bacteria in CRC progression and metastasis remain elusive.

In this study, we compared the differences in the tissue-associated microbiota between tumor tissues from metastatic and non-metastatic CRC patients and analysed the clinical value of differential bacteria in the prognosis of CRC metastasis. Our findings will shed light on fully revealing the characteristics of tissue-associated bacteria and provide an effective foundation for the in-depth study of their role in CRC metastasis.

Materials and methods

Subjects

We selected patients who were surgically treated for colorectal cancer between January 2020 and December 2021. According to the UICC/AJCC TNM Staging System for CRC (8th edition, 2017), 92 patients with CRC were divided into a metastatic group (n = 48) and a non-metastatic group (n = 44) by specialist physicians. Exclusion criteria were anal canal tumors, appendiceal tumors, neuroendocrine tumors, familial adenomatous polyposis and cases of additional surgery for perforation or bleeding complicated by endoscopic treatment, colorectal cancer with antibiotics, glucocorticoids or immunosuppressive drugs used within 1 month at the time of sampling, and other colorectal cancer patients who could not be entered into this cohort.

Clinical samples were collected including tumor and tumor-adjacent tissues from CRC patients (the area surrounding the tumor <3 cm was considered adjacent tissue).

The collection of relevant clinical parameters of CRC patients included general clinical information, routine blood tests, biochemistry, coagulation function, tumor markers, histopathology, and other indicators.

Bacterial DNA extraction

Tissue-associated bacterial DNA was extracted from samples by using the QIAamp DNA Microbiome Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. In brief, approximately 100 mg of intestinal tissue was homogenized, host cells were lysed, and host DNA was digested by benzonase (human DNase) while leaving the bacterial cells intact. Then, bacterial cells were concentrated by centrifugation, and bacterial DNA was extracted.

Abbreviations: CRC, colorectal cancer; 16S rDNA, 16S ribosomal DNA; qPCR, quantitative polymerase chain reaction; ROC, receiver operating characteristic; AUC, area under curve; UICC, International Union Against Cancer; AJCC, American Joint Committee on Cancer; TNM, tumor node metastasis; OTU, operational taxonomic unit; CI, confidence interval; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; AFP, alpha fetoprotein; NMSD, non-metric multidimensional scaling analysis; LDA, linear discriminant analysis; JMJD2B, jumonji domain containing 2B; TLR4, toll-like receptor 4; NFAT5, nuclear factor of activated T cells 5; ETBF, enterotoxigenic *Bacteroides fragilis;* BFT, *Bacteroides fragilis* toxin.

16S rDNA amplicon sequencing and analysis

The sequencing procedure was performed as previously described (Emery et al., 2020). Briefly, the V3–V4 hypervariable region of bacterial 16S rDNA was amplified using universal sequencing primers 341F 5′-CCTAYGGGRBGCASCAG-3′ and 806R 5′-GGACTACNNGGGTATCTAAT-3′ (Yuan et al., 2018). The amplicon was sequenced by the Illumina MiSeq PE300 platform at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw FASTQ files were de-multiplexed and quality-filtered by QIIME1 (V1.9.1).[1] The optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE 7.1[2] with 97% sequence similarity level. The most abundant sequence for each OTU was selected as a representative sequence. To minimize the effects of sequencing depth on alpha- and beta-diversity measure, the number of 16S rRNA gene sequences from each sample were rarefied to 20,000. Bioinformatic analysis of the microbiota was based on the OTUs information. Alpha diversity indices including Chao1 richness and Shannon index were calculated with Mothur v1.30.1 (Emery et al., 2020). The similarity among the microbial communities in different samples was determined by non-metric multidimensional scaling analysis (NMDS) based on Bray-curtis dissimilarity using Vegan v2.5-3 package. The linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al., 2011)[3] was performed to identify the significantly abundant taxa (phylum to genera) of bacteria among the different groups (LDA score > 3, $P < 0.05$).

Real-time quantitative PCR (qPCR) analysis

The main differential bacteria and *Bacteroides fragilis* toxin gene were confirmed by qPCR analysis. Briefly, experiments were performed with a QuantStudio 3 Real-time PCR System (Thermo Fisher Scientific, USA). The qPCR reaction system was: 2 × SYBR Green premix [Takara Bio technology (Beijing) Co., Ltd. Beijing, China] 5 μL, 1 μM forward and reverse primer sets (**Table 1**) 2 μL, 20 ng/μL DNA template 1 μL, ddH_2O 2 μL. The conditions of qPCR reaction were as follows: initial denaturation was done at 95°C for 60 s; amplification by using 45 cycles including denaturation at 95°C for 5 s, annealing and extension at 60°C for 30 s; melting curve was done at 95°C for 15 s, 60°C for 60 s; 95°C for 30 s.

Statistical analysis

SPSS 20.0 statistical software was used for statistical analyses. Patient characteristics were compared using unpaired Student's *t*-test, Wilcoxon rank-sum test, or χ2 test as appropriate. Student's *t*-test was used to analyse the differential bacteria between metastatic and non-metastatic CRC tissue. ROC curve analysis was used to determine the diagnostic value of serum biomarkers or selected bacteria in patients with CRC. Other diagnostic parameters were also evaluated, including sensitivity, specificity, cut-off value, and area under the ROC curve (AUC) with 95% confidence interval (CI), to assess the discrimination power of individual or combined biomarkers. A *p*-value less than 0.05 was considered statistically significant.

1 http://qiime.org/install/index.html
2 http://drive5.com/uparse/
3 http://huttenhower.sph.harvard.edu/LEfSe

Results

Patient characteristics

A total of 92 patients with CRC were included in the study, including 48 patients with metastatic CRC and 44 patients with non-metastatic CRC (**Table 2**). Statistical analysis of the basic clinical data indicated that age, gender, tumor size and location of tumor occurrence were not significantly associated with tumor metastasis. The differentiation degree was significantly related to CRC metastasis ($p = 0.008$), which is consistent with the understanding that if the tumor is less differentiated, it is more malignant and prone to metastasis (Derwinger et al., 2010). In addition, 38 of the 59 ulcerated CRC patients developed metastases, but only six of the 25 protuberant CRC patients were diagnosed with metastases ($p = 0.001$). This is because ulcerated CRC progresses deeper into the intestinal mucosa and is more likely to invade lymphatic and blood vessels, leading to CRC metastases (Bateman, 2022). Among the tumor markers alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9), the level of CEA was significantly associated with CRC metastasis.

The α- or β-diversity of microbial community has no significant difference between metastatic and non-metastatic CRC tissue

To analyse the microbiota characteristics of tumor tissues in the metastatic and non-metastatic groups, we performed 16S rDNA amplicon high-throughput sequencing and subsequent bioinformatics analysis. The α-diversity of microbial communities is described by the Shannon and Chao indices (Ibrahim et al., 2019). The results showed that both indices of the two groups were not significantly different, indicating that the bacterial species diversity and richness were similar between metastatic and non-metastatic CRC tissues (**Supplementary Figures 1A, B**). Then, we applied non-metric multidimensional scaling analysis (NMDS) to analyse the β-diversity of the microbial communities. The results showed that the samples of non-metastatic group were not clustered together, and the β-diversity of two groups were not statistic significant (**Supplementary Figure 1C**).

TABLE 1 Quantitative polymerase chain reaction (qPCR) primers for target bacteria and *Bacteroides fragilis* toxin gene.

Bacteria	Primer sequences (5′–3′)	Product size (bp)	References
Bacteroidota	CATGTGGTTTAATTCGATGAT	126	Queipo-Ortuño et al., 2012
	AGCTGACGACAACCATGCAG		
Proteobacteria	CATGACGTTACCCGCAGAAGAAG	195	Queipo-Ortuño et al., 2012
	CTCTACGAGACTCAAGCTTGC		
Bacteroides	GGTTCTGAGAGGAGGTCCC	106	Queipo-Ortuño et al., 2012
	GCTGCCTCCCGTAGGAGT		
Streptococcus	ACGGTCTTGCTGTCACTTATA	257	Johnson et al., 2016
	TACACATATGTTCTTCCCTAATAA		
Escherichia-Shigella	GAGTAAAGTTAATACCTTTGCTCATTG	206	Kurakawa et al., 2013
	GAGACTCAAGCTKRCCAGTATCAG		
Bacteroides fragilis	TCRGGAAGAAAGCTTGCT	162	Tong et al., 2011
	CATCCTTTACCGGAATCCT		
Bacteroides uniformis	TCTTCCGCATGGTAGAACTATTA	112	Tong et al., 2011
	ACCGTGTCTCAGTTCCAATGTG		
Bacteroides fragilis toxin	TGAAGTTAGTGCCCAGATGC	150	Zamani et al., 2017
	CAGTAAAGCCTTCCAGTCC		

The bacterial composition is different between metastatic and non-metastatic CRC tissues

We further analysed the composition of the microbial community from metastatic and non-metastatic CRC tissues. At the phylum level, the results showed that the intestinal bacteria in all tumor tissues were mainly from Firmicutes, Bacteroidota, Proteobacteria, Actinobacteriota, and Fusobacteriota (accounting for approximately 95%) (**Figure 1A**). The relative abundance of Bacteroidetes was significantly higher in the metastatic group than in the non-metastatic group (30.05 ± 21.20 vs. 18.35 ± 17.25%; $P = 0.013$), while the relative abundance of Proteobacteria was significantly lower in the metastatic group than in the non-metastatic group (9.87 ± 18.07 vs. 19.69 ± 29.13%; $P = 0.009$). In addition, Desulfobacterota, although the abundance was very low, was significantly increased in the metastatic group (0.82 ± 1.55 vs. 0.11 ± 0.35%; $P < 0.001$) (**Figure 1B**).

TABLE 2 Clinical characteristics of 92 patients with colorectal cancer.

Parameter	Metastatic (n = 48)	Non-metastatic (n = 44)	P-value
Gender female (F/M)	15/33	15/29	0.772
Age (years)	64.60 ± 14.50	65.80 ± 10.40	0.655
Tumor size	11.58 ± 12.53	14.00 ± 15.57	0.413
Differentiation	–	–	0.008
Well	27	36	–
Moderate-poor	21	8	–
Proximal location	–	–	0.922
Right	12	10	–
Left	14	12	–
Rectum	22	22	–
Alpha fetoprotein	2.89 ± 1.66	2.97 ± 1.35	0.472
Carcinoembryonic antigen	104.47 ± 337.41	10.20 ± 27.91	0.012
Carbohydrate antigen 19-9	122.10 ± 340.65	62.67 ± 302.28	0.124
Macroscopic classification	–	–	0.001
Protuberant lesions	6	19	–
Ulcerated lesions	42	25	–

Data are presented as the mean ± SD.

At the genus level, the bacterial composition in the tumor tissues was mainly composed of five genera: *Bacteroides*, *Streptococcus*, *Escherichia-Shigella*, *Parvimonas*, and *Fusobacterium* (**Figure 1C**). Among them, the relative abundance of *Bacteroides* was significantly higher in the metastatic group than in the non-metastatic group (21.67 ± 19.39 vs. 12.58 ± 12.93%; $P = 0.049$), while the relative abundances of *Streptococcus* and *Escherichia-Shigella* were significantly decreased in the metastatic group compared to the non-metastatic group (5.10 ± 11.9 vs. 23.12 ± 19.42%; $P = 0.008$ and 5.16 ± 14.65 vs. 11.66 ± 25.35%; $P = 0.027$, respectively) (**Figure 1D**).

The linear discriminant analysis (LDA) effect size (LEfSe; LDA score > 3.0) also found many differential bacterial species between the metastatic and non-metastatic CRC groups (**Supplementary Figure 2**). Interestingly, the metastatic CRC group had more relatively high abundance bacterial species than the non-metastatic CRC group, especially o_Bacteroidales, c_Bacteroidia, p_Bacteroidota, g_*Bacteroides*, f_Bacteroidaceae, g_*Alistipes*, f_Rikenellaceae, f_Oscillospiraceae, p_Desulfobacterota, and c_Desulfovibrionia, which had the highest scores, while c_Gammaproteobacteria, p_Proteobacteria, o_Enterobacterales, g_*Escherichia-Shigella*, f_Enterobacteriaceae, c_Bacilli, o_Lactobacillales, g_*Streptococcus*, f_Streptococcaceae, g_*Curvibacter*, o_Spirochaetales, and p_Spirochaetota were greatly enriched in the non-metastatic CRC group.

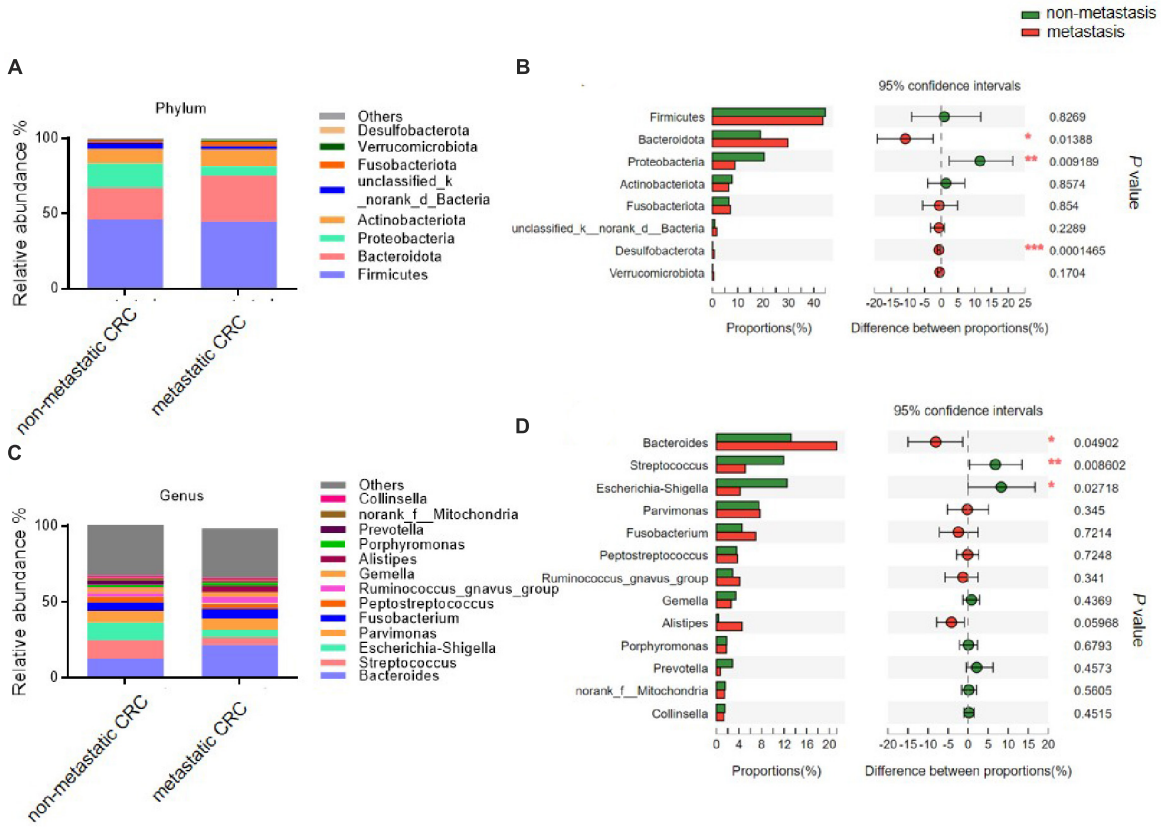

FIGURE 1
The microbial composition of tumor tissues from metastatic and non-metastatic colorectal cancer (CRC) groups. **(A)** Histograms of the predominant bacterial phyla of tumor tissues from metastatic and non-metastatic CRC groups. **(B)** The phylum-level bacterial proportion difference analysis between metastatic and non-metastatic CRC groups. **(C)** Histograms of the predominant bacterial genera of tumor tissues from metastatic and non-metastatic CRC groups. **(D)** The genus-level bacterial proportion difference analysis between metastatic and non-metastatic CRC groups. Metastatic CRC group, n = 48; non-metastatic CRC group, n = 44. The Wilcoxon rank-sum test was used in patterns **(B,D)**. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Bacteroides fragilis and *Bacteroides uniformis* are increased in the metastatic CRC group

Bacterial composition analysis indicated that the abundance of g_*Bacteroides* was the highest among all the bacterial species and was increased in the metastatic CRC group. Then, we tried to identify the specific bacteria at the species level. In total, 37 OTUs were found to belong to g_*Bacteroides*, among which *Bacteroides fragilis* (OTU 2272), unclassified g_*Bacteroides* (OTU5969) and *Bacteroides uniformis* (OTU2249) were the three highest average abundance OTUs (**Supplementary Table 1**). Importantly, *Bacteroides fragilis* and *Bacteroides uniformis* showed a strong increasing trend in the metastatic CRC group compared to the non-metastatic group (p = 0.067 and 0.076, respectively) (**Supplementary Table 1**). As is reported that *Bacteroides fragilis* toxin (BFT) is the potential substance promoting tumorigenesis and metastasis (Zamani et al., 2019; Liu et al., 2020), we tested the *bft* gene frequency in non-metastatic and metastatic CRC tissue samples. The results showed that 28 out of 44 (63.6%) non-metastatic and 41 out of 48 (85.4%) metastatic CRC samples are *bft* gene positive (**Supplementary Figure 3**).

The main differential bacteria are confirmed by qPCR

To confirm the high-throughput sequencing results, we performed a qPCR experiment to quantify the main differential species in tumor tissues. The results showed that at the phylum level, the abundance of Bacteroidota was increased in metastatic CRC tissue, while that of Proteobacteria was decreased (**Figures 2A, B**), but the results of Desulfobacterota were lacking because its abundance was lower than the limit of detection by qPCR in this study; at the genus level, the *Bacteroides* abundance increased, but the *Streptococcus* and *Escherichia-Shigella* abundances significantly decreased in the metastatic CRC group (**Figures 2C–E**). In addition, we tested the abundance of *Bacteroides fragilis* and *Bacteroides uniformis*, and the results showed that they were greatly increased in metastatic CRC tissues (**Figures 2F, G**).

ROC analyses of differential bacteria in diagnostic models for CRC metastasis

First, we examined the diagnostic efficiency of the serum markers AFP, CEA, and CA19-9 in CRC metastasis. As shown

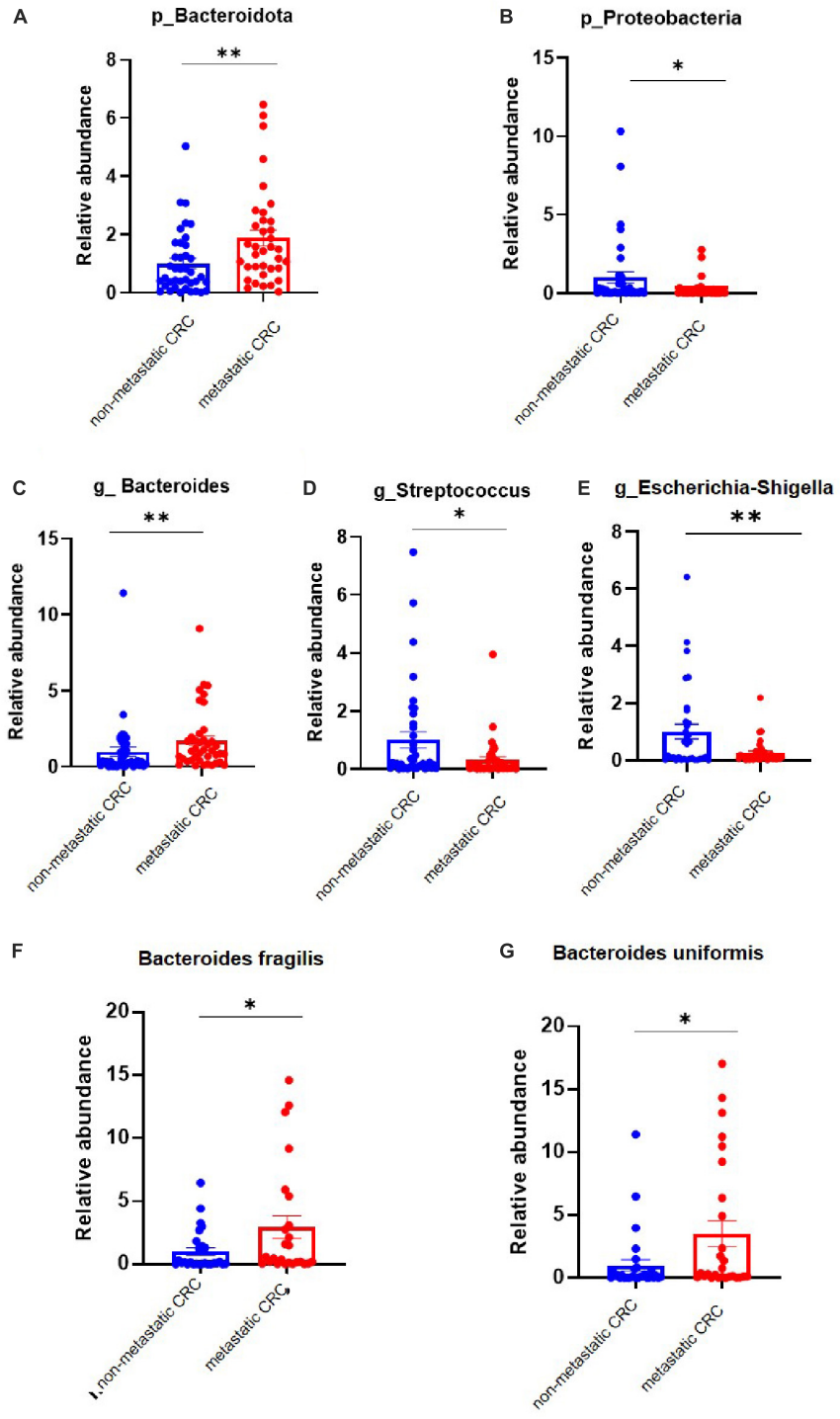

FIGURE 2
The differential bacteria between the metastatic and non-metastatic colorectal cancer (CRC) groups were confirmed by polymerase chain reaction (PCR). **(A,B)** Relative abundance of the Bacteroidetes and Proteobacteria phyla. Metastatic CRC group, n = 48; non-metastatic CRC group, n = 44. **(C–E)** Relative abundance of the *Bacteroides*, *Streptococcus*, and *Escherichia-Shigella* genera. Data below the limit of detection were removed; metastatic CRC group, n = 30; non-metastatic CRC group, n = 27. **(F,G)** Relative abundance of *Bacaeroides fragilis* and *Bacteroides uniformis*. Metastatic CRC group, n = 48; non-metastatic CRC group, n = 44. Data are presented as the mean ± SEM; $^{*}p < 0.05$; $^{**}p < 0.01$ by unpaired Student's t-test.

in Figures 3A–C, the area under the curve (AUC) of CEA (0.652, 95% CI: 0.5387–0.7652, p = 0.012) was the largest, with a sensitivity of 0.479 and specificity of 0.8409 at the optimal cut-off value of 8.875. Next, we examined the diagnostic efficiency of differential bacteria in CRC metastasis. The AUCs of the ROC curves of p_Bacteroidota, p_Proteobacteria, p_Desulfobacterota, g_*Bacteroides* g_*Streptococcus*, and g_*Escherichia-Shigella* were 0.6709 (95% CI: 0.5609–0.7810, P = 0.0048), 0.5978 (95% CI:

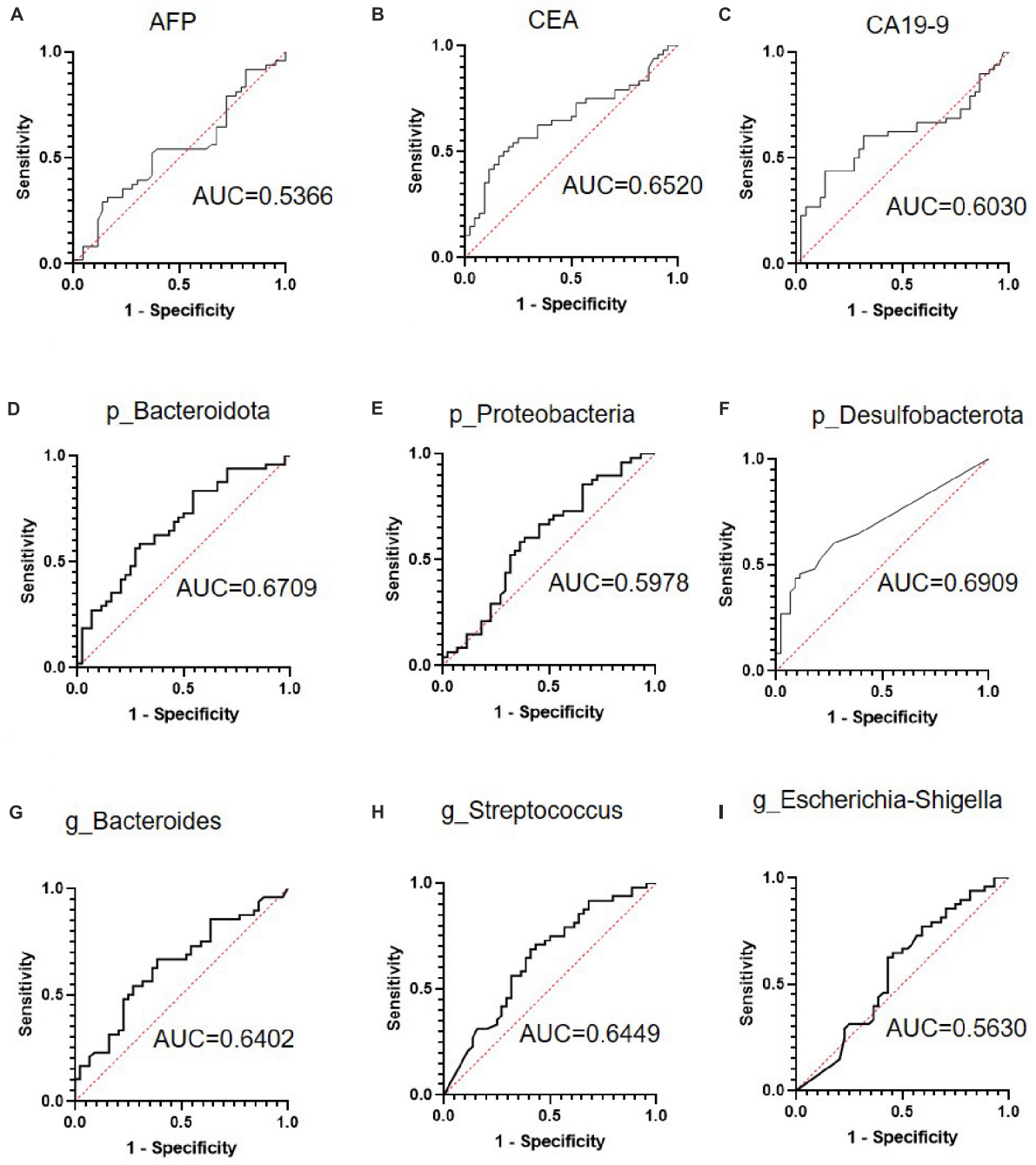

FIGURE 3
The receiver operating characteristic (ROC) curve analysis of serum tumor markers and selected bacteria in predicting the metastasis of colorectal cancer (CRC). **(A–C)** ROC curve analysis of serum tumor markers alpha fetoprotein (AFP), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9) in patients with CRC. **(D–F)** ROC curve analyses of Bacteroidota, Proteobacteria, and Desulfobacterota phyla in patients with CRC. **(G–I)** ROC curve analysis of the *Bacteroides*, *Streptococcus*, and *Escherichia-Shigella* genera in patients with CRC.

0.4801–0.7155 $P = 0.1065$), 0.6906 (95% CI: 0.5822–0.7990, $P = 0.0017$), 0.6402 (95% CI: 0.5269–0.7534, $P = 0.0207$), 0.6449 (95% CI: 0.5313–0.7584, $P = 0.0168$), and 0.5630 (95% CI: 0.4430–0.6829, $P = 0.2985$), respectively (Figures 3D–I). These results indicated that the differential bacterial levels of CRC tissue groups possessed a moderate diagnostic efficiency for CRC metastasis.

Then, we attempted to improve the diagnostic efficacy by combining CEA with selected bacteria. The combination ROC curve of CEA with p_Bacteroidota, p_Proteobacteria, p_Desulfobacterota, g_*Bacteroides* and, or g_*Streptococcus* was drawn (Figures 4A–E), and the AUC was 0.6974 (95% CI: 0.5903–0.8046, $P = 0.0011$), 0.6723 (95% CI: 0.5628–0.7819, $P = 0.0044$), 0.7027 (95% CI: 0.5942–0.8111, $P < 0.001$), 0.6785 (95% CI: 0.5690–0.7880, $P = 0.0032$), 0.7055 (95% CI: 0.5996–0.8114, $P < 0.001$), respectively. Therefore, combination analyses obtained a higher diagnostic efficiency for CRC metastasis.

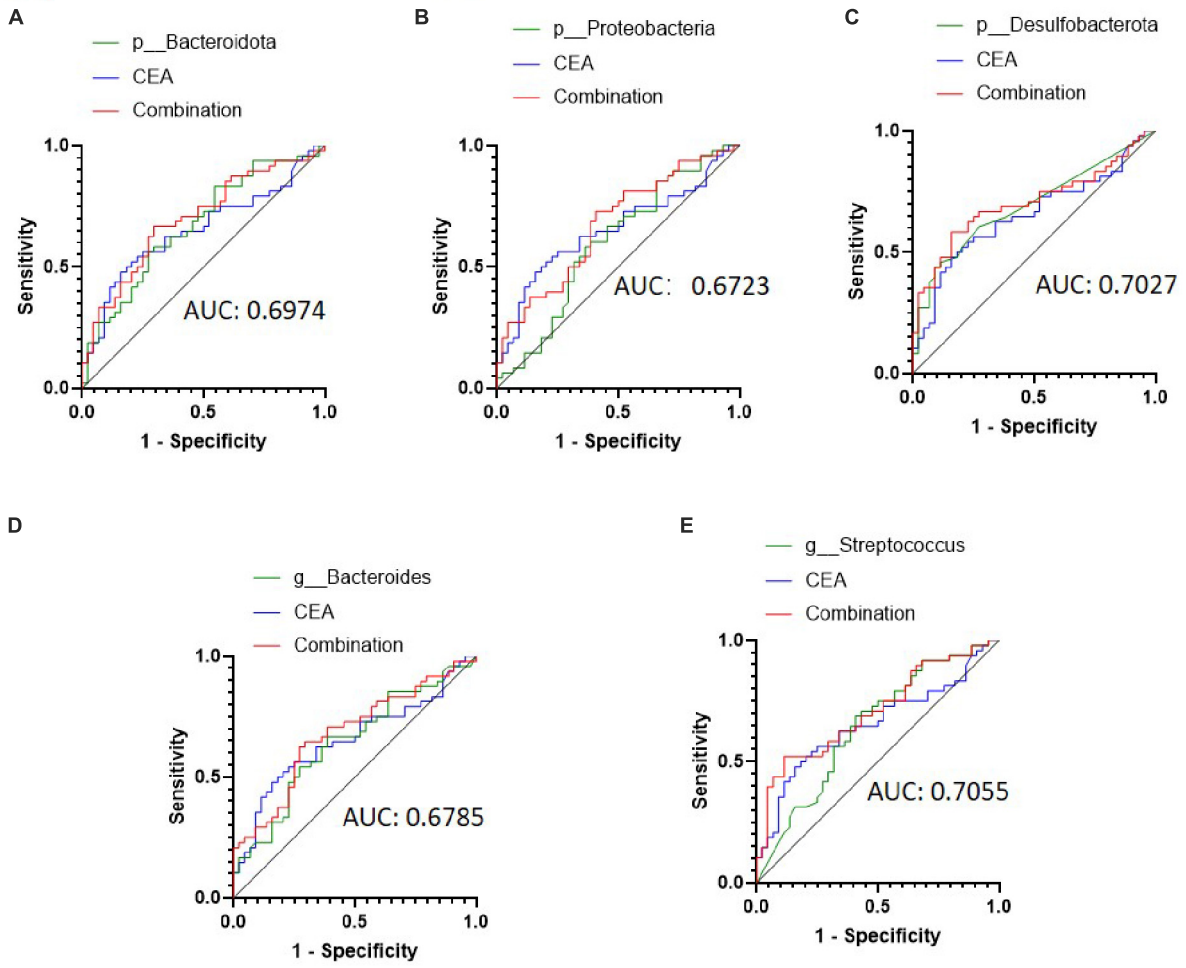

FIGURE 4
Receiver operating characteristic (ROC) curve analysis of carcinoembryonic antigen (CEA) combined with selected bacteria in predicting the metastasis of colorectal cancer (CRC). **(A–C)** ROC curve analysis of CEA combined with patients with Bacteroidota, Proteobacteria, and Desulfobacterota phyla. **(D,E)** ROC curve analysis of CEA combined with patients with *the Bacteroides* and *Streptococcus* genera.

Tumor adjacent tissues of metastatic and non-metastatic CRC show microbial composition differences

To explore whether the differential bacteria only existed in tumor tissue or existed in other normal intestinal tissues, we analysed the microbial composition of tumor-adjacent tissues from these patients. The results showed that at the phylum level, the relative abundances of Bacteroidota and Desulfobacterota were significantly higher in the adjacent tissue of the metastatic CRC group than in the adjacent tissue of the non-metastatic CRC group (30.78 ± 20.13 vs. 21.46 ± 20.42% and 0.56 ± 1.19 vs. 0.17 ± 0.4%, respectively) (**Figures 5A, B**), while the relative abundance of Proteobacteria showed a decreased trend in the adjacent tissue of the metastatic group compared to the non-metastatic group (6.02 ± 6.88 vs. 16.03 ± 25.72%) (**Figures 5A, B**), although there was no statistical significance. At the genus level, the relative abundance of *Bacteroides* in the metastatic group was significantly higher than that in the non-metastatic group (23.89 ± 19.31 vs. 18.16 ± 19.66%) (**Figures 5C, D**), while *Streptococcus* was significantly lower than that in the non-metastatic group (3.44 ± 5.6 vs. 5.9 ± 7.43%) (**Figures 5C, D**). These results indicated that the differential bacteria in the adjacent and tumor tissues of metastatic and non-metastatic CRC were consistent, meaning that the CRC metastasis associated bacteria are not specifically enriched in tumor tissues alone but are present in a larger area of the intestinal tract of CRC patients.

Discussion

The harmonious intestinal microbiota, inhabiting the gut lumen, plays a crucial role in gut health (Thursby and Juge, 2017). However, in pathological situations, certain symbiotic bacteria adhere to or invade the intestinal mucosa, which can affect the progression of intestinal diseases, such as colorectal cancer (Tomkovich et al., 2019). In this study, we revealed the potential bacteria that associate with CRC metastasis, the leading cause of CRC death, by systematically analysing the characteristics of the tissue-associated microbiota collected from the non-metastatic and metastatic CRC groups.

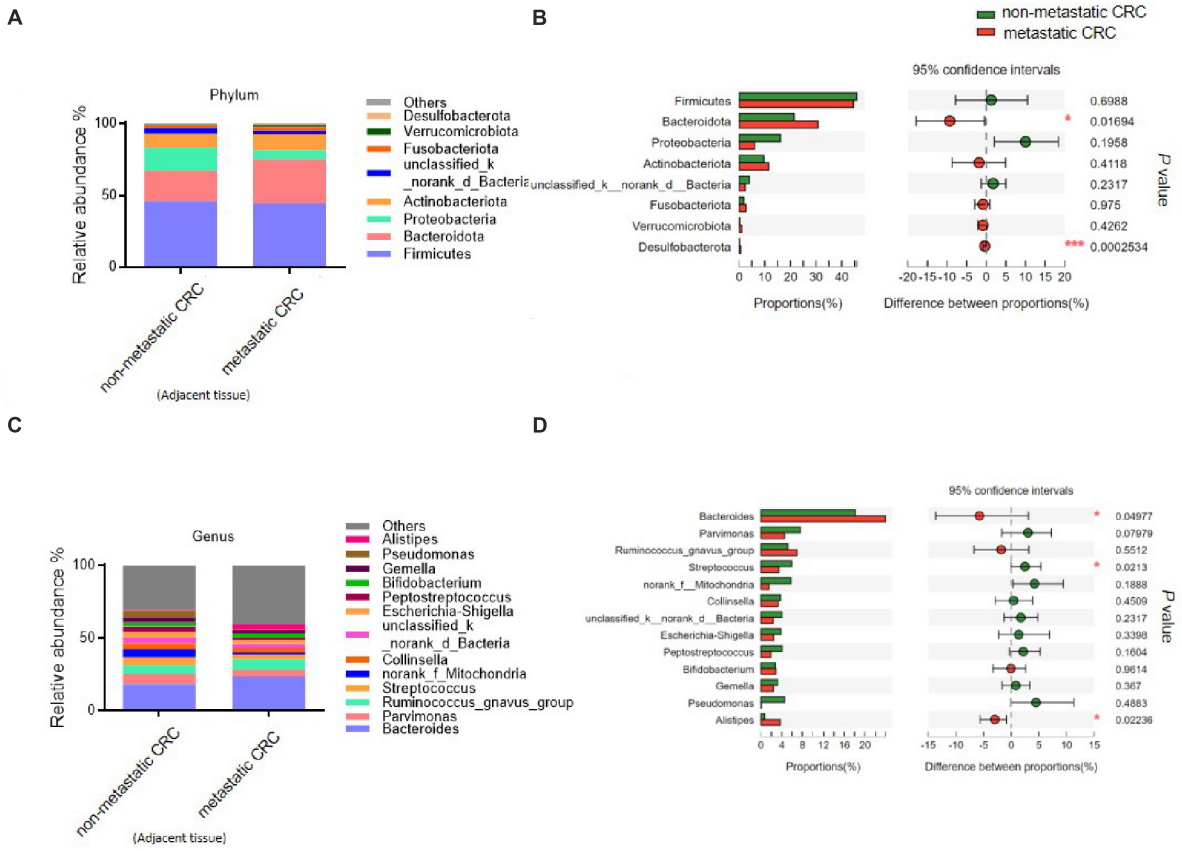

FIGURE 5
The microbial composition of tumor-adjacent tissues from metastatic and non-metastatic colorectal cancer (CRC) groups. **(A)** Histograms of the predominant bacterial phyla of tumor-adjacent tissues from metastatic and non-metastatic CRC groups. **(B)** The phylum-level bacterial proportion difference analysis of tumor-adjacent tissues from metastatic and non-metastatic CRC groups. **(C)** Histograms of the predominant bacterial genera of tumor-adjacent tissues from metastatic and non-metastatic CRC groups. **(D)** The genus-level bacterial proportion difference analysis of tumor-adjacent tissues from metastatic and non-metastatic CRC groups. Tumor-adjacent tissue of the metastatic CRC group, $n = 48$; tumor-adjacent tissue of the non-metastatic CRC group, $n = 44$. The Wilcoxon rank-sum test was used in patterns **(B,D)**. $^{*}P < 0.05$; $^{***}P < 0.001$.

We collected mucosal tissues from CRC patients who underwent surgical operation and extracted the DNA of tissue-associated bacteria. The function of tissue-associated bacteria in CRC progression may differ from that of luminal bacteria (Durbán et al., 2011; Chen, 2018). The gut microbiota in the lumen usually indirectly affects epithelial cells, such as by metabolites (Dalal et al., 2021), but tissue-associated bacteria are believed to stimulate intestinal cells directly and intensely (Chen et al., 2012). Therefore, mucosal bacteria should play more important roles than the gut microbiota in CRC progression. In addition, we found that all the tissues from CRC patients contained more mucosal bacteria than those from healthy individuals (data not shown). The probable reason is that the colon of CRC patients is associated with a reduced intestinal barrier (Sun et al., 2022).

Our study found that the composition of the flora of the two groups showed a great difference. We noted that *Bacteroides* was the most abundant bacterium for tissue adhesion and was significantly enriched in the metastatic group. *Bacteroides fragilis* and *Bacteroides uniformis* were two species that were significantly elevated in the metastatic group. In fact, it was reported that *Bacteroides fragilis* was higher in the stool of CRC patients than in healthy individuals. In addition, *Bacteroides fragilis* has the ability to penetrate the colonic mucus and resides deep within crypt channels (Lee et al., 2013). Thus, its abundance was very high in CRC intestinal tissue (Li S. et al., 2021). Mechanistically, *Bacteroides fragilis* can secrete *B. fragilis* toxin and induce stemness in CRC by upregulating Jumonji domain-containing protein 2B (JMJD2B) levels in a TLR4-NFAT5-dependent pathway (Liu et al., 2020). Recently, Parida et al. (2021) found that enterotoxigenic *Bacteroides fragilis* (ETBF) is present in breast tumor tissue, triggers epithelial hyperplasia and augments breast cancer growth and metastasis via the β-catenin and Notch1 pathways. Our results indicated that the abundance of *Bacteroides fragilis* increased significantly, and *bft* gene was more prevalent in metastatic CRC samples, which is consistent with its role in bowel cancer progression and metastasis. *Bacteroides uniformis* is usually known as a harmless bacterium, but some other studies and our study identified that its abundance increased in the CRC group. Further studies are needed to clarify the potential tumor-promoting function of *Bacteroides uniformis*.

The abundance of *Streptococcus* was decreased in the metastatic CRC group. Many studies have reported that different species of *Streptococcus* play different roles in CRC. Some species, such as *Streptococcus gallolyticus*, strongly associated with the occurrence of colorectal cancer are known as tumor-promoting bacteria

(Aymeric et al., 2018). Nevertheless, Li Q. et al. (2021) reported that *Streptococcus thermophiles*, which is depleted in stool samples of patients with CRC, plays a tumor-suppressive role by secreting β-galactosidase to maintain high galactose content throughout the gastrointestinal tract and then inhibit the Hippo pathway in tumour tissues. In our study, *Streptococcus* may act as a tumor-inhibiting bacterium by an unknown mechanism. Our ongoing work will try to identify specific species and research their antitumor functions.

In addition, our results showed that the main differential bacteria of tumor-adjacent tissue are similar to those of tumor tissues, indicating that in CRC patients, tissue-associated bacteria may be present in a wider range of intestinal tissues rather than only in the tumor. Similarly, Boleij et al. (2015) reported that the *bft* gene, which plays an important role in the pathogenesis of human CRC, is not limited to tumors but spans a larger portion of the colonic mucosa. Therefore, further study is needed to comprehensively evaluate the impact of CRC metastasis associated bacteria on intestinal health.

Data availability statement

The datasets presented in this study can be found in online repositories and accession number can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA916596.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Committee of the Affiliated Hospital of Medical School, Ningbo University (KS202111002). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

DS and YZ conceived and designed the project. PZ, ZD, and TL recruited patients and collected tissue samples. PZ performed qPCR experiments. PZ, ZD, YX, ZX, YH, and DS analysed the data. PZ and DS prepared the manuscript. DS and YZ wrote and reviewed the final version of the text. All authors contributed to finalizing the manuscript, read, and approved the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1133607/full#supplementary-material

References

Aymeric, L., Donnadieu, F., Mulet, C., du Merle, L., Nigro, G., Saffarian, A., et al. (2018). Colorectal cancer specific conditions promote *Streptococcus gallolyticus* gut colonization. *Proc. Natl. Acad. Sci. U. S. A.* 115, E283–E291.

Bateman, A. C. (2022). Pathology of colorectal polyps and cancer. *Surgery* 38, 12–17. doi: 10.1016/j.mpsur.2019.10.012

Biller, L. H., and Schrag, D. (2021). Diagnosis and treatment of metastatic colorectal cancer: A review. *Jama* 325, 669–685. doi: 10.1001/jama.2021.0106

Boleij, A., Hechenbleikner, E. M., Goodwin, A. C., Badani, R., Stein, E. M., Lazarev, M. G., et al. (2015). The *Bacteroides fragilis* toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clin. Infect Dis.* 60, 208–215. doi: 10.1093/cid/ciu787

Chen, G. Y. (2018). The role of the gut microbiome in colorectal cancer. *Clin. Colon. Rectal Surg.* 31, 192–198. doi: 10.1055/s-0037-1602239

Chen, W., Liu, F., Ling, Z., Tong, X., and Xiang, C. (2012). Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. *PLoS One* 7:e39743. doi: 10.1371/journal.pone.0039743

Cheng, Y., Ling, Z., and Li, L. (2020). The intestinal microbiota and colorectal cancer. *Front. Immunol.* 11:615056. doi: 10.3389/fimmu.2020.615056

Clay, S. L., Fonseca-Pereira, D., and Garrett, W. S. (2022). Colorectal cancer: The facts in the case of the microbiota. *J. Clin. Invest.* 132:e155101. doi: 10.1172/JCI155101

Costa, C. P. D., Vieira, P., Mendes-Rocha, M., Pereira-Marques, J., Ferreira, R. M., and Figueiredo, C. (2022). The tissue-associated microbiota in colorectal cancer: A systematic review. *Cancers* 14:3385. doi: 10.3390/cancers14143385

Dalal, N., Jalandra, R., Bayal, N., Yadav, A. K., Harshulika, Sharma, M., et al. (2021). Gut microbiota-derived metabolites in CRC progression and causation. *J. Cancer Res. Clin. Oncol.* 147, 3141–3155. doi: 10.1007/s00432-021-03729-w

Dekker, E., Tanis, P. J., Vleugels, J. L. A., Kasi, P. M., and Wallace, M. B. (2019). Colorectal cancer. *Lancet* 394, 1467–1480. doi: 10.1016/S0140-6736(19)32319-0

Derwinger, K., Kodeda, K., Bexe-Lindskog, E., and Taflin, H. (2010). Tumour differentiation grade is associated with TNM staging and the risk of node metastasis in colorectal cancer. *Acta Oncol.* 49, 57–62. doi: 10.3109/02841860903334411

Durbán, A., Abellán, J. J., Jiménez-Hernández, N., Ponce, M., Ponce, J., Sala, T., et al. (2011). Assessing gut microbial diversity from feces and rectal mucosa. *Microb. Ecol.* 61, 123–133. doi: 10.1007/s00248-010-9738-y

Emery, D. C., Cerajewska, T. L., Seong, J., Davies, M., Paterson, A., Allen-Birt, S. J., et al. (2020). Comparison of blood bacterial communities in periodontal health and periodontal disease. *Front. Cell Infect. Microbiol.* 10:577485. doi: 10.3389/fcimb.2020.577485

Flemer, B., Lynch, D. B., Brown, J. M., Jeffery, I. B., Ryan, F. J., Claesson, M. J., et al. (2017). Tumour-associated and non-tumour-associated microbiota in colorectal cancer. *Gut* 66, 633–643. doi: 10.1136/gutjnl-2015-309595

Fong, W., Li, Q., and Yu, J. (2020). Gut microbiota modulation: A novel strategy for prevention and treatment of colorectal cancer. *Oncogene* 39, 4925–4943. doi: 10.1038/s41388-020-1341-1

Ibrahim, A., Hugerth, L. W., Hases, L., Saxena, A., Seifert, M., Thomas, Q., et al. (2019). Colitis-induced colorectal cancer and intestinal epithelial estrogen receptor beta impact gut microbiota diversity. *Int. J. Cancer* 144, 3086–3098. doi: 10.1002/ijc.32037

Johnson, E. J., Zemanick, E. T., Accurso, F. J., Wagner, B. D., Robertson, C. E., and Harris, J. K. (2016). Molecular identification of *Staphylococcus aureus* in airway samples from children with cystic fibrosis. *PLoS One* 11:e0147643. doi: 10.1371/journal.pone.0147643

Keku, T. O., Dulal, S., Deveaux, A., Jovov, B., and Han, X. (2015). The gastrointestinal microbiota and colorectal cancer. *Am. J. Physiol. Gastrointest Liver Physiol.* 308, G351–G363. doi: 10.1152/ajpgi.00360.2012

Kurakawa, T., Kubota, H., Tsuji, H., Matsuda, K., Takahashi, T., Ramamurthy, T., et al. (2013). Intestinal Enterobacteriaceae and *Escherichia coli* populations in Japanese adults demonstrated by the reverse transcription-quantitative PCR and the clone library analyses. *J. Microbiol. Methods* 92, 213–219. doi: 10.1016/j.mimet.2012.12.008

Lee, S. M., Donaldson, G. P., Mikulski, Z., Boyajian, S., Ley, K., and Mazmanian, S. K. (2013). Bacterial colonization factors control specificity and stability of the gut microbiota. *Nature* 501, 426–429. doi: 10.1038/nature12447

Li, Q., Hu, W., Liu, W. X., Zhao, L. Y., Huang, D., Liu, X. D., et al. (2021). *Streptococcus thermophilus* inhibits colorectal tumorigenesis through secreting β-galactosidase. *Gastroenterology* 160, 1179–1193.e4 doi: 10.1053/j.gastro.2020.09.003

Li, S., Liu, J., Zheng, X., Ren, L., Yang, Y., Li, W., et al. (2021). Tumorigenic bacteria in colorectal cancer: Mechanisms and treatments. *Cancer Biol. Med.* 19, 147–162. doi: 10.20892/j.issn.2095-3941.2020.0651

Liu, Q. Q., Li, C. M., Fu, L. N., Wang, H. L., Tan, J., Wang, Y. Q., et al. (2020). Enterotoxigenic *Bacteroides fragilis* induces the stemness in colorectal cancer via upregulating histone demethylase JMJD2B. *Gut Microbes.* 12:1788900. doi: 10.1080/19490976.2020.1788900

Parida, S., Wu, S., Siddharth, S., Wang, G., Muniraj, N., Nagalingam, A., et al. (2021). A procarcinogenic colon microbe promotes breast tumorigenesis and metastatic progression and concomitantly activates notch and β-catenin axes. *Cancer Discov.* 11, 1138–1157. doi: 10.1158/2159-8290.CD-20-0537

Pretzsch, E., Bösch, F., Neumann, J., Ganschow, P., Bazhin, A., Guba, M., et al. (2019). Mechanisms of metastasis in colorectal cancer and metastatic organotropism: Hematogenous versus peritoneal spread. *J. Oncol.* 2019:7407190. doi: 10.1155/2019/7407190

Queipo-Ortuño, M. I., Boto-Ordóñez, M., Murri, M., Gomez-Zumaquero, J. M., Clemente-Postigo, M., Estruch, R., et al. (2012). Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *Am. J. Clin. Nutr.* 95, 1323–1334. doi: 10.3945/ajcn.111.027847

Rebersek, M. (2021). Gut microbiome and its role in colorectal cancer. *BMC Cancer* 21:1325. doi: 10.1186/s12885-021-09054-2

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., et al. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.* 12:R60. doi: 10.1186/gb-2011-12-6-r60

Sun, J., Zhang, J., Xia, Y., and Zhang, Y.-G. (2022). Abstract IA018: Microbiome and intestinal barriers in colon cancer. *Cancer Res.* 82, IA018–IA018. doi: 10.1158/1538-7445.CRC22-IA018

Sung, H., Ferlay, J., Siegel, R. L., 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. doi: 10.3322/caac.21660

Tabowei, G., Gaddipati, G. N., Mukhtar, M., Alzubaidee, M. J., Dwarampudi, R. S., Mathew, S., et al. (2022). Microbiota dysbiosis a cause of colorectal cancer or not? A systematic review. *Cureus* 14:e30893. doi: 10.7759/cureus.30893

Thursby, E., and Juge, N. (2017). Introduction to the human gut microbiota. *Biochem. J.* 474, 1823–1836. doi: 10.1042/BCJ20160510

Tomkovich, S., Dejea, C. M., Winglee, K., Drewes, J. L., Chung, L., Housseau, F., et al. (2019). Human colon mucosal biofilms from healthy or colon cancer hosts are carcinogenic. *J. Clin. Invest.* 129, 1699–1712. doi: 10.1172/JCI124196

Tong, J., Liu, C., Summanen, P., Xu, H., and Finegold, S. M. (2011). Application of quantitative real-time PCR for rapid identification of *Bacteroides fragilis* group and related organisms in human wound samples. *Anaerobe* 17, 64–68. doi: 10.1016/j.anaerobe.2011.03.004

Wong, S. H., and Yu, J. (2019). Gut microbiota in colorectal cancer: Mechanisms of action and clinical applications. *Nat. Rev. Gastroenterol. Hepatol.* 16, 690–704. doi: 10.1038/s41575-019-0209-8

Yuan, J., Zhao, J., Wen, T., Zhao, M., Li, R., Goossens, P., et al. (2018). Root exudates drive the soil-borne legacy of aboveground pathogen infection. *Microbiome* 6:156. doi: 10.1186/s40168-018-0537-x

Zamani, S., Hesam Shariati, S., Zali, M. R., Asadzadeh Aghdaei, H., Sarabi Asiabar, A., Bokaie, S., et al. (2017). Detection of enterotoxigenic *Bacteroides fragilis* in patients with ulcerative colitis. *Gut Pathog.* 9:53. doi: 10.1186/s13099-017-0202-0

Zamani, S., Taslimi, R., Sarabi, A., Jasemi, S., Sechi, L. A., and Feizabadi, M. M. (2019). Enterotoxigenic *Bacteroides fragilis*: A possible etiological candidate for bacterially-induced colorectal precancerous and cancerous lesions. *Front. Cell Infect. Microbiol.* 9:449. doi: 10.3389/fcimb.2019.00449

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Gut *Streptococcus* is a microbial marker for the occurrence and liver metastasis of pancreatic cancer

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Background: Gut microbiome plays an indispensable role in the occurrence and progression in various diseases. The incidence of pancreatic cancer (PC) and liver metastasis (PCLM) are high, most of them are found in advanced stage. Therefore, it is particularly necessary to search for predictive biomarkers, which are helpful for early detection and treatment, and thus improve the survival rate and quality of life of PC patients.

Methods: We retrospectively analyzed 44 pancreatic cancer patients (P group, n=44) and 50 healthy people (N group, n=50) from March 21, 2021 and August 2, 2022. Among all PC patients, we divided them into liver metastasis group (LM group, n=27) and non-liver metastasis group (non-LM group, n=17). DNA was extracted and 16S ribosomal RNA (16S rRNA) gene sequencing was performed. SPSS was used for statistical analyses and all bioinformatics analyses were based on QIIME2, p<0.05 were considered statistically significant.

Results: The microbial richness and diversity of group P and LM were higher than that of group N and non-LM. LEfSe analysis found that *Streptococcus* was a significantly different microorganism, which was further identified by random forest (RF) model, and its ability to predict PC and PCLM was verified by ROC curve.

Conclusion: We demonstrated significant differences in intestinal microbiome composition between PC patients and healthy people, and found that *Streptococcus* is a potential biomarker for early prediction of PC and PCLM, which is critical for early diagnosis of diseases.

KEYWORDS

***Streptococcus*, pancreatic cancer, liver metastasis, gut microbiome, 16S rRNA sequencing**

Introduction

According to the GLOBOCAN's statistics in 2020 (Sung et al., 2021), there were approximately half million new cases of pancreatic cancer (PC) worldwide (2.6%/19.3 million), however, its mortality rate accounts for 4.7% of all cancer specific deaths. Although in recent years, with the rise of new treatment methods including immunotherapy

and targeted therapy, as well as the deepening understanding of complex mechanisms, the overall 5-year survival rate of PC has not changed much. It is estimated that PC may become one of the main causes of cancer-related deaths in the future (Neoptolemos et al., 2018). The pancreas is a retroperitoneal organ, due to its concealed location, is surrounded by the duodenum, which affects its observation (Zhang et al., 2018), and lacks special symptoms and develops rapidly. Thus, PC is often found in the late stage. Liver metastasis (LM) is one of the most common modes of PC metastasis, and the main cause of treatment failure and death in advanced PC patients (Zheng et al., 2020). At present, there is no effective prediction method for PC. In clinical practice, traditional methods such as serum tumor markers, imaging examination or biopsy are commonly used to screen and diagnose PC. However, when the indicators change, the disease has progressed (Zhou et al., 2017). Therefore, it is very necessary to find convenient, non-invasive and inexpensive PC prediction biomarkers, which will help in the early detection and treatment of PC.

Pancreatic carcinogenesis is related to a variety of risk factors, including genetic factors, inflammatory factors, and stimulating factors, such as smoking, drinking, etc. Currently, researchers have focused on intestinal microorganisms (Klein, 2021). The human intestinal microbes and its metabolites constitute a complex microecology. There are approximately 10^{14} types of bacteria in the human digestive tract, mainly distributed in the colon and rectum (Sender et al., 2016). It plays a crucial role in many life processes, such as promoting metabolism, regulating energy storage, activating immune system, and maintaining intestinal homeostasis (Deschasaux et al., 2018; Hartmann et al., 2019). Under normal circumstances, bacteria in the digestive tract maintain a relative balance of species and quantity through symbiosis, competition and antagonism, and maintain dynamic balance with the host. Once the balance is abnormal or disrupted, it can cause bacteria disorders and lead to a series of diseases (Fu et al., 2022). More importantly, the toxic products of intestinal microorganisms have been identified as possible carcinogens, such as improving the tumorigenic effect by triggering double stranded DNA damage. At the same time, intestinal microorganisms are associated with a variety of risk factors related to pancreatic cancer, such as diabetes, chronic pancreatitis and obesity, which may potentially affect PC and PCLM. Based on this background, this study took pancreatic cancer as the starting point to explore the functional mechanism of intestinal microorganisms in liver metastasis, hoping to find biomarkers that predict PC and PCLM, and provide theoretical basis for prolonging the survival of PC patients.

Abbreviations: PC, pancreatic cancer; LM, liver metastasis; rRNA, Ribosome ribonucleic acid; QIIME, Quantitative Insights into Microbial Ecology; ASVs, Amplicon sequence variants; PERMANOVA, permutational multivariate analysis of variance; PCoA, Principal coordinate analysis; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Methods

Patients

We retrospectively analyzed 44 untreated PC patients (pancreatic cancer group, P group) and 50 matched healthy volunteers (Normal group, N group) between March 21, 2021 to August 2, 2022. All patients were selected through preset inclusion and exclusion criteria, the inclusion criteria were as follows: (1) all cases were confirmed as pancreatic cancer at the cancer center of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology; (2) the patients had not received anti-tumor treatment, except for surgery, and stool samples were collected 3 weeks after the surgery; (3) no antibiotics or other drugs that may affect the intestinal flora were taken before the samples collection; (4) no intestinal invasive operations, such as gastrointestinal endoscopy and enema were performed; (5) without bile duct obstruction; and (6) the patients knew the contents of the study and had signed the informed consent. Patients who do not meet the inclusion criteria will be excluded. Among the PC patients, we divided them into liver metastasis group (LM group, $n = 27$) and non-liver metastasis (non-LM group, $n = 17$) to find the key intestinal microorganisms and related metabolic pathways that distinguish PC and PCLM.

For all PC patients, baseline clinical-pathological characteristics, including age, gender, body mass index (BMI), tumor sites, pathological types, whether metastasis, metastasis sites, whether surgery, lines of treatment, Eastern Cooperative Oncology Group (ECOG) performance and baseline bilirubin value were available for review. This retrospective study was approved by the Ethical Committees of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (No. 2014-041).

Sample collection and 16S rRNA sequencing

Fecal samples of the PC patients were collected by the trained medical staff before anti-tumor treatment, put each sample in a 50 mL sterile specimen collection box, then immediately transfer to a −80°C refrigerator for storage. After all samples were collected, they were sent for examination. The samples' total microbiome DNA was isolated with Omega Mag-Bind Soil DNA kit (Omega Bio-Tek, Norcross, GA, United States), and the concentration and purity of the DNA were determined (Yu et al., 2021). The V3-V4 variable regions of the qualified samples were sequenced using Illumina platform (Illumina, San Diego, CA, United States) and the original data were filtered by the dada2 method of Quantitative Insights into Microbial Ecology2 (QIIME2) software (v2019.4) (Rai et al., 2019), and the effective data were stored in FASTQ format. On this basis, the similarity sequences were clustered as amplicon sequence variants (ASVs), then the Naive Bayes classifier in QIIME2 software was used to cross compare with the Greengenes database (release 13.8)[1] (DeSantis et al., 2006) for species annotation.

1 http://greengenes.secondgenome.com/

TABLE 1 Demographic characteristics of PC patients (n=44).

Variable	n (%)
Age (y)	
<65	27 (61.36%)
≥65	17 (38.64%)
Gender	
Male	21 (47.73%)
Female	23 (52.27%)
BMI	22.43 ± 2.75
Tumor sites	
Head	18 (40.91%)
Body	3 (6.82%)
Tail	5 (11.36%)
Whole	18 (40.91%)
Pathological types	
Adenocarcinoma	36 (81.82%)
Squamous cell carcinoma	3 (6.82%)
Other	5 (11.36%)
Whether metastasis	
Yes	32 (72.73%)
No	12 (27.27%)
Metastasis sites (n = 32)	
Liver	27 (84.38%)
Lung	4 (12.50%)
Distant lymph node	18 (56.25%)
Peritoneum	9 (28.13%)
ECOG performance	
0	43 (97.73%)
1	1 (2.27%)
Whether surgery	
Yes	11 (25.00%)
No	33 (75.00%)
Total bilirubin (TBIL) (μmol/L)	15.90 ± 12.57
Direct bilirubin (DBIL) (μmol/L)	5.63 ± 4.83
Indirect bilirubin (IBIL) (μmol/L)	11.86 ± 12.39

Statistical and bioinformatic analyses

SPSS software (version 22.0, SPSS Inc., Chicago, IL, United States) were used for statistical analysis. Graphpad prism 8.0 (GraphPad Software Inc., San Diego, CA, United States) was used to draw charts. The patient characteristics were compared by Chi-squared test and Student's t-test. All tests were performed by two-sided, and $p < 0.05$ were considered statistically significant. The 16S rRNA sequencing data were analyzed based on QIIME2 software. Principal coordinates analysis (PCoA) (Shi et al., 2020), Linear discriminant analysis (LDA) and effect size (LEfSe) (Segata et al., 2011), Kruskal-Wallis test, permutational multivariate analysis of variance (PERMANOVA), random forest (RF) model (Liu et al., 2020) and other bioinformatic methods were used to perform hypothesis test of intergroup diversity and predict the microbial metabolism function.

Results

Demographic characteristics

A total of 44 GC patients between March 21, 2021 and August 2, 2022 were included in our retrospective study. There was no statistical difference in baseline information between the two groups, the baseline characteristics of 44 PC patients were summarized in Table 1. In the PC population, 61.36% were under 65 years old, and there were 23 (52.27%) females and 21 (47.73%) males. The body mass index (BMI) was 22.43 ± 2.75 kg/cm^2. The tumors mainly located in the whole (40.91%) and head (40.91%) of the pancreas. The majority pathological classification was adenocarcinoma, accounting for 81.82%. Thirty-two (72.73%) PC patients suffered from metastasis, including twenty-seven (27/32, 84.38%) liver metastasis (LM), eighteen (18/32, 56.25%) distant lymph node metastasis, nine (9/32, 28.13%) peritoneal metastasis and four (4/32, 12.50%) lung metastasis, only one quarter patients underwent surgery before treatment.

16S rRNA sequencing results

Sequencing data processing

The rarefaction curve and species accumulation curve indicated that the sample sequencing depth was sufficient and the annotated species were rich, with more than 6*10^4 species, which can better represent the microbial flora information of each group, as shown in Figures 1A,B. Venn diagram showed that 36886 and 21251 ASV/OTUs were clustered in P group and N group, respectively, (Figure 1C).

Microbial diversity analysis

For alpha diversity, Chao1 and Shannon indexes were used to represent the gene richness and number, both of them were higher in the P group than in the N group ($p < 0.001$, $p = 0.37$) (Figure 2A). According to Bray-Curtis distance algorithm, the graphs of principal coordinate analysis (PCoA) and 3D-PCoA[2] were drawn to display beta diversity, β-diversity between P and N group was large ($R^2 = 4.477$, $p = 0.001$), indicating that there were composition differences between two groups (Figures 2B,C). Axis 1, 2, and 3 of 3D-PCoA explain 8.810, 6.599, and 4.659% of variance, respectively.

Compositional analysis between PM and non-PM groups

According to the relative abundance (Figures 1E,G), intragroup cumulative abundance (Figures 1F,H) and phylogenetic tree plot (Figure 1D) of the top 10 species in each sample. At phylum level, *Firmicutes, Bacteroidetes, Proteobacteria* and *Actinobacteria* in both groups rank in the top four (Figure 1D). Among them, the relative abundance of *Verrucomicrobia* in P

2 https://view.qiime2.cn/

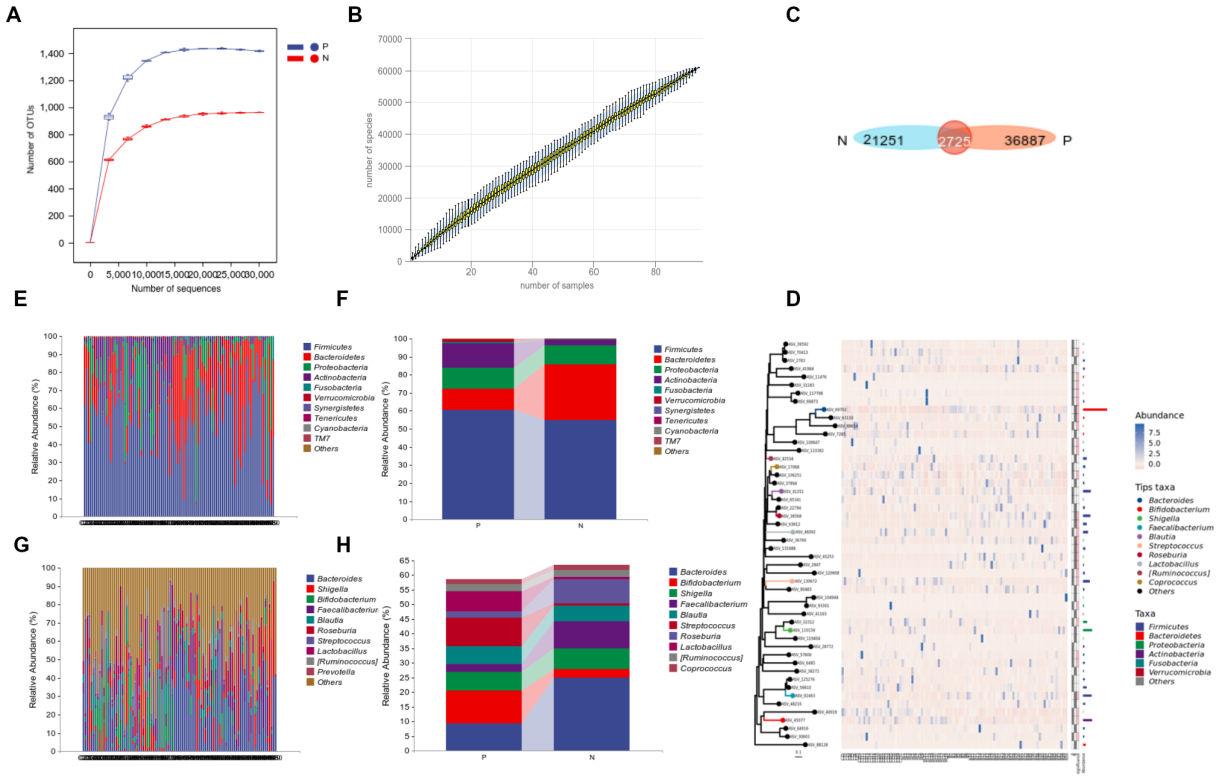

FIGURE 1
16S rRNA sequencing data processing and species composition of P and N groups. **(A)** Refraction curve; **(B)** species accumulation curve; **(C)** Venn gram of ASV/OTUs; **(D)** phylogenetic tree plot; **(E–H)** compositional analysis of each sample **(E,G)** and group **(F,H)** at the phylum level **(E,F)**, and at the genus level **(G,H)**.

group is 9.89% higher than that in N group (Figures 1E,F), and at the genus level, the relative abundance of *Streptococcus*, *Lactobacillus*, and *Bifidobacterium* in the P group was significantly increased, especially *Streptococcus*, which increased by 9.04 times in the N group (Figures 1G,H).

Linear discriminant analysis (LDA) was conducted to estimate the effect size (LEfSe) of each differential flora. The LDA threshold effect value was set to 4, and the *p* value after FDR correction was set to 0.05, so as to find the flora markers with statistical differences between the two groups. It was found that there were 16 significantly different microorganisms in the P group, ranking from high to low according to LDA value were *c_Bacilli, o_Lactobacillales, p_Actinobacteria, f_Streptococcaceae, g_Streptococcus, c_Actinobacteria, o_Bifidobacteriales, f_Bifidobacteriaceae, g_Bifidobacterium, f_Lactobacillaceae, g_Lactobacillus, f_Enterobacteriaceae, o_Enterobacteriales, c_Gammaproteobacteria, f_Enterococcaceae,* and *g_Enterococcus*, while there were 11 significantly microbes in N group: *p_Bacteroidetes, c_Bacteroidia, o_Bacteroidales, f_Bacteroidaceae, g_Bacteroides, c_Clostridia, o_Clostridiales, f_Lachnospiraceae, g_Roseburia, g_Faecalibacterium,* and *f_Veillonellaceae*. Note: here p, c, o, f, g and s represent phylum, class, order, family, genus and species, respectively (Figures 2D,E).

Next, the 16S rRNA sequencing data were predicted by the phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2) software. We used Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database[3] and MetaCyc database[4] annotated the functional path. Figure 3 and Supplementary Tables S1, S2 showed that the gut microbes of PC patients were clustered into six classifications, including metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, and human diseases in KEGG functional pathway analysis, while seven classifications in MetaCyc functional pathway analysis, among them, the carbohydrate metabolism pathway and amino acid biosynthesis pathway were significantly enriched, indicated that metabolic disorder may be related to GC metastasis. At the same time, 28 metabolic pathways were significantly different between P and N groups, the top five pathways ranked by difference from high to low were: mycothiol biosynthesis, mono-trans, poly-cis decaprenyl phosphate biosynthesis, methyl ketone biosynthesis, reductive acetyl coenzyme A pathway and superpathway of L-arginine, putrescine, and 4-aminobutanoate degradation (Table 2).

3 http://www.genome.jp/kegg/pathway.html
4 https://metacyc.org/

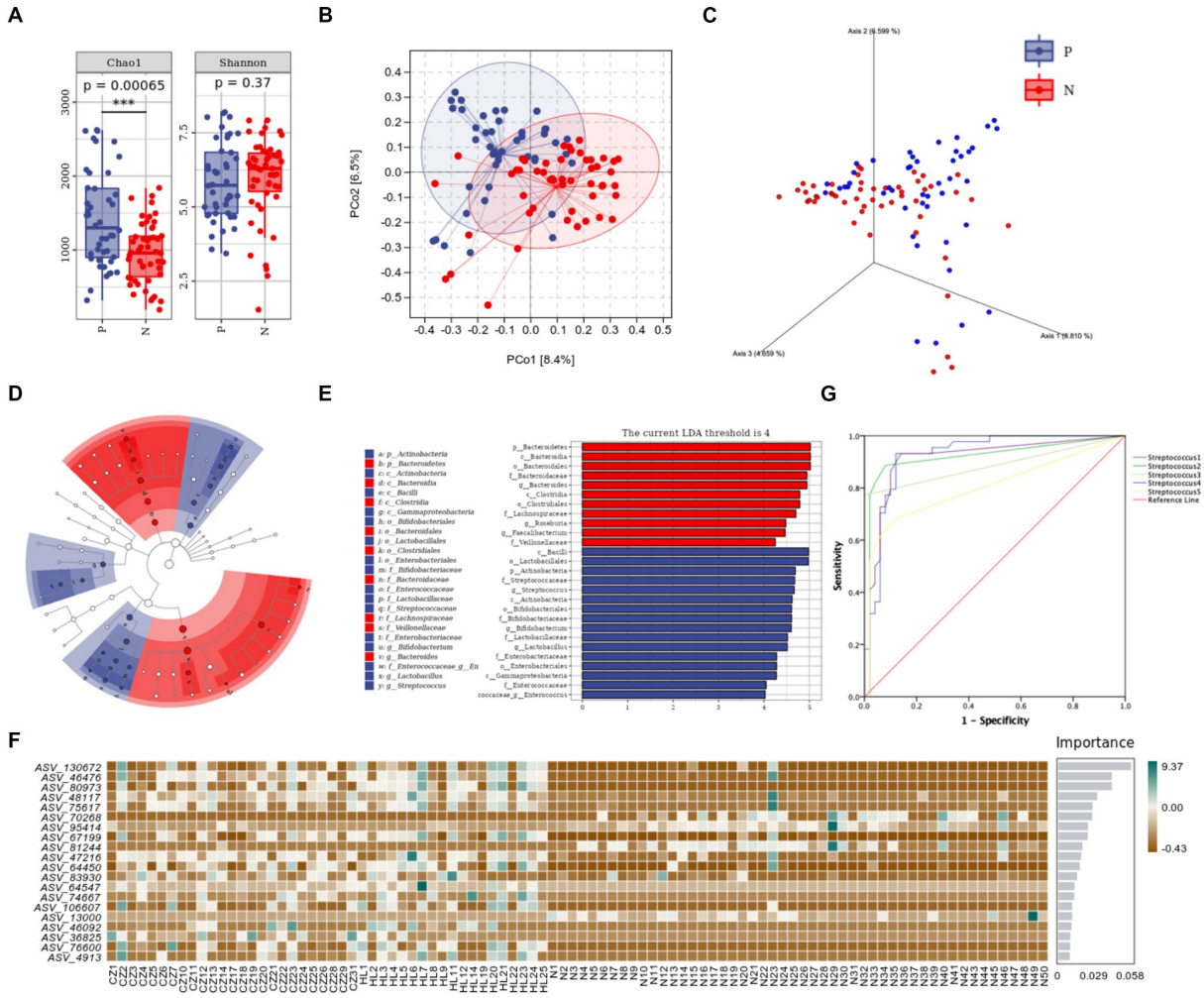

FIGURE 2
Species diversity results of P group and N group. **(A)** Alpha diversity; **(B)** PCoA of beta diversity (R^2=4.477, p =0.001); **(C)** 3D-PCoA; **(D)** taxonomic branch diagram of LEfSe (LDA threshold=4); **(E)** LDA histogram; **(F)** RF model; **(G)** ROC curves. ***p<0.001.

Streptococcus is a gut microbial marker for predicting PC and PCLM

The top 20 important species were screened out by random forest (RF) analysis. The heat map showed the abundance distribution of these species in each sample (Figure 2F). Table 3 lists the top five ASV IDs and corresponding species, including *Streptococcus1, Streptococcus2, Streptococcus3, Streptococcus4, and Streptococcus5*. All of them were enriched in P group by LEfSe analysis, further analysis of PC prediction ability of the above species by ROC curve showed that the area under the curves (AUC) of *Streptococcus1* was 0.927 (p<0.001), indicated that the increase of its number can predict the occurrence of PC (Figure 2G). In other words, *Streptococcus* is a predictive microbiota marker of PC, the more *Streptococcus* is, the higher the incidence of pancreatic cancer.

To further explore the effect of fecal bacteria on PC patients with liver metastasis (LM), we divided them into liver metastasis group (LM group, n=27) and non-liver metastasis group (non-LM group, n=17), respectively. There was no significant difference in clinicopathological features between the two groups (Table 4). The sequencing depth of these two groups is good. The species composition of LM group is different from that of non-LM group, and the gene number and richness in LM group were higher. RF analysis found that *Streptococcus* also played a key role in identifying LM, and ROC curve was verified (AUC=0.796, p=0.001) (Supplementary Figures S1, S2 and Table 3), indicating that *Streptococcus* has great potential in predicting PC as well as PCLM.

Discussion

With the proposal of the Human Microbiome Project (HMP) and the extensive development of high-throughput sequencing, metagenomics, biochip technology and biological information analysis, the relationship between gut microbes and health has received unprecedented attention. Intestinal flora is an indispensable part of the body and plays an important role in human physiological health. It also plays a key role in the occurrence and development of pancreatic cancer (PC) (Riquelme et al., 2019). PC is one of the most

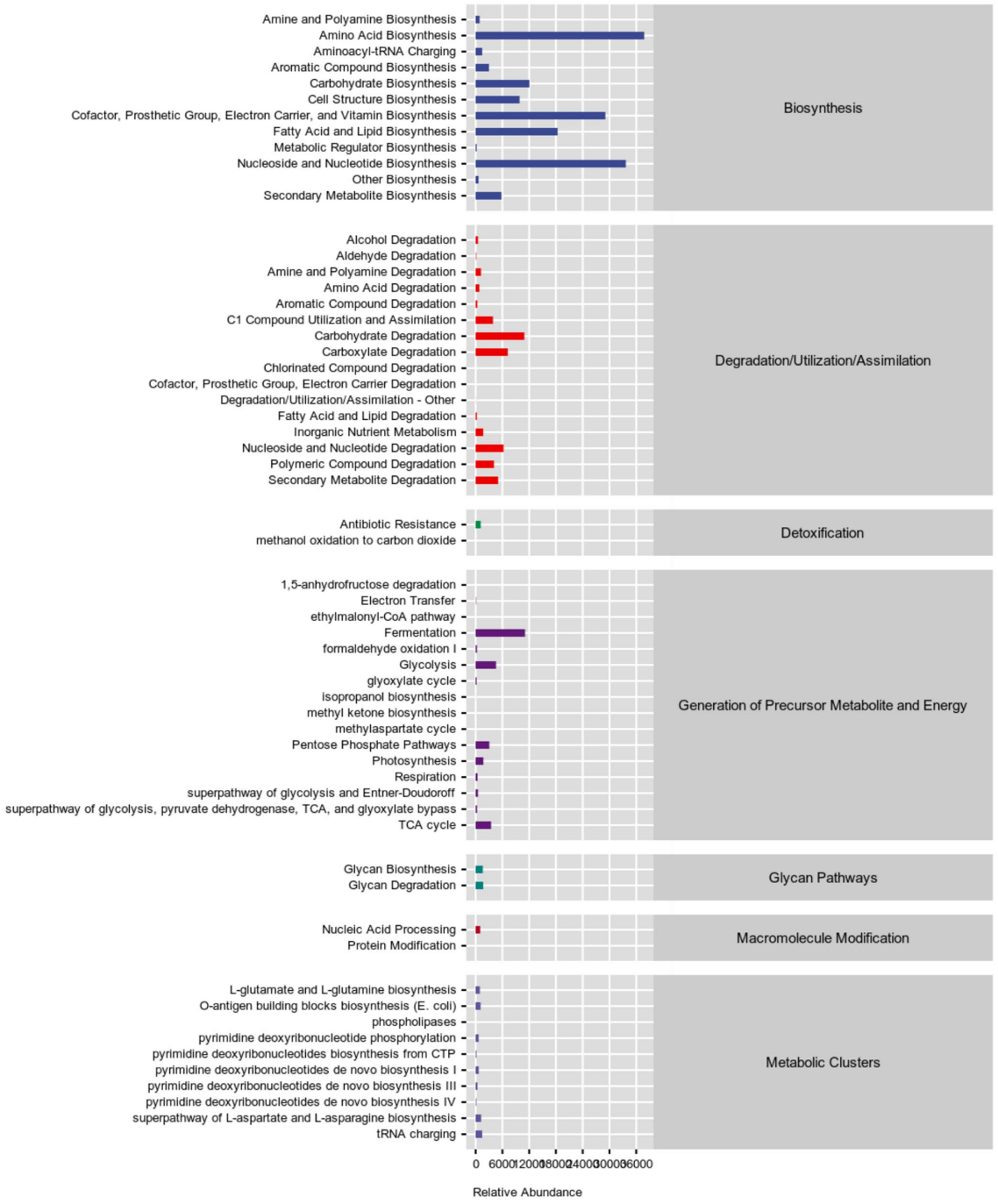

FIGURE 3
Statistics of metabolic pathways in PC patients. Different colors are used to distinguish different pathways, as blue represents biosynthesis, red represents degradation/utilization/assimilation, green represents detoxification, deep-purple represents generation of precursor metabolite and energy, blue-green represents glycan pathways, brown represents macromolecule modification, purple represents metabolic clusters.

lethal malignant tumors and one of the biggest burdens in the world. Because its early symptoms are not obvious and most of them are found in late stage, it is necessary to find convenient and non-invasive biomarkers. Therefore, in order to find microorganisms that may predict PC, we retrospectively collected fecal samples from 44 PC patients and 50 normal people, 16S rRNA sequencing technology and bioinformatic analysis were performed to find the predictive biomarkers.

Our study found that the intestinal microbial richness of PC patients was higher, and the *Streptococcus* content was significantly increased. Through LEfSe, RF analysis and verified by ROC curve, it was found that it had important discrimination ability in the PC group and could specifically predict PC and PCLM. *Streptococcus* belongs to *p__Firmicutes, c__Bacilli, o__Lactobacillales, and f__Streptococcaceae,* is a common pyogenic Gram-positive coccus, which widely exists in human gastrointestinal tract and nasopharynx, mainly causing

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TABLE 2 Differential metabolic pathway between P and N groups ($P<0.05$).

Pathway	Description	LogFC	SE	*P* value	adj *P* value
PWY1G-0	Mycothiol biosynthesis	2.615	0.321	4.44E-16	9.42E-14
PWY-6383	Mono-trans, poly-cis decaprenyl phosphate biosynthesis	2.316	0.284	4.44E-16	9.42E-14
PWY-7007	Methyl ketone biosynthesis	2.315	0.350	3.79E-11	5.35E-09
CODH-PWY	Reductive acetyl coenzyme A pathway	−2.359	0.371	2.05E-10	2.17E-08
ARGDEG-PWY	Superpathway of L-arginine, putrescine, and 4-aminobutanoate degradation	1.772	0.388	4.887E-06	<0.001
ORNARGDEG-PWY	Superpathway of L-arginine and L-ornithine degradation	1.772	0.388	4.887E-06	<0.001
PWY-6071	Superpathway of phenylethylamine degradation	1.763	0.380	3.561E-06	<0.001
PWY-5265	Peptidoglycan biosynthesis II (staphylococci)	1.821	0.408	7.96E-06	<0.001
3-HYDROXYPHENYLACETATE-DEGRADATION-PWY	4-hydroxyphenylacetate degradation	1.522	0.360	<0.001	0.001
PWY-6565	Superpathway of polyamine biosynthesis III	0.145	0.035	<0.001	0.002
PWY0-321	Phenylacetate degradation I (aerobic)	1.458	0.364	<0.001	0.002
PWY-5181	Toluene degradation III (aerobic) (*via* p-cresol)	1.466	0.369	<0.001	0.003
PWY0-1277	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation	1.566	0.405	<0.001	0.004
HCAMHPDEG-PWY	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate	1.725	0.451	<0.001	0.004
PWY-6690	Cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate	1.725	0.451	<0.001	0.004
GALLATE-DEGRADATION-II-PWY	Gallate degradation I	1.570	0.444	<0.001	0.011
PWY-6562	Norspermidine biosynthesis	1.416	0.409	0.001	0.014
GALLATE-DEGRADATION-I-PWY	Gallate degradation II	1.376	0.404	0.001	0.015
METHYLGALLATE-DEGRADATION-PWY	Methylgallate degradation	1.374	0.404	0.001	0.015
PWY-6185	4-methylcatechol degradation (ortho cleavage)	1.457	0.430	0.001	0.015
PWY-7373	Superpathway of demethylmenaquinol-6 biosynthesis II	2.106	0.667	0.002	0.032
ORNDEG-PWY	Superpathway of ornithine degradation	0.758	0.241	0.002	0.032
PWY-6397	Mycolyl-arabinogalactan-peptidoglycan complex biosynthesis	1.927	0.626	0.002	0.038
PWY-6182	Superpathway of salicylate degradation	1.260	0.411	0.002	0.038

(Continued)

TABLE 2 (Continued)

Pathway	Description	LogFC	SE	*P* value	adj *P* value
PWY-5417	Catechol degradation III (ortho-cleavage pathway)	1.262	0.417	0.002	0.040
PWY-5431	Aromatic compounds degradation *via* β-ketoadipate	1.262	0.417	0.002	0.040
CATECHOL-ORTHO-CLEAVAGE-PWY	Catechol degradation to β-ketoadipate	1.285	0.426	0.003	0.040
VALDEG-PWY	L-valine degradation I	−1.354	0.453	0.003	0.042

TABLE 3 Random forest model predicts the biomarkers for PC diagnosis.

Order	ASV	Bacteria	AUC	SE	*P*	Enriched group by LEfSe
1	ASV-130672	*Streptococcus1*	0.927	0.028	<0.001	P
2	ASV-46476	*Streptococcus2*	0.918	0.034	<0.001	P
3	ASV-80973	*Streptococcus3*	0.886	0.039	<0.001	P
4	ASV-48117	*Streptococcus4*	0.912	0.034	<0.001	P
5	ASV-75617	*Streptococcus5*	0.802	0.049	<0.001	P

pyogenic inflammation, hypersensitivity diseases and so on. Nowadays, its role in cancer occurrence and progression is gradually known (Boleij et al., 2011; Zhou et al., 2022). In the previous research of our team, we found that *Streptococcus* played a crucial role in GC and GCLM (Yu et al., 2021), and observed its role in extrahepatic metastasis of liver cancer in unpublished study. Thus, *Streptococcus* can be used as a biomarker for early diagnosis to guide the precise treatment of diseases. What's more, in our preliminary functional and metabolic pathway analysis, we found that mycothiol biosynthesis pathway was significantly different between PC patients and normal people, and its changes may be a potential mechanism for the occurrence and development of PC. Mycothiol (MSH), a major low molecular weight thiol in mycobacteria, is an important cellular antioxidant. At present, a large number of literatures have reported that MSH is a promising antimicrobial target. As MSH only exists in actinomycetes, it is a good microbial target. However, as this study only explores PC and PCLM preliminarily, there is no in-depth mechanism exploration, and further *in vitro* and *in vivo* experiments are needed to prove it.

In addition, there are some limitations in our study: (1) this study is limited to a single center with a small sample size, which needs to be external verified by large sample and multi-center trials; (2) we could not avoid the impact of diet on intestinal microorganisms, we hope that randomized controlled trials can be conducted in the future to eliminate uncontrollable factors; (3) as the patients are still in the treatment stage and have not reached the follow-up time, we have not analyzed the treatment-related information in this study. Our research group has established a complete specimen bank of intestinal microbes of PC patients before and after treatment. We will conduct in-depth research in the future, hoping to reveal the potential synergy between tumor treatment and microbial microbes; (4) our study can only annotate microbial species at species levels, further *in vivo* animal experiments and clinical studies are needed to confirm the specific pathogens or bacteria that cause the differences; and (5) this study does not include the exploration of mechanism, we are looking forward to further exploration of mechanism by scholars based on our research findings in the future. Despite the above defects, this study can still explain the role of *Streptococcus* in PC and PCLM to a great extent.

Conclusion

To sum up, the intestinal microbial structure characteristics of PC and PCLM patients have changed, and the number of *Streptococcus* in these two groups has increased significantly, which can specifically predict PC and PCLM and serve as a predictive microbiota marker.

Data availability statement

The data presented in the study can be found in online repositories. The names of the repository and accession number can be found below: NCBI SRA, PRJNA977486, https://www.ncbi.nlm.nih.gov/sra/.

Ethics statement

The studies involving human participants were reviewed and approved by the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JL and HL: conception and design and administrative support. YM: provision of study materials or patients. JY: collection and

TABLE 4 Demographic characteristics of LM (n=27) and non-LM (n=17) patients.

Variable	LM (n=27)	non-LM (n=17)	χ^2/t	P
Age (y)				
<65	16 (59.26%)	11 (64.71%)	0.131	0.718
≥65	11 (40.74%)	6 (35.29%)		
Gender				
Male	14 (51.85%)	7 (41.18%)	0.477	0.490
Female	13 (48.15%)	10 (58.82%)		
BMI (kg/m^2)	22.55 ± 3.10	22.23 ± 2.14	0.408	0.685
Tumor sites				
Head	9 (33.33%)	9 (52.94%)	1.931	0.587
Body	2 (7.41%)	1 (5.88%)		
Tail	3 (11.11%)	2 (11.76%)		
Whole	13 (48.15%)	5 (29.41%)		
Pathological types				
Adenocarcinoma	21 (77.78%)	15 (88.24%)	0.979	0.613
Squamous cell carcinoma	2 (7.41%)	1 (5.88%)		
Other	4 (14.81%)	1 (5.88%)		
Whether metastasis				
Yes	27 (100.00%)	5 (29.41%)	/	/
No	0 (0.00%)	12 (70.59%)		
Metastasis sites				
Liver	27 (100.00%)	0 (0.00%)	/	/
Lung	2 (7.41%)	2 (11.76%)		
Distant lymph node	15 (55.56%)	3 (17.65%)		
Peritoneum	7 (25.93%)	2 (11.76%)		
ECOG performance				
0	26 (96.30%)	0 (0.00%)	/	/
1	1 (3.70%)	17 (100.00%)		
Whether surgery			/	/
Yes	2 (7.41%)	9 (52.94%)		
No	25 (92.59%)	8 (47.06%)		
Total bilirubin (TBIL) (μmol/L)	16.04 ± 8.15	15.67 ± 17.69	0.088	0.931
Direct bilirubin (DBIL) (μmol/L)	6.12 ± 5.16	4.87 ± 4.33	0.771	0.446
Indirect bilirubin (IBIL) (μmol/L)	12.12 ± 9.79	11.47 ± 15.96	0.157	0.876

assembly of data. JY and YM: data analysis and interpretation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1184869/full#supplementary-material

SUPPLEMENTARY FIGURE S1
16s rRNA sequencing data processing and species composition of LM and non-LM groups. **(A)** refraction curve; **(B)** species accumulation curve; **(C)** Venn gram of ASV/OTUs; **(D)** phylogenetic tree plot; **(E–H)** compositional analysis of each sample **(E,G)** and group **(F,H)** at the phylum level **(E,F)**, and at the genus level **(G,H)**.

SUPPLEMENTARY FIGURE S2
Species diversity results of LM and non-LM groups. **(A)** alpha diversity; **(B)** PCoA of beta diversity (R^2=1.123, P=0.229); **(C)** 3D-PCoA; **(D)** taxonomic branch diagram of LEfSe (LDA threshold=2); **(E)** LDA histogram; **(F)** RF model; **(G)** ROC curves

References

Boleij, A., van Gelder, M. M., Swinkels, D. W., and Tjalsma, H. (2011). Clinical importance of *Streptococcus gallolyticus* infection among colorectal cancer patients: systematic review and meta-analysis. *Clin. Infect. Dis.* 53, 870–878. doi: 10.1093/cid/cir609

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., et al. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* 72, 5069–5072. doi: 10.1128/AEM.03006-05

Deschasaux, M., Bouter, K. E., Prodan, A., Levin, E., Groen, A. K., Herrema, H., et al. (2018). Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nat. Med.* 24, 1526–1531. doi: 10.1038/s41591-018-0160-1

Fu, A., Yao, B., Dong, T., Chen, Y., Yao, J., Liu, Y., et al. (2022). Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer. *Cells* 185, 1356–1372.e26. doi: 10.1016/j.cell.2022.02.027

Hartmann, P., Chu, H., Duan, Y., and Schnabl, B. (2019). Gut microbiota in liver disease: too much is harmful, nothing at all is not helpful either. *Am. J. Physiol. Gastrointest. Liver Physiol.* 316, G563–G573. doi: 10.1152/ajpgi.00370.2018

Klein, A. P. (2021). Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. *Nat. Rev. Gastroenterol. Hepatol.* 18, 493–502. doi: 10.1038/s41575-021-00457-x

Liu, Y., Geng, R., Liu, L., Jin, X., Yan, W., Zhao, F., et al. (2020). Gut microbiota-based algorithms in the prediction of metachronous adenoma in colorectal cancer patients following surgery. *Front. Microbiol.* 11:1106. doi: 10.3389/fmicb.2020.01106

Neoptolemos, J. P., Kleeff, J., Michl, P., Costello, E., Greenhalf, W., and Palmer, D. H. (2018). Therapeutic developments in pancreatic cancer: current and future perspectives. *Nat. Rev. Gastroenterol. Hepatol.* 15, 333–348. doi: 10.1038/s41575-018-0005-x

Rai, S. N., Qian, C., Pan, J., Rai, J. P., Song, M., Bagaitkar, J., et al. (2019). Microbiome data analysis with applications to pre-clinical studies using QIIME2: statistical considerations. *Genes Dis.* 8, 215–223. doi: 10.1016/j.gendis.2019.12.005

Riquelme, E., Zhang, Y., Zhang, L., Montiel, M., Zoltan, M., Dong, W., et al. (2019). Tumor microbiome diversity and composition influence pancreatic cancer outcomes. *Cells* 178, 795–806.e12. doi: 10.1016/j.cell.2019.07.008

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., and Garrett, W. S. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.* 12:R60. doi: 10.1186/gb-2011-12-6-r60

Sender, R., Fuchs, S., and Milo, R. (2016). Revised estimates for the number of human and Bacteria cells in the body. *PLoS Biol.* 14:e1002533. doi: 10.1371/journal.pbio.1002533

Shi, Y., Zhang, L., Do, K. A., Peterson, C. B., and Jenq, R. R. (2020). aPCoA: covariate adjusted principal coordinates analysis. *Bioinformatics* 36, 4099–4101. doi: 10.1093/bioinformatics/btaa276

Sung, H., Ferlay, J., Siegel, R. L., 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. doi: 10.3322/caac.21660

Yu, D., Yang, J., Jin, M., Zhou, B., Shi, L., Zhao, L., et al. (2021). Fecal *Streptococcus* alteration is associated with gastric cancer occurrence and liver metastasis. *mBio* 12:e0299421. doi: 10.1128/mBio.02994-21

Zhang, L., Sanagapalli, S., and Stoita, A. (2018). Challenges in diagnosis of pancreatic cancer. *World J. Gastroenterol.* 24, 2047–2060. doi: 10.3748/wjg.v24.i19.2047

Zheng, B., Qu, J., Ohuchida, K., Feng, H., Chong, S. J. F., Yan, Z., et al. (2020). LAMA4 upregulation is associated with high liver metastasis potential and poor survival outcome of pancreatic cancer. *Theranostics* 10, 10274–10289. doi: 10.7150/thno.47001

Zhou, C. B., Pan, S. Y., Jin, P., Deng, J. W., Xue, J. H., Ma, X. Y., et al. (2022). Fecal signatures of *Streptococcus anginosus* and *Streptococcus constellatus* for noninvasive screening and early warning of gastric cancer. *Gastroenterology* 162, 1933–1947.e18. doi: 10.1053/j.gastro.2022.02.015

Zhou, B., Xu, J. W., Cheng, Y. G., Gao, J. Y., Hu, S. Y., Wang, L., et al. (2017). Early detection of pancreatic cancer: where are we now and where are we going? *Int. J. Cancer* 141, 231–241. doi: 10.1002/ijc.30670

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MV-CVIB: a microbiome-based multi-view convolutional variational information bottleneck for predicting metastatic colorectal cancer

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Introduction: Imbalances in gut microbes have been implied in many human diseases, including colorectal cancer (CRC), inflammatory bowel disease, type 2 diabetes, obesity, autism, and Alzheimer's disease. Compared with other human diseases, CRC is a gastrointestinal malignancy with high mortality and a high probability of metastasis. However, current studies mainly focus on the prediction of colorectal cancer while neglecting the more serious malignancy of metastatic colorectal cancer (mCRC). In addition, high dimensionality and small samples lead to the complexity of gut microbial data, which increases the difficulty of traditional machine learning models.

Methods: To address these challenges, we collected and processed 16S rRNA data and calculated abundance data from patients with non-metastatic colorectal cancer (non-mCRC) and mCRC. Different from the traditional health-disease classification strategy, we adopted a novel disease-disease classification strategy and proposed a microbiome-based multi-view convolutional variational information bottleneck (MV-CVIB).

Results: The experimental results show that MV-CVIB can effectively predict mCRC. This model can achieve AUC values above 0.9 compared to other state-of-the-art models. Not only that, MV-CVIB also achieved satisfactory predictive performance on multiple published CRC gut microbiome datasets.

Discussion: Finally, multiple gut microbiota analyses were used to elucidate communities and differences between mCRC and non-mCRC, and the metastatic properties of CRC were assessed by patient age and microbiota expression.

KEYWORDS

microbiome, multi-view, information bottleneck, metastatic colorectal cancer, risk assessment

1. Introduction

The human intestine is one of the most important organs in the digestive system, which maintains the normal life activities of the human body through metabolism (Cho and Blaser, 2012). Microbes in the gut derive energy from the food we eat and release metabolites and hormones to regulate physical health. As our microbial research continues to deepen, more and more investigations show that the chemical signals released by human gut microbes play a key role in human health and disease (Gilbert et al., 2018). From the perspective of human health, the intestinal flora in the body contributes to the construction of the

immune system and participates in and regulates the physiological processes of various cells (De Sordi et al., 2019). More importantly, a variety of complex diseases have been confirmed to be related to certain intestinal flora, including inflammatory bowel disease, type 2 diabetes, Alzheimer's disease, HIV, autism, obesity, and cardiovascular and cerebrovascular diseases (Schmidt et al., 2018; Shkoporov et al., 2019). Some malignancies, such as colorectal cancer (CRC), have also been shown to be associated with gut microbes (Chen et al., 2022; Wani et al., 2022). CRC is the third leading cause of cancer deaths, and ∼20% of patients develop metastases, known as metastatic colorectal cancer (mCRC). It mainly includes colon cancer liver metastasis, multiple lymph node metastasis, hematogenous metastasis, and implantation metastasis (Enquist et al., 2014). All of this emerging evidence confirms that the gut microbiome can be a potential predictor of a variety of diseases and cancers (Zou et al., 2017).

With the advent of the genome era, the development of high-throughput sequencing technology has provided a new technical platform for the study of microbial community structure (Zhou et al., 2015). In particular, 16S rRNA gene sequencing has become an important means to study the composition and distribution of gut microbial communities (Langille et al., 2013). It fully shows the diversity of human gut flora and reveals potential factors for disease aggravation. Although much evidence suggests that the gut microbiome can be used to predict colorectal cancer, few investigations have used microbial data to identify mCRC. Therefore, effectively extracting key features of the microbiota from gut microbial data faces a series of challenges (Cammarota et al., 2020; Wang and Zou, 2023). Since disease samples are small and more difficult to obtain than healthy samples, a large number of studies use healthy-disease groups rather than disease-disease groups. A small number of samples and many features can lead to the curse of dimensionality, that is, features are highly sparse, such as strain-level informative data containing hundreds of thousands of genetic markers (Somorjai et al., 2003; Akay and Hess, 2019). However, it is almost difficult for traditional machine learning models to mine valuable information from such small sample data. Second, although gene signatures provide more information than microbial abundance data, more feature information also requires huge computational resources, which may lead to overfitting and greatly increase the time cost (Yang et al., 2021).

Considering the metastatic characteristics of CRC, non-metastatic colorectal cancer (non-mCRC) patients are more worried about mCRC with higher mortality (Reyes et al., 2019; Rumpold et al., 2020). Existing microbiome-based CRC prediction methods mainly use species relative abundance or strain-level marker profiles or a combination of the two. With the development of deep learning, it has become feasible to use deep learning to predict CRC from gut microbiome data (Marcos-Zambrano et al., 2021; Salim et al., 2023). The MicroPheno method is based on 16S rRNA sequence data, subsamples it, and computes the k-mer representation of the sequence, and the final k-mer is used to complete disease prediction (Asgari et al., 2018). Oh and Zhang (2020) proposed dimensionality reduction of microbiome abundance data or gene signature profiles with multiple different autoencoders, and then classical machine learning methods were used to complete disease classification. Reiman et al. (2020) took a microbial phylogenetic tree matrix as input and used a convolutional neural network (CNN) for disease prediction. Wirbel et al. (2021) developed SIAMCAT, a multifunctional R toolbox for machine learning-based comparative metagenomics. The toolbox contains a variety of feature matrices such as genes, pathways, and microbial taxa to statistically infer host disease phenotype associations. Grazioli et al. proposed multimodal variational information bottleneck (MVIB), a multimodal representation that can input species relative abundance, strain-level marker profiles, and metabolomic data and learn meaningful joint codes through information bottleneck theory (Grazioli et al., 2022). This study used multiple published microbiome disease phenotype datasets including CRC and achieved excellent predictive performance. However, the relative independence among relative abundances, strain-level marker profiles, and metabolomic data contains rich cross-modal information in addition to the modal information of the microbiome, which may lead to model uncertainty (Holzinger et al., 2022).

Compared with the traditional health-disease classification strategy, we adopt a new disease-disease classification strategy, which identifies more severe diseases among sick patients. Disease-disease sample features are often more difficult to distinguish than healthy-disease sample features, which is also a challenge for predictive models. Figure 1 shows the specific strategy process.

In this study, we propose a multi-view convolutional variational information bottleneck (MV-CVIB) model to specifically address the prediction problem of mCRC. The variational information bottleneck (VIB) can extract all the judgmental information that is helpful for disease prediction while filtering out redundant information (Alemi et al., 2016). For deep neural networks, forgetting details enables the model to form general concepts and improves generalization performance. The Qiime2 tool was used to process and obtain the final relative abundance data (Hall and Beiko, 2018). We calculated the Euclidean distance between each sample based on the relative abundance of the microbiome and took the samples with the closest Euclidean distance as neighbors. Therefore, the nearest neighbor information between each sample can be regarded as a new view. MV-CVIB expands the microbiome input data structure to the maximum capacity while also being insensitive to outliers in the data. Not only that, to test the generalization ability of MV-CVIB, we also performed various experiments on multiple published control-CRC datasets.

The contributions of this study are as follows:

(1) Current studies mainly focus on the prediction of CRC while neglecting the more severe mCRC. We are the first to apply deep learning to the microbiome-based mCRC prediction problem and achieve excellent prediction results.
(2) Compared with the traditional health-disease classification strategy, we adopt a new disease-disease classification strategy, which identifies more severe diseases among sick patients. Identifying more complex diseases from diseases is more conducive to mining the underlying nature of disease exacerbations.
(3) We compute the nearest neighbor information for the relative abundance data and feed it together as a view into the VIB with convolution and pooling modules.

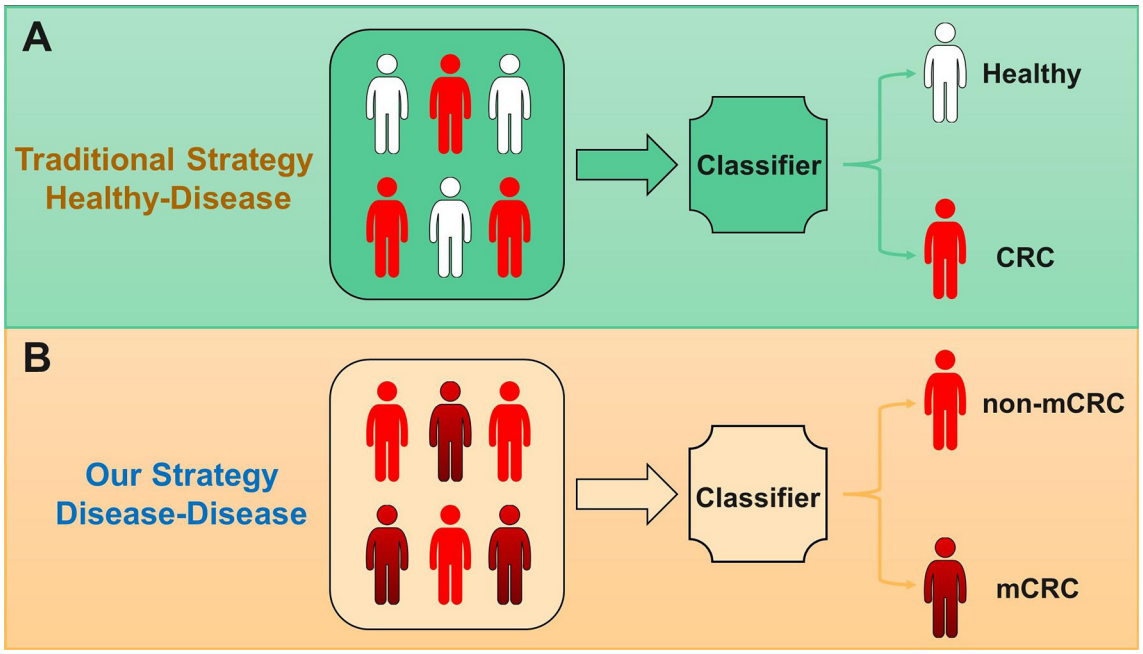

FIGURE 1
(A) Traditional classification strategy for microbiome-based disease prediction is health to disease. **(B)** Our strategy for microbiome-based disease prediction is disease to disease, focusing on predicting malignancy from disease, such as the prediction from non-metastatic colorectal cancer (non-mCRC) to metastatic colorectal cancer (mCRC).

The VIB can extract all the judgmental information that is helpful for disease prediction while filtering out redundant information. Since we introduce convolution and pooling operations into the model, the data in the input stream become smoother, which increases the robustness and generalization ability of the model and avoids overfitting.

2. Materials and methods

2.1. Datasets

To evaluate and analyze predictive models, we collected 16S rRNA data from the gut microbiota of patients with metastatic colorectal cancer (mCRC, $n = 9$) and non-metastatic colorectal cancer (non-mCRC, $n = 7$) from the National Center for Biotechnology Information (NCBI) (Coordinators, 2015). The original data come from the People's Hospital of Wuhan University, and the data type is raw sequence read. Raw sequence data can be accessed through the NCBI Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA531761). Table 1 shows the specific information of all samples. Figure 2 shows that each sample is isolated in the gut. The pre-processing of this dataset will be described in detail in the next section.

In addition, we also collected three control-CRC datasets from published studies to evaluate the generalization ability of the model, specifically including colorectal (Pasolli et al., 2016), colorectal-EMBL, and early-colorectal-EMBL (Zeller et al., 2014). Only the CRC group and the healthy group were included in these datasets and had unique true labels, diseased or healthy. Therefore, this study does not perform predictions on the future health of the samples. Table 2 shows the specific information of the three public datasets.

TABLE 1 Specific information for non-mCRC patients and mCRC patients.

Sample ID	Disease status	Age	Gender
SRR8873486	Non-mCRC	68	Male
SRR8873495	Non-mCRC	74	Male
SRR8873494	Non-mCRC	68	Male
SRR8873483	Non-mCRC	65	Female
SRR8873496	Non-mCRC	66	Female
SRR8873491	Non-mCRC	38	Male
SRR8873490	Non-mCRC	54	Female
SRR8873493	mCRC	44	Female
SRR8873492	mCRC	70	Male
SRR8873487	mCRC	82	Female
SRR8873484	mCRC	85	Male
SRR8873489	mCRC	56	Female
SRR8873488	mCRC	73	Male
SRR8873498	mCRC	32	Male
SRR8873485	mCRC	61	Female
SRR8873497	mCRC	68	Male

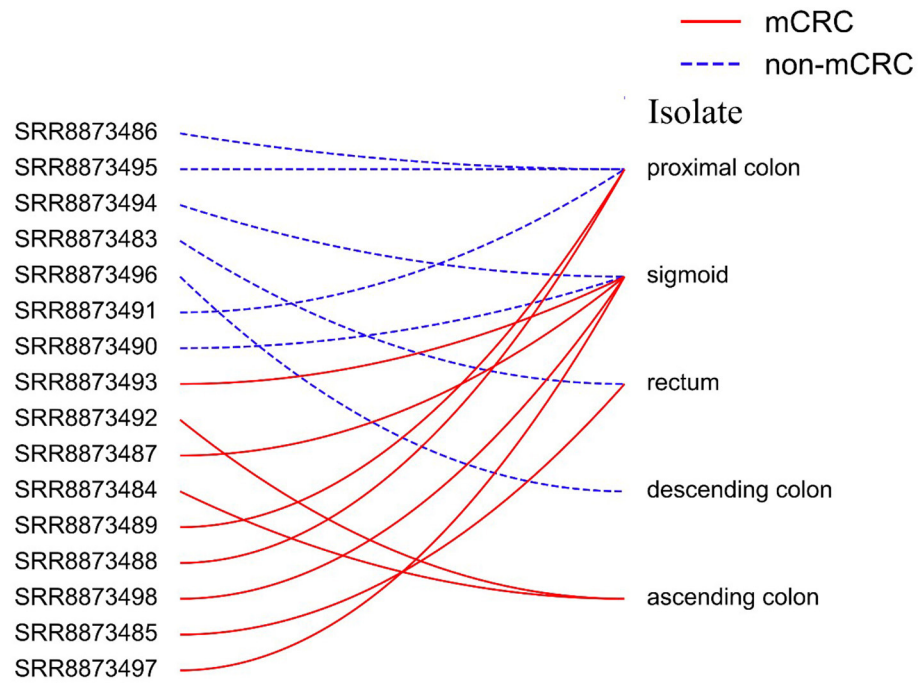

FIGURE 2
Each sample is isolated differently from the gut.

2.2. Pre-processing

First, we converted sra files on NCBI to fastq.gz files using fastq-dump version 2.8.0 in SRA Toolkit and then converted it to multiple fastq files with forward and reverse. The next step is to import these data into Qiime2 and review the data quality. Next, we denoise the data using Deblur with default parameters. The specific role is to filter out noisy sequences, remove chimeric sequences, accidental sequences (sequences that occur only once), and de-redundant these sequences. The purpose is to obtain the signature table and reference sequence.

Finally, we used Qiime2 to analyze the composition of microbial communities from the denoised data. Among them, the feature table represents the relative abundance of species and serves as the input feature vector of the proposed model.

2.3. Multiple types of dimensionality reduction analysis

In this study, to explore the internal structural characteristics of the pre-processed data and the degree of cognitive difference between mCRC and non-mCRC, we used various types of dimensionality reduction analysis methods (He et al., 2023), including principal components analysis (PCA) (Jiang et al., 2022), principal co-ordinates analysis (PCoA) (Wang et al., 2016), t-distributed stochastic neighbor embedding (t-SNE) (Kostic et al., 2015), and non-metric multidimensional scaling (NMDS) (Mekadim et al., 2022). The difference analysis section in Figure 3 shows an outline of the four dimensionality reduction approaches between mCRC and non-mCRC.

TABLE 2 Three CRC datasets from published studies.

Datasets	Total samples	Control sample	Disease samples
Colorectal (CRC1)	121	73	48
Colorectal-EMBL (CRC2)	199	103	96
Early-colorectal-EMBL (CRC3)	96	52	44

For PCA, the original microbial characteristic information of samples is projected into the dimension with the maximum projection information as far as possible. The advantage of PCA is that the loss of feature information after dimension reduction is minimal. The disadvantage is that in the case of complete ignorance of the data, PCA cannot better retain data information. For PCoA, it is a non-constrained dimension reduction method, and PCoA can find the most important coordinates in the distance matrix without changing the mutual position relationship between mCRC and non-mCRC. The disadvantage is that PCoA can only roughly understand the similarity or difference between samples but cannot accurately calculate the degree of difference. For t-SNE, it is a non-linear dimension reduction method, which can preserve the local features of the dataset. The disadvantage of t-SNE is that the setting of hyperparameters is relatively strict, and an improper setting will lead to poor results. Similar to PCoA, NMDS also uses the sample similarity distance matrix for dimension reduction analysis. It is worth noting that NMDS focuses on the ordering relation of values in the distance matrix. When there are more samples, NMDS can more accurately reflect the differences among samples. The disadvantage of NMDS is that it is easy to fall into the local optimal point, and it needs to run several times with different random

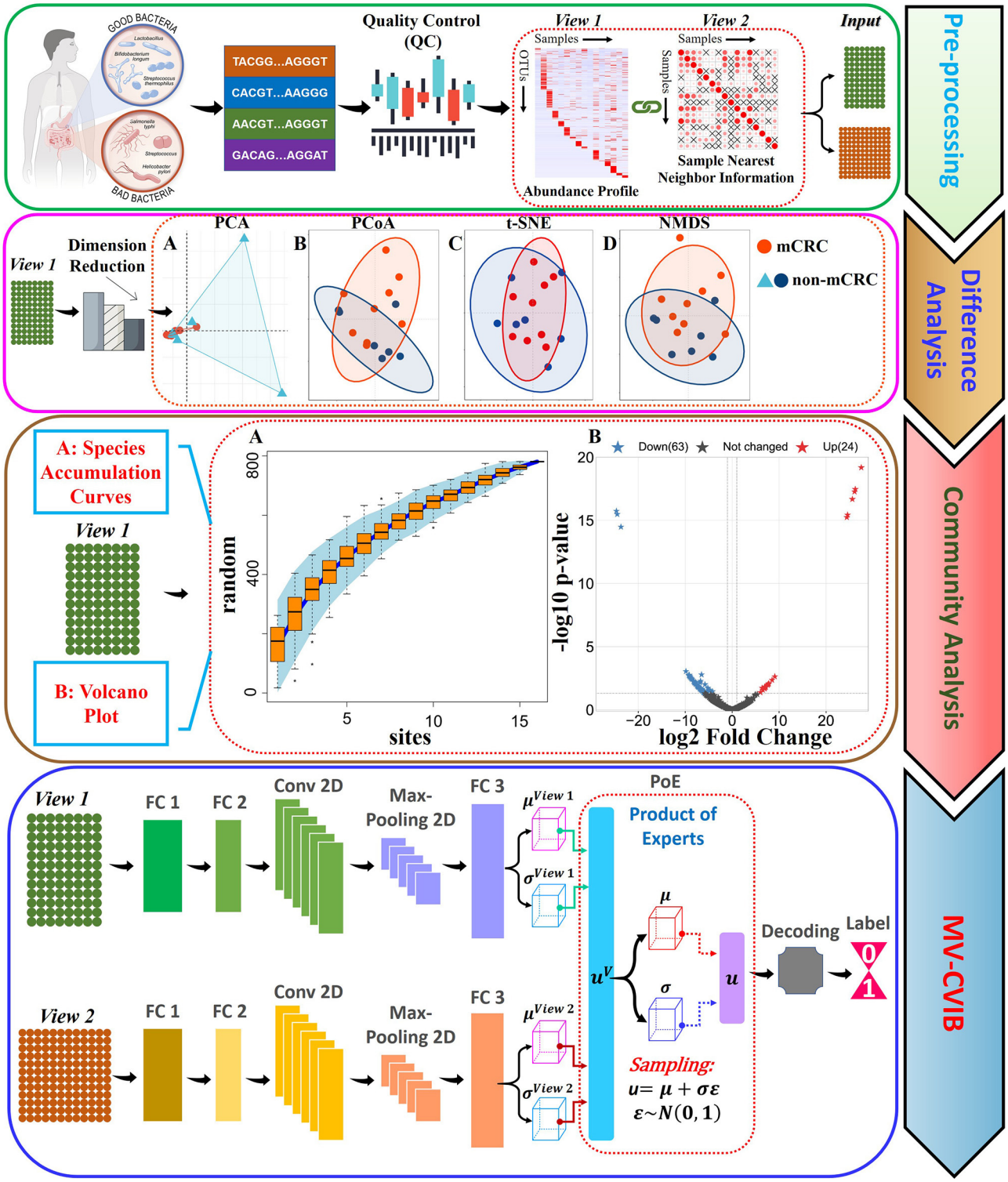

FIGURE 3
Work flowchart. Pre-processing: We collected the sequence information of the samples from NCBI and obtained the species abundance data through a series of quality control and filtering methods, and at the same time, we calculated the nearest neighbor information of the samples. Variance Analysis: We map abundance data into a two-dimensional space through dimensionality reduction. **(A)** PCA is used for dimensionality reduction and visualization. **(B)** PCoA is used for dimensionality reduction and visualization. **(C)** T-SNE is used for dimensionality reduction and visualization. **(D)** NMDS is used for dimensionality reduction and visualization. Community Analysis: **(A)** We used the species accumulation curve (SAC) to describe the real situation of the disease samples. **(B)** We used volcano plots to visualize upregulated and downregulated points. MV-CVIB: Flowchart of the proposed method. The method specifically includes three fully connected layers, a two-dimensional convolutional layer, a maximum pooling layer, a PoE module, a decoding module, and an output layer.

starts to be more likely to obtain the global optimal solution. In addition, we also performed four types of dimensionality reduction visualization analysis on three CRC datasets. Overall, similar to the case of mCRC, there is a large overlap between healthy and diseased sample points. Specific experimental results are included in Supplementary material.

Considering the small number of samples and more feature information in mCRC and non-mCRC, we used four different types of dimensionality reduction methods to mine the distribution rules of samples in the dimensional space. According to the results, the spatial distribution of mCRC and non-mCRC is different, but some samples overlap in space. However, compared with non-mCRC, mCRC is formed by further deterioration on the basis of non-mCRC, so there are differences in microbial abundance features. In addition, to further show the significance index of the difference between the groups, we performed an analysis of similarities (ANOSIM) (Buttigieg and Ramette, 2014) on the mCRC dataset and three CRC datasets, respectively. As a non-parametric test method, ANOSIM has been widely used to evaluate the overall similarity and similar significance of two sets of experimental data. The results are detailed in Supplementary material. From the experimental results, there are statistically significant differences between the groups of the mCRC, CRC1, and CRC2 datasets (R-value of >0 and P-value of <0.05). There is a difference between the groups in the CRC3 dataset but not significant (R-value of >0 and P-value of >0.05), which may be related to the fact that the samples included in the dataset are early CRC patients and late CRC patients.

2.4. Microbial Community Analysis

To measure the species richness status in the community and judge whether the number of samples is sufficient, we used the species accumulation curve (SAC) (Gotelli and Colwell, 2001) to describe the real situation of the disease samples. With the help of the SAC, we can not only estimate the diversity difference between different communities reflected by the number of samples but also estimate the upper limit of community diversity under the condition that the number of samples is sufficient. In addition, based on the principle of statistical testing, we use the volcano plot to show the distribution of abundance level differences between the samples. The detailed results are shown in the Community Analysis section of Figure 3.

From the SAC, we can observe that the curve eventually flattens out, which confirms that the number of samples is reasonable. In other words, the number of mCRC and non-mCRC in the dataset can effectively reflect the species diversity and species richness of the samples. From the volcano plot, there are some significant points, including 24 upregulated points and 63 downregulated points, most of which have no significant difference.

2.5. The multi-view convolutional variational information bottleneck

We set Y to be a random variable. $X^1, X^2, \ldots, X^V$ represent a set of multi-view input random variables, and Y is their ground-truth labels. To make the notation more compact, a collection of data views is represented as a data point $X = \{X^i \mid i^{th}\ view\ present\}$. We set U to be a stochastic encoding of X, defined by the parameter encoder $p(u|x;\theta)$, which comes from the deep neural network of the intermediate layers in the upstream part of the model. Furthermore, in the rest of this study, X, Y, and U are represented as random variables, and x, y, and u are their multidimensional instances, respectively. θ is a parameter vector, and θ is a function parameterized by θ. S is a set.

Referring to the information bottleneck theory (Tishby et al., 2000), the purpose is to learn to encode U, so as to maximize the information provided to Y and maximize the compression to X. Therefore, maximizing the mutual information $I(U,Y;\theta)$ between U and Y can be written as follows:

$$I(U,Y;\theta) = \int p(u,y|\theta) \log \frac{p(u,y|\theta)}{p(u|\theta)\, p(y|\theta)} dydu. \tag{1}$$

Let $I(U,Y;\theta) = \int p(u,y|\theta) \log \frac{p(u,y|\theta)}{p(u|\theta)p(y|\theta)} dydu.$ be a valid solution to maximize (1). However, given the constraint that maximizing compression imposes on U, we need to forget as much information about X as possible. Therefore, the objective function can be written as follows:

$$\max_{\theta} R_{IB}(\theta) = I(U,Y;\theta) - \beta I(U,X;\theta), \tag{2}$$

where $\max_{\theta} R_{IB}(\theta) = I(U,Y;\theta) - \beta I(U,X;\theta)$, is the Lagrange multiplier greater than or equal to 0 and controls the trade-off. $I(U,Y;\theta)$ can make U to predict Y, and $\beta I(U,X;\theta)$ is a constraint that U is the minimal sufficient statistic for X.

We refer to the solution process by Alemi et al. (2016) for the bottleneck of deep variational information. Equation (2) can be rewritten as follows:

$$\begin{aligned} J_{DeepVIB} = & \frac{1}{N} \sum_{n=1}^{N} \mathop{\mathbf{E}}_{\varepsilon \sim p(\varepsilon)} \left[-\log q\left(y_n | f(x_n, \varepsilon)\right) \right] \\ & + \beta KL\left[p(U|x_n), r(U) \right], \end{aligned} \tag{3}$$

where $\varepsilon \sim N(0, I)$ is denoted as the auxiliary Gaussian noise variable, and KL is the Kullback–Leibler divergence. It is worth noting that f is originally an encoding function, but in this study, it is a neural network. The introduction of f has a re-parameterization trick (Kingma and Welling, 2013), that is, $p(u|x;\theta)\, dx = p(\varepsilon)\, d\varepsilon$, where $u = f(x,\varepsilon)$ can be regarded as a deterministic variable, in particular, considering that this formula can make the noise variable independent. Thus, backpropagation is used to optimize the gradient of the objective function of equation (3). Overall, the calculation will be easier. Furthermore, a multivariate Gaussian distribution with a diagonal covariance structure $u = f(x,\varepsilon)$ is the target of the variational approximation posterior, and $u = \mu + \sigma\varepsilon$ is re-parameterized.

Since our model has multi-view input, we take into account the nearest neighbor information between each sample. Therefore, we can further improve the objective function of Deep VIB in equation (3), and$X$ as a multi-view random variable can be

expressed as X. The $p(U|x)$ of equation (3) can be expressed as $p\left(U|x^1, x^2, \ldots, x^V\right)$, with the joint of V available data views as the condition. We refer to the method in multimodal variational autoencoder (MVAE) (Wu and Goodman, 2018), where conditional independence between different modes of U and approximate $p\left(U|x^i\right)$ with $q\left(U|x^i\right) = \widetilde{q}\left(U|x^i\right) p(U)$ is assumed. $\widetilde{q}\left(U|x^i\right)$ is a random encoder for the i-th data view, and $p(U)$ is a prior. Therefore, the product of multiple single-view posteriors can be considered equivalent to the joint posterior, which can be written as follows:

$$p\left(U|x^1, x^2, \ldots, x^V\right) \propto \frac{\prod_{i=1}^{V} p\left(U|x^i\right)}{\prod_{i=1}^{V-1} p(U)} \approx \frac{\prod_{i=1}^{V}\left[q\left(U|x^i\right) = \widetilde{q}\left(U|x^i\right) p(U)\right]}{\prod_{i=1}^{V-1} p(U)} = p(U)\prod_{i=1}^{V}\widetilde{q}\left(U|x^i\right) \quad (4)$$

Equation (4) can be considered the product of experts (PoE). Considering that the product of Gaussian experts is itself a Gaussian distribution (Cao and Fleet, 2014), once the probability distribution is Gaussian, then PoE has a simple solution. Therefore, the objective formulation of the multi-view-based convolutional variational information bottleneck can be written as follows:

$$J_{MV-CVIB} = \frac{1}{N}\sum_{n=1}^{N} \mathrm{E}_{\varepsilon\sim p(\varepsilon)}\left[-\log q\left(y_n|f\left(x_n^1, x_n^2, \ldots, x_n^V, \varepsilon\right)\right)\right] + \beta KL\left[p(U)\prod_{i=1}^{V}\widetilde{q}\left(U|x_n^i\right), r(U)\right]. \quad (5)$$

2.6. Model implementation details

In MV-CVIB, we mainly input two data views, one is the microbial relative abundance matrix and the other is the nearest neighbor information, for each sample generated based on the microbial relative abundance matrix. The sample nearest neighbor information matrix can be written as follows:

$$NN_{Sample}(a, b) = \sqrt{\sum RA\left(a_i - b_i\right)^2}, \quad (6)$$

where NN_{Sample} represents the sample nearest neighbor information matrix, NN_{Sample} represents the relative abundance matrix, and a_i and b_i represent the i-th element of the row vector and column vector, respectively.

To avoid overfitting, dropout and early stopping are applied in this study. Dropout greatly reduces the size of the neural network, allowing the neural network to learn local features in the data. Early stopping can stop training early when overfitting occurs. We used the dedicated stochastic encoder b_i to embed different views of gut microbiome data. f_{mlp} represents a multilayer perceptron (MLP). For the data of both the above views, we used the SiLU (Hendrycks and Gimpel, 2016) activation function for fully connected layers and used dropout ($p = 0.2$) during training.

We used a logistic regression model $q\left(y|u\right) = \sigma\left(f_d(u)\right)$ with a logistic sigmoid function in the decoder and $f_d(u) = w^T u +$ b. The purpose is to perform binary classification operations. y models two diagnostic CRC labels, such as mCRC and non-mCRC. Furthermore, for other published CRC datasets in this study, y models two diagnosed disease labels, such as CRC or healthy. In addition, in equation (5) mentioned above, $r(U)$ and $r(U)$ are spherical Gaussian distributions with K dimensions, where $r(U) = p(U) = N(0, I)$. We set $K = 256$ and $\beta = 10^{-5}$.

All experiments were performed under Windows 10 with NVIDIA GTX 1650 GPU and CUDA 10.2 installed, where the machine's processor is AMD Ryzen7 4800H. The source code and data are available at: https://github.com/cuizhensdws.

2.7. Performance evaluation

To evaluate the classification performance of the model more accurately and comprehensively, inspired by DeepMicro, we design a similar evaluation scheme. The ratio of the training set and test set in the mCRC dataset is adjusted to 8:2. It is worth noting that the random partition seed is also set the same as DeepMicro, which guarantees a fair random training-test split. Furthermore, for the published CRC dataset, we also adopted the same dataset partitioning scheme. The above settings can further reduce the information leakage and improve the efficiency of the response model more accurately.

We used a stratified 5-fold cross-validation applied to the training set and calculated the AUC score through the validation set, and the epoch with the best parameters among all epochs was selected. The AUC can be written as follows:

$$AUC = \frac{\sum_{i\in positive\ class} rank_i - \frac{M*(1+M)}{2}}{M*N} \quad (7)$$

where M is the number of positive samples, and $N$is the number of negative samples.

3. Results

3.1. Predictive performance of MV-CVIB on mcrc datasets

To evaluate the performance of MV-CVIB, we compared the existing advanced methods, including MVIB (Grazioli et al., 2022), PopPhy-CNN (Reiman et al., 2020), DeepMicro (Oh and Zhang, 2020), random forest (RF), multilayer perceptron (MLP), and SVM. To be fair, these methods are executed multiple times. Considering that different devices and parameter settings may affect the prediction results, we can only ensure that each method is relatively optimal rather than absolutely optimal. It is important to note that DeepMicro is a framework consisting mainly of dimension reduction modules and classification modules. The DeepMicro model does not specify which combination has the best predictive performance. Therefore, we compared all the method combinations.

Our method consists of two parts, one is MV-CVIB which contains the nearest neighbor information, and the other is MV-CVIB (single view) which uses only microbiome abundance data.

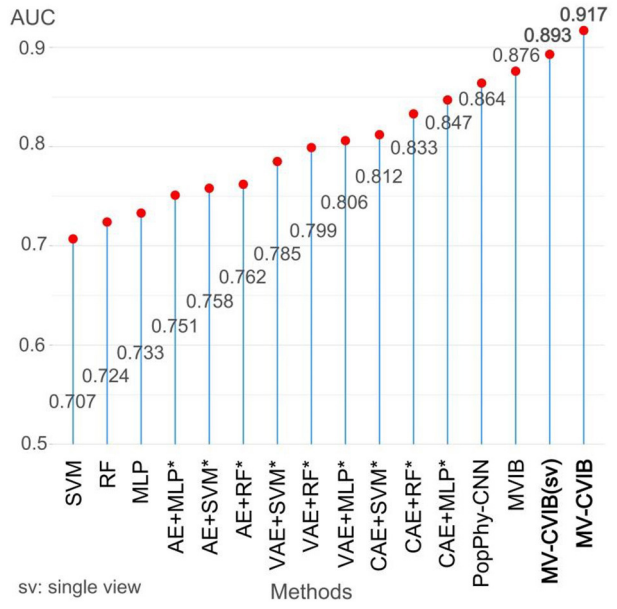

FIGURE 4
Comparison of AUC values of our method with state-of-the-art methods on the mCRC dataset. The methods with asterisks are all from the DeepMicro model, and these methods are a combination of methods in DeepMicro. The best performing AUC is indicated in black bold.

For PopPhy-CNN, we modified the source code so that it is roughly consistent with our model framework. The original PopPhy-CNN provided different experimental procedures. To ensure the consistency of the verification test, we made corresponding framework adjustments. For SVM and RF, we used the same grid search to set the hyperparameters, referring to MetAML (Pasolli et al., 2016). Figure 4 shows the AUC values for each method. Specifically, the AUC value of MV-CVIB reached 0.917, better than 0.893 of MV-CVIB (single view). In other words, the nearest neighbor information helped improve the prediction performance of MV-CVIB by 2.4%. Moreover, the AUC value of MV-CVIB and MV-CVIB (single view) both exceeded that of MVIB, and the AUC value of MV-CVIB is 4.1% higher than that of MVIB. Slightly lower than MVIB is PopPhy-CNN, which has an AUC value of 0.864. Multiple combinations in the DeepMicro framework achieve an AUC value >0.75.

3.2. Predictive performance of MV-CVIB on CRC datasets in published studies

In this study, to more comprehensively evaluate our predictive model, we also performed predictive experiments on three CRC datasets. Figure 5 shows the AUC values for each method.

First, on the Colorectal dataset, the AUC value of the proposed model was 0.818, while the AUC value of MV-CVIB (single view) was 0.814, both of which were superior to other advanced models. Interestingly, RF and PopPhy-CNN both have the same AUC of 0.803. The AUC of MVIB is only 0.78, and the performance of the model is mediocre. In DeepMicro, the AUC value of AE + MLP was 0.799. The predictive effect of CAE+MLP was slightly lower than that of AE + MLP, and the AUC was 0.791.

Second, on the Colorectal-EMBL dataset, as shown in Figure 5, the AUC value for RF is 0.89, which is higher than any other method. This was followed by MV-CVIB and MV-CVIB (single view) with AUC values of 0.825 and 0.821, respectively. Compared with MVIB, the prediction performance of MV-CVIB is improved by 1.1%. Compared with RF, the prediction performance of MV-CVIB is reduced by 6.5% and that of MVIB is reduced by 7.6%. It is worth noting that we cannot get the predicted results of PopPhy-CNN because there is an infinite loop in the experiment process.

Finally, on the Early-Colorectal-EMBL dataset, as shown in Figure 5, none of the methods has an AUC value above 0.6. According to the previous ANOSIM analysis, there are differences between the groups on the Early-Colorectal-EMBL dataset but not significant. Therefore, we speculate that this may be a reason for the AUC of each method to be less than 0.6. Compared with other advanced methods, MV-CVIB achieved an AUC value of 0.589, while MV-CVIB (single view) was slightly lower, with an AUC value of 0.586. Compared with MVIB, MV-CVIB improved performance by 4.6%. Once again, RF (AUC = 0.582) showed excellent performance on this dataset, outperforming all methods except ours. Moreover, in DeepMicro, any autoencoder combined with RF achieved a high AUC value. As on the Colorectal-EMBL dataset, PopPhy-CNN still produces an infinite loop on the Early-Colorectal-EMBL dataset.

3.3. Ablation experiments

Considering that we have introduced multi-view, convolution, and pooling modules in MV-CVIB, to verify the impact of this module on the overall performance of the method, we set up multiple ablation experiments on the mCRC dataset and three CRC datasets. Figure 6 shows the AUC values of different combinations of the proposed method on different datasets. From the results of the ablation experiments, it can be observed that on the mCRC dataset, the impact of multi-view on the performance of the method is slightly higher than that of the convolution module. However, on the three CRC datasets, the impact of the convolution module on the performance of the method is slightly higher than that of the multi-view.

Overall, according to the results of the ablation experiments, we speculate that when the number of samples is small, multi-view may be easier to take advantage of the prediction performance; when the number of samples is large, the convolution module may be more likely to take advantage of the prediction performance.

3.4. The gut microbial diversity is different in mcrc and non-mcrc patients

The stacked bar graphs of the microbiota (phylum level) of the non-mCRC group and the mCRC group are shown in Figure 7A. In their gut, the microbial community was dominated by Firmicutes and Proteobacteria. Among them, a small number

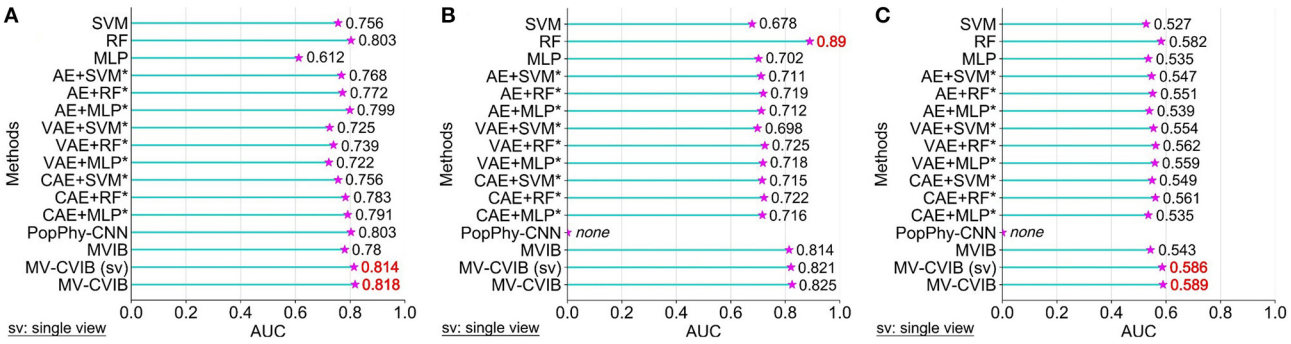

FIGURE 5
Comparison of AUC values of our method with state-of-the-art methods on three CRC datasets. The methods with asterisks are all from the DeepMicro model, and these methods are a combination of methods in DeepMicro. On the Colorectal dataset, we follow the partial AUC results from DeepMicro. Specifically including AE + SVM*, VAE + SVM*, and CAE + RF*, the results of other combinations are derived from this study. Since DeepMicro did not use Colorectal-EMBL and Early-Colorectal-EMBL datasets, the results on these two datasets are also derived from this study. The best performing AUC is indicated in red font. **(A)** The AUC value of each method on the Colorectal dataset. **(B)** The AUC value of each method on the Colorectal-EMBL dataset. **(C)** The AUC value of each method on the Early-Colorectal-EMBL dataset.

of Verrucomicrobia were present in a 61-year-old mCRC patient. It can be observed from the figure that as the age of mCRC patients increases, the relative abundance of Firmicutes tends to decrease overall.

As shown in Figure 7B, to better describe the microbial richness and uniformity of the intestinal tract, we used the alpha diversity index to measure the intestinal ecosystem from different perspectives (Wang et al., 2018). It specifically includes eight indicators: richness, Shannon, Simpson, Pielou, invsimpson, Chao1, ACE, and goods coverage. Taken together, compared with non-mCRC patients, the number and diversity of intestinal communities in mCRC patients tended to increase, and the evenness index of intestinal communities in mCRC patients was significantly increased. In addition, from the perspective of goods coverage, the indices of non-mCRC and mCRC samples are close to 1, which indicates that the sequencing depth is reasonable, that is, the depth has basically covered all species in the sample. We also performed alpha diversity analysis on the three CRC datasets, and the specific analysis results are included in Supplementary material.

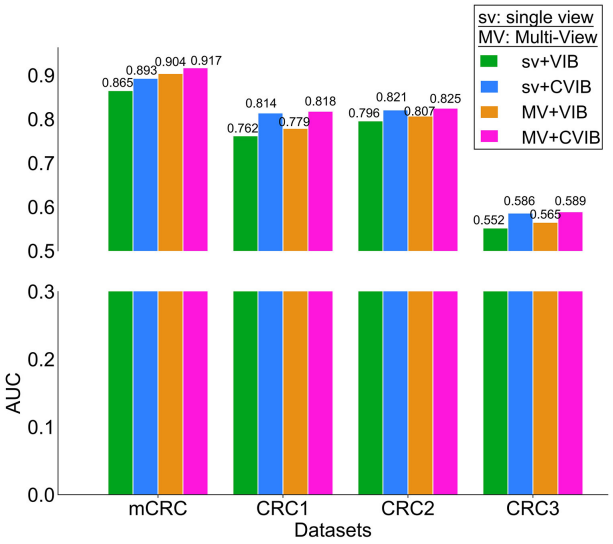

FIGURE 6
AUC comparison of different combinations of the proposed method on different datasets.

3.5. Potential biomarker identification with statistical differences

To further mine the differences between non-mCRC and mCRC samples, we used STAMP to output significantly different OTUs within the 95% confidence interval (Parks et al., 2014). As shown in Figure 7C, the mean proportion value at OTU83 was significantly higher in mCRC patients than in non-mCRC patients. Second, at OTU99, OTU215, and OTU232, mCRC patients were also higher than non-mCRC patients. Interestingly, the taxonomy of OTU215 was accurate to the species, specifically *Propionibacterium acnes*. Not only that, we also used the LDA effect size (LEfSe) (Segata et al., 2011) analysis to discover and explain the biomarkers with statistical differences between non-mCRC and mCRC patients. As shown in the clade diagram of Figure 7D, yellow indicates species without significant differences, and both red and green indicate significant differences. Among them, green nodes represent microbial groups that play an important role in non-mCRC samples, and red nodes represent microbial groups that play an important role in mCRC samples. In the histogram of LDA value distribution in Figure 7E, we can clearly find that there are far more biomarkers with statistical differences in mCRC samples than in non-mCRC samples. Therefore, this may be more conducive to the prediction of metastatic disease in CRC patients, so as to make early diagnosis and treatment.

3.6. Patients' age and metastatic risk assessment

As mentioned earlier in this study, with the development of a standardized multidisciplinary team consultation

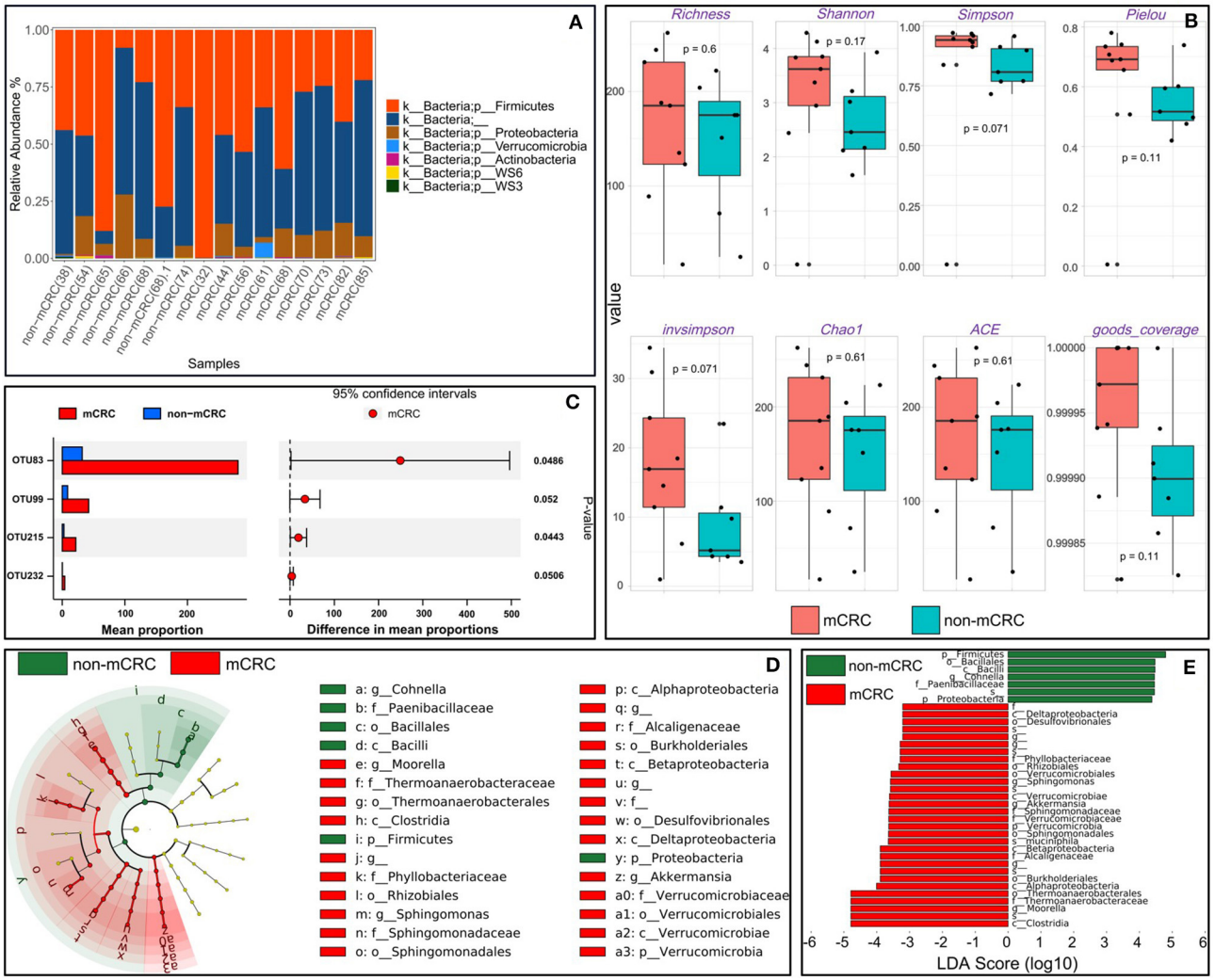

FIGURE 7
(A) Microbiota (phylum level) stacked bar graphs of the non-mCRC group and mCRC group. **(B)** To better describe the microbial richness and uniformity of the intestinal tract, we used the alpha diversity index to measure the intestinal ecosystem from different perspectives. **(C)** To further mine the differences between non-mCRC and mCRC samples, we used STAMP to output significantly different OTUs within the 95% confidence interval. **(D)** LDA effect size (LEfSe) analysis was used to discover and interpret biomarkers that were statistically different between non-mCRC and mCRC patients. **(E)** Histogram of the distribution of LDA values.

model, the survival rate of non-mCRC patients has been significantly improved. However, the metastatic nature of non-mCRC cannot be ignored, and the therapeutic effect of most chemotherapy drugs on mCRC is limited. Therefore, compared with non-mCRC, the survival rate of mCRC is extremely low. To assess the relationship between patient age and metastatic risk of non-mCRC, we constructed a risk model to obtain a risk score. Patients will be divided into high-risk and low-risk groups based on risk scores. Ultimately, we explored the relationship between microbiota expression and patient survival.

As shown in Figure 8, patients with risk scores were divided into high-risk and low-risk groups according to the cutoff value. Combining Figures 8A, B, it can be observed that as the risk score increases, the age of patients presents a downward trend, and the age span of the high-risk group is larger than that of the low-risk group. There is a possibility of cancer metastasis in all segments. It can be observed from Figure 8C that the expression of Desulfovibrionales showed a trend from high to low from left to right, while Thermoanaerobacterales and Actinomycetales showed a trend of gradually increasing expression. From Figures 8A, C, it can be observed that Thermoanaerobacterales and Actinomycetales are positively correlated with risk scores, and Desulfovibrionales are negatively correlated with risk scores. Other flora showed irregular expression trends. Desulfovibrionales produce hydrogen sulfide, a genotoxic compound in the gut. This substance can destabilize the genome or chromosomes (Dahmus et al., 2018; Zhao et al., 2022). Several recent studies have shown that genomic instability is found in more than 80% of sporadic CRCs. Actinomycetales are an important gut flora. Actinomycetales involved in CRC development have different characteristics compared with healthy microbiota (Rebersek, 2021; Li et al., 2023).

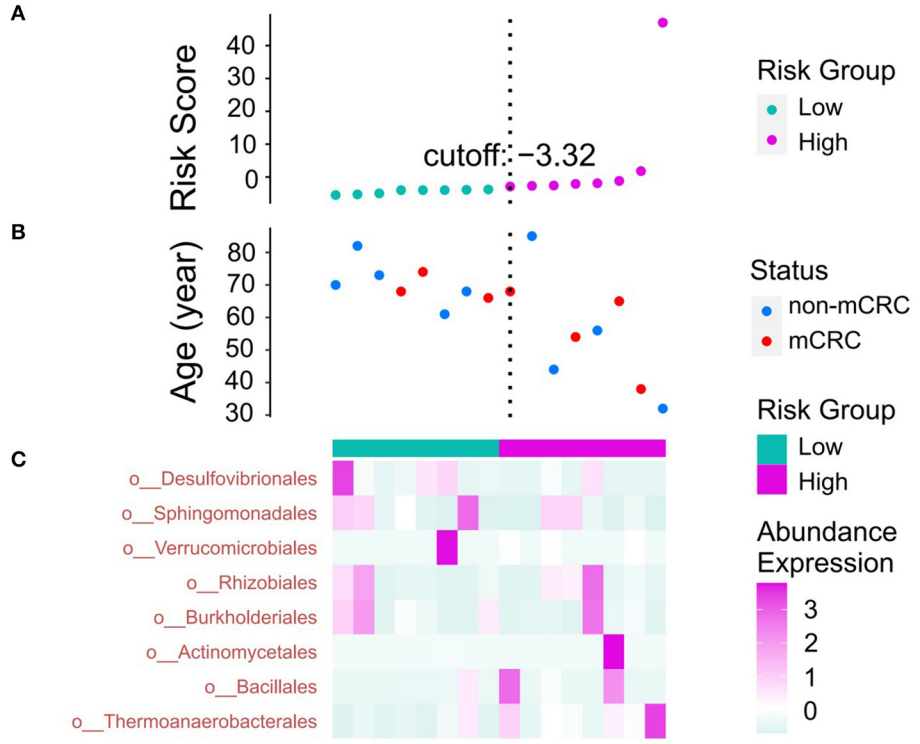

FIGURE 8
(A) According to the risk value, the high risk group and the low risk group are divided. **(B)** Scatter plot of the relationship between patient age and risk status. **(C)** The heat map of the abundance expression of the bacterial group (order level).

Overall, the higher the risk score, the worse the prognosis, and the higher the expression of Desulfovibrionales, the better the prognosis, which may be the beneficial flora before cancer metastasis; while the higher the expression of Thermoanaerobacterales and Actinomycetales, the worse the prognosis, which may be the bad flora after cancer metastasis.

4. Discussion

Many complex reasons and limitations make microbiome-based disease prediction a challenging task. It is mainly reflected as follows: (1) The composition of the human microbial community is very complex, and the boundaries between the bacterial communities are fuzzy. (2) There are various ways to generate microbial community characteristic data, which leads to data heterogeneity. (3) Human health status is dynamic rather than fixed, and healthy samples are not absolute, which may increase data noise and outliers. (4) Conventional microbiome-based disease prediction mostly adopts the health-disease classification method, ignoring the deterioration process of the disease. We adopted a new classification schema: disease-disease instead of health-disease. We focussed on identifying more severe diseases, especially cancer and cancer metastases, from disease samples. This facilitates exploration and reveals the underlying properties of disease exacerbation. It is meaningful for non-mCRC patients. We can obtain potential biomarkers through the analysis of differences in the bacterial flora of patients and explore the biological process and development rules of CRC metastasis on the basis of the microbiome. This is conducive to further expanding the treatment options for non-mCRC patients and improving the prognosis of non-mCRC patients.

We employed a variety of microbiome analysis methods to explore the diversity of non-mCRC and mCRC. From the experimental results, the communities of non-mCRC and mCRC were quite different, the distribution of the flora was complex and diverse, and the flora composition of different samples was different. Compared with the state-of-the-art methods for microbiome-based disease prediction, the proposed method MV-CVIB achieved higher AUC values on the mCRC dataset. In addition, in order to more comprehensively evaluate MV-CVIB and verify its generalization ability, we collected datasets from three published studies and conducted experiments. The number of samples in the three public CRC datasets does not exceed 200, which belongs to high-dimensional small sample data. This limitation may affect the experimental results. Therefore, in future, we can use transfer learning or data augmentation. The experimental results show that MV-CVIB achieves higher AUC values on two of the three datasets. Based on the predicted results, we performed a statistical analysis of potential biomarkers in non-mCRC and mCRC. Finally, we modeled the risk score, explored the age trend of the risk score, and screened out those bacterial orders that had positive and negative effects on patients.

5. Conclusion

In this study, we propose a deep learning approach based on a multi-view convolutional variational information bottleneck for the prediction of mCRC. The multi-view contains species abundance data and sample field information, where the sample neighborhood information is obtained based on the species abundance data, which ensures that our method will not introduce additional noise when inputting multi-view data, and has a better time and space complexity. Our results demonstrate that the method has good predictive performance. However, on the Colorectal-EMBL dataset, all deep learning methods are not as effective as RF, which may be related to the internal structural characteristics of the dataset. We explored the degree of difference between non-mCRC and mCRC from various perspectives, analyzed those statistically significant differences in flora, and constructed an age risk assessment model to explore the rules of age and cancer metastasis. Of course, there are also some deficiencies in this study, mainly two points. First, due to concerns about patient privacy and medical ethics, the number of samples obtained is small, which may cause the model to fall into overfitting to small samples. Second, the prediction model is only for CRC data and does not consider other disease data, which may lead to a lack of generalizability of the model. We will also develop more effective methods for more complex microbiome-based disease phenotype prediction and improve the scalability of the prediction method as much as possible.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, PRJNA531761.

Author contributions

ZC proposed and designed the algorithm. YW and D-SH demonstrated the effectiveness of the method and analyzed the experimental data. Q-HZ, S-GW, and YH drafted the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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References

Akay, A., and Hess, H. (2019). Deep learning: current and emerging applications in medicine and technology. *IEEE J. Biomed. Health Inf.* 23, 906–920. doi: 10.1109/JBHI.2019.2894713

Alemi, A. A., Fischer, I., Dillon, J. V., and Murphy, K. (2016). Deep variational information bottleneck. *arXiv*. [preprint]. doi: 10.48550/arXiv.1612.00410

Asgari, E., Garakani, K., McHardy, A. C., and Mofrad, M. R. K. (2018). MicroPheno: predicting environments and host phenotypes from 16S rRNA gene sequencing using a k-mer based representation of shallow sub-samples. *Bioinformatics* 34, i32–i42. doi: 10.1093/bioinformatics/bty296

Buttigieg, P. L., and Ramette, A. A. (2014). guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses. *FEMS Microbiol. Ecol.* 90, 543–550. doi: 10.1111/1574-6941.12437

Cammarota, G., Ianiro, G., Ahern, A., Carbone, C., Temko, A., Claesson, M. J., et al. (2020). Gut microbiome, big data and machine learning to promote precision medicine for cancer. *Nat. Rev. Gastroenterol. Hepatol.* 17, 635–648. doi: 10.1038/s41575-020-0327-3

Cao, Y., and Fleet, D. J. (2014). Generalized product of experts for automatic and principled fusion of Gaussian process predictions. *arXiv*. [preprint]. doi: 10.48550/arXiv.1410.7827

Chen, Y.-C., Chuang, C.-H., Miao, Z.-F., Yip, K.-L., Liu, C.-J., Li, L.-H., et al. (2022). Gut microbiota composition in chemotherapy and targeted therapy of patients with metastatic colorectal cancer. *Front. Oncol.* 12, 955313. doi: 10.3389/fonc.2022.955313

Cho, I., and Blaser, M. J. (2012). The human microbiome: at the interface of health and disease. *Nat. Rev. Genet.* 13, 260–270. doi: 10.1038/nrg3182

Coordinators, N. R. (2015). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* 44, D7–D19. doi: 10.1093/nar/gkv1290

Dahmus, J. D., Kotler, D. L., Kastenberg, D. M., and Kistler, C. A. (2018). The gut microbiome and colorectal cancer: a review of bacterial

pathogenesis. *J. Gastrointest. Oncol.* 9, 769–777. doi: 10.21037/jgo.2018.04.07

De Sordi, L., Lourenço, M., and Debarbieux, L. (2019). The battle within: interactions of bacteriophages and bacteria in the gastrointestinal tract. *Cell Host Microbe* 25, 210–218. doi: 10.1016/j.chom.2019.01.018

Enquist, I. B., Good, Z., Jubb, A. M., Fuh, G., Wang, X., Junttila, M. R., et al. (2014). Lymph node-independent liver metastasis in a model of metastatic colorectal cancer. *Nat. Commun.* 5, 3530. doi: 10.1038/ncomms4530

Gilbert, J. A., Blaser, M. J., Caporaso, J. G., Jansson, J. K., Lynch, S. V., and Knight, R. (2018). Current understanding of the human microbiome. *Nat. Med.* 24, 392–400. doi: 10.1038/nm.4517

Gotelli, N. J., and Colwell, R. K. (2001). Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol. Lett.* 4, 379–391. doi: 10.1046/j.1461-0248.2001.00230.x

Grazioli, F., Siarheyeu, R., Alqassem, I., Henschel, A., Pileggi, G., and Meiser, A. (2022). Microbiome-based disease prediction with multimodal variational information bottlenecks. *PLoS Comput. Biol.* 18, e1010050. doi: 10.1371/journal.pcbi.1010050

Hall, M., and Beiko, R. G. (2018). "16S rRNA gene analysis with QIIME2," in *Microbiome Analysis: Methods and Protocols*, eds R. G. Beiko, W. Hsiao, and J. Parkinson (New York, NY: Springer New York), 113–129. doi: 10.1007/978-1-4939-8728-3_8

He, S., Ye, X., Sakurai, T., and Zou, Q. (2023). MRMD3.0: a python tool and webserver for dimensionality reduction and data visualization via an ensemble strategy. *J. Mol. Biol.* 435, 168116. doi: 10.1016/j.jmb.2023.168116

Hendrycks, D., and Gimpel, K. (2016). Gaussian error linear units (gelus). *arXiv.* [preprint]. doi: 10.48550/arXiv.1606.08415

Holzinger, A., Dehmer, M., Emmert-Streib, E., Cucchiara, R., Augenstein, I., Del Ser, J., et al. (2022). Information fusion as an integrative cross-cutting enabler to achieve robust, explainable, and trustworthy medical artificial intelligence. *Inf. Fusion* 79, 263–278. doi: 10.1016/j.inffus.2021.10.007

Jiang, L., Elrod, C., Kim, J. J., Swafford, A. D., Knight, R., and Thompson, W. K. (2022). Bayesian multivariate sparse functional principal components analysis with application to longitudinal microbiome multiomics data. *Ann. Appl. Stat.* 16, 2231–2249, 2219. doi: 10.1214/21-AOAS1587

Kingma, D. P., and Welling, M. (2013). Auto-encoding variational bayes. *arXiv.* [preprint]. doi: 10.48550/arXiv.1312.6114

Kostic, A. D., Gevers, D., Siljander, H., Vatanen, T., Hyötyläinen, T., Hämäläinen, A. M., et al. (2015). The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* 17, 260–273. doi: 10.1016/j.chom.2015.01.001

Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. A., et al. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotechnol.* 31, 814–821. doi: 10.1038/nbt.2676

Li, W., Zhou, X., Yuan, S., Wang, L., Yu, L., Sun, J., et al. (2023). Exploring the complex relationship between gut microbiota and risk of colorectal neoplasia using bidirectional mendelian randomization analysis. *Cancer Epidemiol. Biomarkers Prev.* 32, 809–817. doi: 10.1158/1055-9965.EPI-22-0724

Marcos-Zambrano, L. J., Karaduzovic-Hadziabdic, K., Turukalo, T. L., Przymus, P., Trajkovik, V., Aasmets, O., et al. (2021). Applications of machine learning in human microbiome studies: a review on feature selection, biomarker identification, disease prediction and treatment. *Front. Microbiol.* 12, 313. doi: 10.3389/fmicb.2021.634511

Mekadim, C., Skalnikova, H. K., Cizkova, J., Cizkova, V., Palanova, A., Horak, V., et al. (2022). Dysbiosis of skin microbiome and gut microbiome in melanoma progression. *BMC Microbiol.* 22, 63. doi: 10.1186/s12866-022-02458-5

Oh, M., and Zhang, L. (2020). DeepMicro: deep representation learning for disease prediction based on microbiome data. *Sci. Rep.* 10, 6026. doi: 10.1038/s41598-020-63159-5

Parks, D. H., Tyson, G. W., Hugenholtz, P., and Beiko, R. G. (2014). STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* 30, 3123–3124. doi: 10.1093/bioinformatics/btu494

Pasolli, E., Truong, D. T., Malik, F., Waldron, L., and Segata, N. (2016). Machine learning meta-analysis of large metagenomic datasets: tools and biological insights. *PLoS Comput. Biol.* 12, e1004977. doi: 10.1371/journal.pcbi.1004977

Rebersek, M. (2021). Gut microbiome and its role in colorectal cancer. *BMC Cancer* 21, 1325. doi: 10.1186/s12885-021-09054-2

Reiman, D., Metwally, A. A., Sun, J., and Dai, Y. (2020). PopPhy-CNN: a phylogenetic tree embedded architecture for convolutional neural networks to predict host phenotype from metagenomic data. *IEEE J. Biomed. Health Inf.* 24, 2993–3001. doi: 10.1109/JBHI.2020.2993761

Reyes, C., Engel-Nitz, N. M., Byfield, S. D., Ravelo, A., Ogale, S., Bancroft, T., et al. (2019). Cost of disease progression in patients with metastatic breast, lung, and colorectal cancer. *Oncologist* 24, 1209–1218. doi: 10.1634/theoncologist.2018-0018

Rumpold, H., Niedersüß-Beke, D., Heiler, C., Falch, D., Wundsam, H. V., Metz-Gercek, S., et al. (2020). Prediction of mortality in metastatic colorectal cancer in a real-life population: a multicenter explorative analysis. *BMC Cancer* 20, 1149. doi: 10.1186/s12885-020-07656-w

Salim, F., Mizutani, S., Zolfo, M., and Yamada, T. (2023). Recent advances of machine learning applications in human gut microbiota study: from observational analysis toward causal inference and clinical intervention. *Curr. Opin. Biotechnol.* 79, 102884. doi: 10.1016/j.copbio.2022.102884

Schmidt, T. S., Raes, J., and Bork, P. (2018). The human gut microbiome: from association to modulation. *Cell* 172, 1198–1215. doi: 10.1016/j.cell.2018.02.044

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., et al. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.* 12, R60. doi: 10.1186/gb-2011-12-6-r60

Shkoporov, A. N., Clooney, A. G., Sutton, T. D. S., Ryan, F. J., Daly, K. M., Nolan, J. A., et al. (2019). The human gut virome is highly diverse, stable, and individual specific. *Cell Host microbe* 26, 527–541. e525. doi: 10.1016/j.chom.2019.09.009

Somorjai, R. L., Dolenko, B., and Baumgartner, R. (2003). Class prediction and discovery using gene microarray and proteomics mass spectroscopy data: curses, caveats, cautions. *Bioinformatics* 19, 1484–1491. doi: 10.1093/bioinformatics/btg182

Tishby, N., Pereira, F. C., and Bialek, W. (2000). The information bottleneck method. *arXiv*. [preprint]. doi: 10.48550/arXiv.physics/0004057

Wang, C., and Zou, Q. (2023). Prediction of protein solubility based on sequence physicochemical patterns and distributed representation information with DeepSoluE. *BMC Biol.* 21, 12. doi: 10.1186/s12915-023-01510-8

Wang, Y., Xu, L., Gu, Y. Q., and Coleman-Derr, D. (2016). MetaCoMET: a web platform for discovery and visualization of the core microbiome. *Bioinformatics* 32, 3469–3470. doi: 10.1093/bioinformatics/btw507

Wang, Z., Zolnik, C. P., Qiu, Y., Usyk, M., Wang, T., Strickler, H. D., et al. (2018). Comparison of fecal collection methods for microbiome and metabolomics studies. *Front. Cell. Infect. Microbiol.* 8, 301. doi: 10.3389/fcimb.2018.00301

Wani, A. K., Roy, P., Kumar, V., and Mir, T. U. G. (2022). Metagenomics and artificial intelligence in the context of human health. *Infect. Genet. Evol.* 100, 105267. doi: 10.1016/j.meegid.2022.105267

Wirbel, J., Zych, K., Essex, M., Karcher, N., Kartal, E., Salazar, G., et al. (2021). Microbiome meta-analysis and cross-disease comparison enabled by the SIAMCAT machine learning toolbox. *Genome Biol.* 22, 93. doi: 10.1186/s13059-021-02306-1

Wu, M., and Goodman, N. (2018). Multimodal generative models for scalable weakly-supervised learning. *Adv. Neural Inf. Process. Syst.* 31, 5580–5590. doi: 10.5555/3327345.3327461

Yang, C., Chowdhury, D., Zhang, Z., Cheung, W. K., Lu, A., Bian, Z., et al. (2021). A review of computational tools for generating metagenome-assembled genomes from metagenomic sequencing data. *Comput. Struct. Biotechnol. J.* 19, 6301–6314. doi: 10.1016/j.csbj.2021.11.028

Zeller, G., Tap, J., Voigt, A. Y., Sunagawa, S., Kultima, J. R., Costea, P. I., et al. (2014). Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol. Syst. Biol.* 10, 766. doi: 10.15252/msb.20145645

Zhao, Y., Zhong, X., Yan, J., Sun, C., Zhao, X., and Wang, X. (2022). Potential roles of gut microbes in biotransformation of natural products: an overview. *Front. Microbiol.* 13, 956378. doi: 10.3389/fmicb.2022.956378

Zhou, J., He, Z., Yang, Y., Deng, Y., Tringe, S. G., and Alvarez-Cohen, L. (2015). High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. *MBio* 6, e02288–14. doi: 10.1128/mBio.02288-14

Zou, S., Fang, L., and Lee, M.-H. (2017). Dysbiosis of gut microbiota in promoting the development of colorectal cancer. *Gastroenterol. Rep.* 6, 1–12. doi: 10.1093/gastro/gox031

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Application of Mendelian randomization to assess host gene–gut microbiota correlations in patients with esophageal cancer

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Background: Increasing evidence suggests that esophageal cancer (ESCA) may be correlated with gut flora. However, their causal connection remains unclear. This study aimed to evaluate potential causal linkages and gene–gut microbiome associations between the gut microbiota and ESCA using Mendelian randomization (MR).

Methods: We analyzed the data using genome-wide association studies. The exposure factor and outcome variable were the gut microbiota and ESCA, respectively. The MR-Egger method, weighted median, inverse-variance weighted method, heterogeneity test, sensitivity analysis, and multiplicity analysis were used for the MR analysis. And it was validated using an external dataset. Further meta-analysis was performed to validate the robustness of this relationship. Finally, we annotated single nucleotide polymorphisms in the gut microbiota that were causally associated with ESCA to explore possible host gene-gut microbiota correlations in patients with ESCA.

Results: We identified four species with potential associations with ESCA. Three of these species had a negative causal relationship with ESCA (odds ratio (OR): 0.961; 95% confidence interval (CI): 0.923–0.971; $p = 0.047$ for *Romboutsia*; OR: 0.972; 95% CI: 0.921–0.961; $p = 0.018$ for *Lachnospira*; OR: 0.948; 95% CI: 0.912–0.970; $p = 0.032$ for *Eubacterium*). A positive causal relationship was observed between one bacterial group and ESCA (OR: 1.105; 95% CI: 1.010–1.072; $p = 0.018$ for *Veillonella*). External datasets show the same trend. This is further supported by meta-analysis. None of the data showed pleiotropy, and leave-one-out analysis indicated the reliability of these findings. The gut microbiomes of patients with ESCA may correlate with the 19 identified genes.

Conclusion: Our data indicate a potential causal link between these four gut bacteria and ESCA and identify a correlation between host genes and gut microbiota in ESCA, offering novel therapeutic options.

KEYWORDS

esophageal cancer, gut microbiota, Mendelian randomization, single nucleotide polymorphism, meta-analysis

Background

Esophageal cancer (ESCA) is one of the most common cancers globally, with the sixth highest mortality rate according to global cancer data (Sung et al., 2021). Surgery is an effective treatment for ESCA, but in advanced ESCA, the 5-year survival rate of patients remains less than 25% even after surgery (Oppedijk et al., 2014). Chemotherapy is commonly used as a treatment for ESCA but has unavoidable side effects such as toxicity and drug resistance (He et al., 2021). Additionally, epidemiological data indicates that the incidence of ESCA is increasing annually, gravely endangering human health (Uhlenhopp et al., 2020). Therefore, identifying factors potentially associated with ESCA can provide an essential basis for the early prevention of ESCA.

Increasing evidence has shown that the gut microbiota and ESCA are closely related (Cheung et al., 2022; Muszynski et al., 2022; Ohkusa et al., 2022; Baba et al., 2023; Sugimoto et al., 2023). Further, significant variations have been reported in the composition and abundance of fecal microorganisms between patients with ESCA and healthy controls. Notably, these differences are closely correlated with the severity of the disease, suggesting that the gut microbiota may play a significant role in the development of ESCA (Li et al., 2022; Lin et al., 2022). Moreover, gut microbiota can alter genome-wide methylation levels in ESCA, which may be one of the mechanisms influencing the malignant behavior of ESCA cells (Baba et al., 2023). Exposure to antibiotics leads to changes in the gut microbiota, which in turn increase the risk of developing ESCA; the risk of developing the disease increases with the duration of antibiotic exposure (Thanawala et al., 2023). Therefore, investigating the potential link between the gut microbiota and disease to prevent and treat ESCA is crucial.

Although current research reveals that the gut microbiota and ESCA are related, the results are susceptible to confounding factors. Mendelian randomization (MR) is a genetic technique frequently used to investigate causal links between exposures and outcomes and prevents confounding variables in common observational studies because genetic variants are randomly assigned at conception (Smith and Ebrahim, 2003).

We investigated the potential causative relationship between gut microbiota and ESCA using MR to provide a proper theoretical foundation for understanding the interaction between ESCA and gut microbiota. We further identified genes related to the single nucleotide polymorphisms (SNPs) in the gut microbiota obtained by MR analysis. Our results may help identify novel therapeutic options for ESCA treatment.

Methods

Research methods

In this study, heterogeneity, sensitivity, and multiplicity analyses were conducted in addition to MR analyses using genome-wide association studies (GWAS) information to evaluate the causal relationship between gut microbiota and ESCA. MR studies must satisfy three core assumptions of association, independence, and exclusivity: (i) the selected SNPs should be significantly associated with the exposure (intestinal microbiota); (ii) the SNPs must be independent of potential confounders between the exposure and the outcome; and (iii) there is no direct relationship between the SNPs and the outcome (ESCA), and the causal linkage can only be made through the intestinal flora. The workflow is illustrated in Figure 1.

Data sources

Gut microbiota data from the most recent GWAS meta-analysis, comprising 24 cohorts and 18,340 participants, were used in this investigation (Kurilshikov et al., 2021). ESCA data for the experimental and validation groups were obtained from the UK Biospecimen Repository,[1] the experimental group included 372,756 samples and the validation group included 476,306 samples (Table 1).

Instrument selection

A total of 196 bacterial traits, including 9 phyla, 16 classes, 20 orders, 32 families, and 119 genera, were retained after initially removing 15 bacterial qualities without specific names. SNPs significantly related to the gut microbiota were chosen at the genome-wide level ($p < 1.0 \times 10^{-5}$, $R^2 < 0.001$, and clumping distance = 10,000 kb) to fulfill the first MR hypothesis that SNPs must be strongly associated with the gut microbiota. Second, to ensure that the genetic variants were not associated with potential confounders (smoking, heavy alcohol consumption, hot beverages, pickles), a query was performed in the Phenoscanner database.[2] This step was performed to ensure that the SNPs were not associated with known confounders and, ultimately, to obtain SNPs significantly associated with the gut microbiota, serving as instrumental variables. Next, we calculated the proportion of variance (R^2). We calculated the F-statistic using the following formula: $R = 2 \times MAF \times (1\text{-}MAF) \times \beta^2$, $F = R^2\ (n\text{-}k\text{-}1)/K\ (1\text{-}R^2)$, where "MAF" is the minor allele frequency, "N" denotes the exposed GWAS sample size, and "K" is the number of SNPs. $F > 10$ confirmed the absence of a mild instrumental variable bias. The process was completed by annotating the SNPs using an internet database.[3]

Statistical analysis

The weighted median approach, MR-Egger method, and random effects inverse variance weighting (IVW) method were used in the MR analysis. Statistical significance was set at $p < 0.05$.

Abbreviations: BE, Barrett's esophagus; CI, Confidence interval; ESCA, Esophageal cancer; GWAS, Genome-wide association studies; HPV, Human papillomavirus; IVW, Inverse-variance weighted; MAF, Minor allele frequency; ME, Mendelian randomization; OR, Odds ratio; SNPs, Single nucleotide polymorphisms.

1 https://gwas.mrcieu.ac.uk/
2 http://www.phenoscanner.medschl.cam.ac.uk/
3 https://biit.cs.ut.ee/gprofiler/snpense

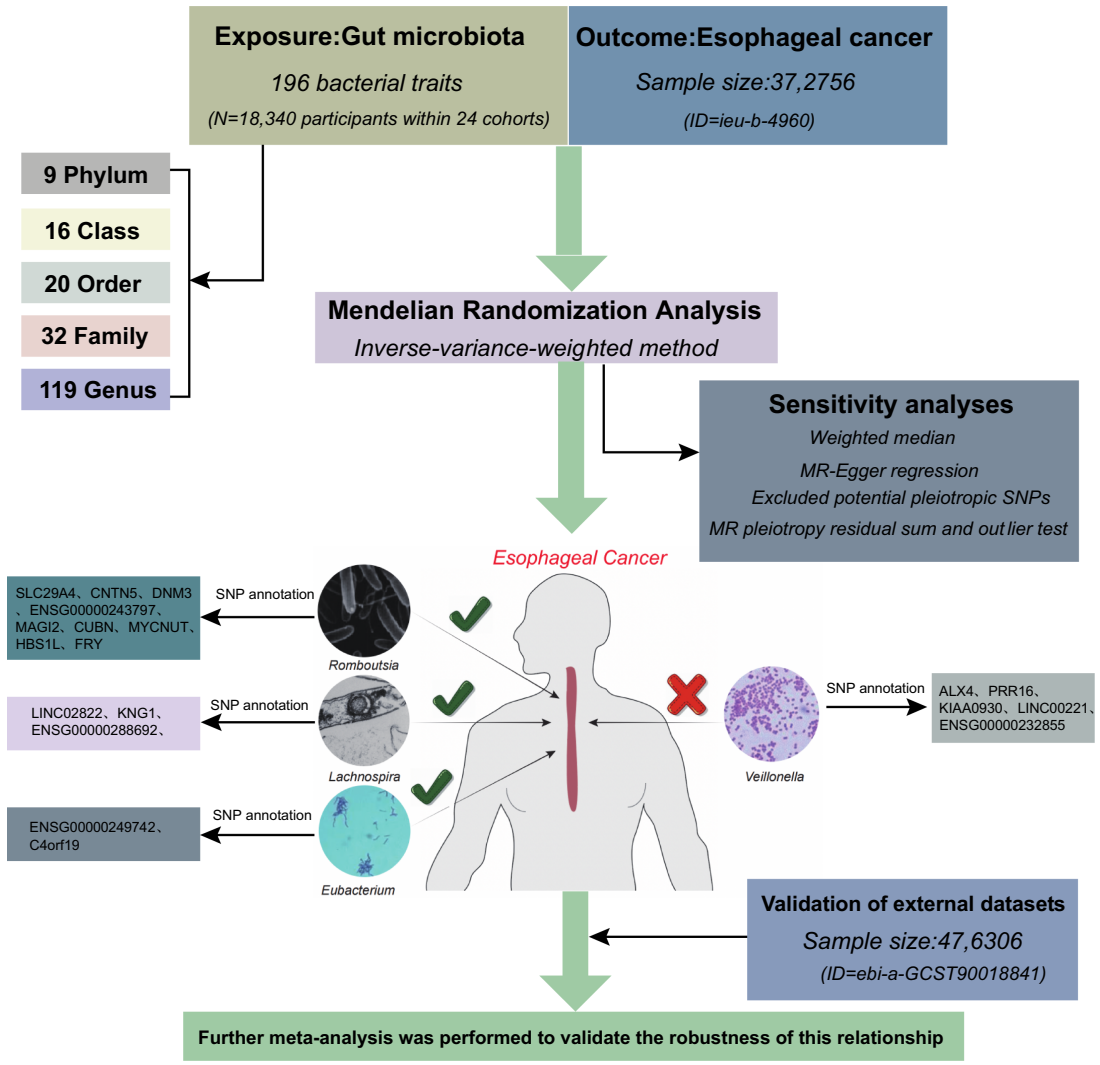

FIGURE 1
The workflow of the present study.

TABLE 1 Details of the genome-wide association studies and datasets used in our analyses.

Exposure or outcome	Sample size	Ancestry	Links for data download	PMID
Human gut microbiome	18,340 participants	Mixed	https://mibiogen.gcc.rug.nl	33462485
Esophageal cancer (Training Group)	3,72,756 participants	European	https://gwas.mrcieu.ac.uk/datasets/ieu-b-4960/	31516927
Esophageal cancer (Validation Group)	4,76,206 participants	European	https://www.ebi.ac.uk/gwas/studies/GCST90018841	34594039

The IVW approach was primarily employed to analyze these studies (Burgess and Thompson, 2017), and methods such as MR-Egger and weighted median were used to complement the IVW method (Verbanck et al., 2018). Additionally, we applied Cochran's Q method to evaluate heterogeneity among SNPs. We performed several heterogeneity tests, including the MR-Egger intercept test and a sensitivity analysis, to ensure the robustness of our results. The leave-one-out test was used for the sensitivity analysis to determine outliers among the final SNPs. All data were analyzed using the R packages "Two-Sample-MR" and "MR-PRESSO" in R software (version 4.3.0).

Results

Main results of the 196 bacterial traits with the risk of ESCA

The F-statistics for the 196 bacterial characteristics, ranging from 21.63 to 144.84 and with mean values exceeding 10, suggested a robust connection with exposure. We screened for SNPs strongly associated with the gut microbiota ($p < 1.0 \times 10^{-5}$), and the linkage disequilibrium parameter was set ($R^2 < 0.001$, kb = 10,000). Thereafter, the data of the final ESCA were extracted

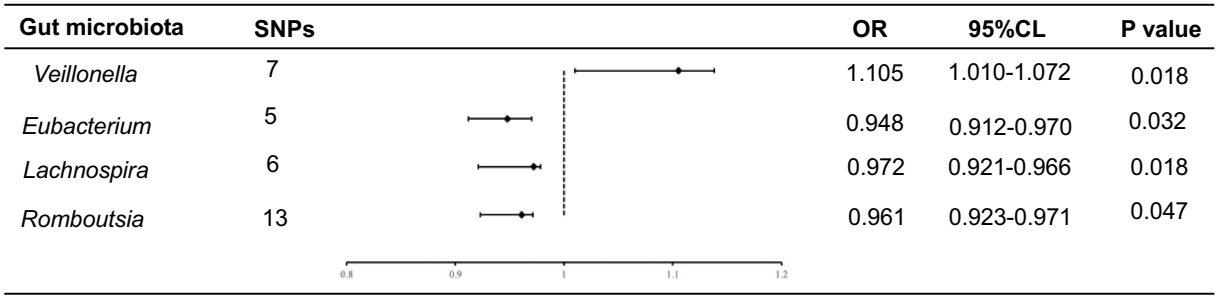

FIGURE 2
Forest plot of the associations between genetically determined 4 bacterial traits with the ESCA.

from GWAS, and 196 intestinal flora were merged with the final ESCA sequentially. SNPs with palindromic sequences were excluded from the analysis. Finally, we obtained four gut microbiota samples with potential associations with ESCA using the IVW method (Figure 2).

Using the IVW method, we found that *Romboutsia* was negatively associated with ESCA (odds ratio (OR): 0.961; $p = 0.047$). After screening for F-statistics and excluding chain imbalances, 13 SNPs related to ESCA were included (Supplementary Table S1). In the weighted median approach, the results for the association between *Romboutsia* and ESCA remained stable ($p = 0.02$). To evaluate the stability of these findings, we performed the MR-Egger test on the loci of the included SNPs. No possible horizontal pleiotropy was found ($p = 0.96$), indicating that instrumental variables did not significantly alter the outcomes through mechanisms other than exposure. Cochran's Q test results showed no significant heterogeneity among the SNPs ($p = 0.45$).

Similarly, *Lachnospira* was negatively associated with ESCA (OR = 0.972; $p = 0.018$). After screening, six SNPs associated with ESCA were included (Supplementary Table S2). In the weighted median approach, the genus *Lachnospira* was weakly associated with ESCA ($p = 0.08$). The SNPs were then subjected to an MR-Egger test, which revealed no apparent level of multiple effects ($p = 0.96$). According to Cochran's Q analysis ($p = 0.45$), no discernible heterogeneity was observed among the selected SNPs. Additionally, using the IVW method, we discovered that the genus *Eubacterium* was adversely linked with ESCA (OR: 0.948; $p = 0.032$). Overall, five SNPs associated with ESCA were included (Supplementary Table S3). Again, no pleiotropy ($p = 0.60$) or heterogeneity ($p = 0.79$) was observed. In contrast, using the IVW method, we found that *Veillonella* was positively associated with ESCA (OR: 1.105; $p = 0.018$). The seven SNPs associated with ESCA identified after screening (Supplementary Table S4) were free of pleiotropy ($p = 0.87$) and heterogeneity ($p = 0.41$).

We conducted a "leave-one-out" sensitivity analysis to confirm the impact of each SNP on the overall causation. The findings revealed that none of the SNPs exhibited significant differences when one SNP was excluded (Supplementary Figure S1). Finally, the results were plotted using a pattern map (Figure 3). Using an online database, we identified 19 genes that may be associated with the gut microbiota of patients with ESCA (Table 2).

We brought the above four intestinal flora into the external dataset for further analysis, and by plotting scatter plots for the training group (Figure 4A) and the validation group (Figure 4B), we found that the four intestinal flora in the validation group had the same tendency to have the same effect on ESCA when compared with the experimental group, which further validated our results. Meanwhile, we further counted the heterozygosity and pleiotropy of the training and validation group analyses (Figure 5), which showed that none of them had pleiotropy, indicating the credibility of our results.

Meta analysis

We further meta-analyzed and plotted and mapped the correlation data of the training and validation groups with the four intestinal flora in a forest plot (Figures 6A–D), which showed that the above relationship was robust. For ease of analysis, we summarized the results into a three-line table (Figure 6E), which showed less statistical heterogeneity and statistically significant results, which further justified our conclusions above.

Discussion

The esophagus in the gastrointestinal tract is colonized by various microorganisms and is not sterile (Laserna-Mendieta et al., 2021). Healthy individuals have a relatively stable pH (approximately 7) that provides a stable environment for microbial survival (Hasan et al., 2021). The gut microbiota can directly or indirectly affect human health and disease and is considered a new "organ" (Baquero and Nombela, 2012). Gut microbes significantly impact cell formation, differentiation, metabolism, and growth. Dysbiotic gut microbiota may contribute to the body's carcinogenic process (Zhou et al., 2021; Gou et al., 2023; Guevara-Ramirez et al., 2023). Notably, the gut microbiota may be involved in the development of ESCA (Lv et al., 2019; Zhou et al., 2021). For example, human papillomavirus (HPV) and alterations in intestinal bacteria may cause ESCA (Meng et al., 2018). Moreover, in regions with a high incidence of ESCA, a high prevalence of HPV has often been reported (Yano et al., 2021). Deng et al. studied 23 patients with ESCA and 23 healthy

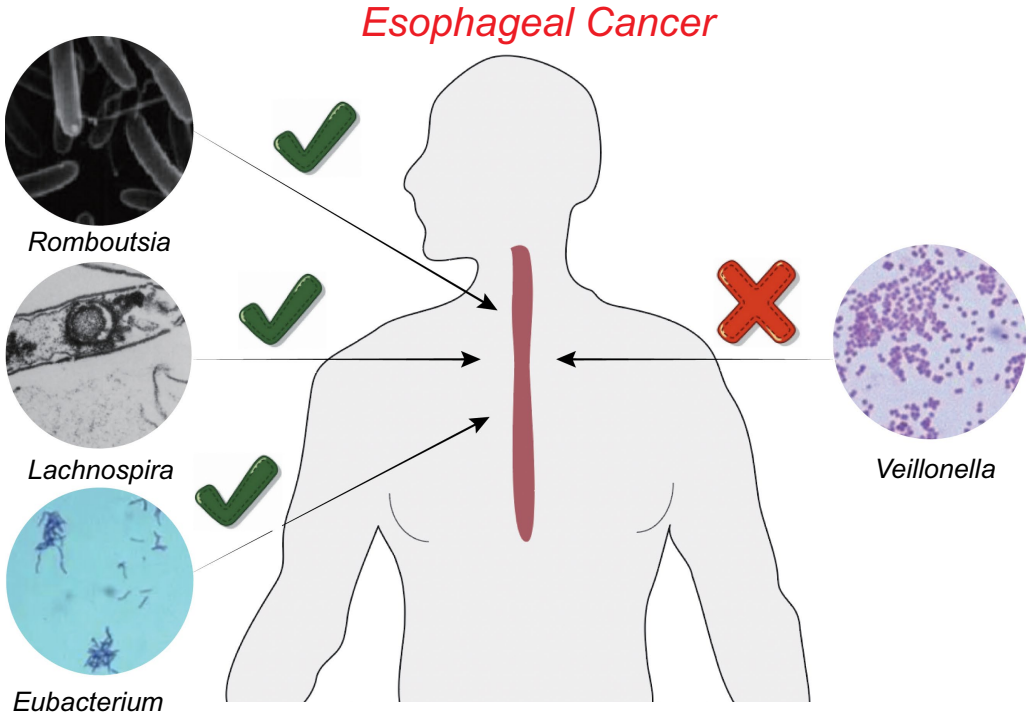

FIGURE 3
Patterns of the relationship between four gut microbiota and ESCA.

individuals and observed that patients with ESCA have higher levels of Firmicutes and Actinobacteria and lower levels of Bacteroidetes (Deng et al., 2021). This suggests that the gut microbiota and development of ESCA are closely related. In addition, changes in the gut microbiota can increase the levels of pro-inflammatory cytokines and immune cells, thereby inducing tumorigenesis (Proano-Vasco et al., 2021). Notably, gut microbiota can induce the overexpression of nitric oxide synthase, potentially leading to ESCA (Gillespie et al., 2021). The gut microbiota can also interact with the host by secreting bioactive substances, such as vitamins (Malesza et al., 2021), that can be beneficial or harmful for the organism (Riwes and Reddy, 2018). These explanations help to clarify how the gut microbiota and ESCA are related.

In this study, MR analysis was used to assess the causal relationship between gut microbiota and ESCA for providing a theoretical foundation for the interactions between ESCA and gut microbiota, given the lack of conclusive evidence to support the potential relationship between gut microbiota and ESCA. The results of this study showed that four intestinal microbiota were potentially associated with ESCA. Specifically, the genera *Romboutsia*, *Lachnospira*, and *Eubacterium* were negatively associated with ESCA, whereas *Veillonella* had a positive causal relationship with ESCA.

The genus *Romboutsia* is a group of gram-positive bacteria first proposed in 2014 by Ricaboni et al. (2016) from the right half of the human colon using colonoscopy. Most studies concluded that *Romboutsia* belongs to the natural gut microbial community and plays an essential role in host health. For example, *Lactobacillus acidophilus* ameliorates colitis by increasing the abundance of *Romboutsia* (Han et al., 2023). Several studies have found that *Romboutsia* is significantly less abundant in patients with inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis) (Qiu et al., 2020; Wang et al., 2023), and is associated with hepatocellular liver cancer and postherpetic neuralgia (Feng et al., 2023; Jiao et al., 2023). Consequently, *Romboutsia* may influence immune regulation and intestinal health (Liu et al., 2023). At the same time *Romboutsia* is able to increase the antioxidant capacity of the body (Zhang et al., 2023), attenuates intestinal inflammatory damage and inhibits endoplasmic reticulum stress, which may be the mechanism by which it affects human health (Li et al., 2023). Moreover, *Romboutsia* is closely associated with esophageal epithelial atrophy (Pan et al., 2021), an essential stage in the development of ESCA. The potential relationship between *Romboutsia* and ESCA identified in this study is consistent with the above findings, which suggests that our analysis is logical.

Lachnospira is integral to the gut microbiota and colonizes the intestinal lumen from birth (Vacca et al., 2020). It is a group of potentially beneficial bacteria involved in the metabolism of various carbohydrates. Fermentation produces acetic and butyric acids, which provide energy to the host (Devillard et al., 2007; Wong et al., 2014). *Lachnospira* has been implicated in various diseases, including obesity (Natividad et al., 2018), liver disease (De Minicis et al., 2014), and chronic kidney disease (Yasuno et al., 2023), and can also lead to depression via the gut–brain axis (Bonaz et al., 2018). The *Lachnospira* spp. have anti-inflammatory and antioxidant effects and can protect the intestinal mucosal barrier by inhibiting inflammatory responses and scavenging free radicals, which may be a mechanism for preventing esophageal diseases (Mukherjee et al., 2020). A cohort study showed that *Lachnospira*, which has anti-inflammatory properties, was

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TABLE 2 SNP annotation of intestinal flora IVs.

		id	chr	Start	End	Strand	Gene_ids	Gene_names
Genus	Romboutsia	rs10279978	7	5279756	5279756	+	ENSG00000164638	SLC29A4
		rs11221428	11	99794319	99794319	+	ENSG00000149972	CNTN5
		rs16843578	1	171934745	171934745	+	ENSG00000197959	DNM3
		rs28603357	7	106766378	106766378	+	ENSG00000243797	ENSG00000243797
		rs34302036	7	78507408	78507408	+	ENSG00000187391	MAGI2
		rs61841503	10	16977560	16977560	+	ENSG00000107611	CUBN
		rs62504452		−1	−1			
		rs7109293		−1	−1			
		rs75200530		−1	−1			
		rs75987356	2	15926583	15926583	+	ENSG00000223850	MYCNUT
		rs77702691		−1	−1			
		rs9389266	6	135090599	135090599	+	ENSG00000112339	HBS1L
		rs9567264	13	32146619	32146619	+	ENSG00000073910	FRY
	Lachnospira	rs13157098		−1	−1			
		rs159484	4	111074471	111074471	+	ENSG00000288692	ENSG00000288692
		rs2520509	12	90612737	90612737	+	ENSG00000286021	LINC02822
		rs4686798	3	186727647	186727647	+	ENSG00000113889	KNG1
		rs4923324		−1	−1			
		rs56791201		−1	−1			
	Eubacterium	rs12129908		−1	−1			
		rs12423772		−1	−1			
		rs1425962	4	186984245	186984245	+	ENSG00000249742	ENSG00000249742
		rs2973294	4	37525057	37525057	+	ENSG00000154274	C4orf19
		rs34561138		−1	−1			
	Veillonella	rs1882878	21	28638351	28638351	+	ENSG00000232855	ENSG00000232855
		rs2013594	11	44280604	44280604	+	ENSG00000052850	ALX4
		rs55807413		−1	-1			
		rs62376424	5	120578590	120578590	+	ENSG00000184838	PRR16
		rs6656807		-1	-1			
		rs7359080	14	106483909	106483909	+	ENSG00000270816	LINC00221
		rs742016	22	45208919	45208919	+	ENSG00000100364	KIAA0930

significantly reduced in ESCA patients compared to the normal group (Cheung et al., 2022). This is consistent with our findings in the current study.

Eubacterium has been found to be associated with age-related macular degeneration (AMD) (Mao et al., 2023), female infertility (Xi et al., 2023), multiple sclerosis (MS) (Vacaras et al., 2023) and other diseases. Although a previous Mendelian analysis done by Yang et al. showed that *Eubacterium* reduced the risk of Barrett's esophagus (Yang et al., 2022), the literature exploring the relationship between *Eubacterium* spp. and ESCA remains scarce to date. This is the innovative finding of our present study, and it also suggests an interesting research direction for us. Now, our experiments on *Eubacterium* for the prevention of ESCA are in progress, and the results will be published in a follow-up study.

The genus *Veillonella* includes gram-negative, anaerobic, non-motile, and non-spore-forming coccus bacteria (Djais et al., 2019). *Veillonella* is strongly associated with the development of several diseases, and has been found to promote the proliferation of lung adenocarcinoma (Zeng et al., 2023), whilst *Veillonella* activates macrophages to promote inflammatory responses via the LPS-TLR4 pathway (Zhan et al., 2022), for example, *Veillonella* correlated with the severity of radiation esophagitis (Lin et al., 2022), and inflammation is one of the factors leading to esophageal cancer, suggesting that *Veillonella* may indirectly contribute to esophageal carcinogenesis through inflammation. In our study, only the genus *Veillonella* positively correlated with ESCA. An extensive body of literature describes the pathogenic role of *Veillonella* in the esophagus. For example, a study using whole genome sequencing (wGS) and RNA sequencing (rNAseq) of tumors from 61 patients with ESCA

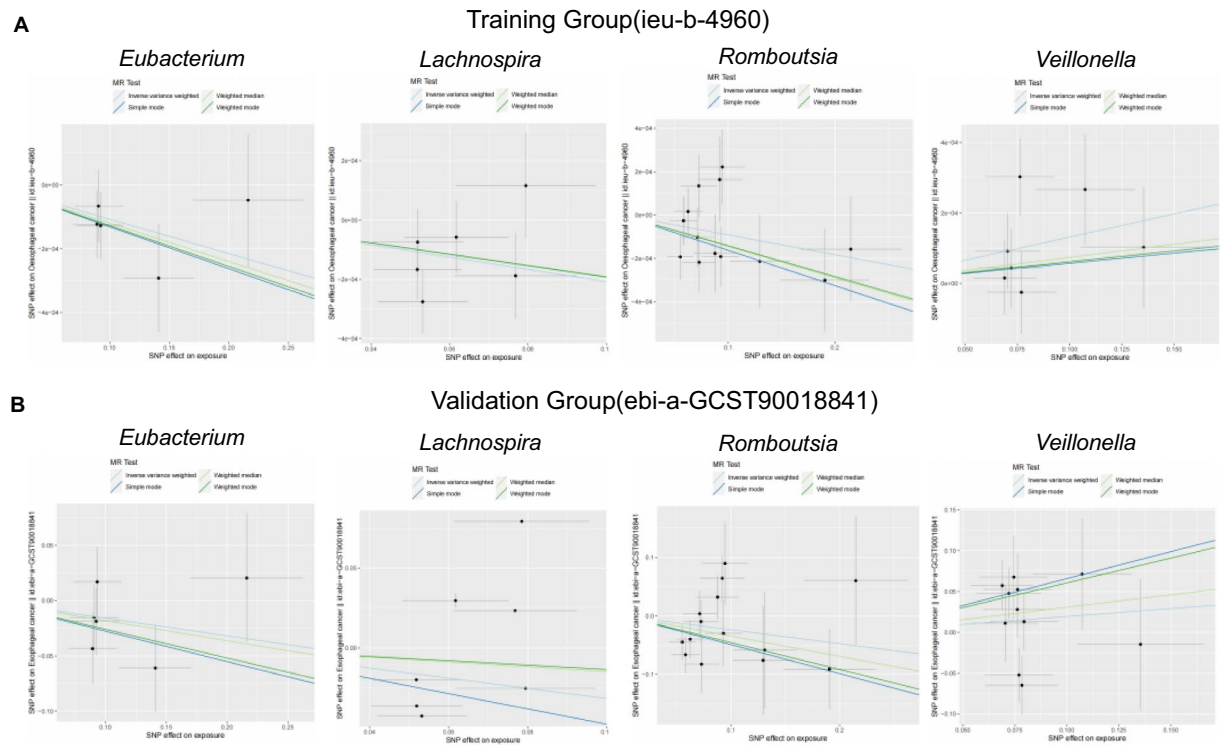


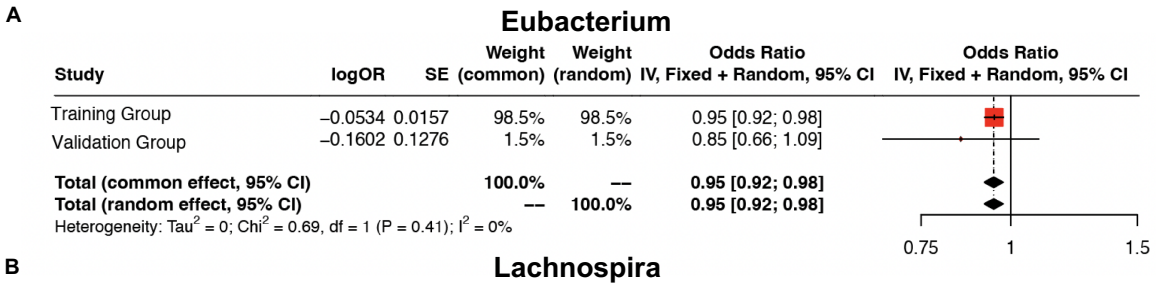
FIGURE 4
Scatterplot of correlation between training and validation groups. **(A)** Scatterplot of the causal relationship between the four intestinal flora and ESCA in the training group. **(B)** Scatterplot of the causal relationship between the four intestinal flora and ESCA in the validation group.

	Heterogeneity		Pleiotropy
	IVW	MR-Egger	
Training Group			
Eubacterium	0.79	0.71	
Lachnospira	0.33	0.41	0.23
Romboutsia	0.45	0.37	0.65
Veillonella	0.29	0.40	0.87
Validation Group			
Eubacterium	0.48	0.59	0.65
Lachnospira	0.53	0.68	0.22
Romboutsia	0.22	0.26	0.26
Veillonella	0.10	0.12	0.54

FIGURE 5
Heterogeneity and pleiotropy results of training and validation group analyses.

found a high abundance of *Veillonella* in ESCA (Nomburg et al., 2022). It has also been shown that *Veillonella* levels gradually increase during the development of esophageal reflux (GR)-Barrett's esophagus (BE)-esophageal adenocarcinoma (EA) (Di Pilato et al., 2016; Hao et al., 2022). Gram-negative bacteria are mainly associated with esophageal abnormalities, e.g., *Veillonella* is mostly associated with BE (Lv et al., 2019; Park and Lee, 2020), which aligns with our findings. These findings have important implications for research in ESCA in the future and may inspire new prevention and treatment strategies for this disease.

Many previous studies have demonstrated a potential relationship between the gut microbiota and ESCA (Baba et al., 2023; Co et al., 2023; Sugimoto et al., 2023). However, most of these studies were observational and susceptible to confounding



E

Outcome	OR	95%CL	P value	Heterogeneity
Eubacterium	0.946	0.918-0.976	<0.001	0.406
Lachnospira	0.971	0.948-0.995	0.017	0.418
Romboutsia	0.958	0.935-0.984	0.001	0.220
Veillonella	1.105	1.073-1.139	<0.001	0.628

FIGURE 6
Meta-analysis of the causal association between host gene–gut microbiota and ESCA. **(A–D)** Meta-analysis of the causal relationship between four intestinal flora and ESCA. **(E)** Meta-analysis results.

factors. In contrast, the present study used a genetic epidemiological approach to minimize the influence of confounding factors and provide a compelling insight into the relationship between gut microbiota and ESCA. And the use of external datasets to validate trends and Meta-analysis of the results ensures maximum stability of the results.

Gut flora plays a dual role in cancer development, and the use of gut flora in conjunction with traditional antitumor treatment strategies, as well as the use of probiotics, FMT, and dietary control, can improve the efficacy of anticancer treatments, while reducing the incidence of side effects and improving prognosis (Sun et al., 2023). However, this study had certain limitations. First, most of the participants were European. Additional research is required to determine whether these conclusions apply to other ethnic groups. Second, the flora in this study was limited to the genus level and was not further subdivided.

Conclusion

We analyzed the potential relationship between 196 common intestinal microbiota and ESCA. We found that the genera *Romboutsia*, *Lachnospira*, *Eubacterium*, and *Veillonella* may be causally associated

with ESCA, which may provide new ideas for ESCA research and treatment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for this study, because data were obtained from publicly available databases and no identifiable data was published.

Author contributions

ZZ: Writing – original draft. GZ: Conceptualization, Writing – review & editing. ZH: Methodology, Writing – review & editing. YS: Investigation, Writing – review & editing. DW: Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1309596/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Leave-one-out analysis forest plot.

SUPPLEMENTARY TABLE 1
13 SNPs associated with ESCA in *Romboutsia*.

SUPPLEMENTARY TABLE 2
6 SNPs associated with ESCA in *Lachnospira*.

SUPPLEMENTARY TABLE 3
5 SNPs associated with ESCA in *Eubacterium*.

SUPPLEMENTARY TABLE 4
7 SNPs associated with ESCA in *Veillonella*.

References

Baba, Y., Hara, Y., Toihata, T., Kosumi, K., Iwatsuki, M., Iwagami, S., et al. (2023). Relationship between gut microbiome fusobacterium nucleatum and LINE−1 methylation level in esophageal cancer. *Esophagus* 20, 704–712. doi: 10.1007/s10388-023-01009-9

Baquero, F., and Nombela, C. (2012). The microbiome as a human organ. *Clin. Microbiol. Infect.* 18, 2–4. doi: 10.1111/j.1469-0691.2012.03916.x

Bonaz, B., Bazin, T., and Pellissier, S. (2018). The Vagus nerve at the Interface of the microbiota-gut-brain Axis. *Front. Neurosci.* 12:49. doi: 10.3389/fnins.2018.00049

Burgess, S., and Thompson, S. G. (2017). Interpreting findings from Mendelian randomization using the MR-egger method. *Eur. J. Epidemiol.* 32, 377–389. doi: 10.1007/s10654-017-0255-x

Cheung, M. K., Yue, G. G. L., Lauw, S., Li, C. S. Y., Yung, M. Y., Ng, S. C., et al. (2022). Alterations in gut microbiota of esophageal squamous cell carcinoma patients. *J. Gastroenterol. Hepatol.* 37, 1919–1927. doi: 10.1111/jgh.15941

Co, E. L., Hameed, M., Sebastian, S. A., Garg, T., Sudan, S., Bheemisetty, N., et al. (2023). Narrative review of probiotic use on the recovery of postoperative patients with esophageal Cancer. *Curr. Nutr. Rep.* doi: 10.1007/s13668-023-00490-z

De Minicis, S., Rychlicki, C., Agostinelli, L., Saccomanno, S., Candelaresi, C., Trozzi, L., et al. (2014). Dysbiosis contributes to fibrogenesis in the course of chronic liver injury in mice. *Hepatology* 59, 1738–1749. doi: 10.1002/hep.26695

Deng, Y., Tang, D., Hou, P., Shen, W., Li, H., Wang, T., et al. (2021). Dysbiosis of gut microbiota in patients with esophageal cancer. *Microb. Pathog.* 150:104709. doi: 10.1016/j.micpath.2020.104709

Devillard, E., McIntosh, F. M., Duncan, S. H., and Wallace, R. J. (2007). Metabolism of linoleic acid by human gut bacteria: different routes for biosynthesis of conjugated linoleic acid. *J. Bacteriol.* 189, 2566–2570. doi: 10.1128/JB.01359-06

Di Pilato, V., Freschi, G., Ringressi, M. N., Pallecchi, L., Rossolini, G. M., and Bechi, P. (2016). The esophageal microbiota in health and disease. *Ann. N. Y. Acad. Sci.* 1381, 21–33. doi: 10.1111/nyas.13127

Djais, A. A., Theodorea, C. F., Mashima, I., Otomo, M., Saitoh, M., and Nakazawa, F. (2019). Identification and phylogenetic analysis of oral Veillonella species isolated from the saliva of Japanese children. *F1000Res* 8:616. doi: 10.12688/f1000research.18506.5

Feng, J., Wu, Y., Dai, P., Wang, D., Liu, L., and Chai, B. (2023). Gut microbial signatures of patients with primary hepatocellular carcinoma and their healthy first-degree relatives. *J. Appl. Microbiol.* 134:221. doi: 10.1093/jambio/lxad221

Gillespie, M. R., Rai, V., Agrawal, S., and Nandipati, K. C. (2021). The role of microbiota in the pathogenesis of esophageal adenocarcinoma. *Biology (Basel)* 10:697. doi: 10.3390/biology10080697

Gou, H., Su, H., Liu, D., Wong, C. C., Shang, H., Fang, Y., et al. (2023). Traditional medicine Pien Tze Huang suppresses colorectal tumorigenesis through restoring gut microbiota and metabolites. *Gastroenterology* 165, 1404–1419. doi: 10.1053/j.gastro.2023.08.052

Guevara-Ramirez, P., Cadena-Ullauri, S., Paz-Cruz, E., Tamayo-Trujillo, R., Ruiz-Pozo, V. A., and Zambrano, A. K. (2023). Role of the gut microbiota in hematologic cancer. *Front. Microbiol.* 14:1185787. doi: 10.3389/fmicb.2023.1185787

Han, M., Liao, W., Dong, Y., Fei, T., and Gai, Z. (2023). Sustained ameliorative effect of *Lactobacillus acidophilus* LA85 on dextran sulfate sodium-induced colitis in mice. *J. Food Sci.* 88, 3893–3904. doi: 10.1111/1750-3841.16723

Hao, Y., Karaoz, U., Yang, L., Yachimski, P. S., Tseng, W., Nossa, C. W., et al. (2022). Progressive dysbiosis of human orodigestive microbiota along the sequence of gastroesophageal reflux, Barrett's esophagus and esophageal adenocarcinoma. *Int. J. Cancer* 151, 1703–1716. doi: 10.1002/ijc.34191

Hasan, A., Hasan, L. K., Schnabl, B., Greytak, M., and Yadlapati, R. (2021). Microbiome of the Aerodigestive tract in health and esophageal disease. *Dig. Dis. Sci.* 66, 12–18. doi: 10.1007/s10620-020-06720-6

He, S., Xu, J., Liu, X., and Zhen, Y. (2021). Advances and challenges in the treatment of esophageal cancer. *Acta Pharm. Sin. B* 11, 3379–3392. doi: 10.1016/j.apsb.2021.03.008

Jiao, B., Cao, X., Zhang, C., Zhang, W., Yu, S., Zhang, M., et al. (2023). Alterations of the gut microbiota in patients with postherpetic neuralgia. *AMB Express* 13:108. doi: 10.1186/s13568-023-01614-y

Kurilshikov, A., Medina-Gomez, C., Bacigalupe, R., Radjabzadeh, D., Wang, J., Demirkan, A., et al. (2021). Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat. Genet.* 53, 156–165. doi: 10.1038/s41588-020-00763-1

Laserna-Mendieta, E. J., FitzGerald, J. A., Arias-Gonzalez, L., Ollala, J. M., Bernardo, D., Claesson, M. J., et al. (2021). Esophageal microbiome in active eosinophilic esophagitis and changes induced by different therapies. *Sci. Rep.* 11:7113. doi: 10.1038/s41598-021-86464-z

Li, N., Bai, C., Zhao, L., Ge, Y., and Li, X. (2022). Characterization of the fecal microbiota in gastrointestinal cancer patients and healthy people. *Clin. Transl. Oncol.* 24, 1134–1147. doi: 10.1007/s12094-021-02754-y

Li, K., Wu, J., Xu, S., Li, X., Zhang, Y., and Gao, X. J. (2023). Rosmarinic acid alleviates intestinal inflammatory damage and inhibits endoplasmic reticulum stress and smooth muscle contraction abnormalities in intestinal tissues by regulating gut microbiota. *Microbiol. Spectr.* 11:1914. doi: 10.1128/spectrum.01914-23

Lin, M. Q., Wu, Y. H., Yang, J., Lin, H. C., Liu, L. Y., Yu, Y. L., et al. (2022). Gut microbiota characteristics are associated with severity of acute radiation-induced esophagitis. *Front. Microbiol.* 13:883650. doi: 10.3389/fmicb.2022.883650

Liu, J., Cai, J., Fan, P., Dong, X., Zhang, N., Tai, J., et al. (2023). Salidroside alleviates dextran sulfate sodium-induced colitis in mice by modulating the gut microbiota. *Food Funct.* 14, 7506–7519. doi: 10.1039/D3FO01929B

Lv, J., Guo, L., Liu, J. J., Zhao, H. P., Zhang, J., and Wang, J. H. (2019). Alteration of the esophageal microbiota in Barrett's esophagus and esophageal adenocarcinoma. *World J. Gastroenterol.* 25, 2149–2161. doi: 10.3748/wjg.v25.i18.2149

Malesza, I. J., Malesza, M., Walkowiak, J., Mussin, N., Walkowiak, D., Aringazina, R., et al. (2021). High-Fat, western-style diet, systemic inflammation, and gut microbiota: A narrative review. *Cells* 10:3164. doi: 10.3390/cells10113164

Mao, D., Tao, B., Sheng, S., Jin, H., Chen, W., Gao, H., et al. (2023). Causal effects of gut microbiota on age-related macular degeneration: a Mendelian randomization study. *Invest. Ophthalmol. Vis. Sci.* 64:32. doi: 10.1167/iovs.64.12.32

Meng, C., Bai, C., Brown, T. D., Hood, L. E., and Tian, Q. (2018). Human gut microbiota and gastrointestinal Cancer. *Genomics Proteomics Bioinformatics* 16, 33–49. doi: 10.1016/j.gpb.2017.06.002

Mukherjee, A., Lordan, C., Ross, R. P., and Cotter, P. D. (2020). Gut microbes from the phylogenetically diverse genus Eubacterium and their various contributions to gut health. *Gut Microbes* 12:1802866. doi: 10.1080/19490976.2020.1802866

Muszynski, D., Kudra, A., Sobocki, B. K., Folwarski, M., Vitale, E., Filetti, V., et al. (2022). Esophageal cancer and bacterial part of gut microbiota – a multidisciplinary point of view. *Front. Cell. Infect. Microbiol.* 12:1057668. doi: 10.3389/fcimb.2022.1057668

Natividad, J. M., Agus, A., Planchais, J., Lamas, B., Jarry, A. C., Martin, R., et al. (2018). Impaired aryl hydrocarbon receptor ligand production by the gut microbiota is a key factor in metabolic syndrome. *Cell Metab.* 28:e734, 737–749.e4. doi: 10.1016/j.cmet.2018.07.001

Nomburg, J., Bullman, S., Nasrollahzadeh, D., Collisson, E. A., Abedi-Ardekani, B., Akoko, L. O., et al. (2022). An international report on bacterial communities in esophageal squamous cell carcinoma. *Int. J. Cancer* 151, 1947–1959. doi: 10.1002/ijc.34212

Ohkusa, T., Nishikawa, Y., and Sato, N. (2022). Gastrointestinal disorders and intestinal bacteria: advances in research and applications in therapy. *Front. Med.* 9:935676. doi: 10.3389/fmed.2022.935676

Oppedijk, V., van der Gaast, A., van Lanschot, J. J., van Hagen, P., van Os, R., van Rij, C. M., et al. (2014). Patterns of recurrence after surgery alone versus preoperative chemoradiotherapy and surgery in the CROSS trials. *J. Clin. Oncol.* 32, 385–391. doi: 10.1200/JCO.2013.51.2186

Pan, F., Zhang, L. L., Luo, H. J., Chen, Y., Long, L., Wang, X., et al. (2021). Dietary riboflavin deficiency induces ariboflavinosis and esophageal epithelial atrophy in association with modification of gut microbiota in rats. *Eur. J. Nutr.* 60, 807–820. doi: 10.1007/s00394-020-02283-4

Park, C. H., and Lee, S. K. (2020). Exploring esophageal microbiomes in esophageal diseases: a systematic review. *J. Neurogastroenterol. Motil.* 26, 171–179. doi: 10.5056/jnm19240

Proano-Vasco, A., Baumeister, T., Metwaly, A., Reitmeier, S., Kleigrewe, K., Meng, C., et al. (2021). High-fructose diet alters intestinal microbial profile and correlates with early tumorigenesis in a mouse model of Barrett's esophagus. *Microorganisms* 9:2432. doi: 10.3390/microorganisms9122432

Qiu, X., Zhao, X., Cui, X., Mao, X., Tang, N., Jiao, C., et al. (2020). Characterization of fungal and bacterial dysbiosis in young adult Chinese patients with Crohn's disease. *Ther. Adv. Gastroenterol.* 13:175628482097120. doi: 10.1177/1756284820971202

Ricaboni, D., Mailhe, M., Khelaifia, S., Raoult, D., and Million, M. (2016). Romboutsia timonensis, a new species isolated from human gut. *New Microbes New Infect.* 12, 6–7. doi: 10.1016/j.nmni.2016.04.001

Riwes, M., and Reddy, P. (2018). Microbial metabolites and graft versus host disease. *Am. J. Transplant.* 18, 23–29. doi: 10.1111/ajt.14443

Smith, G. D., and Ebrahim, S. (2003). 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int. J. Epidemiol.* 32, 1–22. doi: 10.1093/ije/dyg070

Sugimoto, T., Atobe, S., Kado, Y., Takahashi, A., Motoori, M., Sugimura, K., et al. (2023). Gut microbiota associated with the mitigation effect of synbiotics on adverse events of neoadjuvant chemotherapy in patients with esophageal cancer: a retrospective exploratory study. *J. Med. Microbiol.* 72:1723. doi: 10.1099/jmm.0.001723

Sun, J., Chen, F., and Wu, G. (2023). Potential effects of gut microbiota on host cancers: focus on immunity, DNA damage, cellular pathways, and anticancer therapy. *ISME J.* 17, 1535–1551. doi: 10.1038/s41396-023-01483-0

Sung, H., Ferlay, J., Siegel, R. L., 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. doi: 10.3322/caac.21660

Thanawala, S. U., Kaplan, D. E., Falk, G. W., Beveridge, C. A., Schaubel, D., Serper, M., et al. (2023). Antibiotic exposure is associated with a risk of esophageal adenocarcinoma. *Clin. Gastroenterol. Hepatol.* 21, 2817–2824.e4. doi: 10.1016/j.cgh.2023.03.012

Uhlenhopp, D. J., Then, E. O., Sunkara, T., and Gaduputi, V. (2020). Epidemiology of esophageal cancer: update in global trends, etiology and risk factors. *Clin. J. Gastroenterol.* 13, 1010–1021. doi: 10.1007/s12328-020-01237-x

Vacaras, V., Muresanu, D. F., Buzoianu, A. D., Nistor, C., Vesa, S. C., Paraschiv, A. C., et al. (2023). The role of multiple sclerosis therapies on the dynamic of human gut microbiota. *J. Neuroimmunol.* 378:578087. doi: 10.1016/j.jneuroim.2023.578087

Vacca, M., Celano, G., Calabrese, F. M., Portincasa, P., Gobbetti, M., and De Angelis, M. (2020). The controversial role of human gut Lachnospiraceae. *Microorganisms* 8:573. doi: 10.3390/microorganisms8040573

Verbanck, M., Chen, C. Y., Neale, B., and Do, R. (2018). Publisher correction: detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat. Genet.* 50:1196. doi: 10.1038/s41588-018-0164-2

Wang, K., Qin, L., Cao, J., Zhang, L., Liu, M., Qu, C., et al. (2023). Kappa-Selenocarrageenan oligosaccharides prepared by Deep-Sea enzyme alleviate inflammatory responses and modulate gut microbiota in ulcerative colitis mice. *Int. J. Mol. Sci.* 24:4672. doi: 10.3390/ijms24054672

Wong, J., Piceno, Y. M., DeSantis, T. Z., Pahl, M., Andersen, G. L., and Vaziri, N. D. (2014). Expansion of urease-and uricase-containing, indole-and p-cresol-forming and contraction of short-chain fatty acid-producing intestinal microbiota in ESRD. *Am. J. Nephrol.* 39, 230–237. doi: 10.1159/000360010

Xi, Y., Zhang, C., Feng, Y., Zhao, S., Zhang, Y., Duan, G., et al. (2023). Genetically predicted the causal relationship between gut microbiota and infertility: bidirectional Mendelian randomization analysis in the framework of predictive, preventive, and personalized medicine. *EPMA J.* 14, 405–416. doi: 10.1007/s13167-023-00332-6

Yang, Z., Yu, R., Deng, W., and Wang, W. (2022). Roles of 21 genera of human gut microbiota in Barrett's esophagus risk: a Mendelian randomization study. *Front. Genet.* 13:894900. doi: 10.3389/fgene.2022.894900

Yano, Y., Etemadi, A., and Abnet, C. C. (2021). Microbiome and cancers of the esophagus: A Review. *Microorganisms* 9:764. doi: 10.3390/microorganisms9081764

Yasuno, T., Takahashi, K., Tada, K., Hiyamuta, H., Watanabe, M., Ito, K., et al. (2023). Dysbiosis of gut microbiota in patients with chronic kidney disease. *Intern. Med.* doi: 10.2169/internalmedicine.1602-23

Zeng, W., Wang, Y., Wang, Z., Yu, M., Liu, K., Zhao, C., et al. (2023). *Veillonella parvula* promotes the proliferation of lung adenocarcinoma through the nucleotide oligomerization domain 2/cellular communication network factor 4/nuclear factor kappa B pathway. *Discov. Oncol.* 14:129. doi: 10.1007/s12672-023-00748-6

Zhan, Z., Liu, W., Pan, L., Bao, Y., Yan, Z., and Hong, L. (2022). Overabundance of *Veillonella parvula* promotes intestinal inflammation by activating macrophages via LPS-TLR4 pathway. *Cell Death Discov.* 8:251. doi: 10.1038/s41420-022-01015-3

Zhang, P., Jiang, G., Wang, Y., Yan, E., He, L., Guo, J., et al. (2023). Maternal consumption of l-malic acid enriched diets improves antioxidant capacity and glucose metabolism in offspring by regulating the gut microbiota. *Redox Biol.* 67:102889. doi: 10.1016/j.redox.2023.102889

Zhou, J., Sun, S., Luan, S., Xiao, X., Yang, Y., Mao, C., et al. (2021). Gut microbiota for esophageal Cancer: role in carcinogenesis and clinical implications. *Front. Oncol.* 11:717242. doi: 10.3389/fonc.2021.717242

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Gut microbiota and its therapeutic implications in tumor microenvironment interactions

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The development of cancer is not just the growth and proliferation of a single transformed cell, but its tumor microenvironment (TME) also coevolves with it, which is primarily involved in tumor initiation, development, metastasis, and therapeutic responses. Recent years, TME has been emerged as a potential target for cancer diagnosis and treatment. However, the clinical efficacy of treatments targeting the TME, especially its specific components, remains insufficient. In parallel, the gut microbiome is an essential TME component that is crucial in cancer immunotherapy. Thus, assessing and constructing frameworks between the gut microbiota and the TME can significantly enhance the exploration of effective treatment strategies for various tumors. In this review the role of the gut microbiota in human cancers, including its function and relationship with various tumors was summarized. In addition, the interaction between the gut microbiota and the TME as well as its potential applications in cancer therapeutics was described. Furthermore, it was summarized that fecal microbiota transplantation, dietary adjustments, and synthetic biology to introduce gut microbiota-based medical technologies for cancer treatment. This review provides a comprehensive summary for uncovering the mechanism underlying the effects of the gut microbiota on the TME and lays a foundation for the development of personalized medicine in further studies.

KEYWORDS

cancer therapy, gut microbiota, microbiota tumor microenvironment, synthetic biology, therapeutic target

1 Introduction

Cancer is one of the significant causes of death, affecting millions of people globally (Siegel et al., 2019). Only 5–10% of cancer cases are associated with genetics, while most are related to environmental factors (Anand et al., 2008). The tumor microenvironment (TME) has been confirmed to play an essential role in tumor initiation and development, with its interactions with cancer cells well-studied (Kise et al., 2016). Most scientists believe that the TME can offer efficient and cost-effective therapeutics for various cancers, including gastric and colon cancers (Merlo et al., 2006). The TME comprises noncellular components and noncancerous host cells, including endothelial cells, fibroblasts, immune cells, and even microbes (Whisner and Athena

Aktipis, 2019). The chemopathological qualities of the TME were classified into six categories, contributing to an in-depth understanding of its complexity and heterogeneity and guiding anticancer therapy (Jin and Jin, 2020). Considering the strengthened comprehension of the essential effects of TME on tumor growth and therapeutic resistance, therapeutic benefits in cancer patients have been achieved by targeting components of the TME (Xiao and Yu, 2021). Therefore, a comprehensive understanding of the TME provides a framework for preventing and treating cancers.

Microbiota is one of the cellular components in TME that play an essential and irreplaceable role in human systems as well as other factors like genetic due to the microbiota community can modulate various biological processes including cellular metabolism, physiology, and immune responses (Marsland et al., 2015; Andreeva et al., 2020). Disturbances in the human microbiota have been linked to several diseases, such as inflammatory bowel diseases (IBDs), cardiovascular diseases, and cancer (De Martel et al., 2012). Microbiota can form the TME for tumor initiation and development by regulating mucosal immunity and hormonal elements in humans (De Martel et al., 2012). Modulating host-microbiota interactions, especially in the gut, which hosts the most rich and diverse microbiota, has emerged as a state-of-the-art therapeutic approach for cancer treatment (Schwabe and Jobin, 2013). Previous studies have shown that the gut microbiota can regulate the sensitivity and responses of cancer patients to chemotherapy (Liu et al., 2022; Rahman et al., 2022). Furthermore, alterations in gut microbial structure have been reported to serve as potential indicators for early cancer diagnosis and other diseases (Sepich-Poore et al., 2021). Therefore, a comprehensive understanding of the interaction between the gut microbiota and the TME is beneficial for developing effective, safe, and patient-friendly treatments.

In this review, we (1) investigated the effects of microbiota and the TME on host immunity; (2) introduced their mutual effects on cancer prevention and therapy; and (3) discussed various methods for adjusting the TME to maximize the therapeutic effect of cancer, including fecal microbiota transplantation (FMT), dietary adjustments, and synthetic biology design. This study will provide a foundation for cancer-targeted therapies based on the gut microbiota and the TME in future applications and studies.

2 Role of the gut microbiota in cancer

2.1 Human microbiota

The human body hosts various microbes, with over 100 trillion symbiotic microorganisms (Sender et al., 2016). The human microbiota comprises complicated communities of bacteria, archaea, and viruses (Matson et al., 2021). The primary colonizers in these communities belong to six phyla: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, and Cyanobacteria (Ghosh and Pramanik, 2021). However, the relative abundance and load of these phyla, especially the bacterial composition at the genera level, differ significantly among the different communities (Cho and Blaser, 2012). Each anatomical niche, including the skin, gut, vagina, nose, mouth, and conjunctiva, possesses a distinct mixture of microbial populations. Among these, the human microbiota, especially the gut microbiota, has gained more attention due to its significant effect on human health and diseases (O'Hara and Shanahan, 2006). However, the relationship between microbiota and tumorigenesis is complicated as it is influenced by the microbial community and abiotic factors. Studies have reported that changes in the gut microbial community and its homeostasis can influence the development and progression of multiple cancers in humans. Chronic inflammation caused by the gut microbiota is a widely accepted mechanism that promotes tumor development. Furthermore, substances released by gut bacteria have been found to damage DNA, resulting in pathogenic mutations (Kumar et al., 2023). Notably, certain species of gut bacteria exhibited antitumor effects in some animal studies, particularly those involved in short-chain fatty acid (SCFA) synthesis (Yao et al., 2022). Moreover, gut bacteria can enhance the immune response to tumors by activating the immune system (Ge et al., 2021). By studying the tumor-associated gut microbiota, cancer prognosis can be predicted, and thus, stopping the generation of these associated microbes can halt cancer progression. Research on this microbiota would provide a novel and more patient-friendly strategy for cancer treatment.

2.2 Cancer microbiota

The International Association for Cancer Research has identified 11 microbes as human carcinogens or "oncomicrobes," including Human Papillomaviruses, Hepatitis B virus, Hepatitis C virus, Epstein–Barr virus, Human T-cell lymphotropic virus type I, Human immunodeficiency virus type 1, Human herpesvirus 8, Merkel cell polyomavirus, *Helicobacter pylori* (*H. pylori*), *Opisthorchis viverrini*, and *Schistosoma haematobium* (Plummer et al., 2016). Among these, *H. pylori*, regarding gut microbiota modulation, has received significant attention and has been well studied. *H. pylori* is known to cause chronic inflammation of the gastric mucosa, potentially leading to gastric and duodenal ulcers, and is confirmed to be related to mucosa-associated lymphoid tissue lymphoma (extranodal marginal zone B-cell lymphoma) in the stomach (Wang et al., 2014). Consequently, since gastric cancer caused by *H. pylori* infection depends significantly on the long-term inflammatory response of the host immune system, understanding the relationship between *H. pylori* and other gastric bacterial infections and host immune responses at the molecular level during gastric carcinogenesis is of great importance (Kim and Wang, 2021).

The molecular mechanisms underlying the epidemiology of oncomicrobes and their clinical scenarios have been well studied (Sepich-Poore et al., 2021). Although carcinogenic microbiota can colonize various parts of the human body, their detection in microbial-triggered cancer is challenging, mainly due to individual differences in genetic makeup (Ribet and Cossart, 2015). In addition, certain microbiota can cause cancer through genotoxin-mediated mutagenesis, such as colibactin (Wilson et al., 2019) and cytolethal distending toxins, indicating that not all microbiota are carcinogenic or can be conditionally carcinogenic, e.g., prolonged *H. pylori* infection can trigger gastric cancer (Parsonnet, 1993; Matysiak-Budnik and Mégraud, 2006).

Increasing evidence shows that a significant "complicit" microbiota can trigger carcinogenesis through interactions with other abiotic factors. This category encompasses multiple

immunomodulatory roles of microbiota and their bioactive metabolites involved in tumor growth, which might be related to the effect of the immune system on solid tumorigenesis (Bagheri et al., 2022). Tumors located on boundary surfaces-including the oropharynx, skin, and the respiratory, digestive, and genitourinary tracts-contain microbiota, which complicates cancer-microbe causality (Garrett, 2015). The gut microbiota establishes the core factors of the gut microenvironment under healthy and cancerous conditions. Simultaneously, different TMEs show diverse community structures of the central gut microbiota. The different gut microbiota compositions associated with various cancers are summarized in Table 1. Moreover, a decrease in the abundance of specific microorganisms may also increase the cancer risk of the host in areas far from the transfer of such microorganisms (Sears and Garrett, 2014). Therefore, understanding microbes throughout the body is essential for understanding the relationship between the gut microbiota and cancer.

Given the high individual heterogeneity of the gut microbiota due to variations in genetics, diet, and other factors, its performance varies across subtypes of certain cancers. The gut microbiota is highly related to chronic inflammation in multiple organs, which can promote the development and progression of tumorigenesis. However, it is unrelated to cancers resulting from genetic inheritance or mutations (Karki and Kanneganti, 2019). Previous research has found that *Enterobacteriaceae* exhibits high abundance across all subtypes of gastric tumors, whereas *Lachnoclostridium*, *Bifidobacterium*, *Parabacteroides*, and *Barnesiella* are found in patients with adenocarcinoma (Zhou et al., 2021). The gut microbial community and biodiversity significantly depend on the types/subtypes of tumors and different tumor stages (Chen et al., 2022). Although the role of the gut microbiota in subtypes of different cancers requires further study and clarification, its potential for tumor diagnosis and treatment has gained widespread recognition.

3 Relationship between the gut microbiota and the TME

3.1 The microbiome as an ingredient of the tumor microenvironment

The TME comprises malignant and nonmalignant cells and the contents of the tumor (Figure 1). The permanent mutual relationship between tumor cells and the TME significantly affects tumor initiation, progression, metastasis, and therapeutic responses (Xiao and Yu, 2021). Recently, the conventional drugs including aspirin, celecoxib, β-adrenergic antagonist, metformin, and statin with antitumor capability that show potential use in combination therapy by targeting TME components (Jin and Jin, 2020). Due to the different layers of microbial niches, the TME is a complex environment in which the microbiota has been recognized as a novel yet essential element (Turroni et al., 2008; Rowland et al., 2018). The microbiota functionally reduces tumor cell metabolism, such as inflammation, genotoxin generation, and production of bacterial metabolites with various characteristics (Kovács et al., 2020). Accumulating evidence has shown that the interactions between the microbiota and their metabolites in the TME can influence host immunity and the intestinal epithelium, ultimately driving or inhibiting tumor growth (Barry et al., 2018).

The model of the gut microbiota and the TME is complex, including biotic and abiotic drivers from cells, blood vessels, and the extracellular matrix that constitutes the tissues surrounding a tumor (Zhu et al., 2021). Studies have reported that gut bacteria can regulate the activation of human immune cells to migrate to the TME for tumor cell elimination (Buzas, 2023). In addition, the complex interaction between the gut microbiota and the TME can enable tumor cells to evade the immune system and proliferate more efficiently (Kalaora et al., 2022). Understanding

TABLE 1 Gut microbiota compositions are associated with cancers.

Type	Experimental Model	Bacterial species	Virulence factor	Mechanisms	Reference
Colorectal cancer	Mice	*Peptostreptococcus anaerobius* (Firmicutes)	N/A	Promotion of cell proliferation, induction of oxidative damage, TLR2/TLR4 interaction, SREBP2/AMPK activation	Tsoi et al. (2017)
	Mice	*Fusobacterium nucleatum* (Fusobacteria)	FadA	Cell proliferatio induction, modulation of Ecadherin/β-catenin signals, enhanced expression of NF-κB, cyclin D1	Rubinstein et al. (2013)
	Mice	Genotoxic *Escherichia coli*	Colibactin	Phosphorylated H2AX foci formation	Cuevas-Ramos et al. (2010)
	Stool sample	*Enterotoxigenic Bacteroides fragilis* (Bacteroides)	*B. fragilis* toxin, fragilysin	Elevated IL-1 levels, activation of STAT3/β-catenin, Ecadherin cleavage, induction of Th-17 response	Ulger Toprak et al. (2006)
Pancreatic cancer	Mouse and macrophages	*Porphyromonas gingivalis* (Bacteroidetes)	N/A	Apoptosis induction, interaction with TLR2/TLR4, activation of STAT3/NF-κB signaling pathways	Huck et al. (2017)
Liver cancer	Mouse	*Helicobacter hepaticus* (Proteobacteria)	Cytolethaldistending toxin	Promotion of endoreplication, enhanced p-21/Ki-67 expression	Péré-Védrenne et al. (2016)
Gastric cancer	Mouse and epithelial cells	*Helicobacter pylori* (Proteobacteria)	VacA	Autophagy induction, elevated MAPK/ERK1/2 expression, activation of Wnt/β-catenin signals	Meng et al. (2018)

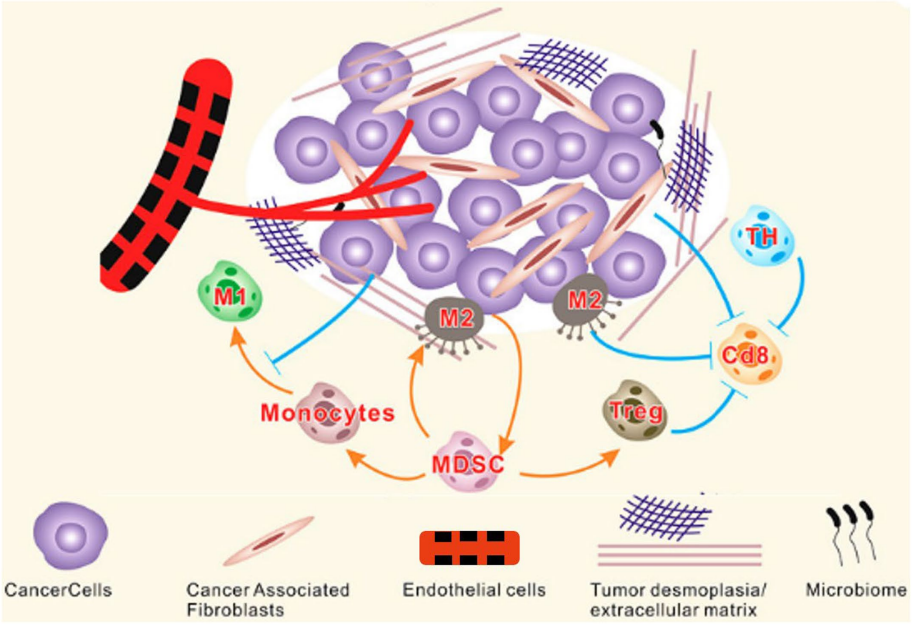

FIGURE 1
Components of TME. The TME is a complex network of stromal cells, microbiome and other cellular entities surrounding tumor cells. Tumor and stromal cells actively interact to support tumor growth by promoting desmoplasia, angiogenesis, and immune suppression. MDSC, Myeloid-derived suppressor cells; TH, T helper cells; M, Macrophages (Zubair et al., 2022).

this system holds promise for cancer prevention, diagnosis, and treatment.

3.2 Effects of the gut microbiota on the TME

The gut microbiota is crucial in the development, maintenance, and growth of the host immune system (Shi et al., 2017). The intestinal ecosystem can affect local and distant neoplasia by influencing the influx of myeloid, immune context, lymphoid cells, and inflammatory and metabolic patterns (Ma et al., 2019). Thus, the gut microbiota is emerging as a critical modulator of the TME in various cancers, such as colorectal, gastric, and liver cancers (Lakritz et al., 2014). For instance, a previous study reported that bacteria such as *Fusobacterium nucleatum* can enhance tumor growth by inhibiting human immune responses (Chattopadhyay et al., 2021). Moreover, breast and ovarian cancers are associated with specific biosignatures of the gut microbiota, such as the abundance of *Lactobacillus crispatus*, which negatively correlates with cancer occurrence (Banerjee et al., 2018).

Furthermore, the secretory components of the gut microbiota are reported to be associated with TME. For example, outer membrane vesicles (OMVs) can reprogram the TME toward a pro-TH1 pattern (CXCL10, IFN-g; Kim et al., 2017). Metabolites produced by the gut microbiota, including butyrate and niacin, can mediate Gpr109a-dependent interleukin (IL)-18 induction in the colonic epithelium, suppressing colitis and colon cancer. Additionally, the TME can regulate tumor development, metastatic progression, and the efficacy of therapeutic interventions (Figure 2; Singh et al., 2014). Studies have found that tumor cells can establish a bidirectional functional relationship with the surrounding stromal cells during malignant progression (Poutahidis et al., 2013). The synthesis and secretion of sonic hedgehog, which selectively reacts with stellate cells, are promoted by the activation of the CXCL12/CXCR4 pathway in pancreatic tumor cells, thereby driving desmoplasia (Singh et al., 2012). The desmoplastic TME affects pancreatic cancer pathobiology and chemoresistance (Özdemir et al., 2014). One study on lung cancer showed that cancer-associated fibroblast (CAF)-derived IL-6 induces epithelial-mesenchymal transition and confers resistance to cisplatin in non-small cell lung carcinoma (Yang et al., 2016). Moreover, CAFs secrete various proinflammatory molecules (IL-6, CCL2, and TGF-β), which enhance immunosuppressive cell recruitment (Dirkx et al., 2006).

High accumulation of tumor-associated macrophages (TAMs) and other immunosuppressive cells in the TME induces cancer progression and therapy resistance (Xiang et al., 2021; Yan and Wan, 2021). The depletion of CD163+ TAMs, which cause immune suppression, leads to robust infiltration of cytotoxic T cells into the TME, resulting in the control of melanoma development (Etzerodt et al., 2019). High $CD163^+$ TAMs in the TME have been linked to worse clinical outcomes in patients with various myelomas (Omatsu et al., 2014). Pancreatic tumors exhibit a growing infiltration of TAMs and a scarcity of cytotoxic T cells in their TME (Lankadasari et al., 2019). Additionally, the effects of TAMs on angiogenesis have been previously reported (Larionova et al., 2021). For example, TAM depletion can induce a significant decrease in vessel density (Yang et al., 2016). Thus, various factors, such as MMPs, ILs, VEGF, PDGF, and TGF-β secreted by TAMs in the TME, can promote vascularization in tumor tissues (Dirkx et al., 2006). MDSCs are used to heavily infiltrate the TME of glioblastoma, activating B-cell–induced immune suppression by inhibiting $CD8^+$ T-cell activation (Lee-Chang et al.,

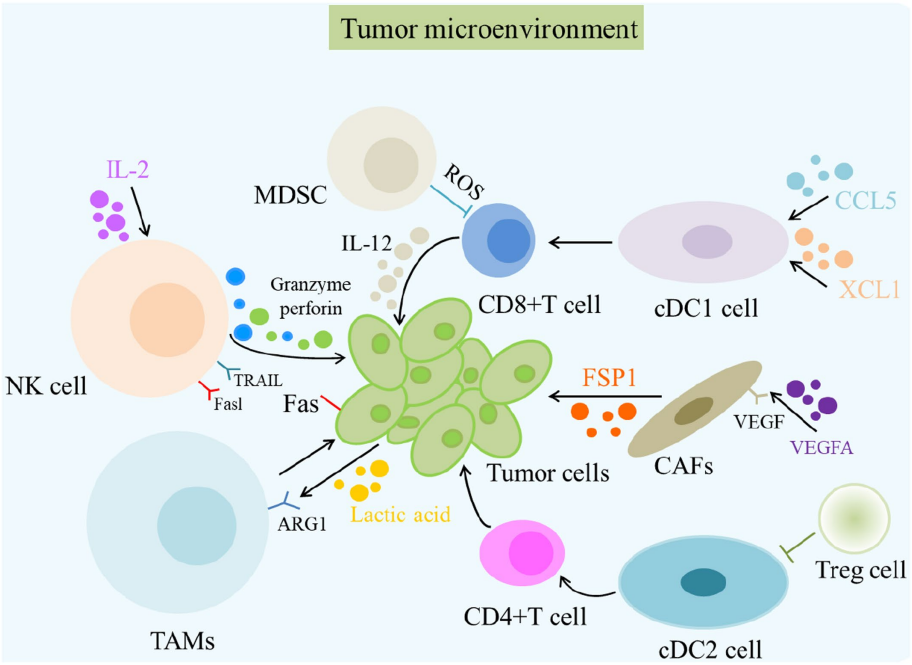

FIGURE 2
The role of TME in cancer and its immunotherapy. The primary cells of the TME in cancer immunity are NK cells, DC cells, CD8 + T cells, Treg cells, fibroblasts, TAMs, and MDSCs. Different cells induce the death of tumor cells in various ways, such as releasing perforin and granzyme and mediating cytotoxicity by TRAIL and Fasl receptors. MDSC, Myeloid-derived suppressor cells; ROS, Reactive oxygen species; NK cells, Natural killer cells.

2019). Furthermore, the high intratumoral burden of *F. nucleatum* correlates with a poor response to neoadjuvant chemotherapy in patients with esophageal squamous cell carcinoma (Yamamura et al., 2019). Therefore, the TME microbiota significantly influences cancer pathogenesis and therapeutic outcomes.

Noncellular components of the TME are also crucial for cancer progression, aggressiveness, and chemoresistance (Schulz et al., 2019). The stiffness of the extracellular matrix promotes tumor cell survival and proliferation while upregulating integrin signaling (Pickup et al., 2014). Hyaluronic acid, a CD44 receptor, is abundant in the TME of various cancers (Mattheolabakis et al., 2015). Their mutual effects activate cancer-promoting signaling pathways and induce the upregulation of noncoding RNA species, such as miR-10b/miR-302/miR-21 and lncRNAs. In pancreatic cancer, the stroma is highly reactive with different hyaluronic acids, resulting in elevated interstitial fluid pressures that lead to vascular collapse and poor chemotherapy outcomes (Provenzano et al., 2012). Targeting enzymes in pancreatic tumors with recombinant hyaluronidase has been shown to degrade hyaluronic acid and enhance therapy by reducing metastasis and improving survival (Kim et al., 2021). In addition, the secretory components of the gut microbiota are related to the TME. For instance, OMVs can reprogram the TME toward a pro-TH1 pattern (CXCL10, IFN-g; Kim et al., 2017), while metabolites, such as butyrate and niacin, can mediate the Gpr109a-dependent induction of IL-18 in the colonic epithelium, suppressing colitis and colon cancer.

Metabolites from the gut microbiota enter host cells and interact with the human immune response, promoting various tumor-inhibitory and immunomodulatory molecules. They also inhibit inflammation by maintaining the integrity of the epithelial barrier and the intestinal tract (Rooks and Garrett, 2016). A previous study found that the products of the metabolic activities of the gut microbiota significantly affect host metabolic pathways related to adiposity, lipids, and energy homeostasis (Poutahidis et al., 2013). Thus, uncovering how metabolites and submetabolites from the gut microbiota affect immune cells and reshape the TME can strongly contribute to the development of tumor therapeutics.

Gut microbiota metabolites, such as SCFAs and inosine, directly or indirectly interact with the TME to reshape it, thereby affecting the cancer process (Min et al., 2005). SCFAs contribute to maintaining intestinal homeostasis and regulating intestinal barrier function (Wang et al., 2017). Moreover, several fatty and cholic acids are associated with inflammation (Min et al., 2005). Butyrate and SCFAs, which can be generated by *Faecalibacterium prausnitzii*, control angiogenesis and reduce the expression of proangiogenic factors. Thus, increasing butyrate concentration is believed to slow down and halt cancer growth (Davie, 2003). Conversely, deoxycholic and petrocholic acids can cause DNA damage by increasing the generation of reactive oxygen species (Payne et al., 2007). Recent studies have shown that the intestinal bacteria *B. pseudolongum* can produce inosine, which drives Th1 cell differentiation in the presence of exogenous IFN-g (Kroemer and Zitvogel, 2020). Moreover, the status of *B. pseudolongum* has been reported to be associated with the response to ICB therapy, such as anti-CTLA-4 and anti-PD-L1, through its interaction with the adenosine A2A receptor on T cells (Mager and Burkhard, 2020). CTLA-4 and PD-L1 are the primary targets of immune checkpoint therapy, which involves membrane-bound molecules that impede unbounded T-cell responses after initial stimulation (Mager et al., 2020). Thus, cancer cells can avoid immune surveillance by employing this mechanism. However, while

reactivating inefficient T cells, immune checkpoint inhibitors (ICIs) can restore the response to tumor antigens (Sharma and Allison, 2015). Clinical research and preclinical trials have revealed that the gut microbiota affects the efficacy of ICIs, thereby explaining significant variations in patients' responses to ICIs (Vétizou et al., 2015). Hence, gaining an in-depth understanding of how the gut microbiota, their metabolites, and the host immune system interact to reshape and regulate the TME holds promise for advancing cancer immunotherapy.

Overall, the effects of the gut microbiota on the TME are complex and not yet fully understood. However, studies on this system have demonstrated its potential application in manipulating gut microbes to influence the effectiveness of cancer treatment and improve patient outcomes.

4 Gut microbiota modulation and their TME target

4.1 Cancer diagnostics based on microbiota

Cancer is typically diagnosed following the identification of a lump through palpation or imaging techniques, followed by a biopsy to confirm cellular malignancy (Fass, 2008). Tomographic detection techniques, including PET, MRI, and CT, efficiently identify macroscopic lesions in the body (Pokharel et al., 2013). Compared to stable genetic characteristics, the homeostasis of the human gut microbiota is more susceptible to tumorigenesis. Furthermore, studies have confirmed that the gut microbiota dynamics can potentially aid in diagnosing and locating malignancies, such as *Streptococcus gallolyticus* bacteremia, based on their gastrointestinal origin (Klein et al., 1977). Most microbial-based cancer diagnostics focus on sequencing tumors within the aerodigestive tract, including colorectal (Flemer et al., 2017), pancreatic (Farrell et al., 2012), and lung cancer (Yan et al., 2015). It has been suggested that different cancer types may host microbiota with unique compositions outside the aerodigestive tract, such as in the oral cavity. Nejman et al. investigated intratumoral microbiota from over 30 cancers, applying their blood-based diagnostics and providing visual evidence of microbial intratumoral spatial distributions and intracellular localization in seven different cancers (Nejman and Livyatan, 2020).

Currently, several bacteria-based strategies have been developed for tumor detection (Panteli et al., 2015), including the use of engineered bacteria that combine the specificity of tumor-targeting bacteria with the sensitivity of biomarker assays (Panteli et al., 2020). Attenuated bacteria were engineered to release an exogenous reporter protein, ZsGreen, using a remotely inducible genetic switch (Kaimala et al., 2018). Both *in vivo* and *in vitro* experiments showed that these bacteria could identify tumors through systemic measurements of the released ZsGreen (Panteli et al., 2020). Although bacteria-based cancer diagnosis is a promising strategy, it faces several challenges, such as low biomass relative to the host and confounding from reagents or environmental pollutants. Thus, combining gut microbiota-based methods with conventional diagnostic techniques, including genome sequencing, qPCR, immunohistochemistry, and electron microscopy, can offer more accurate and efficient cancer diagnoses.

Microbial-based cancer diagnostics have emerged as a new area that is focused on designing or developing novel strategies based on specific biosignatures of the gut microbiota in various cancers or at different tumor stages (Kim and Lee, 2021). Furthermore, deep learning and machine learning algorithms enable the identification of microbial profiles indicative of cancers, which is the basis of precision medicine. Microbial-based cancer diagnostics also hold the potential to improve cancer screening and early detection efforts, promising the development of more accurate and effective diagnostic tools for various cancers and ultimately improving patient outcomes.

4.2 Microbial-based cancer therapy

The human gut microbiota is recognized as a fundamental component of the immune system (Hooper and Macpherson, 2010; Maynard et al., 2012). Further studies have demonstrated that the gut microbiota can regulate immune responses, thus affecting the efficacy of cancer immunotherapy (Roy and Trinchieri, 2017). Several clinical trials have recently been conducted to alter the gut microbiota for cancer therapy (Table 2). Methods such as FMT, probiotics, dietary interventions (discussed in the subsequent section), and microbial engineering based on synthetic biology offer potential anticancer effects by targeting both tumor cells and the TME (Figure 3).

FMT is an artificial strategy for manipulating the gut microbiota and the TME (Zhang et al., 2020). Several clinical conditions, such as *clostridium difficile* infection, ulcerative colitis, and other gastrointestinal conditions, have been successfully treated by transferring fecal material from a donor to a recipient through colonoscopy, enema, or oral administration (Tan and Johnson, 2019). According to ongoing clinical trials, FMT from donors responsive to immunotherapy can enhance antitumor immune responses and potential clinical outcomes (Kang and Cai, 2021). Modifying the gut microbiota through FMT has been found to modulate the composition of the tumor microbiota, antitumor immune responses, and tumor growth kinetics (Matson et al., 2021). However, the long-term effectiveness and stability of FMT remain unclear (McQuade et al., 2019). Although some clinical trials have successfully incorporated the modulation of the gut microbiota using FMT into cancer therapy, the applications of FMT in cancer patients are limited due to antibiotic preconditioning, administration route, and modulation frequency, complicating its clinical use in targeting the gut microbiota (Cheng et al., 2020). Therefore, more specific clinical trials are needed for fecal transplants in cancer patients.

Probiotics are widely used to shift the microbial community (Zaramela et al., 2021). These interventions are being investigated for tumor therapy based on both retrospective studies and prospective clinical trials (Panebianco et al., 2020). Live bacteria are orally administered in probiotics, supplying substrates that stimulate the development or activity of beneficial bacteria in the gut and that further modulate the components of the overall gut microbiota (Markowiak and Śliżewska, 2017). Recent findings have confirmed that the gut microbiota can regulate immune responses, which could potentially affect the efficacy of cancer immunotherapy, indicating that probiotics can reduce the side effects of anticancer therapy (Lu et al., 2021). Several commercially available probiotics have been studied in preclinical models and clinical trials (Helmink et al., 2019). It has been reported that patient outcomes can be influenced by

TABLE 2 Selected clinical trials are modulating the gut microbiota in cancer therapy.

Type	Patient number	Objective	Intervention	Clinical outcomes	NCT number
Melanoma	20	To study concurrent use of FMT and pembrolizumab in patients with PD-1-resistant melanoma	FMT (donor responder to PD-1 therapy) with pembrolizumab	Overall response rate, change in T cell composition and function; change in innate and adaptive immune subsets	NCT03341143
Breast cancer	20	To assess the efficacy of Presurgical antibiotics to influence antitumor immune function	Primal Defense ULTRA Probiotic Formula	Mean number of cytotoxic $CD8^+$ T cells	NCT03358511
Colorectal cancer	35	To investigate the effect of probiotics on gut microbiota and the immune and inflammatory response	Probiotics	The colonic microbiota GI function	NCT00936572
Colon cancer	20	To reactivate the tumor-suppressor genes using probiotics	ProBion Clinica (*Bifidobacterium lactis*, *L. acidophilus*)	Changes in microbiota composition and DNA methylation	NCT03072641
Acute myeloid leukemia	20	To use FMT to prevent complications associated with dysbiosis in patients undergoing intensive treatment	Auto-FMT	Dysbiosis correction, eradication of multidrug resistant bacteria, definition of dysbiosis biosignature	NCT02928523
Hepatocellular carcinoma	64	To assess the role of probiotics in preventing septic and liver functional complications related to bacterial translocation following surgical resection of HCC	Probiotics-Lactibiane Tolerance (*Bifidobacterium lactis*, *L. acidophilus, L. plantarum, L.salivarius*)	Area under the plasma concentration versus time curve of endotoxin circulating levels	NCT02021253
Lung cancer	41	To assess the effects of chemotherapy on microbiome and probiotics on chemotoxicity	*Clostridium butyricum*	Composition of microbiome with probiotics, adverse effects of chemo, change in immunity and nutrition index	NCT02771470
Renal cell cancer	20	To prevent diarrhea in patients treated with sunitinib by probiotics	Micronutrient- fortified probiotic yogurt	Change in levels of *Bifidobacterium* spp. in stool samples	NCT02944617

compositional variations in the gut microbiota or the TME (Helmink et al., 2019).

Anaerobic bacteria play a crucial role in the gastrointestinal tract (Zaramela et al., 2021). A functional gut microbiota Bifidobacterium is commonly used to treat IBDs, including ulcerative colitis (Zhang et al., 2021). The TME creates a suitable growth environment for anaerobic bacteria under low-oxygen conditions (Leppäranta et al., 2008). The antitumor effects of anti-CD47 immunotherapy can be significantly improved by accumulating Bifidobacterium in the TME (Sivan et al., 2015). Current clinical trials primarily focus on the effectiveness of probiotic treatment for colorectal, kidney, breast, gynecologic, and lung cancers.

Microbial metabolites also contribute to regulating antitumor immunity (Sipe et al., 2020). SCFAs are crucial for maintaining gut integrity and serve as the primary energy source for intestinal epithelial cells (Parada Venegas et al., 2019). SCFAs, such as acetate, propionate, and butyrate, are absorbed through the intestinal epithelium and transmitted to T cells through G-coupled protein receptors to influence tumor differentiation (Moniri and Farah, 2021). In the colon, SCFAs protect gut integrity from invading foreign microorganisms by inducing Treg cells or IL-10 (Park et al., 2015). A direct interaction exists between SCFAs and CD8+ T cells in the circulation system, enhancing their antitumor effects (Bachem et al., 2019). Overall, SCFA-producing microbiota contribute to the response to ICIs (Huang et al., 2020). In addition, prebiotics and synbiotics (a combination of probiotics and prebiotics) are ideal for cancer prevention (Raman et al., 2013). Prebiotics are defined as fermentable, nondigestible food ingredients that can improve the health of the host (Legesse Bedada et al., 2020).

Dietary fibers resist digestion and absorption in the small intestine but undergo complete or partial fermentation in the large intestine (Buttriss and Stokes, 2008; Mudgil and Barak, 2013). Most fractions of edible plants or their extracts are carbohydrates and are regarded as prebiotics (Hijová et al., 2019). Fermentation of nondigestible compounds is key to proliferation and apoptosis modulation in tumor cells (Cruz-Bravo et al., 2014). Prebiotics protect cells against cancer through fecal bulking, colonic pH change, carcinogen binding to bacteria, xenobiotic-metabolizing enzymes, gene expression modulation in feces and cecum, and immune response modulation (Harris and Ferguson, 1993). Therefore, diet, lifestyle, and gut microbiota composition are related (Shamekhi et al., 2020). Regarding dysbiosis or bacterial imbalance in the intestine due to variations in diet or the environment, tumors can be induced by virulence factors, microbial metabolites, and inflammatory routes (Dos Reis et al., 2017).

4.3 Synthetic biology application on cancer diagnosis and therapy

Synthetic biology has enabled the modification of living cells through sophisticated decision-making processes to achieve

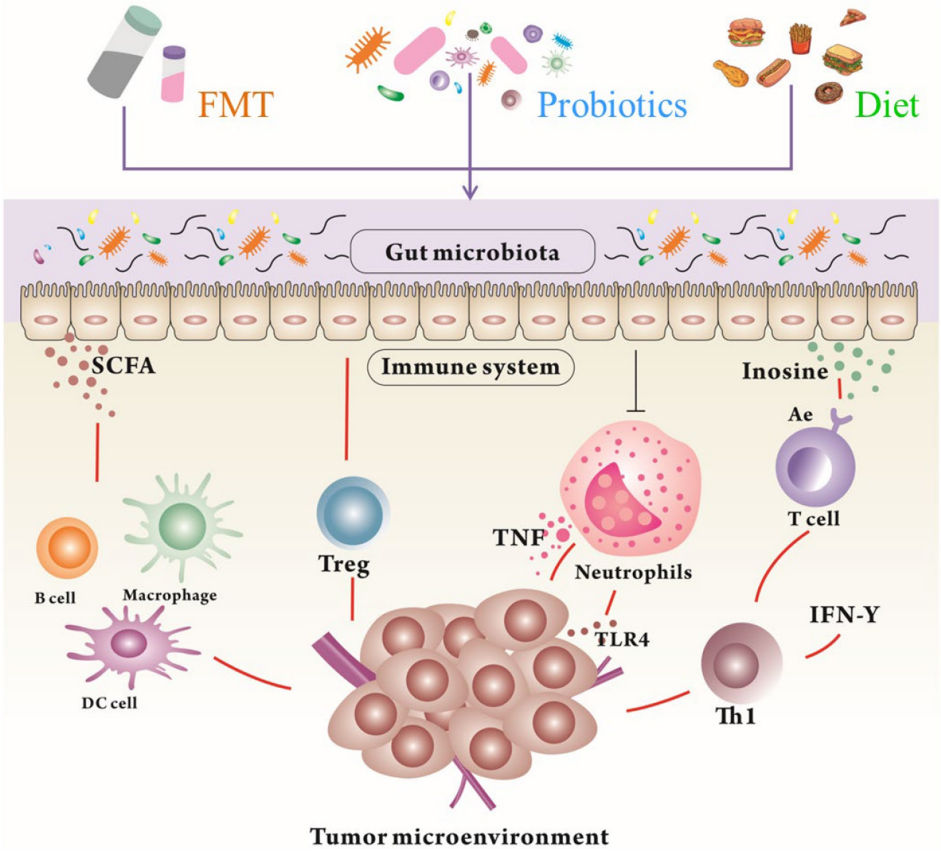

FIGURE 3
The modulation of the gut microbiota and their metabolites by FMT, probiotics, and diet to reshape TME for tumor therapy. Gut microbiota and their metabolites can promote the immunotherapy in humans through different mechanisms. FMT, Fecal Microbial Transplantation; SCFA, Short Chain Fatty Acids; TNF, Tumor necrosis factor.

user-defined outcomes, such as creating sense-and-respond adaptive therapies (Kitada et al., 2018). Some bacterial species selectively proliferate and accumulate at tumor sites, making them suitable candidates for tumor monitoring and targeted therapy (Figure 4; Kramer et al., 2018). The ability of bacteria to target tumors can be improved through synthetic biology, and therapeutic payloads can be delivered with increased precision. For instance, to decrease off-target effects in healthy tissues, bacteria have been engineered with quorum sensing switches that activate effector gene expression only when the bacterial population reaches a certain threshold density (Anderson et al., 2006). Alternatively, bacteria expressing therapeutic payloads can infiltrate tumor cells or utilize the type III secretory system (T3SS). This syringe-like, protein-based structure injects proteins into target cells (Huh et al., 2013). T3SS has been used to engineer *Salmonella* to deliver antiangiogenic proteins to tumor cells for controlling tumor growth *in vivo* or tumor-related antigens to antigen-presenting cells for triggering antitumor immunity (Shi et al., 2016). In addition, bacteria were programmed to degrade adenosine and kynurenine (West et al., 2018), which inhibit antitumor immunity (Siska and Rathmell, 2015) and produce cyclic-di-AMP (Leventhal et al., 2018), thus activating the stimulator of interferon genes pathway to enhance antitumor immunity (Chen et al., 2016).

Several studies have shown a significant decrease in tumor growth using anticancer-related bacteria in preclinical mouse models (Din et al., 2016). However, bacterial susceptibility was not eliminated by the host's immune system (Grushkin, 2012). Thus, the use of anticancer-related bacteria may involve sophisticated engineering to ensure their efficient action before they are released by the host's immune system.

5 Prospective and conclusion

The human gut microbiota plays a critical role in tumor growth, progression, and treatment. The interaction among the gut microbiota, host's immune system, and tumors can offer valuable insights into adjusting the gut microbiota to optimize the TME and enhance cancer immunotherapy.

The human microbiota significantly impacts the overall health of the human host and contributes to the development of various diseases. However, our current understanding of how human microbiota can confer susceptibility to certain cancers remains incomplete. A significant knowledge gap still exists regarding the underlying mechanisms governing bacterial activity as well as the compositions of microbiota due to limitations in culturing many

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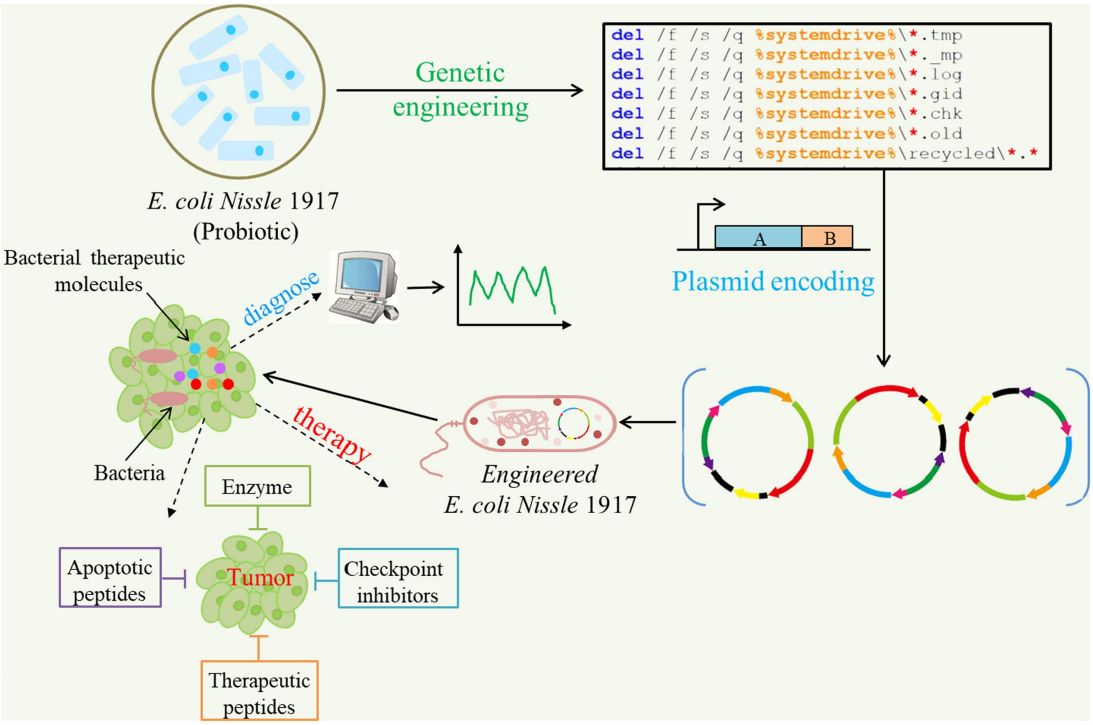

FIGURE 4
Applications of synthetic biology for cancer diagnosis and targeted therapeutics. Some probiotics like *E. coli Nissle* 1917 can be programmed to produce and deliver anti-cancer agents in solid tumors. Multiple drug payloads can be encoded by one or more engineered strains against tumors.

bacterial species, small clinical sample sizes, and a lack of risk assessment data.

Individual diversity in the gut microbiota is the primary challenge for large-scale validation and further intestinal microecology analysis. Therefore, integrating biological information, extensive data, and artificial intelligence into precision medicine would be aid in the future 10 development of novel drugs. However, these methods are not yet widely employed, and further in-depth investigations are necessary to ensure their adoption. In future, personalized medicine will likely incorporate microbiome-based diagnosis and treatment strategies. Despite the current challenges, a powerful new toolkit has been developed by enhancing our understanding of the roles of microbiota in cancer to improve patient care.

Author contributions

PF: Conceptualization, Writing – original draft. XX: Writing – review & editing. IB: Writing – review & editing. CQ: Data curation, Software, Writing – review & editing. YL: Data curation, Supervision, Writing – review & editing. PZ: Conceptualization, Writing – review & editing. YM: Writing – review & editing.

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Conflict of interest

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References

Anand, P., Kunnumakara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., Lai, O. S., et al. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res.* 25, 2097–2116. doi: 10.1007/s11095-008-9661-9

Anderson, J. C., Clarke, E. J., Arkin, A. P., and Voigt, C. A. (2006). Environmentally controlled invasion of cancer cells by engineered bacteria. *J. Mol. Biol.* 355, 619–627. doi: 10.1016/j.jmb.2005.10.076

Andreeva, N. V., Gabbasova, R. R., and Grivennikov, S. I. (2020). Microbiome in cancer progression and therapy. *Curr. Opin. Microbiol.* 56, 118–126. doi: 10.1016/j.mib.2020.09.001

Bachem, A., Makhlouf, C., Binger, K. J., de Souza, D. P., Tull, D., Hochheiser, K., et al. (2019). Microbiota-derived short-chain fatty acids promote the memory potential of antigen-activated CD8+ T cells. *Immunity* 51, 285–297. doi: 10.1016/j.immuni.2019.06.002

Bagheri, Z., Moeinzadeh, L., and Razmkhah, M. (2022). *Roles of microbiota in Cancer: From tumor development to treatment. J. Oncol.* 2022, 3845104.

Banerjee, S., Tian, T., Wei, Z., Shih, N., Feldman, M. D., Peck, K. N., et al. (2018). Distinct microbial signatures associated with different breast Cancer types. *Front. Microbiol.* 9:951. doi: 10.3389/fmicb.2018.00951

Barry, K. C., Hsu, J., Broz, M. L., Cueto, F. J., Binnewies, M., Combes, A. J., et al. (2018). A natural killer–dendritic cell axis defines checkpoint therapy–responsive tumor microenvironments. *Nat. Med.* 24, 1178–1191. doi: 10.1038/s41591-018-0085-8

Buttriss, J., and Stokes, C. (2008). Dietary fibre and health: an overview. *Nutr. Bull.* 33, 186–200. doi: 10.1111/j.1467-3010.2008.00705.x

Buzas, E. I. (2023). The roles of extracellular vesicles in the immune system. *Nat. Rev. Immunol.* 23, 236–250. doi: 10.1038/s41577-022-00763-8

Chattopadhyay, I., Dhar, R., Pethusamy, K., Seethy, A., Srivastava, T., Sah, R., et al. *Exploring the role of gut microbiome in Colon Cancer. 193* (2021) 1780–1799 London: BioMed Central.

Chen, Y., Huang, Y., Ding, X., Yang, Z., He, L., Ning, M., et al. (2022). A multi-omics study of familial lung Cancer: microbiome and host gene expression patterns. *Front. Immunol.* 13:827953. doi: 10.3389/fimmu.2022.1101130

Chen, Q., Sun, L., and Chen, Z. J. (2016). Regulation and function of the cGAS–STING pathway of cytosolic DNA sensing. *Nat. Immunol.* 17, 1142–1149. doi: 10.1038/ni.3558

Cheng, Y.-W., Alhaffar, D., Saha, S., Khanna, S., Bohm, M., Phelps, E., et al. (2020). Fecal microbiota transplantation is safe and effective in patients with Clostridioides difficile infection and cirrhosis. *Clin. Gastroenterol. Hepatol.* 18, 2234–2243.e1. doi: 10.1016/j.cgh.2019.12.029

Cho, I., and Blaser, M. J. (2012). The human microbiome: at the interface of health and disease. *Nat. Rev. Genet.* 13, 260–270. doi: 10.1038/nrg3182

Cruz-Bravo, R. K., Guevara-González, R. G., Ramos-Gómez, M., Oomah, B. D., Wiersma, P., Campos-Vega, R., et al. (2014). The fermented non-digestible fraction of common bean (*Phaseolus vulgaris* L.) triggers cell cycle arrest and apoptosis in human colon adenocarcinoma cells. *Genes Nutr.* 9:359. doi: 10.1007/s12263-013-0359-1

Cuevas-Ramos, G., Petit, C. R., Marcq, I., Boury, M., Oswald, E., and Nougayrède, J.-P. (2010). *Escherichia coli* induces DNA damage in vivo and triggers genomic instability in mammalian cells. *Proc. Natl. Acad. Sci.* 107, 11537–11542. doi: 10.1073/pnas.1001261107

Davie, J. R. (2003). Inhibition of histone deacetylase activity by butyrate. *J. Nutr.* 133, 2485S–2493S. doi: 10.1093/jn/133.7.2485S

De Martel, C., Ferlay, J., Franceschi, S., Vignat, J., Bray, F., Forman, D., et al. (2012). Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol.* 13, 607–615. doi: 10.1016/S1470-2045(12)70137-7

Din, M. O., Danino, T., Prindle, A., Skalak, M., Selimkhanov, J., Allen, K., et al. (2016). Synchronized cycles of bacterial lysis for in vivo delivery. *Nature* 536, 81–85. doi: 10.1038/nature18930

Dirkx, A. E., Egbrink, M. G. O., Wagstaff, J., and Griffioen, A. W. (2006). Monocyte/macrophage infiltration in tumors: modulators of angiogenesis. *J. Leukoc. Biol.* 80, 1183–1196. doi: 10.1189/jlb.0905495

Dos Reis, S. A., da Conceição, L. L., Siqueira, N. P., Rosa, D. D., da Silva, L. L., and do Carmo, G. P. M. (2017). Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. *Nutr. Res.* 37, 1–19. doi: 10.1016/j.nutres.2016.11.009

Etzerodt, A., TsaLkitzi, K., Maniecki, M., Damsky, W., Delfini, M., Baudoin, E., et al. (2019). Specific targeting of CD163(+) TAMs mobilizes inflammatory monocytes and promotes T cell-mediated tumor regression. *J. Exp. Med.* 216, 2394–2411. doi: 10.1084/jem.20182124

Farrell, J. J., Zhang, L., Zhou, H., Chia, D., Elashoff, D., Akin, D., et al. (2012). Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. *Gut* 61, 582–588. doi: 10.1136/gutjnl-2011-300784

Fass, L. (2008). Imaging and cancer: a review. *Mol. Oncol.* 2, 115–152. doi: 10.1016/j.molonc.2008.04.001

Flemer, B., Lynch, D. B., Brown, J. M., Jeffery, I. B., Ryan, F. J., Claesson, M. J., et al. (2017). Tumour-associated and non-tumour-associated microbiota in colorectal cancer. *Gut* 66, 633–643. doi: 10.1136/gutjnl-2015-309595

Garrett, W. S. (2015). Cancer and the microbiota. *Science* 348, 80–86. doi: 10.1126/science.aaa4972

Ge, Y., Wang, X., Guo, Y., Yan, J., Abuduwaili, A., Aximujiang, K., et al. (2021). Gut microbiota influence tumor development and Alter interactions with the human immune system. *J. Experimental & Clin. Cancer Res.: CR* 40:42. doi: 10.1186/s13046-021-01845-6

Ghosh, S., and Pramanik, S. (2021). Structural diversity, functional aspects and future therapeutic applications of human gut microbiome. *Arch. Microbiol.* 203, 5281–5308. doi: 10.1007/s00203-021-02516-y

Grushkin, D. (2012). The new drug circuit. *Nat. Med.* 18, 1452–1454. doi: 10.1038/nm1012-1452

Harris, P. J., and Ferguson, L. R. (1993). Dietary fibre: its composition and role in protection against colorectal cancer. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 290, 97–110. doi: 10.1016/0027-5107(93)90037-G

Helmink, B., Khan, M., Hermann, A., Gopalakrishnan, V., and Wargo, J. (2019). The microbiome, cancer, and cancer therapy. *Nat. Med.* 25, 377–388. doi: 10.1038/s41591-019-0377-7

Hijová, E., Bertková, I., and Štofilová, J. (2019). Dietary fibre as prebiotics in nutrition. *Cent. Eur. J. Public Health* 27, 251–255. doi: 10.21101/cejph.a5313

Hooper, L. V., and Macpherson, A. J. (2010). Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat. Rev. Immunol.* 10, 159–169. doi: 10.1038/nri2710

Huang, J., Jiang, Z., Wang, Y., Fan, X., Cai, J., Yao, X., et al. (2020). Modulation of gut microbiota to overcome resistance to immune checkpoint blockade in cancer immunotherapy. *Curr. Opin. Pharmacol.* 54, 1–10. doi: 10.1016/j.coph.2020.06.004

Huck, O., Al-Hashemi, J., Poidevin, L., Poch, O., Davideau, J.-L., Tenenbaum, H., et al. (2017). Identification and characterization of micro RNA differentially expressed in macrophages exposed to *Porphyromonas gingivalis* infection. *Infect. Immun.* 85, e00771–e00716. doi: 10.1128/IAI.00771-16

Huh, J. H., Kittleson, J. T., Arkin, A. P., and Anderson, J. C. (2013). Modular Design of a Synthetic Payload Delivery Device. *ACS Synth. Biol.* 2, 418–424. doi: 10.1021/sb300107h

Jin, M. Z., and Jin, W. L. (2020). The updated landscape of tumor microenvironment and drug repurposing. *Signal Transduc. Tar. Ther.* 5:166. doi: 10.1038/s41392-020-00280-x

Kaimala, S., Al-Sbiei, A., Cabral-Marques, O., Fernandez-Cabezudo, M. J., and Al-Ramadi, B. K. (2018). Attenuated Bacteria as immunotherapeutic tools for Cancer treatment. *Front. Oncol.* 8:136. doi: 10.3389/fonc.2018.00136

Kalaora, S., Nagler, A., and Wargo, J. A. (2022). Mechanisms of immune activation and regulation: Lessons from melanoma. *Nat. Rev. Cancer.* 22, 195–207.

Kang, Y. B., and Cai, Y. (2021). Faecal microbiota transplantation enhances efficacy of immune checkpoint inhibitors therapy against cancer. *World J. Gastroenterol.* 27, 5362–5375. doi: 10.3748/wjg.v27.i32.5362

Karki, R., and Kanneganti, T.D., *Diverging inflammasome signals in tumorigenesis and potential targeting. 19* (2019). 197–214 Washington, DC: American Society For Microbiology.

Kim, P. K., Halbrook, C. J., Kerk, S. A., Radyk, M., Wisner, S., Kremer, D. M., et al. (2021). Hyaluronic acid fuels pancreatic cancer cell growth. *elife* 10:645. doi: 10.7554/eLife.62645

Kim, J., and Lee, H. K. (2021). Potential role of the gut microbiome in colorectal Cancer progression. *Front. Immunol.* 12:807648. doi: 10.3389/fimmu.2021.807648

Kim, O. Y., Park, H. T., Dinh, N. T. H., Choi, S. J., Lee, J., Kim, J. H., et al. (2017). Bacterial outer membrane vesicles suppress tumor by interferon-γ-mediated antitumor response. *Nat. Commun.* 8:626. doi: 10.1038/s41467-017-00729-8

Kim, J., and Wang, T. C. (2021). Helicobacter pylori and gastric Cancer. *Gastrointest. Endosc. Clin. N. Am.* 31, 451–465. doi: 10.1016/j.giec.2021.03.003

Kise, K., Kinugasa-Katayama, Y., and Takakura, N. (2016). Tumor microenvironment for cancer stem cells. *Adv. Drug Deliv. Rev.* 99, 197–205. doi: 10.1016/j.addr.2015.08.005

Kitada, T., DiAndreth, B., Teague, B., and Weiss, R. (2018). Programming gene and engineered-cell therapies with synthetic biology. *Science* 359:eaad1067. doi: 10.1126/science.aad1067

Klein, R. S., Recco, R. A., Catalano, M. T., Edberg, S. C., Casey, J. I., and Steigbigel, N. H. (1977). Association of *Streptococcus bovis* with carcinoma of the Colon. *N. Engl. J. Med.* 297, 800–802. doi: 10.1056/NEJM197710132971503

Kovács, T., Mikó, E., Ujlaki, G., Sári, Z., and Bai, P. (2020). The microbiome as a component of the tumor microenvironment. *Tumor Microenviron.* 1225, 137–153. doi: 10.1007/978-3-030-35727-6_10

Kramer, M. G., Masner, M., Ferreira, F. A., and Hoffman, R. M. (2018). Bacterial therapy of Cancer: promises, limitations, and insights for future directions. *Front. Microbiol.* 9. doi: 10.3389/fmicb.2018.00016

Kroemer, G., and Zitvogel, L. (2020). Inosine: novel microbiota-derived immunostimulatory metabolite. *Cell Res.* 30, 942–943. doi: 10.1038/s41422-020-00417-1

Kumar, A., Ali, A., Kapardar, R. K., Dar, G. M., Nimisha, A., Sharma, A. K., et al. (2023). Implication of gut microbes and its metabolites in colorectal cancer. *J. Cancer Res. Clin. Oncol.* 149, 441–465. doi: 10.1007/s00432-022-04422-2

Lakritz, J. R., Poutahidis, T., Levkovich, T., Varian, B. J., Ibrahim, Y. M., Chatzigiagkos, A., et al. (2014). Beneficial bacteria stimulate host immune cells to counteract dietary and genetic predisposition to mammary cancer in mice. *Int. J. Cancer* 135, 529–540. doi: 10.1002/ijc.28702

Lankadasari, M. B., Mukhopadhyay, P., Mohammed, S., and Harikumar, K. B. (2019). TAMing pancreatic cancer: Combat with a double edged sword. *Mol. Cancer.* 18, 48.

Larionova, I., Kazakova, E., Gerashchenko, T., and Kzhyshkowska, J. (2021). New angiogenic regulators produced by TAMs: perspective for targeting tumor angiogenesis. *Cancer* 13:3253. doi: 10.3390/cancers13133253

Lee-Chang, C., Rashidi, A., Miska, J., Zhang, P., Pituch, K. C., Hou, D., et al. (2019). Myeloid-derived suppressive cells promote B cell–mediated immunosuppression via transfer of PD-L1 in GlioblastomaIdentification of regulatory B cells in GBM. *Cancer Immunol. Res.* 7, 1928–1943. doi: 10.1158/2326-6066.CIR-19-0240

Legesse Bedada, T., Feto, T. K., Awoke, K. S., Garedew, A. D., Yifat, F. T., and Birri, D. J. (2020). Probiotics for cancer alternative prevention and treatment. *Biomed. Pharmacother.* 129:110409. doi: 10.1016/j.biopha.2020.110409

Leppäranta, O., Vaahtio, M., Peltola, T., Zhang, D., Hupa, L., Hupa, M., et al. (2008). Antibacterial effect of bioactive glasses on clinically important anaerobic bacteria in vitro. *J. Mater. Sci. Mater. Med.* 19, 547–551. doi: 10.1007/s10856-007-3018-5

Leventhal, D., West, K., Fisher, A., Sokolovska, A., Kolodziej, S., Li, N., et al. (2018). Abstract LB-131: activation of innate and adaptive immunity via combinatorial immunotherapy using synthetic biotic™ medicines. *Cancer Res.* 78:LB-131. doi: 10.1158/1538-7445.AM2018-LB-131

Liu, L., Bai, Y., Xiang, L., Qi, W., Gao, L., Li, X., et al. (2022). Interaction between gut microbiota and tumour chemotherapy. *Clin. Transl. Oncol.* 24, 2330–2341. doi: 10.1007/s12094-022-02919-3

Lu, K., Dong, S., Wu, X., Jin, R., and Chen, H. (2021). Probiotics in Cancer. *Front. Oncol.* 11:148. doi: 10.3389/fonc.2021.638148

Ma, W., Mao, Q., Xia, W., Dong, G., Yu, C., and Jiang, F. (2019). Gut microbiota shapes the efficiency of Cancer therapy. *Front. Microbiol.* 10. doi: 10.3389/fmicb.2019.01050

Mager, L. F., and Burkhard, R. (2020). Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Sci*. 369, 1481–1489.

Mager, L. F., Burkhard, R., Pett, N., Cooke, N. C., Brown, K., Ramay, H., et al. (2020). Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science* 369, 1481–1489. doi: 10.1126/science.abc3421

Markowiak, P., and Śliżewska, K. (2017). Effects of probiotics, prebiotics, and Synbiotics on human health. *Nutrients* 9:1021. doi: 10.3390/nu9091021

Marsland, B. J., Trompette, A., and Gollwitzer, E. S. (2015). The gut-lung Axis in respiratory disease. *Ann. Am. Thorac. Soc.* 12, S150–S156. doi: 10.1513/AnnalsATS.201503-133AW

Matson, V., Chervin, C. S., and Gajewski, T. F. (2021). Cancer and the microbiome-influence of the commensal microbiota on Cancer, immune responses, and immunotherapy. *Gastroenterology* 160, 600–613. doi: 10.1053/j.gastro.2020.11.041

Mattheolabakis, G., Milane, L., Singh, A., and Amiji, M. M. (2015). Hyaluronic acid targeting of CD44 for cancer therapy: from receptor biology to nanomedicine. *J. Drug Target.* 23, 605–618. doi: 10.3109/1061186X.2015.1052072

Matysiak-Budnik, T., and Mégraud, F. (2006). *Helicobacter pylori* infection and gastric cancer. *Eur. J. Cancer* 42, 708–716. doi: 10.1016/j.ejca.2006.01.020

Maynard, C. L., Elson, C. O., Hatton, R. D., and Weaver, C. T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. *Nature* 489, 231–241. doi: 10.1038/nature11551

McQuade, J. L., Daniel, C. R., Helmink, B. A., and Wargo, J. A. (2019). Modulating the microbiome to improve therapeutic response in cancer. *Lancet Oncol.* 20, e77–e91. doi: 10.1016/S1470-2045(18)30952-5

Meng, C., Bai, C., Brown, T. D., Hood, L. E., and Tian, Q. (2018). Human gut microbiota and gastrointestinal cancer. *Genomics Proteomics Bioinfo.* 16, 33–49. doi: 10.1016/j.gpb.2017.06.002

Merlo, L. M. F., Pepper, J. W., Reid, B. J., and Maley, C. C. (2006). Cancer as an evolutionary and ecological process. *Nat. Rev. Cancer* 6, 924–935. doi: 10.1038/nrc2013

Min, H., Yao, J., Allinen, M., Cai, L., and Polyak, K. (2005). The role of the tumor microenvironment in breast cancer progression. *Breast Cancer Res.* 7:1. doi: 10.1186/bcr1060

Moniri, N. H., and Farah, Q. (2021). Short-chain free-fatty acid G protein-coupled receptors in colon cancer. *Biochem. Pharmacol.* 186:114483. doi: 10.1016/j.bcp.2021.114483

Mudgil, D., and Barak, S. (2013). Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: a review. *Int. J. Biol. Macromol.* 61, 1–6. doi: 10.1016/j.ijbiomac.2013.06.044

Nejman, D., and Livyatan, I. (2020). The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Sci*. 368, 973–980.

O'Hara, A. M., and Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO Rep.* 7, 688–693. doi: 10.1038/sj.embor.7400731

Omatsu, M., Kunimura, T., Mikogami, T., Shiokawa, A., Nagai, T., Masunaga, A., et al. (2014). Difference in distribution profiles between CD163+ tumor-associated macrophages and S100+ dendritic cells in thymic epithelial tumors. *Diagn. Pathol.* 9:215. doi: 10.1186/s13000-014-0215-7

Özdemir, B. C., Pentcheva-Hoang, T., Carstens, J. L., Zheng, X., Wu, C.-C., Simpson, T. R., et al. (2014). Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas Cancer with reduced survival. *Cancer Cell* 25, 719–734. doi: 10.1016/j.ccr.2014.04.005

Panebianco, C., Latiano, T., and Pazienza, V. (2020). Microbiota manipulation by probiotics administration as emerging tool in Cancer prevention and therapy. *Front. Oncol.* 10. doi: 10.3389/fonc.2020.00679

Panteli, J. T., Forkus, B. A., Van Dessel, N., and Forbes, N. S. (2015). Genetically modified bacteria as a tool to detect microscopic solid tumor masses with triggered release of a recombinant biomarker. *Integr. Biol.* 7, 423–434. doi: 10.1039/c5ib00047e

Panteli, J. T., Van Dessel, N., and Forbes, N. S. (2020). Detection of tumors with fluoromarker-releasing bacteria. *Int. J. Cancer* 146, 137–149. doi: 10.1002/ijc.32414

Parada Venegas, D., De la Fuente, M. K., Landskron, G., González, M. J., Quera, R., Dijkstra, G., et al. (2019). Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front. Immunol.* 10. doi: 10.3389/fimmu.2019.01486

Park, J., Kim, M., Kang, S. G., Jannasch, A. H., Cooper, B., Patterson, J., et al. (2015). Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR–S6K pathway. *Mucosal Immunol.* 8, 80–93. doi: 10.1038/mi.2014.44

Parsonnet, J. (1993). Helicobacter pylori and gastric cancer. *Gastroenterol. Clin. N. Am.* 22, 89–104. doi: 10.1016/S0889-8553(21)00265-X

Payne, C. M., Weber, C., Crowley-Skillicorn, C., Dvorak, K., Bernstein, H., Bernstein, C., et al. (2007). Deoxycholate induces mitochondrial oxidative stress and activates NF-κB through multiple mechanisms in HCT-116 colon epithelial cells. *Carcinogenesis* 28, 215–222. doi: 10.1093/carcin/bgl139

Péré-Védrenne, C., Cardinaud, B., Varon, C., Mocan, I., Buissonnière, A., Izotte, J., et al. (2016). The cytolethal distending toxin subunit CdtB of Helicobacter induces a Th17-related and antimicrobial signature in intestinal and hepatic cells in vitro. *J. Infect. Dis.* 213, 1979–1989. doi: 10.1093/infdis/jiw042

Pickup, M., Mouw, J., and Weaver, V. (2014). The extracellular matrix modulates the hallmarks of cancer. *EMBO Rep.* 15, 1243–1253. doi: 10.15252/embr.201439246

Plummer, M., de Martel, C., Vignat, J., Ferlay, J., Bray, F., and Franceschi, S. (2016). Global burden of cancers attributable to infections in 2012: a synthetic analysis. *Lancet Glob. Health* 4, e609–e616. doi: 10.1016/S2214-109X(16)30143-7

Pokharel, S. S., Macura, K. J., Kamel, I. R., and Zaheer, A. (2013). Current MR imaging lipid detection techniques for diagnosis of lesions in the abdomen and pelvis. *Radio Graphics* 33, 681–702. doi: 10.1148/rg.333125068

Poutahidis, T., Kearney, S. M., Levkovich, T., Qi, P., Varian, B. J., Lakritz, J. R., et al. (2013). Microbial symbionts accelerate wound healing via the neuropeptide hormone oxytocin. *PLoS One* 8:e78898. doi: 10.1371/journal.pone.0078898

Provenzano, P. P., Cuevas, C., Chang, A. E., Goel, V. K., Von Hoff, D. D., and Hingorani, S. R. (2012). Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* 21, 418–429. doi: 10.1016/j.ccr.2012.01.007

Rahman, M. M., Islam, M. R., Shohag, S., Ahasan, M. T., Sarkar, N., Khan, H., et al. (2022). Microbiome in cancer: role in carcinogenesis and impact in therapeutic strategies. *Biomed. Pharmacother.* 149:112898. doi: 10.1016/j.biopha.2022.112898

Raman, M., Ambalam, P., Kondepudi, K. K., Pithva, S., Kothari, C., Patel, A. T., et al. (2013). Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. *Gut Microbes* 4, 181–192. doi: 10.4161/gmic.23919

Ribet, D., and Cossart, P. (2015). How bacterial pathogens colonize their hosts and invade deeper tissues. *Microbes Infect.* 17, 173–183. doi: 10.1016/j.micinf.2015.01.004

Rooks, M. G., and Garrett, W. S. (2016). Gut microbiota, metabolites and host immunity. *Nat. Rev. Immunol.* 16, 341–352. doi: 10.1038/nri.2016.42

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., et al. (2018). Gut microbiota functions: metabolism of nutrients and other food components. *Eur. J. Nutr.* 57, 1–24. doi: 10.1007/s00394-017-1445-8

Roy, S., and Trinchieri, G. (2017). Microbiota: a key orchestrator of cancer therapy. *Nat. Rev. Cancer* 17, 271–285. doi: 10.1038/nrc.2017.13

Rubinstein, M. R., Wang, X., Liu, W., Hao, Y., Cai, G., and Han, Y. W. (2013). *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA Adhesin. *Cell Host Microbe* 14, 195–206. doi: 10.1016/j.chom.2013.07.012

Schulz, M., Salamero-Boix, A., Niesel, K., Alekseeva, T., and Sevenich, L. (2019). Microenvironmental regulation of tumor progression and therapeutic response in brain metastasis. *Front. Immunol.* 10:1713. doi: 10.3389/fimmu.2019.01713

Schwabe, R. F., and Jobin, C. (2013). The microbiome and cancer. *Nat. Rev. Cancer* 13, 800–812. doi: 10.1038/nrc3610

Sears, C. L., and Garrett, W. S. (2014). Microbes, microbiota, and colon cancer. *Cell Host Microbe* 15, 317–328. doi: 10.1016/j.chom.2014.02.007

Sender, R., Fuchs, S., and Milo, R. (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* 164, 337–340. doi: 10.1016/j.cell.2016.01.013

Sepich-Poore, G. D., Zitvogel, L., Straussman, R., Hasty, J., Wargo, J. A., and Knight, R. (2021). The microbiome and human cancer. *Science* 371:552. doi: 10.1126/science.abc4552

Shamekhi, S., Lotfi, H., Abdolalizadeh, J., Bonabi, E., and Zarghami, N. (2020). An overview of yeast probiotics as cancer biotherapeutics: Possible clinical application in colorectal cancer. *Clin. Transl. Oncol.* 22, 1227–1239.

Sharma, P., and Allison, J. P. (2015). The future of immune checkpoint therapy. *Science* 348:56. doi: 10.1126/science.aaa8172

Shi, N., Li, N., Duan, X., and Niu, H. (2017). Interaction between the gut microbiome and mucosal immune system. *Mil. Med. Res.* 4:14. doi: 10.1186/s40779-017-0122-9

Shi, L., Yu, B., Cai, C.-H., and Huang, J.-D. (2016). Angiogenic inhibitors delivered by the type III secretion system of tumor-targeting *Salmonella typhimurium* safely shrink tumors in mice. *AMB Express* 6, 1–10. doi: 10.1186/s13568-016-0226-8

Siegel, R. L., Miller, K. D., and Jemal, A. (2019). Cancer statistics. *CA Cancer J. Clin.* 69, 7–34. doi: 10.3322/caac.21551

Singh, A. P., Arora, S., Bhardwaj, A., Srivastava, S. K., Kadakia, M. P., Wang, B., et al. (2012). CXCL12/CXCR4 protein signaling Axis induces sonic hedgehog expression in pancreatic Cancer cells via extracellular regulated kinase-and Akt kinase-mediated activation of nuclear factor κB: IMPLICATIONS FOR BIDIRECTIONAL TUMOR-STROMAL INTERACTIONS*. *J. Biol. Chem.* 287, 39115–39124. doi: 10.1074/jbc.M112.409581

Singh, N., Gurav, A., Sivaprakasam, S., Brady, E., Padia, R., Shi, H., et al. (2014). Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* 40, 128–139. doi: 10.1016/j.immuni.2013.12.007

Sipe, L. M., Chaib, M., Pingili, A. K., Pierre, J. F., and Makowski, L. (2020). Microbiome, bile acids, and obesity: how microbially modified metabolites shape anti-tumor immunity. *Immunol. Rev.* 295, 220–239. doi: 10.1111/imr.12856

Siska, P. J., and Rathmell, J. C. (2015). T cell metabolic fitness in antitumor immunity. *Trends Immunol.* 36, 257–264. doi: 10.1016/j.it.2015.02.007

Sivan, A., Corrales, L., Hubert, N., Williams, J. B., Aquino-Michaels, K., Earley, Z. M., et al. (2015). Commensal Bifidobacterium promotes antitumor immunity and facilitates anti–PD-L1 efficacy. *Science* 350, 1084–1089. doi: 10.1126/science.aac4255

Tan, X., and Johnson, S. (2019). Fecal microbiota transplantation (FMT) for *C. difficile* infection, just say 'no'. *Anaerobe* 60:102092. doi: 10.1016/j.anaerobe.2019.102092

Tsoi, H., Chu, E. S. H., Zhang, X., Sheng, J., Nakatsu, G., Ng, S. C., et al. (2017). *Peptostreptococcus anaerobius* induces intracellular cholesterol biosynthesis in Colon cells to induce proliferation and causes dysplasia in mice. *Gastroenterology* 152, 1419–1433.e5. doi: 10.1053/j.gastro.2017.01.009

Turroni, F., Ribbera, A., Foroni, E., van Sinderen, D., and Ventura, M. (2008). Human gut microbiota and bifidobacteria: from composition to functionality. *Antonie Van Leeuwenhoek* 94, 35–50. doi: 10.1007/s10482-008-9232-4

Ulger Toprak, N., Yagci, A., Gulluoglu, B. M., Akin, M. L., Demirkalem, P., Celenk, T., et al. (2006). A possible role of *Bacteroides fragilis* enterotoxin in the aetiology of colorectal cancer. *Clin. Microbiol. Infect.* 12, 782–786. doi: doi: 10.1111/j.1469-0691.2006.01494.x

Vétizou, M., Pitt, J. M., Daillère, R., Lepage, P., Waldschmitt, N., Flament, C., et al. (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 350, 1079–1084. doi: 10.1126/science.aad1329

Wang, F., Meng, W., Wang, B., and Qiao, L. (2014). *Helicobacter pylori*-induced gastric inflammation and gastric cancer. *Cancer Lett.* 345, 196–202. doi: 10.1016/j.canlet.2013.08.016

Wang, Y., Mumm, J. B., and Herbst, R. (2017). IL-22 increases permeability of intestinal epithelial tight junctions by enhancing Claudin-2 expression, *J. Immunology*. 199, 3316–3325.

West, K. A., Fisher, A., Leventhal, D., Sokolovska, A., Li, N., Plescia, C., et al. (2018). Abstract 2920: metabolic modulation of the tumor microenvironment using synthetic biotic™ medicines. *Cancer Res.* 78:2920. doi: 10.1158/1538-7445.AM2018-2920

Whisner, C. M., and Athena Aktipis, C. (2019). The role of the microbiome in Cancer initiation and progression: how microbes and Cancer cells utilize excess energy and promote one Another's growth. *Current Nutrit. Reports* 8, 42–51. doi: 10.1007/s13668-019-0257-2

Wilson, M. R., Jiang, Y., Villalta, P. W., Stornetta, A., Boudreau, P. D., Carrá, A., et al. (2019). The human gut bacterial genotoxin colibactin alkylates DNA. *Science* 363:7785. doi: 10.1126/science.aar7785

Xiang, X., Wang, J., Lu, D., and Xu, X. (2021). Targeting tumor-associated macrophages to synergize tumor immunotherapy. *Signal Transduct. Target. Ther.* 6:75. doi: 10.1038/s41392-021-00484-9

Xiao, Y., and Yu, D. (2021). Tumor microenvironment as a therapeutic target in cancer. *Pharmacol. Ther.* 221:107753. doi: 10.1016/j.pharmthera.2020.107753

Yamamura, K., Izumi, D., Kandimalla, R., Sonohara, F., Baba, Y., Yoshida, N., et al. (2019). Intratumoral fusobacterium Nucleatum levels predict therapeutic response to neoadjuvant chemotherapy in esophageal squamous cell carcinoma. *Clin. Cancer Res.* 25:6170. doi: 10.1158/1078-0432.CCR-19-0318

Yan, S., and Wan, G. (2021). Tumor-associated macrophages in immunotherapy. *FEBS J.* 288, 6174–6186. doi: 10.1111/febs.15726

Yan, X., Yang, M., Liu, J., Gao, R., Hu, J., Li, J., et al. (2015). Discovery and validation of potential bacterial biomarkers for lung cancer. *Am. J. Cancer Res.* 5, 3111–3122.

Yang, X., Lin, Y., Shi, Y., Li, B., Liu, W., Yin, W., et al. (2016). FAP promotes immunosuppression by Cancer-associated fibroblasts in the tumor microenvironment via STAT3–CCL2 SignalingFAP via STAT3–CCL2 promote tumor immunosuppression. *Cancer Res.* 76, 4124–4135. doi: 10.1158/0008-5472.CAN-15-2973

Yang, M., Ma, B., Shao, H., Clark, A. M., and Wells, A. (2016). Macrophage phenotypic subtypes diametrically regulate epithelial-mesenchymal plasticity in breast cancer cells. *BMC Cancer* 16:419. doi: 10.1186/s12885-016-2411-1

Yao, Y., Cai, X., Fei, W., Ye, Y., Zhao, M., and Zheng, C. (2022). The role of short-chain fatty acids in immunity, inflammation and metabolism. *Crit. Rev. Food Sci. Nutr.* 62, 1–12. doi: 10.1080/10408398.2020.1854675

Zaramela, L. S., Moyne, O., Kumar, M., Zuniga, C., Tibocha-Bonilla, J. D., and Zengler, K. (2021). The sum is greater than the parts: exploiting microbial communities to achieve complex functions. *Curr. Opin. Biotechnol.* 67, 149–157. doi: 10.1016/j.copbio.2021.01.013

Zhang, P.-P., Li, L.-L., Han, X., Li, Q.-W., Zhang, X.-H., Liu, J. J., et al. (2020). Fecal microbiota transplantation improves metabolism and gut microbiome composition in db/db mice. *Acta Pharmacol. Sin.* 41, 678–685. doi: 10.1038/s41401-019-0330-9

Zhang, C., Zhao, Y., Jiang, J., Yu, L., Tian, F., Zhao, J., et al. (2021). Identification of the key characteristics of *Bifidobacterium longum* strains for the alleviation of ulcerative colitis. *Food Funct.* 12, 3476–3492. doi: 10.1039/D1FO00017A

Zhou, C. B., Zhou, Y. L., and Fang, J. Y. (2021). Gut microbiota in Cancer immune response and immunotherapy. *Trends Cancer* 7, 647–660. doi: 10.1016/j.trecan.2021.01.010

Zhu, R., Lang, T., Yan, W., Zhu, X., Huang, X., Yin, Q., et al. (2021). Gut microbiota: Influence on carcinogenesis and modulation strategies by drug delivery systems to improve Cancer therapy. *Adv. Sci.* 8, 2003542.

Zubair, H., Khan, M. A., Anand, S., Srivastava, S. K., Singh, S., and Singh, A. P. (2022). Modulation of the tumor microenvironment by natural agents: implications for cancer prevention and therapy. *Semin. Cancer Biol.* 80, 237–255. doi: 10.1016/j.semcancer.2020.05.009

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Association between the gut microbiota, inflammatory factors, and colorectal cancer: evidence from Mendelian randomization analysis

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Background: Colorectal cancer (CRC) is one of the most common malignant tumors primarily affecting individuals over the age of 50 years. Recent studies have suggested that the dysbiosis of the gut microbiota, a community of microorganisms in the human gut, is closely associated with the occurrence and development of CRC. Additionally, inflammatory factors (IFs) have also been reported to play a significant role in the development of CRC. However, the causal relationships between the gut microbiota, IFs, and CRC remain unclear.

Methods: In this study, we performed Mendelian randomization (MR) analysis using publicly available genome-wide association study (GWAS) data to explore the causal relationship between the gut microbiota, IFs, and CRC. The gut microbiota GWAS data were obtained from the MiBioGen study, while the IFs GWAS data were derived from the comprehensive analysis of three independent cohorts. Causal relationship analysis was conducted using appropriate instrumental variables (IVs) and statistical models.

Results: MR analysis of the gut microbiota and CRC revealed a negative correlation between the *Lachnospiraceae* species in the gut and CRC risk, while a positive correlation was observed between *Porphyromonadaceae* species, *Lachnospiraceae* UCG010 genus, *Lachnospira* genus, and *Sellimonas* genus in the gut, and CRC risk. Additionally, we observed a causal relationship between IL-10 and CRC risk. These findings suggest that the dysbiosis of the gut microbiota might be associated with an increased risk of CRC and that specific bacterial groups may play a crucial role in the occurrence and development of CRC.

Conclusion: Using MR analysis, this study revealed the causal relationships between the gut microbiota, IFs, and CRC. The negative correlation between the *Lachnospiraceae* species in the gut and CRC risk, as well as the causal relationship between IL-10 and CRC, provide important clues for the potential roles of gut microbiota regulation and inflammatory factor control in the prevention and treatment of CRC.

KEYWORDS

gut microbiota, inflammatory factors, colorectal cancer, Mendelian randomization, cancer prevention

1 Introduction

Colorectal cancer (CRC), a malignant tumor originating in the cells of the colon, is a common cancer typically occurring in individuals aged 50 years and above (Benson et al., 2018; Fabregas et al., 2022). Although the symptoms of CRC vary from person to person, some of the most common symptoms include abdominal pain and discomfort, changes in bowel habits (such as constipation, diarrhea, or increased frequency of bowel movements), presence of blood (either bright or dark red) in the stool, and intestinal obstruction (caused in the advanced stages of CRC when the tumor blocks the intestine, leading to severe abdominal pain, vomiting, and constipation) (Otani et al., 2019; Vogel et al., 2022). The risk factors for CRC include age (more common in individuals aged ≥50 years), genetic factors (individuals with a family history of CRC), gastrointestinal diseases (such as inflammatory bowel disease and familial adenomatous polyposis), high-fat, low-fiber diets, obesity, and diabetes (Giovannucci, 2002; Roslan et al., 2019).

The gut microbiota, which includes bacteria, archaea, viruses, fungi, protozoa, and parasites, plays a crucial role in the development of CRC. Recent research has shown a strong association between gut dysbiosis (imbalanced gut microbiota) and CRC (Garrett, 2019; Bai et al., 2022). Dysbiosis can lead to a reduction in the number of beneficial bacteria and an increase in the count of harmful bacteria, thereby disrupting the balance in the gut microbiota. This imbalance in the gut microbiota can lead to the production of harmful metabolites, such as carcinogens and inflammatory mediators, further promoting the development of CRC (Yang et al., 2022). Dysbiosis can also damage the intestinal mucosal barrier, allowing harmful substances and bacterial toxins to enter the intestinal tissue, thereby triggering an inflammatory response that promotes tumor formation and provides a favorable environment for tumor growth and metastasis (Wong and Yu, 2023).

Dysbiosis is also associated with changes in the tumor microenvironment of CRC (Zheng et al., 2020). Previous studies have suggested an association between specific groups of bacteria in the gut microbiome and CRC occurrence. For example, enrichment of the human gut with bacteria from the *Alistipes* genus has been associated with the development of CRC. These bacteria produce harmful metabolites (Louis et al., 2014), such as nitrosamines (Zhao et al., 2022), which promote the development of CRC (Parker et al., 2020). Therefore, the regulation of the gut microbiota serves as one of the potential strategies for the prevention and treatment of colon cancer (O'Keefe, 2016). Regulation of the composition and function of the gut microbiota can enhance the microbial balance in the gut by reducing the number of harmful bacteria and increasing the number of beneficial bacteria, thereby reducing the risk of CRC (Eslami et al., 2019). Some studies have shown that dietary changes, the use of probiotics and prebiotics, etc., regulate the gut microbiota and aid in the prevention and treatment of CRC (Tomasello et al., 2016; Pushpanathan et al., 2019).

Research has shown that the dysbiosis of the gut microbiota and the resulting inflammatory response play an important role in the occurrence and development of CRC (Fiorentini et al., 2020). Dysbiosis regulates the expression of the host genes associated with inflammation in the gut (Fidelle et al., 2020). Previous studies have shown that the dysbiosis of the gut microbiota can lead to the overexpression of inflammation-related genes, further exacerbating inflammatory responses and promoting the occurrence and development of colon cancer (Fidelle et al., 2020; Hou et al., 2022). Therefore, strategies aimed at regulating the gut microbiota may have the potential to modulate inflammatory responses. Mendelian randomization (MR), a relatively new technique that uses single nucleotide polymorphisms (SNPs) with an associated risk factor as instrumental variables (IVs), is used to determine if a causal relationship exists between a risk factor and a specific disease (Bowden and Holmes, 2019). Since the genetic variations detected in the zygote remain unchanged throughout life, these can be used in MR studies to avoid potential confounding variables or other sources of bias (Birney, 2022). In this study, we aimed to explore the causal relationship between the gut microbiota, inflammatory factors (IFs), and CRC, through the MR analysis of the summary-level data from publicly available genome-wide association studies (GWAS).

2 Materials and methods

2.1 Genome-wide association study data

Gut microbiota GWAS data were obtained from the MiBioGen study[1], which is the most extensive multi-racial study on the gut microbiota thus far. In this study, the fecal microbiota data ($n = 340$) and the 16S genotyping data from 16 cohorts ($n = 24{,}000$) were analyzed to identify the relationship between the gut microbiota and human health. The results showed significant variations in the human gut microbiota across regions, ethnicities, and age groups. The genetic predictors of 41 systemic inflammatory regulators were obtained from a comprehensive cytokine-related GWAS meta-analysis conducted on three independent cohorts. These cohorts included 8,293 Finnish participants from the Cardiovascular Risk in Young Finns Study (YFS) and the "FINRISK" studies (FINRISK1997 and FINRISK2002) (Wang et al., 2022). To normalize the distributions of the 41 cytokines, a two-step inverse transformation was applied.

In order to test the univariable associations between 10.7 million genetic polymorphisms and the concentrations of the 41 cytokines, an additive genetic model was employed. This model took into account adjustments for age, sex, body mass index (BMI), and the first 10 genetic principal components. Lastly, the outcome data were obtained from the Finngen database.

2.2 Selection of instrumental variables

Bacterial classification and analyses were performed at five major taxonomic levels (phylum, class, order, family, and genus). To ensure the accuracy and validity of the causal relationships between the gut microbiota and CRC risk, we added restrictions to the IV inclusion criterion as follows. First, only the SNPs with $p < 1e\text{-}05$ were included as IVs for exposure and outcome analysis in the MR studies. Second, the TwoSampleMR R package was used to assign $r^2 = 0.001$ and kb = 10,000 to ensure the independence of the selected IVs and to minimize the linkage disequilibrium effect that violates random allele assignment.

1 https://mibiogen.rug.nl/

2.3 Statistical analysis

Mendelian randomization (MR) is a method used to investigate causal relationships between a modifiable exposure and an outcome using genetic instruments. There are two key assumptions in MR: assumption 1 states that the genetic instruments are associated with the exposure of interest, and assumption 2 states that any association between the instruments and the outcome is mediated by the exposure (Smith and Ebrahim, 2003). To address these assumptions, five MR methods were used in the analysis. The ratio method involved obtaining individual SNP estimates by dividing the SNP's effect on schizophrenia by its corresponding effect on the biomarker. Standard errors were estimated assuming no measurement error. These estimates were then used for weighted analyses using other methods. Inverse variance weighting (IVW) is a commonly used method in MR (Burgess et al., 2013, 2017). It calculates the inverse variance weighted mean of ratio estimates from multiple instruments. This method assumes that all SNPs are valid instruments or that any bias is balanced across the instruments. Both fixed and random effects IVW methods were used. Weighted generalized linear regression is similar to the IVW method but allows for accounting for the correlation between genetic instruments. It was used when utilizing a conservative set of genetic instruments. The weighted median method calculates the median of the weighted empirical distribution function of individual SNP ratio estimates. This method provides a consistent effect estimate if more than 50% of the information comes from valid SNPs. Mendelian randomization Egger regression is a method that performs a weighted linear regression of SNP schizophrenia against SNP biomarker effect estimates (Bowden et al., 2015). It assumes that horizontal pleiotropic effects and SNP exposure associations are uncorrelated. The intercept of the MR Egger regression can be interpreted as a test for overall unbalanced horizontal pleiotropy. Both fixed and random effects versions of this method were performed. By employing these five MR methods, the researchers aimed to minimize bias and obtain reliable estimates of the causal relationship between the modifiable exposure and the outcome of interest. Different causality analysis models were used in this study. Among them, the inverse-variance weighted (IVW) model and MR-Egger method were used for the analysis of samples with multiple SNPs, while the Wald ratio test was used for the analysis of samples with only one SNP.

For sensitivity analyses, heterogeneity was measured using the Cochran Q method. In case of obvious heterogeneity ($p < 0.05$), MR-Egger regression analysis was used to assess the potential pleiotropic inheritance of the SNPs used as IVs. In MR-Egger regression, the intercept term indicates directed horizontal pleiotropy at $p < 0.05$. All statistical analyses in this study were performed using the R package in the R language application (v4.2.1).

3 Results

3.1 Mendelian randomization analysis of the gut microbiota and colorectal cancer

Our preliminary study revealed that 8 out of the 211 gut bacteria may have a causal relationship with CRC (Figure 1). The IVW analysis results for these 8 bacteria were as follows: family *Clostridiales vadin* BB60 group id.11286 ($p = 2.96E-02$; odds ratio (Fabregas et al., 2022) 95% confidence interval (Benson et al., 2018) = 0.75 (0.58, 0.97)), family *Porphyromonadaceae* id.943 ($p = 3.62E-03$; OR 95% CI = 2.03 (1.26, 3.28)), genus *Lachnospiraceae* UCG008 id.11328 ($p = 1.37E-02$; OR 95% CI = 0.74 (0.58, 0.94)), genus *Lachnospiraceae* UCG010 id.11330 ($p = 1.81E-02$; OR 95% CI = 1.61 (1.08, 2.38)), genus *Lachnospira* id.2004 ($p = 3.03E-02$; OR 95% CI = 4.43 (1.15, 17.02)), genus *Prevotella* 9 id.11183 ($p = 4.37E-02$; OR 95% CI = 0.78 (0.61, 0.99)), genus *Ruminococcaceae* UCG010 id.11367 ($p = 1.49E-02$; OR 95% CI = 0.59 (0.38, 0.90)), and genus *Sellimonas* id.14369 ($p = 1.68E-02$; OR 95% CI = 1.25 (1.04, 1.50)). Among them, family *Porphyromonadaceae* id.943, genus *Lachnospiraceae* UCG010 id.11330, genus *Lachnospira* id.2004, and genus *Sellimonas* id.14369 showed a positive correlation with CRC risk, while the other bacterial classes showed a negative correlation, indicating their protective effects. Detailed information on the MR analysis of the gut microbiota and CRC can be found in the Supplementary material S1.

3.2 Mendelian randomization analysis of inflammatory factors and colorectal cancer

This study revealed a causal relationship between one of the 41 inflammatory factors and CRC (Figure 2). The results obtained from the IVW analysis of interleukin-10 and CRC were as follows: ($p = 4.31E-04$; OR 95% CI = 1.49 (1.20, 1.87)). Detailed information on the MR analysis of the gut microbiota and CRC and the inflammatory factors and CRC can be found in the Supplementary material S1.

3.3 Mendelian randomization analysis of the gut microbiota and inflammatory factors

We conducted an MR analysis of the gut microbiota and inflammatory factors to further elucidate the role of inflammatory factors in the association between gut microbiota and CRC. IVW analysis results showed a causal relationship between genus *Lachnospiraceae* UCG010 id.11330 and IL-10 ($p = 2.63E-02$; OR 95% CI = 0.81 (0.67, 0.97)); no significant association was observed between any of the other bacterial taxa and inflammatory factors (Figure 3).

In sensitivity analysis, we conducted heterogeneity and pleiotropy analyses for the immune cells included in our study and their respective diseases. Our results all yielded p-values greater than 0.05, indicating the absence of heterogeneity and pleiotropy SNPs. Additionally, we performed leave-one-out analysis, which also demonstrated the stability of our results. The leave-one-out plot is Figure 4, while the heterogeneity results are presented in Table 1 and the pleiotropy analysis results in Table 2.

4 Discussion

In this study, we conducted a dual sample MR analysis to investigate the causal relationship between gut microbiota, inflammatory factors, and CRC. We found a potential causal relationship between the Lachnospiraceae UCG010 id.11330 bacterial genus and IL-10, CRC. The results showed that Lachnospiraceae

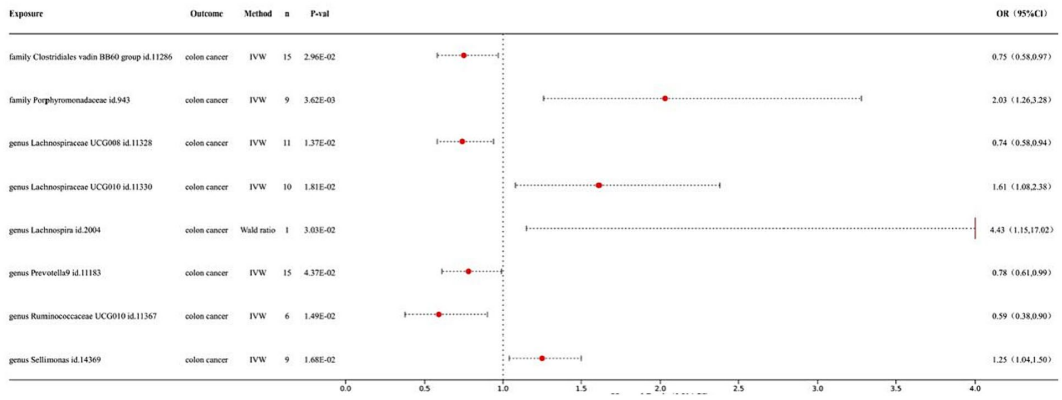

FIGURE 1
Forest map of MR results of gut microbiota and colon cancer.

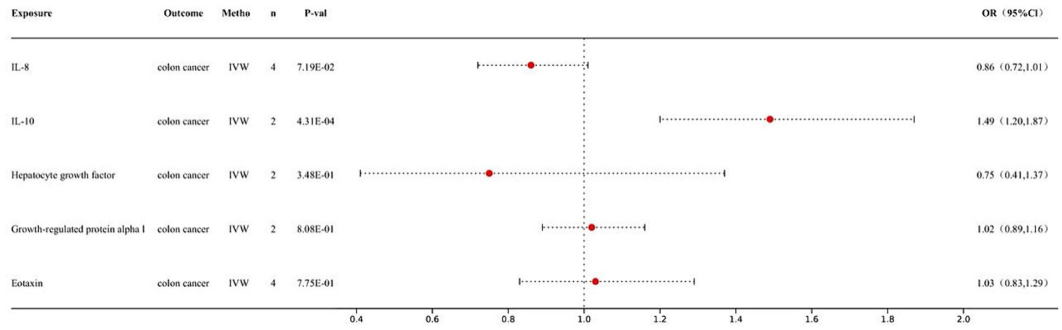

FIGURE 2
Forest map of MR results of inflammatory factors and colon cancer.

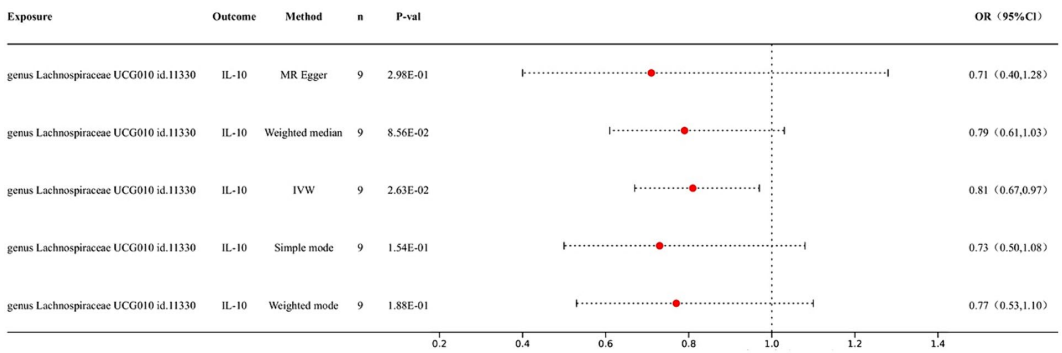

FIGURE 3
Forest map of MR results of gut microbiota and inflammatory factors.

UCG010 id.11330 increased the incidence of CRC, and IL-10 also increased the incidence of CRC. However, further investigation indicated a negative correlation between Lachnospiraceae UCG010 id.11330 and IL-10. Based on these findings, it is hypothesized that the increase in CRC caused by Lachnospiraceae UCG010 id.11330 is not mediated by IL-10. These two processes may be unrelated.

The association between the gut microbiota and CRC has been studied extensively and is supported by a substantial body of evidence. In this context, certain pathogenic bacteria can indirectly induce DNA damage in the host cells or interfere with important cell signaling pathways related to cell proliferation, apoptosis, and inflammation by producing enzymatically active protein toxins, thereby exerting a pro-tumorigenic effect (Chen and Li, 2020; Mirzaei et al., 2021). Bacteria are an important component of the gut microbiota, and several bacterial taxa harbor strains that produce protein toxins with potential pro-carcinogenic properties. Data on the consequences of long-term exposure to these gut bacteria and their toxins is gradually emerging, although research in this field is still relatively limited

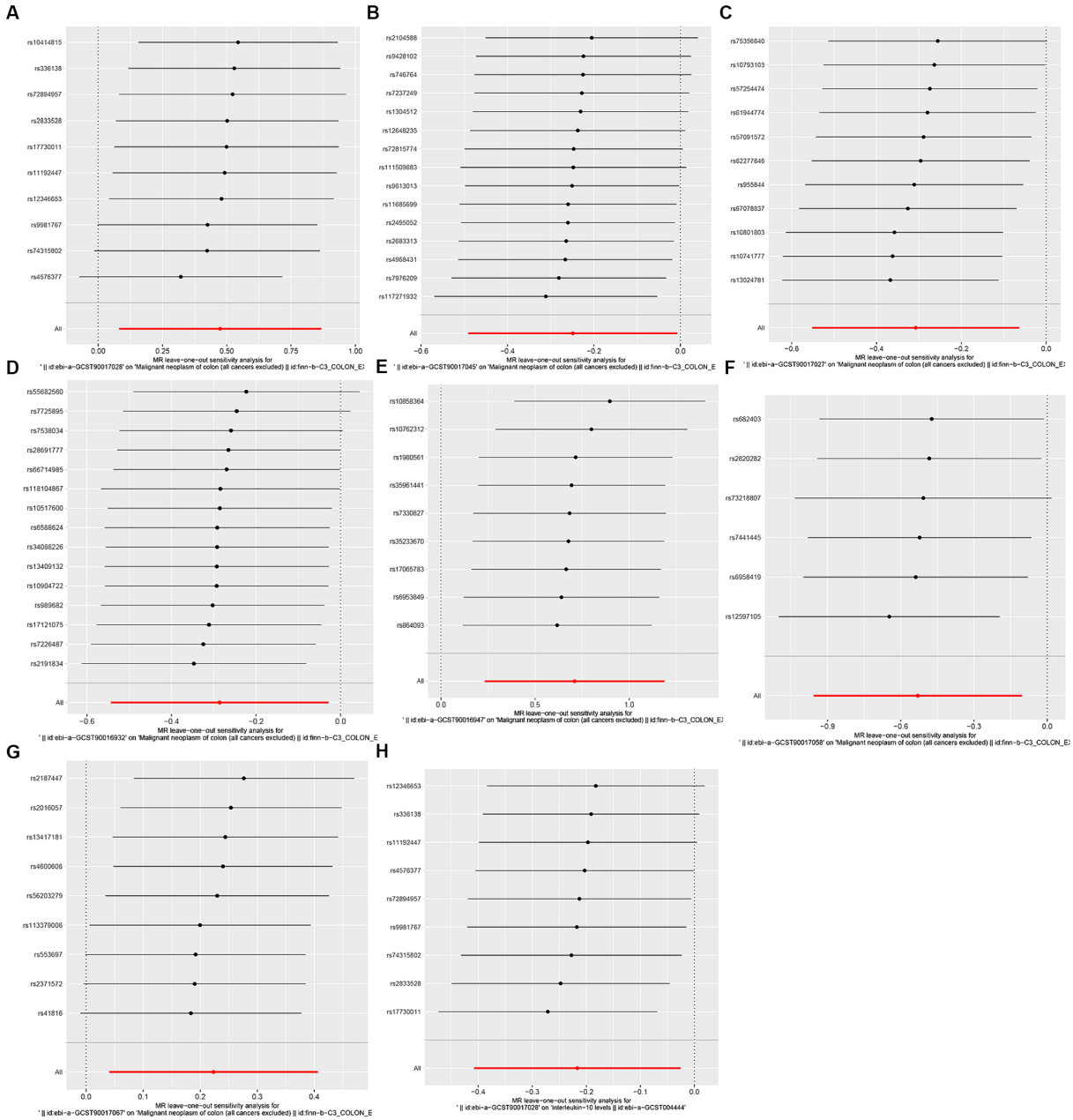

FIGURE 4
leave-one-out plot. **(A)** Leave-one-out plot of genus Lachnospiraceae UCG010 id.11330 and CRC, **(B)** Leave-one-out plot of genus Prevotella9 id.11183 and CRC; **(C)** Leave-one-out plot ofgenus Lachnospiraceae UCG008 id.11328 and CRC; **(D)** Leave-one-out plot of genusfamily Clostridiales vadin BB60 group id.11286 and CRC; **(E)** Leave-one-out plot of family Porphyromonadaceae id.943 and CRC; **(F)** Leave-one-out plot of genus Ruminococcaceae UCG010 id.11367 and CRC; **(G)** Leave-one-out plot of genus Sellimonas id.14369 and CRC; **(H)** Leave-one-out plot of genus Lachnospiraceae UCG010 id.11330 and IL-10.

(Illescas et al., 2021). Previous studies have demonstrated that Lachnospiraceae UCG010 id.11330 is a potential biomarker closely related to oxidative stress and metabolic genes (Qin et al., 2022). Oxidative stress plays an important role in the initiation and promotion stage of colon cancer, which may be the reason for the increased risk of CRC caused by Lachnospiraceae UCG010 id.11330 (Miyamoto et al., 2019).

Inflammation is a significant factor in the development of CRC. Chronic inflammation can lead to abnormal cell proliferation and mutations, increasing the risk of developing cancer. Inflammation can also alter the intestinal microenvironment, promoting tumor growth and metastases (Shawki et al., 2018; Dong et al., 2019). Conditions such as ulcerative colitis (UC) and Crohn's disease (CD) can cause chronic inflammation in the intestine, thereby increasing the risk of CRC. Patients with UC and CD have a higher incidence of CRC and require regular monitoring and screening. There is a complex interaction between inflammation and genetic factors (Goc et al., 2021). Inflammation can alter gene expression, leading to abnormal cell proliferation and mutations, and certain genetic mutations can increase the risk of developing CRC. The interaction between genetic factors and inflammation plays a crucial role in the development of CRC (Goc et al., 2021). There is a close relationship

TABLE 1 The heterogeneity test of gut microbiota, inflammatory factors, and colorectal cancer in this study.

id.exposure	Outcome	Method	Q	Q_df	Q_pval
family Clostridiales vadin BB60 group id.11286	CRC	MR Egger	8.31	13	0.82
family Clostridiales vadin BB60 group id.11286	CRC	IVW	10.41	14	0.73
family Porphyromonadaceae id.943	CRC	MR Egger	6.85	7	0.44
family Porphyromonadaceae id.943	CRC	IVW	7.17	8	0.52
genus Lachnospiraceae UCG008 id.11328	CRC	MR Egger	7.36	9	0.60
genus Lachnospiraceae UCG008 id.11328	CRC	IVW	9.20	10	0.51
genus Lachnospiraceae UCG010 id.11330	CRC	MR Egger	7.86	8	0.45
genus Lachnospiraceae UCG010 id.11330	CRC	IVW	9.85	9	0.36
genus Prevotella9 id.11183	CRC	MR Egger	9.08	13	0.77
genus Prevotella9 id.11183	CRC	IVW	9.49	14	0.80
genus Ruminococcaceae UCG010 id.11367	CRC	MR Egger	2.65	4	0.62
genus Ruminococcaceae UCG010 id.11367	CRC	IVW	2.65	5	0.75
genus Sellimonas id.14369	CRC	MR Egger	5.86	7	0.56
genus Sellimonas id.14369	CRC	IVW	7.26	8	0.51
genus Lachnospiraceae UCG010 id.11330	Interleukin-10	MR Egger	5.00	7	0.66
genus Lachnospiraceae UCG010 id.11330	Interleukin-10	IVW	5.18	8	0.74
Interleukin-10	CRC	IVW	0.00	1	0.97

TABLE 2 The pleiotropy test of gut microbiota, inflammatory factors, and colorectal cancer in this study could not be conducted for some immune cells due to insufficient SNPs being included.

id.exposure	id.outcome	egger_intercept	se	pval
family Clostridiales vadin BB60 group id.11286	CRC	0.05	0.03	0.17
family Porphyromonadaceae id.943	CRC	−0.04	0.06	0.59
genus Lachnospiraceae UCG008 id.11328	CRC	0.09	0.07	0.21
genus Lachnospiraceae UCG010 id.11330	CRC	0.06	0.04	0.20
genus Prevotella9 id.11183	CRC	−0.02	0.04	0.53
genus Ruminococcaceae UCG010 id.11367	CRC	0.00	0.04	0.97
genus Sellimonas id.14369	CRC	0.09	0.08	0.28
genus Lachnospiraceae UCG010 id.11330	Interleukin-10	0.01	0.02	0.69
Interleukin-10	CRC	NA	NA	NA

between inflammation and the immune system. Inflammation can activate the immune system, enhancing its ability to eliminate tumor cells. The expression and function of IL-10, an immune regulatory factor (Zegarra Ruiz et al., 2022) that has a significant impact on CRC development and treatment, have been studied extensively in CRC (Lian et al., 2019). Studies have shown that elevated levels of IL-10 in CRC tissues are closely associated with tumor staging, lymph node metastasis, and poor prognosis. Additionally, increased IL-10 expression is also associated with increased invasiveness and metastatic potential of the tumors (Lian et al., 2019). In CRC, IL-10 primarily affects tumor development by regulating immune and inflammatory responses. It inhibits the activation and functioning of the immune cells, thereby reducing tumor cell clearance by cytotoxic T cells and natural killer cells (Sethi et al., 2018). Furthermore, it suppresses inflammatory responses and cell apoptosis, thereby promoting tumor cell proliferation and survival. The application of IL-10 in CRC treatment is gaining great interest. Some studies have found that the inhibition of IL-10 expression or function enhances the killing effect exerted by the immune cells on the tumors, thereby improving treatment outcomes. Additionally, inhibiting IL-10 expression or function can also reduce tumor invasiveness and metastasis, thereby improving patient prognosis (Cai and Zhang, 2016; Rossowska et al., 2018; Huang et al., 2020).

The relationship between the gut microbiota and digestive tract cancer has been a topic of considerable interest. Increasing evidence suggests that the microbiota may play a significant role in the pathogenesis of digestive tract cancer, including influencing host immune responses, metabolite production, chronic inflammation, and intestinal mucosal barrier function (Zou et al., 2018; Fan et al., 2021; Lee et al., 2023). Factors such as inflammation and bacterial infection may cause a shift from the symbiotic state of the gut microbiota to a pro-carcinogenic configuration (Weinberg and Marshall, 2019). However, our study found a negative correlation between Lachnospiraceae UCG010 id.11330 and IL-10, suggesting that

Lachnospiraceae UCG010 id.11330 does not mediate colon cancer through IL-10. Recent literature has reported that the abundance of LachnospiraceaeUCG-009 is negatively associated with inflammatory factors such as interleukin-12P40, interferon, and DR5 with specific bacterial genera (Xu et al., 2022). In addition, recent literature has reported that Lachnospiraceae UCG-006 may modulate the immune system and gut microbiota through its anti-allergic and anti-inflammatory effects, which also supports the possible anti-inflammatory effects of Lachnospiraceae (Li et al., 2022).

In recent decades, researchers have actively explored the potential connection between the gut microbiota and digestive tract cancer, seeking to understand the role of the microbiota in the occurrence, development, and treatment of cancer. MR is a method used to assess the effects of therapeutic interventions and is commonly employed in clinical trials. The relationship between the gut microbiota and digestive may be utilized to evaluate the impact of specific microbial communities or microbial combinations on the development and treatment of cancer. Numerous similar studies have demonstrated the significant role of MR in research on the gut microbiota and digestive tract cancer (Ni et al., 2022; Li et al., 2023; Long et al., 2023; Xie et al., 2023).

Conclusively, this study has several advantages over other similar studies: The use of Mendelian randomization analysis in this study effectively controlled for confounding factors, while leveraging a large-scale GWAS dataset enhanced the statistical power and generalizability of the findings. The exploration of the relationship between gut microbiota, inflammatory factors, and colorectal cancer not only sheds light on potential prevention and treatment strategies but also contributes to a deeper understanding of the underlying mechanisms. Furthermore, the identification of specific bacterial groups associated with colorectal cancer risk provides promising targets for future interventions and therapeutic approaches aimed at modulating the gut microbiota to mitigate CRC risk. However, it also has some limitations. Firstly, the results of this study can be applied only to specific populations and samples because the participants were predominantly of European descent. Additionally, potential variations in population characteristics and data collection methods exist. Despite efforts to gather data, the lack of comprehensive data hinders further statistical analysis to adjust for potential confounding factors, which is also a common challenge in Mendelian randomization studies. Secondly, gut microbes are diverse and complex, and their potential confounding factors may have some influence on causality. In the future, we will further design prospective controlled experiments to investigate the mechanism of action between gut microbiota and CRC.

5 Conclusion

There is a causal relationship between the gut microbiota, IL-10 and CRC. Regulation of the gut microbiota and anti-inflammatory ability may serve as a potential strategy for the prevention and treatment of CRC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

MM: Conceptualization, Methodology, Writing – original draft. ZZ: Conceptualization, Methodology, Writing – review & editing. JL: Formal analysis, Methodology, Resources, Writing – review & editing. YH: Data curation, Methodology, Writing – original draft. WK: Funding acquisition, Writing – review & editing. XY: Funding acquisition, Investigation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1309111/full#supplementary-material

References

Bai, X., Wei, H., Liu, W., Coker, O. O., Gou, H., Liu, C., et al. (2022). Cigarette smoke promotes colorectal cancer through modulation of gut microbiota and related metabolites. *Gut* 71, 2439–2450. doi: 10.1136/gutjnl-2021-325021

Benson, A. B., Venook, A. P., Al-Hawary, M. M., Cederquist, L., Chen, Y. J., Ciombor, K. K., et al. (2018). NCCN guidelines insights: Colon Cancer, version 2.2018. *J. Natl. Compr. Cancer Netw.* 16, 359–369. doi: 10.6004/jnccn.2018.0021

Birney, E. (2022). Mendelian Randomization. *Cold Spring Harb. Perspect. Med.* 12:a041302. doi: 10.1101/cshperspect.a041302

Bowden, J., Davey Smith, G., and Burgess, S. (2015). Mendelian randomization with invalid instruments: effect estimation and bias detection through egger regression. *Int. J. Epidemiol.* 44, 512–525. doi: 10.1093/ije/dyv080

Bowden, J., and Holmes, M. V. (2019). Meta-analysis and Mendelian randomization: a review. *Res. Synth. Methods* 10, 486–496. doi: 10.1002/jrsm.1346

Burgess, S., Bowden, J., Fall, T., Ingelsson, E., and Thompson, S. G. (2017). Sensitivity analyses for robust causal inference from Mendelian randomization analyses with multiple genetic variants. *Epidemiology* 28, 30–42. doi: 10.1097/EDE.0000000000000559

Burgess, S., Butterworth, A., and Thompson, S. G. (2013). Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet. Epidemiol.* 37, 658–665. doi: 10.1002/gepi.21758

Cai, J., and Zhang, Z. (2016). An analysis of IL-10/IL-10R genetic factors related to risk of Colon Cancer and inflammatory bowel disease in a Han Chinese population. *Clin. Lab.* 62, 1147–1154. doi: 10.7754/Clin.Lab.2015.151120

Chen, C., and Li, H. (2020). The inhibitory effect of gut microbiota and its metabolites on colorectal Cancer. *J. Microbiol. Biotechnol.* 30, 1607–1613. doi: 10.4014/jmb.2002.02032

Dong, J., Liang, W., Wang, T., Sui, J., Wang, J., Deng, Z., et al. (2019). Saponins regulate intestinal inflammation in colon cancer and IBD. *Pharmacol. Res.* 144, 66–72. doi: 10.1016/j.phrs.2019.04.010

Eslami, M., Yousefi, B., Kokhaei, P., Hemati, M., Nejad, Z. R., Arabkari, V., et al. (2019). Importance of probiotics in the prevention and treatment of colorectal cancer. *J. Cell. Physiol.* 234, 17127–17143. doi: 10.1002/jcp.28473

Fabregas, J. C., Ramnaraign, B., and George, T. J. (2022). Clinical updates for Colon Cancer care in 2022. *Clin. Colorectal Cancer* 21, 198–203. doi: 10.1016/j.clcc.2022.05.006

Fan, X., Jin, Y., Chen, G., Ma, X., and Zhang, L. (2021). Gut microbiota Dysbiosis drives the development of colorectal Cancer. *Digestion* 102, 508–515. doi: 10.1159/000508328

Fidelle, M., Yonekura, S., Picard, M., Cogdill, A., Hollebecque, A., Roberti, M. P., et al. (2020). Resolving the paradox of Colon Cancer through the integration of genetics, immunology, and the microbiota. *Front. Immunol.* 11:600886. doi: 10.3389/fimmu.2020.600886

Fiorentini, C., Carlini, F., Germinario, E. A. P., Maroccia, Z., Travaglione, S., and Fabbri, A. (2020). Gut microbiota and Colon Cancer: a role for bacterial protein toxins? *Int. J. Mol. Sci.* 21:6201. doi: 10.3390/ijms21176201

Garrett, W. S. (2019). The gut microbiota and colon cancer. *Science* 364, 1133–1135. doi: 10.1126/science.aaw2367

Giovannucci, E. (2002). Modifiable risk factors for colon cancer. *Gastroenterol. Clin. N. Am.* 31, 925–943. doi: 10.1016/S0889-8553(02)00057-2

Goc, J., Lv, M., Bessman, N. J., Flamar, A. L., Sahota, S., Suzuki, H., et al. (2021). Dysregulation of ILC3s unleashes progression and immunotherapy resistance in colon cancer. *Cell* 184, 5015–5030.e16. doi: 10.1016/j.cell.2021.07.029

Hou, W., Yi, C., and Zhu, H. (2022). Predictive biomarkers of colon cancer immunotherapy: present and future. *Front. Immunol.* 13:1032314. doi: 10.3389/fimmu.2022.1032314

Huang, X. M., Zhang, N. R., Lin, X. T., Zhu, C. Y., Zou, Y. F., Wu, X. J., et al. (2020). Antitumor immunity of low-dose cyclophosphamide: changes in T cells and cytokines TGF-beta and IL-10 in mice with colon-cancer liver metastasis. *Gastroenterol Rep (Oxf)* 8, 56–65. doi: 10.1093/gastro/goz060

Illescas, O., Rodriguez-Sosa, M., and Gariboldi, M. (2021). Mediterranean diet to prevent the development of Colon diseases: a meta-analysis of gut microbiota studies. *Nutrients* 13:2234. doi: 10.3390/nu13072234

Lee, S. Y., Park, H. M., Kim, C. H., and Kim, H. R. (2023). Dysbiosis of gut microbiota during fecal stream diversion in patients with colorectal cancer. *Gut Pathog* 15:40. doi: 10.1186/s13099-023-00566-9

Li, X. J., Gao, M. G., Chen, X. X., Rong, Y. M., Huang, L. L., and Huang, J. S. (2023). Genetically predicted causal effects of gut microbiota and gut metabolites on digestive tract Cancer: a two-sample Mendelian randomization analysis. *World. J. Oncol.* 14, 558–569. doi: 10.14740/wjon1737

Li, R., Huang, X., Yang, L., Liang, X., Huang, W., Lai, K. P., et al. (2022). Integrated analysis reveals the targets and mechanisms in immunosuppressive effect of Mesalazine on ulcerative colitis. *Front. Nutr.* 9:867692. doi: 10.3389/fnut.2022.867692

Lian, G., Chen, S., Ouyang, M., Li, F., Chen, L., and Yang, J. (2019). Colon cancer cell secretes EGF to promote M2 polarization of TAM through EGFR/PI3K/AKT/mTOR pathway. *Technol. Cancer Res. Treat.* 18:1533033819849068. doi: 10.1177/1533033819849068

Long, Y., Tang, L., Zhou, Y., Zhao, S., and Zhu, H. (2023). Causal relationship between gut microbiota and cancers: a two-sample Mendelian randomisation study. *BMC Med.* 21:66. doi: 10.1186/s12916-023-02761-6

Louis, P., Hold, G. L., and Flint, H. J. (2014). The gut microbiota, bacterial metabolites and colorectal cancer. *Nat. Rev. Microbiol.* 12, 661–672. doi: 10.1038/nrmicro3344

Mirzaei, R., Afaghi, A., Babakhani, S., Sohrabi, M. R., Hosseini-Fard, S. R., Babolhavaeji, K., et al. (2021). Role of microbiota-derived short-chain fatty acids in cancer development and prevention. *Biomed. Pharmacother.* 139:111619. doi: 10.1016/j.biopha.2021.111619

Miyamoto, S., Narita, T., Komiya, M., Fujii, G., Hamoya, T., Nakanishi, R., et al. (2019). Novel screening system revealed that intracellular cholesterol trafficking can be a good target for colon cancer prevention. *Sci. Rep.* 9:6192. doi: 10.1038/s41598-019-42363-y

Ni, J. J., Li, X. S., Zhang, H., Xu, Q., Wei, X. T., Feng, G. J., et al. (2022). Mendelian randomization study of causal link from gut microbiota to colorectal cancer. *BMC Cancer* 22:1371. doi: 10.1186/s12885-022-10483-w

O'Keefe, S. J. (2016). Diet, microorganisms and their metabolites, and colon cancer. *Nat. Rev. Gastroenterol. Hepatol.* 13, 691–706. doi: 10.1038/nrgastro.2016.165

Otani, K., Kawai, K., Hata, K., Tanaka, T., Nishikawa, T., Sasaki, K., et al. (2019). Colon cancer with perforation. *Surg. Today* 49, 15–20. doi: 10.1007/s00595-018-1661-8

Parker, B. J., Wearsch, P. A., Veloo, A. C. M., and Rodriguez-Palacios, A. (2020). The genus Alistipes: gut Bacteria with emerging implications to inflammation, Cancer, and mental health. *Front. Immunol.* 11:906. doi: 10.3389/fimmu.2020.00906

Pushpanathan, P., Mathew, G. S., Selvarajan, S., Seshadri, K. G., and Srikanth, P. (2019). Gut microbiota and its mysteries. *Indian J. Med. Microbiol.* 37, 268–277. doi: 10.4103/ijmm.IJMM_19_373

Qin, S., He, Z., Wu, Y., Zeng, C., Zheng, Z., Zhang, H., et al. (2022). Instant dark tea alleviates Hyperlipidaemia in high-fat diet-fed rat: from molecular evidence to redox balance and beyond. *Front. Nutr.* 9:819980. doi: 10.3389/fnut.2022.819980

Roslan, N. H., Makpol, S., and Mohd Yusof, Y. A. (2019). A review on dietary intervention in obesity associated Colon Cancer. *Asian Pac. J. Cancer Prev.* 20, 1309–1319. doi: 10.31557/APJCP.2019.20.5.1309

Rossowska, J., Anger, N., Szczygiel, A., Mierzejewska, J., and Pajtasz-Piasecka, E. (2018). Reprogramming the murine colon cancer microenvironment using lentivectors encoding shRNA against IL-10 as a component of a potent DC-based chemoimmunotherapy. *J. Exp. Clin. Cancer Res.* 37:126. doi: 10.1186/s13046-018-0799-y

Sethi, V., Kurtom, S., Tarique, M., Lavania, S., Malchiodi, Z., Hellmund, L., et al. (2018). Gut microbiota promotes tumor growth in mice by modulating immune response. *Gastroenterology* 155, 33–37.e6. doi: 10.1053/j.gastro.2018.04.001

Shawki, S., Ashburn, J., Signs, S. A., and Huang, E. (2018). Colon Cancer: inflammation-associated Cancer. *Surg. Oncol. Clin. N. Am.* 27, 269–287. doi: 10.1016/j.soc.2017.11.003

Smith, G. D., and Ebrahim, S. (2003). "Mendelian randomization": can genetic epidemiology contribute to understanding environmental determinants of disease? *Int. J. Epidemiol.* 32, 1–22. doi: 10.1093/ije/dyg070

Tomasello, G., Mazzola, M., Leone, A., Sinagra, E., Zummo, G., Farina, F., et al. (2016). Nutrition, oxidative stress and intestinal dysbiosis: influence of diet on gut microbiota in inflammatory bowel diseases. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.* 160, 461–466. doi: 10.5507/bp.2016.052

Vogel, J. D., Felder, S. I., Bhama, A. R., Hawkins, A. T., Langenfeld, S. J., Shaffer, V. O., et al. (2022). The American Society of Colon and Rectal Surgeons clinical practice guidelines for the Management of Colon Cancer. *Dis. Colon Rectum* 65, 148–177. doi: 10.1097/DCR.0000000000002323

Wang, Q., Shi, Q., Lu, J., Wang, Z., and Hou, J. (2022). Causal relationships between inflammatory factors and multiple myeloma: a bidirectional Mendelian randomization study. *Int. J. Cancer* 151, 1750–1759. doi: 10.1002/ijc.34214

Weinberg, B. A., and Marshall, J. L. (2019). Colon Cancer in young adults: trends and their implications. *Curr. Oncol. Rep.* 21:3. doi: 10.1007/s11912-019-0756-8

Wong, C. C., and Yu, J. (2023). Gut microbiota in colorectal cancer development and therapy. *Nat. Rev. Clin. Oncol.* 20, 429–452. doi: 10.1038/s41571-023-00766-x

Xie, N., Wang, Z., Shu, Q., Liang, X., Wang, J., Wu, K., et al. (2023). Association between gut microbiota and digestive system cancers: a bidirectional two-sample Mendelian randomization study. *Nutrients* 15:2937. doi: 10.3390/nu15132937

Xu, M., Tian, P., Zhu, H., Zou, R., Zhao, J., Zhang, H., et al. (2022). *Lactobacillus paracasei* CCFM1229 and *Lactobacillus rhamnosus* CCFM1228 alleviated depression- and anxiety-related symptoms of chronic stress-induced depression in mice by regulating xanthine oxidase activity in the brain. *Nutrients* 14:1294. doi: 10.3390/nu14061294

Yang, J., Wei, H., Zhou, Y., Szeto, C. H., Li, C., Lin, Y., et al. (2022). High-fat diet promotes colorectal tumorigenesis through modulating gut microbiota and metabolites. *Gastroenterology* 162, 135–149.e2. doi: 10.1053/j.gastro.2021.08.041

Zegarra Ruiz, D. F., Kim, D. V., Norwood, K., Saldana-Morales, F. B., Kim, M., Ng, C., et al. (2022). Microbiota manipulation to increase macrophage IL-10 improves colitis and limits colitis-associated colorectal cancer. *Gut Microbes* 14:2119054. doi: 10.1080/19490976.2022.2119054

Zhao, Z. X., Chen, S. Z., Xia, Z. L., Bin Xu, Y., Zhang, L. L., Tian, S. M., et al. (2022). High level nitrosamines in rat faeces with colorectal cancer determined by a sensitive GC-MS method. *J. Pharm. Biomed. Anal.* 210:114576. doi: 10.1016/j.jpba.2021.114576

Zheng, D. W., Li, R. Q., An, J. X., Xie, T. Q., Han, Z. Y., Xu, R., et al. (2020). Prebiotics-encapsulated probiotic spores regulate gut microbiota and suppress Colon Cancer. *Adv. Mater.* 32:e2004529. doi: 10.1002/adma.202004529

Zou, S., Fang, L., and Lee, M. H. (2018). Dysbiosis of gut microbiota in promoting the development of colorectal cancer. *Gastroenterol Rep (Oxf)* 6, 1–12. doi: 10.1093/gastro/gox031

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Global status and trends of gastric cancer and gastric microbiota research: a bibliometric analysis

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Background: Numerous studies have cast light on the relationship between the gastric microbiota and gastric carcinogenesis. In this study, we conducted a bibliometric analysis of the relevant literature in the field of gastric cancer and the gastric microbiota and clarified its research status, hotspots, and development trends.

Materials and methods: Publications were retrieved from the Web of Science Core Collection on 18 July 2023. CiteSpace 6.2.R4, VOSviewer 1.6.19.0, and Biblioshiny were used for the co-occurrence and cooperation analyses of countries, institutions, authors, references, and keywords. A keyword cluster analysis and an emergence analysis were performed, and relevant knowledge maps were drawn.

Results: The number of published papers in this field totaled 215 and showed an increasing trend. The analysis of funding suggested that the input in this field is increasing steadily. China had the highest number of publications, while the United States had the highest betweenness centrality. Baylor College of Medicine published the most articles cumulatively. Both Ferreira RM and Cooker OO had the highest citation frequency. The journal *Helicobacter* showed the most interest in this field, while *Gut* provided a substantial research foundation. A total of 280 keywords were obtained using CiteSpace, which were primarily focused on the eradication and pathogenic mechanisms of *Helicobacter pylori*, as well as the application of the gastric microbiota in the evaluation and treatment of gastric cancer. The burst analysis suggested that in the future, research may focus on the application of gastric microorganisms, particularly *Fusobacterium nucleatum*, in the diagnosis and treatment of gastric cancer, along with their pathogenic mechanisms.

Conclusion: Current studies have been tracking the eradication of *Helicobacter pylori* and its pathogenic mechanisms, as well as changes in the gastric microbiota during gastric carcinogenesis. Future research may focus on the clinical application and pathogenesis of stomach microorganisms through bacteria such as *Fusobacterium nucleatum*.

KEYWORDS

gastric cancer, stomach microbiota, gastric carcinogenesis, bibliometric, CiteSpace

1 Introduction

Gastric cancer (GC) is the fifth most common cancer in the world and the fourth leading cause of cancer death (Sung et al., 2021). In the future, although the incidence rate of GC may show a downward trend, for some countries, incidence and mortality increases have been predicted in people below the age of 50 (Arnold et al., 2020; Qi et al., 2023; Teng et al., 2023). *Helicobacter pylori* (*H. pylori*) is considered a major risk factor for GC and has been classified as a Class I carcinogen. The birth-cohort pattern revealed an epidemic of *H. pylori* in gastrointestinal disease (Sonnenberg, 2022). The sequential development model of GC suggests that *H. pylori* colonizes the gastric mucosa, inducing continuous chronic gastric inflammation, then causes cascade pathogenesis: atrophic gastritis (AG), in which *H. pylori* plays a role, and then intestinal metaplasia (IM), dysplasia, and finally GC (Correa, 1988, 1992). However, the decreasing prevalence of *H. pylori* has been histologically observed with the increasing severity of AG (Correa, 1992; Kuipers, 1998; Liu et al., 2022), and some clinical studies have shown that *H. pylori* eradication in patients with advanced lesions does not eliminate the risk of carcinogenesis (Wong et al., 2004; Rugge et al., 2019), indicating that further progression of precancerous conditions may be independent of *H. pylori* colonization. With the prevalence of *H. pylori* infection decreasing (Li et al., 2023), these facts draw attention to gastric microorganisms other than *H. pylori*.

The gastrointestinal microbiota is the largest and most complex microbial ecosystem in the human body, among which bacteria form major communities. However, given its highly acidic environment, which makes it difficult for general bacteria to colonize, the stomach was considered sterile until *H. pylori* was isolated from the gastric mucosa. With the development of molecular techniques, 128 phylotypes of microorganisms have been identified through phylogenetic analysis from gastric endoscopy biopsy samples, most of which belong to *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria*, and 10 main genera have been classified (Bik et al., 2006; Liu et al., 2022). Multiple case–control studies based on gastric mucosal tissue biopsies have confirmed changes in bacterial diversity during the progression of intestinal-type GC. From non-atrophic gastritis (NAG) to IM and then to GC, the bacterial diversity steadily decreased ($p = 0.004$), and the gastric flora abundance changed continuously during this process (Aviles-Jimenez et al., 2014; Liu et al., 2022). Some studies have put forward different views: compared with functional dyspepsia, the richness and diversity of bacterial flora in GC tissue increased, but their uniformity did not increase. In addition, the serological status of *H. pylori* has a significant impact on the composition and diversity of the gastric microbiome (Castano-Rodriguez et al., 2017). Evidence from germ-free insulin–gastrin (INS-GAS) and human gastric microbiota transplant mouse models further supports the potential causality of the microbiota in gastric carcinogenesis (Lofgren et al., 2011; He C. et al., 2022; Kwon et al., 2022). Accordingly, some researchers have used combinations of genera of gastric flora as microbial markers for the non-invasive diagnosis of GC (Chen et al., 2023).

Bibliometrics combines mathematics, statistics, and literature to explore the structural characteristics and hot trends of disciplines through the quantitative analysis of vast amounts of publications and evaluates and predicts the results. It has been widely applied in various fields of medicine (Dracos and Cognetti, 1995; Chen et al., 2012; Kokol et al., 2021). Compared to traditional systematic reviews, the application of bibliometrics in a research field can help new researchers or researchers in other fields to grasp the development process and status of the field based on cluster labels or topics, rather than reading hundreds of unfamiliar studies to obtain limited information. It might also be limited to reading reviews because they often focus on only one research theme (Donthu et al., 2021). For small data samples, synthetic knowledge synthesis can also be applied to extract, synthesize, and multidimensionally structure the corpus of scholarship (Kokol et al., 2022). At present, scholars are increasingly reporting on GC and the gastric microbiota. However, to the best of our knowledge, there is currently no intuitive visual analysis that explores the hotspots and trends in this field. Therefore, we adopted bibliometric methods to conduct a systematic review of this field. By performing quantitative and qualitative analyses of the relevant literature and utilizing visualization tools, the research status, hotspots, and future trends in this field were analyzed.

2 Materials and methods

2.1 Data source

Data were acquired from the Web of Science Core Collection (WoSCC) on 18 July 2023. The query was ((TS = ("gastric cancer*") OR TS = ("gastric neoplasm*") OR TS = ("gastric malignancy") OR TS = ("gastric adenocarcinoma") OR TS = ("gastric carcinoma") OR TS = ("stomach cancer*") OR TS = ("stomach neoplasm*") OR TS = ("stomach malignancy") OR TS = ("stomach adenocarcinoma") OR TS = ("stomach carcinoma")) AND (TS = ("gastric microbiota*") OR TS = ("gastric microbiome*") OR TS = ("gastric microflora") OR TS = ("gastric flora") OR TS = ("gastric bacteria") OR TS = ("gastric microbial community") OR TS = ("gastric bacterial community") OR TS = ("stomach microbiota*") OR TS = ("stomach microbiome*") OR TS = ("stomach microflora") OR TS = ("stomach flora") OR TS = ("stomach bacteria") OR TS = ("stomach microbial community") OR TS = ("stomach bacterial community"))). The language was limited to English. Two evaluators screened the literature independently by reading the titles and keywords, and differences were settled through discussion. A third researcher would participate in further discussion, and a consensus would be reached if the dispute was still inconclusive.

2.2 Data creation and statistical analysis

2.2.1 Data collection and transformation

To export the retrieved documents, "full record and cited references" was selected. Data were converted to "txt" or "csv" format, named "download_*.txt," and then imported into CiteSpace 6.2.R4 and VOSviewer 1.6.19.0 for analysis (Chen, 2006; van Eck and Waltman, 2010). These bibliometric software programs are the most popular and have powerful features. Biblioshiny is a bibliometric program powered by Bibliometrix in the R language. After importing bibliographic records, it can quickly generate visual graphics based on the data, making it a convenient and comprehensive analysis tool (Aria and Cuccurullo, 2017). By using different software, we can achieve complementary functions and verify the analysis results against each other (Cobo et al., 2011; Moral-Munoz et al., 2020).

2.2.2 Data processing

Using Microsoft Excel, the publication volumes of the literature and the funding information were analyzed. CiteSpace 6.2.R4 was applied to deduplicate the obtained documents, retaining only articles and reviews, and then conduct visual analysis. The time span was selected from 2013 to 2023, and the time slice was selected as 1 year. As we described later, we adjusted the k-value appropriately to perform co-occurrence analyses on countries, institutions, and authors and co-citation analyses on journals. Moreover, keywords were used for co-occurrence, clustering, and emergence analyses. Different nodes represent different elements. The color of the ring corresponds to the time when the element appears, the width of the ring represents the frequency of the element at that time, and the size of the whole node reflects the total frequency of the element. The connecting lines between nodes represent co-occurrence, cooperation, or co-citation. The color of the links represents the time when the association first appeared, and its thickness represents the strength of the association. Other parameters were default.

In addition, VOSviewer 1.6.19.0 and Pajek were employed to analyze keyword clusters. Biblioshiny was used to carry out supplementary analyses of the cooperation network, important citing documents, cited source journals, as well as the frequency of keywords and research topic evolution. It is worth noting that in Biblioshiny, we used Keywords Plus for topic analysis due to missing Author Keywords in some literature. Similarly, we detected Author Keywords and Keywords Plus in CiteSpace and VOSviewer. Keywords Plus is as effective as Author Keywords in terms of bibliometric analysis investigating the knowledge structure but is less comprehensive in representing an article's content (Zhang et al., 2016). Therefore, we combined the analysis results of different software programs and read the relevant literature to better describe the topics in the field.

2.2.3 Relative statistical indicators and parameters

2.2.3.1 Parameters in CiteSpace

The g-index is a parameter that can better measure the influence of an author. In CiteSpace, it belongs to the selection criteria and is calculated as $g^2 \leq {}^{k}\sum_{i\leq g} c_i$, k∈Z. Therefore, by adjusting the k-value, the number of nodes can be changed. In order to include as many nodes as possible and exclude less important nodes to ensure the reliability of the analysis, we adopted different k-value settings. The k-value was set to 25 in the co-occurrence analysis of countries, institutions, and keywords; 15 in the authors' co-occurrence analysis and the journal's co-citation analyses.

Betweenness centrality is one of the main metrics in network analysis. It refers to a node's ability to carry information between unconnected groups of nodes, wherein each node represents a research constituent (Donthu et al., 2021). If a node has a centrality greater than 0.1, it is a critical node, as shown by the purple outer ring. The higher the centrality, the more important the bridge function of the node in the whole network.

The emergence detection analysis can reflect the development of an element. For the keywords burst detection, we set γ to 0.45 and the minimum duration to 1.

2.2.3.2 Parameters in Biblioshiny

Callon centrality and density are two parameters that determine the position of bubbles. Centrality is the degree of correlation among different topics. The higher the number of relations a node has with others in the thematic network, the higher the centrality and importance are. Density measures the cohesiveness among a node, which represents the theme's development and delineates its capability to develop and sustain itself (Esfahani et al., 2019; Singh et al., 2023). In the thematic map, density is represented on the vertical axis, while centrality takes the horizontal axis, dividing the map into four quadrants. The upper right quadrant (Q1) contains motor themes, which are important to the research field and have the potential to develop. The upper left quadrant (Q2) contains highly developed and isolated themes, which have abundant internal bonds but less contribution to the development of the field. It means that these themes are potential themes to establish contacts with themes in Q1. The lower left quadrant (Q3) contains emerging or declining themes, which have weak development and are marginal. The lower right quadrant (Q4) contains basic and transversal themes, which have a great value to be discussed in the future.

3 Results

3.1 Research situation analysis

3.1.1 Analysis of publication volume

Analysis of the number of publications is helpful for initially determining whether a research field has received continued attention from researchers and whether it is on the rise. Based on the search results, there were a total of 215 documents, of which 204 were articles and reviews. The statistical diagram of the number of annual publications and annual total publications showed that research in the field of gastric microbiota and gastric cancer first began in 1993. Since 2013, the annual number of publications in this field has shown a significant upward trend. The rapid growth rate from 2018 to 2022 indicated that research in this field has gradually gained more attention in recent years. In 2023, the annual number of publications decreased, which may be due to the fact that the search only ended on 18 July of that year (Figure 1A). The equation fitted according to the annual cumulative number of publications is $y = 1.348e^{0.2847x}$, $R^2 = 0.9818$, which has good fitting properties and conforms to Price's curve. Overall, the total number of publications and the annual publications in this field have grown exponentially.

3.1.2 Analysis of funding

Analysis of the funding information for these publications shows that the cumulative number of funded publications has continued to increase since Web of Science began collecting funding information in 2008. It can be seen that the cumulative funding ratio has also increased steadily. From the analysis of annual funding, we can see that the annual funding rate for publications in this field has been rising steadily since 2013. This shows the attention and investment of researchers, indicating that this field has good development prospects (Figure 1A). According to our analysis, the top eight productive countries were selected for funding analysis. The stacked area chart of annually funded publications shows that in the early years, countries such as the United States and Korea had more funds invested in this field, while since 2021, China has had the largest proportion of funds invested in the field and the greatest output, indicating that China attaches more importance to research in the fields of gastric microbiota and gastric cancer (Figure 1B).

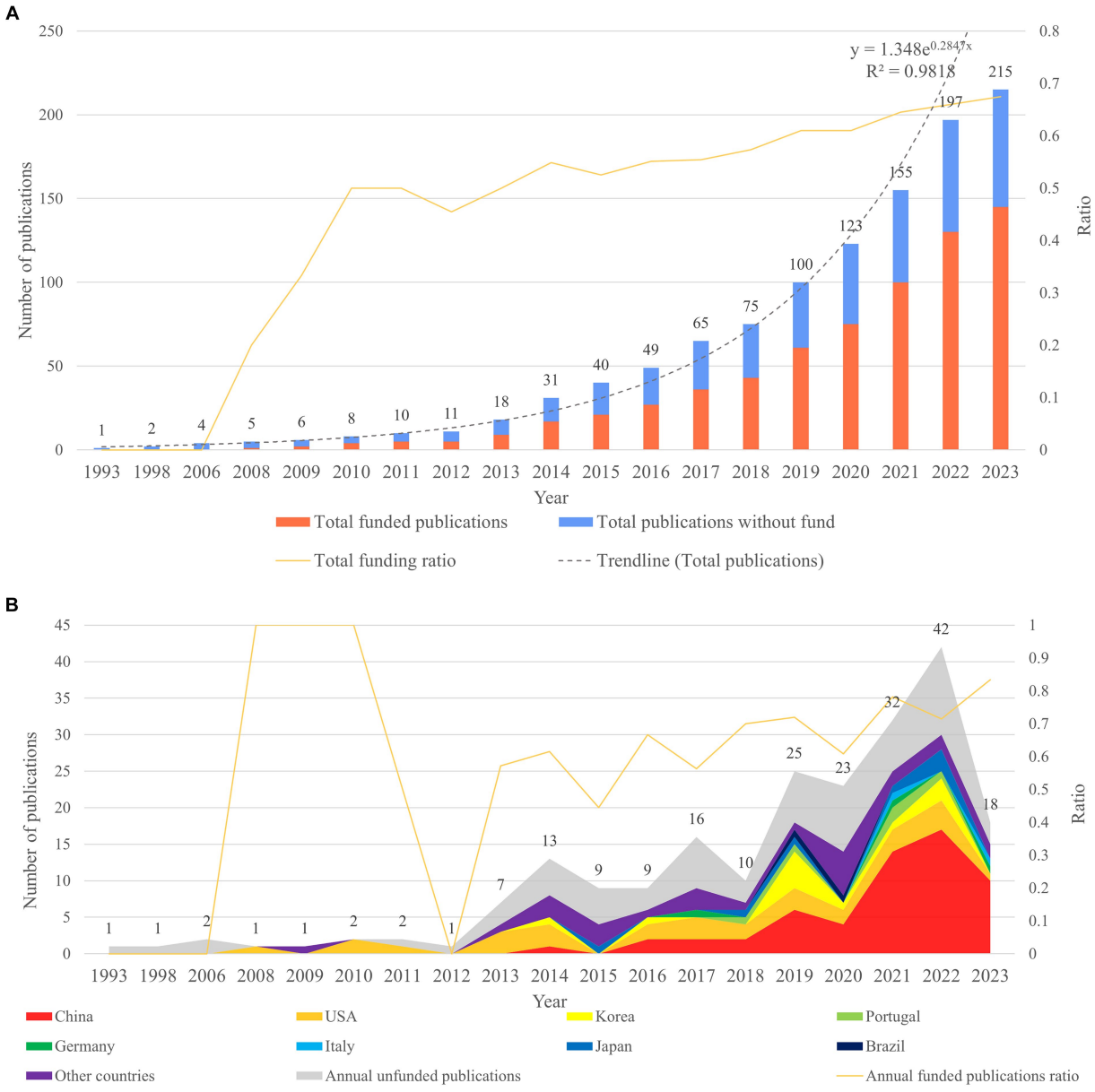

FIGURE 1
The analysis of publication volume and funding information in the field of gastric microbiota and gastric cancer from 1993 to 2023. **(A)** Total cumulative publication volume and cumulative funding situation productions. **(B)** Annual publication volume and annual funding situation.

3.2 Researchers' analysis

3.2.1 Country analysis

The analysis of countries with relevant research from 2013 to 2023 showed that a total of 39 countries followed up on this field. Among them, China and the United States were the most productive countries (Table 1). In the co-occurrence graph, it can be seen that the United States started its research in this field earlier, and China has had an increasing number of prominent publications in recent years (Figure 2A). The betweenness centrality of the United States was the highest, reaching 0.43, which means the United States has a high influence in this field (Table 1). By analyzing the national cooperation network, it can be seen that China and the United States occupy a dominant position in the cooperation network. However, in general, the links between countries are thin and their colors are relatively dark, showing that the intensity of cooperation between countries in this field is low, and there has been poor cooperation in recent years (Figures 2A,B).

3.2.2 Institution analysis

An analysis of institutions showed that there was no prominent institution with a high publication volume. Among all the institutions, Baylor College of Medicine has published the most papers and has the highest betweenness centrality at 0.1. Except for Baylor College of Medicine, there is no institution with a centrality over 0.1, which means the bridge effect of each institution and the cooperation network are weak (Table 2). This might be due to the number of institutions being too high, while the difference in co-occurrence

TABLE 1 The count of publications and the betweenness centrality of the top eight countries.

Rank	Count	Country	Rank	Centrality	Country
1	72	People's Republic of China	1	0.43	United States
2	41	United States	2	0.29	Australia
3	15	Germany	3	0.23	Germany
4	14	Italy	4	0.15	Italy
5	13	Japan	5	0.15	United Kingdom
6	13	South Korea	6	0.14	Japan
7	11	Australia	7	0.11	Sweden
8	8	United Kingdom	8	0.1	Ireland
9	8	Portugal	9	0.06	Greece

frequency is small. The institution cooperation network analysis showed that Vanderbilt University, the US Department of Medicine, Baylor College of Medicine, Universidade do Porto, etc. have cooperated to a certain extent (Figures 2C,D).

3.2.3 Author analysis

Through statistics on the co-occurrence of the first author, it was found that the outputs of each single researcher in the field of gastric cancer and the gastric microbiota were even and relatively small, and the individual researchers exhibited a lack of centrality (Figure 2E). A major research group included Fox James G, Wilson Keith T, Feng Yan, Peek Richard M, etc. Researchers such as Figueiredo C and Ferreira RM, Kim J and Choi IJ, Dong QJ, and Wang LL also occupied a dominant position (Figures 2E,F). There was relatively less cooperation between authors. This result suggests that this field is in its infancy and has broad prospects. Co-citation network analysis of authors can reveal those who are under the spotlight and have made original contributions to a certain field. The CiteSpace analysis results show that Ferreira RM, Bik EM, and Coker OO had higher citation

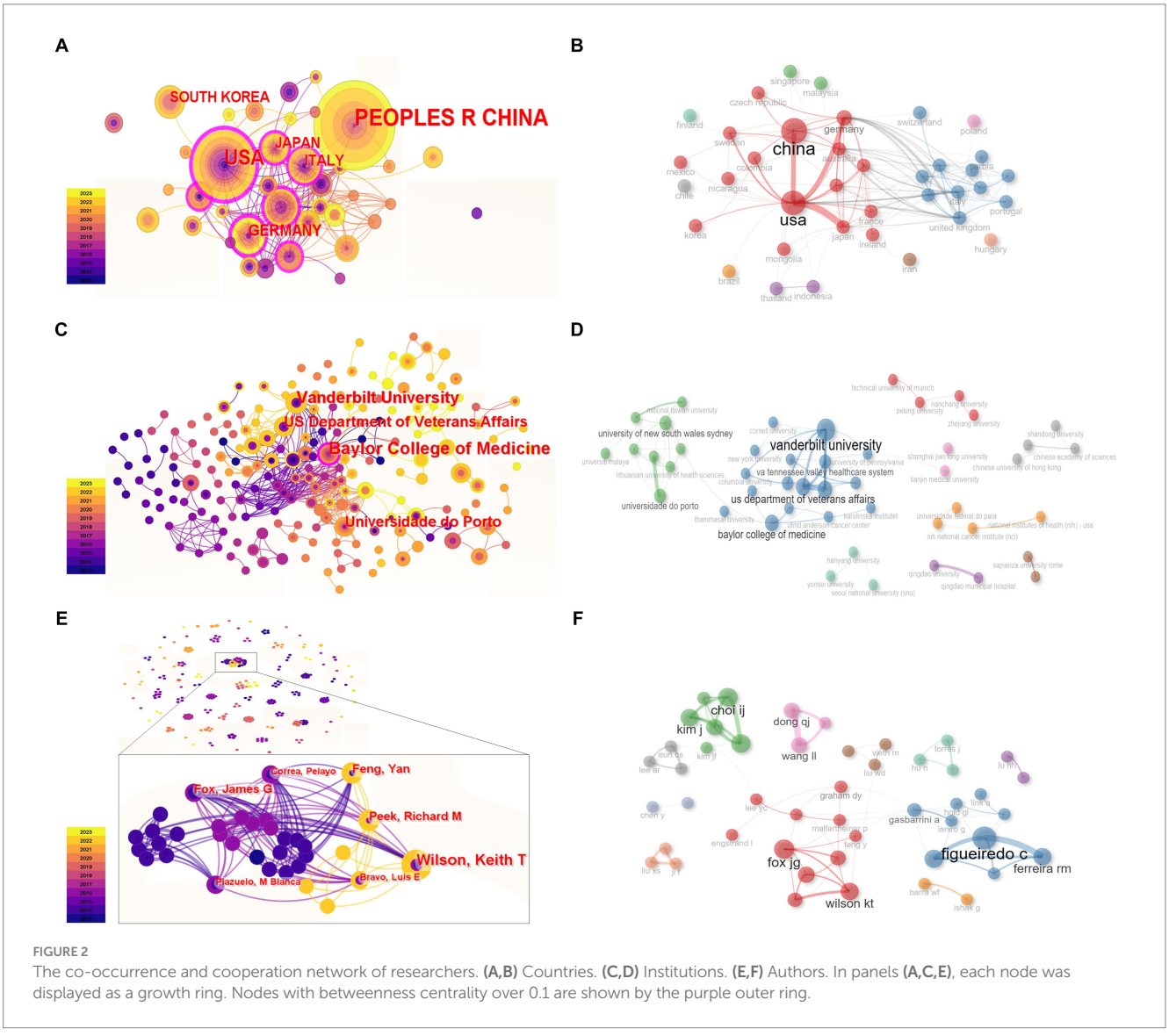

FIGURE 2
The co-occurrence and cooperation network of researchers. **(A,B)** Countries. **(C,D)** Institutions. **(E,F)** Authors. In panels **(A,C,E)**, each node was displayed as a growth ring. Nodes with betweenness centrality over 0.1 are shown by the purple outer ring.

frequency, while Maldonado-Contreras A and Lofgren JL had high betweenness centrality (Table 3).

3.3 Analysis of journals and documents

3.3.1 Document analysis

By analyzing citing documents, the latest research content and the research frontiers can be quickly found. LCS (local citation score) and GCS (global citation score) are two indicators of citing documents. After analyzing the publications retrieved from the WoSCC, it was found that the nodes representing studies by Ferreira RM, Eun CS, Aviles-Jimenez F, Lofgren JL, and Dicksved J were larger and had more pointed arrows, and these articles had the top five LCSs and GCSs, indicating that they were recognized by peers and researchers from other fields, which reflected the focus of this field to a certain extent (Figure 3A; Table 4).

Document co-citation analysis refers to analyzing the references of retrieved documents to find documents cited by different documents at the same time, which helps to identify classic documents and reveal the knowledge basis of the research field. A co-citation analysis of references showed that documents published by Cocker OO and Ferreira RM in *Gut* in 2018 had the highest citation frequency, while an article by Lertpiriyapong K in *Gut* in 2014 had the highest betweenness centrality (Table 5).

3.3.2 Journal analysis

Relevant journal analysis reflects which journals are more interested in this field. Through our retrieval, the documents obtained were published in 109 journals and mainly distributed in the categories of Gastroenterology, Hepatology, Microbiology, and Oncology. The journal *Helicobacter* included the most articles in this field, reaching 16 (Figure 3B). The journal co-citation network shows that articles published in *Gut*, *Gastroenterology*, and *Helicobacter* had higher citation frequencies in this research field, and *Scientific Reports* had an important role with a centrality over 0.1 (Figure 3C). The analysis of the most locally cited sources showed the citation frequency of the source journals from which the references in our retrieval documents came. *Gut*, *Gastroenterology*, and *Helicobacter* had the highest citation frequency, which means they provided most of the research foundation (Figure 3D).

3.4 Keyword and research hotspot analysis

3.4.1 Keyword frequency and co-occurrence analysis

Biblioshiny was used to conduct word frequency analysis of the top 50 keywords from 2013 to 2023. It can be seen that "*helicobacter-pylori*" appeared the most frequently. Other keywords with the highest word frequency included "infection", "cancer", "risk", "gut microbiota", "intestinal metaplasia", "colonization", "eradication", "inflammation", and so on (Figures 4A,B).

We also chose CiteSpace to analyze keywords from 2013 to 2023, with a total of 280 nodes and 1,557 links. Among the 280 keywords, the keywords with the highest frequency included "*Helicobacter pylori*", "gastric cancer", "infection", etc., and the words with the highest betweenness centrality included "*Helicobacter pylori* infection", "gut microbiota", "atrophic gastritis", etc. (Table 6). Our results show that in addition to search terms such as "gastric cancer" and "gastric microbiota", there were keywords reflecting the precancerous conditions of GC, such as "atrophic gastritis", "chronic gastritis", "intestinal metaplasia"; keywords reflecting the eradication treatment

TABLE 2 The count of publications and the betweenness centrality of the top 12 institutions.

	Count	Centrality	Institution
1	9	0.1	Baylor College of Medicine
2	8	0.03	Vanderbilt University
3	7	0	Nanchang University
4	6	0	US Department of Veterans Affairs
5	6	0	Zhejiang University
6	6	0.01	Universidade do Porto
7	5	0	Chinese Academy of Sciences
8	5	0	Hanyang University
9	5	0	University of New South Wales Sydney
10	5	0	Veterans' Health Administration (VHA)
11	5	0.02	Otto von Guericke University
12	5	0.03	Massachusetts Institute of Technology (MIT)

TABLE 3 The cited frequency and betweenness centrality of co-cited authors.

	Count	Year	Name		Centrality	Year	Name
1	97	2018	Ferreira RM	1	0.16	2013	Maldonado-Contreras A
2	92	2013	Bik EM	2	0.15	2013	Lofgren JL
3	91	2018	Coker OO	3	0.12	2014	Lertpiriyapong K
4	83	2013	Correa P	4	0.12	2016	Jo HJ
5	81	2015	Aviles-Jimenez F	5	0.11	2013	Correa P
6	80	2015	Eun CS	6	0.09	2017	Li TH
7	70	2013	Dicksved J	7	0.09	2014	Malfertheiner P
8	67	2013	Lofgren JL	8	0.08	2013	Bik EM
9	66	2014	Lertpiriyapong K	9	0.08	2013	Dicksved J
10	58	2016	Yang I	10	0.08	2014	El-Omar EM

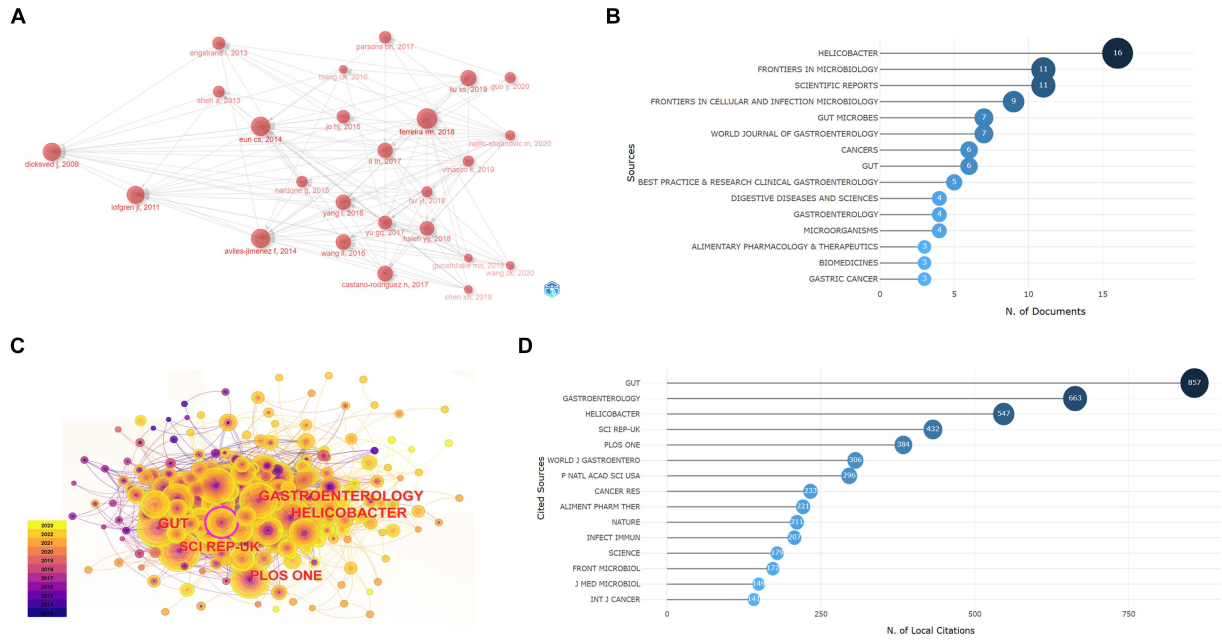

FIGURE 3
The documents and journal analysis. **(A)** Historiograph of citing documents. **(B)** Most relevant sources reflect the journal distribution of retrieval documents. **(C)** Co-citation network of reference journals. **(D)** Most locally cited sources show the source journal of references in the retrieval documents.

TABLE 4 The citing documents with the top five LCS and GCS.

	Paper	Year	LCS		Paper	Year	GCS
1	Ferreira RM, 2018, Gut	2018	96	1	Ferreira RM, 2018, Gut	2018	338
	DOI: 10.1136/gutjnl-2017-314205				DOI: 10.1136/gutjnl-2017-314205		
2	Eun CS, 2014, Helicobacter	2014	82	2	Lofgren JL, 2011, Gastroenterology	2011	238
	DOI: 10.1111/hel.12145				DOI: 10.1053/j.gastro.2010.09.048		
3	Aviles-Jimenez F, 2014, Sci Rep-UK	2014	81	3	Dicksved J, 2009, J Med Microbiol	2009	214
	DOI: 10.1038/srep04202				DOI: 10.1099/jmm.0.007302-0		
4	Dicksved J, 2009, J Med Microbiol	2009	70	4	Aviles-Jimenez F, 2014, Sci Rep-UK	2014	205
	DOI: 10.1099/jmm.0.007302-0				DOI: 10.1038/srep04202		
5	Lofgren JL, 2011, Gastroenterology	2011	68	5	Eun CS, 2014, Helicobacter	2014	180
	DOI: 10.1053/j.gastro.2010.09.048				DOI: 10.1111/hel.12145		

LCS, local citation score; GCS, global citation score.

of *H. pylori*, such as "*Helicobacter pylori*", "eradication", "proton pump inhibitors", "antibiotics", "clarithromycin"; keywords related to gastric microbiota research, such as "gut microbiota", "colonization", "*Fusobacterium nucleatum*", "*Bifidobacterium*", "mucosa associated microbiota", "gastric non-*Helicobacter pylori* helicobacter"; keywords reflecting research technology, such as "16 s rRNA sequencing", "next-generation sequencing"; and keywords reflecting pathogenic mechanisms, such as "inflammation", "regulatory t cells", "immune response", "n nitroso compounds", "DNA methylation", "dendritic cells", "kappa b activation", "cdk12", "CagA", "e cadherin", "ecl cell", etc. (Supplementary Table 1).

3.4.2 Keywords cluster analysis

Cluster analysis can be used to correlate keywords that appear at the same time in documents and cluster highly relevant words into categories so as to mine hidden information. Modularity Q (Q, value interval [0, 1]) and Weighted Mean Silhouette S (S, value interval [−1, 1]) are two important parameters of the clustering map. The Q-value can evaluate the quality of the clustering network. Q > 0.3 indicates that the network structure is persuasive. The S-value can measure the uniformity of cluster members. S > 0.5 indicates that the clustering results are reasonable.

We selected the log-likelihood ratio (LLR) algorithm to cluster 280 keywords from 2013 to 2023. The Q-value was 0.3971, and the S-value was 0.7278, indicating that the clustering results were informative and had reference significance. A total of 11 meaningful clusters were formed (Table 7). The smaller the cluster number, the more keywords were included. The keywords of each cluster partially overlap, which indicates that there is a correlation between the clusters. Through the artificial division of clusters, it can be seen that

TABLE 5 The cited frequency and betweenness centrality of co-cited publications.

	Count	Author and DOI		Centrality	Author and DOI
1	95	Ferreira RM, 2018, Gut	1	0.33	Lertpiriyapong K, 2014, Gut
		DOI: 10.1136/gutjnl-2017-314205			DOI: 10.1136/gutjnl-2013-305178
2	90	Coker OO, 2018, Gut	2	0.18	Coker OO, 2018, Gut
		DOI: 10.1136/gutjnl-2017-314281			DOI: 10.1136/gutjnl-2017-314281
3	50	Liu XS, 2019, Ebiomedicine	3	0.15	Lofgren JL, 2011, Gastroenterology
		DOI: 10.1016/j.ebiom.2018.12.034			DOI: 10.1053/j.gastro.2010.09.048
4	42	Li TH, 2017, Sci Rep-UK	4	0.14	Eun CS, 2014, Helicobacter
		DOI: 10.1038/srep44935			DOI: 10.1111/hel.12145
5	38	Hsieh YY, 2018, Sci Rep-UK	5	0.14	Li TH, 2017, Sci Rep-UK
		DOI: 10.1038/s41598-017-18596-0			DOI: 10.1038/srep44935
6	38	Castano-Rodriguez N, 2017, Sci Rep-UK	6	0.12	Yang I, 2016, Sci Rep-UK
		DOI: 10.1038/s41598-017-16289-2			DOI: 10.1038/srep18594
7	35	Yang I, 2016, Sci Rep-UK	7	0.11	Maldonado-Contreras A, 2011, ISME J
		DOI: 10.1038/srep18594			DOI: 10.1038/ismej.2010.149
8	35	Aviles-Jimenez F, 2014, Sci Rep-UK	8	0.11	Wang LL, 2016, Eur J Gastroen Hepat
		DOI: 10.1038/srep04202			DOI: 10.1097/MEG.0000000000000542
9	32	Lertpiriyapong K, 2014, Gut	9	0.11	Ferreira RM, 2018, Gut
		DOI: 10.1136/gutjnl-2013-305178			DOI: 10.1136/gutjnl-2017-314205
10	31	Eun CS, 2014, Helicobacter	10	0.1	Yu GQ, 2017, Front Cell Infect MI
		DOI: 10.1111/hel.12145			DOI: 10.3389/fcimb.2017.00302
11	31	Yu GQ, 2017, Front Cell Infect MI	11	0.09	Aviles-Jimenez F, 2014, Sci Rep-UK
		DOI: 10.3389/fcimb.2017.00302			DOI: 10.1038/srep04202
12	31	Schulz C, 2018, Gut	12	0.09	Liu XS, 2019, Ebiomedicine
		DOI: 10.1136/gutjnl-2016-312904			DOI: 10.1016/j.ebiom.2018.12.034

in the past 10 years, researchers in this field have mainly specialized in the mechanisms by which the gastric microbiota causes GC, including immunity (cluster #0), metabolism (clusters #2, #3), environmental factors (cluster #6), tumor microenvironment (cluster #7), and paying attention to the types of bacterial flora (clusters #1, #8) and research methods (clusters #4, #9). In addition, *H. pylori* eradication treatment (cluster #5, #10) has been one direction of research. Furthermore, *H. pylori* infection and its eradication treatment are closely related to changes in gastric flora richness and the occurrence of GC. Overall, these clusters are related to each other. In terms of research progress, the timeline map can reflect the time span of each cluster and the correlation between different clusters, reflecting the evolution of research. This showed that clusters #0, #1, #4, #6, and #8 already had important keywords in 2013. Among them, the keywords in clusters #0 and #1 had high frequency and wide relationships with other keywords. Cluster #7 had its first important keyword, "adverse prognosis", in 2018, which indicates that these fields are just beginning to develop and need more complete and thorough research (Figure 4D).

VOSviewer and Pajek were also used to generate clusters by analyzing keywords that appeared over 3 times. A total of 128 keywords were displayed, and 10 clusters were obtained (Figure 4C). Each color represents a thematic cluster with different numbers of keywords. The bigger the node, the greater the frequency of the keyword. Links between nodes signal the relationship between topics. The thicker the link, the greater the occurrence of co-occurrence between keywords. The red cluster contained keywords reflecting *H. pylori* infection in the process of gastric carcinogenesis and had tight links with other clusters through keywords such as "*Helicobacter pylori*", "intestinal metaplasia", and "expression." The green cluster mainly focused on the diagnosis and treatment of patients with precancerous status but had few links with other clusters. The dark blue cluster reflected the treatment of *H. pylori* infection. The yellow cluster included keywords with respect to identifying different members of the gastric microbiota. The violet cluster consisted of keywords reflecting the pathogenic mechanism of *H. pylori*. The light blue cluster contained keywords for factors that belong to gastric microbiota members or influence their components. The orange cluster reflected the *in vitro* research techniques for *H. pylori* infection. Other clusters consisted of keywords reflecting research regarding other microbiota associated with the gastric microbiota. Among all the keywords, except for "gastric cancer" and "gastric microbiota", other keywords making associations with other clusters were "*Helicobacter pylori*", "infection", "risk", "intestinal metaplasia", "inflammation", "colonization", and "eradication", which reflected that the eradication of *H. pylori* and its pathogenic mechanisms got noticed. In addition, keywords such as "proton pump inhibitor", "*Fusobacterium nucleatum*", "autoimmune gastritis", "atrophic

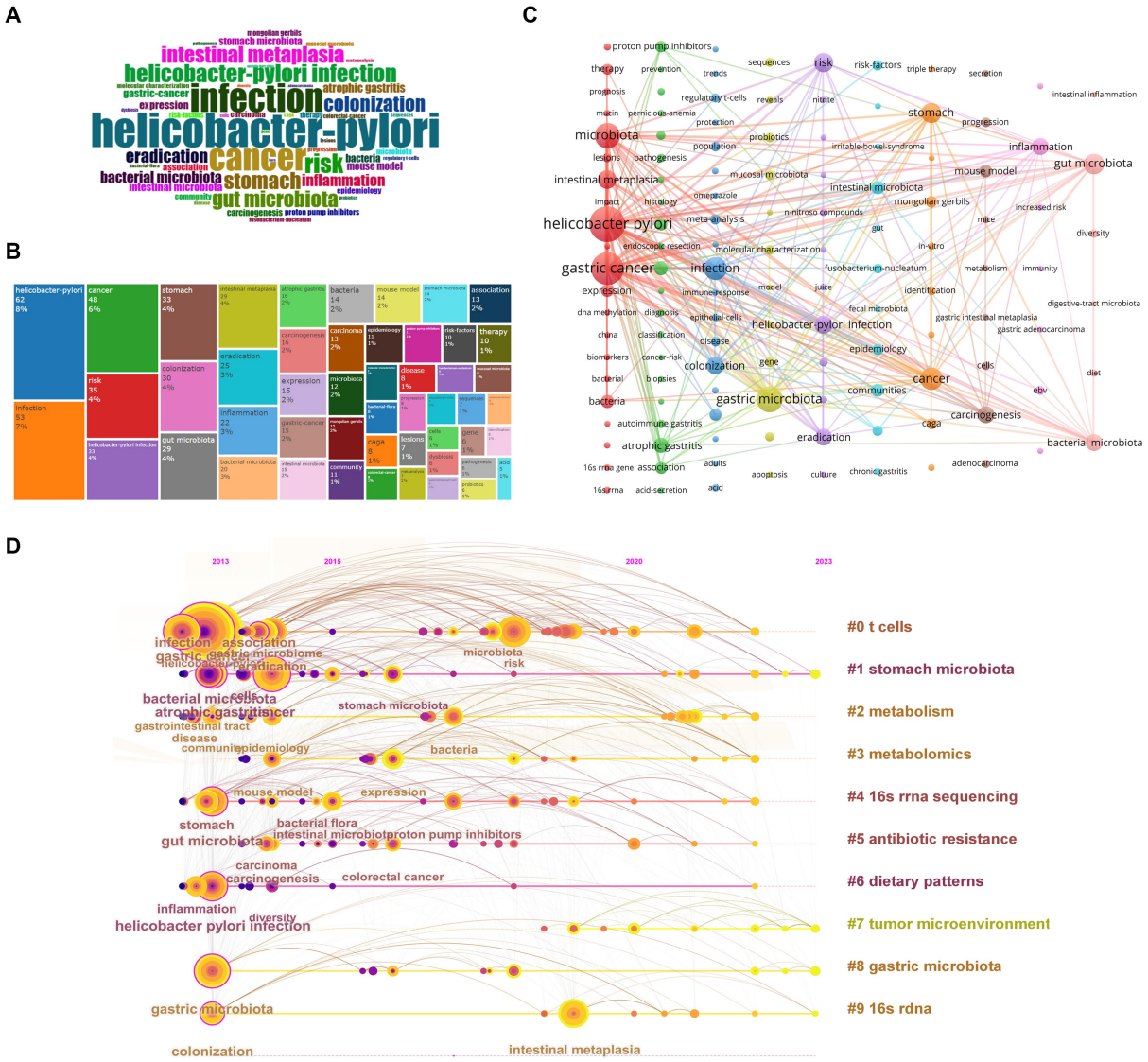

FIGURE 4
Keywords and cluster analysis. **(A)** Word cloud map. **(B)** Tree map of keyword frequency. **(C)** Cluster network of keywords produced by VOSviewer and Pajek. **(D)** Timeline cluster map of keywords.

gastritis", "mucosal microbiota", and "regulatory T cells" were closely related to those important keywords, which may represent new research directions.

3.4.3 Research hotspot evolution

Keywords can reflect the core topics of research to some degree, helping to quickly elucidate the hotspots and progress in the research field. CiteSpace was used to undertake a burst analysis of keywords in the literature in this field from 2013 To 2023. A light blue line indicates that the keyword has not yet appeared, the dark blue line represents that the keyword has begun to appear, and the red line represents that the keyword has emerged (Table 8). Biblioshiny was applied to analyze the thematic evolution and trend topics. According to the distribution of publications per year, a Sankey diagram was drawn using the years 2013 and 2017 as the cutting points, and the thematic maps in each time slice were also exported (Figures 5A–D). In the thematic maps, each bubble represents an emerging topic that moves toward mainstream themes. The names of bubbles are keywords with the highest occurrence in the clusters. The bubble size is proportional to the word occurrences, and the position is determined by its centrality and density. in the Sankey diagram, each block represents a keyword, and its width represents its frequency. The width of links between blocks represents the strength of the association. By detecting the frequency of keywords plus in a certain period, a trend topic scatter diagram was drawn (Figure 5E). The horizontal axis displays the time when high-frequency keywords appear, and the vertical axis displays the first three topics for each year in decreasing order of frequency. Each bubble on the graph represents a topic. The reference year for each topic is identified using the median of the distribution of occurrence over the time period considered, while the bar indicates the first and third quartiles of the occurrence distribution.

In short, these knowledge mappings lead to the same conclusion. Early research focused on *H. pylori* infection and eradication as well as GC progression, and the research content was relatively simple,

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TABLE 6 The frequency and betweenness centrality of the top 15 keywords.

	Count	Year	Keyword		Centrality	Year	Keyword
1	118	2013	*Helicobacter pylori*	1	0.18	2013	*Helicobacter pylori* infection
2	99	2013	Gastric cancer	2	0.17	2013	Gut microbiota
3	49	2013	Infection	3	0.15	2013	Atrophic gastritis
4	45	2014	Cancer	4	0.14	2014	Cancer
5	38	2013	Gastric microbiota	5	0.14	2013	Gastric microbiota
6	33	2013	Gut microbiota	6	0.13	2014	Association
7	33	2013	*Helicobacter pylori* infection	7	0.13	2013	Bacterial microbiota
8	31	2018	Risk	8	0.11	2013	Colonization
9	29	2019	Intestinal metaplasia	9	0.11	2013	Gastric cancer
10	28	2013	Colonization	10	0.11	2013	Infection
11	27	2013	Stomach	11	0.09	2019	Intestinal metaplasia
12	23	2014	Eradication	12	0.08	2013	Inflammation
13	22	2013	Atrophic gastritis	13	0.07	2014	Carcinoma
14	20	2013	Bacterial microbiota	14	0.07	2014	Eradication
15	18	2014	Gastric microbiome	15	0.06	2015	Digestive tract microbiota

TABLE 7 Keywords co-occurrence network clustering table.

Cluster ID	Size	Silhouette	Mean (year)	LLR
#0 T cells	56	0.61	2017	T cells (5.41, 0.05); gastrointestinal microbiota (5.41, 0.05); Barrett's esophagus (5.41, 0.05); 16 s rRNA gene (4.23, 0.05); stomach cancer (4.17, 0.05)
#1 Stomach microbiota	36	0.59	2016	Stomach microbiota (16.2, 1.0E-4); bile acids (8.07, 0.005); bacterial microbiota (5.96, 0.05); accuracy rate (4.03, 0.05); identification (4.03, 0.05)
#2 Metabolism	33	0.707	2018	Metabolism (8.89, 0.005); disease (7.09, 0.01); epidemiology (5.3, 0.05); cytokines (5.3, 0.05); peptic ulcers (4.44, 0.05)
#3 Metabolomics	28	0.708	2018	Metabolomics (11.95, 0.001); pathogenesis (5.95, 0.05); distal gastric cancer (5.95, 0.05); association analysis (5.95, 0.05); thioredoxin (trxA; 5.95, 0.05)
#4 16 s rRNA sequencing	27	0.795	2016	16 s rRNA sequencing (12.9, 0.001); stomach (8.75, 0.005); s (8.59, 0.005); intestinal microbiota (8.59, 0.005); children (8.59, 0.005)
#5 Antibiotic resistance	25	0.782	2017	Antibiotic resistance (10.91, 0.001); chronic gastritis (9.94, 0.005); carcinoma (7.22, 0.01); chronic intestinal inflammation (5.44, 0.05); alternative treatments (5.44, 0.05)
#6 Dietary patterns	19	0.9	2014	Dietary patterns (5.03, 0.05); 16 s ribosomal RNA (5.03, 0.05); prevention (5.03, 0.05); altered Schaedler flora (5.03, 0.05); migrating motor complex (5.03, 0.05)
#7 Tumor microenvironment	17	0.843	2021	Tumor microenvironment (10.55, 0.005); microbiota (microorganism; 7.95, 0.005); treatment (7.95, 0.005); host–microbe interactions (7.95, 0.005); TREGS (regulatory T cells; 7.95, 0.005)
#8 Gastric microbiota	16	0.893	2018	Gastric microbiota (11.05, 0.001); supplementation (5.37, 0.05); gastric non-*Helicobacter pylori* helicobacter (5.37, 0.05); Bifidobacterium (5.37, 0.05); prognosis (5.37, 0.05)
#9 16 s rDNA	14	0.754	2020	16 s rDNA (10.03, 0.005); animal models (5, 0.05); dysplasia (5, 0.05); *candida albicans* (5, 0.05); Epstein-Barr virus (5, 0.05)
#10 Containing triple therapy	6	0.963	2017	Containing triple therapy (9.17, 0.005); *Saccharomyces boulardii* supplementation (9.17, 0.005); containing quadruple therapy (9.17, 0.005); proton pump inhibitor (9.17, 0.005); low dose aspirin (9.17, 0.005)

LLR, log-likelihood ratio.

TABLE 8 Emergent analysis of the top 64 keywords with the strongest citation bursts.

Keywords	Year	Strength	Begin	End	2013–2023
Bacterial microbiota	2013	4.47	2013	2017	
Disease	2013	1.53	2013	2014	
Gastric acid secretion	2013	1.49	2013	2016	
Atrophic gastritis	2013	1.4	2013	2013	
Endoscopic resection	2013	1.28	2013	2017	
Colitis	2013	1.2	2013	2014	
Flora	2014	2.12	2014	2016	
Cells	2014	2.08	2014	2017	
Diversity	2014	1.98	2014	2018	
Mouse model	2014	1.48	2014	2014	
Peptic ulcer	2014	1.27	2014	2014	
Molecular analysis	2014	1.27	2014	2014	
Dendritic cells	2014	1.16	2014	2015	
Immune response	2014	1.14	2014	2014	
Epithelial cells	2014	1.14	2014	2014	
Helicobacter pylori infection	2013	1.13	2014	2015	
Digestive tract microbiota	2015	1.57	2015	2017	
H. pylori	2015	1.37	2015	2018	
Stomach microbiota	2016	2.87	2016	2017	
Colorectal cancer	2016	1.63	2016	2018	
Mongolian gerbils	2016	1.5	2016	2019	
Identification	2016	1.29	2016	2016	
Mice	2016	1.29	2016	2016	
Cancer risk	2017	1.32	2017	2020	
China	2017	1.27	2017	2019	
Pathology	2017	1.25	2017	2017	
H. pylori	2017	1.25	2017	2017	
Kegg modules	2017	1.25	2017	2017	
Gastric cancer risk	2017	1.25	2017	2017	
Proton pump inhibitors	2017	1.21	2017	2020	
Intestinal microbiota	2015	1.13	2017	2017	
Association	2014	2.27	2018	2019	
Eradication	2014	2.24	2018	2020	
Risk	2018	1.74	2018	2023	
Meta-analysis	2018	1.58	2018	2019	
Autoimmune gastritis	2018	1.3	2018	2020	
Pernicious anemia	2018	1.26	2018	2018	
Trends	2018	1.26	2018	2018	
Adults	2018	1.2	2018	2018	
Progression	2019	2.79	2019	2020	
Molecular characterization	2019	2.18	2019	2021	
Diagnosis	2019	1.85	2019	2020	
Tumor microenvironment	2019	1.41	2019	2019	
Sequences	2020	1.79	2020	2021	

(Continued)

TABLE 8 (Continued)

Keywords	Year	Strength	Begin	End	2013–2023
Juice	2020	1.2	2020	2020	
Next-generation sequencing	2020	1.2	2020	2020	
Nitrite	2020	1.2	2020	2020	
N nitroso compounds	2020	1.2	2020	2020	
Dysbiosis	2021	2.12	2021	2023	
Gastric microbiome	2014	1.87	2021	2023	
Fusobacterium nucleatum	2021	1.77	2021	2023	
Mucosa associated microbiota	2021	1.63	2021	2021	
Mucosal microbiota	2021	1.55	2021	2023	
Risk factors	2021	1.41	2021	2023	
Gastric carcinogenesis	2021	1.41	2021	2023	
Community	2013	1.12	2021	2023	
Intestinal metaplasia	2019	2.9	2022	2023	
Therapy	2019	1.97	2022	2023	
Inflammation	2013	1.85	2022	2023	
Expression	2016	1.57	2022	2023	
Gene	2018	1.35	2022	2023	
Apoptosis	2022	1.23	2022	2023	
Beta catenin	2022	1.23	2022	2023	
Adenocarcinoma	2016	1.14	2022	2023	

Light blue line: the year that keywords have not appeared; dark blue line: the year that keywords began to appear; red line: the year that keywords emerged.

containing keywords such as "*Helicobacter pylori* infection", "gastric acid secretion", and "atrophic gastritis", and "peptic ulcer". the keyword "bacterial microbiota" had the highest burst strength and lasted for a long time, which reflected that the concept of the microbiota began to receive significant and sustained attention. At this time, the topic of this research field was homogeneous, and the frequency of each keyword was low.

In the mid-term, the topic was still limited, but the word frequency increased. Research regarding *H. pylori* infection was still at the center position. There were still some keywords indicating studies on *H. pylori* eradication regimen, such as "eradication" and "proton pump inhibitors". The burst detection showed that researchers began to increase their research on the gastrointestinal microbiota, with keywords such as "gastric microbiome", "mucosa associated microbiota", "community", and the strength of the keyword "stomach microbiota" reaching 2.87. There were also keywords reflecting related research methods such as "molecular characterization", "kegg modules", and "next-generation sequencing". In this period, "bacterial microbiota" served as the basis of research. In addition, some new research topics appeared, for example, "dendritic cells", which reflected the focus on the mechanisms of gastric carcinogenesis caused by the gastric microbiota. The pathogenic mechanisms of the gastric microbiota in GC mainly included immune and "inflammation" mechanisms, reflected in keywords such as "dendritic cells", "immune response", and "inflammation".

In recent years, the research topics have become heterogeneous, which is evidenced by the increasing number of topic words with high Callon centrality. In combination with burst detection, thematic evolution, and hotspot trend analysis, it can be inferred that researchers have paid more attention to the richness of bacterial flora and the mechanisms by which different microorganisms cause GC during this stage. Specifically, topics generated included those reflecting the pathogenic mechanisms of *H. pylori*, such as "*Helicobacter pylori*", "n nitroso compounds" and "caga", with burst keywords including "n nitroso compounds", "nitrite", "inflammation", "apoptosis", "epithelial cells", "beta catenin"; those reflecting different members of the gastric microbiome, such as "epstein barr virus", "*Fusobacterium nucleatum*", with burst keywords including "tumor microenvironment", "mucosa associated microbiota", "community", "dysbiosis", "*Fusobacterium nucleatum*"; those reflecting the safety of *H. pylori* eradication therapy, such as "adverse events"; and those reflecting the clinical treatment of the precancerous stage of GC, such as "peptic ulcer", "intestinal metaplasia", "risk factors", and "probiotics", with keywords such as "gastric carcinogenesis". Notably, the burst keywords "dysbiosis", "*Fusobacterium nucleatum*", "mucosal microbiota", "risk factors", "gastric carcinogenesis", "community", "intestinal metaplasia", "therapy", "inflammation", "expression", "gene", "apoptosis", "beta catenin", and "adenocarcinoma" are still in a burst period, leading researchers' attention to changes in the gastric microbiota and its clinical application, and the pathogenic mechanisms of *H. pylori* and other non-*helicobacter* bacteria. Among them, the thematic map of the time slice 2018 to 2023 suggested that topics such as "*Fusobacterium nucleatum*", "mucosal microbiota" and "intestinal metaplasia" in the lower right quadrant are basic and transversal themes and play an important role in the development of this field, but they were understudied and may therefore become hotspots in the future.

Generally speaking, research in this field at this stage is mainly divided into two aspects: the eradication and pathogenic mechanisms

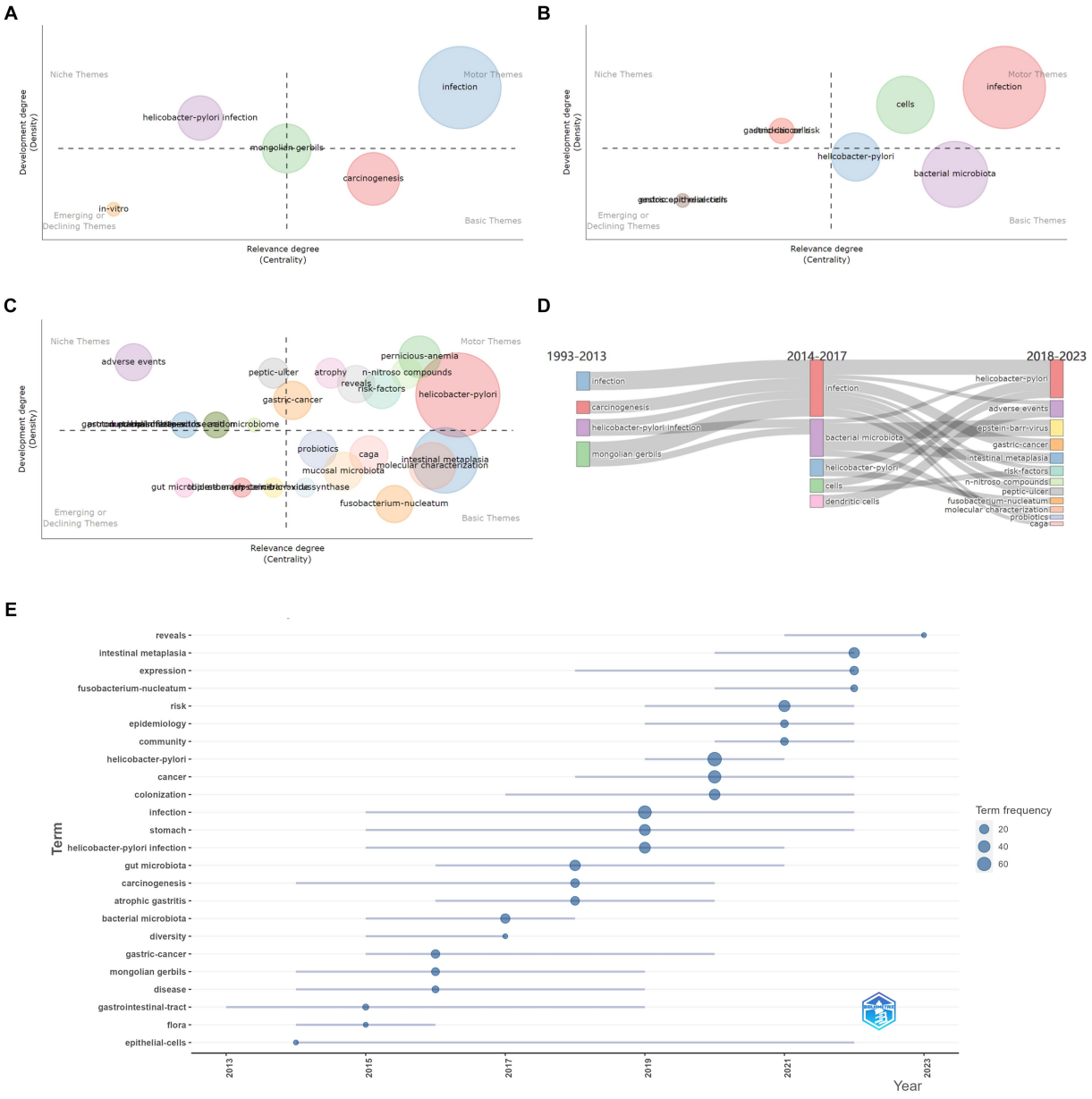

FIGURE 5
Research hotspot analysis. Panels **(A–C)** are the thematic maps of each time slice and describe the evolution trend of keywords. **(A)** represents the period of 1993–2013, **(B)** is for 2014–2017, and **(C)** is for 2018–2023. **(D)** Thematic evolution: the Sankey diagram shows the flow of research themes that merged or split. **(E)** Trend topic scatter diagram.

of *H. pylori*, which were well studied and becoming more important, and the role of different gastric microorganisms in the diagnosis and treatment of GC and their pathogenic mechanisms, which were less studied but important to this field. Among these topics, *H. pylori*, *Fusobacterium nucleatum*, immunity, and inflammation may be future research hotspots.

4 Discussion

This article is the first to identify the current research status, research hotspots, and trends in the field of gastric flora and gastric cancer based on bibliometric methods. The application of bibliometric analysis and visualization can display various topics and trends in basic or clinical research in order for researchers to carry out their work. In addition, our research shows that this research field has broad prospects and that there are still many clinical problems left to be solved.

While the annual cumulative number of papers has shown an exponential growth trend, in the past 3 years, the annual publications in this field have continued to grow rapidly, which suggests that the topic of gastric microbiota and GC has become popular. The analysis of funding showed that many countries are increasing capital input in this field, especially those that are productive. It can be predicted that

in the future, the number of publications in this field will continue to grow exponentially, and funding support may also increase, making this field attractive for researchers.

Among the countries that published articles from 2013 to 2023, the total number of articles published by the top eight countries accounted for more than 90% of all articles published in this field, indicating that these countries were the main contributors to research in this field. Developed countries such as the United States and Germany published papers earlier, and their research results also had a relatively prominent influence. These countries often have advanced medical research institutions, outstanding scientific researchers, and sufficient financial support. As a developing country, China has been increasing its publication volume significantly since 2015, and the total number of articles quickly exceeded that of other countries. This may be due to the large population base, the large number of *H. pylori* infections and GC patients, and the popularization of endoscopic biopsy technology (Fan et al., 2021; Wang et al., 2022), as well as the rapid growth of national GDP and increased investment in scientific research (Lei et al., 2020; Zhou et al., 2021). However, although China is an important representative with respect to investment in this field, China's betweenness centrality was not outstanding enough, indicating that Chinese researchers need to improve the quality of their research. The institutions that contributed the most to this field were mainly universities, mostly from the United States and European countries. Among them, Baylor College of Medicine has made prominent contributions and is considered one of the most outstanding medical schools in the United States. Through the analysis of authors and institutions, it can be seen that Fox JG and Wilson KT from Vanderbilt University, Figueiredo C and Ferreira RM from Universidade do Porto, have made great contributions in this field and published a series of highly influential articles, as well as actively participating in follow-up research. In recent years, with the largest number of publications, China's output mainly came from Zhejiang University and Nanchang University. However, most researchers in this field currently tend to collaborate with domestic research institutions or within research groups in the same institution, even in productive countries. This might be due to the fact that this research field is only in its infancy. Therefore, as the number of publications increases, countries and institutions need to strengthen cooperation to promote the exchange of academic ideas and innovative development in the research field, which will also help to expand their influence.

In terms of journal analysis, the impact factor (IF) of a journal to some extent represents the influence of research in the field and the quality of the research results. The reference source journals have high IFs, indicating that the research foundation in this field is reliable. *Gut* was the most cited journal, indicating that the results published in this journal have an important influence in this research field, with an IF of 24.5. *Helicobacter* was the main journal publishing research in this field, with an IF of 4.4. Recently, journals such as *Frontiers in Microbiology*, *Scientific Reports*, and *Gut Microbes* have published numerous articles, among which the IF of *Gut Microbes* reached 12.2. However, compared to the cited literature, the current IFs of publications in this field are still lower in general. This may be due to the fact that research in this field has not attracted widespread attention, suggesting that researchers need to improve the quality of their research through reasonable experimental design and advanced research technology to produce more influential products and propose more novel perspectives.

A large number of studies have taken various directions, leading to pathogenesis research and clinical practical applications. By reading the key literature identified via the historiograph of citing documents, the research progression can be revealed. After identifying differences in the gastric microbiota characteristics between GC patients and those with dyspepsia, as the technique developed, the gradual shifting of the gastric microbiota in Correa's cascade was confirmed, and genotoxic colonies were finally identified. In this process, the correlations between the gastric microbiota, gastric cancer, and specific pathogenic bacteria were gradually identified. The application of specific microbiota in the clinical identification of GC patients was gradually supported. Subsequently, through PICRUSt analysis and other means, the role of different gastric microorganisms in the progression of GC was gradually revealed. The article published by Ferreira RM et al. in *Gut* in 2018 (doi: 10.1136/gutjnl-2017-314205) had the highest citation frequency and the highest LCS and GCS, indicating that it was widely recognized by researchers as an important research basis. The researchers conducted a retrospective analysis of the gastric microbiota of patients with GC and chronic gastritis. They found that the diversity of the gastric microbiota was reduced in GC patients, and other bacterial genera, mainly intestinal commensal bacteria, were enriched, showing community characteristics different from chronic gastritis patients. This article revealed that gastric microbiota dysbiosis is related to GC and proved that the microbial dysbiosis index (MDI) can be used to identify GC, with the area under the curve (AUC) being 0.91 and 0.89, respectively (Ferreira et al., 2018). "Mucosal microbiome dysbiosis in gastric carcinogenesis," published by Coker OO et al. (doi: 10.1136/gutjnl-2017-314281) close to the same period, also proved that there are differences in the gastric microbial composition and bacterial interactions during the progression from chronic gastritis to GC, and the correlation strengths between enriched groups and reduced groups increased ($p < 0.001$; Coker et al., 2018).

The visualization and clustering of keywords also reveal the evolution of the research topic. The timeline graph and thematic evolution analysis showed that although many important keywords such as "*Helicobacter pylori*" and "gastric cancer" have emerged in the early years, this research field is still developing and the topics described by these keywords are still being studied. In addition, topics that have begun to receive attention in recent years, such as "tumor microenvironment" and "stomach microbiota", may be the focus of future research. In this research field, *H. pylori*, as a major member of the gastric microbiota, receives constant attention. Keyword analysis showed that "*helicobacter-pylori*", "infection", "eradication", "proton pump inhibitors", "regulatory T cells", "CagA", etc. were important keywords. As *H. pylori* is associated with GC and has a high infection rate in the general population, the diagnostic approaches, eradication methods, and carcinogenesis mechanisms for *H. pylori* have been extensively studied (Yang et al., 2014; Ansari and Yamaoka, 2022). However, current *H. pylori* eradication therapies face challenges involving antibiotic resistance (Ansari and Yamaoka, 2022; Suzuki et al., 2022), dysbiosis (Gotoda et al., 2018, 2020), the potential danger of long-term PPI use, and other shortcomings (Kuipers et al., 1996; Xu et al., 2013; Jiang et al., 2019; McCarthy, 2020; Seo et al., 2021; Arai et al., 2023). Recent studies have shown that *H. pylori* eradication regimens based on Vonoprazan (VPZ) are effective and safe (Kakiuchi et al., 2023) and have a higher *H. pylori* eradication rate than PPI-containing triple or quadruple therapy (Chey et al., 2022). The

dual therapy consisting of VPZ and amoxicillin has a low incidence of adverse reactions and can also avoid unnecessary use of antibiotics, reducing the incidence of dysbiosis of the intestinal microbiota (Ouyang et al., 2022; Zhang J. et al., 2023). The dosage regimen of VPZ and the adverse events associated with its strong acid-suppressive effect still need further evaluation (Hu et al., 2022; Arai et al., 2023). In addition, the eradication of *H. pylori* might not be necessary for children because infection by *H. pylori* has a protective effect against asthma and inflammatory bowel disease via the systemic immune tolerance induced by dendritic cells (DCs) and regulatory T cells (Treg cells; Ravikumara, 2023). *Helicobacter pylori* infection or seropositivity have a detrimental impact on the efficacy of cancer immunotherapies, and the eradication of *H. pylori* infection by antibiotherapy does not revert the *H. pylori*-induced hyporesponsiveness to cancer immunotherapy (Che et al., 2022; Oster et al., 2022a,b; Gong et al., 2023). However, another meta-analysis has suggested that GC patients with *H. pylori* infection may respond better to PD-1/PD-L1 blockade therapy (Zhu et al., 2023). These divergent findings may be explained by differences in patients and treatment characteristics, as well as potential confounding factors. Thus, many experts are calling for a more individualized eradication approach in the context of additional risk factors rather than unconditionally eradicating *H. pylori* in every case. Clarifying the pathogenesis of *H. pylori* is still an important task. *Helicobacter pylori* infection triggers complex chronic immune responses, leading to the occurrence of a variety of diseases. *Helicobacter pylori* virulence factors such as cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA), and *Helicobacter pylori* neutrophil-activating protein (HP-NAP) significantly affect the function of DCs in tumor microenvironment or bone marrow-derived DCs and play an important role in the induction of GC (Fu and Lai, 2023; Raspe et al., 2023; Yuan et al., 2023). The Treg cell-mediated inflammatory response caused by *H. pylori* infection (Bagheri et al., 2016, 2018; Owyang et al., 2020) and the genome instability caused by CagA (Bagheri et al., 2018; Takahashi-Kanemitsu et al., 2020; Alipour, 2021; Imai et al., 2021; Murata-Kamiya and Hatakeyama, 2022; Marshall, 2023) have been extensively studied. Recent research has shown that *H. pylori* can inhibit miRNA-375 expression in the stomach. Downregulated miR-375 activates the JAK2-STAT3 pathway, which then promotes the secretion of IL-6, IL-10, and VEGF, leading to the immature differentiation of DCs and the induction of GC (Zhang et al., 2021). Notch signaling regulates the function and phenotype of DCs, thus mediating the differentiation of $CD4^+$ T cells during *H. pylori* infection (Liu et al., 2023).

As 16s rRNA sequencing techniques developed and gut microbiome research boomed with the confirmation of the relationship between the gastric microbiota and gastric carcinogenesis, researchers began to turn their attention to the study of the gastric microbiota. In this respect, "gut microbiota", "colonization", "16s rRNA sequencing", "risk", "atrophic gastritis", and "intestinal metaplasia" are important keywords, indicating that the clinical application of gastric microbiota in GC risk assessment and treatment are also current hotspots. As *H. pylori* is a bacterium that affects and is affected by the gastric microbiota, it is closely related to other bacteria in the progression of GC. Non-*H. pylori* microorganisms interact with *H. pylori* in gastric carcinogenesis (Guo et al., 2023). Successful *H. pylori* eradication can reverse gastric microbiota dysbiosis (Guo et al., 2020; Guo Q. et al., 2022), and its high eradication rate is related to specific flora members (Niu et al., 2021). The intestinal microbiota of *H. pylori*-positive GC patients is also transformed, and this may further contribute to GC (Gao et al., 2018; Dash et al., 2019; Seol et al., 2019; Iino and Shimoyama, 2021). Modulation of the gastrointestinal microbiota is beneficial to the eradication of *H. pylori* and the treatment of gastric diseases related to microbial dysbiosis (Viazis et al., 2022; Musazadeh et al., 2023; Zhang L. et al., 2023). The study of specific strains or pathogenic pathways of non-*H. pylori* bacteria in GC progression is helpful in identifying relevant treatment measures accordingly. Using 16s rRNA sequencing technology to analyze gastric epithelial bacteria at different stages of GC progression, it was found that some bacterial taxa, such as *Peptostreptococcus stomatis*, *Streptococcus anginosus*, *Parvimonas micra*, etc., were significantly enriched in GC patients and could be used to identify precancerous lesions and GC (Coker et al., 2018; Liu et al., 2022). Microbial taxonomic features (MTFs) can be used to predict early gastric neoplasia (EGN; Png et al., 2022) and may improve the accuracy of the polygenic risk score (PRS) model in predicting GC (Wang et al., 2023). As for the mechanism, many studies have revealed that non-*H. pylori* microorganisms promote GC by inducing inflammation, modulating the immune response, triggering DNA damage, and promoting epithelial–mesenchymal transformation (Yang et al., 2022; Liao et al., 2023). Gastric non-*H. pylori* microorganisms may participate in the progression of GC by affecting host DNA methylation (Yue et al., 2023). Different bacterial taxa are related to certain types of infiltrating immune cells (Liao et al., 2023). In the gastric microbiota associated with atrophy/intestinal metaplasia, functional pathways such as amino acid metabolism and inositol phosphate metabolism are enriched, while folate biosynthesis and NOD-like receptor signaling are reduced, which may explain the ongoing progression of precancerous conditions even after *H. pylori* eradication (Sung et al., 2020). Research on the intestinal microbiota continuously activating host immunity and producing a variety of metabolites has illuminated its effect on GC (Nasr et al., 2020; Guo Y. et al., 2022), while the GC microflora can modulate macrophages and enhance gastric tumor development by suppressing antitumor immunity, activating oncogenic signaling pathways, and producing protumor metabolites (Zhang W. et al., 2023).

Through emergent analysis, we can speculate that the role of *Fusobacterium nucleatum* (*F. nucleatum*) in the Correa cascade of GC development may become a research hotspot in the future. *Fusobacterium nucleatum*, which exists in the oral cavity and gastrointestinal tract of humans, is an opportunistic pathogen causing systematic diseases, for example, gastrointestinal cancers (Chen et al., 2022; He Z. et al., 2022). A number of studies have demonstrated the potential pathogenic role of *F. nucleatum* in colorectal cancer (CRC; Lee et al., 2019). *Fusobacterium nucleatum* causes CRC by adhering and forming biofilm, invading host cells, producing metabolites, and releasing vesicles (Chen et al., 2022). *Fusobacterium nucleatum* promotes CRC metastasis through M2 polarization of macrophages in the tumor microenvironment (Chen et al., 2018; Xu et al., 2021). However, its roles in GC are not so clear. In a study using *Clostridium* and *Fusobacterium nucleatum* in biopsy tissue to diagnose GC, the sensitivity reached 100%, the specificity was 68.8%, and the AUC was 0.875 (Hsieh et al., 2018). The combined colonization of *F. nucleatum* and *Helicobacter pylori* has also been associated with a poor survival rate in late-stage GC patients treated with gastrectomy, suggesting that it may promote the progression or metastasis of GC by synergizing with *H. pylori* (Hsieh et al., 2021, 2023). *Fusobacterium nucleatum* has

strong interactions with *Porphyromonas*, *Prevotella*, etc., which may lead to shortened survival (Nie et al., 2021; Lehr et al., 2023). A bioinformatic analysis suggested that neutrophil transcriptional activation induced by *F. nucleatum* may be implicated in the occurrence of GC through several candidate genes, including DNAJB1, EHD1, IER2, CANX, and PH4B. Functional analysis showed that membrane-bound organelle dysfunction, intracellular trafficking, transcription factors ER71 and Sp1, and miR580 and miR155 were other candidate mechanisms (Zhou et al., 2022). The metabolic function analysis showed that *F. nucleatum*-positive GC tissues were significantly enriched in the biosynthesis of lysine, peptidoglycan, and tRNA metabolic functions (Nie et al., 2021). These results await further verification. Existing studies have demonstrated the role of *F. nucleatum* in the ERBB2-PIK3-AKT–mTOR pathway and the miR-885-3p/EphB2/PI3K/AKT axis (Hsieh et al., 2023; Xin et al., 2023). However, some researchers have questioned the actual role of *F. nucleatum* in gastric carcinogenesis. An *F. nucleatum*-positive result was associated with poor prognosis in patients with Lauren's diffuse type GC but had no association with the prognosis of intestinal-type GC. These results still remain to be confirmed (Boehm et al., 2020; Nie et al., 2021). *Fusobacterium nucleatum* may promote carcinogenesis via *Fusobacterium* adhesionA (FadA), which binds to E-cadherin, activating Wnt/β-catenin signaling and various inflammatory and oncogenic properties of the cells (Rubinstein et al., 2013, 2019). Since the diffuse type of GC is strongly associated with E-cadherin deregulation, one may speculate on the potential molecular mimicry and specific prognostic relevance of *F. nucleatum* to the diffuse type of GC (Boehm et al., 2020). Furthermore, the interaction of *F. nucleatum* with non-*H. pylori* gastric microorganisms also requires more explanation.

This study has some limitations. Only relevant studies published by WoSCC were included in this study; cutting-edge research with high quality from other databases such as PubMed and Scopus might have been ignored. Second, the language was restricted to English, which may have excluded high-quality literature in other languages. In addition, the co-citation frequency of the literature is time-dependent, and since the research in this field is still in the developing stage, the number of citations cannot accurately reflect the importance of the document, especially important documents published in recent years. With the exponential growth of publications in this field, our research needs to be constantly updated to keep up with the latest research developments. Finally, due to missing keywords reported by Biblioshiny in some literature, the outcome of the CiteSpace keyword analysis might be a little inaccurate; however, we used different bibliometric software to conduct our analysis and verify the results.

5 Conclusion

To the best of our knowledge, this study is the first to use visualization software and data mining methods to conduct a bibliometric analysis of publications in the field of gastric microbiota and gastric cancer and to determine the research status, hotspots, and development trends in this field. Research in this field has mainly focused on the eradication therapy and pathogenic mechanisms of *Helicobacter pylori*, as well as the utilization of gastric microbiota in the evaluation and treatment of gastric cancer. Future research hotspots may include the use of the gastric microorganisms represented by *Fusobacterium nucleatum* in the diagnosis of and for therapeutic effects on gastric cancer. Their mechanisms of action need to be further explored in order to provide a theoretical basis for clinical application. Relevant researchers or researchers outside this field can use this study to improve their awareness and understanding of the field and to gain some perspectives for further research.

Data availability statement

All data used for our research is available through our retrieval query. Further inquiries can be directed to the corresponding author.

Author contributions

YK: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. CT: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. JZ: Formal analysis, Investigation, Methodology, Writing – review & editing. WD: Conceptualization, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1341012/full#supplementary-material

References

Alipour, M. (2021). Molecular mechanism Of*helicobacter pylori*-induced gastric Cancer. *J. Gastrointest. Cancer* 52, 23–30. doi: 10.1007/s12029-020-00518-5

Ansari, S., and Yamaoka, Y. (2022). *Helicobacter pylori* infection, its laboratory diagnosis, and antimicrobial resistance: a perspective of clinical relevance. *Clin. Microbiol. Rev.* 35:e0025821. doi: 10.1128/cmr.00258-21

Arai, J., Hayakawa, Y., Niikura, R., Ihara, S., Aoki, T., Honda, T., et al. (2023). Letter: potassium-competitive acid blockers may increase the risk of gastric cancer after *Helicobacter pylori* eradication a retrospective multicentre-cohort analysis. *Aliment. Pharmacol. Ther.* 57, 1196–1198. doi: 10.1111/apt.17461

Aria, M., and Cuccurullo, C. (2017). *Bibliometrix*: an R-tool for comprehensive science mapping analysis. *J. Informet.* 11, 959–975. doi: 10.1016/j.joi.2017.08.007

Arnold, M., Park, J. Y., Camargo, M. C., Lunet, N., Forman, D., and Soerjomataram, I. (2020). Is gastric cancer becoming a rare disease? A global assessment of predicted incidence trends to 2035. *Gut* 69, 823–829. doi: 10.1136/gutjnl-2019-320234

Aviles-Jimenez, F., Vazquez-Jimenez, F., Medrano-Guzman, R., Mantilla, A., and Torres, J. (2014). Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer. *Sci. Rep.* 4:4202. doi: 10.1038/srep04202

Bagheri, N., Azadegan-Dehkordi, F., Rahimian, G., Rafieian-Kopaei, M., and Shirzad, H. (2016). Role of regulatory T-cells in different clinical expressions of *Helicobacter pylori* infection. *Arch. Med. Res.* 47, 245–254. doi: 10.1016/j.arcmed.2016.07.013

Bagheri, N., Salimzadeh, L., and Shirzad, H. (2018). The role of T helper 1-cell response in *Helicobacter pylori*-infection. *Microb. Pathog.* 123, 1–8. doi: 10.1016/j.micpath.2018.06.033

Bik, E. M., Eckburg, P. B., Gill, S. R., Nelson, K. E., Purdom, E. A., Francois, F., et al. (2006). Molecular analysis of the bacterial microbiota in the human stomach. *Proc. Natl. Acad. Sci. U. S. A.* 103, 732–737. doi: 10.1073/pnas.0506655103

Boehm, E. T., Thon, C., Kupcinskas, J., Steponaitiene, R., Skieceviciene, J., Canbay, A., et al. (2020). *Fusobacterium nucleatum* is associated with worse prognosis in Lauren's diffuse type gastric cancer patients. *Sci. Rep.* 10:16240. doi: 10.1038/s41598-020-73448-8

Castano-Rodriguez, N., Goh, K., Fock, K. M., Mitchell, H. M., and Kaakoush, N. O. (2017). Dysbiosis of the microbiome in gastric carcinogenesis. *Sci. Rep.* 7:15957. doi: 10.1038/s41598-017-16289-2

Che, H., Xiong, Q., Ma, J., Chen, S., Wu, H., Xu, H., et al. (2022). Association of *Helicobacter pylori* infection with survival outcomes in advanced gastric cancer patients treated with immune checkpoint inhibitors. *BMC Cancer* 22:904. doi: 10.1186/s12885-022-10004-9

Chen, C. M. (2006). CiteSpace II: detecting and visualizing emerging trends and transient patterns in scientific literature. *J. Am. Soc. Inf. Sci. Technol.* 57, 359–377. doi: 10.1002/asi.20317

Chen, C., Hu, Z., Liu, S., and Tseng, H. (2012). Emerging trends in regenerative medicine: a scientometric analysis in CiteSpace. *Expert Opin. Biol. Ther.* 12, 593–608. doi: 10.1517/14712598.2012.674507

Chen, Y., Huang, Z., Tang, Z., Huang, Y., Huang, M., Liu, H., et al. (2022). More than just a periodontal pathogen -the research Progress on *Fusobacterium nucleatum*. *Front. Cell. Infect. Microbiol.* 12:5318. doi: 10.3389/fcimb.2022.815318

Chen, T., Li, Q., Wu, J., Wu, Y., Peng, W., Li, H., et al. (2018). *Fusobacterium nucleatum* promotes M2 polarization of macrophages in the microenvironment of colorectal tumours via a TLR4-dependent mechanism. *Cancer Immunol. Immunother.* 67, 1635–1646. doi: 10.1007/s00262-018-2233-x

Chen, J., Nie, S., Qiu, X., Zheng, S., Ni, C., Yuan, Y., et al. (2023). Leveraging existing 16S rRNA microbial data to identify diagnostic biomarker in Chinese patients with gastric cancer: a systematic meta-analysis. *MSystems* 8:e74723:e0074723. doi: 10.1128/msystems.00747-23

Chey, W. D., Megraud, F., Laine, L., Lopez, L. J., Hunt, B. J., and Howden, C. W. (2022). Vonoprazan triple and dual therapy for *Helicobacter pylori* infection in the United States and Europe: randomized clinical trial. *Gastroenterology* 163, 608–619. doi: 10.1053/j.gastro.2022.05.055

Cobo, M. J., Lopez-Herrera, A. G., Herrera-Viedma, E., and Herrera, F. (2011). Science mapping software tools: review, analysis, and cooperative study among tools. *J. Am. Soc. Inf. Sci. Technol.* 62, 1382–1402. doi: 10.1002/asi.21525

Coker, O. O., Dai, Z., Nie, Y., Zhao, G., Cao, L., Nakatsu, G., et al. (2018). Mucosal microbiome dysbiosis in gastric carcinogenesis. *Gut* 67, 1024–1032. doi: 10.1136/gutjnl-2017-314281

Correa, P. (1988). A human model of gastric carcinogenesis. *Cancer Res.* 48, 3554–3560.

Correa, P. (1992). Human gastric carcinogenesis: a multistep and multifactorial process--first American Cancer Society award lecture on Cancer epidemiology and prevention. *Cancer Res.* 52, 6735–6740.

Dash, N. R., Khoder, G., Nada, A. M., and Al Bataineh, M. T. (2019). Exploring the impact of *Helicobacter pylori* on gut microbiome composition. *PLoS One* 14:e0218274. doi: 10.1371/journal.pone.0218274

Donthu, N., Kumar, S., Mukherjee, D., Pandey, N., and Lim, W. M. (2021). How to conduct a bibliometric analysis: an overview and guidelines. *J. Bus. Res.* 133, 285–296. doi: 10.1016/j.jbusres.2021.04.070

Dracos, A., and Cognetti, G. (1995). Scientific literature: bibliometric and bibliographic indicators as integrative criteria for an objective evaluation of research activity. *Annali dell'Istituto Superiore Di Sanita* 31, 381–390.

Esfahani, H., Tavasoli, K., and Jabbarzadeh, A. (2019). Big data and social media: a scientometrics analysis. *Int J Data Netw Sci* 3, 145–164. doi: 10.5267/j.ijdns.2019.2.007

Fan, X., Qin, X., Zhang, Y., Li, Z., Zhou, T., Zhang, J., et al. (2021). Screening for gastric cancer in China: advances, challenges and visions. *Chin. J. Cancer Res.* 33, 168–180. doi: 10.21147/j.issn.1000-9604.2021.02.05

Ferreira, R. M., Pereira-Marques, J., Pinto-Ribeiro, I., Costa, J. L., Carneiro, F., Machado, J. C., et al. (2018). Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. *Gut* 67, 226–236. doi: 10.1136/gutjnl-2017-314205

Fu, H., and Lai, Y. (2023). The role of *Helicobacter pylori* neutrophil-activating protein in the pathogenesis of *H. Pylori* and beyond: from a virulence factor to therapeutic targets and therapeutic agents. *Int. J. Mol. Sci.* 24:91. doi: 10.3390/ijms24010091

Gao, J., Zhang, Y., Gerhard, M., Mejias-Luque, R., Zhang, L., Vieth, M., et al. (2018). Association between gut microbiota and *Helicobacter pylori*-related gastric lesions in a high-risk population of gastric Cancer. *Front. Cell. Infect. Microbiol.* 8:202. doi: 10.3389/fcimb.2018.00202

Gong, X., Shen, L., Xie, J., Liu, D., Xie, Y., and Liu, D. (2023). *Helicobacter pylori* infection reduces the efficacy of cancer immunotherapy: a systematic review and meta-analysis. *Helicobacter* 28:e13011. doi: 10.1111/hel.13011

Gotoda, T., Kusano, C., Suzuki, S., Horii, T., Ichijima, R., and Ikehara, H. (2020). Clinical impact of vonoprazan-based dual therapy with amoxicillin For*h. pylori*infection in a treatment-naive cohort of junior high school students in Japan. *J. Gastroenterol.* 55, 969–976. doi: 10.1007/s00535-020-01709-4

Gotoda, T., Takano, C., Kusano, C., Suzuki, S., Ikehara, H., Hayakawa, S., et al. (2018). Gut microbiome can be restored without adverse events after *Helicobacter pylori* eradication therapy in teenagers. *Helicobacter* 23:e12541. doi: 10.1111/hel.12541

Guo, Y., Cao, X. S., Guo, G. Y., and Zhou, M. G. Y. (2022). Effect of *Helicobacter Pylori* eradication on human gastric microbiota: a systematic review and Meta-analysis. *Front. Cell. Infect. Microbiol.* 12:899248. doi: 10.3389/fcimb.2022.899248

Guo, Y., Cao, X., Zhou, M., and Yu, B. (2023). Gastric microbiota in gastric cancer: different roles of Helicobacter pylori and other microbes. *Front. Cell. Infect. Microbiol.* 12:5811. doi: 10.3389/fcimb.2022.1105811

Guo, Q., Qin, H., Liu, X., Zhang, X., Chen, Z., Qin, T., et al. (2022). The emerging roles of human gut microbiota in gastrointestinal Cancer. *Front. Immunol.* 13:5047. doi: 10.3389/fimmu.2022.915047

Guo, Y., Zhang, Y., Gerhard, M., Gao, J., Mejias-Luque, R., Zhang, L., et al. (2020). Effect of *Helicobacter pylori* on gastrointestinal microbiota: a population-based study in Linqu, a high-risk area of gastric cancer. *Gut* 69, 1598–1607. doi: 10.1136/gutjnl-2019-319696

He, C., Peng, C., Xu, X., Li, N., Ouyang, Y., Zhu, Y., et al. (2022). Probiotics mitigate *Helicobacter pylori*-induced gastric inflammation and premalignant lesions in INS-GAS mice with the modulation of gastrointestinal microbiota. *Helicobacter* 27:e12898. doi: 10.1111/hel.12898

He, Z., Tian, W., Wei, Q., and Xu, J. (2022). Involvement of *Fusobacterium nucleatum* in malignancies except for colorectal cancer: a literature review. *Front. Immunol.* 13:649. doi: 10.3389/fimmu.2022.968649

Hsieh, Y., Kuo, W., Hsu, W., Tung, S., and Li, C. (2023). *Fusobacterium Nucleatum*-induced tumor mutation burden predicts poor survival of gastric Cancer patients. *Cancers* 15:269. doi: 10.3390/cancers15010269

Hsieh, Y., Tung, S., Pan, H., Chang, T., Wei, K., Chen, W., et al. (2021). *Fusobacterium nucleatum* colonization is associated with decreased survival of *helicobacter pylori*-positive gastric cancer patients. *World J. Gastroenterol.* 27, 7311–7323. doi: 10.3748/wjg.v27.i42.7311

Hsieh, Y., Tung, S., Pan, H., Yen, C., Xu, H., Lin, Y., et al. (2018). Increased abundance of Clostridium and Fusobacterium in gastric microbiota of patients with gastric Cancer in Taiwan. *Sci. Rep.* 8:158. doi: 10.1038/s41598-017-18596-0

Hu, Y., Xu, X., Ouyang, Y., He, C., Li, N., Xie, C., et al. (2022). Optimization of vonoprazan-amoxicillin dual therapy for eradicating *Helicobacter pylori*infection in China: a prospective, randomized clinical pilot study. *Helicobacter* 27:e12896. doi: 10.1111/hel.12896

Iino, C., and Shimoyama, T. (2021). Impact of *Helicobacter pylori* infection on gut microbiota. *World J. Gastroenterol.* 27, 6224–6230. doi: 10.3748/wjg.v27.i37.6224

Imai, S., Ooki, T., Murata-Kamiya, N., Komura, D., Tahmina, K., Wu, W., et al. (2021). *Helicobacter pylori* CagA elicits BRCAness to induce genome instability that may underlie bacterial gastric carcinogenesis. *Cell Host Microbe* 29, 941–958.e10. doi: 10.1016/j.chom.2021.04.006

Jiang, K., Jiang, X., Wen, Y., Liao, L., and Liu, F. (2019). Relationship between long-term use of proton pump inhibitors and risk of gastric cancer: a systematic analysis. *J. Gastroenterol. Hepatol.* 34, 1898–1905. doi: 10.1111/jgh.14759

Kakiuchi, T., Matsuo, M., Endo, H., Sakata, Y., Esaki, M., Noda, T., et al. (2023). Efficacy and safety of vonoprazan-based regimen for *Helicobacter pylori* eradication therapy in Japanese adolescents: a prospective multicenter study. *J. Gastroenterol.* 58, 196–204. doi: 10.1007/s00535-022-01942-z

Kokol, P., Blazun Vosner, H., and Zavrsnik, J. (2021). Application of bibliometrics in medicine: a historical bibliometrics analysis. *Health Info. Libr. J.* 38, 125–138. doi: 10.1111/hir.12295

Kokol, P., Kokol, M., and Zagoranski, S. (2022). Machine learning on small size samples: a synthetic knowledge synthesis. *Sci. Prog.* 105:003685042110297. doi: 10.1177/00368504211029777

Kuipers, E. J. (1998). Review article: relationship between *Helicobacter pylori*, atrophic gastritis and gastric cancer. *Aliment. Pharmacol. Ther.* 12, 25–36. doi: 10.1111/j.1365-2036.1998.00009.x

Kuipers, E. J., Lundell, L., KlinkenbergKnol, E. C., Havu, N., Festen, H., Liedman, B., et al. (1996). Atrophic gastritis and *Helicobacter pylori* infection in patients with reflux esophagitis treated with omeprazole or fundoplication. *N. Engl. J. Med.* 334, 1018–1022. doi: 10.1056/NEJM199604183341603

Kwon, S., Park, J. C., Kim, K. H., Yoon, J., Cho, Y., Lee, B., et al. (2022). Human gastric microbiota transplantation recapitulates premalignant lesions in germ-free mice. *Gut* 71, 1266–1276. doi: 10.1136/gutjnl-2021-324489

Lee, S. A., Liu, F., Riordan, S. M., Lee, C. S., and Zhang, L. (2019). Global investigations of *Fusobacterium nucleatum* in human colorectal Cancer. *Front. Oncol.* 9:566. doi: 10.3389/fonc.2019.00566

Lehr, K., Nikitina, D., Vilchez-Vargas, R., Steponaitiene, R., Thon, C., Skieceviciene, J., et al. (2023). Microbial composition of tumorous and adjacent gastric tissue is associated with prognosis of gastric cancer. *Sci. Rep.* 13:4640. doi: 10.1038/s41598-023-31740-3

Lei, R., Zhang, J., Zhou, H., Liu, Q., Wang, H., and Liu, J. (2020). Analysis of population health projects funded by joint Fund of the National Natural Science Foundation of China between 2015 and 2019. *Annals Transl Med* 8:1477. doi: 10.21037/atm-20-6495

Li, Y., Choi, H., Leung, K., Jiang, F., Graham, D. Y., and Leung, W. K. (2023). Global prevalence of *Helicobacter pylori* infection between 1980 and 2022: a systematic review and meta-analysis. *Lancet Gastroenterol. Hepatol.* 8, 553–564. doi: 10.1016/S2468-1253(23)00070-5

Liao, O., Ye, G., Du, Q., and Ye, J. (2023). Gastric microbiota in gastric cancer and precancerous stages: mechanisms of carcinogenesis and clinical value. *Helicobacter* 28:e12964. doi: 10.1111/hel.12964

Liu, Q., Chen, C., He, Y., Mai, W., Ruan, S., Ning, Y., et al. (2023). Notch signaling regulates the function and phenotype of dendritic cells in *Helicobacter pylori* infection. *Microorganisms* 11:2818. doi: 10.3390/microorganisms11112818

Liu, C., Ng, S. K., Ding, Y., Lin, Y., Liu, W., Wong, S. H., et al. (2022). Meta-analysis of mucosal microbiota reveals universal microbial signatures and dysbiosis in gastric carcinogenesis. *Oncogene* 41, 3599–3610. doi: 10.1038/s41388-022-02377-9

Lofgren, J. L., Whary, M. T., Ge, Z., Muthupalani, S., Taylor, N. S., Mobley, M., et al. (2011). Lack of commensal Flora in *Helicobacter pylori*-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia. *Gastroenterology* 140, 210–220.e4. doi: 10.1053/j.gastro.2010.09.048

Marshall, B. (2023). Epidemiology of Helicobacter in Chinese families: a foundation for cost-effective eradication strategies? *Gut*:29:gutjnl-2023-329786. doi: 10.1136/gutjnl-2023-329786

McCarthy, D. M. (2020). Proton pump inhibitor use, Hypergastrinemia, and gastric carcinoids-what is the relationship? *Int. J. Mol. Sci.* 21:662. doi: 10.3390/ijms21020662

Moral-Munoz, J. A., Herrera-Viedma, E., Santisteban-Espejo, A., and Cobo, M. J. (2020). Software tools for conducting bibliometric analysis in science: an up-to-date review. *Profesional e La Informacion* 29:3. doi: 10.3145/epi.2020.ene.03

Murata-Kamiya, N., and Hatakeyama, M. (2022). *Helicobacter pylori*-induced DNA double-stranded break in the development of gastric cancer. *Cancer Sci.* 113, 1909–1918. doi: 10.1111/cas.15357

Musazadeh, V., Nazari, A., Faghfouri, A. H., Emami, M., Kavyani, Z., Zokaei, M., et al. (2023). The effectiveness of treatment with probiotics in *Helicobacter pylori* eradication: −−/esults from an umbrella meta-analysis on meta-analyses of randomized controlled trials. *Food Funct.* 14, 7654–7662. doi: 10.1039/d3fo00300k

Nasr, R., Shamseddine, A., Mukherji, D., Nassar, F., and Temraz, S. (2020). The crosstalk between microbiome and immune response in gastric Cancer. *Int. J. Mol. Sci.* 21:6586. doi: 10.3390/ijms21186586

Nie, S., Wang, A., and Yuan, Y. (2021). Comparison of clinicopathological parameters, prognosis, micro-ecological environment and metabolic function of gastric Cancer with or without Fusobacterium sp. infection. *J. Cancer* 12, 1023–1032. doi: 10.7150/jca.50918

Niu, Z., Li, S., Shi, Y., and Xue, Y. (2021). Effect of gastric microbiota on quadruple *Helicobacter pylori* eradication therapy containing bismuth. *World J. Gastroenterol.* 27, 3913–3924. doi: 10.3748/wjg.v27.i25.3913

Oster, P., Vaillant, L., McMillan, B., and Velin, D. (2022a). The efficacy of Cancer immunotherapies is compromised by *Helicobacter pylori* infection. *Front. Immunol.* 13:161. doi: 10.3389/fimmu.2022.899161

Oster, P., Vaillant, L., Riva, E., McMillan, B., Begka, C., Truntzer, C., et al. (2022b). *Helicobacter pylori* infection has a detrimental impact on the efficacy of cancer immunotherapies. *Gut* 71, 457–466. doi: 10.1136/gutjnl-2020-323392

Ouyang, Y., Wang, M., Xu, Y., Zhu, Y., Lu, N., and Hu, Y. (2022). Amoxicillin-vonoprazan dual therapy for *Helicobacter pylori* eradication: a systematic review and meta-analysis. *J. Gastroenterol. Hepatol.* 37, 1666–1672. doi: 10.1111/jgh.15917

Owyang, S. Y., Zhang, M., El-Zaatari, M., Eaton, K. A., Bishu, S., Hou, G., et al. (2020). Dendritic cell-derived TGF-β mediates the induction of mucosal regulatory T-cell response to Helicobacter infection essential for maintenance of immune tolerance in mice. *Helicobacter* 25:e12763. doi: 10.1111/hel.12763

Png, C. W., Lee, W. J. J., Chua, S. J., Zhu, F., Yeoh, K. G., and Zhang, Y. (2022). Mucosal microbiome associates with progression to gastric cancer. *Theranostics* 12, 48–58. doi: 10.7150/thno.65302

Qi, J., Li, M., Wang, L., Hu, Y., Liu, W., Long, Z., et al. (2023). National and subnational trends in cancer burden in China, 2005-20: an analysis of national mortality surveillance data. *Lancet Public Health* 8, E943–E955. doi: 10.1016/S2468-2667(23)00211-6

Raspe, J., Contoyannis, A., Schmitz, M., Uebner, H., Cover, T., Mueller, A., et al. (2023). *Helicobacter pylori* protein vacuolating Cytotoxin a (VacA) modulates the murine and human interaction between dendritic cells and T cells. *Pneumologie* 771:S71. doi: 10.1055/s-0043-1761034

Ravikumara, M. (2023). *Helicobacter pylori* in children: think before you kill the bug! *Therap. Adv. Gastroenterol.* 16:610. doi: 10.1177/17562848231177610

Rubinstein, M. R., Baik, J. E., Lagana, S. M., Han, R. P., Raab, W. J., Sahoo, D., et al. (2019). *Fusobacterium nucleatum* promotes colorectal cancer by inducing Wnt/β-catenin modulator Annexin A1. *EMBO Rep.* 20:638. doi: 10.15252/embr.201847638

Rubinstein, M. R., Wang, X., Liu, W., Hao, Y., Cai, G., and Han, Y. W. (2013). *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA Adhesin. *Cell Host Microbe* 14, 195–206. doi: 10.1016/j.chom.2013.07.012

Rugge, M., Meggio, A., Pravadelli, C., Barbareschi, M., Fassan, M., Gentilini, M., et al. (2019). Gastritis staging in the endoscopic follow-up for the secondary prevention of gastric cancer: a 5-year prospective study of 1755 patients. *Gut* 68, 11–17. doi: 10.1136/gutjnl-2017-314600

Seo, S. I., Park, C. H., You, S. C., Kim, J. Y., Lee, K. J., Kim, J., et al. (2021). Association between proton pump inhibitor use and gastric cancer: a population-based cohort study using two different types of nationwide databases in Korea. *Gut* 70, 2066–2075. doi: 10.1136/gutjnl-2020-323845

Seol, M., Lee, Y. R., Kim, K. M., Shin, C. M., Yoon, H., Cho, J. H., et al. (2019). The difference of the gut microbiota of gastric cancer in relation to *Helicobacter pylori* negativity and positivity. *J. Clin. Oncol.* 37S. doi: 10.1200/JCO.2019.37.4_suppl.10

Singh, V., Singh, H., Dhiman, B., Kumar, N., and Singh, T. (2023). Analyzing bibliometric and thematic patterns in the transition to sustainable transportation: uncovering the influences on electric vehicle adoption. *Res. Transp. Bus. Manag.* 50:101033. doi: 10.1016/j.rtbm.2023.101033

Sonnenberg, A. (2022). Epidemiology of *Helicobacter pylori*. *Aliment. Pharmacol. Ther.* 55, S1–S13. doi: 10.1111/apt.16592

Sung, J., Coker, O. O., Chu, E., Szeto, C. H., Luk, S., Lau, H., et al. (2020). Gastric microbes associated with gastric inflammation, atrophy and intestinal metaplasia 1 year after *Helicobacter pylori* eradication. *Gut* 69, 1572–1581. doi: 10.1136/gutjnl-2019-319826

Sung, H., Ferlay, J., Siegel, R. L., 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. doi: 10.3322/caac.21660

Suzuki, S., Kusano, C., Horii, T., Ichijima, R., and Ikehara, H. (2022). The ideal *Helicobacter pylori* treatment for the present and the future. *Digestion* 103, 62–68. doi: 10.1159/000519413

Takahashi-Kanemitsu, A., Knight, C. T., and Hatakeyama, M. (2020). Molecular anatomy and pathogenic actions of *Helicobacter pylori* CagA that underpin gastric carcinogenesis. *Cell. Mol. Immunol.* 17, 50–63. doi: 10.1038/s41423-019-0339-5

Teng, Y., Xia, C., Li, H., Cao, M., Yang, F., Yan, X., et al. (2023). Cancer statistics for young adults aged 20 to 49 years in China from 2000 to 2017: a population-based registry study. *Sci. China Life Sci.* doi: 10.1007/s11427-023-2445-1

van Eck, N. J., and Waltman, L. (2010). Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* 84, 523–538. doi: 10.1007/s11192-009-0146-3

Viazis, N., Argyriou, K., Kotzampassi, K., Christodoulou, D. K., Apostolopoulos, P., Georgopoulos, S. D., et al. (2022). A four-probiotics regimen combined with a standard *Helicobacter pylori*-eradication treatment reduces side effects and increases eradication rates. *Nutrients* 14:632. doi: 10.3390/nu14030632

Wang, Z., Han, W., Xue, F., Zhao, Y., Wu, P., Chen, Y., et al. (2022). Nationwide gastric cancer prevention in China, 2021-2035: a decision analysis on effect, affordability and cost-effectiveness optimisation. *Gut* 71, 2391–2400. doi: 10.1136/gutjnl-2021-325948

Wang, X., Wang, L., Xu, L., Liang, S., Yu, M., Zhang, Q., et al. (2023). Evaluation of polygenic risk score for risk prediction of gastric cancer. *World J Gastrointest Oncol* 15, 276–285. doi: 10.4251/wjgo.v15.i2.276

Wong, B. C., Lam, S. K., Wong, W. M., Chen, J. S., Zheng, T. T., Feng, R. E., et al. (2004). *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 291, 187–194. doi: 10.1001/jama.291.2.187

Xin, Y., Li, X., Zhang, M., Shang, Z., Luo, Z., Wang, Y., et al. (2023). *Fusobacterium nucleatum*-induced exosomal HOTTIP promotes gastric cancer progression through the microRNA-885-3p/EphB2 axis. *Cancer Sci.* 114, 2360–2374. doi: 10.1111/cas.15781

Xu, W., Chen, G., Shao, Y., Li, X., Xu, H., Zhang, H., et al. (2013). Gastrin acting on the cholecystokinin2 receptor induces cyclooxygenase-2 expression through JAK2/STAT3/PI3K/Akt pathway in human gastric cancer cells. *Cancer Lett.* 332, 11–18. doi: 10.1016/j.canlet.2012.12.030

Xu, C., Fan, L., Lin, Y., Shen, W., Qi, Y., Zhang, Y., et al. (2021). *Fusobacterium nucleatum* promotes colorectal cancer metastasis through miR-1322/CCL20 axis and M2 polarization. *Gut Microbes* 13:347. doi: 10.1080/19490976.2021.1980347

Yang, J., Liu, X., Cheng, Y., Zhang, J., Ji, F., and Ling, Z. (2022). Roles of Plasmacytoid dendritic cells in gastric Cancer. *Front. Oncol.* 12:8314. doi: 10.3389/fonc.2022.818314

Yang, J., Lu, C., and Lin, C. (2014). Treatment of *Helicobacter pylori* infection: current status and future concepts. *World J. Gastroenterol.* 20, 5283–5293. doi: 10.3748/wjg.v20.i18.5283

Yuan, X., Zhang, Y., Zhao, X., Chen, A., and Liu, P. (2023). IL-1β, an important cytokine affecting *Helicobacter pylori*-mediated gastric carcinogenesis. *Microb. Pathog.* 174:105933. doi: 10.1016/j.micpath.2022.105933

Yue, K., Sheng, D., Xue, X., Zhao, L., Zhao, G., Jin, C., et al. (2023). Bidirectional mediation effects between Intratumoral microbiome and host DNA methylation changes contribute to stomach adenocarcinoma. *Microbiol Spectr* 11:e0090423. doi: 10.1128/spectrum.00904-23

Zhang, Z., Chen, S., Fan, M., Ruan, G., Xi, T., Zheng, L., et al. (2021). *Helicobacter pylori* induces gastric cancer via down-regulating miR-375 to inhibit dendritic cell maturation. *Helicobacter* 26:e12813. doi: 10.1111/hel.12813

Zhang, J., Hu, C., Zhang, R., Xu, J., Zhang, Y., Yuan, L., et al. (2023). The role of macrophages in gastric cancer. *Front. Immunol.* 14:2176. doi: 10.3389/fimmu.2023.1282176

Zhang, W., Lin, B., Li, Y., Ding, Y., Han, Z., and Ji, R. (2023). Efficacy and safety of Vonoprazan and amoxicillin dual therapy for *Helicobacter pylori* eradication: a systematic review and Meta-analysis. *Digestion* 104, 249–261. doi: 10.1159/000529622

Zhang, J., Yu, Q., Zheng, F., Long, C., Lu, Z., and Duan, Z. (2016). Comparing keywords plus of WOS and author keywords: a case study of patient adherence research. *J. Assoc. Inf. Sci. Technol.* 67, 967–972. doi: 10.1002/asi.23437

Zhang, L., Zhao, M., and Fu, X. (2023). Gastric microbiota dysbiosis and *Helicobacter pylori* infection. *Front. Microbiol.* 14:3269. doi: 10.3389/fmicb.2023.1153269

Zhou, T., Meng, X., Wang, D., Fu, W., and Li, X. (2022). Neutrophil transcriptional deregulation by the periodontal pathogen *Fusobacterium nucleatum* in gastric Cancer: a Bioinformatic study. *Dis. Markers* 2022, 9584507–9584510. doi: 10.1155/2022/9584507

Zhou, H., Yang, X., Liu, Q., Pu, J., and Lei, R. (2021). Distribution of the population and health projects of the joint Fund in China between 2006 and 2019. *Annals Transl Med* 9:1388. doi: 10.21037/atm-21-4364

Zhu, Y., Zhu, F., Ba, H., Chen, J., and Bian, X. (2023). *Helicobacter pylori* infection and PD-L1 expression in gastric cancer: a meta-analysis. *Eur. J. Clin. Invest.* 53:3880. doi: 10.1111/eci.13880

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Gut microbiome alterations during gastric cancer: evidence assessment of case–control studies

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Objectives: The study aims to systematically identify the alterations in gut microbiota that observed in gastric cancer through comprehensive assessment of case–control studies.

Methods: The systematic literature search of PubMed, Embase, Cochrane Library, and Web of Science was conducted to identify case–control studies that compared the microbiomes of individuals with and without gastric cancer. Quality of included studies was evaluated with the Newcastle-Ottawa Quality Assessment Scale (NOS). Meta-analyses utilized a random-effects model, and subgroup and sensitivity analyses were performed to assess study heterogeneity. All data analyses were performed using the "metan" package in Stata 17.0, and the results were described using log odds ratios (log ORs) with 95% confidence intervals (CIs).

Results: A total of 33 studies involving 4,829 participants were eligible for analysis with 29 studies provided changes in α diversity and 18 studies reported β diversity. Meta-analysis showed that only the Shannon index demonstrated statistical significance for α-diversity [−5.078 (−9.470, −0.686)]. No significant differences were observed at the phylum level, while 11 bacteria at genus-level were identified significant changed, e.g., increasing in *Lactobacillus* [5.474, (0.949, 9.999)] and *Streptococcus* [5.095, (0.293, 9.897)] and decreasing in *Porphyromonas* and *Rothia* with the same [−8.602, (−11.396, −5.808)]. Sensitivity analysis indicated that the changes of 9 bacterial genus were robust. Subgroup analyses on countries revealed an increasing abundance of *Helicobacter* and *Streptococcus* in Koreans with gastric cancer, whereas those with gastric cancer from Portugal had a reduced *Neisseria*. Regarding the sample sources, the study observed an increase in *Lactobacillus* and *Bacteroides* in the gastric mucosa of people with gastric cancer, alongside *Helicobacter* and *Streptococcus*. However, the relative abundance of *Bacteroides* decreased compared to the non-gastric cancer group, which was indicated in fecal samples.

Conclusion: This study identified robust changes of 9 bacterial genus in people with gastric cancer, which were country-/sample source-specific. Large-scale studies are needed to explore the mechanisms underlying these changes.

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KEYWORDS

gastric cancer, gastric microbiota, stomach, carcinogenesis, meta-analysis

1 Introduction

Gastric cancer, a prevalent and malignant tumor, is a major global health concern and one of the leading causes of cancer-related death (Sung et al., 2021). According to World Health Organization (2020), it ranked third in terms of cancer-related mortality worldwide. The development of gastric cancer involves multiple factors, including smoking, alcohol consumption, genetics, and alterations in the gut microbiota (Aviles-Jimenez et al., 2014; Rawla and Barsouk, 2019). The gut microbiome consists of a community of microorganisms that reside in the intestines, including bacteria, fungi, viruses, and other types of microorganisms. These communities of microbes perform crucial functions in human physiology and metabolism, including digestion and regulation of the immune system. Furthermore, they are closely linked to human health. In healthy individuals, the gut microbiota tends to remain stable. However, dysbiosis, an imbalance in the gut microbiota, can arise due to medication use, environmental changes, and dietary variation. Dysbiosis of the gut microbiota has been linked to the development of diverse ailments (Fan and Pedersen, 2021).

The relationship between gastric cancer and the gut microbiota has been a primary subject of investigation. Several studies indicate variations in the composition of the gut microbiota between gastric cancer and those without, implying a crucial role of the gut microbiota in the development of gastric cancer. However, the specific changes in bacterial composition vary between studies. Some studies suggest a decrease in microbial diversity (Coker et al., 2018; Peng et al., 2023), whereas others suggest an increase in diversity (Wang et al., 2016; Castaño-Rodríguez et al., 2017). Besides, the specific microbial species implicated in different studies also vary. For example, Castaño-Rodríguez et al. (2017) research detected an enrichment of *Lactococcus*, *Fusobacterium*, and *Veillonella* in gastric cancer compared to precancerous stages. Wei et al. (2023) found notable variations in the prevalence of *Streptococcus*, *Rhodococcus*, and *Ochrobactrum* between individuals with gastric cancer and healthy individuals. Meanwhile, Peng et al. (2023) indicated an increase in some genera such as *Lautropia* and *Lactobacillus*, and a decrease in others notably *Peptostreptococcus* and *Parvimonas* among the gastric cancer group in contrast to the control group. Additionally, the exact role of the gut microbiota in the development of gastric cancer remains the subject of ongoing debate. Although some researchers contend that alterations in the gut microbiota may be an independent risk factor for gastric cancer, others argue that it is a secondary factor. Lastly, differences in the source of samples, gene regions selected for sequencing, sequencing platforms, reference databases, and data analysis methods lead to variations in the results of different studies (Nearing et al., 2022). Thus, further research is essential to investigate the mentioned issues thoroughly. Meta-analysis is a possible method to address above issues by synthesizing published studies and combining the effects of different factors to produce more effective results.

Therefore, this study aims to fill the gaps of previous studies by meta-analysis to summarize research on changes in the gut microbiota of people with gastric cancer and without gastric cancer to elucidate microbial changes during gastric cancer development.

2 Materials and methods

2.1 Registration

The systematic review and meta-analysis was registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the registration number CRD42023437426, which was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (Liberati et al., 2009).

2.2 Data sources and search strategy

A systematic search was executed utilizing computerized bibliographic databases such as PubMed, Embase, Cochrane Library, and Web of Science, covering all records up until April 4, 2023. The search strategy combined MeSH and free terms using the Boolean operators "AND" and "OR." For instance, PubMed was searched with the following query: (microbio*[Title/Abstract]) AND ("stomach neoplasms"[MeSH Terms] OR "cancer of stomach"[Title/Abstract] OR "stomach cancers"[Title/Abstract] OR "gastric cancer*"[Title/Abstract]). The detailed search protocols for each scientific database are shown in Supplementary Table S1.

2.3 Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) adult diagnosed with gastric cancer through gastroscopic biopsy; (2) the control group consisted of non-gastric cancer individuals undergoing either endoscopy or biopsy, including healthy individuals and those with precancerous lesions such as chronic gastritis or intestinal metaplasia; (3) reporting the changes in gut microbiota composition/diversity; and (4) case–control study.

Study was excluded if it met any of the following situations: (1) people had undergone gastric cancer-related treatments, such as surgery, chemotherapy, radiation therapy, and immunotherapy; (2) pregnant women were involved; (3) samples were from oral, skin, or oropharyngeal; and (4) changes in the gut microbiota cultured in specific media were excluded since the culture conditions exert a significant influence on microbiota data (Goodrich et al., 2014; Widder et al., 2016). Additionally, abstracts, editorials, comments, and studies written in languages other than English were also excluded.

2.4 Study selection and data extraction

Two researchers (Zhang and Wu) screened the searching results of databases according to the inclusion and exclusion criteria independently. Titles and abstracts were screened firstly, and then the full texts were reviewed to identify eligible studies. Four researchers (Zhang, Wu, Ju, and Wang) independently exacted the following

information from each eligible study: the study ID (first author and publication year), country, sample size, age, gender, *H. pylori* infection status, sample source, method for measuring the microbiome, DNA extraction methods, annotation database, composition and diversity of the gut microbiome in people with and without gastric cancer, and the differences in the gut microbiome between the two groups. The exacted data were cross-checked by four investigators. Any disagreement during study selection and data extraction was settled by consultation with the fifth researcher (Zhu) to reach a consensus.

2.5 Quality assessment

The quality of the included studies was evaluated using the Newcastle-Ottawa Quality Assessment Scale (NOS) (Wells et al., 2000). The NOS consists of selection, comparability, and measurement of exposure factors. Each study can receive a maximum of nine points. Two researchers (Zhang and Ju) assessed each study independently, and any discrepancies were resolved through consensus or with the assistance of a third researcher (Zhu) if necessary.

2.6 Data synthesis

Meta-analysis was conducted using the "metan" package in Stata 17.0 with a random-effects model, and heterogeneity was assessed using the I^2 statistic. Based on the alterations in the diversity and abundance of microbiota between people with and without gastric cancer, these results were transposed into a binary format to indicate whether there was an increase. The results of meta-analyses were presented as log odds ratios (log OR) and their 95% confidence intervals (CI). A log OR significantly less than 0 indicated a decrease in the abundance of a certain microbial community in people with gastric cancer compared to those without gastric cancer, while a log OR significantly greater than 0 indicated an increase in the abundance of a certain microbial community in people with gastric cancer. For a more intuitive evaluation, Forest plots were utilized. Meta-regression and subgroup analyses were performed to investigate potential heterogeneity, considering the country, sample source, amplification region of the 16S rRNA gene, and microbial database. Sensitivity analysis was performed on studies with a sample size exceeding 50. Funnel plot, Egger's and Begg's test were conducted to detect potential publication bias which was corrected by trim-and-fill analysis (Mavridis and Salanti, 2014). All p-values were two-tailed, and those $p < 0.05$ were considered statistically significant.

3 Results

3.1 Literature search and studies overview

A total of 2,364 studies were identified from PubMed, Embase, Cochrane Library, and Web of Science. After duplicates removal, 1,582 studies remained for screening the title and abstract. Out of the 1,582 studies, 1,491 studies were excluded. The excused studies included meta-analyses, reviews, protocols, meeting abstracts, experiments and non-English articles, and those that did not focus on gastric cancer or provide the required results. As a result, 91 articles entered the full-text review stage. Finally, 33 studies met the eligible criteria and were included in the meta-analysis. The selection process is illustrated in Figure 1.

Table 1 shows the main characteristics of the included studies which published between 2014 and 2023. The majority of studies were conducted in Asian countries, including China ($n = 21$), Korea ($n = 8$), and Mongolia ($n = 1$). Three studies were conducted in Europe, two in Portugal and one in Lithuania. In addition, one study was conducted in several countries. A total of 4,829 participants were included in these studies, with males outnumbering females. Fourteen studies reported on people infected with *Helicobacter pylori*. A total of 25 studies collected samples from gastric mucosal biopsies during gastroscopy, while four studies used fecal samples (Liang et al., 2019; Qi et al., 2019; He et al., 2022; Kim et al., 2022), and four studies used gastric juice samples (Park et al., 2022; Sun et al., 2022; Peng et al., 2023; Wei et al., 2023). Twenty-seven studies used 16S gene sequencing technology, but with different amplified regions. Of these, one study amplified the V1–V2 (Nikitina et al., 2023), V1–V4 (Wei et al., 2023), V1–V8 (Pimentel-Nunes et al., 2021), V4–V5 (Chen et al., 2019), V5 (Eun et al., 2014), and V5–V6 (Ferreira et al., 2018) regions, respectively. Two studies amplified V1–V3 (Jo et al., 2016; Sohn et al., 2017) and six studies amplified V4 (Coker et al., 2018; Wang et al., 2020; He et al., 2022; Li et al., 2022; Miao et al., 2022; Peng et al., 2023). The most commonly amplified region was V3–V4, with thirteen studies using this region. Three studies did not specify the region amplified (Wang et al., 2016; Castaño-Rodríguez et al., 2017; Deng et al., 2021; Wu et al., 2021). To study the fungal composition of the gut microbiota, one study used the ITS2 region for PCR amplification (Yang et al., 2022). Nine studies did not report the specific gene sequence database used, while the remaining studies mainly relied on databases such as SILVA ($n = 9$), Greengenes ($n = 7$), NCBI ($n = 6$), Ezbio ($n = 2$), EzTaxon-e ($n = 2$), and RDP ($n = 1$).

The NOS was used to assess the quality of the included studies. Three studies scored nine points, 10 studies scored eight points, 15 studies scored seven points, and the remaining studies scored six points or less. The detailed quality assessment scores can be found in the Supplementary Table S2.

3.2 Primary outcomes

3.2.1 Biodiversity

Out of the 33 studies analyzed, 29 focused on investigating the α-diversity of the gastrointestinal microbiota and 18 studies explicitly reported differences in β-diversity between people with and without gastric cancer (refer to Supplementary Table S3). However, due to the diverse use of different indices and variations in expression across studies, quantitative analysis of β-diversity was not available. Meta-analysis showed that only the Shannon index demonstrated statistical significance for α-diversity [−5.078 (−9.470, −0.686)] (Supplementary Figure S1).

3.2.2 Differences in the microbial composition

Eighteen studies with five phylum-level gut microbiotas were available for meta-analysis: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Clostridia*, and *Proteobacteria*. No statistically significant differences between people with and without gastric cancer in terms of these five phylum-level gut microbiotas were identified by meta-analysis.

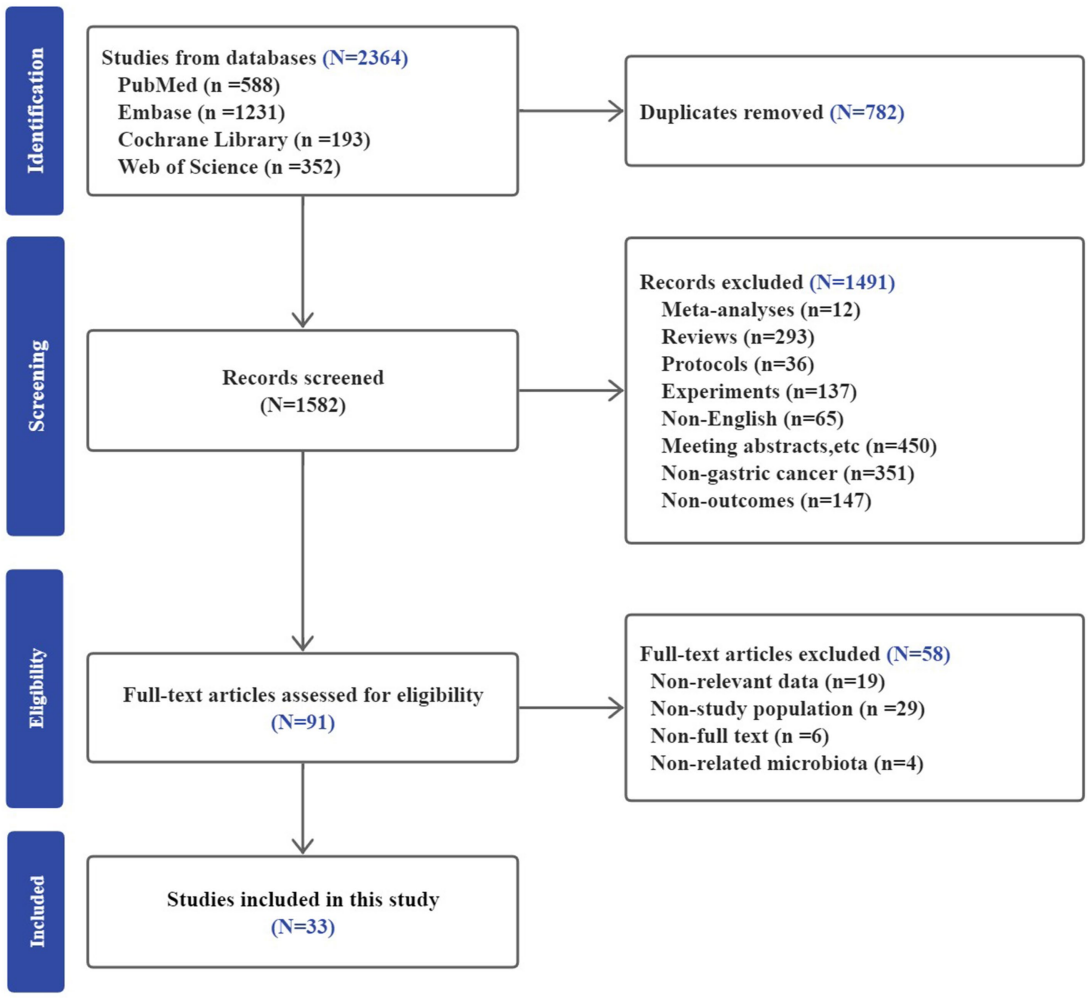

FIGURE 1
Description of the selection of the included studies.

Supplementary Table S4 presents the changes in relative abundance at the phylum level for people with and without gastric cancer in individual studies.

A total of 30 studies reported data on the relative abundance of bacteria at genus-level in people with gastric cancer compared to those without it (Supplementary Table S5). The most frequently reported genera in gastric cancer patient samples were *Lactobacillus* and *Helicobacter*. Meta-analysis of the data from these studies indicated significant changes in the abundance of 11 out of 32 evaluated genera, with nine exhibiting an increase and two exhibiting a decrease. The increased abundance of genera such as *Lactobacillus* and *Streptococcus* was characterized by log odds ratio (95% CI) of 5.474 (0.949, 9.999) and 5.095 (0.293, 9.897), respectively. In contrast, *Porphyromonas* and *Rothia* exhibited a significant and identical decrease in people with gastric cancer, with −8.602 (−11.396, −5.808). Table 2 presents a detailed summary of the findings.

3.3 Subgroup analyses

Subgroup analyses in gastric cancer microbiome research revealed significant findings, highlighting the impact of methodological and geographical variables (Table 3). At the phylum level, *Actinobacteria* exhibited significant abundance changes across different 16S rRNA regions, with a pronounced increase in the V1–V3 region (6.748, 95% CI, 3.608, 9.889) and a decrease in the V4 region when annotated with Greengenes (−10.334, 95% CI: −13.116, −7.552). Subgroup analysis on geographical regions found a higher prevalence of *Helicobacter* and *Streptococcus* in the Korean population, with 9.936 (4.611, 15.261) and 5.651 (2.795, 8.508), respectively, at genus-level. In contrast, the Portuguese population exhibited a reduced prevalence of *Neisseria* with −9.006 (−11.795, −6.218). The prevalence of *Lactobacillus* varied across different 16S rRNA gene amplification regions, with 8.365 (5.567, 11.162) for the V4 region and 7.449 (4.642, 10.257) for the NR region. In gastric biopsy samples, *Lactobacillus* was less prevalent with a log OR of −5.939 (0.300, 11.578), while *Bacteroides* showed a higher abundance, evidenced by a log OR of 11.154 (8.227, 14.082). The analysis of gastric acid samples showed a higher prevalence of *Helicobacter* and *Streptococcus*, with 8.552 (5.757, 11.348) and 8.598 (5.803, 11.393), respectively. Those results suggested that the bacteria were country-/sample source-specific. Database analysis revealed a notable increase in the prevalence of *Lactobacillus* among individuals diagnosed with gastric cancer in studies utilizing Greengenes, with a mean of 9.598

TABLE 1 Characteristics of the included studies.

Study ID	Country	Age	Sample size (male/female)	*H. pylori* status	Sample source	DNA extraction method (region amplified) database used	NOS
Eun et al. (2014)	Korea	Chronic gastritis: 50.4 ± 11.5 IM: 57.5 ± 7.3 GC: 65.7 ± 11.3	Chronic gastritis:10 (4/6) IM: 10 (7/3) GC: 11 (6/5)	Chronic gastritis: 70% IM: 40% GC: 64%	Gastric mucosal biopsies	Phenol/chloroform method and a DNA clean-up kit; 16S rRNA(V5); SILVA database	8
Jo et al. (2016)	Korea	GC: Hp (−), 61.8 ± 10.92; Hp (+), 54.1 ± 12.50; Control: Hp (−), 62.3 ± 13.61; Hp (+), 55.9 ± 10.97	GC: 34 (24/10) Control: 29 (12/17)	GC: 44.1% Control: 55.1%	Gastric mucosal biopsies	iNtRON Biotechnology; 16S rRNA(V1–V3); EzTaxon-e database	8
Wang et al. (2016)	China	55.8 ± 13.5	Chronic gastritis: 212 GC: 103 (200/115)	Chronic gastritis: 45.3% GC: 49.5%	Gastric mucosal biopsies	Qiagen Dneasy blood and tissue kit; 16S rRNA(region NR); Ribosomal database	7
Castaño-Rodríguez et al. (2017)	China	GC: 62.08 GU: 64.75 FD: 49.55	GC: 12 (5/7) GU: 4 (4/0) FD: 20 (13/7)	GC: 91.7% GU: 100% FD: 50%	Antral gastric biopsies	Isolate II RNA mini kit and Tetro cDNA synthesis kit; 16S rRNA (region NR); –	6
Li et al. (2017)	China	Normal: 49.13 Gastritis: 48 IM: 53.22 GC: 53.43 Eradication: 52.18	Normal: 8 (3/5) Gastritis: 9 (2/7) IM: 18 (8/10) GC: 14 (10/4) Eradication: 11 (3/8)	Normal: 0% Gastritis: 100% IM: 66.7% GC: 28.6% Eradication: 9%	Antrum and corpus gastric biopsies	QIAGEN DNeasy Kit; 16S rDNA(V3–V4); Greengene database	8
Sohn et al. (2017)	Korea	GC: Hp (−), 68; Hp (+): 52.8 Control: Hp (−), 53.5; Hp (+), 55.67	GC: Hp (−), 2; Hp (+),5; (3/4) Control: Hp (−), 2; Hp (+), 3; (2/3)	GC: 28.57% Control: 40%	Gastric mucosal (antrum and body) biopsies	iNtRON Biotechnology Kit; 16S rRNA(V1–V3); EzTaxon-e database	6
Yu et al. (2017)	China Mexico	Non-malignant: China, 60.8; Mexico, 64.5; Tumor, NA	non-malignant: China, 77; Mexico, 80. (27/130) tumor: China, 80; Mexico, 54. (62/72)	–	Tumor tissue and matched non-malignant tissue	Allprep RNA/DNA/Protein mini kit (QIAGEN) and QIAamp DNA mini kit (QIAGEN); 16S rRNA(V3–V4); Greengenes and BioProject database	8
Coker et al. (2018)	China	–	AG: 77 SG: 74 IM: 17 GC: 39	Xi'an: 50.8–53.9% Mongolia:44.8–47.5%	Antrum, body and fundus for SG, AG and IM. Biopsies cancer lesions and adjacent non-cancerous tissues of GC.	QIAamp DNA Mini Kit; 16S rRNA(V4); SIL VA database	7

(Continued)

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TABLE 1 (Continued)

Study ID	Country	Age	Sample size (male/female)	*H. pylori* status	Sample source	DNA extraction method (region amplified) database used	NOS
Ferreira et al. (2018)	Portugal	Chronic gastritis: 43.6 ± 7.0 GC: 58.8 ± 13.2	Chronic gastritis:81 (79/2); GC: 54 (32/22)	–	Gastric biopsies or surgical specimens of non-neo plastic gastric mucosa adjacent to the tumour	– 16S rRNA(V5–V6); Greengenes database	7
Hsieh et al. (2018)	China	Gastritis: 32.2 IM: 46.3 GC: 68.6	Gastritis:9 (3/6) IM: 7 (4/3) GC: 11 (5/6)	Gastritis: 55.5% IM: 85.7% GC: 0	Gastric biopsies	TRI Reagent®; 16S rRNA(V3–V4); NCBI database	7
Chen et al. (2019)	China	60	(*N* = 62, gastric adenocarcinoma) 124 gastric tissue samples (cancerous and paired non-cancerous tissues)	29%	Subtotal gastrectomy	Lysozyme, proteinase K and SDS, phenol chloroform isoamyl alcohol, glycogen, sodium acetate and cold isopropanol; 16S rRNA(V4–V5); NCBI and SIL VA database	5
Gunathilake et al. (2019)	Korea	Control: 51.53 ± 7.21 GC: 53.68 ± 9.60	Control: 288 (181/107) GC: 268 (172/96)	Control: 93.4% GC: 99.6%	Gastric mucosa biopsy	MagAttract DNA Blood M48 kit; 16S rRNA(V3–V4); NCBI database	7
Liang et al. (2019)	China	GC: 52.3 ± 11.2 HC: 53.4	GC: 20 HC: 22	–	Fecal samples	E.Z.N.A Stool DNA Kit; – –	8
Qi et al. (2019)	China	GC: 58.06 ± 11.24 HC: 45.58 ± 8.86	GC: 116 (96/20) HC: 88 (53/35)	–	Fecal samples	E.Z.N.A.® Stool DNA Kit; 16S rDNA(V3–V4); NCBI and SIL VA database	9
Gantuya et al. (2020)	Mongolian	46.4	GC: 48 Normal: 22 Gastritis: 20 Atrophy: 66 IM: 40 (59/137)	–	Gastric mucosal biopsies	DNeasy Blood & Tissue Kit; 16S rRNA(V3–V4); SIL VA database	7
Gunathilake et al. (2020)	Korea	Control: 51.53 ± 7.21 GC: 53.68 ± 9.60	Control:288 GC:268 (353/203)	Control: 93.4% GC:99.63%	Gastric mucosal biopsy	MagAttract DNA Blood M48 kit; 16S rRNA(V3–V4); Ezbio database	9

(Continued)

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TABLE 1 (Continued)

Study ID	Country	Age	Sample size (male/female)	*H. pylori* status	Sample source	DNA extraction method (region amplified) database used	NOS
Wang et al. (2020)	China	HC: 45.63 CG: 49.04 IM: 56.93 IN: 62.16 GC:57.35	HC: 30 (15/15) CG: 21 (10/11) IM: 27 (16/11) IN: 25 (18/7) GC:29 (18/11)	HC: 0% CG: 28.57% IM: 29.63% IN: 60% GC:58.62%	Gastric mucosal biopsy	QIAamp DNA Mini Kit; 16S rRNA(V4); Greengenes database	7
Deng et al. (2021)	China	Chronic superficial Gastritis: 45–70 GC: 46–75	Chronic superficial gastritis: 25 (13/12) GC: 34 (24/10)	Chronic superficial gastritis: 0% GC: 26.47%	Chronic superficial gastritis: the antrum ($n = 10$), corpus ($n = 7$) and cardia ($n = 8$) GC: cancer in the antrum ($n = 19$) and corpus ($n = 15$).	– 16S rRNA(region NR); RDP and NCBI database	9
Gunathilake et al. (2021)	Korea	–	GC: 268 HC: 288 (353/203)	–	Gastric mucosal biopsies	MagAttract DNA Blood M48 kit; 16 S rRNA(V3–V4); Ezbio database	8
Kadeerhan et al. (2021)	China	Normal/SG: 53.8 ± 7.8 CAG: 53.4 ± 9.3 IM: 58.5 ± 7.7 DYS/GC: 57.6 ± 6.4	Normal/SG: 35 (13/22) CAG: 52 (29/23) IM: 67 (43/24) DYS/GC: 25 (20/5)	Normal/SG: 74.3% CAG: 92.3% IM: 94.0% DYS/GC: 60.0%	Gastric mucosal biopsies	QIAamp DNA Mini Kit; 16S rRNA(V3–V4); Greengenes and SIL VA database	7
Pimentel-Nunes et al. (2021)	Portugal	Controls: 53 (27–82) Extensive atrophy/metaplasia: 63 (53–87) Early gastric cancer: 70 (43–89)	Controls: 17 (11/6) Extensive atrophy/metaplasia: 12 (5/7) Early gastric cancer: 31 (17/14)	Controls: 41% Extensive atrophy/metaplasia: 25% Early gastric cancer: 13%	Biopsy fragment from the antrum and the corpus	NZY Tissue gDNA isolation kit; 16S rRNA(V1–V8); –	7
Wu et al. (2021)	China	GC: 62.50 ± 6.64 SG: 61.78 ± 6.25	GC: 18 (15/3) SG: 32 (24/8)	–	Gastric mucosa biopsy samples, samples were collected from the greater curvature of the antrum, the lesser curvature of the antrum, the greater curvature of the stomach body, the lesser curvature of the stomach body, and the fundus.	E.Z.N.A.® Stool DNA Kit; 16S rRNA(region NR); SIL VA database	7
Zhang et al. (2021)	China	SG: 56.00 ± 10.25 AG: 63.58 ± 6.69 GIN: 64.80 ± 9.93 GC: 69.87 ± 11.57	SG: 17 AG: 10 GIN: 5 GC: 15 (20/27)	–	Gastric mucosal biopsies	E.Z.N.A ®Stool DNA Kit; 16S rRNA(V3–V4); SILVA database	8

(Continued)

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TABLE 1 (Continued)

Study ID	Country	Age	Sample size (male/female)	*H. pylori* status	Sample source	DNA extraction method (region amplified) database used	NOS
He et al. (2022)	China	–	Gastric cancer: 30 Healthy people: 30	–	Fecal samples	CTAB method; 16S rDNA(V4); –	7
Kim et al. (2022)	Korea	GC: 62.9 ± 10.2 Control: 50.7 ± 13.6	GC: 45 (31/14) CG:49 IM:43 (Control:47/45)	0%	Histological evaluation using endoscopic biopsy tissues.	DNeasy PowerSoil Kit; 16S rRNA(V3–V4); NCBI and taxonomy databases	7
Li et al. (2022)	China	GC: 63.5 HC: 55	GI Cancer: 130 (93/37) HC: 147 (84/63)	–	Fecal samples	NucleoSpin Soil DNA Kit; 16S rRNA(V4); Greengenes database	6
Miao et al. (2022)	China	SG: 47.40 ± 12.37 AG: 45.77 ± 13.62 GMAH: 64.00 ± 11.83 GC: 69.60 ± 6.91	SG: 15 (7/8) AG: 13 (8/5) GMAH: 8 (5/3) GC: 15 (11/4)	SG: 26.7% AG: 61.5% GMAH: 100% GC: 73.3%	Gastric mucosal biopsies	QIAamp PowerFecal Pro DNA Kit; 16S rRNA(V4); Greengenes database	7
Park et al. (2022)	Korea	Gastritis: 59.8 ± 12.5 Gastric adenoma: 65.3 ± 9.6 EGC: 62.7 ± 10.8 AGC: 58.8 ± 15.8	Gastritis: 16 (6/10) Gastric adenoma: 16 (12/4) EGC: 36 (25/11) AGC: 20 (14/6)	–	Gastric juice	DNeasy PowerSoil kit; 16S rRNA(V3–V4); SILVA database	8
Sun et al. (2022)	China	SG: 50.29 ± 14.31 AG: 60.67 ± 10.71 IM: 60.27 ± 14.89 Dys: 62.71 ± 12.21 GC: 71.67 ± 9.87	SG: 56 (27/29) AG: 9 (5/4) IM: 27 (12/15) Dys: 29 (15/14) GC: 13 (7/6)	0%	Gastric mucosal biopsies and Gastric juice	E.Z.N.A® Soil DNA Kit; 16S rRNA(V3–V4); –	7
Yang et al. (2022)	China	GC: 60.59 ± 12.73 HC: 52.64 ± 10.92	GC: 22 (16/6) HC: 11 (4/7)	–	Gastric mucosal biopsies	E.Z.N.A. R soil DNA Kit; ITS2 rRNA PCR; –	6
Nikitina et al. (2023)	Lithuania	–	GC: 76 HC: 29	–	Gastric mucosal biopsies	AllPrep DNA/RNA Mini kit; 16S rRNA(V1–V2); –	8
Peng et al. (2023)	China	HC: 49.5 (32–60) GPL: 48.5 (32–59) GC: 59.5 (44–81)	HC: 22 (13/9) GPL: 22 (10/12) GC: 16 (10/6)	HC: 27.3% GPL: 40.9% GC: 68.8%	Gastric juice	QIAamp® FAST DNA Stool Mini Kit; 16S rRNA(V4); –	8

(Continued)

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TABLE 1 (Continued)

Study ID	Country	Age	Sample size (male/female)	*H. pylori* status	Sample source	DNA extraction method (region amplified) database used	NOS
Wei et al. (2023)	China	Healthy: 51.61 ± 11.68 GC: 67.97 ± 9.24	Healthy: 61 (37/24) GC: 78 (58/20)	–	Gastric juice	– 16S rDNA(V1–V4); –	7

IM, intestinal metaplasia; HP, *Helicobacter pylori*; GU, gastric ulcers; SG, superficial gastritis; AG, atrophic gastritis; AGC, advanced gastric cancer; CAG, chronic atrophic gastritis; CG, chronic gastritis; CSG, chronic superficial gastritis; DYS, dysplasia; EGC, early gastric cancer; F, female; FD, functional dyspepsia; F/M, female to male ratio; GA, gastritis; GC, gastric cancer; GAD, gastric adenoma; GI, gastrointestinal; GIN, gastric intraepithelial neoplasia; GMAH, gastric mucosal atypical hyperplasia; GPL, gastric precancerous lesions; HC, healthy control; IN, intraepithelial neoplasia; M, male; NCBI, National Center for Biotechnology Information; NOS, Newcastle-Ottawa Scale; RDP, ribosomal database project; SIL VA, small subunit rRNA database.

(6.813, 12.383). Conversely, studies referencing NCBI indicated an increase in *Fusobacterium*, with a mean of 8.163 (5.200, 11.127).

3.4 Meta-regression

Meta-regression analysis aimed to identify sources of heterogeneity in gastric cancer microbiome studies. Results showed that geographic differences significantly affect *Bacteroidetes* and *Firmicutes* abundance (Supplementary Table S6). Specifically, the analysis indicated a strong negative association of *Bacteroidetes* with country (−21.91816, $p < 0.001$) and a positive association for *Firmicutes* (9.307176, $p = 0.018$). Methodological factors, such as the choice of 16S rRNA gene amplification regions and databases for annotation, significantly impacted the abundance of *Actinobacteria*. The method used showed a negative coefficient (−20.59842, $p = 0.009$), while the database used showed a positive coefficient (18.17374, $p = 0.008$). Additionally, sample sources were found to contribute to the heterogeneity of Firmicutes (−19.25102, $p = 0.006$).

3.5 Sensitivity analysis and publication bias

After excluding studies with a sample size of less than 50, sensitivity analysis revealed trends in changes to microbial diversity indices as well as microbial community structure at the genus and phylum classification levels (Supplementary Table S7). As a result, changes in 9 out of the 11 bacterial genera identified by overall analysis were found to be robust. Notably, the analysis of the genus *Clostridium* showed a slight increase in the log OR from 7.994 to 8.227, and the *p*-value changed from an extremely low 7.55E-12 to 1.80E-07 when small-sample studies were excluded. Although the result remained statistically significant, the increase in heterogeneity to 17.70% suggested some inconsistency between studies. Regarding the Shannon index of α-diversity, the log odds ratio slightly decreased after exclusion, while the *p*-value rose from 0.023 to 0.048. This implied that the negative association's statistical significance was somewhat strengthened. Overall, excluding small-sample studies caused only limited changes in the log odds ratios and *p*-values.

The funnel plots indicated possible publication bias in the meta-analysis of microbial diversity and abundance related to gastric cancer. Asymmetries were observed for several bacteria. The funnel plot for Shannon appears symmetrical, indicating minimal bias, which was supported by a non-significant Egger's test. However, a significant Begg's test for Shannon suggested that further scrutiny might be necessary. For *Actinobacteria*, both Egger's and Begg's tests showed a low probability of bias. The plot for *Proteobacteria* displayed slight asymmetry, but only the trim-and-fill method indicated the need for adjustment, adding three studies to the left. *Helicobacter*, *Lactobacillus*, and *Streptococcus* exhibited asymmetrical plots. Begg's test suggested potential bias for the latter two, although Egger's test results did not align with this for all (Supplementary Table S8).

4 Discussion

This meta-analysis aggregated data from 33 studies and explored the evolution of the gut microbiome from pre-cancerous conditions to

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TABLE 2 Meta-analysis of changes on genus level between gastric and non-gastric cancer patients.

Genus	No. of studies	Simple size	Log odds ratio (95% CI)	*p*-value	I^2
Lactobacillus	11	1027	5.474 (0.949, 9.999)	0.020	93.60%
Streptococcus	11	969	5.095 (0.293, 9.897)	0.038	93.80%
Achromobacter	2	184	8.716 (5.923, 11.510)	1.995e-09	0.00%
Bacillus	2	303	9.661 (6.876, 12.446)	1.058e-10	0.00%
Capnocytophaga	2	226	8.643 (5.847, 11.439)	1.995e-09	0.00%
Clostridium	3	204	7.994 (5.706, 10.283)	7.553e-12	0.00%
Dialister	2	257	8.995 (6.204, 11.787)	1.350e-09	0.00%
Klebsiella	2	253	9.141 (6.203, 12.080)	1.995e-09	9.70%
Slackia	2	254	8.909 (6.116, 11.703)	1.362e-09	0.00%
Porphyromonas	2	182	−8.602 (−11.396, −5.808)	1.995e-09	0.00%
Rothia	2	182	−8.602 (−11.396, −5.808)	1.995e-09	0.00%

TABLE 3 Statistically significant bacterial groups identified by the meta-analysis in subgroup analysis.

Outcome	Subgroup	Bacterial groups	*N*	Sample size	Log odds ratio (95% CI)	*p*-value	I^2
Phylum	**Method**						
	16S rRNA(V1–V3)	*Actinobacteria*	2	75	6.748 (3.608, 9.889)	2.54E-05	18.50%
	16S rRNA(V4)	*Bacteroidetes*	2	409	10.334 (7.552, 13.116)	6.62E-13	97.20%
		Actinobacteria	2	409	−10.334 (−13.116, −7.552)	6.62E-13	0.00%
	Database						
	EzTaxon-e	*Actinobacteria*	2	75	6.748 (3.608, 9.889)	2.54E-05	18.50%
	Greengenes	*Proteobacteria*	3	469	9.531 (7.254, 11.808)	1.87E-15	0.00%
		Actinobacteria	2	409	−10.334 (−13.116, −7.552)	6.62E-13	0.00%
	NCBI and SILVA	*Actinobacteria*	2	328	10.143 (7.362, 12.923)	1.17E-12	0.00%
		Firmicutes	2	328	−10.143(−12.923, −7.362)	1.17E-12	0.00%
Genus	**Country**						
	Korea	*Helicobacter*	2	592	9.936 (4.611, 15.261)	2.78E-04	72.50%
		Streptococcus	2	33	5.651 (2.795, 8.508)	1.27E-04	0.00%
	Portugal	Neisseria	2	195	−9.006 (−11.795, −6.218)	7.33E-10	0.00%
	Method						
	16S rRNA (V4)	*Lactobacillus*	2	170	8.365 (5.567, 11.162)	7.90E-09	0.00%
	16S rRNA (region NR)	*Lactobacillus*	2	86	7.449 (4.642, 10.257)	2.64E-07	0.00%
	Sample source						
	Stomach	*Bacteroides*	12	680	11.154 (8.227, 14.082)	4.88E-13	9.90%
		Lactobacillus	8	718	5.939 (0.300, 11.578)	0.039	93.80%
	Gastric juice	*Helicobacter*	2	175	8.552 (5.757, 11.348)	4.04E-09	0.00%
	Feces	*Streptococcus*	2	177	8.598 (5.803, 11.393)	3.95E-09	0.00%
		Bacteroides	3	306	−8.800 (−11.079, −6.520)	4.66E-13	0.00%
	Database						
	Greengenes	*Lactobacillus*	2	267	9.598 (6.813, 12.383)	5.69E-11	0.00%
	NCBI	*Fusobacterium*	2	151	8.163 (5.200, 11.127)	1.01E-07	10.60%

N, the number of studies.

the development of gastric cancer. In comparison to previous studies, our analysis was more comprehensive. Initially, we conducted a meta-analysis, followed by subgroup analysis, sensitivity analysis, and meta-regression. Additionally, we conducted a detailed analysis of publication bias. According to our study, a pattern of reduced microbial diversity was found, which is consistent with earlier studies (Liu et al.,

2022; Li et al., 2023). However, it is important to note that earlier studies may have been limited by the scope of their sample selection, potentially not capturing the full spectrum of microbiome variability associated with gastric cancer. Our analysis builds upon and expands these findings by incorporating a broader and more diverse datasets, enhancing the comprehensiveness and generalizability of our conclusions. The reduction in microbial diversity observed in various studies emphasizes its potential impact on the immune system's ability to respond to cancer. This highlights the critical role of the gut microbiome in the progression of gastric cancer.

The Shannon index, often used to measure species richness and evenness (Lozupone and Knight, 2008), was significantly reduced in people with gastric cancer compared to pre-cancerous conditions in this study, which implies a decrease in the diversity of the gastric microbial ecosystem when gastric cancer develops. The reasons for the decrease are not yet clear. A previous study suggested that it might be a result of factors such as gastric acid and *Helicobacter pylori* infection reshaping the microbial community during the carcinogenic process (Wang et al., 2018). However, changes in diet, use of antibiotics or other medications (David et al., 2014; Altveş et al., 2020) were reported to be associated with the reduction in Shannon index.

At the genus level, an increase in *Lactobacillus* and *Streptococcus*, alongside a decrease in *Rothia* and *Porphyromonas*, were identified in this meta-analysis. The variation in bacterial abundance is thought to influence the immune system's ability to detect and eliminate cancer cells. For example, an increase in *Lactobacillus* correlates with higher counts of $CD3^+$ T cells (Qi et al., 2019), suggesting a complex relationship between microbiome composition and immune function. Furthermore, experimental evidence from studies such as Lertpiriyapong et al. (2014) highlighted how specific bacterial presences could trigger inflammation and promote cancer development. Interestingly, interventions such as post-surgical supplementation with *Clostridium butyricum* have been shown to modulate immune responses favorably, indicating potential therapeutic pathways (Cao et al., 2022). Epidemiological studies have suggested a correlation between the occurrence of gastric cancer and periodontal disease (Lo et al., 2021). *Porphyromonas* is one of the pathogens that cause periodontal disease (Darveau, 2010). Experiments have shown that lipopolysaccharide (LPS) from *Porphyromonas* can damage the gastric mucosal barrier, which is considered a promoting factor for cancer-related gastritis. Furthermore, LPS from *Porphyromonas* can regulate the host's immune response (Oriuchi et al., 2024). Although direct research linking *Rothia* with gastric cancer is limited, it is important to note that *Rothia* is part of the core microbiota in the stomachs of healthy individuals (Nardone and Compare, 2015). The gut microbiota can produce butyrate, a short-chain fatty acid that has been shown to suppress the expression of PD-L1 and IL-10 in immune cells and demonstrate tumor growth inhibition potential in mouse models (Lee et al., 2024). It is speculated that *Rothia* may influence the progression of gastric cancer through its metabolic products. Future studies will likely focus on elucidating the specific mechanisms of these associations.

The roles of *Lactobacillus* and *Streptococcus* in gastric cancer are nuanced, with *Lactobacillus* associated with both anti-inflammatory effects and cancer progression, potentially serving as a biomarker for the disease (Bali et al., 2021). Similarly, *Streptococcus* adheres to gastric mucosa, influencing cancer development through metabolic and immune modulation (Spiegelhauer et al., 2020). Notably, *Streptococcus anginosus* has been implicated in exacerbating gastric inflammation and cancer progression (Fu et al., 2024). *Helicobacter pylori*'s role in gastric cancer development is significant (Plottel and Blaser, 2011). The involvement of *Helicobacter pylori*, a well-documented factor in gastric cancer, showed variability in our analysis, contrasting with findings by Liu et al. (2022), which could be attributed to methodological and sample size differences.

Due to the high heterogeneity observed in certain microbial communities in the overall analysis, a series of analyses including subgroup analysis, sensitivity analysis, and meta-regression were conducted to identify the sources of heterogeneity. Subgroup analyses revealed regional variations in bacterial communities, suggesting that dietary or environmental factors contributed to a higher prevalence of *Streptococcus* in Asian populations compared to Europeans. Geographic differences had a significant impact on the levels of *Bacteroidetes* and *Firmicutes*. Research showed that there were significant geographic differences in the composition of the gut microbiota between populations from the United States, Chile, South Africa, Kuwait and Malaysia, particularly in the distribution of *Bacteroidetes* and *Firmicutes*. In samples from the United States, *Firmicutes* dominate, followed by other regions such as South Africa. In Chilean samples, however, *Bacteroidetes* took the lead. Moreover, by calculating the ratio of *Firmicutes* to *Bacteroidetes* (F:B), it was found that the F:B in US samples was the highest, reaching 4.15, while the F:B in Chilean samples was the lowest (Kumar and Bhadury, 2023). Other studies in the Asian population also showed that geographic differences significantly affect the abundance of *Bacteroidetes* and *Firmicutes* in the gut. These differences were related to the unique dietary habits, cultural customs and environmental conditions of each region (Lim et al., 2021; Taha et al., 2023).

Additionally, methodological choices and sample sources introduced variability in the detection of bacteria such as *Bacteroides* and *Lactobacillus*. It became clear that methodological differences, including the choice of sample sources and DNA sequencing techniques, were the main cause of inconsistencies in microbiota research (Hiergeist et al., 2016; Tang et al., 2020). The choice between using feces or endoscopic biopsies as samples significantly affected the outcomes (Jalanka et al., 2015; Tropini et al., 2018), highlighting the nuanced impact of sample origin on research findings. Furthermore, variations in DNA isolation and sequencing methodologies, as well as the choice of database platforms, posed challenges in accurately differentiating microbial communities. These methodological considerations were crucial in microbiome studies, emphasizing the need for a rigorous and standardized approach to mitigate inconsistencies and enhance the comparability of results across studies. This comprehensive approach ensured that the complexities of microbial ecosystems were accurately interpreted, fostering advancements in our understanding of the microbiome's role in health and disease. Variations in DNA isolation and sequencing methodologies, as well as database platforms, could introduce errors in microbial differentiation. Moreover, meta-regression also confirmed that geographic, methodological, and sample origin differences were the sources of heterogeneity.

Sensitivity analysis on studies with sample size no less than 50 revealed an increase in the *p*-values for *Lactobacillus* and *Streptococcus*. This change suggested that smaller studies might have influenced the results due to their high variability or specific biases. These small-sample studies sometimes showed a more significant association because of greater statistical variation or because of selective reporting and publication bias. Because most study samples were collected during health examinations, it was difficult to collect a large number of samples, which limited the ability to conduct large-scale research.

Therefore, future studies should aim to expand the research scale and include a broader population to explore the potential association between these bacteria and gastric cancer.

This meta-analysis presented a detailed examination of the changes in the gut microbiome that are linked to the development of gastric cancer. It highlighted the intricate relationship between microbial diversity and cancer, the potential of microbiome-focused therapies, and the need for methodological rigor in future research. The limitations of this study, including lack of data, potential bias, and inability to include all relevant factors, highlight the need for large-scale studies. These limitations underscore the need for large-scale studies to confirm these findings and further explore the role of the microbiome in gastric cancer. Specifically, future research should focus on conducting long-term cohort studies to explore the dynamic changes in the gut microbiome during the development and progression of gastric cancer. In parallel, pathogenic mechanism studies should be conducted to understand how specific microbes promote or influence the development of gastric cancer. In addition, interventional studies could be conducted to evaluate the efficacy of specific microbes in the prevention and treatment of gastric cancer. Through these studies, we can gain a more complete understanding of the role of the microbiome in gastric cancer and provide guidance for targeted prevention and treatment strategies in the clinical setting.

5 Conclusion

This study identified robust changes of nine bacterial genus in people with gastric cancer, which were country-/sample source-specific, with lower α-diversity observed in individuals with gastric cancer. Large-scale studies are needed to explore the mechanisms underlying these changes.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

RZ: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. YW: Data curation, Writing – original draft, Writing – review & editing. WJ: Data curation, Writing – review & editing. SW: Data curation, Writing – review & editing. YL: Conceptualization, Writing – review & editing. HZ: Conceptualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1406526/full#supplementary-material

References

Altveş, S., Yildiz, H. K., and Vural, H. C. (2020). Interaction of the microbiota with the human body in health and diseases. *Biosci. Microbiota Food Health* 39, 23–32. doi: 10.12938/bmfh.19-023

Aviles-Jimenez, F., Vazquez-Jimenez, F., Medrano-Guzman, R., Mantilla, A., and Torres, J. (2014). Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer. *Sci. Rep.* 4:4202. doi: 10.1038/srep04202

Bali, P., Coker, J., Lozano-Pope, I., Zengler, K., and Obonyo, M. (2021). Microbiome signatures in a fast- and slow-progressing gastric cancer murine model and their contribution to gastric carcinogenesis. *Microorganisms* 9:189. doi: 10.3390/microorganisms9010189

Cao, W., Zheng, C., Xu, X., Jin, R., Huang, F., Shi, M., et al. (2022). *Clostridium butyricum* potentially improves inflammation and immunity through alteration of the microbiota and metabolism of gastric cancer patients after gastrectomy. *Front. Immunol.* 13:1076245. doi: 10.3389/fimmu.2022.1076245

Castaño-Rodríguez, N., Goh, K. L., Fock, K. M., Mitchell, H. M., and Kaakoush, N. O. (2017). Dysbiosis of the microbiome in gastric carcinogenesis. *Sci. Rep.* 7:15957. doi: 10.1038/s41598-017-16289-2

Chen, X. H., Wang, A., Chu, A. N., Gong, Y. H., and Yuan, Y. (2019). Mucosa-associated microbiota in gastric cancer tissues compared with non-cancer tissues. *Front. Microbiol.* 10:1261. doi: 10.3389/fmicb.2019.01261

Coker, O. O., Dai, Z., Nie, Y., Zhao, G., Cao, L., Nakatsu, G., et al. (2018). Mucosal microbiome dysbiosis in gastric carcinogenesis. *Gut* 67, 1024–1032. doi: 10.1136/gutjnl-2017-314281

Darveau, R. P. (2010). Periodontitis: a polymicrobial disruption of host homeostasis. *Nat. Rev. Microbiol.* 8, 481–490. doi: 10.1038/nrmicro2337

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505, 559–563. doi: 10.1038/nature12820

Deng, Y., Ding, X., Song, Q., Zhao, G., Han, L., Ding, B., et al. (2021). Alterations in mucosa-associated microbiota in the stomach of patients with gastric cancer. *Cell. Oncol.* 44, 701–714. doi: 10.1007/s13402-021-00596-y

Eun, C. S., Kim, B. K., Han, D. S., Kim, S. Y., Kim, K. M., Choi, B. Y., et al. (2014). Differences in gastric mucosal microbiota profiling in patients with chronic gastritis, intestinal metaplasia, and gastric cancer using pyrosequencing methods. *Helicobacter* 19, 407–416. doi: 10.1111/hel.12145

Fan, Y., and Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. *Nat. Rev. Microbiol.* 19, 55–71. doi: 10.1038/s41579-020-0433-9

Ferreira, R. M., Pereira-Marques, J., Pinto-Ribeiro, I., Costa, J. L., Carneiro, F., Machado, J. C., et al. (2018). Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. *Gut* 67, 226–236. doi: 10.1136/gutjnl-2017-314205

Fu, K., Cheung, A. H. K., Wong, C. C., Liu, W., Zhou, Y., Wang, F., et al. (2024). *Streptococcus anginosus* promotes gastric inflammation, atrophy, and tumorigenesis in mice. *Cell* 187, 882–896.e17. doi: 10.1016/j.cell.2024.01.004

Gantuya, B., el Serag, H. B., Matsumoto, T., Ajami, N. J., Uchida, T., Oyuntsetseg, K., et al. (2020). Gastric mucosal microbiota in a Mongolian population with gastric cancer and precursor conditions. *Aliment. Pharmacol. Ther.* 51, 770–780. doi: 10.1111/apt.15675

Goodrich, J. K., di Rienzi, S. C., Poole, A. C., Koren, O., Walters, W. A., Caporaso, J. G., et al. (2014). Conducting a microbiome study. *Cell* 158, 250–262. doi: 10.1016/j.cell.2014.06.037

Gunathilake, M. N., Lee, J., Choi, I. J., Kim, Y. I., Ahn, Y., Park, C., et al. (2019). Association between the relative abundance of gastric microbiota and the risk of gastric cancer: a case-control study. *Sci. Rep.* 9:13589. doi: 10.1038/s41598-019-50054-x

Gunathilake, M., Lee, J., Choi, I. J., Kim, Y. I., and Kim, J. (2021). Association between bacteria other than Helicobacter pylori and the risk of gastric cancer. *Helicobacter* 26:e12836. doi: 10.1111/hel.12836

Gunathilake, M., Lee, J., Choi, I. J., Kim, Y. I., Yoon, J., Sul, W. J., et al. (2020). Alterations in gastric microbial communities are associated with risk of gastric cancer in a Korean population: a case-control study. *Cancers* 12:2619. doi: 10.3390/cancers12092619

He, F., Wang, F., Yang, J., and Yang, S. (2022). Explore and analyze the composition and characteristics of intestinal microbiota between gastric cancer patients and healthy people. *Evid. Based Complement. Alternat. Med.* 2022, 1–13. doi: 10.1155/2022/5834293

Hiergeist, A., Reischl, U., and Gessner, A. (2016). Multicenter quality assessment of 16S ribosomal DNA-sequencing for microbiome analyses reveals high inter-center variability. *Int. J. Med. Microbiol.* 306, 334–342. doi: 10.1016/j.ijmm.2016.03.005

Hsieh, Y. Y., Tung, S. Y., Pan, H. Y., Yen, C. W., Xu, H. W., Lin, Y. J., et al. (2018). Increased abundance of Clostridium and Fusobacterium in gastric microbiota of patients with gastric cancer in Taiwan. *Sci. Rep.* 8:158. doi: 10.1038/s41598-017-18596-0

Jalanka, J., Salonen, A., Salojärvi, J., Ritari, J., Immonen, O., Marciani, L., et al. (2015). Effects of bowel cleansing on the intestinal microbiota. *Gut* 64, 1562–1568. doi: 10.1136/gutjnl-2014-307240

Jo, H. J., Kim, J., Kim, N., Park, J. H., Nam, R. H., Seok, Y. J., et al. (2016). Analysis of gastric microbiota by pyrosequencing: minor role of bacteria other than *Helicobacter pylori* in the gastric carcinogenesis. *Helicobacter* 21, 364–374. doi: 10.1111/hel.12293

Kadeerhan, G., Gerhard, M., Gao, J. J., Mejías-Luque, R., Zhang, L., Vieth, M., et al. (2021). Microbiota alteration at different stages in gastric lesion progression: a population-based study in Linqu, China. *Am. J. Cancer Res.* 11, 561–575

Kim, H. N., Kim, M. J., Jacobs, J. P., and Yang, H. J. (2022). Altered gastric microbiota and inflammatory cytokine responses in patients with *Helicobacter pylori*-negative gastric cancer. *Nutrients* 14:4981. doi: 10.3390/nu14234981

Kumar, G., and Bhadury, P. (2023). Exploring the influences of geographical variation on sequence signatures in the human gut microbiome. *J. Genet.* 102:102. doi: 10.1007/s12041-023-01448-4

Lee, S. Y., Jhun, J., Woo, J. S., Lee, K. H., Hwang, S. H., Moon, J., et al. (2024). Gut microbiome-derived butyrate inhibits the immunosuppressive factors PD-L1 and IL-10 in tumor-associated macrophages in gastric cancer. *Gut Microbes* 16:2300846. doi: 10.1080/19490976.2023.2300846

Lertpiriyapong, K., Whary, M. T., Muthupalani, S., Lofgren, J. L., Gamazon, E. R., Feng, Y., et al. (2014). Gastric colonisation with a restricted commensal microbiota replicates the promotion of neoplastic lesions by diverse intestinal microbiota in the *Helicobacter pylori* INS-GAS mouse model of gastric carcinogenesis. *Gut* 63, 54–63. doi: 10.1136/gutjnl-2013-305178

Li, N., Bai, C., Zhao, L., Ge, Y., and Li, X. (2022). Characterization of the fecal microbiota in gastrointestinal cancer patients and healthy people. *Clin. Transl. Oncol.* 24, 1134–1147. doi: 10.1007/s12094-021-02754-y

Li, Y., Hu, Y., Zhan, X., Song, Y., Xu, M., Wang, S., et al. (2023). Meta-analysis reveals *Helicobacter pylori* mutual exclusivity and reproducible gastric microbiome alterations during gastric carcinoma progression. *Gut Microbes* 15:2197835. doi: 10.1080/19490976.2023.2197835

Li, T. H., Qin, Y., Sham, P. C., Lau, K. S., Chu, K. M., and Leung, W. K. (2017). Alterations in gastric microbiota after *H. pylori* eradication and in different histological stages of gastric carcinogenesis. *Sci. Rep.* 7:44935. doi: 10.1038/srep44935

Liang, W., Yang, Y., Wang, H., Wang, H., Yu, X., Lu, Y., et al. (2019). Gut microbiota shifts in patients with gastric cancer in perioperative period. *Medicine* 98:e16626. doi: 10.1097/MD.0000000000016626

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., et al. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med.* 6:e1000100. doi: 10.1371/journal.pmed.1000100

Lim, M. Y., Hong, S., Bang, S. J., Chung, W. H., Shin, J. H., Kim, J. H., et al. (2021). Gut microbiome structure and association with host factors in a Korean population. *mSystems* 6:e0017921. doi: 10.1128/mSystems.00179-21

Liu, C., Ng, S. K., Ding, Y., Lin, Y., Liu, W., Wong, S. H., et al. (2022). Meta-analysis of mucosal microbiota reveals universal microbial signatures and dysbiosis in gastric carcinogenesis. *Oncogene* 41, 3599–3610. doi: 10.1038/s41388-022-02377-9

Lo, C. H., Kwon, S., Wang, L., Polychronidis, G., Knudsen, M. D., Zhong, R., et al. (2021). Periodontal disease, tooth loss, and risk of oesophageal and gastric adenocarcinoma: a prospective study. *Gut* 70, 620–621. doi: 10.1136/gutjnl-2020-321949

Lozupone, C. A., and Knight, R. (2008). Species divergence and the measurement of microbial diversity. *FEMS Microbiol. Rev.* 32, 557–578. doi: 10.1111/j.1574-6976.2008.00111.x

Mavridis, D., and Salanti, G. (2014). How to assess publication bias: funnel plot, trim-and-fill method and selection models. *Evid. Based Ment. Health* 17:30. doi: 10.1136/eb-2013-101699

Miao, Y., Tang, H., Zhai, Q., Liu, L., Xia, L., Wu, W., et al. (2022). Gut microbiota dysbiosis in the development and progression of gastric cancer. *J. Oncol.* 2022, 1–15. doi: 10.1155/2022/9971619

Nardone, G., and Compare, D. (2015). The human gastric microbiota: is it time to rethink the pathogenesis of stomach diseases? *United European Gastroenterol J* 3, 255–260. doi: 10.1177/2050640614566846

Nearing, J. T., Douglas, G. M., Hayes, M. G., MacDonald, J., Desai, D. K., Allward, N., et al. (2022). Microbiome differential abundance methods produce different results across 38 datasets. *Nat. Commun.* 13:342. doi: 10.1038/s41467-022-28034-z

Nikitina, D., Lehr, K., Vilchez-Vargas, R., Jonaitis, L. V., Urba, M., Kupcinskas, J., et al. (2023). Comparison of genomic and transcriptional microbiome analysis in gastric cancer patients and healthy individuals. *World J. Gastroenterol.* 29, 1202–1218. doi: 10.3748/wjg.v29.i7.1202

Oriuchi, M., Lee, S., Uno, K., Sudo, K., Kusano, K., Asano, N., et al. (2024). *Porphyromonas gingivalis* lipopolysaccharide damages mucosal barrier to promote gastritis-associated carcinogenesis. *Dig. Dis. Sci.* 69, 95–111. doi: 10.1007/s10620-023-08142-6

Park, J. Y., Seo, H., Kang, C. S., Shin, T. S., Kim, J. W., Park, J. M., et al. (2022). Dysbiotic change in gastric microbiome and its functional implication in gastric carcinogenesis. *Sci. Rep.* 12:4285. doi: 10.1038/s41598-022-08288-9

Peng, X., Yao, S., Huang, J., Zhao, Y., Chen, H., Chen, L., et al. (2023). Alterations in bacterial community dynamics from noncancerous to gastric cancer. *Front. Microbiol.* 14:1138928. doi: 10.3389/fmicb.2023.1138928

Pimentel-Nunes, P., Barros, A., Pita, I., Miranda, I., Conceição, G., Borges-Canha, M., et al. (2021). Gastric microbiome profile throughout gastric carcinogenesis: beyond helicobacter. *Scand. J. Gastroenterol.* 56, 708–716. doi: 10.1080/00365521.2021.1902560

Plottel, C. S., and Blaser, M. J. (2011). Microbiome and malignancy. *Cell Host Microbe* 10, 324–335. doi: 10.1016/j.chom.2011.10.003

Qi, Y. F., Sun, J. N., Ren, L. F., Cao, X. L., Dong, J. H., Tao, K., et al. (2019). Intestinal microbiota is altered in patients with gastric cancer from Shanxi province, China. *Dig. Dis. Sci.* 64, 1193–1203. doi: 10.1007/s10620-018-5411-y

Rawla, P., and Barsouk, A. (2019). Epidemiology of gastric cancer: global trends, risk factors and prevention. *Prz. Gastroenterol.* 14, 26–38. doi: 10.5114/pg.2018.80001

Sohn, S. H., Kim, N., Jo, H. J., Kim, J., Park, J. H., Nam, R. H., et al. (2017). Analysis of gastric body microbiota by pyrosequencing: possible role of bacteria other than *Helicobacter pylori* in the gastric carcinogenesis. *Journal Cancer Prevent.* 22, 115–125. doi: 10.15430/JCP.2017.22.2.115

Spiegelhauer, M. R., Kupcinskas, J., Johannesen, T. B., Urba, M., Skieceviciene, J., Jonaitis, L., et al. (2020). Transient and persistent gastric microbiome: adherence of bacteria in gastric cancer and dyspeptic patient biopsies after washing. *J. Clin. Med.* 9:1882. doi: 10.3390/jcm9061882

Sun, Q. H., Zhang, J., Shi, Y. Y., Zhang, J., Fu, W. W., and Ding, S. G. (2022). Microbiome changes in the gastric mucosa and gastric juice in different histological stages of *Helicobacter pylori*-negative gastric cancers. *World J. Gastroenterol.* 28, 365–380. doi: 10.3748/wjg.v28.i3.365

Sung, H., Ferlay, J., Siegel, R. L., 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. doi: 10.3322/caac.21660

Taha, S. F. M., Bhassu, S., Omar, H., Raju, C. S., Rajamanikam, A., Govind, S. K. P., et al. (2023). Gut microbiota of healthy Asians and their discriminative features revealed by metagenomics approach. *3 Biotech* 13:275. doi: 10.1007/s13205-023-03671-3

Tang, Q., Jin, G., Wang, G., Liu, T., Liu, X., Wang, B., et al. (2020). Current sampling methods for gut microbiota: a call for more precise devices. *Front. Cell. Infect. Microbiol.* 10:151. doi: 10.3389/fcimb.2020.00151

Tropini, C., Moss, E. L., Merrill, B. D., Ng, K. M., Higginbottom, S. K., Casavant, E. P., et al. (2018). Transient osmotic perturbation causes long-term alteration to the gut microbiota. *Cell* 173, 1742–54.e17. doi: 10.1016/j.cell.2018.05.008

Wang, Z., Gao, X., Zeng, R., Wu, Q., Sun, H., Wu, W., et al. (2020). Changes of the gastric mucosal microbiome associated with histological stages of gastric carcinogenesis. *Front. Microbiol.* 11:997. doi: 10.3389/fmicb.2020.00997

Wang, L. L., Liu, J. X., Yu, X. J., Si, J. L., Zhai, Y. X., and Dong, Q. J. (2018). Microbial community reshaped in gastric cancer. *Eur. Rev. Med. Pharmacol. Sci.* 22, 7257–7264. doi: 10.26355/eurrev_201811_16260

Wang, L., Zhou, J., Xin, Y., Geng, C., Tian, Z., Yu, X., et al. (2016). Bacterial overgrowth and diversification of microbiota in gastric cancer. *Eur. J. Gastroenterol. Hepatol.* 28, 261–266. doi: 10.1097/MEG.0000000000000542

Wei, Q., Zhang, Q., Wu, Y., Han, S., Yin, L., Zhang, J., et al. (2023). Analysis of bacterial diversity and community structure in gastric juice of patients with advanced gastric cancer. *Discov. Oncol.* 14:7. doi: 10.1007/s12672-023-00612-7

Wells, G., Shea, B. J., O'Connell, D., Peterson, J., Welch, V., Losos, M., et al. (2000). The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. (2000). Available at: https://api.semanticscholar.org/CorpusID:79550924.

Widder, S., Allen, R. J., Pfeiffer, T., Curtis, T. P., Wiuf, C., Sloan, W. T., et al. (2016). Challenges in microbial ecology: building predictive understanding of community function and dynamics. *ISME J.* 10, 2557–2568. doi: 10.1038/ismej.2016.45

World Health Organization. Global health estimates 2020: Deaths by cause, age, sex, by country and by region, 2000–2019 (2020). Available at: http://who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-leading-causes-of-death.

Wu, Z. F., Zou, K., Wu, G. N., Jin, Z. J., Xiang, C. J., Xu, S., et al. (2021). A comparison of tumor-associated and non-tumor-associated gastric microbiota in gastric cancer patients. *Dig. Dis. Sci.* 66, 1673–1682. doi: 10.1007/s10620-020-06415-y

Yang, P., Zhang, X., Xu, R., Adeel, K., Lu, X., Chen, M., et al. (2022). Fungal microbiota dysbiosis and ecological alterations in gastric cancer. *Front. Microbiol.* 13:889694. doi: 10.3389/fmicb.2022.889694

Yu, G., Torres, J., Hu, N., Medrano-Guzman, R., Herrera-Goepfert, R., Humphrys, M. S., et al. (2017). Molecular characterization of the human stomach microbiota in gastric cancer patients. *Front. Cell. Infect. Microbiol.* 7:302. doi: 10.3389/fcimb.2017.00302

Zhang, X., Li, C., Cao, W., and Zhang, Z. (2021). Alterations of gastric microbiota in gastric cancer and precancerous stages. *Front. Cell. Infect. Microbiol.* 11:559148. doi: 10.3389/fcimb.2021.559148

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