

Understanding the role of microbiome in alteration of cellular metabolism and cancer development

Edited by

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Understanding the role of microbiome in alteration of cellular metabolism and cancer development

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Editorial: Understanding the role of microbiome in alteration of cellular metabolism and cancer development

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microbiome-cancer interactions, metabolic dysregulation, microbial dysbiosis, multi-omics analysis, precision oncology

Editorial on the Research Topic

Understanding the role of microbiome in alteration of cellular metabolism and cancer development

Introduction

Cancer is not just a genetic disease but is deeply entangled with trillions of microbes that live in and on our bodies. The human microbiome has emerged as a critical regulator of cellular metabolism and, increasingly, as a driver of carcinogenesis across multiple organ systems. In this editorial, we provide a concise overview of the intricate relationship between the microbiome and carcinogenesis, underscoring several unifying insights crucial for understanding this emerging field. The ten papers published in frontiers research editorial topic titled “Understanding the Role of Microbiome in Alteration of Cellular Metabolism and Cancer Development,” explored the surprising connection between human microbiome and cancer—ranging from narrative and systematic reviews to original mendelian randomization and multi-omics studies. These studies guide us, how microbial communities and their metabolites modulate tumor initiation, progression, and treatment response. Here, we have summarized each contribution and suggested future directions for this rapidly evolving field.

Sabour et al. comprehensively reviewed, how anaerobic bacteria including *Fusobacterium nucleatum*, *Enterotoxigenic Bacteroides fragilis*, *Peptostreptococcus*, *Prevotella*, *Clostridium* spp., and others initiate and exacerbate colorectal cancer (CRC). Under low-oxygen conditions of a tumor, these bacteria release toxins and metabolites that activate multiple mechanisms by which anaerobic bacteria drive carcinogenesis: activation of Wnt/ β -catenin signaling, disruption of epithelial architecture, proinflammatory cytokine induction, and direct genotoxic DNA damage in hypoxic tumor niches, essentially providing a local environment

to favor tumor growth. The authors underscore the role of dysbiosis and aberrant microbial metabolites (e.g., secondary bile acids, short-chain fatty acids) in shaping a pro-tumorigenic microenvironment.

Chen et al. provided a comprehensive review that links the gut and intratumorally microbiota dysbiosis and gastric cancer (GC). They have highlighted the pivotal role of microbial dysbiosis in the initiation and progression of GC, wherein; imbalance in the microbial community notably *Helicobacter pylori* alongside enrichment of *Citrobacter*, *Clostridium*, *Fusobacterium*, and others contribute to GC by promoting genomic instability, impaired DNA repair, exacerbating tumor hypoxia, and fostering an immunosuppressive microenvironment. The authors evaluate the translational aspect of potential microbiota-based interventions including probiotics, prebiotics, antibiotics, fecal transplantation, and traditional Chinese medicine to improve the chemotherapeutic and immunotherapeutic outcomes. The authors also highlighted the need for rigorous preclinical and clinical validation of these strategies.

Jin and Zhong integrated the transcriptomic profiles with gut microbiome data to colorectal cancer. Based on their analysis they classified colorectal cancer into two biologically and clinically distinct tumor subtypes and constructed a risk score model. The one group, marked by favorable prognosis, in contrasts to another group, which harbors a richer gut microbiota and poorer outcomes. The authors risk-scoring model effectively stratifies survival and links microbial composition to immune microenvironment features and therapeutic sensitivity. Distinct taxa including *Robiginitomaculum* and *Myxococcus* in the low-risk group, and *Sutterella* and *Zymomonas* in the high-risk group emerge as potential biomarkers. This work represents a meaningful step toward the critical importance of personalized treatment.

A mendelian randomization study investigates whether gut microbes can causally influence the risk of ovarian cancer through metabolic changes. Using large-scale Genome-Wide Association Study (GWAS) datasets for gut microbiota, blood metabolites, and ovarian cancer, the authors identify turicibacter sp001543345 as a protective player. Its presence appears to affect lipid metabolism by (1) lowering free cholesterol in small HDL and raising saturated fatty acid ratios to total fatty acids, (2) increasing total cholesterol and cholesteryl esters to total lipids ratio in very-small VLDL particles changes that collectively reduce cancer risk. Additionally, they mapped the genetic-microbial-metabolic chain that pinpoints lipoprotein subclasses and fatty acid ratios as key mediators. Such specificity suggests a future where microbial profiling and targeted lipid modulation could form part of ovarian cancer prevention strategies. By bridging genetics, microbiology, and lipid biochemistry, the study offers a compelling glimpse of precision cancer prevention grounded in the microbiome (Zhang et al.).

Gu et al. use mendelian randomization to show a causal link between specific oral bacteria and CRC risk using data from two large east Asian databanks including China national gene bank and biobank Japan. Using multi-omics approach, they identify 19 taxa that exhibit strong causal associations, with some such as RUG343 appearing protective and others like HOT-345_umgs_976 and W5053_sp000467935_mgs_712 increasing susceptibility. Single-

cell RNA sequencing reveals that high-risk microbial profiles are associated with activation of JAK-STAT signaling and tyrosine metabolism in tumor-adjacent endothelial cells. These findings suggest that oral microbes may influence CRC development through metabolic and inflammatory pathways. They performed *in silico* drug screening and predicted potential therapeutic agents, including menadione sodium bisulfite and raloxifene, that could target these mechanisms and interrupt the microbe driven pathways. This integrated genetic, cellular, and drug-prediction approach opens new possibilities for CRC prevention and treatment.

In a study, Han and Fan unfold the complexity of how shifts in vaginal microecology align with human papillomavirus (HPV) infection and cervical lesion progression. Using Illumina high-throughput sequencing targeting the V4 region of the 16S rRNA gene, they characterized microbial communities across healthy women, high-risk HPV carriers, and those with cervical intraepithelial neoplasia (CIN). Their data reveal that although overall species richness of vaginal microbes doesn't change, cervical cancer (CC) patients exhibit significantly higher microbial diversity compared to high-risk HPV carriers or those with CIN. CC patients showed decline in *Lactobacillus* and Cyanobacteria and a rise in genera such as *Dialister* and *Peptoniphilus*. Crucially, the research identifies stage-specific biomarkers, including *Varibaculum* in healthy women, *Saccharopolyspora* in individuals with high-risk HPV, and several taxa such as *Coprococcus*, *Peptococcus*, and *Ruminococcus* in cervical cancer. These biomarkers suggest that vaginal microbial composition could serve as a diagnostic adjunct for distinguishing between infection stages and cervical lesions. The findings hint that restoring a *Lactobacillus*-dominated microbiome might not only help manage HPV infection but also slow or prevent progression to cervical cancer. This study stands out by providing a proposing tangible microbial target for early intervention of cancer.

Cao et al. presents a narrative review on evidence linking gut microbial alterations to benign prostatic hyperplasia and prostate cancer. The authors synthesize mounting observational and animal data linking gut dysbiosis to chronic prostatitis, benign prostatic hyperplasia, and prostate cancer. Dysbiosis induced changes in circulating short-chain fatty acids (SCFAs), androgen metabolism, and systemic inflammation are proposed as drivers of prostate cell proliferation and malignant transformation. The review highlights emerging data on fecal metabolite signatures as noninvasive biomarkers and therapeutic frontier in men's urological health. Lastly, the review advocates for controlled trials of microbiota-modulating agents in prostatic disease.

Biliary atresia, devastating cholangiopathy remains one of the most challenging pediatric liver diseases, with a complex interplay of genetic, immune, infectious, and environmental factors contributing to its onset and progression. Feng et al. reviewed the role of gut microbiota and their metabolites in biliary atresia in children. Recent research has brought the gut microbiome into sharper focus, revealing how microbial imbalance and altered metabolites may disrupt bile acid metabolism, trigger inflammation, and influence post-surgical outcomes. This review highlights how interventions targeting gut bacteria through probiotics, prebiotics, or microbial metabolites like

butyrate could complement current surgical treatments such as Kasai hepatoportoenterostomy. While promising, these strategies require robust clinical validation to confirm their safety, efficacy, and long-term benefits. Understanding the gut-liver axis in biliary atresia could pave the way for more personalized, effective, and preventative approaches.

Yan et al. used 16S rRNA sequencing and LC-MS metabolomics to profile gut microbiota and serum metabolites in postmenopausal women with normal and reduced bone mineral density (BMD). They divided the individuals into normal and osteoporosis (OS) group. They found higher microbial richness in low-BMD women, with *Bacteroides*, *Blautia*, *Ruminococcus*, and *Anaerostipes* linked to higher BMD, while *Agathobacter* and *Lactobacillus* were enriched in low-BMD cases. Metabolomics revealed alterations in tryptophan metabolism, fatty acid degradation, and steroid hormone biosynthesis. Notably, *Bacteroides* abundance correlated with the microbial *tnaA* gene, connecting gut metabolism to bone health. A combined microbial-metabolite model accurately predicted low-BMD status. These findings highlight multi-omics biomarkers and pathways that may guide microbiome-targeted strategies for osteoporosis prevention in postmenopausal women.

Liang et al. employed bidirectional mendelian randomization approach to test causal relations between gut microbiota changes and lymphoma risk. They used robust method utilizing large-scale GWAS data and identify specific bacterial taxa that appear to increase or reduce the risk of distinct lymphoma subtypes, from diffuse large B-cell lymphoma to Hodgkin lymphoma. microbes like *Alistipes*, *Turicibacter*, *Lactobacillus*, and *Akkermansia* showing protective effects, while *Ruminococcaceae* UCG002, *Eubacterium ventriosum* group, and *Phascolarctobacterium* increased risk. These effects may act through immune modulation, including regulation of immune cell balance, influencing inflammation and antitumor immunity. Microbial metabolites such as short-chain fatty acids particularly butyrate were implicated in key pathways like NF- κ B inhibition, histone acetylation, and apoptosis induction. These findings pave the way for microbiome-based preventive strategies in hematologic malignancies.

As we look ahead, the convergence of high-throughput multi-omics, causal inference methods, and precision microbiology will be

critical for harnessing the microbiome in cancer prevention, diagnosis, and therapy. The studies summarized here lay a robust foundation and chart a clear path for the next wave of microbiome-centric oncology research.

Author contributions

LT: Writing – original draft, Writing – review & editing. ST: Writing – review & editing, Writing – original draft. VR: Writing – review & editing. SV: Writing – review & editing. PK: Writing – review & editing, Supervision, Writing – original draft.

Conflict of interest

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Causal relationships between gut microbiota and lymphoma: a bidirectional Mendelian randomization study

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Background: Multiple studies have suggested a possible connection between the gut microbiota and the development of lymphoma, though the exact nature of this relationship remains unclear. This study aimed to explore whether a causal association exists between gut microbiota and lymphoma.

Methods: A bidirectional two-sample Mendelian randomization (MR) approach was conducted to investigate potential causal effects between gut microbiota and various lymphoma subtypes. The primary method employed for MR analysis was inverse variance weighted (IVW), supplemented by additional methods including MR-Egger, weighted median, and weighted mode approaches. The Cochrane Q test, MR-PRESSO global test and MR-Egger intercept test were performed to assess pleiotropy and heterogeneity. Furthermore, a reverse MR analysis was performed to explore potential reverse causal effect.

Results: The primary MR analysis identified 36 causal relationships between genetic liabilities in gut microbiota and different lymphoma subtypes. Neither the MR-PRESSO test nor the MR-Egger regression detected any pleiotropy, and Cochran's Q test indicated no significant heterogeneity.

Conclusions: Our MR analysis revealed substantial causal associations between gut microbiota and lymphoma, offering new insights into lymphoma prevention and management microbiota.

KEYWORDS

gut microbiota, Hodgkin lymphoma, non-Hodgkin lymphoma, Mendelian randomization, causal association

Introduction

Lymphoma, a type of neoplasia characterized by significant heterogeneity, is commonly classified as Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). These cancers are known for their varying degrees of immune evasion (Swerdlow et al., 2016). Over the past decade, changes in population growth and age structure have contributed to a continued increase in lymphoma incidence (Huang et al., 2022; Zhang et al., 2023). Despite significant advancements in treatments in recent years, the pathogenesis mechanisms of lymphoma remain incompletely elucidated. Currently, the lack of effective treatment options for refractory or drug-resistant lymphomas remains a persistent challenge (Brice et al., 2021; Bishop et al., 2022; Sehgal et al., 2022; Luan et al., 2024). Therefore, it is imperative to unravel the key mechanisms that govern tumor behavior and to develop clinically relevant biomarkers and therapeutic targets. These efforts aim to reduce the incidence of lymphoma and improve prognostic outcomes for patients.

Recently, the emerging paradigm of the microbiota-gut-lymphoma axis has been employed to explore potential correlations between the abundance of gut microbiota and a predisposition to lymphoma (Shi and Zhang, 2021; Upadhyay Banskota et al., 2023). Often described as our “second genome”, the gut microbiota plays a crucial role in shaping our immune response, educating it, and providing protection against pathogen overgrowth (Lynch and Pedersen, 2016). Its influence has been noted in conditions such as NHL and acute lymphoblastic leukemia (Rajagopala et al., 2016; Diefenbach et al., 2021; Shi et al., 2023). A classifier, developed using gut metagenomes for the natural killer/T-cell lymphoma cohort, achieved an accuracy of 0.813 area under the receiver operating characteristic curve (AUROC) in cross-validation (Shi et al., 2023). However, these studies primarily rely on analyzing the abundance and fluctuations of gut microbiota in patients’ fecal samples, and conducting experiments that involve transplanting microbiota into germ-free mice. Despite these advances, the precise correlation between gut microbiota and

lymphoma remains indeterminate, underscoring the need for further research to thoroughly explore this relationship.

Mendelian randomization (MR) is a robust method that utilizes comprehensive data from genome-wide association study (GWAS) to investigate genetic associations. The main benefit of adopting this strategy lies in its capacity to effectively minimize the impact of confounders, including environmental variables, on the outcome. MR analysis involves using single nucleotide polymorphisms (SNPs), derived from independent GWAS, as instrumental variables (IVs). These SNPs are integrated with relevant health outcome data, facilitating the estimation of causal relationships within a unified framework. Additionally, this method enables the distinction between causal and non-causal associations using cross-sectional data (Burgess et al., 2015).

By employing a bidirectional two-sample MR analysis, we sought to investigate the causal association between gut microbiota and lymphoma, with the objective of providing novel insights into approaches for lymphoma prevention and management.

Materials and methods

Study design

Relevant GWAS summary data were employed to probe the plausible causal correlation between gut microbiota and malignant lymphoma, facilitated by a bidirectional two-sample MR analysis (Visscher et al., 2012). Initially, our study focus on determining whether gut microbiota exhibits a preventive or promotive role in lymphoma development. Moreover, we performed a reverse MR analysis to examine whether lymphoma might causally affect gut microbiota. The workflow of this study is underpinned by three fundamental IVs assumptions that support the primary MR analysis, as illustrated in Figure 1. To ensure the robustness of the findings, three hypotheses must be satisfied in the two-sample MR (Bowden et al., 2015): (1) Relevance, demonstrating a significant

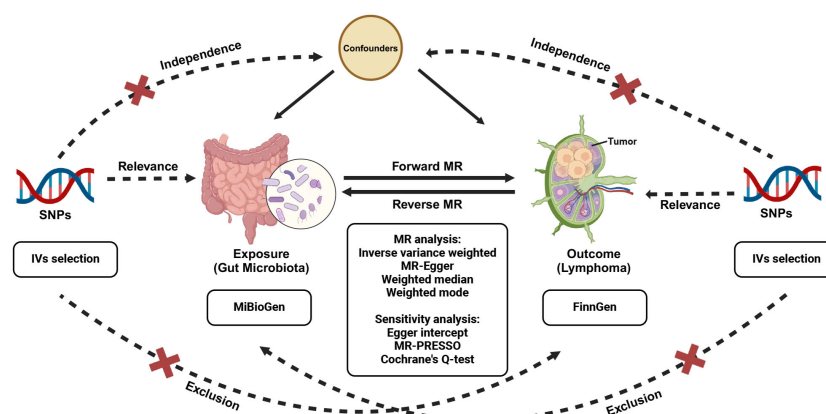


FIGURE 1

Study design of the bidirectional MR study of the correlation between gut microbiota and lymphoma. MR, Mendelian randomization; SNPs, single nucleotide polymorphisms.

association between genetic variations and exposure; (2) Independence, ensuring no relationship between genetic variants and confounding factors; and (3) Exclusion, stipulating that the genetic variants influence the outcome solely through exposure, without involving any other pathways. Genetic variants that fulfill these three hypotheses can be utilized as IVs.

Data selection

Gut microbiota GWAS

Information was obtained from an exhaustive microbiome GWAS conducted by the MiBioGen consortium (Kurilshikov et al., 2021). This multi-ethnic GWAS comprised 18,340 individuals from 24 study cohorts. The analysis demonstrated a connection between autosomal human genetic variants and gut microbiota, taking into account variables such as age, gender, study-specific factors, and genetic principal components, utilizing profiles obtained through 16S ribosomal RNA gene sequencing. Our analysis encompassed a total of 196 taxa, consisting of 119 genera, 32 families, 20 orders, 16 classes and 9 phyla, excluding those unable to be definitively classified or named. Detailed information was shown in [Supplementary Table S1](#).

Lymphoma GWAS

We retrieved data on lymphomas from the FinnGen database (<https://www.finnngen.fi/en>). The GWAS for HL comprised 780 cases and 299952 controls. For NHL, the GWAS data covered various subtypes: diffuse large B-cell lymphoma (DLBCL) with 1010 cases and 287137 controls, follicular lymphoma (FL) with 1081 cases and 299952 controls, mature T/NK-cell lymphomas with 335 cases and 299952 controls, and other and unspecified types of NHL with 1088 cases and 299952 controls. This comprehensive categorization provides more accurate and extensive data on genetic variances, aiding our exploration of causal associations between gut microbiota and different malignant lymphoma pathological subtypes within our MR investigation. Detailed information can be found in [Supplementary Table S1](#).

Selection of eligible IVs

To guarantee the accuracy and authenticity of our findings on the possible association between gut microbiota and lymphoma susceptibility, we employed a range of quality control measures to identify optimal independent IVs. First, SNPs selected to serve as IVs were required to exhibit a significant correlation with the gut microbiota. To explore potential causal associations, we adopted a locus-wide significance threshold at $p = 1 \times 10^{-5}$, consistent with thresholds frequently used in previous analyses (Liu et al., 2022; Lopera-Maya et al., 2022). Additionally, in reverse MR analysis, we employed a lenient genome-wide significance threshold at $p = 5 \times 10^{-6}$ to identify SNPs correlated with lymphoma (Su et al., 2023; Xie et al., 2023). Second, to mitigate potential biases due to strong linkage disequilibrium (LD), we conducted an LD analysis with a threshold set at $r^2 < 0.001$ and a clumping distance of 10,000 kb,

employing the 1000 Genomes Project European samples as a reference panel (Purcell et al., 2007). Third, to minimize any potential confounders, each SNP was assessed in the PhenoScanner website (Kamat et al., 2019). Fourth, GWAS summary data for the chosen SNPs were retrieved from the outcome dataset, and SNPs strongly associated with the outcome ($p < 5 \times 10^{-5}$) were excluded. Fifth, to evaluate the potential influence of horizontal pleiotropy, we performed MR-Egger regression tests and MR-PRESSO analyses. Simultaneously, we excluded palindromic SNPs to avoid potential biases related to strand orientation or allele coding, and removed ambiguous and duplicated SNPs. Finally, IVs were omitted if the F -statistic fell below 10, calculated utilizing the subsequent equation: $F = R^2(n - k - 1)/k(1 - R^2)$. Here R^2 denotes the proportion of variance accounted for by all SNPs, n stands for the total sample size, and k denotes the number of SNPs.

Mendelian randomization analyses

To investigate causality, the inverse variance weighted (IVW) method was employed as the principal approach to synthesize effect estimates (Burgess et al., 2013). Supplemental calculations were conducted utilizing various methods, among them MR-Egger, weighted mode, and weighted median, each accounting for varying assumptions regarding potential pleiotropy (Bowden et al., 2015, 2016). The consistency of results from these complementary methods with the IVW estimates enhances the credibility of our findings. For significance evaluation, a Bonferroni correction was applied, setting the significance thresholds for each taxonomic level by dividing 0.05 by the total number of independent bacterial taxa present at each level: phylum ($p < 5.6 \times 10^{-3}$), class ($p < 3.1 \times 10^{-3}$), order ($p < 2.5 \times 10^{-3}$), family ($p < 1.6 \times 10^{-3}$), and genus ($p < 4.2 \times 10^{-4}$). Additionally, p -values that fell between the established significance threshold and 0.05 were interpreted as suggestive of a potential causal relationship.

We calculated the heterogeneity statistic Q to assess effect estimates. Outlier SNPs were identified using the MR pleiotropy residual sum and outlier (MR-PRESSO) method (Verbanck et al., 2018). Furthermore, we implemented the leave-one-out technique to evaluate the potential influence of a single instrument on our MR findings.

We performed all analyses using the statistical software R (version 4.2.2), employing the TwoSampleMR (version 0.5.6) and MR-PRESSO (version 1.0) packages.

Ethical approval

The summary datasets are freely accessible through OPEN GWAS. We utilized data from participating studies that had received ethical clearance from committees overseeing human experimentation standards. This eliminated the need for additional ethical approval for this study.

Results

Instrumental variables selection

In our analysis, we initially identified appropriate IVs based on predefined criteria. Details about the SNPs utilized in the two-sample MR analysis can be found in [Supplementary Table S2-S6](#). After data harmonization, we determined that more than one SNP was associated with each bacterial taxon and lymphoma subtype. Furthermore, the *F*-statistics for all selected SNPs exceeded 10, alleviating concerns about the strength of the IVs.

MR analysis

The causal effects of gut microbiota on lymphoma

Four MR methods were employed to investigate the causal associations between specific bacterial taxa and various lymphoma subtypes, as illustrated in [Supplementary Table S7-S11](#).

The IVW analysis indicated that four gut microbiota taxa had causal effects on DLBCL, as illustrated in [Figure 2](#). We found that the genus *Ruminococcaceae* UCG002 (odds ratio (OR): 1.43, 95% confidence interval (CI): 1.01–2.01, *p* = 0.043) and the genus *Coprobacter* (OR: 1.41, 95% CI: 1.01–1.96, *p* = 0.044) were positively correlated with the risk of DLBCL. On the contrary, the genus *Alistipes* (OR: 0.57, 95% CI: 0.33–0.98, *p* = 0.043) and the genus *Turicibacter* (OR: 0.60, 95% CI: 0.38–0.96, *p* = 0.034) were negatively correlated with DLBCL risk. Both weighted median and weighted mode analyses demonstrated consistent trends in ORs. Visual representations of the causal relationships between significant bacteria and DLBCL are demonstrated in scatter plots ([Supplementary Figure S1](#)).

The IVW analysis indicated that eight gut microbiota taxa had causal effects on FL, as illustrated in [Figure 3](#). We found that the order *Bacillales* (OR: 1.32, 95% CI: 1.02–1.73, *p* = 0.038), the family *Bacteroidales* S24 7group (OR: 1.50, 95% CI: 1.03–2.20, *p* = 0.036), the family *Family XIII* (OR: 1.99, 95% CI: 1.03–3.83, *p* = 0.040), the genus *Eubacterium ventriosum* group (OR: 1.53, 95% CI: 1.02–2.29, *p* = 0.040) and the genus *Ruminiclostridium9* (OR: 1.83, 95% CI: 1.00–3.32, *p* = 0.048) were positively correlated with the risk of FL, while the family *Peptostreptococcaceae* (OR: 0.63, 95% CI: 0.43–0.93, *p* = 0.019), the genus *Haemophilus* (OR: 0.70, 95% CI: 0.49–0.99, *p* = 0.049) and the genus *Ruminococcaceae* NK4A214 group (OR: 0.55, 95% CI: 0.32–0.93, *p* = 0.025) showed a negative correlation with FL risk. Both the weighted median and weighted mode demonstrated consistent trends in ORs. Visual representations of the causal relationships between significant bacteria and FL are demonstrated in scatter plots ([Supplementary Figure S1](#)).

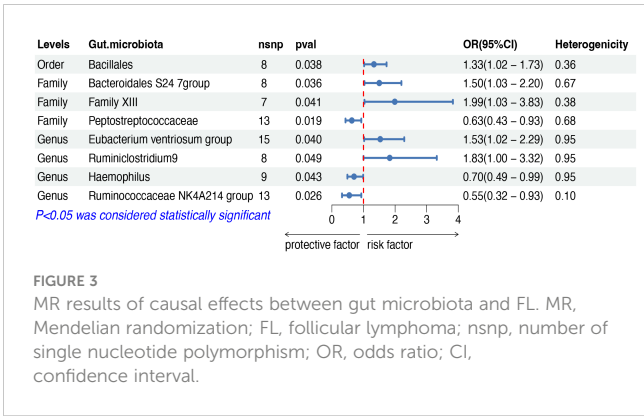
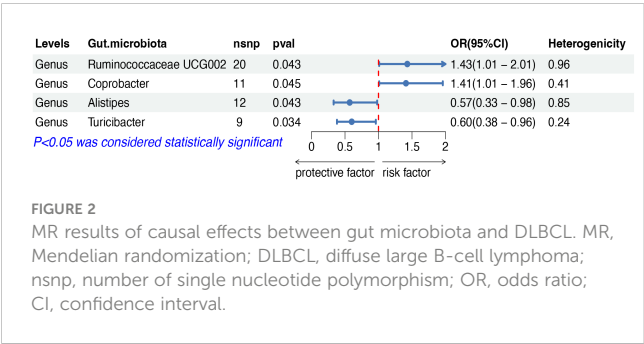
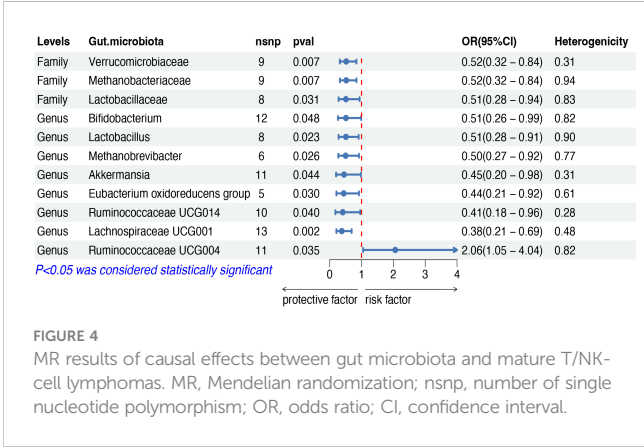


FIGURE 3
MR results of causal effects between gut microbiota and FL. MR, Mendelian randomization; FL, follicular lymphoma; nsnp, number of single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

The IVW analysis indicated that eleven gut microbiota taxa had causal effects on mature T/NK-cell lymphomas, as illustrated in [Figure 4](#). We found that the genus *Ruminococcaceae* UCG004 (OR: 2.06, 95% CI: 1.05–4.04, *p* = 0.035) was positively correlated with the risk of mature T/NK-cell lymphomas. Conversely, several taxa showed negative correlations with the risk, including the family *Methanobacteriaceae* (OR: 0.51, 95% CI: 0.32–0.84, *p* = 0.007) and the genus *Methanobrevibacter* (OR: 0.50, 95% CI: 0.27–0.92, *p* = 0.026), the family *Lactobacillaceae* (OR: 0.51, 95% CI: 0.28–0.94, *p* = 0.031) and the genus *Lactobacillus* (OR: 0.51, 95% CI: 0.28–0.91, *p* = 0.023), the family *Verrucomicrobiaceae* (OR: 0.44, 95% CI: 0.20–0.98, *p* = 0.044) and the genus *Akkermansia* (OR: 0.45, 95% CI: 0.20–0.98, *p* = 0.044), the genus *Bifidobacterium* (OR: 0.51, 95% CI: 0.26–0.99, *p* = 0.047), the genus *Eubacterium oxidoreducens* group (OR: 0.44, 95% CI: 0.21–0.92, *p* = 0.030), the genus *Ruminococcaceae* UCG014 (OR: 0.41, 95% CI: 0.28–0.96, *p* = 0.040) and the genus *Lachnospiraceae* UCG001 (OR: 0.38, 95% CI: 0.20–0.69, *p* = 0.002). Both the weighted median and weighted mode demonstrated consistent trends in ORs. Visual representations of the causal relationships between significant bacteria and FL are demonstrated in scatter plots ([Supplementary Figure S1](#)).



between significant bacteria and mature T/NK-cell lymphomas are demonstrated in scatter plots (Supplementary Figure S1).

The IVW analysis indicated that seven gut microbiota taxa had causal effects on other and unspecified types of NHL, as illustrated in Figure 5. We found that the order Clostridiales (OR: 1.71, 95% CI: 1.07–2.76, $p = 0.026$), the family Deffluvitaleaceae (OR: 1.47, 95% CI: 1.03–2.11, $p = 0.034$), the genus Flavonifractor (OR: 1.82, 95% CI: 1.02–3.25, $p = 0.042$) and the genus Phascolarctobacterium (OR: 1.64, 95% CI: 1.00–2.69, $p = 0.048$) showed a positive correlation with the risk of other and unspecified types of NHL. Conversely, the phylum Lentisphaerae (OR: 0.72, 95% CI: 0.53–0.98, $p = 0.038$), the order Bacillales (OR: 0.75, 95% CI: 0.58–0.97, risk of lymphoma, while the phylum $p = 0.027$), and the genus Slackia (OR: 0.60, 95% CI: 0.39–0.92, $p = 0.018$) were negatively correlated with the risk. Both the weighted median and weighted mode demonstrated consistent trends in ORs. Visual representations of the causal relationships between significant bacteria and other and unspecified types of NHL are demonstrated in scatter plots (Supplementary Figure S1).

The IVW analysis indicated that six gut microbiota taxa had causal effects on HL, as illustrated in Figure 6. We found that the family Bifidobacteriaceae (OR: 1.85, 95% CI: 1.08–3.16, $p = 0.025$) and the genus Eubacterium ventriosum group (OR: 1.68, 95% CI: 1.00–2.80, $p = 0.049$) were positively correlated with the risk of HL, while the family Desulfovibrionaceae (OR: 0.53, 95% CI: 0.29–0.99, $p = 0.045$), the family Lactobacillaceae (OR: 0.65, 95% CI: 0.44–0.97, $p = 0.035$), the genus Candidatus Soleaferrea (OR: 0.58, 95% CI: 0.40–0.86, $p = 0.007$) and the genus Coprobacter (OR: 0.63, 95% CI: 0.42–0.96, $p = 0.031$) showed a negative correlation with HL risk. Both the weighted median and weighted mode demonstrated consistent trends in ORs. Visual representations of the causal relationships between significant bacteria and HL are demonstrated in scatter plots (Supplementary Figure S1).

In the sensitivity analyses, we assessed horizontal pleiotropy and heterogeneity, as detailed in Supplementary Table S12–S13 and Supplementary Figure S2. The MR-PRESSO test revealed no signs of horizontal pleiotropy among these SNPs, as indicated by a p -value exceeding 0.05 in the global test. Additionally, according to Cochran’s Q statistics, we detected no pleiotropy due to cross-instrument effects, with the Cochran’s Q for IVW being exceeding 0.05. The MR-Egger analysis confirmed the absence of directional pleiotropy, with its p -value intercept also being greater than 0.05.

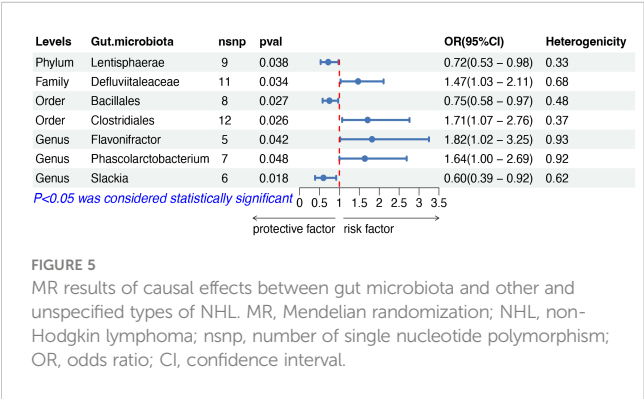


FIGURE 5
MR results of causal effects between gut microbiota and other and unspecified types of NHL. MR, Mendelian randomization; NHL, non-Hodgkin lymphoma; nsnp, number of single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

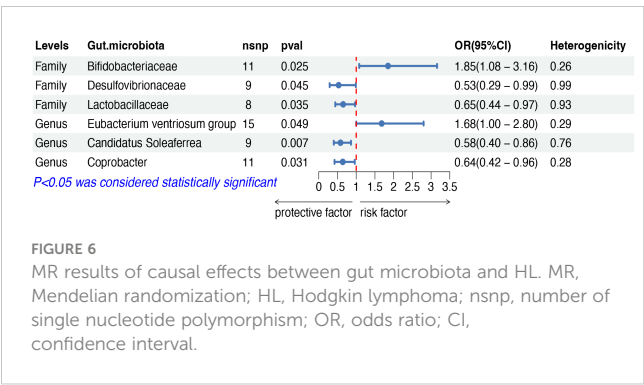


FIGURE 6
MR results of causal effects between gut microbiota and HL. MR, Mendelian randomization; HL, Hodgkin lymphoma; nsnp, number of single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Moreover, the leave-one-out analysis demonstrated the robustness and stability of the results. The sensitivity analyses of gut microbiota, which demonstrated significant causal relationships with lymphoma subtypes, are presented in Table 1.

The causal effects of lymphoma on gut microbiota

We performed reverse MR analyses to explore potential causal associations between lymphoma subtypes and forward significant bacteria. Considering the limited identification of lymphoma associated SNPs identified employing the strict threshold at $p < 5 \times 10^{-8}$, we adopted a more lenient threshold to mitigate potential inaccuracies arising from an insufficient number of SNPs. Except for a reverse causal relationship between the other and unspecified types of NHL and the phylum Lentisphaerae, which was excluded to guarantee the robustness of our results, we generally found no statistically significant associations using the IVW method, as detailed in Supplementary Table S14–S16.

Discussion

To our knowledge, this study is believed to be the first MR analysis to investigate the possible causal link between gut microbiota and lymphoma, representing a pre-lymphoma longitudinal study of the microbiota. We investigated the potential involvement of 196 distinct microbial taxa in the etiology of malignant lymphoma, utilizing the most comprehensive microbiome GWAS summary data available. Our results indicate causal associations between changes in the abundance of certain microbial groups and the development of lymphoma.

Emerging studies suggested that gut microbiota could regulate the formation of lymphoma through various mechanisms, including aberrant activation of the immune system, generation of both pro-inflammatory and anti-inflammatory responses, and modulation of metabolic processes (Shi and Zhang, 2021). In our study, the genus Phascolarctobacterium was associated with an increased risk of lymphoma, while the phylum Lentisphaerae, the family Desulfovibrionaceae and the genus Haemophilus within the phylum Proteobacteria, along with the family Methanobrevibacter and the genus Methanobrevibacter within the phylum Euryarchaeota, were found to be protective factors against lymphoma. Interestingly, the microbiota within the phylum

TABLE 1 Sensitivity analysis of the causal association between gut microbiota and lymphoma.

Subtype	Microbiota	Cochran Q test	MR- PRESSO	MR-Egger	
		p_value	p_value	Intercept	p_value
Diffuse large B-cell lymphoma	Genus Ruminococcaceae UCG002	0.941	0.970	-0.012	0.731
	Genus Coprobacter	0.413	0.450	-0.031	0.686
	Genus Alistipes	0.854	0.870	-0.110	0.197
	Genus Turicibacter	0.241	0.299	-0.016	0.889
Follicular lymphoma	Order Bacillales	0.362	0.398	0.047	0.612
	Family Bacteroidales S24 7group	0.673	0.696	-0.016	0.847
	Family XIII	0.382	0.415	0.014	0.885
	Family Peptostreptococcaceae	0.678	0.689	0.036	0.334
	Genus Eubacterium ventriosum group	0.946	0.946	0.029	0.677
	Genus Ruminiclostridium9	0.703	0.727	0.068	0.495
	Genus Haemophilus	0.472	0.467	-0.011	0.823
	Genus Ruminococcaceae NK4A214 group	0.105	0.131	-0.030	0.659
Mature T/NK-cell lymphomas	Family Verrucomicrobiaceae	0.310	0.312	0.108	0.334
	Family Methanobacteriaceae	0.943	0.955	-0.011	0.945
	Family Lactobacillaceae	0.832	0.806	0.010	0.922
	Genus Bifidobacterium	0.818	0.828	-0.069	0.320
	Genus Lactobacillus	0.903	0.891	0.009	0.923
	Genus Methanobrevibacter	0.773	0.796	0.079	0.455
	Genus Akkermansia	0.309	0.322	0.107	0.340
	Genus Eubacterium oxidoreducens group	0.606	0.651	-0.187	0.304
	Genus Ruminococcaceae UCG014	0.280	0.372	0.063	0.489
	Genus Lachnospiraceae UCG001	0.477	0.521	-0.050	0.696
	Genus Ruminococcaceae UCG004	0.824	0.834	0.080	0.632
Other and unspecified types of non- Hodgkin lymphoma	Phylum Lentisphaerae	0.330	0.378	-0.065	0.492
	Family Defluviitaleaceae	0.844	0.691	0.073	0.282
	Order Bacillales	0.478	0.538	-0.011	0.899
	Order Clostridiales	0.366	0.419	0.045	0.348
	Genus Flavonifractor	0.927	0.933	-0.056	0.606
	Genus Phascolarctobacterium	0.924	0.934	0.101	0.373
	Genus Slackia	0.624	0.704	-0.189	0.251
Hodgkin lymphoma	Family Bifidobacteriaceae	0.262	0.322	0.065	0.337
	Family Desulfovibrionaceae	0.994	0.995	0.004	0.963
	Family Lactobacillaceae	0.928	0.928	0.002	0.973

(Continued)

TABLE 1 Continued

Subtype	Microbiota	Cochran Q test	MR- PRESSO	MR-Egger	
		p_value	p_value	Intercept	p_value
	Genus Eubacterium ventriosum group	0.288	0.285	0.060	0.511
	Genus Candidatus Soleaferrea	0.759	0.772	0.008	0.966
	Genus Coprobacter	0.282	0.311	0.020	0.833

Firmicutes, Bacteroidetes, Actinobacteria and Verrucomicrobia can act as either risk or protective factors depending on the lymphoma subtypes. This phenomenon may be attributed to distinct pathogenic mechanisms among different tumor subtypes, heterogeneous immune responses of tumor cells to microorganisms across subtypes, and the influence of the tumor microenvironment, necessitating further validation through animal experiments and clinical trials.

In this study, the order Clostridiales within the class Clostridia was identified as a risk factor for lymphoma, although its suborder’s microbiota partly promotes tumorigenesis and partly inhibits tumor formation. Research studies have shown that the class Clostridia promotes the differentiation of CD4⁺Foxp3⁺Tregs cells, which subsequently induce the production of IgA⁺ B-cells in the intestinal tract (Atarashi et al., 2011). These B-cells can decrease the absorption of antigens derived from the microbiota in mucosal tissues and reduce the activation of systemic T-cell activation (Cong et al., 2009). Meanwhile, butyric acid, a metabolite of the class Clostridia (Vital et al., 2014), helps inhibit the activation of the NF-κB signaling pathway (Inan et al., 2000). These mechanisms collectively contribute to maintaining immune homeostasis, suppressing deleterious inflammation, and thereby inhibiting tumor formation. Importantly, inflammatory lymphomas are characterized by significant immune cell infiltration, particularly of T cells, frequent mutations that lead to persistent activation of the NF-κB pathway, and a heightened sensitivity to immune checkpoint blockade therapy (Kline et al., 2020). Consistent with these studies, family Peptostreptococcaceae, genus Eubacterium oxidoreducens group, Ruminococcaceae UCG014 and Ruminococcaceae NK4A214 group, Lachnospiraceae UCG001, Slackia, and Turicibacter were found to inhibit tumorigenesis.

Recently, numerous studies have focused on investigating the correlation between gut microbiota along with its metabolites and lymphoma. Gut microbiota, often referred to as the “new virtual metabolic organ”, regulates various metabolic pathways in the host (Evans et al., 2013). Some microbial metabolites can promote or inhibit carcinogenesis. For instance, most short-chain fatty acids (SCFAs), produced by the fermentation of dietary fibers by the two main phyla, Firmicutes and Bacteroidetes, are considered to have anticancer effects. SCFAs act as ligands for G protein-coupled receptors found throughout the gastrointestinal tract and on immune cells, and have been implicated in regulating inflammation and cancer progression (Zhang and Davies, 2016). Additionally, butyrate, a histone deacetylase inhibitor, initiates apoptosis and prevents tumor cell proliferation through the Warburg effect,

enhancing histone 3 acetylation and the expression of target genes such as Fas, P21, P27, etc (Vander Heiden et al., 2009; Wei et al., 2016). Lu et al. found that a decrease in Fusobacterium rectum led to butyrate deficiency in patients with lymphoma, failed to inhibit lymphomagenesis by suppressing the TNF-induced TLR4/MyD88/NF-κB axis (Lu et al., 2022). Interestingly, butyrate can also promote tumor formation by facilitating the extra-thymic production of Treg cells (Arpaia et al., 2013). Consistent with previous observational and animal studies, our study showed that group Eubacterium oxidoreducens, Ruminococcaceae UCG014, Ruminococcaceae NK4A214 group, and Lachnospiraceae UCG001 within the phylum Firmicutes, and the genus Alistipes and Coprobacter within the phylum Bacteroidetes function as protective factors for lymphoma. We also found genus Eubacterium ventriosum Group, Flavonifractor, Ruminococcaceae UCG002, Ruminococcaceae UCG004, and Ruminiclostridium 9 within the phylum Firmicutes to be risk factors for different types of lymphoma, suggesting that different genera of family Eubacteriaceae, Ruminococcaceae, and Lachnospiraceae may have distinct mechanisms of action in different lymphoma types.

Akkermansia muciniphila, a representative species of the phylum Verrucomicrobia in the human intestine, along with genus Lactobacillus and genus Alistipes, are considered important probiotic microorganisms in the human gut (Cani et al., 2022). These probiotics are thought to enhance antitumor activity by improving host metabolism, modulating the immune response and increasing efficacy of immune checkpoint inhibitors in patients with FL (Routy et al., 2018; Merryman et al., 2023). Certain strains of genus Lactobacillus and Bifidobacterium can inhibit the growth of Helicobacter pylori by releasing bacteriocins or organic acids, and may reduce its attachment to gastric epithelial cells, thereby lowering the risk of gastric adenocarcinoma and lymphoma (Gotteland et al., 2006). Furthermore, castalagin, which is enriched in bacteria associated with effective immunotherapeutic responses (e.g., family Ruminococcaceae and genus Alistipes), improves the ratio of CD8⁺ cells to FOXP3⁺CD4⁺ cells in the tumor microenvironment (Messaoudene et al., 2022).

Above all, our research contributes new perspectives on the potential causality between gut microbiota and lymphoma, which have not previously been reported. One major strength of this study lies in its utilization of a MR approach, which helps minimize confounding factors and biases commonly observed in observational studies, thereby enhancing the credibility of the results. Although the MR approach offers several benefits over traditional epidemiological research, interpreting the results

requires considerable caution. This caution is necessary due to potential variability in methodologies used across different cohorts within the MiBioGen consortium, as well as the dynamic and complex nature of the gut microbiota within its ecosystem. Consequently, further epidemiological studies and clinical trials are essential to more definitively determine the causal relationship between gut microbiota and lymphoma. Additionally, the resolution at the genus level provided by 16S sequencing is limited; therefore, alternative approaches, such as shotgun metagenomics, metatranscriptomics, proteomic analysis, and metabolomic profiling are recommended. These methods will enable better harmonization of GWAS data and lead to a more comprehensive understanding of the microbiome's involvement in lymphoma.

In conclusion, our study provides evidence for potential associations between alterations in the composition of gut microbiota and different subtypes of lymphoma. We discovered that several microbial taxa have causal effects on lymphoma, offering valuable insights into prophylactic and therapeutic targets against lymphoma. These findings suggest that microbial prophylaxis or interventions such as probiotic administration, fecal microbiota transplantation, or dietary modifications warrant further exploration.

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 the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

JL: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. GL: Conceptualization, Data curation,

Methodology, Software, Supervision, Writing – original draft, Writing – review & editing. WW: Data curation, Formal Analysis, Project administration, Writing – review & editing. HX: Formal Analysis, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, 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/fcimb.2024.1374775/full#supplementary-material>

References

- Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., et al. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504, 451–455. doi: 10.1038/nature12726
- Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., et al. (2011). Induction of colonic regulatory T cells by indigenous clostridium species. *Science* 331, 337–341. doi: 10.1126/science.1198469
- Bishop, M. R., Dickinson, M., Purtil, D., Barba, P., Santoro, A., Hamad, N., et al. (2022). Second-line tisagenlecleucel or standard care in aggressive B-cell lymphoma. *N Engl. J. Med.* 386, 629–639. doi: 10.1056/NEJMoa2116596
- 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., Davey Smith, G., Haycock, P. C., and Burgess, S. (2016). Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet. Epidemiol.* 40, 304–314. doi: 10.1002/gepi.21965
- Brice, P., Kerviler, E. de, and Friedberg, J. W. (2021). Classical hodgkin lymphoma. *Lancet (London England)* 398, 1518–1527. doi: 10.1016/S0140-6736(20)32207-8
- 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
- Burgess, S., Timpson, N. J., Ebrahim, S., and Davey Smith, G. (2015). Mendelian randomization: where are we now and where are we going? *Int. J. Epidemiol.* 44, 379–388. doi: 10.1093/ije/dyv108
- Cani, P. D., Depommier, C., Derrien, M., Everard, A., and de Vos, W. M. (2022). Akkermansia muciniphila: paradigm for next-generation beneficial microorganisms. *Nat. Rev. Gastroenterol. Hepatol.* 19, 625–637. doi: 10.1038/s41575-022-00631-9
- Cong, Y., Feng, T., Fujishashi, K., Schoeb, T. R., and Elson, C. O. (2009). A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. *Proc. Natl. Acad. Sci. United States America* 106, 19256–19261. doi: 10.1073/pnas.0812681106
- Diefenbach, C. S., Peters, B. A., Li, H., Raphael, B., Moskovits, T., Hymes, K., et al. (2021). Microbial dysbiosis is associated with aggressive histology and adverse clinical outcome in B-cell non-Hodgkin lymphoma. *Blood Adv.* 5, 1194–1198. doi: 10.1182/bloodadvances.2020003129
- Evans, J. M., Morris, L. S., and Marchesi, J. R. (2013). The gut microbiome: the role of a virtual organ in the endocrinology of the host. *J. Endocrinol.* 218, R37–R47. doi: 10.1530/JOE-13-0131
- Gotteland, M., Brunser, O., and Cruchet, S. (2006). Systematic review: are probiotics useful in controlling gastric colonization by *Helicobacter pylori*? *Aliment Pharmacol. Ther.* 23, 1077–1086. doi: 10.1111/j.1365-2036.2006.02868.x
- Huang, J., Pang, W. S., Lok, V., Zhang, L., Lucero-Prisno, D. E., Xu, W., et al. (2022). Incidence, mortality, risk factors, and trends for Hodgkin lymphoma: a global data analysis. *J. Hematol. Oncol.* 15, 57. doi: 10.1186/s13045-022-01281-9
- Inan, M. S., Rasoulpour, R. J., Yin, L., Hubbard, A. K., Rosenberg, D. W., and Giardina, C. (2000). The luminal short-chain fatty acid butyrate modulates NF- κ B activity in a human colonic epithelial cell line. *Gastroenterology* 118, 724–734. doi: 10.1016/S0016-5085(00)70142-9
- Kamat, M. A., Blackshaw, J. A., Young, R., Surendran, P., Burgess, S., Danesh, J., et al. (2019). PhenoScanner V2: an expanded tool for searching human genotype–phenotype associations. *Bioinformatics* 35, 4851–4853. doi: 10.1093/bioinformatics/btz469
- Kline, J., Godfrey, J., and Ansell, S. M. (2020). The immune landscape and response to immune checkpoint blockade therapy in lymphoma. *Blood* 135, 523–533. doi: 10.1182/blood.2019000847
- 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
- Liu, X., Tong, X., Zou, Y., Lin, X., Zhao, H., Tian, L., et al. (2022). Mendelian randomization analyses support causal relationships between blood metabolites and the gut microbiome. *Nat. Genet.* 54, 52–61. doi: 10.1038/s41588-021-00968-y
- Lopera-Maya, E. A., Kurilshikov, A., van der Graaf, A., Hu, S., Andreu-Sánchez, S., Chen, L., et al. (2022). Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project. *Nat. Genet.* 54, 143–151. doi: 10.1038/s41588-021-00992-y
- Lu, H., Xu, X., Fu, D., Gu, Y., Fan, R., Yi, H., et al. (2022). Butyrate-producing *Eubacterium rectale* suppresses lymphomagenesis by alleviating the TNF-induced TLR4/MyD88/NF- κ B axis. *Cell Host Microbe* 30, 1139–1150.e7. doi: 10.1016/j.chom.2022.07.003
- Luan, Y., Li, X., Luan, Y., Luo, J., Dong, Q., Ye, S., et al. (2024). Therapeutic challenges in peripheral T-cell lymphoma. *Mol. Cancer* 23, 2. doi: 10.1186/s12943-023-01904-w
- Lynch, S. V., and Pedersen, O. (2016). The human intestinal microbiome in health and disease. *N Engl. J. Med.* 375, 2369–2379. doi: 10.1056/NEJMra1600266
- Merryman, R. W., Redd, R. A., Freedman, A. S., Ahn, I. E., Brown, J. R., Crombie, J. L., et al. (2024). A multi-cohort phase 1b trial of rituximab in combination with immunotherapy doublets in relapsed/refractory follicular lymphoma. *Ann. Hematol.* 103, 185–198. doi: 10.1007/s00277-023-05475-0
- Messaoudene, M., Pidgeon, R., Richard, C., Ponce, M., Diop, K., Benlaifaoui, M., et al. (2022). A natural polyphenol exerts antitumor activity and circumvents anti-PD-1 resistance through effects on the gut microbiota. *Cancer Discovery* 12, 1070–1087. doi: 10.1158/2159-8290.CD-21-0808
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., et al. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575. doi: 10.1086/519795
- Rajagopala, S. V., Yooseph, S., Harkins, D. M., Moncera, K. J., Zabokrtsky, K. B., Torralba, M. G., et al. (2016). Gastrointestinal microbial populations can distinguish pediatric and adolescent Acute Lymphoblastic Leukemia (ALL) at the time of disease diagnosis. *BMC Genomics* 17, 635. doi: 10.1186/s12864-016-2965-y
- Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P. M., Alou, M. T., Daillère, R., et al. (2018). Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 359, 91–97. doi: 10.1126/science.aan3706
- Sehgal, A., Hoda, D., Riedell, P. A., Ghosh, N., Hamadani, M., Hildebrandt, G. C., et al. (2022). Lisocabtagene maraleucel as second-line therapy in adults with relapsed or refractory large B-cell lymphoma who were not intended for haematopoietic stem cell transplantation (PILOT): an open-label, phase 2 study. *Lancet Oncol.* 23, 1066–1077. doi: 10.1016/S1470-2045(22)00339-4
- Shi, Z., Hu, G., Li, M. W., Zhang, L., Li, X., Li, L., et al. (2023). Gut microbiota as non-invasive diagnostic and prognostic biomarkers for natural killer/T-cell lymphoma. *Gut* 72, 1999–2002. doi: 10.1136/gutjnl-2022-328256
- Shi, Z., and Zhang, M. (2021). Emerging roles for the gut microbiome in lymphoid neoplasms. *Clin. Med. Insights Oncol.* 15, 117955492110241. doi: 10.1177/11795549211024197
- Su, T., Yin, X., Ren, J., Lang, Y., Zhang, W., and Cui, L. (2023). Causal relationship between gut microbiota and myasthenia gravis: a bidirectional mendelian randomization study. *Cell Biosci.* 13, 204. doi: 10.1186/s13578-023-01163-8
- Swerdlow, S. H., Campo, E., Pileri, S. A., Harris, N. L., Stein, H., Siebert, R., et al. (2016). The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood* 127, 2375–2390. doi: 10.1182/blood-2016-01-643569
- Upadhyay Banskota, S., Skupa, S. A., El-Gamal, D., and D'Angelo, C. R. (2023). Defining the role of the gut microbiome in the pathogenesis and treatment of lymphoid Malignancies. *Int. J. Mol. Sci.* 24, 2309. doi: 10.3390/ijms24032309
- Vander Heiden, M. G., Cantley, L. C., and Thompson, C. B. (2009). Understanding the warburg effect: the metabolic requirements of cell proliferation. *Science* 324, 1029–1033. doi: 10.1126/science.1160809
- Verbanck, M., Chen, C.-Y., Neale, B., and Do, R. (2018). Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat. Genet.* 50, 693–698. doi: 10.1038/s41588-018-0099-7
- Visscher, P. M., Brown, M. A., McCarthy, M. I., and Yang, J. (2012). Five years of GWAS discovery. *Am. J. Hum. Genet.* 90, 7–24. doi: 10.1016/j.ajhg.2011.11.029
- Vital, M., Howe, A. C., and Tiedje, J. M. (2014). Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *mBio* 5, e00889. doi: 10.1128/mBio.00889-14
- Wei, W., Sun, W., Yu, S., Yang, Y., and Ai, L. (2016). Butyrate production from high-fiber diet protects against lymphoma tumor. *Leuk. Lymphoma* 57, 2401–2408. doi: 10.3109/10428194.2016.1144879
- 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
- Zhang, L. S., and Davies, S. S. (2016). Microbial metabolism of dietary components to bioactive metabolites: opportunities for new therapeutic interventions. *Genome Med.* 8, 1–18. doi: 10.1186/s13073-016-0296-x
- Zhang, N., Wu, J., Wang, Q., Liang, Y., Li, X., Chen, G., et al. (2023). Global burden of hematologic Malignancies and evolution patterns over the past 30 years. *Blood Cancer J.* 13, 82. doi: 10.1038/s41408-023-00853-3



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Characteristics of the gut microbiota and serum metabolites in postmenopausal women with reduced bone mineral density

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Introduction: Emerging evidence suggests that the gut microbiota is closely associated with bone homeostasis. However, little is known about the relationships among the bone mineral density (BMD) index, bone turnover markers, and the gut microbiota and its metabolites in postmenopausal women.

Methods: In this study, to understand gut microbiota signatures and serum metabolite changes in postmenopausal women with reduced BMD, postmenopausal individuals with normal or reduced BMD were recruited and divided into normal and OS groups. Feces and serum samples were collected for 16S rRNA gene sequencing, liquid chromatography coupled with mass spectrometry (LC-MS)-based metabolomics and integrated analysis.

Results: The results demonstrated that bacterial richness and diversity were greater in the OS group than in the normal group. Additionally, distinguishing bacteria were found among the two groups and were closely associated with the BMD index and bone turnover markers. Metabolomic analysis revealed that the expression of serum metabolites, such as etiocholanolone, testosterone sulfate, and indole-3-pyruvic acid, and the corresponding signaling pathways, especially those involved in tryptophan metabolism, fatty acid degradation and steroid hormone biosynthesis, also changed significantly. Correlation analysis revealed positive associations between normal group-enriched *Bacteroides* abundance and normal group-enriched etiocholanolone and testosterone sulfate abundances; in particular, *Bacteroides* correlated positively with BMD. Importantly, the tryptophan-indole metabolism pathway was uniquely metabolized by the gut bacteria-derived *tnaA* gene, the predicted abundance of which was significantly greater in the normal group than in the control group, and the abundance of *Bacteroides* was strongly correlated with the *tnaA* gene.

Discussion: Our results indicated a clear difference in the gut microbiota and serum metabolites of postmenopausal women. Specifically altered bacteria and derived metabolites were closely associated with the BMD index and bone turnover markers, indicating the potential of the gut microbiota and serum metabolites as modifiable factors and therapeutic targets for preventing osteoporosis.

KEYWORDS

osteoporosis, gut microbiota, serum metabolites, postmenopausal women, tryptophan-indole metabolism

Introduction

As a metabolic bone disease, osteoporosis is characterized by decreased bone mineral density (BMD) and microarchitectural deterioration (Gopinath, 2023). In elderly adults with impaired bone metabolism, fractures are more likely to occur, causing pain, disability, and loss of function (Liang et al., 2022). It is estimated that 10% of the global population suffers from osteoporosis, with 30% of those suffering from it being postmenopausal women older than 50 years of age (Black and Rosen, 2016; Yu and Xia, 2019). However, the current understanding of the pathogenesis of osteoporosis is insufficient for developing drugs that can completely prevent the occurrence and development of osteoporosis (Reid and Billington, 2022). As a result, identifying effective preventive intervention strategies to reduce the risk of fracture and diagnosing osteopenia early are important clinical issues (Song et al., 2022).

The gut microbiome inhabits the gastrointestinal tract and consists of approximately 10 trillion bacteria. The gut microbiota can interact with various organs and systems and is an important medium between the host and microorganisms (Brown et al., 2023). New high-throughput sequencing technologies have made it possible to analyze metabolic characteristics of intestinal microbial communities on a large scale, providing new directions for treating various metabolic disorders. There is growing evidence that the gut microbiota or its metabolites have an impact on bone health by regulating immune, vascular, endocrine, intestinal, and nervous systems (Ding et al., 2020; Song et al., 2022; Xu et al., 2022). Postmenopausal women exhibit altered gut microbiomes, as well as osteopenia and osteoporosis (Rettedal et al., 2021). The gut microbiota-bone axis suggests that *Prevotella histicola* can specifically protect against estrogen deficiency-induced bone loss (Wang et al., 2021). Bioavailable isoflavone and probiotic treatment improve bone status and estrogen metabolism in postmenopausal osteopenic women, according to a randomized controlled trial (Lambert et al., 2017). It follows that gut microbes play a direct role in postmenopausal osteoporosis bone metabolism regulation. However, the specific mechanisms involved in the interaction

between the gut microbiota, serum metabolites, and bone metabolism remain unknown.

This project used an integrated approach combining 16S rRNA gene sequencing with LC-MS-based metabolomics analysis of blood and feces to determine whether specific gut microbiota and their

TABLE 1 Clinical information of the participants for gut microbiota analysis.

Characteristics	Normal (n=38)	OS (n=25)	P-value
BMI (kg/m ²)	25.17 ± 3.51	23.35 ± 2.82	NS
Age (years)	65.21 ± 7.55	63.76 ± 6.63	NS
LS BMD (g/cm ²)	1.24 ± 0.18	0.99 ± 0.11	<0.001
LS T-score	1.24 ± 1.54	-1.02 ± 0.94	<0.001
LS Z-score	2.02 ± 1.57	0.46 ± 1.01	<0.001
Neck BMD (mg/cm ²)	955.16 ± 172.41	778.04 ± 165.85	<0.001
Neck T-score	0.67 ± 1.45	-0.72 ± 1.37	<0.001
Neck Z-score	1.67 ± 1.70	0.56 ± 1.38	<0.01
Hip BMD (mg/cm ²)	1015.63 ± 116.46	806.44 ± 156.85	<0.001
Hip T-score	0.65 ± 0.92	-0.94 ± 1.21	<0.001
Hip Z-score	1.16 ± 1.14	-0.08 ± 1.67	<0.001
ALP (U/L)	74.10 ± 21.13	93.43 ± 25.91	<0.01
VD (ng/mL)	27.74 ± 9.58	21.78 ± 6.18	<0.01
PTH (pg/mL)	33.20 ± 11.67	32.28 ± 10.73	NS
T-PINP (ng/mL)	46.22 ± 16.85	71.52 ± 29.51	<0.001
β-CITX (pg/mL)	504.07 ± 192.87	739.78 ± 263.93	<0.001
N-MID (ng/mL)	14.98 ± 5.21	23.38 ± 10.74	<0.001
E2(pmol/L)	52.11 ± 16.59	42.89 ± 10.37	<0.05

Values are mean ± SD for continuous variables and the P value is based on the Wilcoxon rank-sum test.

NS, nonsignificance.

TABLE 2 Clinical information of the participants for serum metabolites analysis.

Characteristics	Normal (n=61)	OS (n=61)	P-value
BMI (kg/m ²)	25.52 ± 3.42	23.82 ± 3.15	NS
Age (years)	64.25 ± 7.25	67.41 ± 7.94	NS
LS BMD (g/cm ²)	1.24 ± 0.15	0.96 ± 0.12	<0.001
LS T-score	1.18 ± 1.33	-1.27 ± 0.98	<0.001
LS Z-score	2.07 ± 1.42	0.51 ± 1.04	<0.001
Neck BMD (mg/cm ²)	949.30 ± 159.88	714.18 ± 143.89	<0.001
Neck T-score	0.67 ± 1.34	-1.25 ± 1.21	<0.001
Neck Z-score	1.68 ± 1.57	0.23 ± 1.21	<0.001
Hip BMD (mg/cm ²)	1007.59 ± 120.66	755.21 ± 135.88	<0.001
Hip T-score	0.60 ± 0.95	-1.33 ± 1.05	<0.001
Hip Z-score	1.17 ± 1.08	-0.24 ± 1.01	<0.001
ALP (U/L)	73.51 ± 23.86	91.32 ± 23.68	<0.001
VD (ng/mL)	20.17 ± 10.68	20.23 ± 8.30	<0.01
PTH (pg/mL)	31.93 ± 12.45	34.72 ± 12.12	NS
T-PINP (ng/mL)	49.98 ± 27.34	70.75 ± 30.21	<0.001
β-CTX (pg/mL)	507.54 ± 193.38	725.53 ± 300.86	<0.001
N-MID (ng/mL)	15.62 ± 5.07	22.28 ± 9.51	<0.001
E2(pmol/L)	56.08 ± 19.40	45.61 ± 15.09	<0.01

Values are mean ± SD for continuous variables and the P value is based on the Wilcoxon rank-sum test.

NS, nonsignificance.

metabolites are associated with reduced BMD in postmenopausal women. In this study, we evaluated the species and abundance of the gut microbiota and their interactions with metabolites in postmenopausal women with reduced BMD, thus providing new insights into the pathogenesis and therapeutic targets of postmenopausal osteoporosis.

Materials and methods

Study subjects

This study was approved by the Ethics Committee of The Second People's Hospital of Dalian (No.2022.174X). For gut microbiota analysis, postmenopausal subjects with normal BMD (normal group, n=38) or reduced BMD (OS group, n=25) were recruited. Clinical information was presented in Table 1. For metabolomics analysis, normal group (n=61) and OS group (n=61) were recruited. All subjects participating in the study were over 50 years of age and had undergone menopause. Detailed clinical information is presented in Table 2. The study did not include participants with cancer, kidney disease, metabolic or

genetic bone disease, digestive system disease, psychiatric illness, or those who had taken antibiotics within 3 months or those taking medications that might affect bone metabolism.

Clinical data and sample collection

All subjects were interviewed and measured for age, height, and weight, as well as their body mass index (BMI). Fresh feces and blood samples were collected after more than six hours of fasting in the morning.

After finishing serological testing, all collected serum and feces samples were transported to -80°C freezer for further analysis. Serum levels of total 25 (OH) vitamin D (VD), alkaline phosphatase (ALP), osteocalcin (N-MID), C-terminal peptide of type I collagen (β-CTX), N-terminal propeptide of type I procollagen (T-PINP), and parathyroid hormone (PTH) were measured. BMD, T-score and Z-score in the lumbar spine (L1-4, LS), femoral neck (Neck) and total hip joint (femoral neck, trochanteric and intertrochanteric areas, Hip) were measured using a dual-energy X-ray absorptiometry scanner.

Microbiota 16S rRNA gene sequencing

Total genome DNA from feces samples was extracted using CTAB/SDS method. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ug/μL using sterile water. Mix same volume of IX loading buffer (contained SYB green) with PCR products and operate electrophoresis on 2% agarose gel for detection. PCR products was mixed in equidensity ratios. Then, mixture PCR products was purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Fecal DNA was isolated and the V4 hyper variable regions of the bacterial 16S rRNA gene was amplified by primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT). After denaturation for 1 minute at 98°C, 30 cycles were performed, each lasting 10 seconds, followed by annealing at 50°C for 30 seconds and elongation at 72°C for 30 seconds. As soon as PCR products were purified, TruSeq DNA PCR-free sample preparation kits (Illumina, USA) and index codes were added to create sequencing libraries. At last, the libraries were sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated. The QIIME V.2.0 pipeline was used after barcodes were taken off the sequences (Hall and Beiko, 2018). Random selection of reads was used to reduce ASVs of each sample to 10000 (Valles-Colomer et al., 2022). ASV taxonomy was based on the Silva 138 SSURef NR99 16S rRNA gene reference database (Quast et al., 2013; Zhao et al., 2022).

Analysis of gut microbiota profile

Alpha diversity of gut microbiota was estimated by R package Vegan. And the beta diversity was estimated via vegdist function of Vegan R package based on the genus levels' Bray-Curtis distance

matrix (Lloyd-Price et al., 2019). The significant difference of the beta diversity was statistic by PERMANOVA test (adonis function). And the bacterial composition was analyzed by phyloseq R package (McMurdie and Holmes, 2013). Further, the LEfSe (Linear Discriminant Analysis Effect Size) analyze was performed via microbiomeMarker R package (Cao et al., 2022). Moreover, the predicted function of microbiota was an estimated by PICRUST2 (Liu et al., 2016; Douglas et al., 2020).

Analysis of serum metabolomics

A difference in serum metabolite signatures was detected between normal and OS groups by LC-MS. For the identification of metabolites, processed data, such as m/z, RT, and normalized peak areas, were imported into SIMCA. Metabolites were identified using the HMDB (Human Metabolome Database). The HMDB database was adopted to map and identify the metabolites. By using R package ropls to identify the metabolites changes within groups, partial least squares discriminant analysis (PLS-DA) was used to analyze the abundance of significant metabolites with projection (VIP) > 1 and p value (Wilcoxon test) < 0.05. The enrichment pathway between normal and OS serum metabolites profiles was analyzed by MetaboAnalyst 5.0 (Pang et al., 2021).

Correlation analysis

Using Spearman's correlation analysis, more than 20 variables including clinical variables and differences in microbiome were analyzed for the correlation between predicted function and changes in bacteria, microbiota, and metabolites. R package "psych", version 2.4.1 was used in the correlation analysis.

Marker panel for OS individuals

In the discovery dataset, linear regression model was built based on the signatures of microbiota or metabolites of OS subjects (Liang et al., 2020). And the possibility of model was estimated by predict function in both discovery and validation sets. Besides, the accuracy of marker panel for discriminate the OS participants from normal cases in both discovery and validation sets by R package pROC (Robin et al., 2011).

Co-occurrence network analysis

The co-occurrence network of gut microbiota in normal and OS groups was established by R package ggClusterNet based on top 500 ASVs (Wen et al., 2022). And the network vulnerability was estimated via ggClusterNet R package. Besides, the average variation degree (AVD) of the network was analyzed as previous described (Xun et al., 2021).

Statistical analyses

All statistical analyses and graphical representations of the study were performed via R. Data were expressed in mean $\pm$ SEM unless otherwise stated. We utilized Wilcoxon and chi-squared test to evaluate the difference between two groups for continuous and categorical variables, respectively. Significant difference between two groups at a confidence level of 0.05.

Results

Characteristics of the gut microbiota profile and metabolic function of OS patients

As shown in Figures 1A, B, the gut bacterial ACE and Chao1 indices of OS participants were greater than those of the healthy individuals. In addition, PCoA of the genus abundance of the gut microbiota revealed clear differences between the two groups (PERMANOVA test, $P < 0.05$) (Figure 1C). The difference arose from PCoA1 ($P < 0.05$). At the phylum level, there was no significant difference between the two groups (Figure 1D). At the genus level, the proportions of *Bacteroides*, *Blautia*, *Fusicatenibacter*, *Ruminococcus*, and *Anaerostipes* in the OS group were lower than those in the normal group (Figures 1E–I). However, higher proportions of the *Agathobacter* and *Lactobacillus* genera were observed in OS patients (Figures 1K, L).

Identification of the signatures of the gut microbiota profile and metabolic function in OS patients

To identify the key gut bacteria for distinguishing the two groups, LEfSe was performed to analyze the taxa. As shown in Figure 2A, the OS individuals were characterized by enrichment of the genera *Agathobacter*, *Lactobacillus*, *Oscillibacter*, and *Prevotellaceae_UGG-001*. The *Agathobacter* and *Lactobacillus* genera were negatively correlated with the bone density index (Figure 2B). Notably, the *Agathobacter* and *Lactobacillus* genera strongly correlated with the bone loss markers β -CTX and ALP (Figure 2B). However, enrichment of the genera *Bacteroides*, CAG-352, and *[Eubacterium]_siraenum_group* in normal individuals was positively correlated with BMD (Figure 2B). Furthermore, the predicted metabolic function analysis of the gut microbiota showed that the biosynthesis of terpenoids and steroids, photosynthesis-antenna proteins and carotenoid biosynthesis pathways were enhanced in OS patients (Figure 2C). Importantly, the metabolic functions of terpenoid and steroid biosynthesis and photosynthesis-antenna proteins were positively correlated with the *Agathobacter* and *Lactobacillus* genera, which dominate in OS individuals (Figure 2D). The genera *Bacteroides* and *[Eubacterium]_siraenum_group* were negatively associated with the abovementioned pathways (Figure 2D). However, the genera

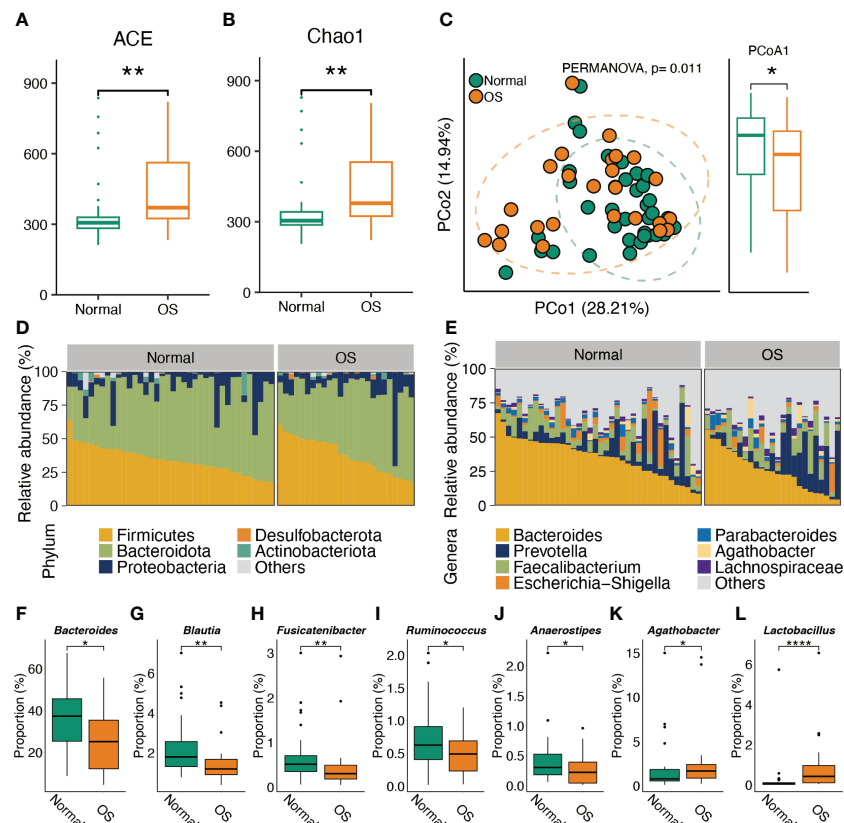


FIGURE 1

Characteristic the gut microbiota composition. (A, B) The alpha diversity comparison of microbiota between two groups. (C) PCoA analysis of gut microbes at genus level. (D, E) The gut microbiota composition in normal and OS cases at phylum and genus levels, respectively. (F–L) The significant difference of gut bacteria at genus level. *: $p < 0.05$, **: $p < 0.01$.

Bacteroides and *[Eubacterium]_siraeum_group* were strongly correlated with galactose metabolism, steroid hormone biosynthesis, cyanoamino acid metabolism, phenylpropanoid biosynthesis, and one carbon pool generated by folate pathways.

Characteristic gut bacterial associations and community stability of OS individuals

We next analyzed the associations of gut bacteria. As shown in Figures 3A, B, a clear difference in the correlation network at the genus level was observed between the two groups. Specifically, *Bacteroides* was correlated with *Parabacteroides* only in the normal group (Figure 3A). However, in the OS group, the genus *[Eubacterium]_coprostanoligenes_group* was negatively correlated with *Bacteroides* (Figure 3B). In addition, the enrichment of *Lactobacillus* and *Agathobacter* in OS patients was positively correlated with that of *Clostridia_UCG-014*, *Lachnospiraceae_NK4A136_group*, and *Roseburia* (Figure 3B). Furthermore, we analyzed the stability of the gut microbiota community at the ASV level (Figures 3C, D). Compared to that in the normal group, the vulnerability of the microbiota network in the OS group was increased (Figure 3E). Consistently, the average variation degree was also significantly greater in the OS group (Figure 3F).

The serum metabolome revealed a distinct metabolism in OS patients

We further analyzed the serum metabolites by analyzing the untargeted metabolome. As shown in Figures 4A, B, the NIM and PIM showed distinct clustering patterns compared to samples from individuals with normal survival. The VIP scores for the metabolites revealed that epitestosterone and 4-methyl-2-oxopentanoic acid contributed significantly to the separation of the PIM and NIM groups, respectively (Figures 4C, D). In addition, a total of 49 (PIM: 24, NIM: 25) metabolites were downregulated, and 39 (PIM: 16, NIM: 23) metabolites were upregulated in the OS group (Figure 4E, F). Specifically, the levels of epitestosterone, etiocholanolone, testosterone sulfate, indole-3-pyruvic acid and isovaleric acid were significantly decreased in OS patients (Figures 4E, F). However, the levels of stiripentol, 7-ketocholesterol and adipic acid were markedly increased in OS patients (Figures 4E, F). Analyses of metabolic sets revealed that altered metabolites contained a considerable amount of fatty acids, conjugates, tryptamines, indolyl carboxylic acids, amino acids, and peptides (Figure 4G). Differentially abundant metabolites were enriched in pathways such as tryptophan metabolism, unsaturated fatty acid biosynthesis, steroid hormone biosynthesis, fatty acid degradation, and valine, leucine, and isoleucine biosynthesis in OS patients (Figure 4H).



FIGURE 2

Characteristic function of gut microbiota. (A) LDA analysis of gut microbiota between two groups at genus level. (B) Spearman's correlation between significantly altered bacteria and clinical index. (C) The remarkably difference predicted function of bacteria between two groups. (D) The correlation analysis of predicted function and bacteria abundance. *: $p < 0.05$.

Integrated analysis of the host- and gut bacteria-derived serum metabolites.

Next, we analyzed the origins of the serum metabolites that were significantly different between the two groups. As shown in Figure 5A, 19 and 36 plasma metabolites were identified as originating from the host and gut microbiomes, respectively. Sixteen metabolites were derived from the host and gut bacteria (Figure 5B). Moreover, pathway enrichment analysis of the metabolites indicated that steroid hormones were uniquely metabolized by the host (Figures 5C, D). In addition, fatty acid degradation occurs via cometabolism by the host and gut microbiota (Figures 5C, E). Importantly, host- and gut microbiota-derived metabolites jointly participate in the Trp metabolism pathway (Figures 5C, F). The plasma-derived indole was uniquely metabolized by the gut bacteria-derived *tnaA* gene (Figure 5F). In addition, the gut microbiota *Tam1* and host *IL411* and *TAA1* commonly facilitate the production of indole-3-pyruvic

acid from tryptophan (Figure 5F). In addition, the gut bacteria *fldH*, *porB* and *porC* promote the conversion of indole-3-pyruvic acid to indole-3-lactic acid (Figure 5F).

Association analysis of the gut microbiota, serum metabolites and BMD indices

Moreover, the integrated analysis of pathways enriched in genes related to altered gut bacteria and serum metabolites showed that only the galactose metabolism and steroid hormone biosynthesis pathways overlapped between the microbiome and metabolites (Figure 6A). In addition, the predicted abundance of the *tnaA* gene in the gut microbiota of OS patients was significantly lower than that in that of normal individuals (Figure 6B). The *Bacteroides* genus was strongly correlated with the *tnaA* gene (Figure 6C). We further compared the abundance of gut microbes that belong to the *Bacteroides* genus at the species level. As shown in Figure 6D, the

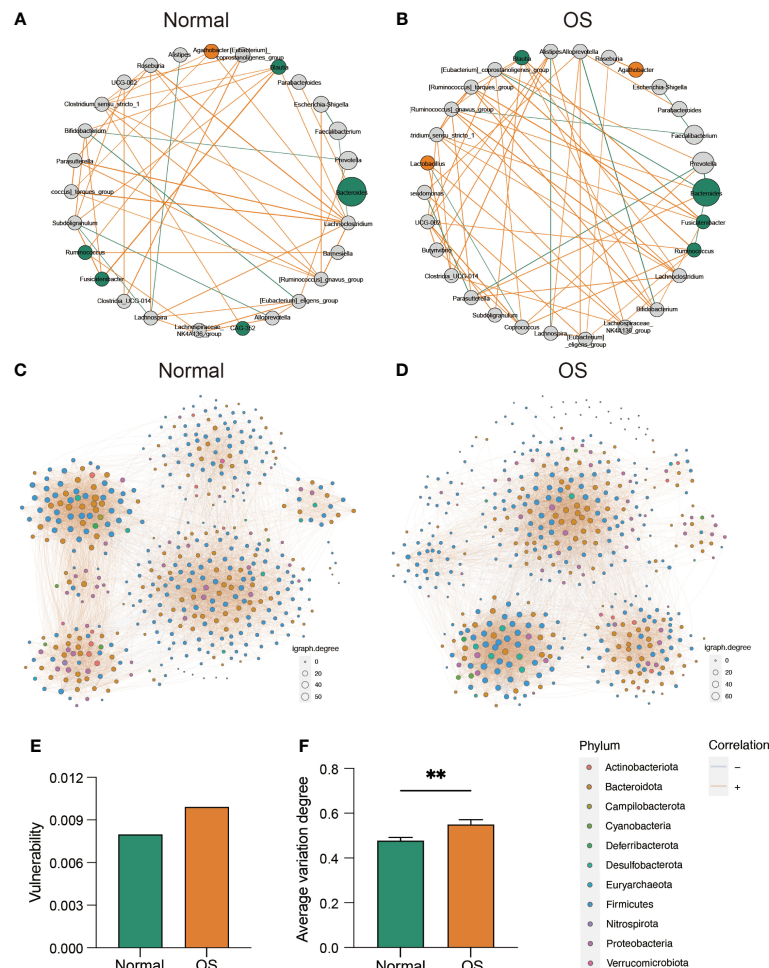


FIGURE 3

The evaluation of gut microbiota interaction and community stability. (A, B) The correlation ship among normal and OS cases, respectively. The orange and green dot indicated bacterial which dominated in OS and normal cases, respectively. (C, D) The bacterial co-occurrence network in two groups at ASVs level. (E) The vulnerability of co-occurrence network between two groups. (F) The comparison of average variation degree between two groups. **: $p < 0.01$.

abundance of *Bacteroides stercoris*, *Bacteroides plebeius*, and *Bacteroides coprocola* significantly decreased in the OS group. However, only *Bacteroides stercoris* was positively correlated with the *tnaA* gene (Figure 6E).

In addition, the integrated correlation analysis showed that *Bacteroides* was positively correlated with epitestosterone, etiocholanolone, testosterone sulfate, 5 α -dihydrotestosterone glucuronide and indole-3-lactic acid (Figure 6F). However, hydrocortisone, palmitic acid and palmitoylcarnitine were negatively correlated with *Bacteroides* genera (Figure 6F). Importantly, the *Bacteroides* genus was positively correlated with neck BMD and hip BMD (Figure 6F). In addition, epitestosterone, etiocholanolone, 5 α -dihydrotestosterone glucuronide, indole-3-lactic acid, indole, tryptophan, and serotonin were positively correlated with palmitoylcarnitine but negatively correlated with palmitic acid (Figure 6F). The level of epitestosterone strongly correlated with the levels of etiocholanolone, 5 α -dihydrotestosterone glucuronide, indole-

3-lactic acid, indole, tryptophan, and serotonin (Figure 6F). The serum epitestosterone concentration was positively correlated with VD and LS BMD (Figure 6F).

Combined biomarkers for discriminating OS patients from normal participants

We further constructed a linear regression model based on the OS signature-related gut microbiota (*Bacteroides*, *Agathobacter*, *Blautia*, *Ruminococcus*, *Fusicatenibacter*, *Lactobacillus* and *Anaerostipes*) and serum metabolites (NIM: 4-Methyl-2-Oxopentanoic Acid, 2-Hydroxycaproic acid, Isovaleric acid, Testosterone sulfate, Dihydrotestosterone glucuronid, and PIM: Epitestosterone, Etiocholanolone, Cotinine, Indole-3-lactic acid, Mentha-1,3,8-triene) to predict reduced BMD. As shown in Figures 7A–C, the individual marker panels could discriminate

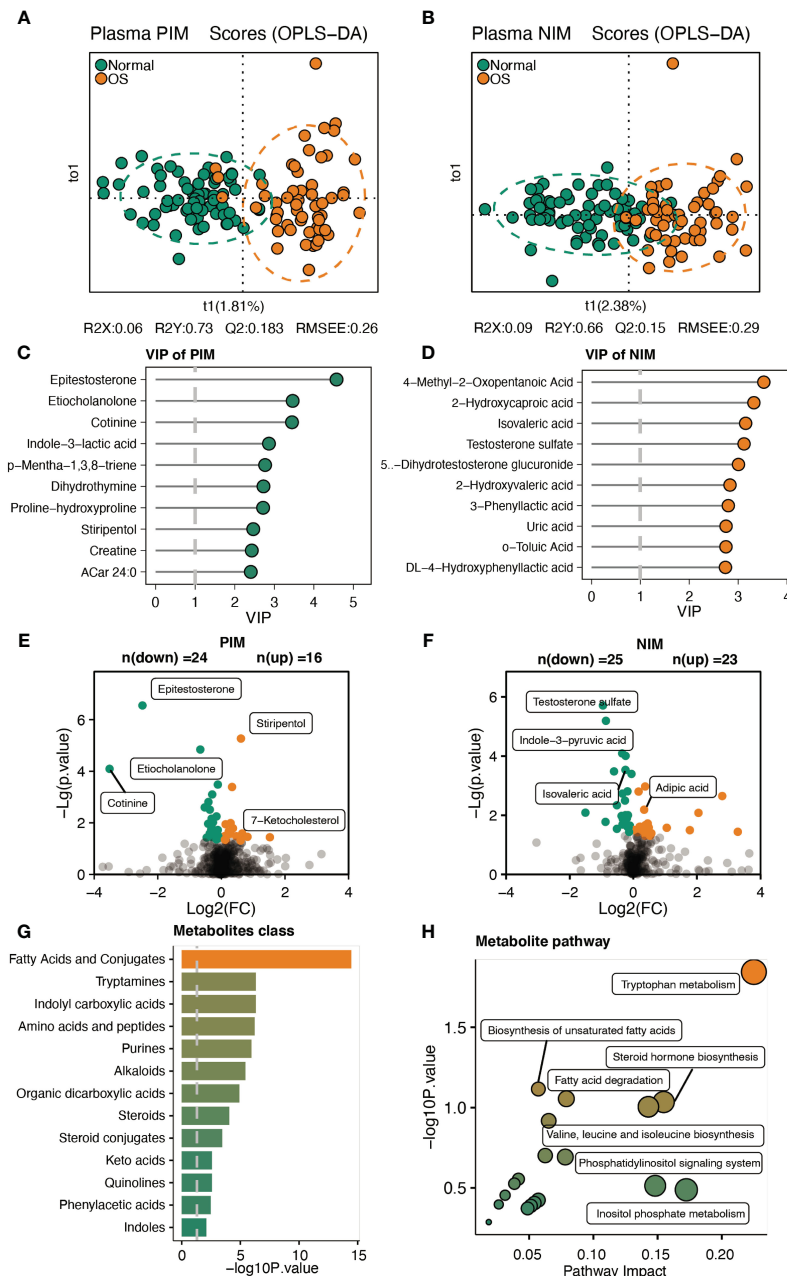


FIGURE 4

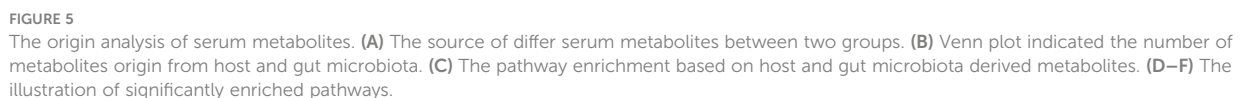
Characteristic of the serum metabolites. (A, B) The OPLS-DA analysis of serum metabolites between two groups in PIM and NIM, respectively. (C, D) The VIP score of metabolites in PIM and NIM, respectively. (E, F) Volcano plots indicated the different metabolites in PIM and NIM, respectively. (G, H) The metabolites' sets and KEGG pathway enrichment based on differ metabolites.

individuals with reduced BMD from normal individuals in both the discovery and validation sets. Importantly, the combination of the gut microbiota and serum metabolites has good diagnostic performance (Figure 7D).

Discussion

Accumulated evidence has shown that dysbiosis of the gut microbiota and metabolites contributes to multiple metabolic diseases in older people, but less is known about the gut

microbiota and serum metabolite signatures of postmenopausal women with reduced BMD (He et al., 2020). Over the age of 50 years, the high incidence of osteoporotic fracture has led to a significant burden for patients and health care providers. It is essential to find ideal targets for researching the risk factors and developing reduced BMD for intervention and treatment in advance. Here, for the first time, we characterized the gut microbiota profile and signatures of serum metabolites in postmenopausal women with reduced BMD. Our results indicated a clear difference in the gut bacterial composition and serum metabolite abundance between the OS group and the normal



Alterations in the gut microbiota were associated with a reduction in bone mineral density (Das et al., 2019). The findings of our study suggested that the α diversity of the gut microbiota differed between the OS and normal groups, and the results indicated that bacterial richness and diversity were increased in the OS group. We observed that some bacteria at the genus level, such as *Bacteroides*, *Blautia*, *Fusicatenibacter*, *Ruminococcus*, and *Anaerostipes*, were enriched in the normal group and that *Agathobacter* and *Lactobacillus* were more abundant in the OS

group. *Bacteroides*, which was decreased in OS patients, has been reported to ameliorate bone loss in ovariectomized mice (Yuan and Shen, 2021). Consistent with our findings, beneficial bacteria, such as *Anaerostipes* and *Blautia*, which can inhibit inflammation, were also decreased in OS patients (Benitez-Paez et al., 2020; Bui et al., 2021). A beneficial anti-inflammatory association of *Blautia* was also found in other clinical settings, including in colorectal cancer (Chen et al., 2012), cirrhosis (Qin et al., 2014), and inflammatory pouchitis following ileal pouch-anal anastomosis (Tyler et al., 2013). Furthermore, the depletion of the butyrate-producing *Firmicutes* bacterium *Fusicatenibacter* in OS patients could produce a beneficial effect on maintaining the stability of the intestinal lumen environment (Tian et al., 2023). *Ruminococcus* was regarded as a beneficial bacterium related to the prevention of

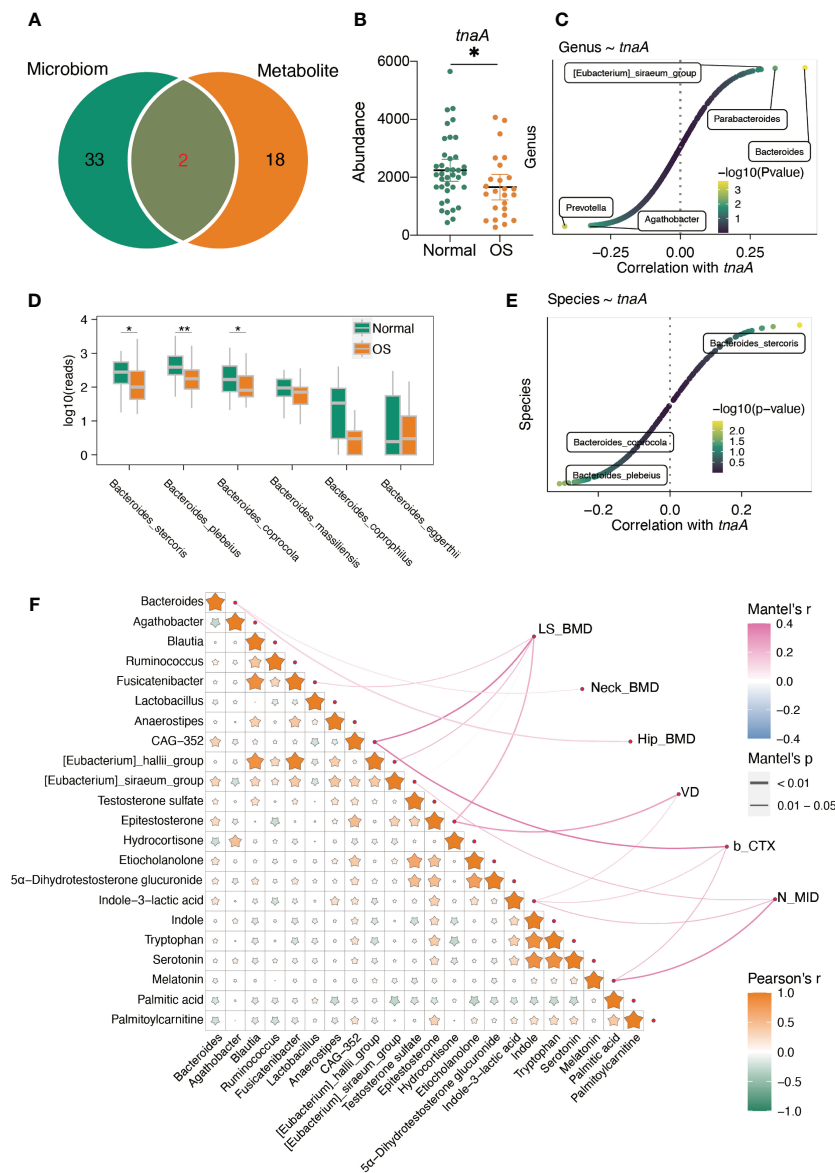


FIGURE 6

The interaction of gut microbiota and serum metabolites. (A) The altered predicted function of gut microbiota and significantly enriched pathway based on metabolites. (B) The predicted gut microbiota *tnaA* gene abundance based on PICRUTS2. (C) The correlation between *tnaA* gene abundance and gut bacteria. (D) The comparison of *Bacteroides* at species level. (E) The correlation between *tnaA* gene abundance and *Bacteroides* species. (F) Mantel's analysis the interaction of differ gut microbiota, serum metabolites and clinical index. *: $p < 0.05$, **: $p < 0.01$.

osteoporosis in studies focusing on the anti-osteoporosis mechanism of active ingredients (Han et al., 2023; Xiao et al., 2023). To the best of our knowledge, *Agathobacter* has not been reported to be associated with osteoporosis. In a study identifying diagnostic biomarkers for compensatory liver cirrhosis, *Agathobacter* was elevated in patients with compensatory liver cirrhosis (Chen et al., 2023). In addition, previous studies have shown that *Lactobacillus* was more abundant in the osteoporosis group than in the control group (He et al., 2020), which was consistent with our results. Moreover, another study showed that *Lactobacillus* supplementation could reduce bone loss in older women with low bone mineral density. We also found a significant alteration in *Lactobacillus* abundance in OS individuals, but the opposite effect was observed (Nilsson et al.,

2018; Li P. et al., 2022). Conflicting results may be explained by the number of specimens and populations included in these studies. As our study demonstrated, the gut microbiota has a close relationship with bone turnover markers (Chen et al., 2021), which is consistent with the increase in bone turnover markers observed in the OS group.

In this study, T-PINP, β -CTX and N-MID were increased in the OS group compared with the normal group, consistent with the findings of previous studies (He et al., 2020; Wang et al., 2023). In addition, our data demonstrated a positive association between the *Bacteroides*/CAG-352/[*Eubacterium*]_siraum_group and BMD and a negative association between the *Agathobacter*/*Lactobacillus* ratio and BMD. By LEfSe analysis, we also observed that *Bacteroides*/CAG-352/[*Eubacterium*]_siraum_group were increased in the normal

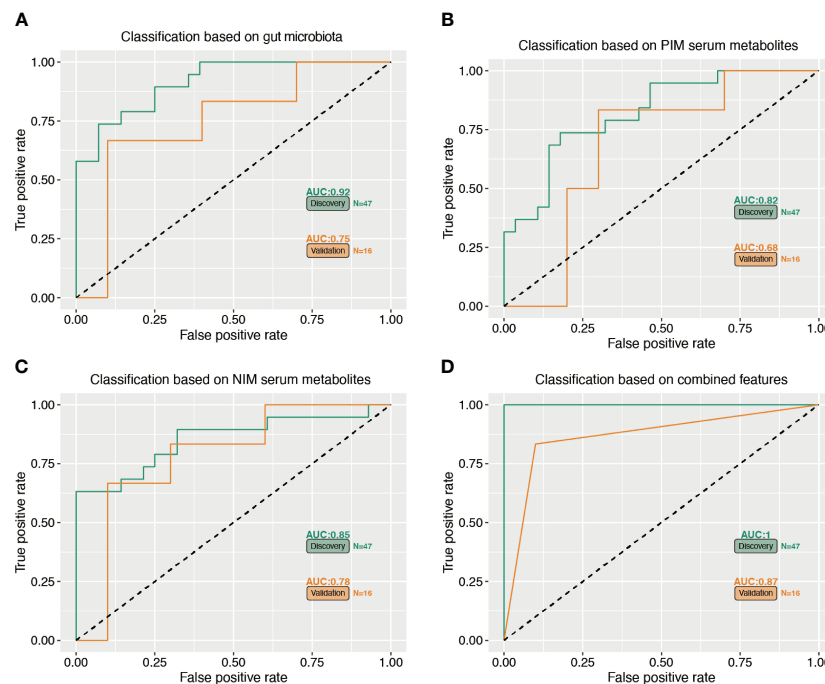


FIGURE 7

Multiple markers for diagnosis of reduced BMD. (A–C) The separated gut microbiota, serum metabolites of PIM and NIM diagnose OS from normal subjects. (D) The accuracy of combined marker panels for diagnose OS from normal subjects.

group, and *Agathobacter/Lactobacillus* was increased in the OS group, which correlated well with the correlation analysis of the BMD index and gut microbiota. In the present study, we also performed a functional prediction analysis of the gut microbiota and found that the pathways involved in the biosynthesis of terpenoids and steroids, photosynthesis–antenna proteins and carotenoid biosynthesis were enhanced in the OS group. Further correlation analysis of the gut microbiota and metabolic pathways revealed that the biosynthesis of terpenoids and steroids and the metabolic function of photosynthesis–antenna proteins were positively correlated with *Agathobacter* and *Lactobacillus*, which were increased in the OS group; moreover, *Bacteroides* and *[Eubacterium]_siraenum_group*, which were increased in the normal group; and maintained a negative association with the abovementioned pathways. In addition, many studies have demonstrated that the gut microbiota regulates the immune system in a similar way to bone metabolism (D'Amelio et al., 2008; Hsu and Pacifici, 2018). A decrease in microbial dysbiosis and downregulation of inflammatory signaling pathways have been associated with increased *Bacteroides* abundance, and all these factors could influence the immune system, leading to the amelioration of bone loss and microstructural destruction (Yuan and Shen, 2021). These results indicated that some gut bacteria are closely associated with bone metabolism, but further studies are needed to determine the underlying mechanisms involved in bone loss.

Moreover, untargeted serum metabolism analysis revealed lower levels of epitestosterone, etiocholanolone, testosterone sulfate, indole-3-pyruvic acid and isovaleric acid and higher levels of stiripentol, 7-ketocholesterol and adipic acid in the OS group than in the normal group. In addition, the altered metabolites were

mainly enriched in fatty acids and conjugates, tryptamines, indolyl carboxylic acids, amino acids and peptides. Furthermore, the enrichment of relevant metabolic pathways that differed significantly between the two groups was based on differentially abundant metabolites, which indicated the enrichment of tryptophan metabolism, the biosynthesis of unsaturated fatty acids, steroid hormone biosynthesis, fatty acid degradation and the biosynthesis of valine, leucine and isoleucine. It has previously been demonstrated that etiocholanolone can enhance osteoblast proliferation (Wu and Zhang, 2018), and the present study confirms these findings. The metabolites of dehydroepiandrosterone (DHEA), particularly estrogen and testosterone, play an important role in bone homeostasis, and a significant link has been found between DHEA and increased bone mineral density. In part, this is because DHEA increases the activity of osteoblasts and the level of insulin-like growth factor 1 (IGF-1) in the blood (Kirby et al., 2020). Interestingly, IGF-1 is also known to improve fracture healing. Isovaleric acid improved ovariectomy-induced osteoporosis by inhibiting osteoclast differentiation in another study, in agreement with our findings (Cho et al., 2021). 7-Ketocholesterol, which was the most common serum metabolite in the OS group, was recently reported to induce oxipoptophagy and inhibit osteogenic differentiation (Ouyang et al., 2022). In terms of metabolic pathways, tryptophan and its metabolites in the regulation of bone metabolism have been well investigated in numerous studies (Michalowska et al., 2015; Al Saedi et al., 2022; Tsuji et al., 2023), and some tryptophan metabolites could become important targets for developing new pharmacological treatments for osteoporosis. Additionally, a multiomics study in a large cohort identified an amino acid metabolism-mediated association between

gut microbiota and osteoporosis, suggesting that gut dysbiosis and amino acid metabolism could be a target for osteoporosis treatment (Ling et al., 2021). Recent research suggested that puerarin may promote the biosynthesis of unsaturated fatty acids and regulate phospholipid metabolism in OVX-induced osteoporosis (Li B. et al., 2022), and the results showed that biosynthesis of unsaturated fatty acids was important in undermining adipogenic differentiation and promoting osteogenic differentiation of BMSCs (bone marrow mesenchymal stem cells) in ovariectomized rats. Although the relationship between steroid hormones and osteoporosis has long been established, relevant research has indicated that probiotics can prevent sex steroid deficiency-induced bone loss (Li et al., 2016). These findings appear to be consistent with our results, and much of the relevant literature has clarified the important role of the gut microbiota in osteoporosis progression.

Among the mentioned metabolic pathways, steroid hormones are uniquely metabolized by the host, and fatty acid degradation is cometabolized by the host and gut microbiota. Importantly, host- and gut microbiota-derived metabolites jointly participate in the tryptophan metabolism pathway, and the plasma-derived indole is uniquely metabolized by the gut bacteria-derived *tnaA* gene. Moreover, the predicted abundance of the *tnaA* gene in the OS gut microbiota was significantly lower than that in the normal gut microbiota, and the *Bacteroides* genus was strongly correlated with the *tnaA* gene. By comparing the abundances of gut microbes that belong to the *Bacteroides* genus at the species level, we found that the abundances of *Bacteroides stercoris*, *Bacteroides plebeius*, and *Bacteroides coprocola* were significantly decreased in the OS group. Among them, only the abundance of *Bacteroides stercoris* was positively correlated with the abundance of the *tnaA* gene. To the best of our knowledge, the *Bacteroides stercoris* and *tnaA* genes have never been reported to be associated with osteoporosis, indicating that further studies are needed to investigate the potential action and underlying mechanisms of these genes on osteoporosis.

Correlation analysis allowed us to identify the associations between gut bacteria and serum metabolites and bone mineral density indices. We found positive associations between normal group-enriched *Bacteroides* abundance and normal group-enriched epitestosterone, etiocholanolone and testosterone sulfate. In addition, *Bacteroides* correlated positively with femoral neck and hip BMD, and a worthwhile indicator, testosterone, correlated positively with lumbar spine BMD. In this study, we also focused on the early diagnostic value of gut bacteria/serum metabolites for reduced BMD. Using the linear regression model, we confirmed that the presence of gut bacteria combined with serum metabolites could be a better indicator for predicting reduced BMD. A mathematical regression model was recently used as a practical method for the early diagnosis of postmenopausal osteoporosis after screening with multiple feature selection methods (Kwon et al., 2022). Individual marker panels of gut bacteria or serum metabolites could discriminate individuals with OS from healthy individuals in both the discovery and validation sets. Importantly, the combination of gut bacteria and serum metabolites had a greater diagnostic performance. Over the age of 50 years, the incidence of osteoporotic fracture is approximately 1/3 and 1/5 in female and male populations, respectively, during their lifetime, representing a

significant burden for patients and health care providers. Therefore, it is highly important to effectively predict the occurrence of osteoporosis at the early stage (Munoz et al., 2020).

Conclusion

Collectively, our results suggested that postmenopausal women with reduced BMD have significant changes in the gut microbiota and serum metabolites, which are significantly correlated with the BMD index and bone turnover markers. This correlation provides potential directions for exploring the mechanism of osteoporosis development and potential early diagnostic indicators for reduced BMD. This study might lead to the use of novel interventions to improve the level of bone health in postmenopausal women.

Data availability statement

The data presented in this study is deposited in the NCBI repository, accession number PRJNA1084172.

Ethics statement

The studies involving humans were approved by Ethics Committee of The Second People's Hospital of Dalian. The studies were conducted in accordance with the local legislation and institutional requirements. The 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

LY: Funding acquisition, Writing – original draft. XW: Writing – original draft. TY: Investigation, Software, Writing – review & editing. ZQ: Data curation, Writing – review & editing. HL: Investigation, Software, Writing – review & editing. HN: Formal analysis, Writing – review & editing. KW: Methodology, Project administration, Writing – review & editing. DL: Resources, Validation, Writing – review & editing. FH: Conceptualization, Investigation, Supervision, Writing – review & editing. WW: Funding acquisition, Resources, 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.

References

- Al Saedi, A., Chow, S., Vogrin, S., Guillemin, G. J., and Duque, G. (2022). Association between tryptophan metabolites, physical performance, and frailty in older persons. *Int. J. tryptophan research: IJTR* 15, 11786469211069951. doi: 10.1177/11786469211069951
- Benitez-Paez, A., Gomez Del Pugar, E. M., Lopez-Almela, I., Moya-Perez, A., Codoner-Franch, P., and Sanz, Y. (2020). Depletion of blautia species in the microbiota of obese children relates to intestinal inflammation and metabolic phenotype worsening. *mSystems* 5 (2), e00857-19. doi: 10.1128/mSystems.00857-19
- Black, D. M., and Rosen, C. J. (2016). Clinical practice. Postmenopausal osteoporosis. *New Engl. J. Med.* 374, 254–262. doi: 10.1056/NEJMcP1513724
- Brown, E. M., Clardy, J., and Xavier, R. J. (2023). Gut microbiome lipid metabolism and its impact on host physiology. *Cell Host Microbe* 31, 173–186. doi: 10.1016/j.chom.2023.01.009
- Bui, T. P. N., Manneras-Holm, L., Puschmann, R., Wu, H., Troise, A. D., Nijse, B., et al. (2021). Conversion of dietary inositol to propionate and acetate by commensal Anaerostipes associates with host health. *Nat. Commun.* 12, 4798. doi: 10.1038/s41467-021-25081-w
- Cao, Y., Dong, Q., Wang, D., Zhang, P., Liu, Y., and Niu, C. (2022). microbiomeMarker: an R/Bioconductor package for microbiome marker identification and visualization. *Bioinformatics* 38, 4027–4029. doi: 10.1093/bioinformatics/btac438
- Chen, L., Yan, S., Yang, M., Yu, F., Wang, J., Wang, X., et al. (2021). The gut microbiome is associated with bone turnover markers in postmenopausal women. *Am. J. Trans. Res.* 13, 12601–12613.
- 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
- Chen, Y., Chen, S., Xu, C., Yu, L., Chu, S., Bao, J., et al. (2023). Identification of diagnostic biomarkers for compensatory liver cirrhosis based on gut microbiota and urine metabolomics analyses. *Mol. Biotechnol.* 24. doi: 10.1007/s12033-023-00922-9
- Cho, K. M., Kim, Y. S., Lee, M., Lee, H. Y., and Bae, Y. S. (2021). Isovaleric acid ameliorates ovariectomy-induced osteoporosis by inhibiting osteoclast differentiation. *J. Cell. Mol. Med.* 25, 4287–4297. doi: 10.1111/jcmm.16482
- D'Amelio, P., Grimaldi, A., Di Bella, S., Brianza, S. Z. M., Cristofaro, M. A., Tamone, C., et al. (2008). Estrogen deficiency increases osteoclastogenesis up-regulating T cells activity: a key mechanism in osteoporosis. *Bone* 43, 92–100. doi: 10.1016/j.bone.2008.02.017
- Das, M., Cronin, O., Keohane, D. M., Cormac, E. M., Nugent, H., Nugent, M., et al. (2019). Gut microbiota alterations associated with reduced bone mineral density in older adults. *Rheumatology* 58, 2295–2304. doi: 10.1093/rheumatology/kez302
- Ding, K., Hua, F., and Ding, W. (2020). Gut microbiome and osteoporosis. *Aging Dis.* 11, 438–447. doi: 10.14336/AD.2019.0523
- Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., et al. (2020). PICRUSt2 for prediction of metagenome functions. *Nat. Biotechnol.* 38, 685–688. doi: 10.1038/s41587-020-0548-6
- Gopinath, V. (2023). Osteoporosis. *Med. Clinics North America* 107, 213–225. doi: 10.1016/j.mcna.2022.10.013
- Hall, M., and Beiko, R. G. (2018). 16S rRNA gene analysis with QIIME2. *Methods Mol. Biol.* 1849, 113–129. doi: 10.1007/978-1-4939-8728-3_8
- Han, X., Fu, Y., Wang, K., Li, S., Jiang, C., Wang, S., et al. (2023). Epigallocatechin gallate alleviates osteoporosis by regulating the gut microbiota and serum metabolites in rats. *Food Funct.* 14 (23), 10564–10580. doi: 10.1039/D3FO03233G
- He, J., Xu, S., Zhang, B., Xiao, C., Chen, Z., Si, F., et al. (2020). Gut microbiota and metabolite alterations associated with reduced bone mineral density or bone metabolic indexes in postmenopausal osteoporosis. *Aging* 12, 8583–8604. doi: 10.18632/aging.v12i9
- Hsu, E., and Pacifici, R. (2018). From osteoimmunology to osteomicrobiology: how the microbiota and the immune system regulate bone. *Calcified Tissue Int.* 102, 512–521. doi: 10.1007/s00223-017-0321-0
- Kirby, D. J., Buchalter, D. B., Anil, U., and Leucht, P. (2020). DHEA in bone: the role in osteoporosis and fracture healing. *Arch. osteoporosis* 15, 84. doi: 10.1007/s11657-020-00755-y
- Kwon, Y., Lee, J., Park, J. H., Kim, Y. M., Kim, S. H., Won, Y. J., et al. (2022). Osteoporosis pre-screening using ensemble machine learning in postmenopausal Korean women. *Healthcare* 10 (6), 1107. doi: 10.3390/healthcare10061107
- Lambert, M. N. T., Thybo, C. B., Lykkeboe, S., Rasmussen, L. M., Frette, X., Christensen, L. P., et al. (2017). Combined bioavailable isoflavones and probiotics improve bone status and estrogen metabolism in postmenopausal osteopenic women: a randomized controlled trial. *Am. J. Clin. Nutr.* 106, 909–920. doi: 10.3945/ajcn.117.153353
- Li, J. Y., Chassaing, B., Tyagi, A. M., Vaccaro, C., Luo, T., Adams, J., et al. (2016). Sex steroid deficiency-associated bone loss is microbiota dependent and prevented by probiotics. *J. Clin. Invest.* 126, 2049–2063. doi: 10.1172/JCI86062
- Li, P., Ji, B., Luo, H., Sundh, D., Lorentzon, M., and Nielsen, J. (2022). One-year supplementation with Lactobacillus reuteri ATCC PTA 6475 counteracts a degradation of gut microbiota in older women with low bone mineral density. *NPJ biofilms microbiomes* 8, 84. doi: 10.1038/s41522-022-00348-2
- Li, B., Wang, Y., Gong, S., Yao, W., Gao, H., Liu, M., et al. (2022). Puerarin improves OVX-induced osteoporosis by regulating phospholipid metabolism and biosynthesis of unsaturated fatty acids based on serum metabolomics. *Phytomedicine: Int. J. phytotherapy phytopharmacology* 102, 154198. doi: 10.1016/j.phymed.2022.154198
- Liang, B., Burley, G., Lin, S., and Shi, Y. C. (2022). Osteoporosis pathogenesis and treatment: existing and emerging avenues. *Cell. Mol. Biol. Lett.* 27, 72. doi: 10.1186/s11658-022-00371-3
- Liang, L., Rasmussen, M. H., Piening, B., Shen, X., Chen, S., Rost, H., et al. (2020). Metabolic dynamics and prediction of gestational age and time to delivery in pregnant women. *Cell* 181, 1680–1692 e1615. doi: 10.1016/j.cell.2020.05.002
- Ling, C. W., Miao, Z., Xiao, M. L., Zhou, H., Jiang, Z., Fu, Y., et al. (2021). The association of gut microbiota with osteoporosis is mediated by amino acid metabolism: multionics in a large cohort. *J. Clin. Endocrinol. Metab.* 106, e3852–e3864. doi: 10.1210/clinem/dgab492
- Liu, H. X., Rocha, C. S., Dandekar, S., and Wan, Y. J. (2016). Functional analysis of the relationship between intestinal microbiota and the expression of hepatic genes and pathways during the course of liver regeneration. *J. Hepatol.* 64, 641–650. doi: 10.1016/j.jhep.2015.09.022
- Lloyd-Price, J., Arze, C., Ananthakrishnan, A. N., Schirmer, M., Avila-Pacheco, J., Poon, T. W., et al. (2019). Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 569, 655–662. doi: 10.1038/s41586-019-1237-9

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcimb.2024.1367325/full#supplementary-material>

- McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8, e61217. doi: 10.1371/journal.pone.0061217
- Michalowska, M., Znorko, B., Kaminski, T., Oksztulska-Kolanek, E., and Pawlak, D. (2015). New insights into tryptophan and its metabolites in the regulation of bone metabolism. *J. Physiol. pharmacology: an Off. J. Polish Physiol. Soc.* 66, 779–791.
- Munoz, M., Robinson, K., and Shibli-Rahhal, A. (2020). Bone health and osteoporosis prevention and treatment. *Clin. obstetrics gynecology* 63, 770–787. doi: 10.1097/GRF.0000000000000572
- Nilsson, A. G., Sundh, D., Backhed, F., and Lorentzon, M. (2018). Lactobacillus reuteri reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial. *J. Internal Med.* 284, 307–317. doi: 10.1111/joim.12805
- Ouyang, J., Xiao, Y., Ren, Q., Huang, J., Zhou, Q., Zhang, S., et al. (2022). 7-ketocholesterol induces oxiaoptophagy and inhibits osteogenic differentiation in MC3T3-E1 cells. *Cells* 11 (18), 2882. doi: 10.3390/cells11182882
- Pang, Z., Chong, J., Zhou, G., de Lima Morais, D. A., Chang, L., Barrette, M., et al. (2021). MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res.* 49, W388–W396. doi: 10.1093/nar/gkab382
- Qin, N., Yang, F., Li, A., Prifti, E., Chen, Y., Shao, L., et al. (2014). Alterations of the human gut microbiome in liver cirrhosis. *Nature* 513, 59–64. doi: 10.1038/nature13568
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks1219
- Reid, I. R., and Billington, E. O. (2022). Drug therapy for osteoporosis in older adults. *Lancet* 399, 1080–1092. doi: 10.1016/S0140-6736(21)02646-5
- Rettedal, E. A., Ilesanmi-Oyelere, B. L., Roy, N. C., Coad, J., and Kruger, M. C. (2021). The gut microbiome is altered in postmenopausal women with osteoporosis and osteopenia. *J. Bone Min. Res.* 36, 10452. doi: 10.1002/jbm.b.410452
- Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, J. C., et al. (2011). pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinf.* 12, 77. doi: 10.1186/1471-2105-12-77
- Song, S., Guo, Y., Yang, Y., and Fu, D. (2022). Advances in pathogenesis and therapeutic strategies for osteoporosis. *Pharmacol. Ther.* 237, 108168. doi: 10.1016/j.pharmthera.2022.108168
- Tian, H., Cui, J., Ye, C., Zhao, J., Yang, B., Xu, Y., et al. (2023). Depletion of butyrate-producing microbes of the Firmicutes predicts nonresponse to FMT therapy in patients with recurrent *Clostridium difficile* infection. *Gut Microbes* 15, 2236362. doi: 10.1080/19490976.2023.2236362
- Tsuji, A., Ikeda, Y., Yoshikawa, S., Taniguchi, K., Sawamura, H., Morikawa, S., et al. (2023). The tryptophan and kynurenine pathway involved in the development of immune-related diseases. *Int. J. Mol. Sci.* 24 (6), 5742. doi: 10.3390/ijms24065742
- Tyler, A. D., Knox, N., Kabakchiev, B., Milgrom, R., Kirsch, R., Cohen, Z., et al. (2013). Characterization of the gut-associated microbiome in inflammatory pouch complications following ileal pouch-anal anastomosis. *PLoS One* 8, e66934. doi: 10.1371/journal.pone.0066934
- Valles-Colomer, M., Bacigalupe, R., Vieira-Silva, S., Suzuki, S., Darzi, Y., Tito, R. Y., et al. (2022). Variation and transmission of the human gut microbiota across multiple familial generations. *Nat. Microbiol.* 7, 87–96. doi: 10.1038/s41564-021-01021-8
- Wang, Z., Chen, K., Wu, C., Chen, J., Pan, H., Liu, Y., et al. (2021). An emerging role of *Prevotella histicola* on estrogen deficiency-induced bone loss through the gut microbiota-bone axis in postmenopausal women and in ovariectomized mice. *Am. J. Clin. Nutr.* 114, 1304–1313. doi: 10.1093/ajcn/nqab194
- Wang, H., Liu, J., Wu, Z., Zhao, Y., Cao, M., Shi, B., et al. (2023). Gut microbiota signatures and fecal metabolites in postmenopausal women with osteoporosis. *Gut Pathog.* 15, 33. doi: 10.1186/s13099-023-00553-0
- Wen, T., Xie, P., Yang, S., Niu, G., Liu, X., Ding, Z., et al. (2022). ggClusterNet: An R package for microbiome network analysis and modularity-based multiple network layouts. *iMeta* 1, e32. doi: 10.1002/imt2.32
- Wu, X., and Zhang, M. (2018). Effects of androgen and progestin on the proliferation and differentiation of osteoblasts. *Exp. Ther. Med.* 16, 4722–4728. doi: 10.3892/etm
- Xiao, X., Wang, J., Zhu, Y., Deng, B., Liu, Y., Wang, S., et al. (2023). Phytosterols protect against osteoporosis by regulating gut microbiota. *J. Agric. Food Chem.* 71, 14539–14549. doi: 10.1021/acs.jafc.3c01489
- Xu, Q., Li, D., Chen, J., Yang, J., Yan, J., Xia, Y., et al. (2022). Crosstalk between the gut microbiota and postmenopausal osteoporosis: Mechanisms and applications. *Int. Immunopharmacol.* 110, 108998. doi: 10.1016/j.intimp.2022.108998
- Xun, W., Liu, Y., Li, W., Ren, Y., Xiong, W., Xu, Z., et al. (2021). Specialized metabolic functions of keystone taxa sustain soil microbiome stability. *Microbiome* 9, 35. doi: 10.1186/s40168-020-00985-9
- Yu, F., and Xia, W. (2019). The epidemiology of osteoporosis, associated fragility fractures, and management gap in China. *Arch. osteoporosis* 14, 32. doi: 10.1007/s11657-018-0549-y
- Yuan, S., and Shen, J. (2021). *Bacteroides vulgatus* diminishes colonic microbiota dysbiosis ameliorating lumbar bone loss in ovariectomized mice. *Bone* 142, 115710. doi: 10.1016/j.bone.2020.115710
- Zhao, S. S., Chen, L., Yang, J., Wu, Z. H., Wang, X. Y., Zhang, Q., et al. (2022). Altered gut microbial profile accompanied by abnormal fatty acid metabolism activity exacerbates endometrial cancer progression. *Microbiol. Spectr.* 10, e0261222. doi: 10.1128/spectrum.02612-22



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Biliary atresia: the role of gut microbiome, and microbial metabolites

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Biliary atresia (BA) is a progressive fibroinflammatory disease affecting both the extrahepatic and intrahepatic bile ducts, potentially leading to chronic cholestasis and biliary cirrhosis. Despite its prevalence, the exact mechanisms behind BA development remain incompletely understood. Recent research suggests that the gut microbiota and its metabolites may play significant roles in BA development. This paper offers a comprehensive review of the changing characteristics of gut microbiota and their metabolites at different stages of BA in children. It discusses their influence on the host's inflammatory response, immune system, and bile acid metabolism. The review also explores the potential of gut microbiota and metabolites as a therapeutic target for BA, with interventions like butyrate and gut microbiota preparations showing promise in alleviating BA symptoms. While progress has been made, further research is necessary to untangle the complex interactions between gut microbiota and BA, paving the way for more effective prevention and treatment strategies for this challenging condition.

KEYWORDS

biliary atresia, gut microbiota, metabolites, probiotics, children

Introduction

Biliary atresia (BA) is a severe hepatobiliary disease of unknown etiology and pathogenesis in infancy. It is characterized by varying degrees of fibrotic atresia of the extrahepatic and intrahepatic bile ducts, resulting in obstructive jaundice and liver fibrosis (Hartley et al., 2009). BA is a rare disease with incidence rates ranging from 1 in 18,000 live births in European and American countries to 1 in 8,000 in Asian countries. Females are 3.5–4.0 times more likely to be affected than males (Sanchez-Valle et al., 2017; Schreiber et al., 2022). The current treatment of choice for BA is Kasai hepatopuertoenterostomy

Abbreviations: ADD3, adducin three gene; BA, biliary atresia; CMV, Cytomegalovirus; LGG, *Lactobacillus casei rhamnosus*; LPS, lipopolysaccharide; LT, liver transplantation; KPE, Kasai hepatopuertoenterostomy; SCFAs, short-chain fatty acid.

(KPE), which is successful in about 50% of patients (Hartley et al., 2009). Despite advancements in surgical techniques for BA, the clinical outcomes for these patients remain unsatisfactory. Recurrent cholangitis and progressive liver fibrosis often occur within a short time period after KPE, necessitating liver transplantation (LT). Therefore, a clear understanding of the etiology and molecular pathogenesis of BA is crucial for gaining insight into disease progression and identifying potential intervention strategies.

The term “gut microbiota” encapsulates the intricate and diverse assembly of microorganisms, including bacteria, fungi, archaea, and viruses, that reside within the gastrointestinal tract. The resident gut microbiota and its host engage in a symbiotic relationship, wherein each party derives significant benefits from the other. The gut microbiota, in a state of mutual dependence, flourishes within the stable and nutrient-rich environment furnished by the host. In turn, it generates bioactive metabolites that are pivotal for sustaining intestinal homeostasis and bolstering the host's overall health (Valdes et al., 2018; Jaswal et al., 2023). It also influences the physiological functioning of distant organs through several routes, including liver, endocrine, immune, and metabolic pathways. This is particularly well documented regarding the gut-liver axis (Delzenne et al., 2019; Tilg et al., 2022; Rager and Zeng, 2023). Typically, the composition and structure of gut microbiota and metabolites are relatively balanced, and they communicate bi-directionally with the gut and its microbiome through the gut-liver axis (Milosevic et al., 2019; Tilg et al., 2022; Rager and Zeng, 2023). Breaking this balance, termed dysbiosis, can trigger an excessive intestinal immune response and metabolic disorders, which leads to the disease (Milosevic et al., 2019; Hou et al., 2022). Recent research has suggested that the gut microbiota and their metabolites, the diverse community of microorganisms residing in the gastrointestinal tract, may play a crucial role in the pathogenesis of hepatobiliary diseases, including nonalcoholic fatty liver disease, as well as liver fibrosis, cholestatic liver disease and BA (Kobayashi et al., 1988; Aron-Wisnewsky et al., 2020; Schneider et al., 2021). Understanding the relationship between the gut microbiota and their metabolites and hepatobiliary diseases shows great potential in treating liver disease.

Growing evidence suggests that gut microbiota and metabolites are associated with BA (Song et al., 2021b; Yang et al., 2022). A few cohort studies with few subjects have found that individuals with BA have different gut bacterial communities from healthy individuals (Han, 2019; Chen, 2022). Moreover, A 2-year randomized, double-blind, placebo-controlled trial found that supplementing *Lactobacillus casei rhamnosus* (LGG) significantly reduced frequency of bacterial cholangitis in 30 patients with BA (Orłowska et al., 2021). However, the systemic and functional link between gut microbiota and their metabolites, and BA has remained unexplored. Therefore, this review summarizes the possible etiology of BA, the correlation between the gut microbiota and its metabolites and BA, the possible mechanisms for BA, and the causal effect of gut microbiota on BA. We also discuss the importance of gut microbiota and metabolites (i.e., probiotics, butyrate) as a plausible therapeutic administration strategy.

Etiology of biliary atresia

The pathogenesis of BA is highly complex, with the precise etiology and pathogenesis still not fully understood. Current research suggests that a multifactorial origin is likely, with viral infections, toxins, genetic variants, and immune dysregulation being the most commonly implicated factors in the development of the disease (Hartley et al., 2009; Bezerra et al., 2018). Additionally, imbalances in the gut microbiota and its metabolites are considered as potential contributors to the pathogenesis of BA (Figure 1).

Viral infection

Viral infections have been postulated to play a role in the etiology of the disease (Averbukh and Wu, 2018). However, the mechanism through which virus infection causes these severe abnormalities during human pregnancy is not clear (Averbukh and Wu, 2018). In the 1970s, Altshuler first suggested finding evidence of an association between BA and viral perinatal infections (Altshuler, 1973). Several studies have detected a notably high prevalence of Cytomegalovirus (CMV) infection among pediatric patients with BA. The prevalence of active CMV infection within this demographic is reported to range from approximately 30% to as high as 64%, underscoring the potential significance of CMV in the pathogenesis of BA (Shen et al., 2008; Fischler et al., 2022). Significant evidence suggests a potential role for rotavirus in the development of BA. However, definitive proof is yet to be established. Infection of Ross River virus (RRV) soon after birth is a well-established biliary epithelium injury model and shares human BA's phenotypic features, which demonstrates perinatal viral infection as the possible cause of its pathogenesis (Averbukh and Wu, 2018). In addition to CMV and rotavirus, rhinovirus, human herpesvirus, human oncovirus, adenovirus, Epstein-Barr virus, hepatitis B virus, and parvovirus B-19 are also involved in the pathologic process of BA (Saito et al., 2015). While the specific mechanisms of viral-induced BA remain elusive, one central pathophysiological concept involves immune dysregulation triggered by viral infection. Viruses may provoke an immune response by directly harming bile duct epithelial cells or indirectly by stimulating bile ducts and the liver, leading to the release of inflammatory mediators such as pro-inflammatory cytokines (TNF- α , IL-6, IL-1 β) and chemokines (CX3CR1), which are thought to contribute to the development of BA (Bezerra et al., 2018; Wang et al., 2020b).

Immune dysregulation

The pathogenesis of BA implicates a complex interplay between immune dysfunction, autoimmunity, and the inherent susceptibility of the neonatal immune system, which is notably immature (Klemann et al., 2016; Wang et al., 2020b). While research has begun to elucidate the dynamic interplay between innate and

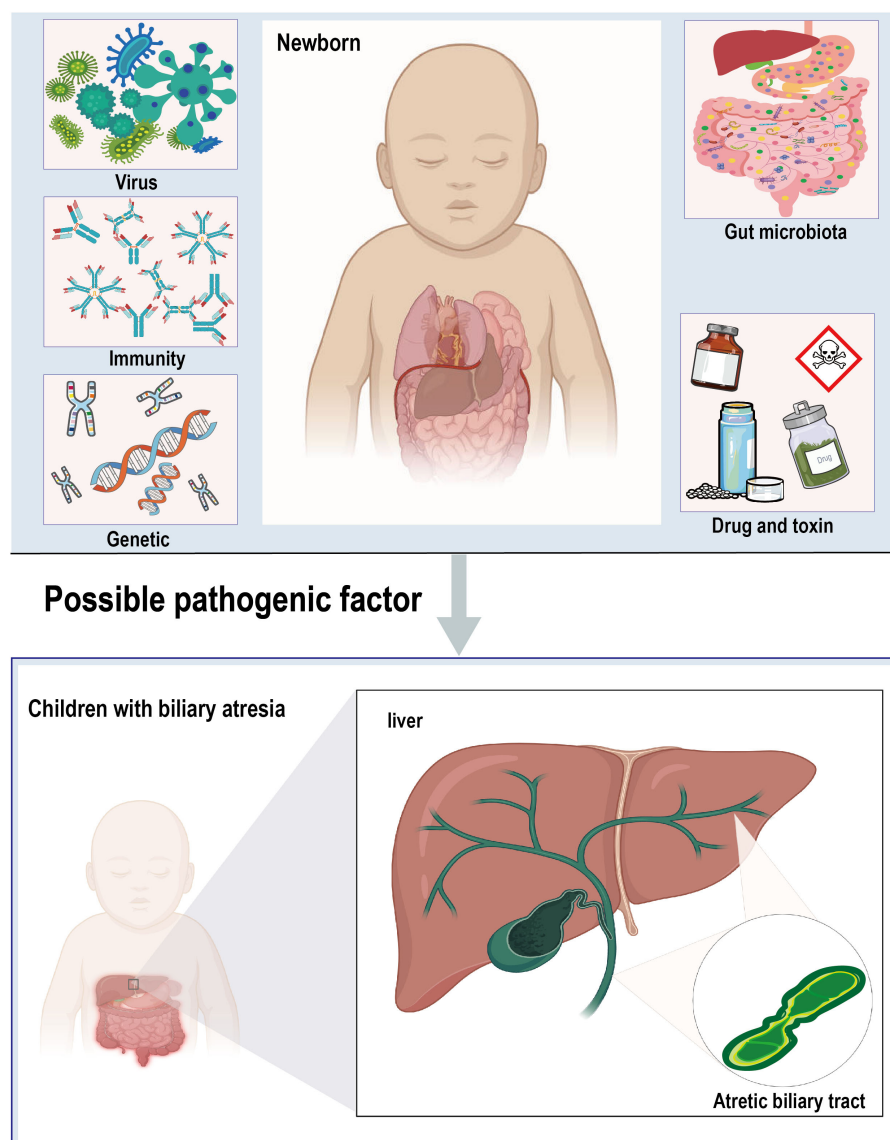


FIGURE 1
Diagrammatic representation of biliary atresia and multi-factorial etiology.

adaptive immune responses in the development of BA, the precise regulatory mechanisms still need to be fully understood (Wang et al., 2020b). BA, a severe pediatric liver disease, has been linked to perinatal viral infections that can disrupt immune regulation (Saito et al., 2015). Following such infections, a cascade of chemokines, including CXCL2, is released (Jee et al., 2022; Wang et al., 2023). These chemokines facilitate the differentiation of T helper 1 (Th1) cells and stimulate the secretion of interleukin-2 (IL-2) and interferon-gamma (IFN- γ) (Brindley et al., 2012). The subsequent activation of cytotoxic T lymphocytes (CTLs) can directly destroy the bile duct epithelium (Brindley et al., 2012). Additionally, these events can activate Kupffer cells, resulting in a significant infiltration of inflammatory factors within the liver (Wang et al., 2020b). This inflammatory response can cause damage to both the biliary epithelium and hepatocytes (Wang et al., 2023). Concurrently, the activation of hepatic stellate cells (HSCs) is triggered, leading to the

secretion of collagen and the promotion of liver fibrosis (Davenport et al., 2001). Moreover, viral infections can activate the complement system, mediating direct damage to the biliary epithelium by natural killer (NK) cells (Miethke et al., 2010; Wang et al., 2020b). Recent studies have identified a role for B-lymphocytes in the pathogenesis of BA, with histopathological examinations in pediatric patients revealing an accumulation of IgG-associated antibodies within the liver (Wang et al., 2020b). This finding suggests a contribution of autoimmunity to the disease process. Regulatory T (Treg) cells are increasingly recognized as pivotal in the pathogenesis of BA (Brindley et al., 2012). In a murine model constructed using the RRV, a depletion of Treg cells in the liver was observed. However, the prophylactic intraperitoneal injection of Treg cells from wild-type mice into neonates prior to RRV infection significantly improved both the pathological phenotype and the survival rate of the mice. Subsequent research has elucidated that

Treg cells ameliorate BA by curbing the overactivation of CD8⁺ T lymphocytes (Muraji et al., 2008; Miethke et al., 2010). Furthermore, Treg cells have been shown to mitigate BA symptoms by reducing the number of NK cells and through the secretion of IL-17 and TNF- α (Zhang and Kang, 2022). Concurrently, $\gamma\delta$ T cells have been implicated in this immunomodulatory process (Klemann et al., 2016). These findings suggest that the immune profile of children with BA may include the innate immunity, adaptive immunity, and graft-versus-host immunity, among others. Understanding these immune features may reveal potential targets for novel therapeutic interventions for BA.

Genes and heredity

Numerous clinical studies have revealed that racial disparities have long been recognized in BA and are explained by intrinsic genetic predisposition and other environmental factors (Hartley et al., 2009). The incidence of BA is less prevalent in Europe and The United States, but it is relatively more common in Asia (Sanchez-Valle et al., 2017; Schreiber et al., 2022). Multiple genome-wide association studies have identified a risk locus on chromosome 10q24.2 in Asian and white cohorts (Garcia-Barceló et al., 2010). The adducin three gene (ADD3), which is located in this region, has been investigated, and it has been found that variants that alter ADD3 expression affect the outcomes of BA. Furthermore, the deletion of ADD3 in a zebrafish model leads to abnormal biliary tract (Cui et al., 2023). These findings suggest that the occurrence of BA might be related to ethnogenetic factors. It has also been observed that BA runs in families. In clinical cohort studies of BA, a significant familial clustering of BA has been observed, with three out of five children in a given family developing BA (Tran et al., 2021). Unfortunately, the mechanisms by which genetic factors contribute to the development of BA are still unknown. Genetic mutations also play a crucial role in the development of BA. Defects in specific genes, such as AMER1, INVS, and OCRL, have been detected in children with BA combined with malformations (Tran et al., 2021). Additionally, a recessive mutation in the *inv* gene was found to cause persistent jaundice followed by atresia of the extrahepatic bile ducts in mice, which is highly similar to the pathologic changes of BA in human beings (Mazziotti et al., 1999). This finding suggests that genes play an essential role in the development of BA.

Drug and toxin

Drugs and toxins are also involved in the occurrence of BA. In several outbreaks of BA in lambs and calves in Australia, it was found that pregnant lambs and calves were exposed to a new type of isoflavone toxin (bilitresone) (Harper et al., 1990; Lorent et al., 2015). Subsequently, in animal models of mice, it was confirmed that bilitresone could cause serious destruction of the extrahepatic biliary tree and lead to the loss of cilia in the bile duct cells of newborn mice (Yang et al., 2020). This suggests that environmental

toxins may be associated with certain BA cases. Hosoda et al. (Hosoda et al., 1997). gave pregnant Wistar rats intraperitoneal phalloidin (an actin-binding toxin). Hepatic histology showed fibrosis, thickening of the extrahepatic bile duct wall, and stenosis and atresia of the duct lumen. The liver showed interlobular fibrosis, and, like human BA, complete occlusion was found only in rats exposed to the drug during the fetus. In mouse cholangiocytes, the toxin bilitresone was found to reduce glutathione levels by inhibiting GSH synthesis. This reduction in GSH levels and the presence of thione and SOX17 led to extrahepatic cholangiocyte injury and fibrosis in mice, as observed in three-dimensional spheroid cultures and neonatal extrahepatic duct explants (Waisbourd-Zinman et al., 2016). In summary, drugs and toxins play an important role in the development of the BA.

Gut microorganisms and their metabolites

An increasing body of evidence has shown that gut microbiome can impact the occurrence of BA because of its induction of persistent intestinal inflammation and intestinal barrier function (Jee et al., 2017; Wu et al., 2024). A clinical study demonstrated marked differences in the gut microbiome structure between patients with BA and healthy controls, especially regarding microbial abundance and diversity (Wang et al., 2020a; van Wessel et al., 2021; Chen, 2022; Sun et al., 2022). BA showed lower diversity and significant structural segregation in the microbiome. At the phylum level, *Proteobacteria* numbers increased, whereas those of *Bacteroidetes* decreased in BA. At the genus level, several potential pathogens, such as *Streptococcus* and *Klebsiella*, thrived in BA, while numbers for *Bifidobacterium* and several butyrate-producing bacteria declined (Han, 2019; Chen, 2022). Increases in harmful bacteria elevate the probability of direct interaction between the microbes and host tissue. Moreover, recent reports have shown that BA is associated with gut microbiota dysbiosis, disrupted intestinal barrier, and chronic inflammation (Alexander et al., 2023; Wu et al., 2024). In several experimental studies related to BA, it has been found that the intestinal barrier is differentially damaged in children with BA (Abu Faddan et al., 2017; Yan et al., 2022). This is evidenced by increased lipopolysaccharide (LPS) levels in the whole body and liver and significant hepatic inflammatory responses triggered by LPS (Isayama et al., 2006; Yan et al., 2022). In the RRV mouse model, signs of intestinal barrier damage were observed, including decreased mucosal thickness, glandular crypt depth, and villus height. In the rat model of BA with choledochal ligation, the intestine showed edema and inflammatory lesions, formation of intestinal epithelial gaps, reduction of tight junctions, decreased secretory immune proteins, and increased bacterial adherence to the mucosa. These data suggest that BA destroyed the gut microbiome's structure, damaged the intestinal barrier's function, and increased intestinal permeability (Jee et al., 2017). Cholangitis following KPE also offers compelling evidence for the translocation of gut bacteria (Wang et al., 2023). The isolation of systemic bacteria with intestinal origins, such as *Klebsiella* and *Escherichia coli*, in pediatric cholangitis cases underscores the link between the gut microbiota and the pathogenesis of BA (Song et al., 2021b; Zheng et al., 2022). This suggests that the gut microbiota

may play a pivotal role in the development of BA, potentially through the disruption of the gut barrier function, leading to bacterial and PAMP translocation.

Not only is the gut microbiota closely related to the host's health, but the metabolites of the gut microbiota also play an important role. short-chain fatty acids (SCFAs), particularly butyrate, have shown positive effects such as promoting anti-inflammation and enhancing intestinal epithelial barrier function through binding and activating SCFAs receptors (Sokal et al., 1996). Xu et al (Xu et al., 2023), found that changes in the gut microbiota composition in BA, especially the butyrate-producing microbiota, and butyrate levels in BA were negatively correlated with jaundice clearance and cholangitis (Sokal et al., 1996). A recent large-scale metabolomics analysis showed that BA is associated with a signature in amino acid metabolites (Zhou et al., 2015). Unfortunately, the current study did not examine the crosstalk between metabolites and gut microbiota for the BA. These findings suggest that the composition of the gut microbiota and their associated metabolites play an essential role in the pathogenesis of BA, and gut microbiota alterations may serve as potential therapeutic targets in the future.

Other factors

Other factors could also play a role in the procession of these particular BA. Vascular abnormalities may be one of the causes of BA. An analysis conducted by Ho et al (Ho et al., 1993), on 11 cases of BA revealed hyperplasia and hypertrophic tortuous hepatic artery branches in all patients' extrahepatic and intrahepatic parts. Histological examination of the liver of BA children suggested ischemic and hypoxic factors in the portal area of the liver. Vascular endothelial growth factor was found to promote BA and positively correlated with the degree of liver fibrosis. This indicates that vascular abnormalities may be involved in the development of BA. Additionally, some scholars have suggested that maternal microchimerism may be a pathogenesis of BA (Muraji et al., 2008). Some studies have shown that maternal cells containing X chromosomes have been found in the liver of male BA children. These X chromosome-containing cells also express CD8⁺ or cytokeratin, suggesting that maternal cells may be immune cells that can participate in developing biliary epithelium. Thus, maternal microchimerism may be a potential pathogenesis of BA (Muraji et al., 2008).

Alterations in the gut microbiota and metabolites associated with biliary atresia

BA is an occlusive fibroinflammatory biliary disease affecting the bile ducts in infants. It is characterized by rapid liver fibrosis progression, with histopathological changes including ductal reaction, portal fibrosis, and bile thrombi (Bezerra et al., 2018; Shen et al., 2019). Ductal reaction manifests as small bile duct

proliferation around portal areas, while portal fibrosis is defined by the formation of fibrous septa between liver nodules. Liver fibrosis in BA involves the production of extracellular matrix (ECM) by activated hepatic stellate cells (HSCs), a wound-healing response (Shen et al., 2019). Bile acids have cytotoxic effects that activate HSCs and stimulate the secretion of profibrogenic mediators, such as transforming growth factor beta, reactive oxygen species, TNF- α , and platelet-derived growth factor (Waisbourd-Zinman et al., 2016). These factors enhance HSC activation and ECM synthesis, exacerbating fibrosis beyond that seen in other pediatric and adult diseases (Kisseleva and Brenner, 2021). Timely intervention is crucial as untreated BA can progress to cirrhosis within weeks of birth (Bezerra et al., 2018). However, due to persistent jaundice and recurrent cholangitis, liver fibrosis continues to progress swiftly, resulting in a long-term survival rate of approximately 50% for children with BA (Schreiber et al., 2022). Post-surgery progressive liver fibrosis has become the primary constraint on the efficacy of KPE. Consequently, many patients eventually require an LT as a life-saving procedure (Hartley et al., 2009; Sanchez-Valle et al., 2017; Bezerra et al., 2018). The international shortfall between available donor organs and the number of patients requiring a transplant is significant, meaning patients often deteriorate and succumb while awaiting a transplantation (Zarrinpar and Busuttil, 2013).

Gut microbiota and their metabolites has been suggested to play a role in almost all major diseases including hepatobiliary diseases. The gut microbiota interacts with the liver and gallbladder through different mechanisms, such as diet, bile acids, immune factors, and metabolites, including SCFAs, to maintain intestinal homeostasis (Jain et al., 2021). The gut microflora has been implicated in the development and progression of BA. Particular microbiome diversity is highly variable in patients with BA, and the characteristics of the gut microbiota vary at different stages of BA (Figure 2) (Han, 2019; Qin et al., 2021; Chen, 2022; Fu et al., 2022a). Gut microbiota and their metabolites may participate in regulating immune response during inflammation, tissue repair following damage and autoimmunity and release a variety of pro-inflammatory and chemotactic factors to promote hepatic fibrosis through the degradation of stromal collagen and regulation of HSCs, thus improving the course of BA (Davenport et al., 2001; Wang et al., 2020b). Understanding the gut microbiota and metabolic changes in different BA stages could lead to novel therapeutic approaches for this condition (Tables 1, 2).

Gut microbiota and metabolic in patients with biliary atresia

Studies have confirmed altered gut microbiota richness and diversity indices in BA patients (Han, 2019; Wang et al., 2020a). There were significant differences in the composition of intestinal microorganisms between the BA group and the control group. From the phylum level perspective, the relative abundance of Bacteroidetes in children with BA was significantly lower than that in the control group. In contrast, the abundance of Firmicutes was increased considerably. At the genus level, the

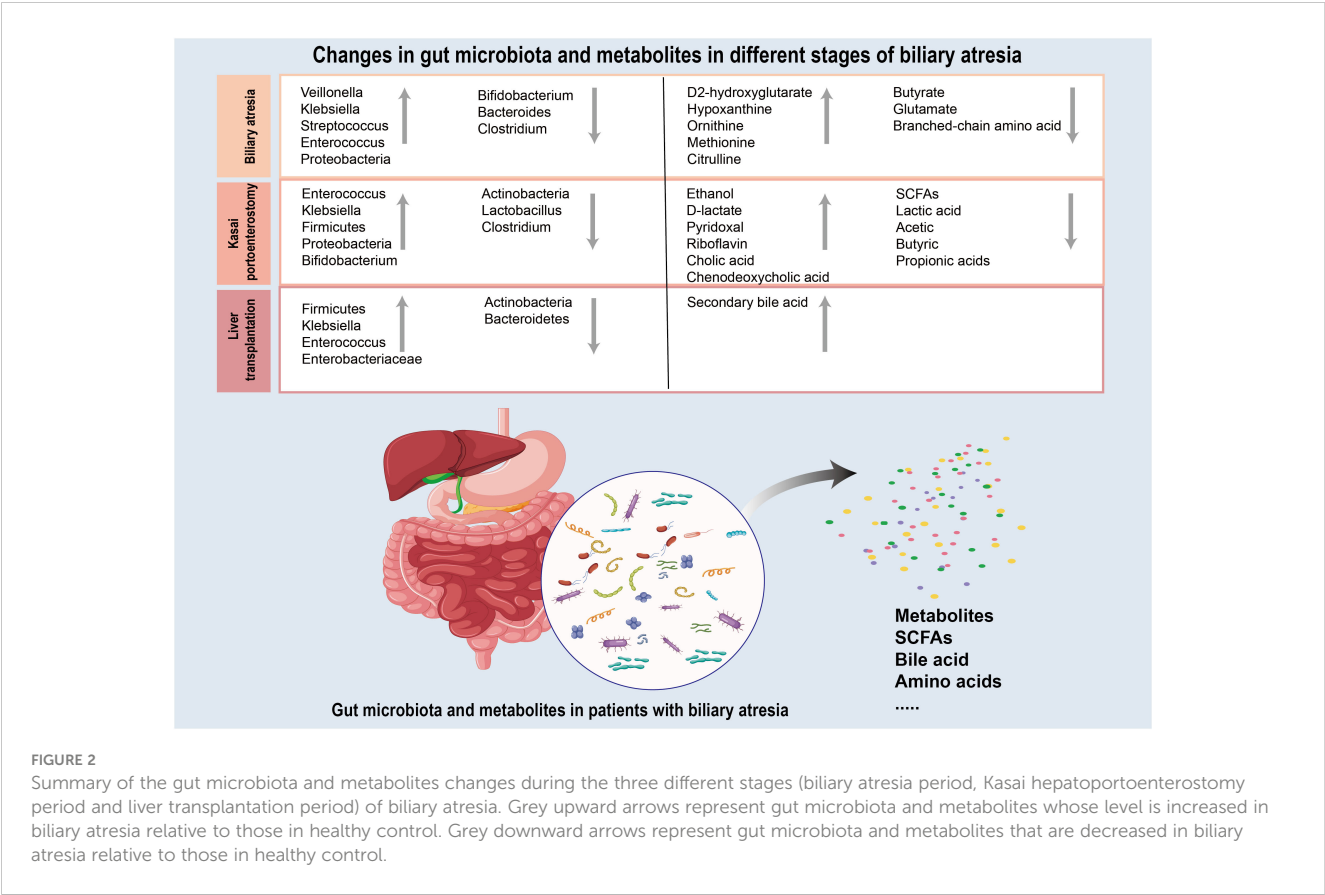


TABLE 1 Dysbiosis of gut microbes and metabolite in biliary atresia.

Patient/Model	Dysbiosis of the Microbes	Metabolite Changes	Ref
Biliary atresia without treatment			
43 BA patients and 22 HC	<i>Streptococcus</i> , <i>Klebsiella</i> and <i>Haemophilus</i> ↑↑; <i>Bifidobacterium</i> , <i>Bacteroides</i> and <i>Lactobacillus</i> ↓↓	NA	(Chen, 2022)
11 BA patients and 10 HC	<i>Veillonella</i> ↑↑; <i>Bacteroides</i> and <i>Clostridium</i> ↓↓	NA	(Han, 2019)
55 BA infants, 19 HC and 21 cholestatic control	<i>Enterococcus</i> and <i>Clostridium</i> ↑↑; <i>Bifidobacterium</i> ↓	NA	(Jain et al., 2023)
102 BA infants and HC	<i>Proteobacteria</i> , <i>Bacilli</i> (<i>Lactobacillus</i>), <i>Fusobacteria</i> , and <i>Streptococcus</i> , <i>Klebsiella</i> , and <i>Enterococcus</i> ↑↑ <i>Bacteroides</i> and <i>Clostridium</i> ↓↓	Hypoxanthine ↑; glutamate/glutamine ↓	(Jee et al., 2022)
9 bottle-fed infants with BA and HC	<i>Bifidobacteria</i> , lecithinase-negative <i>clostridia</i> , <i>Streptococci</i> , and <i>Staphylococci</i> ↓↓	NA	(Kobayashi et al., 1988)
35 BA patients, 35 patients with neonatal cholestasis rather than BA, and 35 HC.	NA	Ornithine, methionine, and citrulline ↑; Glycine, branched-chain amino acid ↓	(Raouf et al., 2016)
16 BA (early stage) patients, 16 HC; 16 BA (later stage) patients, 10 HC	<i>Proteobacteria</i> , <i>Klebsiella</i> , <i>Streptococcus</i> , <i>Veillonella</i> , and <i>Enterococcus</i> ↑↑ <i>Bifidobacterium</i> , <i>Actinobacteria</i> , <i>Verrucomicrobia</i> and <i>Blautia</i> ↓↓	NA	(Song et al., 2021b)
46 BA patients and 22 HC	NA	D2-hydroxyglutarate	(Tian et al., 2022)

(Continued)

TABLE 1 Continued

Patient/Model	Dysbiosis of the Microbes	Metabolite Changes	Ref
Biliary atresia without treatment			
34 BA patients and 34 HC	<i>Streptococcus</i> and <i>Klebsiella</i> ↑↑ <i>Bifidobacterium</i> ↓↓	Butyrate ↓	(Wang et al., 2020a)
43 BA patients, 33 other cholestatic diseases and 42 HC	<i>Proteobacteria</i> , <i>Streptococcus</i> and <i>Lactobacillus</i> ↑↑	Butyrate ↓	(Xu et al., 2023)
Biliary atresia with KPE			
30 BA children and 23 HC	<i>Enterococcus</i> ↑↑	SCFAs, lactic acid, acetic, butyric, and propionic acids↓↓	(Orłowska et al., 2021)
12 pre-LT, 12 BA post-LT and 22 HC	<i>Firmicutes</i> ↑↑ <i>Actinobacteria</i> ↓↓	NA	(Chen, 2022)
40 BA patients	<i>Bifidobacterium</i> and <i>Lactobacillus</i> ↑↑ <i>Faecalibacterium</i> ↓↓	NA	(Fu et al., 2022a)
40 BA patients	<i>Escherichia coli</i> . ↑↑ <i>Bifidobacterium</i> and <i>Lactobacillus</i> ↓↓	NA	(Fu et al., 2022b)
55 BA infants pre-KPE	NA	Ethanol and D-lactate ↑↑	(Alexander et al., 2023)
4 BA patients	<i>Proteobacteria</i> ↑↑	NA	(Elaine Chen et al., 2020)
55 BA infants, 19 HC and 21 cholestatic control	<i>Enterococcus</i> ↑↑ <i>Bifidobacterium</i> ↓↓	Acetate↓↓	(Jain et al., 2023)
39 BA patients	<i>Klebsiella</i> ↑↑	NA	(Meng et al., 2021)
16 BA patients (8 non-KPE, 8 post-KPE)	<i>Bacteroides</i> , <i>Prevotella</i> , <i>Barnesiella</i> , <i>Parabacteroides</i> , <i>Heliobacterium</i> , <i>Erysipelatoclostridium</i> , and <i>Diaporthe</i>	Pyridoxal and riboflavin ↑↑ cholic acid, chenodeoxycholic acid, and β-muricholic acid↑↑	(Song et al., 2021a)
8 BA patients and 7 cholestasis	<i>Bifidobacterium</i> and <i>Enterobacter</i> ↑↑ <i>Enterococcus</i> ↓↓	NA	(Tessier et al., 2020)
Biliary atresia with LT			
12 pre-LT, 12 BA post-LT and 22 HC	<i>Firmicutes</i> ↑↑ <i>Actinobacteria</i> and <i>Bacteroidetes</i> ↓↓	NA	(Chen, 2022)
16 BA patients and 10 HC	<i>Klebsiella</i> , <i>Enterococcus</i> , <i>Enterobacteriaceae</i> <i>Bacterium</i> ↑↑	NA	(Song et al., 2021c)
10 BA patients	<i>Klebsiella</i> ↑↑	Secondary bile acid↑↑	(Waldner et al., 2023)

↑↑ and ↓↓ indicate increases and decreases, respectively, compared to the reference or baseline value. 'NA' stands for 'Not Applicable', indicating missing or not relevant data for the specific item.

relative abundance of *Bacteroides* and *Clostridium* in the feces of children with BA was significantly reduced. In contrast, the relative abundance of *Veillonella* was considerably higher than that of the control group (Wang et al., 2020a; Chen, 2022). One of the typical characteristics of BA is a decrease in fecal bile acids, which are synthesized in the liver from cholesterol, converted into primary bile acids, and metabolized in the intestine via the enterohepatic circulation (Yan et al., 2022; Yang et al., 2022; He et al., 2023). Yang et al (Yang et al., 2022) found that *Bifidobacterium* showed a significant negative correlation with conjugated bile acids (GCA, TCA, TCDCA, and GCDCA; all $p < 0.05$), and *Rothia* was negatively correlated with secondary bile acids (DCA and LCA; both $p < 0.05$). Unfortunately, current research cannot confirm the relationship between bile acids and gut microbiota. The interaction

between the gut microbiota and bile acids is complex and bidirectional in the host and is referred to as the gut microbiota-bile acid axis (Cai et al., 2022). The gut microbiota converts primary bile acids into secondary bile acids, suggesting that the gut microbiota can affect the composition of secondary bile acids. Changing the secondary bile acid profile could reshape the intestinal bacterial composition and maintain gut homeostasis (Tilg et al., 2022; Zhang et al., 2022; He et al., 2023). The current view is that BA may result from a combination of factors, including genetics, the environment, infections during pregnancy, and immunologic factors (Ho et al., 1993; Bezerra et al., 2018). Dysbiosis of the gut microbiota and abnormal bile acid metabolism may be two of these complex factors, which may play a superimposed contributory role in the development of BA but are

TABLE 2 Metabolites and their impact on functions in biliary atresia.

Metabolite	Source Bacteria	Impact on Functions	Proposed Role in BA	Ref
Butyrate	<i>Proteobacteria</i> , <i>Streptococcus</i> and <i>Lactobacillus</i>	Jaundice clearance and cholangitis	Noninvasive diagnostic i indicator	(Xu et al., 2023)
Secondary bile acid	<i>Klebsiella</i>	Cholangitis and Complications after LT	Guidance on the use of immunosuppressive agents	(Waldner et al., 2023)
Acetate	<i>Bifidobacterium</i>	Cholangitis	Possible protective factor	(Jain et al., 2023)
Propionate	<i>Clostridium</i>	Total bilirubin	Possible protective factor	(Jain et al., 2023)
glutamate/glutamine and hypoxanthine	<i>Bacteroidetes</i> and <i>Clostridia</i>	Promoting survival of bile duct epithelial cells	Potential Treatment Options	(Jee et al., 2022)
Tauroursodeoxycholic acid	<i>Campylobacter</i> and <i>Rikenellaceae</i>	Jaundice clearance	Potential Treatment Options	(Yang et al., 2022)
Bile acids	<i>Faecalibacterium prausnitzii</i> and <i>Escherichia coli</i>	Maintaining the intestinal barrier	Possible protective factor	(Song et al., 2021a)
Tryptophan	<i>V. atypica</i>	Total bilirubin	Noninvasive diagnostic i indicator	(Song et al., 2021b)
Lithocholic acid	<i>Enterococcus faecium</i>	Total bilirubin	Noninvasive diagnostic i indicator	(Song et al., 2021b)
Branched chain amino acids	NA	Correct growth, nitrogen retention, and body composition	Potential Treatment Options	(Sokal et al., 2021b)

unlikely to be the sole cause. Therefore, it is not accurate enough to consider dysbiosis of the gut microbiota or abnormal bile acid metabolism alone as a single cause of BA. Future studies need to elucidate further how these factors interact and their specific roles in the pathogenesis of BA.

Not only is the gut microbiota closely related to the health of the host, but the metabolites of the gut microbiota also play an essential role in the process of BA (Cai et al., 2022). Recently, owing to the development of transcriptomics and metabolomics technology, more research has reported changes in the production of bacterial metabolites, including SCFAs, bile acids, and amino acids in BA patients (Song et al., 2021a; Yang et al., 2022). SCFAs, such as butyric, propionic, acetic, and valeric acids, are carboxylic acids produced through aerobic fermentation of dietary fiber in the intestine (Rauf et al., 2022). It is well known that SCFAs have an essential function in maintaining human health, including gut homeostasis, and serving as the primary fuel for the colonic epithelial cells (Qin et al., 2021). SCFAs display anti-inflammatory activity and are responsible for strengthening the intestinal barrier, which prevents gut leaks (Yan et al., 2022). Due to these properties, they are particularly beneficial in patients with intestinal inflammation. Among SCFAs, butyric acid has the best proven beneficial effect, whereas the importance of the other acids still needs to be understood. In addition to SCFAs, the leading bacterial products, the gut microbiota forms other organic acids. Their role has yet to be discovered. Among them, we distinguish isobutyric and isovaleric acids, collectively called branched short-chain fatty acids(BCFAs), produced through the fermentation of branched-chain amino acids (Rauf et al., 2022). In contrast to SCFAs, increased BCFAs unfavorable affect gut health (Sokal et al., 1996). However, the relationship between the role of

individual bacterial-derived acids and intestinal inflammation remains unclear. Moreover, the association between factors influencing BA onset and course, such as eating habits, lifestyle, medications, and the profile of bacterial-derived acids, still needs to be fully understood.

Gut microbiota and metabolites of biliary atresia patients treated with Kasai surgery

Since 1959, KPE has been recognized as a first-line treatment option for BA. This procedure involves removing a portion of the fibrous mass caused by hepatic portal BA and establishing a hepatico-jejunal anastomosis to restore bile drainage (Sanchez-Valle et al., 2017). KPE has demonstrated prompt relief of bile flow obstruction and improved chances of long-term survival without the need for LT (Bezerra et al., 2018). Clinical studies have shown that an imbalance of gut microbiota exists in children before KPE, with a decrease in the abundance of *Bifidobacteria* and LGG and an increase in the abundance of aerobic bacteria such as *Escherichia coli* and *Enterococci* spp. which is even more pronounced in the early postoperative period (Han, 2019; Song et al., 2021b; Chen, 2022; Jain et al., 2023). Fu et al (Fu et al., 2022b). studied the effect of early nutritional support on the intestinal flora of children after KPE. They found that 7 d after KPE, the levels of *Bifidobacteria* and LGG decreased significantly, and the levels of *E. coli* and *Enterococci* spp. increased significantly compared with those before KPE. In 2022, Fu et al (Fu et al., 2022a). conducted another study on the intestinal flora of children after KPE. They found that the levels of *Bifidobacteria* in children with BA were higher than that of pre-KPE, the levels of *E. faecalis* were lower than

that of pre-KPE, and the levels of *E. faecalis* were lower than that of pre-KPE in children with BA. The LGG level was lower than pre-KPE, and the level of LGG gradually increased in the late postoperative period after KPE. This shows a significant difference in the composition of the gut microbiota at different stages after KPE. This difference may be due to surgical stress, postoperative application of antibiotics and hormones, and changes in bile acid metabolism in the intestinal tract. Anesthesia and surgery (especially gastrointestinal surgery) are potent stimulants for the body, which can induce a severe peripheral inflammatory response that can affect various organs and systems throughout the body, including intestinal tissues. Anesthetic drugs and surgical trauma can affect the gut microbiota (Çitoğlu et al., 2012; Micó-Carnero et al., 2020). In addition, antibiotics play an essential role in preventing postoperative infections. However, it is also an important factor in the dysbiosis of the gut microbiota after KPE.

While KPE can effectively alleviate symptoms of cholestasis and improve short-term prognosis in certain children with BA, it is important to note that progressive hepatic fibrosis and recurrent cholangitis are common complications that may arise post-surgery (Bezerra et al., 2018). Cholangitis, in particular, is a frequent occurrence post-KPE, and its underlying cause in BA is still unclear. However, retrograde infection by intestinal bacteria, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella*, *Acinetobacter baumannii*, and *Salmonella typhimurium*, are believed to be the primary cause (Madadi-Sanjani et al., 2021; van Wessel et al., 2021).

Metabolites of the gut microbiota have been shown to play an vital role in the progress of BA disease. Recent studies have shown significant metabolic abnormalities in post-KPE in BA (Zhou et al., 2015; Jain et al., 2023). Orowska et al (Orłowska et al., 2021). analyzed the postoperative fecal composition of patients undergoing KPE surgery. They found significant differences in the expression of several metabolites in patients treated with LGG compared to those in the placebo group. Moreover, patients with postoperative cognitive impairment showed decreased levels of SCFAs, lactic acid, acetic, butyric and propionic acids. Previous studies have confirmed that increased levels of SCFAs alleviate the extent of liver injury (Rauf et al., 2022). Alexander et al (Alexander et al., 2023). found that Ethanol, at three months post-KPE, is associated with LT in BA. Ethanol and D-lactate production are linked, suggesting a role for gut microbiota-ethanol and D-lactate production in BA. Jain et al (Jain et al., 2023). observed 55 cases of BA. They found that a decrease in *blautia*, *bifidobacteria*, and subsequent dysregulation of SCFAs in the early post-KPE period related to poorer clinical outcomes. In addition, increased intestinal permeability and decreased Acetate levels were found in the early post-KPE. Although several studies have described the current status of alterations in the gut microbiota and its metabolites in children with BA treated with KPE, the specific mechanisms involved in their occurrence remain to be investigated, and thus, more basic and clinical studies are still needed in the future to more systematically investigate the potential mechanisms of action of the gut microbiota and its metabolites in children with BA treated with KPE.

Gut microbiota and metabolites in biliary atresia patients undergoing liver transplantation

KPE effectively alleviates the symptoms of cholestasis and improves the quality of life of patients with BA. Despite these benefits, BA remains the primary reason for liver transplantation (LT) in the pediatric population due to the persistence of progressive cholestasis and recurrent cholangitis even after KPE treatment (Hartley et al., 2009; Bezerra et al., 2018). Following LT in children with BA, various factors such as ischemia/reperfusion injury, postoperative infections, and chronic rejection can have an impact on the microbiota composition (Micó-Carnero et al., 2020). The administration of immunosuppressants, antibiotics, antifungal and antiviral drugs post-transplantation can further disturb the balance of gut microbiota, gastrointestinal epithelial barrier function and increased gut permeability, and result in bacterial translocation (Micó-Carnero et al., 2020; Qin et al., 2021; Sucu et al., 2023). Furthermore, Studies have confirmed that gut microbiota and their metabolites correlate with prognosis and complications after LT (Madadi-Sanjani et al., 2021; Wirth et al., 2023). These findings underscore the critical role of microbiota composition in LT outcomes.

The diversity and number of microorganisms in the gut microbiota of children with BA undergoing LT are significantly altered, with a decrease in the number of beneficial bacteria and an increase in the number of conditional pathogens in the early stages, and a gradual recovery of the microbiome diversity in the late stages, with a decrease in the *Enterobacteriaceae* and a gradual increase in the number of potentially beneficial bacteria, such as the *Rumabacteriaceae* (Yao et al., 2023). Chen et al (Chen, 2022). conducted a prospective study analyzing the microbiome diversity of 12 patients underwent LT in preoperative and early postoperative periods. There is a significant shift in the diversity of gut microbiota during the early post-LT period, with an increase in the abundance of the *Firmicutes* and a decrease in the abundance of the *Actinobacterial* and *Anaplastic bacterial phyla* compared to the preoperative period. In an animal model of LT, the researchers found that rats had a significant decrease in the abundance of probiotics, such as *Bifidobacterium* and LGG, and a significant increase in the abundance of conditional pathogens, such as *Enterobacteriaceae* and *Enterococcus* spp., after undergoing LT. This change in microbiota composition was associated with functional changes related to increased pathogenic toxins in the early postoperative period and perioperative stress. Song et al (Song et al., 2021b). evaluated fecal samples from 16 children with BA who underwent LT pre-transplantation, as well as six months post-transplantation and the fecal microbiomes of 10 healthy controls and found that the microbiome diversity of the children with BA increased at six months post-LT. The differences in microbiota composition were not statistically significant in the post-LT group when compared to healthy controls, suggesting that LT restores, at least partially, the microbiota composition. Similar results were obtained by Waldner et al (Waldner et al., 2023). analyzed the microbiome diversity in patients before and 3, 12, and 24 months

after LT and found that at three months after LT, the intestinal alpha diversity of the gut microbiota was significantly reduced at three months after LT. In contrast, no significant difference was observed at 12 and 24 months compared to healthy controls. In addition, changes in the microbiome diversity were associated with alterations in bile acid synthesis secondary to LT and the choice of different immunosuppressive agents two years after LT. These results suggest that the diversity of gut microbiota in children with BA after LT is associated with the prognosis of LT. Current clinical and basic trials of the gut microbiota and its metabolites in children with BA treated with LT are ongoing, and it needs to be clarified how the metabolites of the gut microbiota of children with BA before and after LT are characterized.

Roles of the gut microbiota dysbiosis on biliary atresia occurrence and development

The exact cause of BA is unknown; it is a multifactorial disease, such genetic, environmental factors, and intestinal immune dysfunction (Hartley et al., 2009). Recent research has shown that BA is caused by changes in the biliary, hepatic, and intestinal tracts, leading to an immune-mediated inflammatory response that causes liver injury and liver fibrosis (Han, 2019). The causes of liver immune imbalance and excessive inflammatory response can be endogenous or exogenous, ranging from hereditary factors to viral infections, environmental toxins, and adverse drug reactions.

Recent clinical studies indicate that gut microbiota and their metabolites, such as SCFAs (acetate and butyrate) and ethanol, are crucial in the pathogenesis of BA (Figure 3) (Jee et al., 2017).

Gut microbiota and metabolites participate in the development of biliary atresia by regulating inflammation

Inflammatory response plays an essential role in the development of BA. Pathological examination of the liver in children with BA reveals that the hepatic portal vein area is infiltrated with many inflammatory cells, including NK cells, CD4⁺ T cells, CD8⁺ T cells, B cells, and macrophages (Han, 2019; Chen, 2022). Activated inflammatory cells secrete many inflammatory mediators, such as interferon-gamma, tumor necrosis factor- α (TNF- α), and interleukins (IL). These cytokines further damage the bile duct epithelial cells, causing obstruction, liver fibrosis, and cirrhosis (Brindley et al., 2012). Studies have confirmed that gut microbiota can influence the condition of children with BA by participating in the body's inflammatory response (Çitoğlu et al., 2012). Meng et al (Meng et al., 2021). found that the postoperative KPE induced gut microbiota dysbiosis, as reflected by reduced diversity. Moreover, the relative abundance of *Klebsiella* increased in the fecal. Gene expression patterns in BA-like organs transfected with *Klebsiella*. Infections were enriched in pathways associated with inflammation, apoptosis and fibrosis. Further studies revealed IL-13/TGF- β 1 mediated fibrosis in postoperative cholangitis in KPE. This study

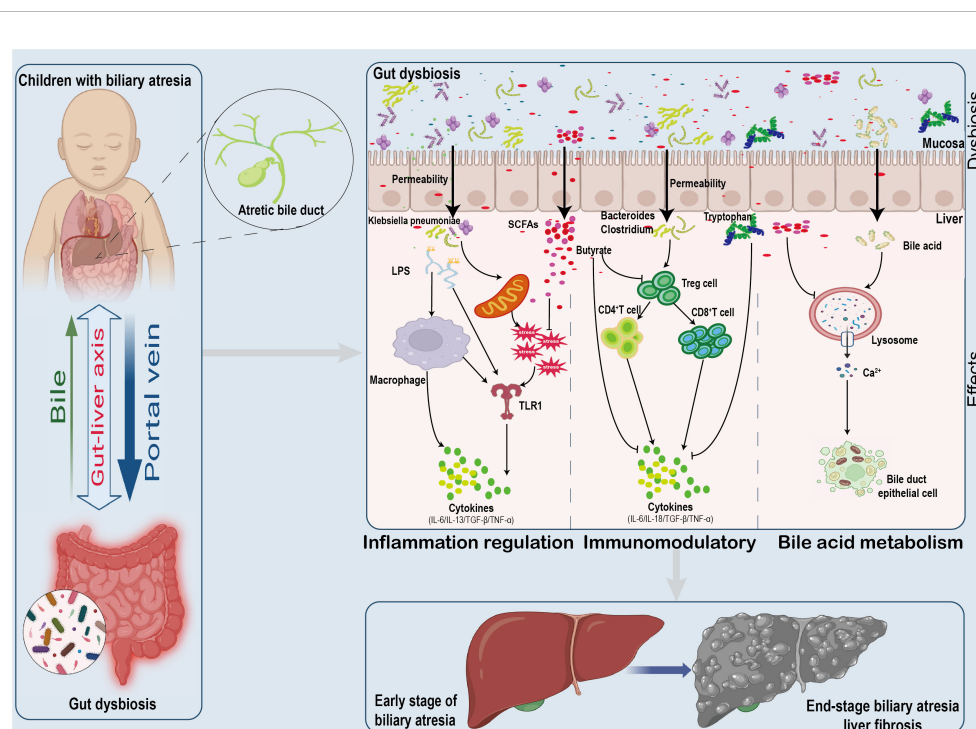


FIGURE 3

Mechanisms Linking Gut Microbiome Alterations to Biliary Atresia Progression. The gut microbiome's impact on biliary atresia involves: (1) Increased pathogen presence and metabolite release disrupting gut integrity. (2) Metabolic and cytokine shifts affecting oxidative stress and inflammation. (3) Reduced butyrate levels leading to immune imbalances and cytokine surges. (4) Bile acid metabolism alterations contributing to cellular damage and apoptosis.

suggests the involvement of *Klebsiella* in regulating the inflammatory response, which further influences the process of BA.

Metabolites of gut microbiota are directly involved in energy metabolism locally but also reach the liver via the liver-gut axis to modulate the inflammatory response (Milosevic et al., 2019; Tilg et al., 2022). Therefore, disturbed gut microbiota can alter hepatic inflammation by affecting metabolite production, among which SCFAs, metabolites of gut microbiota, have been widely recognized. SCFAs were found to exert anti-inflammatory properties by activating G protein-coupled receptors GPR41 and GPR43 and also regulate the production of pro-inflammatory factors, such as IL-6 and TNF- α , through the TLR1 pathway in the Toll-like receptor, to alleviate the systemic inflammatory response (Rauf et al., 2022). *Eicosapentaenoic acid* inhibited liver fibrosis in patients without jaundice six months after KPE. Further studies found that periductal inflammation was reduced by *Eicosapentaenoic acid* supplementation (Zhou et al., 2015). Thus, metabolites of the gut microbiota can be involved in inflammatory regulation, thereby influencing the pathological process of BA. In addition, structural dysregulation of the gut microbiota is also involved in the regulation of inflammation in the bile ducts in the Cxcr2^{-/-} mouse model of RRV infection model showed further enrichment of *Corynebacterium*, *Anaerococcus* and *Streptococcus* (Jee et al., 2017). Among these, *Anaerococcus lactolyticus* was significantly associated with a suppression of biliary injury, cholestasis, and survivability, leading to an inflammatory response and oxidative stress, which are involved in the pathological process of BA.

Gut microbiota and metabolites participate in the progression of biliary atresia by regulating immune response

The modulation of abnormal immune regulation is one of the possible mechanisms in the pathogenesis of BA. Many immune cells are involved in the development of BA. Brindley et al (Brindley et al., 2012). found that the number of Treg cells in children with BA decreased and had a significant negative correlation with the degree of liver fibrosis. Fu et al (Fu et al., 2022a). studied liver biopsies from patients with BA and found that CD4⁺ and CD8⁺ infiltrates were predominantly present in perihepatic bile duct lymphocytes. In addition, the abundance of *Bifidobacterium bifidum* increased significantly, and the abundance of *E. faecalis* decreased pre-KPE. In comparison, the level of CD4⁺ and CD8⁺ was higher than pre-KPE, and the level of IL-18 was lower. Jee et al (Jee et al., 2022). found that hepatic immune cell activation and survival traits were associated with fecal characteristics of *Mycobacterium anomalies* and *Clostridium perfringens* in the RRV-induced BA mouse model. Together, these findings illustrate the essential role of immune response in the BA course. However, the studies mentioned above did not conduct in-depth research on the regulatory mechanism, and the specific mechanism still needs to be clarified. The findings of Jee et al (Jee et al., 2017). also illustrate that butyrate can reduce liver and bile duct inflammation by defending against oxidative

stresses in humans and the BA mouse model. Butyrate is a microbial metabolite that has been shown to affect the liver-gut axis, including directly scavenging free radicals and indirect antioxidant activity through modulation of the pathways involved in expressing cytoprotective enzymes and molecules. In addition to butyrate, tryptophan, which is associated with *Klebsiella*, *Veillonella*, and *Enterococcus* spp., has been shown to exacerbate liver injury in BA, either through direct or indirect effects.

Gut microbiota and metabolites participate in the progression of biliary atresia by regulating bile acid metabolism

Bile acid metabolism disorders in BA is one of the most important causes of liver injury (Liu et al., 2015). Due to bile duct obstruction, children with BA have higher total bile acid levels and significantly elevated hydrophobic bile acid salt concentrations, which can lead to lysis of the endoplasmic reticulum of hepatocytes and bile duct epithelial cells and uncontrolled release of intracellular Ca²⁺ into the cytoplasm, thereby inducing cell damage (Wang et al., 2022). Yang et al (Yang et al., 2022). analyzed the faeces of 84 children with BA. They found that patients with BA had different gut microbiota and bile acid composition characteristics, and their interactions were involved in the process of liver injury in BA, which was closely related to the development of postoperative cholangitis and clearance of jaundice. Wang et al (Wang et al., 2020a). also found that the presence of bile acids was dramatically decreased in BA, and *Clostridium XI/Va* positively correlated with the ratio of primary/secondary bile acids. Unfortunately, previous studies have not conducted an in-depth exploration of the issue. With more awareness of the relationship between gut microbiota and BA, we expect that more studies will be conducted to reveal the exact mechanism and provide new insights into the treatment of BA from the point of view of gut microbiota regulating bile acid metabolism.

Therapeutic strategies targeting gut microbiota in biliary atresia

The current management of BA in children involves a combination of surgical and pharmacological interventions. Surgical treatment, known as KPE, aims to restore bile flow, while pharmacological therapies, including antibiotics, glucocorticosteroids, hepatoprotective agents, and nutritional support, are used with surgery (Bezerra et al., 2018; Schreiber et al., 2022). Recent clinical and experimental studies have highlighted the potential benefits of modulating the gut microbiota to enhance BA treatment outcomes (Orłowska et al., 2021; Jee et al., 2022). This includes strategies like administering probiotics, prebiotics, synbiotics, and metabolites like SCFAs. The cognition of gut microbiota and its metabolites in the progression of BA offers a new possibility to improve its therapeutic efficacy by targeting the gut microbiota.

Probiotics and their potential benefits in biliary atresia management

The main genus of probiotics being studied is LGG, which has shown beneficial effects on BA in children when given in adequate doses. Two small clinical trials have investigated LGG supplementation. One small randomized controlled trial found no difference between LGG (n=14) and placebo (n=16) regarding jaundice, cholangitis or graft requirements over two years (Orłowska et al., 2021). A second trial randomly assigned patients to the LGG (n=10) or neomycin (n=10) groups for cholangitis prophylaxis and found no significant difference in the frequency of cholangitis between the LGG and neomycin groups (Lien et al., 2015). The above results illustrate that the use of probiotics increased the abundance of beneficial bacteria and decreased the abundance of conditional pathogens, maintaining microbiome diversity balance in BA patients while decreasing the risk of cholangitis and LT. Therefore, probiotic treatment could be a promising new therapeutic approach for treating BA patients.

The metabolites of the gut microbiota as a novel approach in biliary atresia treatment

With the in-depth study of gut microbiota and its metabolites, more and more studies have confirmed that gut microbiota metabolites can regulate BA. The SCFAs are the major metabolites produced by gut microbiota fermenting dietary fibers, proteins, and peptides. The SCFAs, including acetate, propionate, and butyrate, constitute a significant class of bacterial metabolites derived from colonic carbohydrate fermentation (Rauf et al., 2022). Studies have shown that butyrate affects microbiome diversity and metabolites and phenotypic expression of experimental BA in neonatal mice and that glutamine promotes the survival of bile duct epithelial cells (Jee et al., 2022). In another study, eicosapentaenoic acid administration reduced Mac-2 binding protein sugar chain modified isomer and hyaluronic acid levels in the liver after KPE and improved liver fibrosis (Sumida et al., 2018). Other gut microbiota metabolic derivatives, such as D-2-hydroxyglutarate, can alleviate the processing of BA by regulating bile acid metabolism with the liver microenvironment and mammalian target of rapamycin signaling (Tian et al., 2022). Therefore, SCFAs and other gut microbiota metabolic derivatives may be considered novel and viable therapeutic agents for preventing and mitigating BA.

Future perspective

Over the past decade, although partial studies on BA and gut microbiota and their metabolites have been conducted, we are still in the beginning stages of demonstrating the relevant role of gut microbiota in BA (Bezerra et al., 2018). So far, our theories are often

based on retrospective and observational studies of small cohorts, and limited animal models restrict the research on BA and gut microbiota (Wang et al., 2020a; Schreiber et al., 2022). We do not have enough evidence to definitively prove that ecological dysbiosis causes BA or whether it only occurs during BA, which results from disease-related metabolic changes. Testing this hypothesis will require longitudinal, interventional, and prospective multicenter studies. Second, the specific mechanism by which specific bacterial strains or metabolic derivatives are associated with the onset of BA is unknown, and whether it can be used as an effective treatment has yet to be mechanistically determined. Further in-depth study of this specific molecular mechanism is needed in the future, which is vital for understanding the gut microbiota in the clinical diagnosis and treatment of BA. Thirdly, other microbiological factors still need to be better understood, such as the crosstalk between gut microbiota, which needs improvement. To date, we still need to confirm the sequential order of bile acids and gut microbiota alterations. The results of the current study are even contradictory. This may be due to the relatively small number of patients and the need for harmonized methods and criteria. Therefore, in-depth studies of these metabolites and their microbial sources are needed, as well as refinement of the characterization of the gut microbiota and their metabolites at different stages of BA. Technological and research developments may facilitate the use of gut microbiota in BA intervention or treatment, and in-depth exploration of the composition and function of gut microbiota will lead to new diagnostic tools and personalized treatments for BA.

Conclusion

BA is a progressive fibroinflammatory disorder of infants involving the extrahepatic and intrahepatic biliary tree. Even with successful KPE, most patients diagnosed with BA progress to end-stage liver disease, necessitating an LT for survival. Numerous studies have reported that gut microbiota are involved in the pathogenesis of many diseases, especially liver disease. Therefore, human biology should not neglect the gut microbiota, which produce or regulate various chemicals and trigger host responses that affect various functions, including inflammation, immunity, and metabolism. This review provides some evidence that further enhances our understanding of BA from the perspective of gut microbiota and their metabolites while simultaneously exploring the pathogenesis of BA. We also summarize the characteristics of changes in the microbiota and its metabolites at different stages in patients with BA, which may be related to the body's inflammatory response, abnormalities in immune regulation, or the severity and progression. However, research on the in-depth mechanism of an intricate interplay between gut microbiota, metabolites, and BA pathophysiological progression remains limited, which makes any conclusive or pathogenic statements about the composition of the gut microbiota and metabolite profiles of BA patients challenging. The current limitations of studying gut flora and its metabolites in BA should motivate scientists to elucidate newer approaches with

modern technologies and explore more options regarding the interactions of gut microbiota, its metabolites, and BA in pathogenesis. The treatment of the gut microbiota and its metabolites in BA patients seems promising in the near future. Large-scale randomized controlled trials in patient are needed to evaluate the beneficial properties of probiotics, prebiotics, and synbiotics, their ideal dosages, duration of supplementation, persistence of their beneficial effects, and their safety in preventing and treating BA.

Author contributions

SF: Investigation, Supervision, Writing – original draft, Writing – review & editing. YC: Conceptualization, Investigation, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. CS: Software, Supervision, Writing – review & editing. CY: Supervision, Validation, Writing – review & editing. YL: Conceptualization, Funding acquisition, Resources, Writing – review & editing.

References

- Abu Faddan, N. H., Sherif, T. M. K., Mohammed, O. A., Nasif, K. A., and El Gezawy, E. M. (2017). Intestinal barrier integrity and function in infants with cholestasis. *Intest Res.* 15, 118–123. doi: 10.5217/ir.2017.15.1.118
- Alexander, E., Burford, C., Davenport, M., Makin, E., Verma, A., Dhawan, A., et al. (2023). THU-332 - Endogenous ethanol production in biliary atresia and its association with clinical outcomes, liver disease severity, fibrosis, bacterial translocation and intestinal permeability. *J. Hepatol.* 78, S999. doi: 10.1016/S0168-8278(23)03064-7
- Altshuler, G. (1973). Letter: Cytomegalovirus and biliary atresia. *Lancet Lond Engl.* 2, 1206. doi: 10.1016/S0140-6736(73)92970-X
- Aron-Wisniewsky, J., Vigliotti, C., Witjes, J., Le, P., Holleboom, A. G., Verheij, J., et al. (2020). Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat. Rev. Gastroenterol. Hepatol.* 17, 279–297. doi: 10.1038/s41575-020-0269-9
- Averbukh, L. D., and Wu, G. Y. (2018). Evidence for viral induction of biliary atresia: A review. *J. Clin. Transl. Hepatol.* 6, 410–419. doi: 10.14218/JCTH.2018.00046
- Bezerra, J. A., Wells, R. G., Mack, C. L., Karpen, S. J., Hoofnagle, J. H., Doo, E., et al. (2018). Biliary atresia: clinical and research challenges for the twenty-first century. *Hepatol. Baltim Md.* 68, 1163–1173. doi: 10.1002/hep.29905
- Brindley, S. M., Lanham, A. M., Karrer, F. M., Tucker, R. M., Fontenot, A. P., and Mack, C. L. (2012). Cytomegalovirus-specific T-cell reactivity in biliary atresia at the time of diagnosis is associated with deficits in regulatory T cells. *Hepatol. Baltim Md.* 55, 1130–1138. doi: 10.1002/hep.24807
- Cai, J., Rimal, B., Jiang, C., Chiang, J. Y. L., and Patterson, A. D. (2022). Bile acid metabolism and signaling, the microbiota, and metabolic disease. *Pharmacol. Ther.* 237, 108238. doi: 10.1016/j.pharmthera.2022.108238
- Chen, B. (2022). Intestinal Microbiota in Patients with Biliary Atresia. Chongqing Medical University.
- Çitoğlu, G. S., Acikara, O. B., Yilmaz, B. S., and Ozbek, H. (2012). Evaluation of analgesic, anti-inflammatory and hepatoprotective effects of lycorine from *Sternbergia fischeriana* (Herbert) Rupr. *Fitoterapia* 83, 81–87. doi: 10.1016/j.fitote.2011.09.008
- Cui, M. M., Gong, Y. M., Pan, W. H., Pei, H. Y., Bai, M. R., Song, H. L., et al. (2023). Contribution of ADD3 and the HLA genes to biliary atresia risk in chinese. *Int. J. Mol. Sci.* 24, 14719. doi: 10.3390/ijms241914719
- Davenport, M., Gonde, C., Redkar, R., Koukoulis, G., Tredger, M., Mieli-Vergani, G., et al. (2001). Immunohistochemistry of the liver and biliary tree in extrahepatic biliary atresia. *J. Pediatr. Surg.* 36, 1017–1025. doi: 10.1053/jpsu.2001.24730
- Delzenne, N. M., Knudsen, C., Beaumont, M., Rodriguez, J., Neyrinck, A. M., and Bindels, L. B. (2019). Contribution of the gut microbiota to the regulation of host metabolism and energy balance: a focus on the gut-liver axis. *Proc. Nutr. Soc* 78, 319–328. doi: 10.1017/S0029665118002756
- Elaine Chen, Y. F., Lai, M. W., Tsai, C. N., Lai, J. Y., Yang, Y. C., and Chen, S. Y. (2020). Association of gut microbiota composition and copy number variation with

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- Kasai procedure outcomes in infants with biliary atresia. *Pediatr. Neonatol.* 61, 238–240. doi: 10.1016/j.pedneo.2019.12.011
- Fischler, B., Czubkowski, P., Dezsofi, A., Liliemark, U., Socha, P., Sokol, R. J., et al. (2022). Incidence, impact and treatment of ongoing CMV infection in patients with biliary atresia in four european centres. *J. Clin. Med.* 11, 945. doi: 10.3390/jcm11040945
- Fu, C. J., Chen, Z. T., Wu, L. Z., and Lin, H. (2022a). Effects of Kasai procedure on immune function and intestinal microbiota in children with biliary atresia. *Med. Theory Pr.* 35, 2263–2265. doi: 10.19381/j.issn.1001-7585.2022.13.041
- Fu, C. J., Wu, L. Z., Chen, Z. T., and Lin, H. (2022b). Early enteral nutrition after Kasai procedure affects immune function and intestinal microbiota in children with biliary atresia. *Chin. Foreign Med. Treat.* 41, 8–11. doi: 10.16662/j.cnki.1674-0742.2022.04.008
- Garcia-Barceló, M. M., Yeung, M. Y., Miao, X. P., Tang, C. S. M., Cheng, G., So, M. T., et al. (2010). Genome-wide association study identifies a susceptibility locus for biliary atresia on 10q24.2. *Hum. Mol. Genet.* 19, 2917–2925. doi: 10.1093/hmg/ddq196
- Han, M. (2019). Preliminary Study on Intestinal Microbiota Diversity in Children with Biliary Atresia. Shanghai Jiao Tong University.
- Harper, P., Plant, J. W., and Unger, D. B. (1990). Congenital biliary atresia and jaundice in lambs and calves. *Aust. Vet. J.* 67, 18–22. doi: 10.1111/j.1751-0813.1990.tb07385.x
- Hartley, J. L., Davenport, M., and Kelly, D. A. (2009). Biliary atresia. *Lancet Lond Engl.* 374, 1704–1713. doi: 10.1016/S0140-6736(09)60946-6
- He, S., Li, L., Yao, Y., Su, J., Lei, S., Zhang, Y., et al. (2023). Bile acid and its bidirectional interactions with gut microbiota: A review. *Crit. Rev. Microbiol.* 1–18. doi: 10.1080/1040841X.2023.2262020
- Ho, C. W., Shioda, K., Shirasaki, K., Takahashi, S., Tokimatsu, S., and Maeda, K. (1993). The pathogenesis of biliary atresia: a morphological study of the hepatobiliary system and the hepatic artery. *J. Pediatr. Gastroenterol. Nutr.* 16, 53–60. doi: 10.1002/j.1536-4801.1993.tb10801.x
- Hosoda, Y., Miyano, T., and Fujimoto, T. (1997). Assay of gamma-glutamyl transpeptidase activity in amniotic fluid offers a possible prenatal diagnosis of biliary atresia in the rat model. *Prenat. Diagn.* 17, 9–12. doi: 10.1002/(ISSN)1097-0223
- Hou, K., Wu, Z. X., Chen, X. Y., Wang, J. Q., Zhang, D., Xiao, C., et al. (2022). Microbiota in health and diseases. *Signal Transduct Target Ther.* 7, 135. doi: 10.1038/s41392-022-00974-4
- Isayama, F., Hines, I. N., Kremer, M., Milton, R. J., Byrd, C. L., Perry, A. W., et al. (2006). LPS signaling enhances hepatic fibrogenesis caused by experimental cholestasis in mice. *Am. J. Physiol-Gastrointest Liver Physiol.* 290, G1318–G1328. doi: 10.1152/ajpgi.00405.2005
- Jain, V., Alexander, E. C., Burford, C., Verma, A., and Dhawan, A. (2021). Gut microbiome: A potential modifiable risk factor in biliary atresia. *J. Pediatr. Gastroenterol. Nutr.* 72, 184–193. doi: 10.1097/MPG.0000000000002973

- Jain, V., Burford, C., Alexander, E., Gerasimidis, K., Verma, A., Davenport, M., et al. (2023). Longitudinal assessment of gut microbiota, metabolome and intestinal barrier dysfunction in biliary atresia. *J. Hepatol.* 78, S987. doi: 10.1016/S0168-8278(23)03045-3
- Jaswal, K., Todd, O. A., and Behnsen, J. (2023). Neglected gut microbiome: interactions of the non-bacterial gut microbiota with enteric pathogens. *Gut Microbes* 15, 2226916. doi: 10.1080/19490976.2023.2226916
- Jee, J., Mourya, R., Shivakumar, P., Fei, L., Wagner, M., and Bezerra, J. A. (2017). Klebsiella. *PLoS One* 12, e0182089. doi: 10.1371/journal.pone.0182089
- Jee, J. J., Yang, L., Shivakumar, P., Xu, P. P., Mourya, R., Thanekar, U., et al. (2022). Maternal regulation of biliary disease in neonates via gut microbial metabolites. *Nat. Commun.* 13, 18. doi: 10.1038/s41467-021-27689-4
- Kisseleva, T., and Brenner, D. (2021). Molecular and cellular mechanisms of liver fibrosis and its regression. *Nat. Rev. Gastroenterol. Hepatol.* 18, 151–166. doi: 10.1038/s41575-020-00372-7
- Klemann, C., Schröder, A., Dreier, A., Möhn, N., Dippel, S., Winterberg, T., et al. (2016). Interleukin 17, produced by $\gamma\delta$ T cells, contributes to hepatic inflammation in a mouse model of biliary atresia and is increased in livers of patients. *Gastroenterology* 150, 229–241. doi: 10.1053/j.gastro.2015.09.008
- Kobayashi, A., Kawai, S., Ohbe, Y., and Benno, Y. (1988). Fecal flora of infants with biliary atresia: effects of the absence of bile on fecal flora. *Am. J. Clin. Nutr.* 48, 1211–1213. doi: 10.1093/ajcn/48.5.1211
- Lien, T. H., Bu, L. N., Wu, J. F., Chen, H. L., Chen, A. C., Lai, M. W., et al. (2015). Use of *Lactobacillus casei rhamnosus* to Prevent Cholangitis in Biliary Atresia After Kasai Operation. *J. Pediatr. Gastroenterol. Nutr.* 60, 654–658. doi: 10.1097/MPG.0000000000000676
- Liu, H. X., Keane, R., Sheng, L., and Wan, Y. J. (2015). Implications of microbiota and bile acid in liver injury and regeneration. *J. Hepatol.* 63, 1502–1510. doi: 10.1016/j.jhep.2015.08.001
- Lorent, K., Gong, W., Koo, K. A., Waisbourd-Zinman, O., Karjoo, S., Zhao, X., et al. (2015). Identification of a plant isoflavonoid that causes biliary atresia. *Sci. Transl. Med.* 7, 286ra67. doi: 10.1126/scitranslmed.aaa1652
- Madadi-Sanjani, O., Schukfeh, N., Uecker, M., Eckmann, S., Dingemann, J., Ure, B. M., et al. (2021). The intestinal flora at kasai procedure in children with biliary atresia appears not to affect postoperative cholangitis. *Eur. J. Pediatr. Surg. Off J. Austrian Assoc. Pediatr. Surg. Al Z Kinderchir.* 31, 80–85. doi: 10.1055/s-0040-1715614
- Mazziotti, M. V., Willis, L. K., Heuckeroth, R. O., LaRegina, M. C., Swanson, P. E., Overbeek, P. A., et al. (1999). Anomalous development of the hepatobiliary system in the Inv mouse. *Hepatology* 30, 372–378. doi: 10.1002/(ISSN)1527-3350
- Meng, L., Liu, J., Wang, J., Du, M., Zhang, S., Huang, Y., et al. (2021). Characteristics of the gut microbiome and IL-13/TGF- β 1 mediated fibrosis in post-kasai cholangitis of biliary atresia. *Front. Pediatr.* 9, 751204. doi: 10.3389/fped.2021.751204
- Micó-Carnero, M., Rojano-Alfonso, C., Álvarez-Mercado, A. I., Gracia-Sancho, J., Casillas-Ramírez, A., and Peralta, C. (2020). Effects of gut metabolites and microbiota in healthy and marginal livers submitted to surgery. *Int. J. Mol. Sci.* 22, 44. doi: 10.3390/ijms22010044
- Miethe, A. G., Saxena, V., Shivakumar, P., Sabla, G. E., Simmons, J., and Choungnet, C. A. (2010). Post-natal paucity of regulatory T cells and control of NK cell activation in experimental biliary atresia. *J. Hepatol.* 52, 718–726. doi: 10.1016/j.jhep.2009.12.027
- Milosevic, I., Vujovic, A., Barac, A., Djelic, M., Korac, M., Radovanovic Spurnic, A., et al. (2019). Gut-liver axis, gut microbiota, and its modulation in the management of liver diseases: A review of the literature. *Int. J. Mol. Sci.* 20, 395. doi: 10.3390/ijms20020395
- Muraji, T., Hosaka, N., Irie, N., Yoshida, M., Imai, Y., Tanaka, K., et al. (2008). intestinal barrier. *Pediatrics* 121, 517–521. doi: 10.1542/peds.2007-0568
- Orłowska, E., Czubkowski, P., Wołochowska, K., Jarzębicka, D., Motyl, I., and Socha, P. (2021). Assessment of *Lactobacillus casei rhamnosus* (LGG) therapy in children with biliary atresia - Randomized placebo controlled trial. *Clin. Res. Hepatol. Gastroenterol.* 45, 101753. doi: 10.1016/j.clinre.2021.101753
- Qin, T., Fu, J., and Verkade, H. J. (2021). BCFAs. *Hum. Genet.* 140, 709–724. doi: 10.1007/s00439-020-02221-8
- Rager, S. L., and Zeng, M. Y. (2023). The gut-liver axis in pediatric liver health and disease. *Microorganisms* 11, 597. doi: 10.3390/microorganisms11030597
- Raouf, A. A., El-Fert, A. Y., El-Sebaay, H. M., Ismail, I. M., and El-Gendy, M. A. (2016). Metabolomic profile in biliary atresia compared with cholestasis in pediatric patients: a comparative study. *Menoufia Medical Journal* 29, 881. doi: 10.4103/1110-2098.202522
- Rauf, A., Khalil, A. A., Rahman, U. U., Khalid, A., Naz, S., Shariati, M. A., et al. (2022). Recent advances in the therapeutic application of short-chain fatty acids (SCFAs): An updated review. *Crit. Rev. Food Sci. Nutr.* 62, 6034–6054. doi: 10.1080/10408398.2021.1895064
- Saito, T., Terui, K., Mitsunaga, T., Nakata, M., Ono, S., Mise, N., et al. (2015). Evidence for viral infection as a causative factor of human biliary atresia. *J. Pediatr. Surg.* 50, 1398–1404. doi: 10.1016/j.jpedsurg.2015.04.006
- Sanchez-Valle, A., Kassira, N., Varela, V. C., Radu, S. C., Paidas, C., and Kirby, R. S. (2017). Biliary atresia: epidemiology, genetics, clinical update, and public health perspective. *Adv. Pediatr.* 64, 285–305. doi: 10.1016/j.yapd.2017.03.012
- Schneider, K. M., Candels, L. S., Hov, J. R., Myllys, M., Hassan, R., Schneider, C. V., et al. (2021). Gut microbiota depletion exacerbates cholestatic liver injury via loss of FXR signalling. *Nat. Metab.* 3, 1228–1241. doi: 10.1038/s42255-021-00452-1
- Schreiber, R. A., Harpavat, S., Hulscher, J. B. F., and Wildhaber, B. E. (2022). Biliary atresia in 2021: epidemiology, screening and public policy. *J. Clin. Med.* 11, 999. doi: 10.3390/jcm111040999
- Shen, W. J., Chen, G., Wang, M., and Zheng, S. (2019). Liver fibrosis in biliary atresia. *World J. Pediatr.* 15, 117–123. doi: 10.1007/s12519-018-0203-1
- Shen, C., Zheng, S., Wang, W., and Xiao, X. M. (2008). Relationship between prognosis of biliary atresia and infection of cytomegalovirus. *World J. Pediatr. WJP.* 4, 123–126. doi: 10.1007/s12519-008-0024-8
- Sokal, E. M., Baudoux, M. C., Collette, E., Hausleithner, V., Lambotte, L., and Buts, J. P. (1996). Branched chain amino acids improve body composition and nitrogen balance in a rat model of extra hepatic biliary atresia. *Pediatr. Res.* 40, 66–71. doi: 10.1203/00006450-199607000-00012
- Song, W., Sun, L. Y., and Zhu, Z. J. (2021a). Effects of previous kasai surgery on gut microbiota and bile acid in biliary atresia with end-stage liver disease. *Front. Med.* 8, 704328. doi: 10.3389/fmed.2021.704328
- Song, W., Sun, L. Y., Zhu, Z. J., Wei, L., Qu, W., and Zeng, Z. G. (2021c). Effect of liver transplantation on intestinal microbiota in children with biliary atresia. *Organ Transpl.* 12, 692–699. doi: 10.3389/fphys.2021.704313
- Song, W., Sun, L. Y., Zhu, Z. J., Wei, L., Qu, W., Zeng, Z. G., et al. (2021b). Association of gut microbiota and metabolites with disease progression in children with biliary atresia. *Front. Immunol.* 12, 698900. doi: 10.3389/fimmu.2021.698900
- Sucu, S., Basarir, K. E., Mihaylov, P., Balik, E., Lee, J. T. C., Fridell, J. A., et al. (2023). Impact of gut microbiota on liver transplantation. *Am. J. Transplant. Off J. Am. Soc. Transplant. Am. Soc. Transpl Surg.* 23, 1485–1495. doi: 10.1016/j.ajt.2023.05.030
- Sumida, W., Uchida, H., Tainaka, T., Shirota, C., Hinoki, A., Kato, T., et al. (2018). Oral administration of eicosapentaenoic acid suppresses liver fibrosis in postoperative patients with biliary atresia. *Pediatr. Surg. Int.* 34, 1059–1063. doi: 10.1007/s00383-018-4313-6
- Sun, X., Cai, Y., Dai, W., Jiang, W., and Tang, W. (2022). The difference of gut microbiome in different biliary diseases in infant before operation and the changes after operation. *BMC Pediatr.* 22, 502. doi: 10.1186/s12887-022-03570-1
- Tessier, M. E. M., Cavallo, L., Yeh, J., Harpavat, S., Hoffman, K. L., Petrosino, J. F., et al. (2020). The fecal microbiome in infants with biliary atresia associates with bile flow after kasai portoenterostomy. *J. Pediatr. Gastroenterol. Nutr.* 70, 789–795. doi: 10.1097/MPG.0000000000002686
- Tian, X., Wang, Y., Lu, Y., Wu, B., Chen, S., Du, J., et al. (2022). Metabolic regulation of cholestatic liver injury by D-2-hydroxyglutarate with the modulation of hepatic microenvironment and the mammalian target of rapamycin signaling. *Cell Death Dis.* 13, 1001. doi: 10.1038/s41419-022-05450-z
- Tilg, H., Adolph, T. E., and Trauner, M. (2022). Gut-liver axis: Pathophysiological concepts and clinical implications. *Cell Metab.* 34, 1700–1718. doi: 10.1016/j.cmet.2022.09.017
- Tran, K. T., Le, V. S., Dao, L. T. M., Nguyen, H. K., Mai, A. K., Nguyen, H. T., et al. (2021). Novel findings from family-based exome sequencing for children with biliary atresia. *Sci. Rep.* 11, 21815. doi: 10.1038/s41598-021-01148-y
- Valdes, A. M., Walter, J., Segal, E., and Spector, T. D. (2018). Role of the gut microbiota in nutrition and health. *BMJ* 361, k2179. doi: 10.1136/bmj.k2179
- van Wessel, D., Nomden, M., Bruggink, J., de Kleine, R., Kurilshikov, A., Verkade, H., et al. (2021). Gut microbiota composition of biliary atresia patients before kasai portoenterostomy associates with long-term outcome. *J. Pediatr. Gastroenterol. Nutr.* 73, 485–490. doi: 10.1097/MPG.0000000000000324
- Waisbourd-Zinman, O., Koh, H., Tsai, S., Lavrut, P. M., Dang, C., Zhao, X., et al. (2016). The toxin bilatresone causes mouse extrahepatic cholangiocyte damage and fibrosis through decreased glutathione and SOX17. *Hepatology* 64, 880–893. doi: 10.1002/hep.28599
- Waldner, B., Aldrian, D., Zöggeler, T., Oberacher, H., Oberhuber, R., Schneeberger, S., et al. (2023). The influence of liver transplantation on the interplay between gut microbiome and bile acid homeostasis in children with biliary atresia. *Hepatology* 77, e0151. doi: 10.1097/HCG.0000000000000151
- Wang, Y. W., Lin, C. I., Chen, H. W., Wu, J. C., and Chuang, Y. H. (2023). Apoptotic biliary epithelial cells and gut dysbiosis in the induction of murine primary biliary cholangitis. *J. Transl. Autoimmun.* 6, 100182. doi: 10.1016/j.jtauto.2022.100182
- Wang, Z., Liu, H., Li, L., Li, Y., Yan, H., and Yuan, Y. (2022). Modulation of disordered bile acid homeostasis and hepatic tight junctions using salidroside against hepatocyte apoptosis in furan-induced mice. *J. Agric. Food Chem.* 70, 10031–10043. doi: 10.1021/acs.jafc.2c04654
- Wang, J., Qian, T., Jiang, J., Yang, Y., Shen, Z., Huang, Y., et al. (2020a). Gut microbial profile in biliary atresia: a case-control study. *J. Gastroenterol. Hepatol.* 35, 334–342. doi: 10.1111/jgh.14777
- Wang, J., Xu, Y., Chen, Z., Liang, J., Lin, Z., Liang, H., et al. (2020b). Liver immune profiling reveals pathogenesis and therapeutics for biliary atresia. *Cell* 183, 1867–1883.e26. doi: 10.1016/j.cell.2020.10.048
- Wirth, U., Jiang, T., Schardey, J., Kratz, K., Li, M., Schirren, M., et al. (2023). The role of microbiota in liver transplantation and liver transplantation-related biliary complications. *Int. J. Mol. Sci.* 24, 4841. doi: 10.3390/ijms24054841

- Wu, J. F., Tseng, P. H., Chang, H. H., Chiang, C. M., Lin, W. H., Hsu, W. M., et al. (2024). The prevalence and impact of small intestine bacterial overgrowth in biliary atresia patients. *Pediatr. Res.* 95, 302–307. doi: 10.1038/s41390-023-02818-5
- Xu, X., Zhao, Y., Wang, X., Zhang, R., Liu, S., Sun, R., et al. (2023). Diagnostic and prognostic value of the gut microbiota and its metabolite butyrate in children with biliary atresia. *Pediatr. Surg. Int.* 40, 24. doi: 10.1007/s00383-023-05606-8
- Yan, M., Hou, L., Cai, Y., Wang, H., Ma, Y., Geng, Q., et al. (2022). Effects of intestinal FXR-related molecules on intestinal mucosal barriers in biliary tract obstruction. *Front. Pharmacol.* 13, 906452. doi: 10.3389/fphar.2022.906452
- Yang, Y., Wang, J., Zhan, Y., Chen, G., Shen, Z., Zheng, S., et al. (2020). The synthetic toxin biliatresone causes biliary atresia in mice. *Lab. Investig. J. Tech Methods Pathol.* 100, 1425–1435. doi: 10.1038/s41374-020-0467-7
- Yang, T., Yang, S., Zhao, J., Wang, P., Li, S., Jin, Y., et al. (2022). Comprehensive analysis of gut microbiota and fecal bile acid profiles in children with biliary atresia. *Front. Cell Infect. Microbiol.* 12, 914247. doi: 10.3389/fcimb.2022.914247
- Yao, S., Yagi, S., Hirata, M., Miyachi, Y., Ogawa, E., Uozumi, R., et al. (2023). Chronological changes in the gut microbiota and intestinal environment in recipients and donors of living donor liver transplantation. *J. Hepato-Biliary-Pancreat Sci.* 30, 439–452. doi: 10.1002/jhbp.1241
- Zarrinpar, A., and Busuttil, R. W. (2013). Liver transplantation: past, present and future. *Nat. Rev. Gastroenterol. Hepatol.* 10, 434–440. doi: 10.1038/nrgastro.2013.88
- Zhang, H., and Kang, Q. (2022). Analysis of immune microenvironment in different pathological stages of liver in biliary atresia. *Chin. J. Immunol.* 38, 656–660. doi: 10.3969/j.issn.1000-484X.2022.06.003
- Zhang, Y. L., Li, Z. J., Gou, H. Z., Song, X. J., and Zhang, L. (2022). The gut microbiota-bile acid axis: A potential therapeutic target for liver fibrosis. *Front. Cell Infect. Microbiol.* 12, 945368. doi: 10.3389/fcimb.2022.945368
- Zheng, L., Wu, Y., Gong, Z., Lv, Z., Xu, W., Sheng, Q., et al. (2022). The composition of the gut microbiota is altered in biliary atresia with cholangitis. *Front. Surg.* 9, 990101. doi: 10.3389/fsurg.2022.990101
- Zhou, K., Xie, G., Wang, J., Zhao, A., Liu, J., Su, M., et al. (2015). Metabonomics reveals metabolite changes in biliary atresia infants. *J. Proteome Res.* 14, 2569–2574. doi: 10.1021/acs.jproteome.5b00125



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Gut microbiome: a novel preventive and therapeutic target for prostatic disease

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The human gut microbiome (GM) impacts various physiological processes and can lead to pathological conditions and even carcinogenesis if homeostasis is disrupted. Recent studies have indicated a connection between the GM and prostatic disease. However, the underlying mechanisms are still unclear. This review aims to provide a summary of the existing information regarding the connection between the GM and various prostatic conditions such as chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), benign prostatic hyperplasia (BPH), and prostate cancer (PCa). Furthermore, the review aims to identify possible pathogenic mechanisms and suggest potential ways of targeting GM to prevent and treat prostatic disease. Due to the complexity of the mechanism between GM and prostatic diseases, additional research is required to comprehend the association between the two. This will lead to more effective treatment options for prostatic disease.

KEYWORDS

gut microbiome, prostate cancer, benign prostatic hyperplasia, prostatitis, therapy

1 Introduction

Prostatic disease, such as noncancerous prostate conditions (NPC), which encompass prostatitis and benign prostate hyperplasia (BPH), along with prostate cancer (PCa), are common male urinary system issues globally. These conditions pose a significant economic burden to society (Fang et al., 2021). According to the NIH classification, prostatitis is classified into types I-IV (Krieger et al., 1999). The following analysis centers on Type III prostatitis, alternatively referred to as chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), making up the majority of cases at 90-95% and having the potential to impact males across all age groups. Signs of this condition may consist of discomfort in the lower abdomen, frequent urination, a sudden need to urinate, nighttime urination, problems with sexual function, and different mental health issues (Krieger et al., 2008; Schaeffer, 2008). As

individuals age, the frequency of BPH increases, leading to worsening urinary symptoms like trouble starting urination, weak urine flow, urgent need to urinate, and more frequent urination. In severe cases, these symptoms may lead to urinary retention and secondary chronic renal dysfunction (Kahokehr and Gilling, 2014; Kim et al., 2016). PCa is the second most prevalent cancer in men globally and the most frequent in the male reproductive system. Individuals with PCa may show signs of urethral pressure, such as difficulties with urination, sudden inability to urinate, blood in the urine, and loss of bladder control. As the disease progresses, the tumor may invade the bones, resulting in bone pain, pathological fracture, anemia, spinal cord compression, and other poor prognoses (Nguyen-Nielsen and Borre, 2016; Teo et al., 2019; Sandhu et al., 2021). Despite the high frequency of prostatic disease and their significant effect on men's well-being, the underlying causes are still not well understood (Schaeffer, 2008; Kahokehr and Gilling, 2014; Sandhu et al., 2021). This lack of understanding has resulted in inadequate prevention and prognosis of prostatic disease. Therefore, searching for new preventive therapeutic targets is necessary to improve the situation.

Various microorganisms, such as bacteria, archaea, fungi, viruses, and protozoa, are present in the intricate ecosystem of the human gut. These microorganisms are collectively referred to as gut microbiota. The gut microbiome (GM) refers to the surroundings, including the microbiota, any proteins or metabolites produced by them, their metagenome, and host proteins and metabolites present in this setting (Whiteside et al., 2015; Porter et al., 2018). The GM is essential in overseeing different physiological functions, including intestinal homeostasis, intestinal mucosal barrier function, inflammation, immune regulation, metabolic balance, and endocrine equilibrium in the body (Kuziel and Rakoff-Nahoum, 2022). Research has shown that an imbalance in the microbial species composition, known as dysbiosis, plays a role in the development of several diseases, such as Parkinson's disease, Alzheimer's disease, hypertension, atherosclerosis, obesity, diabetes mellitus, gestational diabetes mellitus, non-alcoholic fatty liver disease, inflammatory bowel disease, and colon cancer (Illiano et al., 2020). Additionally, the microbiota composition can affect the effectiveness and tolerability of disease treatments and medications (Badgeley et al., 2021). Furthermore, recent studies suggest that the microbiome, particularly that of the gut, may play a role in the development and progression of disease within the urinary tract, including prostatic disease, and as such, the 'gut-prostate axis' should be considered when treating patients (Jones-Freeman et al., 2021). To the end, we summarized research on the association between GM and prostatic disease, including possible pathogenic mechanisms and potential ways to target GM for prevention and treatment.

2 Observational and experimental evidence indicating a strong link between GM and prostatic disease

Recent developments in biological techniques, including high-throughput sequencing like 16S rRNA sequencing, transcriptomics,

and metabolomics, offer a chance to examine the GM pattern and its relationship with the advancement of prostatic disease (Jones-Freeman et al., 2021). Recent observational studies show that the GM and metabolites of men with prostate disease are significantly different from those of healthy men. This indicates that specific intestinal microorganisms could play a role in the development, advancement, and modified treatment outcomes of prostate conditions. Additionally, several animal studies have reported similar findings. Table 1 shows clinical studies and animal experiments related to prostate disease, showing notable variations in gut microbiota and metabolites between the experimental and control groups.

2.1 The GM and CP/CPPS

A study comparing 25 individuals with CP/CPPS to 25 controls found that those with CP/CPPS had a lower diversity of gut microbiota, which formed distinct clusters compared to controls. Additionally, they had significantly fewer *Prevotella* bacteria, suggesting a possible biomarker for the condition (Shoskes et al., 2016). A different research study in China included 41 patients with CP/CPPS and 43 healthy controls, indicating notable variations in gut microbiota composition among the groups. The research developed an innovative diagnostic approach for CP/CPPS using microbiomes, showing potential for future treatment options and non-invasive diagnostic markers for patients with CP/CPPS (Wang et al., 2023). In addition, a comprehensive analysis examined CNP's effects on the gut microbiota, gene expression, and DNA methylation in rats. The results showed strong associations between changes in gene expression, DNA methylation, and gut microbiota with various biological processes such as intestinal immunity, metabolism, and epithelial barrier function (Liu et al., 2021b). Observative research has noticed significant gut microbiota differences in CP/CPPS patients; however, there is no specific microbiota associated with CP/CPPS in different studies. Moreover, a large cohort is needed to prove this association.

2.2 The GM and BPH

A study involving 66 individuals with prostate enlargement (PE) and 62 controls found that the PE group had a higher percentage of *Bacillota* (Firmicutes) and Actinomycetota (*Actinobacteria*) and a lower percentage of *Bacteroidota* (*Bacteroidetes*). In the PE group, the ratio of *Bacillota* to *Bacteroidota* was notably more excellent compared to the non-PE group (Takezawa et al., 2021). In addition, there is also a significant difference between BPH and controls in animal models. Certain gut microbiota levels have been noted to vary in rat models with BPH, including *Lactiplantibacillus*, *Flavonifractor*, *Acetatifactor*, *Oscillibacter*, *Pseudoflavonifractor*, *Intestinimonas*, *Butyricimonas*, *Muribaculaceae*, *Turicibacteraceae*, *Turicibacter*, and *Coprococcus*. *Lactiplantibacillus* and *Acetatifactor*, two types of microbiotas, were linked to supporting and preventing prostate cell apoptosis,

TABLE 1 Observational and experimental studies reveal the association between GM and prostatic disease.

Prostatic disease	First author/ Year	Country	Subject	Sample size	Test method	Main outcomes	Reference
CP/CPPS	Daniel A Shoskes, 2016	America	human	50	16S rRNA gene sequencing	(1) Patients with CP/CPPS having decreased alpha diversity (p=0.001); (2) 3 taxa were overrepresented in cases and 12 were underrepresented, eg <i>Prevotella</i> .	(Shoskes et al., 2016)
	Shuai Wang, 2023	China	human	84	16S rRNA gene sequencing	(1) Alpha-diversity makes no difference, but Beta-diversity does; (2) three bacterial species were highly representative and seven bacterial species were low.	(Wang et al., 2023)
	Junsheng Liu, 2021	China	rat	12	16S rDNA sequencing, transcriptomic analysis, methyl C-capture sequencing,	(1) an altered structure of the GM; (2) 185 genes of intestinal epithelium associated with immune system, digestive system, metabolic process, changed; (3) 73,232 differentially methylated sites in the DNA of intestinal epithelium.	(Liu et al., 2021b)
BPH	Kentaro Takezawa, 2021	Japan	human	128	16S rRNA metagenomic analyses	(1) a higher proportion of <i>Bacillota</i> (<i>Firmicutes</i>) and <i>Actinomycetota</i> (<i>Actinobacteria</i>) and a lower proportion of <i>Bacteroidota</i> (<i>Bacteroidetes</i>); (2) the <i>Firmicutes/Bacteroidetes</i> (F/B) ratio was significantly higher.	(Takezawa et al., 2021)
	Jinho An, 2023	Korea	rat	7	next-generation sequencing (NGS)	(1) an altered abundance of <i>Lactiplantibacillus</i> , <i>Flavonifractor</i> , <i>Acetatifactor</i> , <i>Oscillibacter</i> , <i>Pseudoflavonifractor</i> , <i>Intestinimonas</i> , and <i>Butyricimonas</i> genera; (2) finasteride treatment altered the abundance of <i>Barnesiella</i> , <i>Acetatifactor</i> , <i>Butyricimonas</i> , <i>Desulfovibrio</i> , <i>Anaerobacterium</i> , and <i>Robinsoniella</i> genera.	(An et al., 2023)
	Lu-Yao Li, 2022	China	rat	10	16S rDNA sequencing and liquid chromatography tandem mass spectrometry (LC-MS/MS)	(1) beta-diversity increased; (2) <i>Muribaculaceae</i> , <i>Turicibacteraceae</i> , <i>Turicibacter</i> and <i>Coprococcus</i> were decreased; (3) <i>Mollicutes</i> and <i>Prevotella</i> were increased; (4) the differential metabolites were enriched in steroid hormone biosynthesis, ovarian steroidogenesis, biosynthesis of unsaturated fatty acids and bile secretion; (5) <i>Prevotellaceae</i> , <i>Corynebacteriaceae</i> , <i>Turicibacteraceae</i> , <i>Bifidobacteriaceae</i> took part in metabolism.	(Li LY. et al., 2022)
Pca	Shaheen Alanee, 2019	USA	human	30	16 S rRNA gene sequencing	no significant GM difference found in two groups.	(Alanee et al., 2019)
	Weibo Zhong, 2022	China	Mouse and human	NA and 35	16 S rRNA gene sequencing	(1) the abundance of <i>Pseudomonadota</i> (<i>Proteobacteria</i>) was higher after antibiotic exposure; (2) intratumoral lipopolysaccharide (LPS) increased under the elevation of gut permeability, and the NF-κB-IL6-STAT3 axis was activated; (3) <i>Pseudomonadota</i> was enriched in patients with mPca and was positively linked to plasma IL6 level, regional lymph node metastasis status, and distant metastasis status.	(Zhong et al., 2022)
	K S Smith, 2021	USA	human	16	16 S rRNA gene sequencing	(1) no differences in alpha diversity; (2) Beta-diversity metrics were different.	(Smith et al., 2021)
		USA	human	30	16 S rDNA gene sequencing		

(Continued)

TABLE 1 Continued

Prostatic disease	First author/ Year	Country	Subject	Sample size	Test method	Main outcomes	Reference
	Karen S Sfanos, 2018					(1) a significant difference in alpha diversity; (2) compositional differences of men taking ATT, such as <i>Akkermansia muciniphila</i> and <i>Ruminococcaceae spp</i> ; (3) an enriched gene pathways involved in steroid biosynthesis and steroid hormone biosynthesis of men taking oral ATT.	(Sfanos et al., 2018)
	David M Golombos, 2018	USA	human	20	next-generation sequencing (NGS)	(1) higher abundance of <i>Bacteriodes massiliensis</i> ; (2) <i>Faecalibacterium prausnitzii</i> and <i>Eubacterium rectalie</i> had lower abundance; (3) relative gene, pathway, and enzyme abundance were different.	(Golombos et al., 2018)
	Joseph K M Li, 2021	China	human	86	16 S rRNA gene sequencing	(1) alpha/beta-diversity were significantly different after ADT; (2) <i>Ruminococcus gnavus</i> and <i>Bacterioides spp</i> had higher abundance while <i>Lachnospira</i> and <i>Roseburia</i> were reduced after ADT; (3) the F/B was lower after ADT; (4) biosynthesis of lipopolysaccharide (endotoxin) and propanoate and the energy cycle pathways were enriched after ADT.	(Li et al., 2021)
	Pin-Yu Huang, 2021	China	mouse	6	16 S rRNA gene sequencing	(1) <i>Akkermansiaceae</i> , <i>Bifidobacteriaceae</i> and <i>Enterococcaceae</i> were separately higher at different time in the progress of PCa; (2) bacterial associated with steroid biosynthesis and butirosin and neomycin biosynthesis were increased.	(Huang et al., 2021)
	Yufei Liu, 2020	China	human	21	16 S rRNA gene sequencing	(1) increased abundance of genus <i>Phascolarctobacterium</i> and <i>Ruminococcus</i> in CRPC; (2) bacterial gene pathways involved in terpenoids/polyketides metabolism and ether lipid metabolism were activated in CRPC.	(Liu and Jiang, 2020)
	Makoto Matsushita, 2021	Japan	human	152	16 S rRNA gene sequencing	(1) the relative abundances of <i>Rikenellaceae</i> , <i>Alistipes</i> , and <i>Lachnospira</i> , all SCFA-producing bacteria, were significantly increased in high-risk PCa;	(Matsushita et al., 2021)
	Michael A Liss, 2018	USA	human	105	16 S rRNA gene sequencing	(1) <i>Bacterioides</i> and <i>Streptococcus</i> species were higher in PCa; (2) Folate and argininewere the most significantly altered pathways.	(Liss et al., 2018)

GM, Gut microbiome; CP/CPPS, chronic prostatitis/chronic pelvic pain syndrome; BPH, benign prostatic hyperplasia; PCa, Prostate cancer; ATT, Androgen receptor axis-targeted therapies; ADT, Androgen deprivation therapy; SCFA, Short chain fatty acid.

respectively. These effects were reversed by finasteride, a medication often prescribed for BPH. Additionally, analysis of intestinal contents using LC-MS/MS showed that various metabolites linked to the production of steroid hormones, ovarian steroid synthesis, creation of unsaturated fatty acids, and release of bile were primarily related to cellular functions, processing environmental information, metabolism, and organismal systems, potentially linked to *Prevotellaceae*, *Corynebacteriaceae*, *Turicibacteraceae*, and *Bifidobacteriaceae* (Li LY. et al., 2022; An et al., 2023). These changes suggest their potential utility in diagnosing, preventing, and treating BPH.

2.3 The GM, androgen deprivation therapy, and PCa

Research examined the gut microbiota makeup and abundance, pinpointed specific metabolites and metabolic routes linked to PCa, and subsequently created a microbiome risk assessment for the disease. Research by K S Smith found that beta-diversity metrics significantly differed in PCa cases (Smith et al., 2021). A pilot study comparing men with BPH or clinically localized PCa at intermediate or high risk found that those with PCa had a more significant presence of *Phocaeicola massiliensis* (*Bacteroides massiliensis*) (Matsushita et al., 2021). Moreover, some specific microbiotas are abnormal in patients with PCa, such as *Bacteroides*, *Streptococcus* species (Liss et al., 2018), *Akkermansiaceae*, *Bifidobacteriaceae*, and *Enterococcaceae* (Huang et al., 2021). In addition, Weibo Zhong et al. discovered an abundance of *Pseudomonadota* (*Proteobacteria*) in individuals with metastatic prostate cancer (mPCa), which showed a positive association with plasma interleukin-6 (IL-6) levels, regional lymph node metastasis, and distant metastasis status (Zhong et al., 2022). Furthermore, it was found that the levels of *Rikenellaceae*, *Alistipes*, and *Lachnospira*, which are all bacteria that produce short-chain fatty acids (SCFAs), were notably higher in the high-risk group (Golombos et al., 2018). Apart from the microbiota difference, a discrepancy in metabolites derived from GM, such as folate and arginine, has also been found (Liss et al., 2018). Nonetheless, a future investigation discovered that examination of the bacterial classifications in the stool samples did not show any grouping corresponding to benign or malignant prostate biopsies (Alane et al., 2019). Overall, while most studies indicate a notable distinction between PCa and men in good health, additional data is required to support these results.

Androgen deprivation therapy (ADT), using medications such as bicalutamide, enzalutamide, and abiraterone acetate, or surgical castration, is the mainstay of conventional care for locally advanced PCa or mPCa. Following a period of ADT, all individuals with PCa will eventually develop castration-resistant prostate cancer (CRPC), a fatal phase of the disease (Desai et al., 2021). Recent studies have suggested that ADT may interact with the GM and change the composition and abundance of specific microbiota, resulting in a shorter period in the process of CRPC. Following ADT, there was a notable change in the alpha/beta-diversity, with variations observed

in *Mediterraneibacter gnavus* (*Ruminococcus gnavus*), *Bacteroides*, *Lachnospira*, *Roseburia* (Li et al., 2021), *Akkermansia muciniphila* and *Oscillospira* (*Ruminococcaceae*) (Sfanos et al., 2018), *Phascolarctobacterium* and *Ruminococcus* (Liu and Jiang, 2020). Bacterial gene pathways in fecal microbiota play a role in lipopolysaccharide (endotoxin), propanoate, terpenoids/polyketides metabolism, lipid metabolism, and steroid hormone biosynthesis, as shown in functional analyses (Sfanos et al., 2018; Liu and Jiang, 2020; Li et al., 2021).

Apart from epidemiological evidence, results from Mendelian randomization studies also suggested a causal link between gut microbiota and prostatic disease, which is an approach that employs single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to assess causal relationships, similar to randomized controlled trials (Xia et al., 2023; Xie and Hu, 2023; Shen et al., 2024).

3 The GM influences prostatic disease through multiple different potential mechanisms

Although current observational studies have pointed to changes in the GM of patients with prostatic disease, and these characteristic differences have the potential to be targets for prevention and treatment, the exact mechanisms of their influence still need to be comprehensively clear. This part outlines the possible underlying principles associated with GM and how it affects the development of prostatic disease. This impact could be due to direct infections in the prostate caused by a known microbial cause, as well as indirect effects like immune system regulation, changes in metabolism, increased androgen activity, and effects on ADT. In many cases, frequent interactions with the GM, both direct and indirect, are in play (Figure 1).

3.1 Distant dissemination of gut pathogens

Experts and medical professionals acknowledge the harmful presence of bacteria in urinary tract infections (UTIs), which could play a role in causing prostate inflammation and prostatic disease development (Wagenlehner et al., 2014). Intestinal bacteria can enter the prostate through the urinary tract or travel through the bloodstream, resulting in infections that contribute to inflammatory conditions like bacterial prostatitis and chronic bacterial prostatitis, ultimately increasing the risk of BPH and PCa (Cai et al., 2019; Jin et al., 2024). Historically, urine bacteriological culture has been utilized for the purpose of isolating and identifying pathogens responsible for UTIs, including aerobic, rapidly multiplying organisms like *Escherichia coli* (80%) and *Enterococcus faecalis*, which primarily come from the intestinal tract (Ronald, 2003). Furthermore, various research has found and recognized bacteria in prostate tissues (Miyake et al., 2022). All of this evidence suggests that gut bacteria may cause pathological states of the prostate by directly infecting and interacting with the prostate.

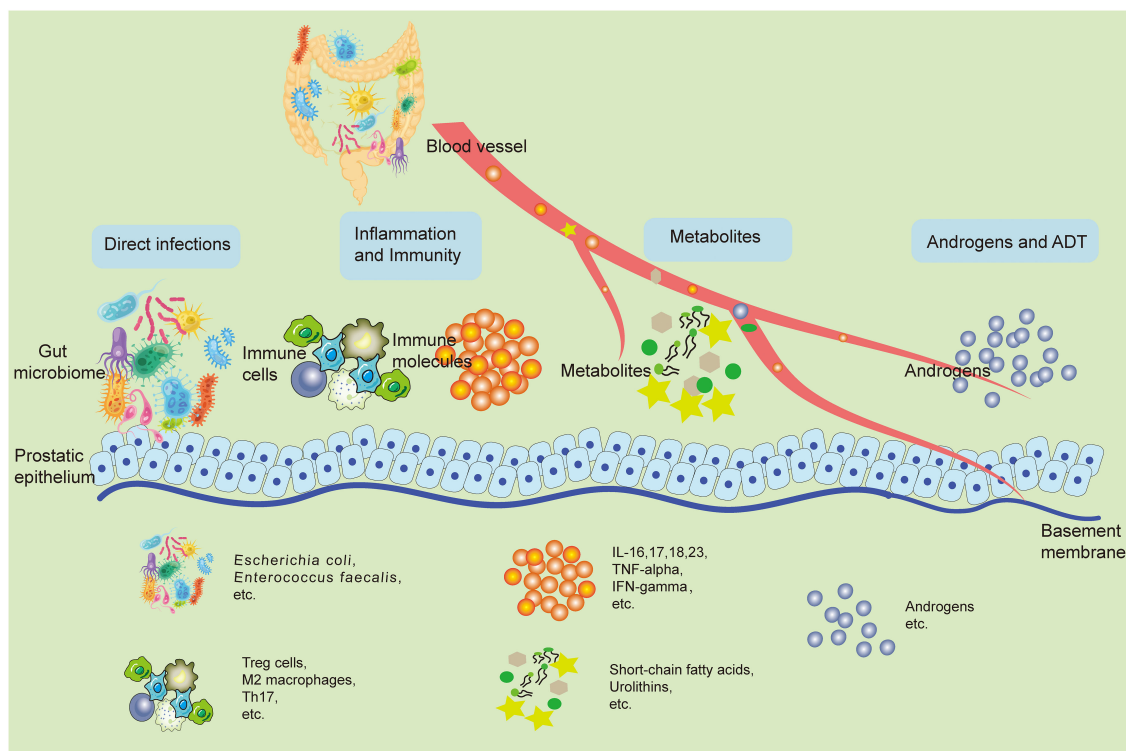


FIGURE 1

The potential mechanisms involved with the GM and its impact on prostatic disease pathogenesis. The GM can shadow prostate health in both direct and indirect ways. Bacteria from the gut can retroactively infect the prostate through the urethra and directly cause pathological states of the prostate. Changes in the local immune inflammatory state of the intestine can spread inflammatory factors and other immune molecules to the prostate through the bloodstream, causing changes in the local immune inflammatory environment of the prostate, leading to prostate disease. Metabolites from the gut microbiota like SCFAs, urolithins can also travel through the blood stream to the prostate, play a variety of bioactive functions, and directly lead to the activation of a variety of pathologic mechanisms of prostate cells. In addition, some gut microbiotas can change the synthesis and breakdown of androgens in the body, which directly affects the development of prostatic hyperplastic diseases, especially PCa. Accordingly, ADT treatment is also regulated by the GM. GM, Gut microbiome; SCFA, Short-chain fatty acids; PCa, Prostate cancer; ADT, Androgen deprivation therapy.

3.2 Inflammation and immune dysregulation

The digestive system is not just important for breaking down food and taking in nutrients, it is also the primary immune system in the body, containing 60–80% of the body's immune cells and structures that help regulate the immune system when faced with bacteria (Zhou et al., 2021). Dysbiosis can lead to the production of inflammatory cytokines like IL-17, IL-23, TNF- α , and IFN- γ by the gut microbiota, which can then travel through the bloodstream to other parts of the body, such as the prostate, causing systemic inflammation. This inflammation can indirectly alter the local environment of the prostate, affecting immune molecules and cells, and potentially contributing to the development or progression of diseases (Porter et al., 2018; Russo et al., 2023).

In addition, SCFAs produced through the breakdown of carbohydrates by gut bacteria are vital in controlling the body's functions and are seen as a key group of compounds responsible for this impact. Acetate, propionate, and butyrate play important roles in a variety of biological functions (Duan et al., 2023). New studies have uncovered the important roles they play in immune and inflammatory reactions. Butyrate inhibits the formation of

interferon- γ (IFN- γ) producing cells and enhances the formation of regulatory T (Treg) cells, as an example. Propionate hinders the start of a Th2 immune reaction by dendritic cells. SCFAs notably inhibit the polarization of M2 macrophages, highlighting their immunomodulatory characteristics and therapeutic potential. Furthermore, an imbalance in gut bacteria resulting in changes in SCFA production has been linked to the advancement of prostate diseases. SCFAs induce autophagy in cancer cells and stimulate M2 polarization in macrophages, hastening tumor progression. Moreover, SCFAs boost the activation of hypoxia-inducible factor 1 (HIF-1) through inhibition of histone deacetylase, leading to elevated synthesis of antimicrobial agents and enhanced macrophage-driven eradication of pathogens. This emphasizes the ability of SCFAs to fight against microbes and their importance in protecting the host. SCFAs are linked to the production of IL-6 and IL-18 in the prostate, and the balance of gut bacteria can influence the inflammatory environment in the prostate gland (Ratajczak et al., 2023). In a laboratory setting, SCFAs increased the movement and penetration of PCa cells by stimulating autophagy through TLR3 activation, subsequently triggering NF- κ B and MAPK pathways. Concurrently, the autophagy process in PCa cells led to an increased secretion of

chemokine CCL20, which could alter the tumor microenvironment by attracting additional macrophage infiltration and converting them into M2 type, ultimately enhancing the invasiveness of PCa cells. Additionally, among 362 patients with PCa, there was a positive association between the expression of CCL20 in prostate tissue and Gleason score, pre-surgery PSA levels, and invasion of neural/seminal vesicles. There was an inverse relationship with post-surgery biochemical recurrence-free survival (Liu et al., 2023). Moreover, a group created a mouse model of experimental autoimmune prostatitis (EAP) through subcutaneous immunization and found an imbalance in the frequency of Th17/Treg cells. Levels of propionic acid were lower in EAP mice than in control mice. Supplementing with propionic acid decreased susceptibility to EAP and restored the balance of Th17/Treg cell differentiation both *in vivo* and *in vitro*. Additionally, the impact of propionic acid on Th17 and Treg cells was assessed, including the regulation of SCFA receptor G-protein-coupled receptor (GPCR) 43 and intracellular histone deacetylase 6 (Du et al., 2022).

Matsushita et al. utilized *Pten*-knockout mice with prostate-specific characteristics to serve as a model for PCa and explored the cause of inflammatory cancer growth induced by a high-fat diet (HFD) and the role of the gut microbiome. In HFD mice with large prostate tumors, the levels of histamine and the expression of *Hdc* gene, which is responsible for histamine production, were increased, leading to an increase in mast cells surrounding the tumor foci. Fexofenadine administration, an H1 receptor antagonist, inhibited tumor growth in mice fed a HFD by decreasing myeloid-derived suppressor cells and inhibiting IL6/STAT3 signaling. Consuming a HFD led to an imbalance in gut bacteria, causing an increase in levels of lipopolysaccharide (LPS) in the bloodstream. PCa showed increased *Hdc* expression following intraperitoneal injection of LPS. Blocking the activation of LPS/Toll-like receptor 4 pathway reduced the growth of tumors induced by high-fat diet. In total prostatectomy specimens of severely obese patients, there was a rise in the quantity of mast cells surrounding the cancer foci (Matsushita et al., 2022b).

Immune elimination and immune escape are hallmarks of cancer; both can be partly bacteria-dependent in shaping immunity by mediating host immunomodulation. In addition, host immunity regulates the microbiome by altering bacteria-associated signaling to influence tumor surveillance. Cancer immunotherapy, including immune checkpoint blockade (ICB), appears to have heterogeneous therapeutic effects in different individuals, partially attributed to the microbiota. Thus, the microbiome signature can predict clinical outcomes, prognosis, and immunotherapy responses (Zhou et al., 2021).

3.3 Metabolites derived from the GM

Host metabolism is controlled by the gut microbiota. In recent years, it has been indicated that metabolites derived from GM play a vitally crucial role in gut homeostasis and could be transported to other body sites, including the prostate, exerting various functions. In addition to the high immune activity as mentioned in 3.1, SCFAs has many other biological activities involved in regulating prostate

health. In a research study with 183 elderly males (103 with BPH and 80 without), researchers examined the types and amounts of SCFA in stool samples. The study revealed that patients with BPH had notably elevated levels of branched SCFAs, such as isobutyric acid and isovaleric acid (Ratajczak et al., 2021). In a research study, it was discovered that the amount of G protein-coupled estrogen receptor (GPER) expression, known for its ability to prevent prostate hyperplasia, was notably reduced in cases of prostate enlargement caused by ulcerative colitis (UC). Sodium butyrate could be up-regulated in the prostate when treated with sodium butyrate and increase the expression of GPER, which shows that SCFA could be a target for BPH (Dong et al., 2022). Furthermore, there was a decrease in propionic acid levels in the EAP mouse model when compared to controls. The addition of propionic acid supplementation decreased susceptibility to EAP, indicating that propionic acid could potentially serve as a protective factor for prostate health (Du et al., 2022). In addition, SCFAs may serve as intermediaries connecting dysbiosis of the microbiota in CRPC and the advancement of PCa. By using a transgenic TRAMP mouse model, *in vitro* PCa cell transwell, and macrophage recruitment assays, Yufei Liu et al. discovered that transferring fecal microbiota from patients with CRPC to TRAMP mice increased levels of SCFAs-producing gut bacteria like *Ruminococcus*, *Alistipes*, and *Phascolarctobacterium*, resulting in higher levels of gut SCFAs (acetate and butyrate). Supplementation with CRPC FMT or SCFA notably hastened the progression of PCa in mice. In a laboratory setting, SCFAs increased the movement and penetration of PCa cells by stimulating autophagy through TLR3, subsequently activating NF- κ B and MAPK pathways (Liu et al., 2023). Makoto Matsushita's study revealed that there is a higher presence of SCFA-producing bacteria like *Alistipes* in the gut microbiome of individuals with advanced PCa, leading to elevated levels of insulin-like growth factor 1 (IGF-1) in the prostate. IGF-1 has the ability to speed up the growth of PCa by activating phosphoinositide 3-kinase and mitogen-activated protein kinase, as well as enhancing sensitivity to sex hormones (Matsushita et al., 2022a). One review also noted the importance of SCFAs in maintaining cell balance by influencing histone deacetylases (HDACs), which impacts cell adhesion, immune cell recruitment, cytokine release, chemotaxis, and apoptosis (Mirzaei et al., 2021). Hence, changing the composition of gut bacteria to affect SCFA levels may be a viable option for preventing or treating prostatic disease.

Urolithins (Uros), which are derived from the metabolism of polyphenols, ellagitannins, and ellagic acid by gut microbiota, are able to be absorbed by the body. Iwona et al. found that Uros inhibited proliferation and induced apoptosis of LNCaP PCa cells (Stanisławska et al., 2019; García-Villalba et al., 2022). Combining bicalutamide with UroA and UroB resulted in an increased anti-proliferative impact. UroA and UroC reduced the secretion of prostate-specific antigen (PSA) induced by DHT (Stanisławska et al., 2018). Pioneering studies indicate that urolithins may play a role in the health benefits associated with consuming foods high in ellagitannins, such as pomegranates, walnuts, and strawberries. Uros and their associated metabolites that have been conjugated (glucuronides, sulfates, etc.) Various Uros can be found in a variety

of human fluids and tissues, including the prostate (García-Villalba et al., 2022). This result indicated their potential use in complementary therapy of prostatic disease.

3.4 Androgens and ADT

Androgens are essential for the development and maintenance of healthy prostate cells, as well as hormone-responsive prostate cancer. ADT is a standard treatment for proliferative prostatic disease, including BPH and particularly PCa (Cui and Zhang, 2013). Nevertheless, through ADT, mPCa will ultimately become independent of androgens, leading to lethal CRPC, suggesting the existence of another origin of androgen (Zhang et al., 2022). Additionally, the GM can synthesize or decompose androgens, thereby affecting at least part of the systemic levels of the androgen, which could impact the effectiveness of ADT and lead to the emergence of CRPC. A study conducted in Japan included 54 male participants and identified certain bacteria from the *Bacillota* (*Clostridiales* etc.) were found to be increased in the PCa patients with serum high-testosterone expression, suggesting the GM may affect testosterone metabolism in older men (Matsushita et al., 2022c). Pernigoni et al. discovered that ADT in mice and humans supports the growth of specific beneficial bacteria, leading to the development of resistance to castration in mice. The GM in mice and CRPC patients was found to have an abundance of species that can transform androgen precursors into active androgens. Antibiotic treatment delayed the development of castration resistance in immunodeficient mice by eliminating the gut microbiota. Treatment from CRPC mice and patients made mice with PCa resistant to castration. Conversely, the progression of tumors was managed through the use of fecal microbiota transplantation (FMT) from patients with hormone-sensitive prostate cancer (HSPCa) and the administration of *Leyella stercorea* (*Prevotella stercorea*) (Fenner, 2021; Gut microbiota drive androgen resistance in prostate cancer, 2021; Pernigoni et al., 2021). *Clostridium scindens*, part of the intestinal microbiome, has the ability to transform cortisol into 11 β -hydroxy androstenedione (11 β -OHA), a strong precursor of androgens. Studies indicated that cortisol byproducts from *Clostridium scindens*-conditioned medium stimulated growth and increased movement of androgen-responsive PCa cells (LNCaP). Additionally, cells exposed to these compounds showed activated androgen receptor (AR) and induced AR-controlled genes (Bui et al., 2023). Lindsey K Ly et al. also found that certain bacteria, such as *Clostridium scindens*, could convert androgen precursors from the adrenal gland into potent androgens (Ly et al., 2020; Bui et al., 2023). In summary, these findings emphasize the importance of the GM in promoting CRPC tumor growth through stimulating androgen production and reveal a potential fecal bacterial pattern in patients that could serve as a useful indicator for identifying PCa patients who could potentially benefit from microbiome-focused treatment.

Thauera sp. strain GDN1 is an atypical beta proteobacterium, with the ability to break down androgen in both aerobic and anaerobic environments. Hsiao et al. gave C57BL/6 mice strain

GDN1 orally, leading to a decrease of about 50% in serum androgen levels. The researchers hypothesized that it inhibited the reuse of androgens via enterohepatic circulation (Hsiao et al., 2023). Additionally, gut microbes that break down medications prescribed for androgen deprivation therapy may impact the effectiveness of the treatment (Terrisse et al., 2022b). The data suggest that androgen-degrading intestinal bacteria could be effective probiotics for treating hyperandrogenism in alternative therapy.

Conversely, oral drugs used for ADT like bicalutamide, enzalutamide, and abiraterone acetate may be associated with alterations in the gut microbiota, potentially leading to an increase in species known to influence response to anti-PD-1 immunotherapy, such as *Akkermansia muciniphila* and *Oscillospiraceae* spp. Additionally, a more detailed depiction of bacterial genetic pathways related to the production of steroids and steroid hormones was identified within the fecal microbiome (Sfanos et al., 2018). In research involving 23 individuals with PCa, it was found that the diversity of gut bacteria decreased notably after 24 weeks of ADT, with significant alterations in the levels of *Pseudomonadota*, *Gammaproteobacteria*, *Pseudomonadales*, *Pseudomonadota* (*Pseudomonas*), and concentrations (Kure et al., 2023). ADT can reduce the population of *Corynebacterium* spp. that utilize androgens. In patients with PCa, oral Abiraterone acetate enhances the presence of the beneficial bacteria *Akkermansia muciniphila*. Additional research shows that Abiraterone acetate is broken down by bacteria in a laboratory setting, with specific components affecting growth in a way that could influence how patients with castrate-resistant conditions respond to treatment (Daisley et al., 2020). ADT administration in mice with PCa led to an increase in the number of cells in the thymus and the production of cells. Furthermore, the effectiveness of ADT was diminished by the depletion of the GM caused by oral antibiotics. PCa reduced the relative abundance of *Akkermansia muciniphila* in the gut, and ADT reversed this effect. Furthermore, housing PCa-bearing mice with tumor-free mice or administering *Akkermansia* orally enhanced the effectiveness of ADT. This is relevant for individuals with PCa as prolonged ADT leads to higher thymic production, as evidenced by elevated levels of recent thymic emigrant cells in the bloodstream. Healthy volunteer feces successfully restored ADT efficacy, but feces from PCa patients did not have the same effect. These findings suggest the potential clinical utility of reversing intestinal dysbiosis and repairing acquired immune defects in PCa patients (Terrisse et al., 2022a).

4 Potential therapies targeting the GM for prostatic disease

Dysbiosis of the gut microbiota is a result of the imbalance between beneficial and harmful microorganisms. Several factors, including diet, traditional Chinese medicine, antibiotics, probiotics and prebiotics, fecal transplantation, and certain natural compounds, contribute to prostatic disease development and progression by improving this imbalance in the GM, which should be considerable when preventing and treating prostatic disease (Pernigoni et al., 2023) (Figure 2).

4.1 Diet

Dietary components directly and profoundly impact the GM and diet-derived metabolites. Rational dietary patterns and applying nutritional supplements can help regulate intestinal microbiota homeostasis, thereby preventing and treating prostatic disease. However, the specific diet or supplements that are effective still need to be determined. Recent research has shown that consuming a diet high in fat can increase the risk of developing prostate disease, with the gut microbiota potentially playing a role as a contributing factor (Labbé et al., 2019; Zmora et al., 2019). High-fat diet (HFD) is known to result in an imbalance of gut bacteria and their metabolites, which can contribute to a leaky gut. This allows various metabolites and bacterial components, like SCFAs and phospholipids, to enter the bloodstream, leading to conditions such as endotoxemia. This phenomenon can thus orchestrate the inflammatory response, promoting the initiation and progress of prostatic disease (Zmora et al., 2019).

A rat model of CP/CPPS was created by administering subcutaneous testosterone and 17 β -oestradiol (E (2)) hormone pellets, followed by providing either regular tap water or tap water enriched with 2% galactoglucomannan-rich hemicellulose

extract (GGM group) from Norway spruce. Rats with hormone-induced CP/CPPS exhibit distinct alterations in gut microbiota, leading to subsequent changes in short-chain fatty acids and lipopolysaccharides (Konkol et al., 2019). Rats with HFD-induced BPH exhibited morphological abnormalities in their prostate tissues. Metagenomic analysis of the gut revealed that *Bacillota*, *Bacteroidota*, and *Ruminococcus spp* exhibited greater abundance in the HFD group. The KEGG analysis revealed that the genes with altered expression were predominantly enriched in pathways such as NOD-like receptor (NLR) signaling, PI3K-Akt signaling, estrogen-signaling, GABAergic synapse-related signaling, and pantothenate and CoA biosynthesis (Gu et al., 2021; Gu et al., 2024).

A study recruited 40 PCa patients who were grouped into weight-loss diet and control, then test the GM of feces. *Pseudomonadota* were plentiful, and the Gleason sum correlated with *Clostridium* and *Blautia*, as discovered by the team. Moreover, a rise in the intake of red meat compared to the initial level was linked to the presence of *Prevotella* and *Blautia bacteria*. Individuals who raised their consumption of poultry experienced a reduction in the abundance of *Eubacteriales (Clostridiales)* (Frugé et al., 2018). A PCa mouse model raised with HFD also showed that HFD could promote prostate carcinogenesis, and microbiota-mediated equol

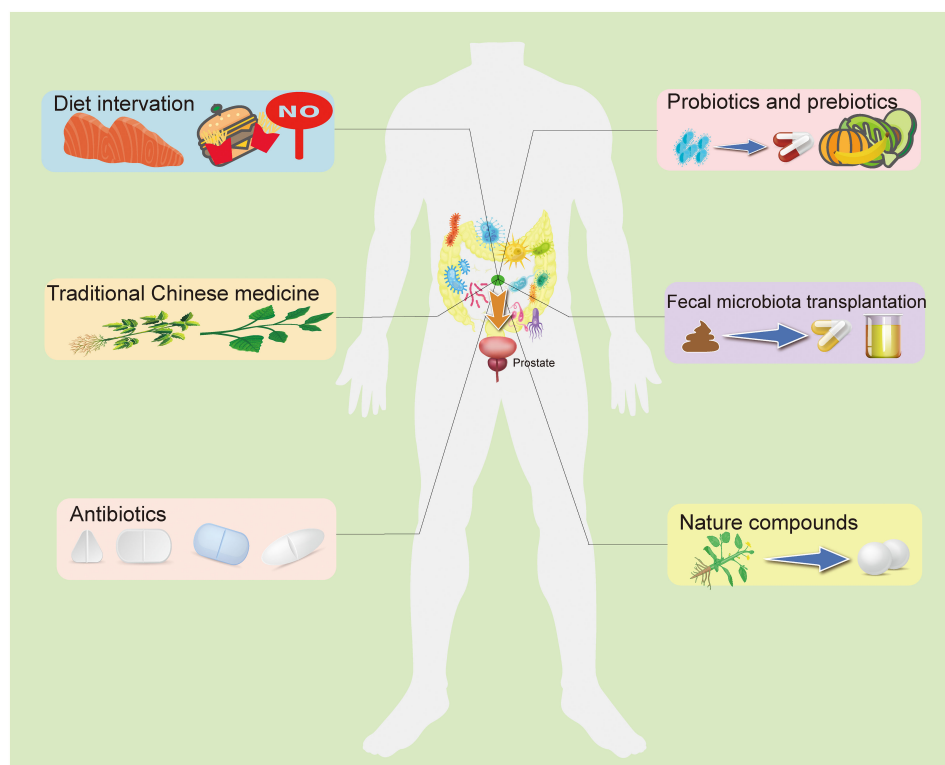


FIGURE 2

The potential preventive and therapeutic methods target the GM for prostatic disease. The current study suggests that targeting the GM to regulate prostate status is a promising treatment. A high-fat diet is believed to be an important threat to prostatic disease, and fat metabolites from the gut microbiota can influence prostate health in a number of ways, as do other dietary components. Recent research indicated that some TCM used to treat prostatic disease may work by targeting gut microbiota. In addition, antibiotics, probiotics, prebiotics, and fecal bacteria transplantation directly alter the composition of the intestinal flora and the abundance of specific bacteria, improving intestinal disorders. Some natural compounds have been found to interact with the GM to play a role in anti-prostatic disease, and with further research and development, these drug candidates are gradually being understood and applied. GM, Gut microbiome; TCM, Traditional Chinese medicine.

significantly decreased because of the decreased abundance of equol-producing bacterium *Adlercreutzia*. Thus, the authors hypothesized that HFD might promote PCa by adversely affecting equol-producing bacterium (Liu et al., 2019). Studies indicated that diets high in saturated and monounsaturated fatty acids, such as the Lard diet, may increase the risk of developing and progressing PCa. Additionally, the composition of gut bacteria, specifically *Eubacteriales* s and *Lactobacillales*, was significantly different in mice fed the Lard diet compared to those fed a fish oil diet. Furthermore, the regulation of lipid processing and cholesterol production pathways involves three and seven frequently altered genes in PCa tissues, some of which are associated with the prevalence of the *Lactobacillales* order in the mouse intestinal tract. Thus, SMFA could potentially enhance the advancement of PCa by increasing the presence of certain gut bacteria and upregulating lipogenic genes in PCa (Sato et al., 2022). Evidence suggests that indole-3-carbinol (I3C), a compound found in cruciferous vegetables, could potentially provide protection against PCa. The impact of dietary I3C on the GM of mice may be attributed to its modulatory effect (Wu et al., 2019).

4.2 Traditional Chinese medicine

Traditional Chinese medicine (TCM) is a unique medical treatment in China, some of which have demonstrated promising efficacy in treating prostatic disease. Recent research indicates that they are able to function by focusing on the GM. *Poria cocos* polysaccharides (PPs) is an antiandrogenic drug used to treat BPH and CP/CPPS. A study using rats demonstrated that the metabolites of PPs, 7-keto deoxycholic acid and haloperidol glucuronide, were significantly enriched by *Parabacteroides*, *Fusicatenibacter*, and *Parasutterella* after fermentation by human fecal microbiota. These metabolites could potentially act as signal molecules to alleviate CP/CPPS (Yu et al., 2022). When compared to finasteride in treating CP/CPPS, PPs and finasteride both significantly improved the histological damage in the inflamed prostate. Additionally, PPs and finasteride suppressed the synthesis of inflammatory cytokines (TNF- α , IL-2, and IL-8) as well as androgens (dihydrotestosterone and testosterone). 16S rDNA sequencing revealed that PPs and finasteride induced unique changes in the composition of the gut microbiota. Additional examination indicated that PPs, as opposed to finasteride, reversed alterations in the intestinal microbiota caused by CP/CPPS, specifically affecting *Oscillospiraceae* NK4A214 group, an unidentified bacterium from *Oscillospiraceae*, *Ruminiclostridium* 9, *Phascolarctobacterium*, *Coriobacteriaceae* UCG-002, and *Oribacterium*. LDA effect size analysis revealed that PPs recovered the gut microbiota by targeting the *Oscillospiraceae* NK4A214 group (Liu et al., 2021a). Additional examination of sex hormones showed that PPs may help reduce CP/CPPS by controlling the levels of testosterone (T), dihydrotestosterone (DTH), and estradiol (E2). PPs may help reduce CNP by controlling the levels of inducible nitric oxide synthase, malonaldehyde, and superoxide dismutase in the inflamed prostate, thereby boosting antioxidant activity (Liu et al., 2020).

Rapeseed bee pollen is considered an important remedy for CP/CPPS, as well as having the ability to regulate gut microbiota and enhance intestinal health. Rapeseed bee pollen has the ability to reduce symptoms of CP/CPPS by specifically controlling the gut microbiota, with increased amounts and broken cell wall pollen demonstrating better results. Administering a large amount of rapeseed bee pollen with disrupted walls resulted in a decrease of around 32% in prostate wet weight and 36% in prostate index. Treatment with rapeseed bee pollen that had been disrupted by the wall also led to a significant decrease in the levels of proinflammatory cytokines, including IL-6, IL-8, IL-1 β , and TNF- α . Additionally, rapeseed bee pollen has the ability to suppress harmful bacteria and improve beneficial bacteria, especially in the ratio of *Bacillota* to *Bacteroidota* and the quantity of *Prevotella* (genus) (Qiao et al., 2023).

Traditionally, it is a common practice in medicine to use *Epilobium* sp. for treating the initial phases of BPH and inflammation. Certain components from the extracts (endothelin B, quercetin-3-O-glucuronide, myricetin-3-O-rhamnoside) demonstrated activity in LNCaP cells. Further analysis revealed that ellagitannins from *Epilobium hirsutum* herbs (a kind of *Epilobium* sp.) Human gut microbiota was shown to convert extracts into urolithins. Urolithin C exhibited strong effects in suppressing cell growth, PSA release, and arginase function (Stolarczyk et al., 2013; Piwowarski et al., 2017).

4.3 Antibiotics

Antibiotics are seen as a key factor in causing dysbiosis in the gut microbiota, and short-term antibiotic exposure for patients undergoing prostate biopsy skews the GM composition, leading to long-lasting changes in the gut microbiota that are difficult to reverse (Li JKM. et al., 2022; Tóth et al., 2022). Antibiotics are primarily utilized for treating upper respiratory tract infections (URIs) and urinary tract infections (UTIs), particularly in elderly males who often experience complications such as PCa and BPH in clinical settings (Ong et al., 2008). In Korea, a retrospective cohort study based on the population found that over one million people who used antibiotics for 180 days or longer had an increased risk of PCa compared to those who did not use antibiotics. Furthermore, those who utilized four or more types of antibiotics were at an increased risk of PCa compared to those who did not use antibiotics (Park et al., 2023). Research examined the link between antibiotic usage rates and cancer susceptibility across 30 European nations, finding that countries with elevated consumption of specific types of penicillin and tetracycline had a greater likelihood of developing PCa (Ternák et al., 2020). Weibo Zhong's study found that disturbing the GM with broad-spectrum antibiotics in water led to the development of subcutaneous and orthotopic tumors in mice. Subsequent analysis of mouse feces using 16S rRNA sequencing revealed a notable increase in *Pseudomonadota* levels following antibiotic treatment, leading to heightened gut permeability and intertumoral LPS, ultimately facilitating the progression of PCa through the NF- κ B-IL6-STAT3 pathway in mice (Zhong et al., 2022). Given that antibiotics have such a strong ability to regulate

the GM, it is also essential to consider how structural changes in the microbiota might affect the treatment of disease when using antibiotics in the clinic.

4.4 Probiotics and prebiotics

Probiotics, which are live microorganisms that promote health, primarily consist of *Bifidobacterium* and *Lactiplantibacillus* strains, along with some *Enterococcus* and *Streptococcus* strains (Wilkins and Sequoia, 2017). The presence of beneficial bacteria in PCa patients, like *Prevotella* spp., has been linked to higher rates of patient survival. Live probiotics could potentially be utilized to postpone the development of aggressive PCa, as shown in previous studies on preclinical mice (Pernigoni et al., 2021). Changes in the gut microbiota of the host can impact multiple organisms, suggesting that a carefully planned group of bacteria with diverse effects from various strains could be more effective than giving just one type of live bacteria as a probiotic. A potential combination of bacteria for treating PCa could consist of various species of *Prevotella*. They are positively correlating with patients' survival (Pernigoni et al., 2023). Moreover, androgen catabolic intestinal bacteria may be helpful as a potent biogenic organism in hyper-androgen replacement therapy.

Prebiotics are indigestible components of food that have a positive impact on human health by promoting the growth of certain beneficial gut bacteria. Studies have shown that prebiotics can impact immune regulation, microbial protection, as well as bowel and metabolic processes (Sanders et al., 2019). In a study involving 30 patients with endometrial, cervical, colon, rectal, or PCa undergoing pelvic radiotherapy, a randomized, double-blind controlled trial was conducted. The patients were either given partially hydrolyzed guar gum or a placebo. The results indicated a decrease in diarrhea frequency and an increase in *Bifidobacterium* count in the group that received partially hydrolyzed guar gum. However, there was no notable variation in quality-of-life scores (Rosli et al., 2021). Therefore, utilizing routines that support healthy gut microbes with probiotics/prebiotics could reduce the likelihood of prostate cancer development in high-risk men.

4.5 Fecal microbiota transplantation

FMT, a recent technique, involves transferring fecal material from a healthy donor into the recipient's intestines to balance microbial populations. FMT can be administered in fresh or frozen samples, as well as in capsule form, through either an upper or lower gastrointestinal route. Some research indicates that the lower gastrointestinal route may be more effective for this purpose, possibly due to the presence of colonic pathology (Baunwall et al., 2020; Walter and Shanahan, 2023). Research conducted on FMT using stool samples from healthy donor mice or HSPC patients has demonstrated a delay in the development of castration resistance when compared to FMT from CRPC mice or patient donors (Pernigoni et al., 2021). The combined research suggests that FMT could be a viable approach to transferring

beneficial bacteria from those who respond well to those who do not, in order to address resistance to treatment. Weimin Dong et al. discovered that ulcerative colitis led to an enlargement of the prostate, with elevated levels of GPER expression that could be reversed by FMT. Furthermore, prostate tissues exhibited higher butyric acid levels after they were treated with FMT. *In vitro* experiments showed that the fecal filtrate (FF) from healthy mice increased the expression of GPER, suppressed cell proliferation, and triggered apoptosis in BPH-1 cells (Dong et al., 2022). A previous study showed that *Ruminococcus* correlated with phospholipid metabolism was more abundant in CRPC than in HSPC individuals. Treatment with CRPC feces hastened the progression of prostate cancer in mice and elevated the levels of *Ruminococcus* in their intestines. CRPC FMT treated mice showed increased levels of most fecal lipids, such as lysophosphatidylcholine and phosphatidylcholine, along with higher levels of LPCAT1, RAD51, and DNA-PKcs in the prostate, indicating that *Ruminococcus* may promote PCa progression by upregulating LPCAT1 and DNA repair proteins (Liu Y. et al., 2021). The results showed that fecal transplantation with certain beneficial bacteria is a crucial question that needs consideration, and future research to explore beneficial ones is essential.

4.6 Natural compound

In addition to the standard therapies that target the gut microbiota mentioned above, some natural compound candidates have shown promising therapeutic potential. Astaxanthin is a naturally occurring substance that possesses anti-inflammatory and immunomodulatory effects, as well as probiotic properties. AST administered orally led to an increase in the proportion of *Akkermansia muciniphila*, resulting in higher levels of SCFAs acetate in the blood, enhanced expression of colonic tight junction markers, reduced levels of serum lipopolysaccharide, ultimately reducing inflammation and pain in EAP mice (Liu et al., 2024). The extract from blackberry seeds boosted the overall quantity of intestinal bacteria. It altered the prevalence of particular bacteria and demonstrated abilities for reducing inflammation and inhibiting cell growth by suppressing the expression of IL-1 β mRNA induced by LPS in the proliferation of LNCaP cells (Choe et al., 2020). Phytoestrogens called lignans, obtained from a variety of plants, can be metabolized by the gut microbiota in humans to produce enterodiols (END) or enterolactone (ENL). Lignans have been identified for their antioxidant and anti-inflammatory effects, as well as their involvement in estrogen receptor-related pathways. Additionally, lignans have shown effectiveness in inhibiting tumor growth in different types of cancer cells, including PCa. The molecular pathways of lignans in treating PCa include the suppression of inflammatory signals, such as the NF- κ B pathway (Rowland et al., 2003; Hålldin et al., 2019; Jang et al., 2022). Polyphenols from berries are active compounds produced and released by various types of berry fruits. These substances hinder the growth of harmful bacteria and support helpful bacteria, which helps reduce inflammation by blocking NF- κ B and preventing the development

of PCa. Research findings indicated that polyphenols found in berries have the potential to be a valuable source of bioactive substances that can influence the GM and help in the treatment of PCa (Bouyahya et al., 2022). Green tea catechins (GTCs) could influence the molecular pathways involved in the development of PCa. Orally administering the GM enzyme changes the structure of GTC, impacting its availability, activity, and toxicity while also controlling inflammation and hormone pathways (Stanisławska et al., 2019; Kumar et al., 2022). Pomegranate juice contains ellagitannins (ETs), which are polyphenols that have the potential to prevent PCa through their bioactive properties. Extraterrestrials undergo partial hydrolysis in the digestive system, transforming into ellagic acid (EA) and further into Uro A. These compounds travel through the bloodstream to various parts of the body, including the prostate, where they inhibit the proliferation of prostate cancer cells and stimulate their programmed cell death (Seeram et al., 2007; González-Sarrias et al., 2010; Vicinanza et al., 2013). As more drug candidates become available, approaches for regulating the microbiome will diversify, and it will be critical to explore whether combined regulation between these drugs can enhance or influence each other.

In conclusion, current observational and experimental data suggest that some gut microbiotas differ between prostatic disease patients and healthy people. Additionally, basic experiments indicate that the GM can affect prostate health through a variety of mechanisms, including direct pathogen invasion, immune regulation, metabolite and androgen regulation, and so on. This evidence suggested that targeting the gut microbiota in the treatment of prostatic disease may have a positive effect. The initial studies of the aforementioned treatments have confirmed this idea, but these treatments have not yet been used in the clinic. In the future, we will need to conduct more experiments to confirm the possibility of targeting the GM to treat prostate disease.

5 Conclusion and future directions

There is a small but growing body of work investigating the GM and its relationship with prostatic health and disease. This review provides an overview of the observational and animal data that demonstrate the relationship between GM and prostatic disease. Then, we specifically analyze the potential mechanisms mediating this phenomenon, involving direct infection, inflammation, immunity, metabolites derived from the GM, androgens, and ADT. With this in mind, we propose six potential ways to target GM to prevent or treat prostatic disease, including diet intervention, TCM, antibiotics, probiotics and prebiotics, FMT, and natural compounds. Overall, targeting the GM to maintain prostate health is a promising approach. However, it should be noted that current research into the GM and prostate is limited in certain respects. Initially, the current study suggests possible pathogenesis,

but the mechanisms do not fully explain the occurrence of prostate disease. How important the role of the GM in the pathogenesis of prostate disease is still unclear, as is the extent of benefit that can be obtained from targeted therapy. Furthermore, previous studies on the mechanism are based on animal studies, and whether it exists in humans is still unknown. In addition, the question of whether these treatments have side effects and whether the combination of these potential treatments can achieve greater efficacy is also worth further investigation. Last, in light of the diverse range of prostate diseases affecting patients, it is crucial to investigate further the efficacy of various treatment methods. We hope to have more research to reveal the specific gut microbiota and its effect mechanism, in order to develop more precise treatment to protect prostate health.

Author contributions

HC: Writing – original draft, Writing – review & editing. DZ: Writing – original draft. PW: Writing – original draft. YW: Writing – original draft, Writing – review & editing. CS: Writing – original draft. HW: Writing – original draft. HD: Writing – original draft. WZ: Writing – original draft. ZG: Writing – original draft. HZ: Writing – review & editing. SW: Writing – review & editing.

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Conflict of interest

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References

- (2021). Gut microbiota drive androgen resistance in prostate cancer. *Cancer Discovery* 11, 2959. doi: 10.1158/2159-8290.CD-RW2021-153
- Alanee, S., El-Zawahry, A., Dynda, D., Dabaja, A., McVary, K., Karr, M., et al. (2019). A prospective study to examine the association of the urinary and fecal microbiota with prostate cancer diagnosis after transrectal biopsy of the prostate using 16sRNA gene analysis. *Prostate* 79, 81–87. doi: 10.1002/pros.23713
- An, J., Song, Y., Kim, S., Kong, H., and Kim, K. (2023). Alteration of gut microbes in benign prostatic hyperplasia model and finasteride treatment model. *Int. J. Mol. Sci.* 24. doi: 10.3390/ijms24065904
- Badgeley, A., Anwar, H., Modi, K., Murphy, P., and Lakshmikuttyamma, A. (2021). Effect of probiotics and gut microbiota on anti-cancer drugs: Mechanistic perspectives. *Biochim. Biophys. Acta Rev. Cancer*. 1875, 188494. doi: 10.1016/j.bbcan.2020.188494
- Baunwall, S. M. D., Lee, M. M., Eriksen, M. K., Mullish, B. H., Marchesi, J. R., Dahlerup, J. F., et al. (2020). Fecal microbiota transplantation for recurrent *Clostridioides difficile* infection: An updated systematic review and meta-analysis. *EClinicalMedicine* 29–30, 100642. doi: 10.1016/j.eclinm.2020.100642
- Bouyahya, A., Omari, N. E., El Hachlafi, N., Jemly, M. E., Hakkour, M., Balahbib, A., et al. (2022). Chemical compounds of berry-derived polyphenols and their effects on gut microbiota, inflammation, and cancer. *Molecules* 27. doi: 10.3390/molecules27103286
- Bui, N. N., Li, C. Y., Wang, L. Y., Chen, Y. A., Kao, W. H., Chou, L. F., et al. (2023). *Clostridium* scindens metabolites trigger prostate cancer progression through androgen receptor signaling. *J. Microbiol. Immunol. Infect.* 56, 246–256. doi: 10.1016/j.jmii.2022.12.009
- Cai, T., Santi, R., Tamanini, L., Galli, I. C., Perletti, G., Bjerklund Johansen, T. E., et al. (2019). Current knowledge of the potential links between inflammation and prostate cancer. *Int. J. Mol. Sci.* 20. doi: 10.3390/ijms20153833
- Choe, U., Li, Y., Yu, L., Gao, B., Wang, T. T. Y., Sun, J., et al. (2020). Chemical composition of cold-pressed blackberry seed flour extract and its potential health-beneficial properties. *Food Sci. Nutr.* 8, 1215–1225. doi: 10.1002/fsn3.1410
- Cui, Y., and Zhang, Y. (2013). The effect of androgen-replacement therapy on prostate growth: a systematic review and meta-analysis. *Eur. Urol.* 64, 811–822. doi: 10.1016/j.eururo.2013.03.042
- Daisley, B. A., Chanyi, R. M., Abdur-Rashid, K., Al, K. F., Gibbons, S., Chmiel, J. A., et al. (2020). Abiraterone acetate preferentially enriches for the gut commensal *Akkermansia muciniphila* in castrate-resistant prostate cancer patients. *Nat. Commun.* 11, 4822. doi: 10.1038/s41467-020-18649-5
- Desai, K., McManus, J. M., and Sharifi, N. (2021). Hormonal therapy for prostate cancer. *Endocr. Rev.* 42, 354–373. doi: 10.1210/edrv/bnab002
- Dong, W., Zheng, J., Huang, Y., Tan, H., Yang, S., Zhang, Z., et al. (2022). Sodium butyrate treatment and fecal microbiota transplantation provide relief from ulcerative colitis-induced prostate enlargement. *Front. Cell Infect. Microbiol.* 12, 1037279. doi: 10.3389/fcimb.2022.1037279
- Du, H. X., Yue, S. Y., Niu, D., Liu, C., Zhang, L. G., Chen, J., et al. (2022). Gut microflora modulates th17/treg cell differentiation in experimental autoimmune prostatitis via the short-chain fatty acid propionate. *Front. Immunol.* 13, 915218. doi: 10.3389/fimmu.2022.915218
- Duan, H., Wang, L., Huangfu, M., and Li, H. (2023). The impact of microbiota-derived short-chain fatty acids on macrophage activities in disease: Mechanisms and therapeutic potentials. *BioMed. Pharmacother.* 165, 115276. doi: 10.1016/j.biopha.2023.115276
- Fang, C., Wu, L., Zhu, C., Xie, W. Z., Hu, H., and Zeng, X. T. (2021). A potential therapeutic strategy for prostatic disease by targeting the oral microbiome. *Med. Res. Rev.* 41, 1812–1834. doi: 10.1002/med.21778
- Fenner, A. (2021). Intestinal microbiota drive CRPC. *Nat. Rev. Urol.* 18, 705. doi: 10.1038/s41585-021-00537-2
- Frugé, A. D., Ptacek, T., Tsuruta, Y., Morrow, C. D., Azrad, M., Desmond, R. A., et al. (2018). Dietary changes impact the gut microbe composition in overweight and obese men with prostate cancer undergoing radical prostatectomy. *J. Acad. Nutr. Diet.* 118, 714–723.e1. doi: 10.1016/j.jand.2016.10.017
- García-Villalba, R., Giménez-Bastida, J. A., Cortés-Martín, A., Ávila-Gálvez, M., Tomás-Barberán, F. A., Selma, M. V., et al. (2022). Urolithins: a comprehensive update on their metabolism, bioactivity, and associated gut microbiota. *Mol. Nutr. Food Res.* 66, e2101019. doi: 10.1002/mnfr.202101019
- Golombos, D. M., Ayangbesan, A., O'Malley, P., Lewicki, P., Barlow, L., Barbieri, C. E., et al. (2018). The role of gut microbiome in the pathogenesis of prostate cancer: A prospective, pilot study. *Urology* 111, 122–128. doi: 10.1016/j.urology.2017.08.039
- González-Sarriás, A., Giménez-Bastida, J. A., García-Conesa, M. T., Gómez-Sánchez, M. B., García-Talavera, N. V., Gil-Izquierdo, A., et al. (2010). Occurrence of urolithins, gut microbiota ellagic acid metabolites and proliferation markers expression response in the human prostate gland upon consumption of walnuts and pomegranate juice. *Mol. Nutr. Food Res.* 54, 311–322. doi: 10.1002/mnfr.200900152
- Gu, L., Alemasi, A., Chen, Y., Li, X., Mei, Y., Zhou, J., et al. (2024). Gut microbiota disorders in obesity-associated benign prostatic hyperplasia in rats. *Biochem. Genet.* doi: 10.1007/s10528-023-10634-z
- Gu, M., Liu, C., Yang, T., Zhan, M., Cai, Z., Chen, Y., et al. (2021). High-fat diet induced gut microbiota alterations associating with ghrelin/jak2/stat3 up-regulation to promote benign prostatic hyperplasia development. *Front. Cell Dev. Biol.* 9, 615928. doi: 10.3389/fcell.2021.615928
- Hålldin, E., Eriksen, A. K., Brunius, C., da Silva, A. B., Bronze, M., Hanhineva, K., et al. (2019). Factors explaining interpersonal variation in plasma enterolactone concentrations in humans. *Mol. Nutr. Food Res.* 63, e1801159. doi: 10.1002/mnfr.201801159
- Hsiao, T. H., Chou, C. H., Chen, Y. L., Wang, P. H., Brandon-Mong, G. J., Lee, T. H., et al. (2023). Circulating androgen regulation by androgen-catabolizing gut bacteria in male mouse gut. *Gut Microbes* 15, 2183685. doi: 10.1080/19490976.2023.2183685
- Huang, P. Y., Yang, Y. C., Wang, C. L., Hsiao, P. W., Chiang, H. I., and Chen, T. W. (2021). Increase in *akkermansia* in gut microbiota of prostate cancer-bearing mice. *Int. J. Mol. Sci.* 22. doi: 10.3390/ijms22179626
- Illiano, P., Brambilla, R., and Parolini, C. (2020). The mutual interplay of gut microbiota, diet and human disease. *FEBS J.* 287, 833–855. doi: 10.1111/febs.15217
- Jang, W. Y., Kim, M. Y., and Cho, J. Y. (2022). Antioxidant, anti-inflammatory, anti-menopausal, and anti-cancer effects of lignans and their metabolites. *Int. J. Mol. Sci.* 23. doi: 10.3390/ijms232415482
- Jin, B. R., Kim, H. J., Na, J. H., Lee, W. K., and An, H. J. (2024). Targeting benign prostate hyperplasia treatments: AR/TGF- β /NOX4 inhibition by apocynin suppresses inflammation and proliferation. *J. Adv. Res.* 57, 135–147. doi: 10.1016/j.jare.2023.04.006
- Jones-Freeman, B., Chonwerawong, M., Marcelino, V. R., Deshpande, A. V., Forster, S. C., and Starkey, M. R. (2021). The microbiome and host mucosal interactions in urinary tract diseases. *Mucosal Immunol.* 14, 779–792. doi: 10.1038/s41385-020-00372-5
- Kahokehr, A., and Gilling, P. J. (2014). Landmarks in BPH—from etiology to medical and surgical management. *Nat. Rev. Urol.* 11, 118–122. doi: 10.1038/nrurol.2013.318
- Kim, E. H., Larson, J. A., and Andriole, G. L. (2016). Management of benign prostatic hyperplasia. *Annu. Rev. Med.* 67, 137–151. doi: 10.1146/annurev-med-063014-123902
- Konkol, Y., Kesitalo, A., Vuorikoski, H., Pietilä, S., Elo, L. L., Munukka, E., et al. (2019). Chronic nonbacterial prostate inflammation in a rat model is associated with changes of gut microbiota that can be modified with a galactoglucomannan-rich hemicellulose extract in the diet. *BJU Int.* 123, 899–908. doi: 10.1111/bju.14553
- Krieger, J. N., Lee, S. W., Jeon, J., Cheah, P. Y., Liong, M. L., and Riley, D. E. (2008). Epidemiology of prostatitis. *Int. J. Antimicrob. Agents*. 31 Suppl 1, S85–S90. doi: 10.1016/j.ijantimicag.2007.08.028
- Krieger, J. N., Nyberg, L. Jr., and Nickel, J. C. (1999). NIH consensus definition and classification of prostatitis. *Jama* 282, 236–237. doi: 10.1001/jama.282.3.236
- Kumar, N. B., Hogue, S., Pow-Sang, J., Poch, M., Manley, B. J., Li, R., et al. (2022). Effects of green tea catechins on prostate cancer chemoprevention: the role of the gut microbiome. *Cancers (Basel)* 14. doi: 10.3390/cancers14163988
- Kure, A., Tsukimi, T., Ishii, C., Aw, W., Obana, N., Nakato, G., et al. (2023). Gut environment changes due to androgen deprivation therapy in patients with prostate cancer. *Prostate Cancer Prostatic Dis.* 26, 323–330. doi: 10.1038/s41391-022-00536-3
- Kuziel, G. A., and Rakoff-Nahoum, S. (2022). The gut microbiome. *Curr. Biol.* 32, R257–Rr64. doi: 10.1016/j.cub.2022.02.023
- Labbé, D. P., Zadra, G., Yang, M., Reyes, J. M., Lin, C. Y., Cacciatore, S., et al. (2019). High-fat diet fuels prostate cancer progression by rewiring the metabolome and amplifying the MYC program. *Nat. Commun.* 10, 4358. doi: 10.1038/s41467-019-12298-z
- Li, L. Y., Han, J., Wu, L., Fang, C., Li, W. G., Gu, J. M., et al. (2022). Alterations of gut microbiota diversity, composition and metabolomics in testosterone-induced benign prostatic hyperplasia rats. *Mil Med. Res.* 9, 12. doi: 10.1186/s40779-022-00373-4
- Li, J. K. M., Wang, L. L., Lau, B. S. Y., Tse, R. T. H., Cheng, C. K. L., Leung, S. C. H., et al. (2022). Oral antibiotics perturbation on gut microbiota after prostate biopsy. *Front. Cell Infect. Microbiol.* 12, 959903. doi: 10.3389/fcimb.2022.959903
- Li, J. K. M., Wang, L. L., Wong, C. Y. P., Chiu, P. K. F., Teoh, J. Y. C., Kwok, H. S. W., et al. (2021). A cross-sectional study on gut microbiota in prostate cancer patients with prostatectomy or androgen deprivation therapy. *Prostate Cancer Prostatic Dis.* 24, 1063–1072. doi: 10.1038/s41391-021-00360-1
- Liss, M. A., White, J. R., Goros, M., Gelfond, J., Leach, R., Johnson-Pais, T., et al. (2018). Metabolic biosynthesis pathways identified from fecal microbiome associated with prostate cancer. *Eur. Urol.* 74, 575–582. doi: 10.1016/j.eururo.2018.06.033
- Liu, Y., and Jiang, H. (2020). Compositional differences of gut microbiome in matched hormone-sensitive and castration-resistant prostate cancer. *Transl. Androl Urol.* 9, 1937–1944. doi: 10.21037/tau
- Liu, J., Liu, L., Zhang, G., and Peng, X. (2021a). Poria cocos polysaccharides attenuate chronic nonbacterial prostatitis by targeting the gut microbiota: Comparative study of Poria cocos polysaccharides and finasteride in treating chronic prostatitis. *Int. J. Biol. Macromol.* 189, 346–355. doi: 10.1016/j.jbiomac.2021.08.139
- Liu, J., Wang, Y., Zhang, G., Liu, L., and Peng, X. (2021b). Multi-omics analysis reveals changes in the intestinal microbiome, transcriptome, and methylome in a rat

model of chronic non-bacterial prostatitis: indications for the existence of the gut-prostate axis. *Front. Physiol.* 12, 753034. doi: 10.3389/fphys.2021.753034

Liu, Y., Wu, X., and Jiang, H. (2019). High dietary fat intake lowers serum equol concentration and promotes prostate carcinogenesis in a transgenic mouse prostate model. *Nutr. Metab. (Lond)*. 16, 24. doi: 10.1186/s12986-019-0351-x

Liu, Y. F., Xie, W. J., Xi, P., Zhang, Z. C., Chen, R., Fu, S. Q., et al. (2024). Astaxanthin alleviates chronic prostatitis/chronic pelvic pain syndrome by increasing colonization of Akkermansia muciniphila in the intestine. *Phytomedicine* 123, 155249. doi: 10.1016/j.phymed.2023.155249

Liu, Y., Yang, C., Zhang, Z., and Jiang, H. (2021). Gut microbiota dysbiosis accelerates prostate cancer progression through increased LPCAT1 expression and enhanced DNA repair pathways. *Front. Oncol.* 11, 679712. doi: 10.3389/fonc.2021.679712

Liu, J., Yu, J., and Peng, X. (2020). Poria cocos polysaccharides alleviates chronic nonbacterial prostatitis by preventing oxidative stress, regulating hormone production, modifying gut microbiota, and remodeling the DNA methylome. *J. Agric. Food Chem.* 68, 12661–12670. doi: 10.1021/acs.jafc.0c05943

Liu, Y., Zhou, Q., Ye, F., Yang, C., and Jiang, H. (2023). Gut microbiota-derived short-chain fatty acids promote prostate cancer progression via inducing cancer cell autophagy and M2 macrophage polarization. *Neoplasia* 43, 100928. doi: 10.1016/j.neo.2023.100928

Ly, L. K., Rowles, J. L. 3rd, Paul, H. M., Alves, J. M. P., Yemm, C., Wolf, P. M., et al. (2020). Bacterial steroid-17,20-desmolase is a taxonomically rare enzymatic pathway that converts prednisone to 1,4-androstenedione-3,11,17-trione, a metabolite that causes proliferation of prostate cancer cells. *J. Steroid Biochem. Mol. Biol.* 199, 105567. doi: 10.1016/j.jsbmb.2019.105567

Matsushita, M., Fujita, K., Hatano, K., De Velasco, M. A., Uemura, H., and Nonomura, N. (2022a). Connecting the dots between the gut-IGF-1-prostate axis: A role of IGF-1 in prostate carcinogenesis. *Front. Endocrinol. (Lausanne)*. 13, 852382. doi: 10.3389/fendo.2022.852382

Matsushita, M., Fujita, K., Hatano, K., Hayashi, T., Kayama, H., Motooka, D., et al. (2022b). High-fat diet promotes prostate cancer growth through histamine signaling. *Int. J. Cancer*. 151, 623–636. doi: 10.1002/ijc.34028

Matsushita, M., Fujita, K., Motooka, D., Hatano, K., Fukae, S., Kawamura, N., et al. (2021). The gut microbiota associated with high-Gleason prostate cancer. *Cancer Sci.* 112, 3125–3135. doi: 10.1111/cas.14998

Matsushita, M., Fujita, K., Motooka, D., Hatano, K., Hata, J., Nishimoto, M., et al. (2022c). Firmicutes in gut microbiota correlate with blood testosterone levels in elderly men. *World J. Mens Health* 40, 517–525. doi: 10.5534/wjmh.210190

Mirzaei, R., Afaghi, A., Babakhani, S., Sohrabi, M. R., Hosseini-Fard, S. R., Babolhavaei, 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

Miyake, M., Tatsumi, Y., Ohnishi, K., Fujii, T., Nakai, Y., Tanaka, N., et al. (2022). Prostate diseases and microbiome in the prostate, gut, and urine. *Prostate Int.* 10, 96–107. doi: 10.1016/j.pnrl.2022.03.004

Nguyen-Nielsen, M., and Borre, M. (2016). Diagnostic and therapeutic strategies for prostate cancer. *Semin. Nucl. Med.* 46, 484–490. doi: 10.1053/j.smnucmed.2016.07.002

Ong, D. S., Kuyvenhoven, M. M., van Dijk, L., and Verheij, T. J. (2008). Antibiotics for respiratory, ear and urinary tract disorders and consistency among GPs. *J. Antimicrob. Chemother.* 62, 587–592. doi: 10.1093/jac/dkn230

Park, S. J., Hong, J., Park, Y. J., Jeong, S., Choi, S., Chang, J., et al. (2023). Association between antibiotic use and subsequent risk of prostate cancer: A retrospective cohort study in South Korea. *Int. J. Urol.* doi: 10.12103/rs.3.rs-2501566/v1

Pernigoni, N., Guo, C., Gallagher, L., Yuan, W., Colucci, M., Troiani, M., et al. (2023). The potential role of the microbiota in prostate cancer pathogenesis and treatment. *Nat. Rev. Urol.* 20, 706–718. doi: 10.1038/s41585-023-00795-2

Pernigoni, N., Zagato, E., Calcinotto, A., Troiani, M., Mestre, R. P., Cali, B., et al. (2021). Commensal bacteria promote endocrine resistance in prostate cancer through androgen biosynthesis. *Science* 374, 216–224. doi: 10.1126/science.abf8403

Piwowarski, J. P., Bobrowska-Korczak, B., Stanisławska, I., Bielecki, W., Wrzesien, R., Granica, S., et al. (2017). Evaluation of the effect of epilobium angustifolium aqueous extract on LNCaP cell proliferation in *in vitro* and *in vivo* models. *Planta Med.* 83, 1159–1168. doi: 10.1055/s-0043-109372

Porter, C. M., Shrestha, E., Peiffer, L. B., and Sfanos, K. S. (2018). The microbiome in prostate inflammation and prostate cancer. *Prostate Cancer Prostatic Dis.* 21, 345–354. doi: 10.1038/s41391-018-0041-1

Qiao, J., Xiao, X., Wang, K., Haubruge, E., Dong, J., and Zhang, H. (2023). Rapeseed bee pollen alleviates chronic non-bacterial prostatitis via regulating gut microbiota. *J. Sci. Food Agric.* 103, 7896–7904. doi: 10.1002/jsfa.12878

Ratajczak, W., Laszczyńska, M., Rył, A., Dołęgowska, B., Sipak, O., Stachowska, E., et al. (2023). Tissue immunoexpression of IL-6 and IL-18 in aging men with BPH and MetS and their relationship with lipid parameters and gut microbiota-derived short chain fatty acids. *Aging (Albany NY)*. 15, 10875–10896. doi: 10.18632/aging.205091

Ratajczak, W., Mizerski, A., Rył, A., Słojewski, M., Sipak, O., Piasecka, M., et al. (2021). Alterations in fecal short chain fatty acids (SCFAs) and branched short-chain

fatty acids (BCFAs) in men with benign prostatic hyperplasia (BPH) and metabolic syndrome (MetS). *Aging (Albany NY)*. 13, 10934–10954. doi: 10.18632/aging.v13i8

Ronald, A. (2003). The etiology of urinary tract infection: traditional and emerging pathogens. *Dis. Mon.* 49, 71–82. doi: 10.1067/mda.2003.8

Rosli, D., Shahar, S., Manaf, Z. A., Lau, H. J., Yusof, N. Y. M., Haron, M. R., et al. (2021). Randomized controlled trial on the effect of partially hydrolyzed guar gum supplementation on diarrhea frequency and gut microbiome count among pelvic radiation patients. *J. Parenter Enteral Nutr.* 45, 277–286. doi: 10.1002/jpen.1987

Rowland, I., Faughnan, M., Hoey, L., Wähälä, K., Williamson, G., and Cassidy, A. (2003). Bioavailability of phyto-estrogens. *Br. J. Nutr.* 89 Suppl 1, S45–S58. doi: 10.1079/BJN2002796

Russo, G. I., Bongiorno, D., Bonomo, C., Musso, N., Stefani, S., Sokolakis, I., et al. (2023). The relationship between the gut microbiota, benign prostatic hyperplasia, and erectile dysfunction. *Int. J. Impot Res.* 35, 350–355. doi: 10.1038/s41443-022-00569-1

Sanders, M. E., Merenstein, D. J., Reid, G., Gibson, G. R., and Rastall, R. A. (2019). Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nat. Rev. Gastroenterol. Hepatol.* 16, 605–616. doi: 10.1038/s41575-019-0173-3

Sandhu, S., Moore, C. M., Chiong, E., Beltran, H., Bristow, R. G., and Williams, S. G. (2021). Prostate cancer. *Lancet* 398, 1075–1090. doi: 10.1016/S0140-6736(21)00950-8

Sato, H., Narita, S., Ishida, M., Takahashi, Y., Mingguo, H., Kashima, S., et al. (2022). Specific gut microbial environment in lard diet-induced prostate cancer development and progression. *Int. J. Mol. Sci.* 23. doi: 10.3390/ijms23042214

Schaeffer, A. J. (2008). Epidemiology and evaluation of chronic pelvic pain syndrome in men. *Int. J. Antimicrob. Agents.* 31 Suppl 1, S108–S111. doi: 10.1016/j.jantimicag.2007.08.027

Seeram, N. P., Aronson, W. J., Zhang, Y., Henning, S. M., Moro, A., Lee, R. P., et al. (2007). Pomegranate ellagitannin-derived metabolites inhibit prostate cancer growth and localize to the mouse prostate gland. *J. Agric. Food Chem.* 55, 7732–7737. doi: 10.1021/jf071303g

Sfanos, K. S., Markowski, M. C., Peiffer, L. B., Ernst, S. E., White, J. R., Pienta, K. J., et al. (2018). Compositional differences in gastrointestinal microbiota in prostate cancer patients treated with androgen axis-targeted therapies. *Prostate Cancer Prostatic Dis.* 21, 539–548. doi: 10.1038/s41391-018-0061-x

Shen, C., Chen, Z., Zhang, W., Chen, X., Zheng, B., and Shi, C. (2024). Preliminary study of the effect of gut microbiota on the development of prostatitis. *BMC Med. Genomics* 17, 35. doi: 10.1186/s12920-024-01812-y

Shoskes, D. A., Wang, H., Polackwich, A. S., Tucky, B., Altemus, J., and Eng, C. (2016). Analysis of gut microbiome reveals significant differences between men with chronic prostatitis/chronic pelvic pain syndrome and controls. *J. Urol.* 196, 435–441. doi: 10.1016/j.juro.2016.02.2959

Smith, K. S., Frugé, A. D., van der Pol, W., Caston, N. E., Morrow, C. D., Demark-Wahnefried, W., et al. (2021). Gut microbial differences in breast and prostate cancer cases from two randomized controlled trials compared to matched cancer-free controls. *Benef. Microbes* 12, 239–248. doi: 10.3920/BM2020.0098

Stanisławska, I. J., Granica, S., Piwowarski, J. P., Szawkało, J., Wiązecki, K., Czarnocki, Z., et al. (2019). The activity of urolithin A and M4 valerolactone, colonic microbiota metabolites of polyphenols, in a prostate cancer *in vitro* model. *Planta Med.* 85, 118–125. doi: 10.1055/a-0755-7715

Stanisławska, I. J., Piwowarski, J. P., Granica, S., and Kiss, A. K. (2018). The effects of urolithins on the response of prostate cancer cells to non-steroidal antiandrogen bicalutamide. *Phytomedicine* 46, 176–183. doi: 10.1016/j.phymed.2018.03.054

Stolarczyk, M., Piwowarski, J. P., Granica, S., Stefańska, J., Naruszewicz, M., and Kiss, A. K. (2013). Extracts from *Epilobium* sp. herbs, their components and gut microbiota metabolites of *Epilobium* ellagitannins, urolithins, inhibit hormone-dependent prostate cancer cells (LNCaP) proliferation and PSA secretion. *Phytother. Res.* 27, 1842–1848. doi: 10.1002/ptr.4941

Takezawa, K., Fujita, K., Matsushita, M., Motooka, D., Hatano, K., Banno, E., et al. (2021). The Firmicutes/Bacteroidetes ratio of the human gut microbiota is associated with prostate enlargement. *Prostate* 81, 1287–1293. doi: 10.1002/pros.24223

Teo, M. Y., Rathkopf, D. E., and Kantoff, P. (2019). Treatment of advanced prostate cancer. *Annu. Rev. Med.* 70, 479–499. doi: 10.1146/annurev-med-051517-011947

Ternák, G., Berényi, K., Sümegi, A., Szczeni, Á., Fodor, B., Németh, B., et al. (2020). Antibiotic consumption patterns in European countries may be associated with the incidence of major carcinomas. *Antibiot. (Basel)* 9. doi: 10.3390/antibiotics9100643

Terrisse, S., Goubet, A. G., Ueda, K., Thomas, A. M., Quiniou, V., Thelemaque, C., et al. (2022a). Immune system and intestinal microbiota determine efficacy of androgen deprivation therapy against prostate cancer. *J. Immunother. Cancer* 10. doi: 10.1200/JCO.2022.40.6_suppl.168

Terrisse, S., Zitvogel, L., and Kroemer, G. (2022b). Effects of the intestinal microbiota on prostate cancer treatment by androgen deprivation therapy. *Microb. Cell.* 9, 202–206. doi: 10.15698/mic

Tóth, Z., Bezzegh, A., Tordé, Á., Vársárhelyi, B., and Gyarmati, B. (2022). Short term ciprofloxacin and clindamycin combination antibiotic therapy before and after transrectal ultrasound scan and prostate biopsy: Its impact on major components of gut microbiome. *Mol. Cell Probes* 66, 101874. doi: 10.1016/j.mcp.2022.101874

Vicinanza, R., Zhang, Y., Henning, S. M., and Heber, D. (2013). Pomegranate juice metabolites, ellagic acid and urolithin A, synergistically inhibit androgen-independent

- prostate cancer cell growth via distinct effects on cell cycle control and apoptosis. *Evid Based Complement Alternat Med.* 2013, 247504. doi: 10.1155/2013/247504
- Wagenlehner, F. M., Weidner, W., Pilatz, A., and Naber, K. G. (2014). Urinary tract infections and bacterial prostatitis in men. *Curr. Opin. Infect. Dis.* 27, 97–101. doi: 10.1097/QCO.0000000000000024
- Walter, J., and Shanahan, F. (2023). Fecal microbiota-based treatment for recurrent *Clostridioides difficile* infection. *Cell* 186, 1087. doi: 10.1016/j.cell.2023.02.034
- Wang, S., Zang, M., Yang, X., Lv, L., Chen, L., Cui, J., et al. (2023). Gut microbiome in men with chronic prostatitis/chronic pelvic pain syndrome: profiling and its predictive significance. *World J. Urol.* 41, 3019–3026. doi: 10.1007/s00345-023-04587-6
- Whiteside, S. A., Razvi, H., Dave, S., Reid, G., and Burton, J. P. (2015). The microbiome of the urinary tract—a role beyond infection. *Nat. Rev. Urol.* 12, 81–90. doi: 10.1038/nrurol.2014.361
- Wilkins, T., and Sequoia, J. (2017). Probiotics for gastrointestinal conditions: A summary of the evidence. *Am. Fam. Phys.* 96, 170–178.
- Wu, Y., Li, R. W., Huang, H., Fletcher, A., Yu, L., Pham, Q., et al. (2019). Inhibition of tumor growth by dietary indole-3-carbinol in a prostate cancer xenograft model may be associated with disrupted gut microbial interactions. *Nutrients* 11. doi: 10.3390/nu11020467
- Xia, D., Wang, J., Zhao, X., Shen, T., Ling, L., and Liang, Y. (2023). Association between gut microbiota and benign prostatic hyperplasia: a two-sample mendelian randomization study. *Front. Cell Infect. Microbiol.* 13, 1248381. doi: 10.3389/fcimb.2023.1248381
- Xie, Q., and Hu, B. (2023). Effects of gut microbiota on prostatic cancer: a two-sample Mendelian randomization study. *Front. Microbiol.* 14, 1250369. doi: 10.3389/fmicb.2023.1250369
- Yu, J., Hu, Q., Liu, J., Luo, J., Liu, L., and Peng, X. (2022). Metabolites of gut microbiota fermenting *Poria cocos* polysaccharide alleviates chronic nonbacterial prostatitis in rats. *Int. J. Biol. Macromol.* 209, 1593–1604. doi: 10.1016/j.ijbiomac.2022.04.029
- Zhang, H., Zhou, Y., Xing, Z., Sah, R. K., Hu, J., and Hu, H. (2022). Androgen metabolism and response in prostate cancer anti-androgen therapy resistance. *Int. J. Mol. Sci.* 23. doi: 10.3390/ijms232113521
- Zhong, W., Wu, K., Long, Z., Zhou, X., Zhong, C., Wang, S., et al. (2022). Gut dysbiosis promotes prostate cancer progression and docetaxel resistance via activating NF- κ B-IL6-STAT3 axis. *Microbiome* 10, 94. doi: 10.1186/s40168-022-01289-w
- 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
- Zmora, N., Suez, J., and Elinav, E. (2019). You are what you eat: diet, health and the gut microbiota. *Nat. Rev. Gastroenterol. Hepatol.* 16, 35–56. doi: 10.1038/s41575-018-0061-2



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Analysis of the correlation between cervical HPV infection, cervical lesions and vaginal microecology

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Background: Vaginal microbiota is involved in human papillomavirus (HPV) infection and cervical cancer (CC) progression, and the specific changes in vaginal microbial composition during this process remains uncertain.

Objective: This study aimed to observe the changes in the specific composition of vaginal microorganisms in different cervical lesions and identify biomarkers at different stages of lesions.

Methods: In this study we used the illumina high-throughput gene sequencing technology to determine the V4 region of 16SrRNA and observed the vaginal microbial composition in different cervical lesions.

Results: The vaginal microbiota of patients with high-risk HPV infection and cervical lesions is significantly different from that of the normal population, but there is no significant difference in the richness of vaginal microbes. The diversity of vaginal species in CC patients is higher than that in high-risk HPV infection or CIN patients. The main manifestation is an increase in the diversity of vaginal microbes, a decrease in the relative abundance of cyanobacteria and Lactobacillus, and an increase in the relative abundance of dialister, peptoniphila and other miscellaneous bacteria. There are characteristic vaginal biomarker in normal women, high risk HPV patients and CC patients. In detail, the biomarker in the normal group was varibaculum, the biomarker in the high-risk HPV group was saccharopolyspora, the biomarker of the CC group was the Proteobacteria, Corynebacterium, Coprococcus, Peptococcus and Ruminococcus.

Conclusions: The study indicated that the compositions of vaginal microbes in different cervical lesions is different. The vaginal microbial composition has a certain diagnostic effect on healthy women, patients with high-risk HPV infection and cervical lesions. These microbes may serve as potential biomarkers for CC. It also provided an effective way for the treatment of HPV infections and cervical lesions.

KEYWORDS

HPV infection, cervical intraepithelial neoplasia, cervical cancer, vaginal microecology, 16srRNA

1 Background

Cervical cancer (CC) is one of the most common malignancies in women which associated with HPV infection in different regions and populations (Crow, 2012). HPV infection is a common female reproductive tract disease, 70% of women may be infected with this disease in their lifetime (Xia et al., 2023), but so far there is still a lack of effective therapy. How to effectively control the replication of HPV virus has become a challenge for clinicians.

The human microbiota is considered to be the “second human genome” (Integrative HMP (iHMP) Research Network Consortium, 2019). As an important part of microbiota, vaginal microbiome plays a vital role in preventing various infectious diseases of the reproductive tract. Recent studies have shown that there is a relatively relationship between vaginal microbiome, HPV infection and cervical lesions (Chao et al., 2019).

The vaginal microecology is a complex and dynamic system, mainly composed of related anatomical structures, human immunity, internal and external environments, and vaginal microbiome. The normal vaginal micro-ecosystem is parasitized by a variety of bacteria such as lactobacillus, streptococcus, and a various microorganisms such as mycoplasma and viruses, and jointly resist the invasion of external pathogenic bacteria (Liang et al., 2019). When the vaginal microecological balance is broken, it will cause a decrease in the number of lactobacilli, an increase in the number of other miscellaneous bacteria, anaerobic bacteria, and changes in vaginal microbial diversity (Borgdorff et al., 2016), which may lead to a series of reproductive tract diseases. A meta-analysis based on related articles published from 2003 to 2017 showed that among sexually active women, those with vaginal microecological imbalance are more likely to develop persistent HPV infections and increase the risk of cervical intraepithelial neoplasia (CIN) (Brussels et al., 2019). Conversely, patients with HPV infection and cervical lesions are also more likely to develop vaginal infectious diseases. The essence of HPV infection is an infectious disease of the reproductive tract. In addition to symptomatic treatment of pathogenic bacteria, attention should be paid to the microecological balance. Therefore, restoring the balance of micro-ecology may be another important way to

prevent and treat CC in the future. V4 region is one of the 9 variable regions of 16SrRNA which has a moderate length and high coverage of bacteria. It has been widely used in microbial sequencing and analysis in recent years (Caporaso et al., 2012). In this study we used the illumina high-throughput gene sequencing technology to determine the V4 region of 16SrRNA and observed the vaginal microbial composition in different cervical lesions. The purpose is to explore the causes of HPV infection and cervical lesions, which will provide relevant theoretical basis for the development of vaginal microecological therapy and microecological preparations, and provide new methods for the diagnosis and treatment of HPV infections and cervical lesions.

2 Materials and methods

2.1 Patients

This study was approved by the Ethics Committee of Suzhou Sci-tech City Hospital. According to the Declaration of Helsinki, written informed consent was obtained from all patients.

All patients were screened strictly according to the following criteria: (1) Having a history of sexual life; (2) Age 20-65 years old; (3) Race is a Han nationality; (4) No history of sexual life, vaginal treatment and flushing 3 days before sampling; (5) No history of antibiotic use within 2 weeks before sampling; (6) No history of radiotherapy and chemotherapy; (7) No history of cervical and vaginal surgery; (8) No sexually transmitted diseases; (9) No abnormal vaginal bleeding or vaginal prolapse and other vaginal diseases; (10) No long-term use of sex hormones, immunosuppressants and steroids; (11) No immune diseases, other malignant tumors and mental diseases; (12) Not during menstruation, pregnancy or lactation. If any criterion is not met, it cannot be included in our study.

125 patients were enrolled in our study who underwent HPV, Thinprep cytologic test (TCT), and colposcopy in the Second Hospital of Dalian Medical University and Suzhou Sci-tech City Hospital from June 2020 to December 2020. HPV typing was

performed by PCR and reverse point hybridization which can detect 28 HPV types, including high-risk HPV: HPV16, 18, 31, 33, 35, 39, 45, 59, 52, 53, 56, 58, 59, and 66, common low-risk HPV: HPV68, CP8304, 6, 11, 43, 73, 82, 26, 40, 42, 44, 54, 61, and 83. According to the HPV and TCT results, they were divided into the normal group (n=27, N: HPV and TCT are negative) and the high-risk HPV group (n=40, H: high-risk HPV positive for at least 1 year, TCT negative). Based on postoperative pathological results, patients with shunt colposcopy, colposcopy biopsy or cervical conization were divided into CIN group (n=40, C: CIN2 or 3) and CC group (n=18, Ca: cervical cancer).

The types of HPV infection, age, menopausal history, pregnancy, birth history, and contraceptive methods in each group of patients were analyzed. Using 16S rRNA-V4 region gene amplification technology and Illumina high-throughput sequencing technology to detect vaginal secretions retained in the GUHE Flora Storage buffer (Zhejiang Hangzhou Equipment Preparation 20190682, GUHE Laboratories, Hangzhou, China), the composition of the vaginal microbiota in each sample is verified. Using bioinformatics analysis methods to analyze the composition of the microbes, diversity of vaginal microbes, microbial markers and microbial predictive ability in each group.

2.2 Sampling method

The patients were maintained the lithotomy position, a sterile vaginal speculum without lubricant was used to gently and fully expose the cervix, rotate the sterile cotton swab on the upper 1/3 section of the vagina and the lower section of the vagina for 10-15 seconds, put the cotton swab with vaginal secretion into the storage solution (Zhejiang Hangzhou equipment preparation 20190682), stir and rinse for 30 seconds, discard the cotton swab and mix the storage solution, the specimen bottles were stored in the refrigerator at -20°C.

2.3 DNA extraction

DNA samples from vaginal secretions were extracted using DNA isolation kit (GUHE Laboratories, Hangzhou, China). Agarose gel electrophoresis and Nano Drop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) were used to measure and detect DNA.

2.4 16S rRNA amplicon pyrosequencing

The forward primer 515F(5'-GTGCCAGCMGCCGCGGTAA-3') and the reverse primer 806R(5'-GGACTACHVGGGTWTCTAAT-3') were used for PCR amplification of the bacterial 16S rRNA gene V4 region. The PCR components contained Phusion High-Fidelity PCR Master Mix (25 µl), which include DNA Template (10 µl), Forward primer(3 µl), Reverse primer(3 µl), DMSO(3 µl), and ddH₂O(6 µl). Thermal cycling includes

initial denaturation for 30 s (98°C), 25 cycles of denaturation for 15 s (98°C), annealing for 15 s (58°C), extension for 15 s (72°C), final extension for 60 s (72°C). PCR amplicons were purified and quantified using AgencourtAMPure XP Beads (Beckman Coulter, Indianapolis, IN, USA) and the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). The Illumina NovoSeq6000 platform (GUHE Info technology Co., Ltd, Hangzhou, China) was used for paired-end 2×150 bp sequencing.

2.5 Sequence analysis

Sequencing data were processed by Quantitative Insights Into Microbial Ecology (QIIME, v1.9.1) (Caporaso et al., 2010). Low-quality sequences were filtered by specific criteria (Chen and Jiang, 2014). Operational taxonomic unit(OTU) was selected by Vsearch v2.4.4 (Rognes et al., 2016) and classified by VSEARCH to search representative sequences in the SILVA132 database (Quast et al., 2013). To prevent environmental contamination, all samples in the project were processed side by side using the same batches of reagents, at the same time, we have added a sample with a control check and a native sample of a standard strain. Decontam was used to remove contamination sequences.

2.6 Bioinformatics and statistical analysis

Sequence data were analyzed by R packages (v3.2.0) and QIIME. The OTU table was used for the calculation of OTU-level alpha diversity and generation of OTU-level ranked abundance curves. Taxa abundance at the phylum, class, order, family, genus and species levels were compared among samples or groups by Kruskal test from R stats package. Kruskal.test and Wilcox.test were used to analyze the alpha diversity of each group. Anosim test was used to determine whether the grouping was meaningful. Linear discriminant analysis was used for effect size (LEfSe) to detect significantly different taxa across groups (Segata et al., 2011).

2.7 Data analysis

SPSS 21.0 (SPSS, Chicago, Illinois, USA) was used for statistical analysis of clinical data. Continuous variables and categorical variables was analyzed by the t-test and χ^2 test or Fisher's test separately. P values <0.05 were considered to be statistically significant.

3 Results

3.1 Clinical data

97 of the 125 patients, were infected with HPV. In those 97 HPV patients, the most HPV subtype is type 16, accounting for 47.4% (n=46), followed by type 52 (22.7%) (n=22), type 58 (16.5%) (n=16)

TABLE 1 Clinical data.

characteristics	N (n=27)	H (n=40)	C (n=40)	Ca (n=18)	χ^2 value	P value
age					7.15	0.067
≤50	24	32	31	10		
>50	3	8	9	8		
menopausal history					6.94	0.074
premenopausal	24	28	31	10		
postmenopausal	3	12	9	8		
fertility status					5.88	0.105*
had not given birth	6	6	3	0		
had given birth	21	34	37	18		
contraceptive methods					14.5	0.192*
Non-contraception	9	20	13	12		
safe period	0	1	0	0		
condom	13	16	17	4		
Intrauterine device	5	3	9	2		
Sterilization	0	0	1	0		
BMI					3.354	0.773*
≤18.5	7	11	8	7		
18.5-25	15	22	27	9		
≥25	5	7	5	2		

P value is calculated using χ^2 and Fishers test (*).

and type 18 (12.4%) (n=12) respectively. In the high-risk HPV group, HPV16 and/or 18 infections accounted for 52.5% (n=21). In the CIN group, HPV16 and/or 18 infections accounted for 50% (n=20), and 1 case without HPV infection, accounting for 2.5%. In the CC group, HPV16 and/or 18 infection accounted for 94.4% (n=17). The ages of the enrolled patients were 21 to 65 years old. There were no statistical differences in general clinical data among the groups including age, menopausal history, fertility status, contraceptive methods and BMI (Table 1).

3.2 Microbial composition

The Relative abundance of phyla in four groups was shown in Figure 1. The Phylum Firmicutes occupied the highest proportion. The relative abundances of Firmicutes in the normal group, high-risk HPV group, CIN group and CC group were 60.1%, 68.7%, 68.6%, and 59.2%, respectively and there was no significant difference among the groups. The other top 10 phyla in vagina are Actinobacteria, Bacteroides, Proteobacteria, Fusobacteria, Tenericutes, Chlamydiae, TM7, Thermi, and Cyanobacteria (Figure 1A). The Wilcoxon test results showed that compared to the normal group, the relative abundance of Cyanobacteria and Proteobacteria in the CIN group was significantly decreased, and that of Thermi was significantly

increased, while that of Cyanobacteria and Thermi in the CC group was significantly decreased. Compared with the CIN group, the relative abundance of Fusobacteria in the CC group was significantly decreased while that of Proteobacteria was significantly increased. In addition, the relative abundance of Cyanobacteria in the CIN group was significantly lower than that of high-risk HPV group (Figures 1B–E).

The Relative abundance of genera in all groups was shown in Figure 2. The Lactobacillus occupied the highest proportion. The relative abundances in the normal group, high-risk HPV group, CIN group, and CC group were 48.9%, 57.4%, 53.8%, 33.6% and the remaining top 10 bacterial genera included Prevotella, Megasphaera, Streptococcus, Sneathia, Dialister, Anaerococcus, Ureaplasma, Peptoniphilus, and Mycoplasma (Figure 2A). The Wilcoxon test results showed that there were significant differences in bacterial genera among groups. For the top 10 bacterial genera, there are significant differences between the high-risk HPV group and the CC group or between the CIN group and the CC group. Compared with the high-risk HPV group, the relative abundance of Lactobacillus in the CC group was significantly reduced while that of Dialister was significantly increased. Compared with the CIN group, the relative abundance of Lactobacillus in the CC group was also significant reduced, while that of Dialister and Peptoniphilus were significantly increased (Figures 2B–G).

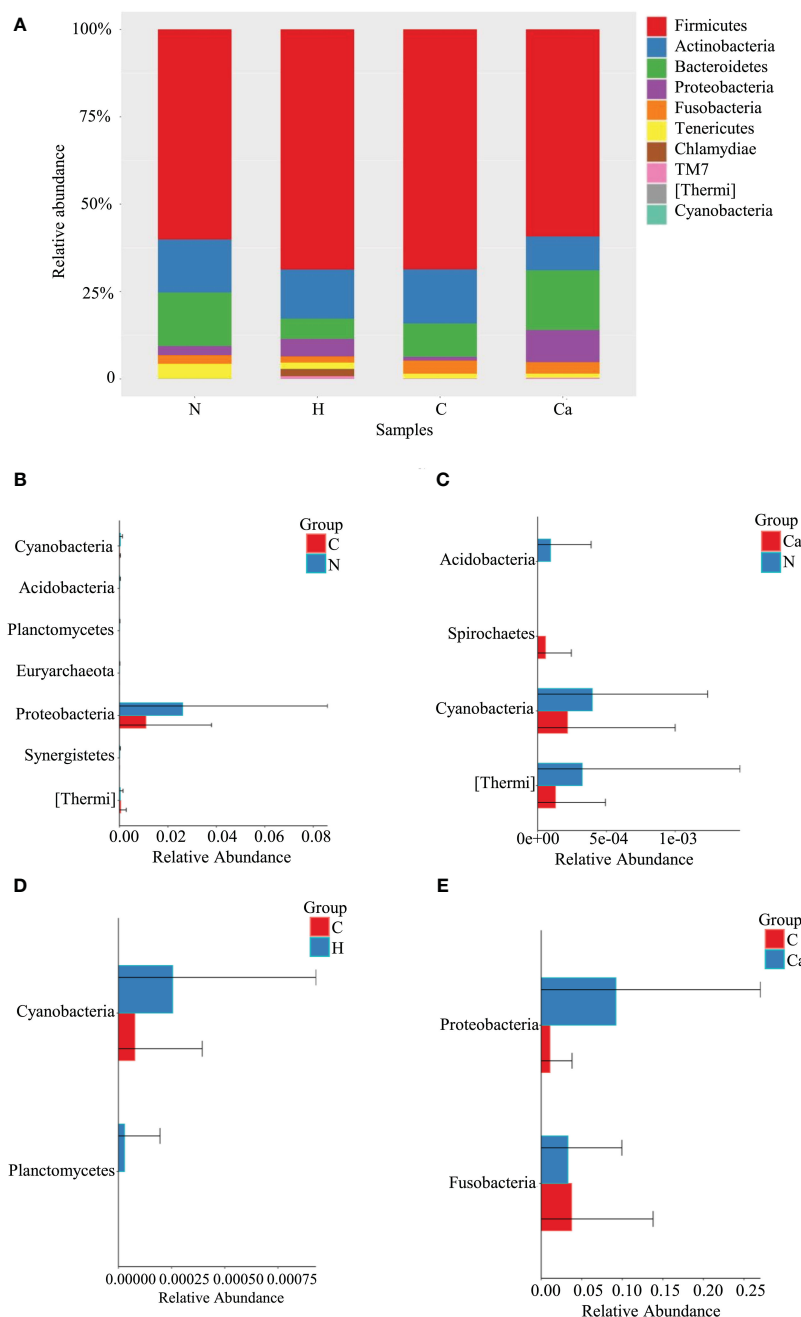


FIGURE 1

Comparative analysis of the relative abundance of phyla in four groups. (A) Bar chart of relative abundance of top 10 phyla in four groups. (B) Histogram of differential phylum between Group N and Group C. (C) Histogram of differential phylum between Group N and Group Ca. (D) Histogram of differential phylum between Group H and Group C. (E) Histogram of differential phylum between Group C and Group Ca.

3.3 Microbial Alpha diversity

First, we described the Alpha diversity of the bacterial community by measuring Chao1 index and Shannon index. Second, we created the rarefaction curves to test the sequencing depth and the result showed that the amount of sampling is reasonable (Figure 3A). We found the Shannon index of the CC group was significantly higher than that of the HPV group ($P=0.034$) and the CIN group ($P=0.019$) (Figure 3B). But there

was no significant difference of the Chao1 index between groups ($P>0.05$) (Figure 3C).

3.4 Biomarkers

In order to fully determine the role of vaginal microbiota and the differences in microbial between the groups, we performed the Anosim test. The results showed that there were significant

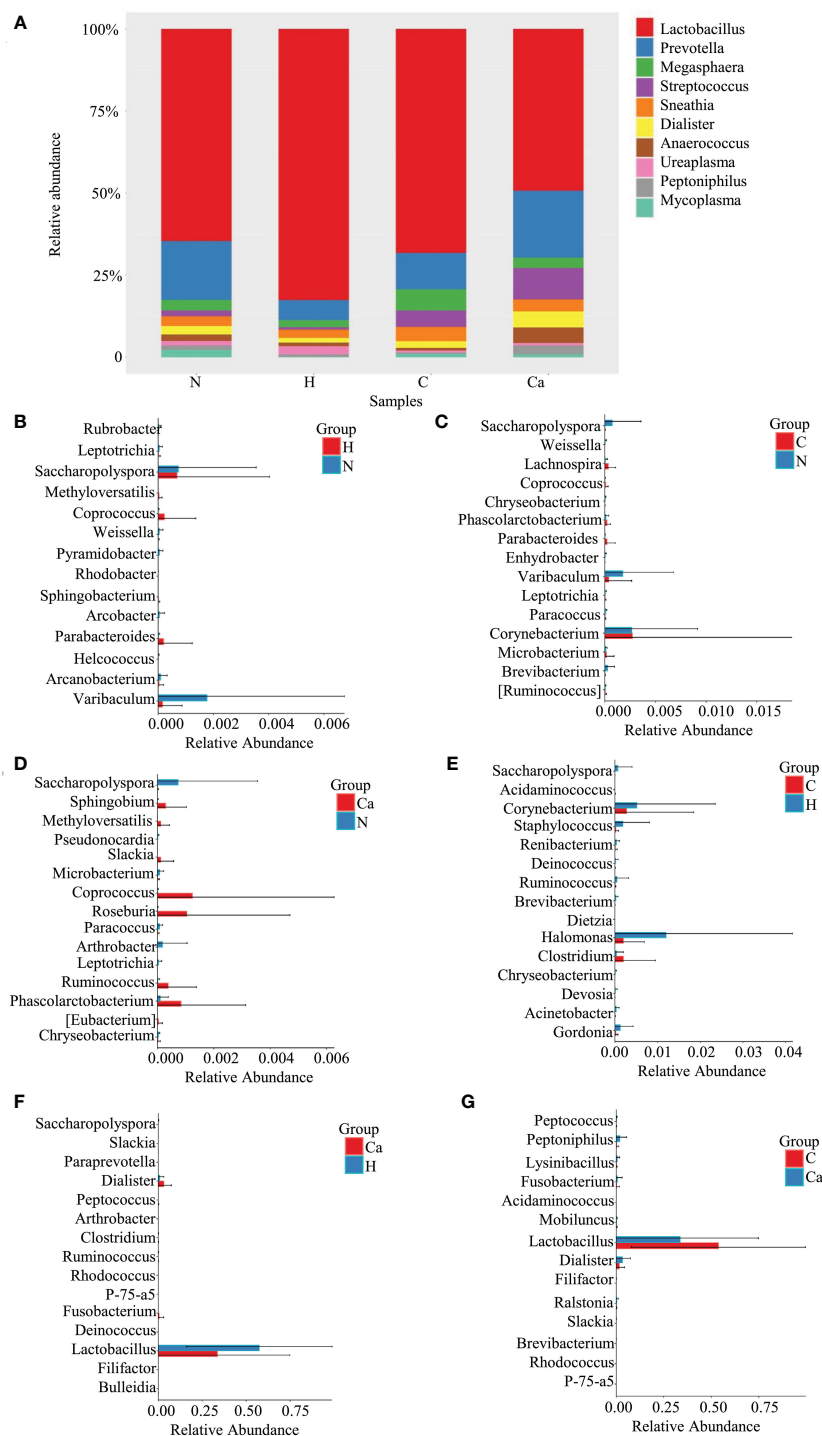


FIGURE 2

Comparative analysis of the relative abundance of genera in four groups. (A) Bar chart of relative abundance of top 10 genera in four groups. (B) Histogram of differential genera between Group N and Group H. (C) Histogram of differential genera between Group N and Group C. (D) Histogram of differential genera between Group N and Group Ca. (E) Histogram of differential genera between Group H and Group C. (F) Histogram of differential genera between Group H and Group Ca. (G) Histogram of differential genera between Group C and Group Ca.

differences between the groups ($P=0.044$) (Figure 4). Furthermore, the composition of vaginal microbes in each group was analyzed by LEfSe, and Figure 5 showed the clade diagram and bar graph of LDA value (only microbes with $LDA>2$). In this way, biomarkers

(species with significant differences between groups) were screened between groups. The difference markers between groups selected by LEfSe are the dominant species, and these markers are distinguished by specific significant differences between this group and other

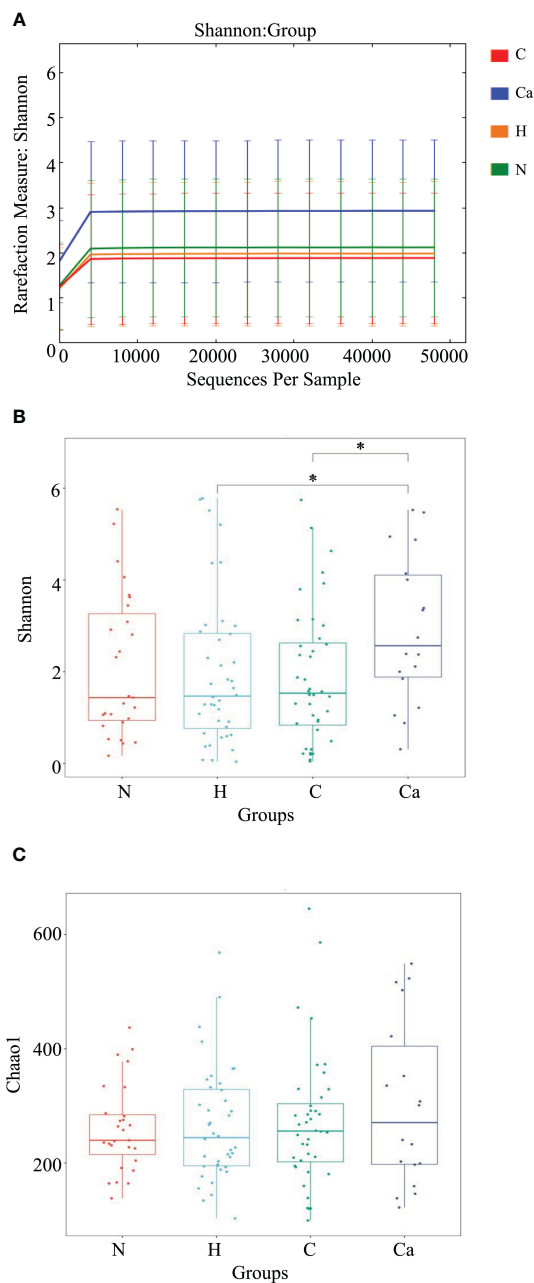


FIGURE 3
The microbial Alpha diversity analysis in different groups. (A) Shannon-Winner curve in different groups. (B) Shannon index box chart for each group. (C) Chao1 index box chart for each group. (* $P < 0.05$).

groups. At the phylum level, the biomarker of the CC group was the Proteobacteria, and at the genus level, the biomarkers of the CC group were *Corynebacterium* (LDA=3.16), *Coprococcus* (LDA=2.27), *Peptococcus* (LDA=2.25) and *Ruminococcus* (LDA=2.08), the biomarker in the high-risk HPV group was *saccharopolyspora* (LDA=2.04), the biomarker in the normal group was *varibaculum* (LDA=2.34), and no biomarker have been found in the CIN group (Figure 5).

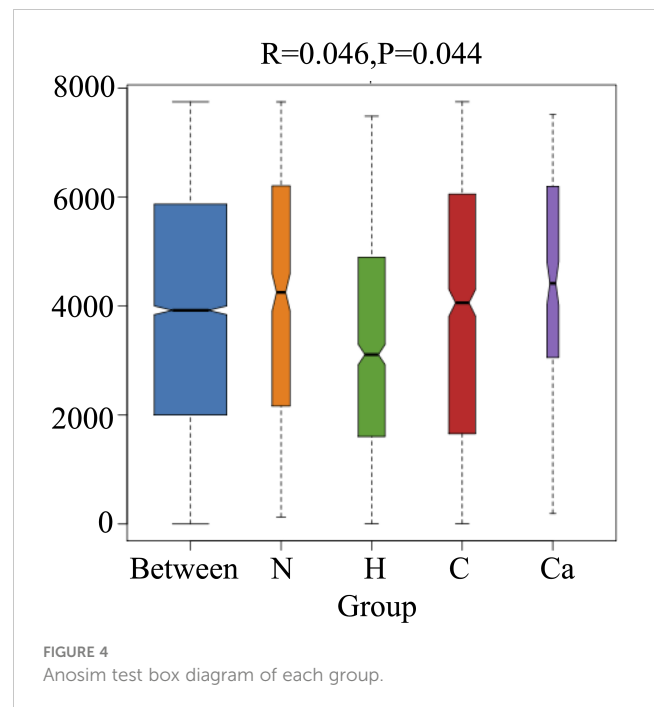


FIGURE 4
Anosim test box diagram of each group.

3.5 Microbial prediction ability

In this study, 50 different bacterial communities were screened, a random forest graph was made (Figure 6), and an ROC curve was plotted (Figure 7). The results showed that the vaginal microbial composition had a predictive effect on each group. The prediction accuracy of HPV group (AUC=0.51) and CC group (AUC=0.68) was lower than that of the normal group (AUC=0.75) and CIN group (AUC=0.71).

4 Discussion

The female reproductive tract is a channel that communicates with the outside world which is easily invaded by external microorganisms, leading to HPV infections and other infectious diseases. The infection of high-risk HPV subtypes may lead to the CC (Yang et al., 2020). Consistent with the previous study, this study showed that more than 90% of patients with CIN or cervical cancer are infected with high-risk HPV. Among all HPV subtypes, types 16 and 18 are considered to have the highest carcinogenic effects (de Sanjosé et al., 2007) (Torre et al., 2015). However, most HPV infections subside within a short period of time, and only a few patients will gradually cause CC after prolonged infection. It is known that the vaginal microecology has played an important role in the process of HPV infections-induced cervical lesions (Kovachev, 2020). Vaginal microecology is affected by the factors such as age, menstruation, medication, vaginal lavage, contraceptive methods and other factors. It is a dynamic equilibrium system formed by the mutual restriction and coordination of microorganisms and the environment in the host (Mittra et al.,

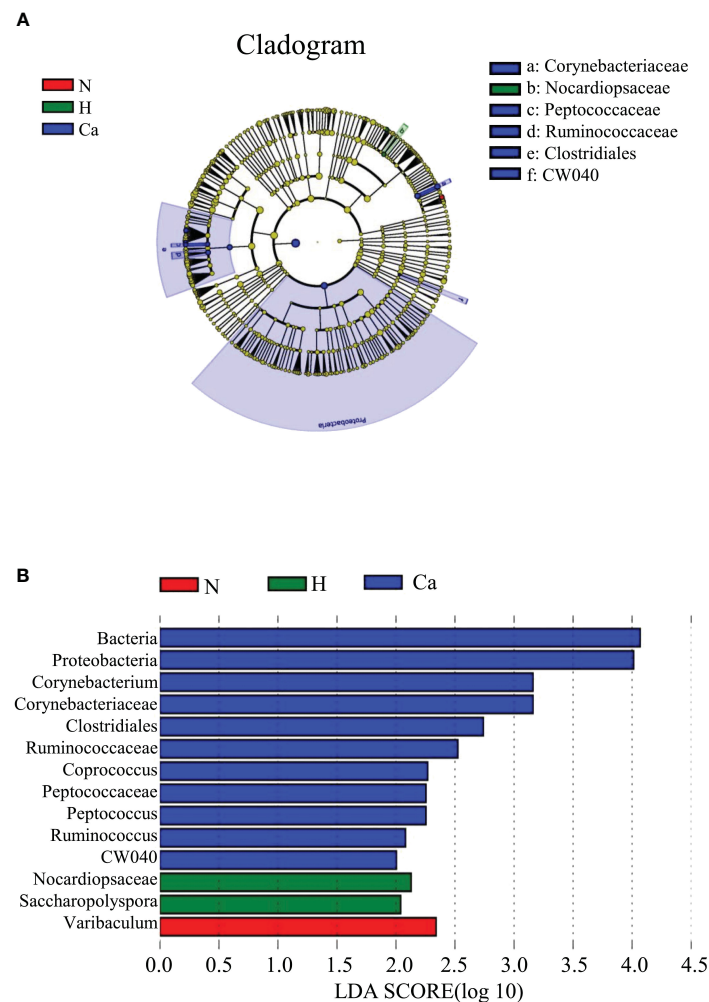


FIGURE 5

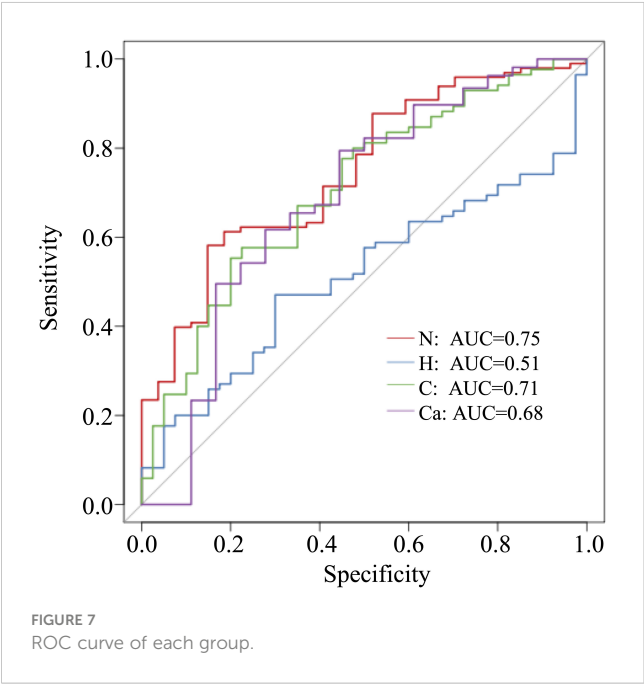
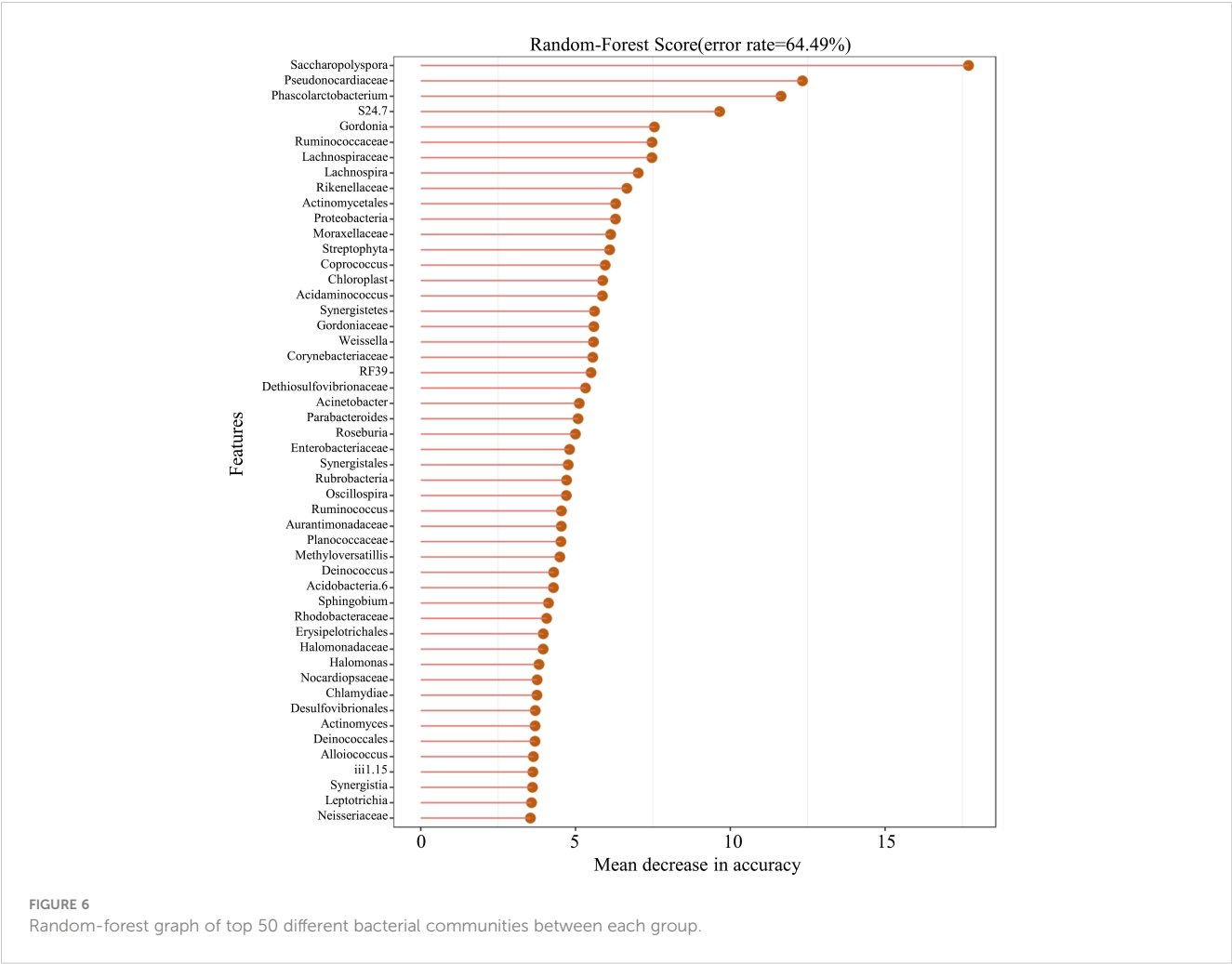
Biomarkers of each group. (A) The evolutionary branch diagram of microorganism with significant differences in each group. (B) LDA value histogram of microorganism with significant differences in each group.

2016). If the vaginal microecological balance is broken and the immune system is compromised, foreign microorganisms will invade the reproductive system and reduce the immune system's clearance rate of HPV and other pathogens (Chehoud et al., 2017).

Firmicutes is the dominant phyla and *Lactobacillus* is the dominant genera for normal vaginal microbiome. Similar to previous studies (Walther-Antônio et al., 2016; Chao et al., 2019; Norenhag et al., 2020; So et al., 2020; Xie et al., 2020), our study found that Firmicutes did not differ significantly between the groups. Compared with the cervical lesions group, *Lactobacillus* were significantly reduced in the CC group, while the relative abundance of *Dialister* and *Peptoniphilus* was significantly increased. It has been suggested that pathogenic mechanism of *Peptoniphilus* may be related to its strong adhesion to the vaginal epithelium and the formation of biofilms (Patterson et al., 2010), while the pathogenic mechanism of *Lactobacillus* may vary with different strains (Ravel et al., 2011; Tekla et al., 2023). The research on Proteobacteria is inconclusive (Chao et al., 2019; Cheng et al., 2020). Recent study has found that Proteobacteria in colon cancer are positively correlated with tumor burden (Lee et al., 2021). Our study also showed that

Proteobacteria in CC patients has significantly increased compared with CIN patients. It indicated that Proteobacteria may be involved in tumor progression. Fusobacteria is another one of the common bacterial phyla in the vagina. Harrandah et al. found that Fusobacteria potentially enhanced the invasiveness and survival rate of cancer cells in the oral cavity (Harrandah et al., 2020), but our results demonstrated that the relative abundance of Fusobacteria has decreased significantly in the development of cervical lesions, The reasons for this difference may due to the selection of different cases, different geographic region and microenvironment. Therefore, it needs to be further explored.

There are few studies on the distribution and function of cyanobacteria in human microecology all over the world. Previous studies have shown that cyanobacteria are one of the common intestinal microorganisms of small intestinal adenocarcinoma (Pan et al., 2021). Cyanobacteria are also described in the breast. Some studies show that the relative abundance of cyanobacteria in Chinese women is significantly higher than that in Slovak women and it indicates that cyanobacteria are related to the geographical area. The relative abundance of cyanobacteria in primary breast cancer is lower



than that of healthy women in China (Hadzega et al., 2021) and this result similar to ours. Although its mechanism is unclear, it suggests that cyanobacteria may play an inhibitory role in tumor progression. However, there are few studies on the physiological role and mechanism of cyanobacteria in the vaginal. Our findings may lay a foundation for future research. Previous studies have shown that thermi may be related to HPV16 (Huang et al., 2018). Other studies have shown that the relative abundance of thermi is higher in advanced lung cancer than in early lung cancer (Yu et al., 2016). Our study also suggests that the relative abundance of thermi in CIN group is high. It indicates that thermi may be related to tumors development. However, the specific mechanism remains to be further explored.

In recent years, the research on the diversity of the vaginal microbiome has gradually become a hot topic, and Alpha diversity is the main indicator to measure the diversity and richness of microbial communities. The diversity index is mainly used to measure the heterogeneity of the community, and the richness is mainly used to measure the number of species in a single sample. A study on the change of vaginal microecological under different cervical lesions showed that patients with normal cervix had a higher vaginal microbiome richness than patients with cervical

lesions, but the difference was not statistically significant (Wu et al., 2020). Another study has also shown that there is no significant difference in the abundance and diversity of vaginal bacteria under different cervical lesions, but HPV infection significantly increases the abundance and diversity of vaginal bacteria (Chen et al., 2020). Mitra et al. found that following the degree of cervical lesions increases, the richness and diversity of vaginal bacteria also increase (Tekka et al., 2023). In current study, the Shannon index was used to estimate the microbial diversity, and the Chao1 index was used to estimate the microbial richness. The results showed that there is no significant difference in microbial richness but the diversity of vaginal microbiome in patients with CC was significantly higher than that in patients with high-risk HPV infection and CIN patients. These different results in various studies on the diversity of vaginal microbiome may be related to different geographic regions, races, sample numbers, and sample collection techniques (Kovachev, 2020). A study involving almost all ethnic females showed that the risk of increased microbiota diversity and vaginal microecological imbalance in Caribbean African women is four times than that of European/Caucasian and African women (Mortaki et al., 2020), indicating that race is a key factor affecting the vaginal bacterial community. All the research objects of this study are from the Han nationality, it only represent the characteristics of vaginal microorganisms of the Han nationality. Based on the above studies, a worldwide multi-center study may be quite necessary.

It has been believed that there are characteristic biomarkers in patients with HPV infection and different degrees of cervical lesions, the biomarkers derived from the results of various studies are different. In recent years, studies found that *Delftia* and *Gardnerella* may be biomarkers of cervical precancerous lesions (Usyk et al., 2020; Wu et al., 2020) and bacteria such as *Gardnerella vaginalis*, *Gardnerella*, *Peptostreptococcus anaerobius*, *Mobiluncus curtisii*, *Prevotella* genus and *Fusobacterium nucleatum* may be characteristic biomarkers of HPV16-positive patients (Yang et al., 2020). This study used LEfSe analysis to explore the characteristic biomarkers and showed that *Proteobacteria*, *Corynebacterium*, *Coprococcus*, *Peptococcus* and *Ruminococcus* may be used as biomarkers for CC patients. The biomarker for high-risk HPV patients is *Saccharopolyspora*, and the biomarker for normal patients is *varibaculum*. These characteristic bacterial genera are not only related to the degree of different cervical lesions, but may also be related to certain reproductive tract infections. Previous studies have shown that *Proteobacteria* (Wei et al., 2023), *Corynebacterium* (Wang et al., 2014) and *Peptococcus* (Lefèvre et al., 1985) are related to bacterial vaginosis. The relationship between CC and bacterial vaginosis is also worthy of further study. *Varibaculum* is a kind of actinomycete and previous studies have shown that its specific strain may exist in the intestine of premature infants (Brown et al., 2013). And a data searched in HMP showed that *Varibaculum* in healthy human microecology were mainly distributed in the vagina (Integrative HMP (iHMP) Research Network Consortium, 2019) and it is consistent with our results. Recently, a new strain of *varibaculum* has been extracted from the vagina of healthy Senegalese women (Fall et al., 2019). Therefore, the

distribution and mechanism of *varibaculum* and its subordinate species may be a new research field of vaginal microecology. The composition of the vaginal microbiome is different between patients with CIN1 and CIN2, and this different composition has extremely high accuracy in predicting the severity of CIN (Lee et al., 2020). Our results also demonstrated that the composition of vaginal microbes may predict high-risk HPV infection and cervical lesions.

5 Conclusion

This study found that the vaginal microecology is different under various pathological conditions of the cervix, but no difference in the richness of vaginal microbes. The diversity of vaginal species in CC patients is mainly manifested as a reduction in the number of *Cyanobacteria* and *Lactobacillus*, accompanied by an increase in the number of *Dialister*, *Peptoniphilus* and other miscellaneous bacteria. Different cervical lesions have specific biomarkers, so the composition of the vaginal microecological flora has a diagnostic effect on cervical lesions. These results may provide new evidences for the diagnosis of HPV infection and cervical lesions. Due to the changes in the vaginal microecology under cervical lesions, restoring the balance of vaginal microecology may be an effective way for the treatment of HPV infection and cervical lesions.

Data availability statement

The raw data used to support the findings of this study are provided in the following links: https://www.jianguoyun.com/p/DdRG93UQ_svSDBjQidkFIAA.

Ethics statement

The studies involving humans were approved by The Ethical Committee of Suzhou Hospital, Affiliated Hospital of Medical School, Nanjing University approved the study protocol under the No. of IRB202110002RI. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

ZF: Conceptualization, Investigation, Project administration, Supervision, Validation, Writing – review & editing, Writing – original draft. DH: Data curation, Investigation, Writing – review & editing. XF: Project administration, Resources, Supervision, Validation, Writing – review & editing. YZ: Data curation, Investigation, Visualization, Writing – original draft, Writing – review & editing. LZ: Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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References

- Borgdorff, H., Armstrong, S. D., Tytgat, H. L., Ndayisaba, G. F., Wastling, J. M., van de Wijgert, J. H., et al. (2016). Unique Insights in the Cervicovaginal *Lactobacillus* iners and *L. crispatus* Proteomes and Their Associations with Microbiota Dysbiosis. *PLoS One* 11, e0150767. doi: 10.1371/journal.pone.0150767
- Brown, C. T., Sharon, I., Thomas, B. C., Castelle, C. J., Morowitz, M. J., and Banfield, J. F. (2013). Genome resolved analysis of a premature infant gut microbial community reveals a *Varibaculum cambriense* genome and a shift towards fermentation-based metabolism during the third week of life. *Microbiome* 1, 30. doi: 10.1186/2049-2618-1-30
- Brusselsaers, N., Shrestha, S., van de Wijgert, J., and Verstraelen, H. (2019). Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. *Am. J. Obstet Gynecol.* 221, 9–18.e8. doi: 10.1016/j.ajog.2018.12.011
- 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, 335–336. doi: 10.1038/nmeth.f.303
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., et al. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1621–1624. doi: 10.1038/ismej.2012.8
- Chao, X. P., Sun, T. T., Wang, S., Fan, Q. B., Shi, H. H., Zhu, L., et al. (2019). Correlation between the diversity of vaginal microbiota and the risk of high-risk human papillomavirus infection. *Int. J. Gynecol. Cancer.* 29, 28–34. doi: 10.1136/ijgc-2018-000032
- Chehoud, C., Stieh, D. J., Bailey, A. G., Laughlin, A. L., Allen, S. A., McCotter, K. L., et al. (2017). Associations of the vaginal microbiota with HIV infection, bacterial vaginosis, and demographic factors. *AIDS.* 31, 895–904. doi: 10.1097/QAD.0000000000001421
- Chen, H., and Jiang, W. (2014). Application of high-throughput sequencing in understanding human oral microbiome related with health and disease. *Front. Microbiol.* 5, 508. doi: 10.3389/fmicb.2014.00508
- Chen, Y., Qiu, X., Wang, W., Li, D., Wu, A., Hong, Z., et al. (2020). Human papillomavirus infection and cervical intraepithelial neoplasia progression are associated with increased vaginal microbiome diversity in a Chinese cohort. *BMC Infect. Dis.* 20, 629. doi: 10.1186/s12879-020-05324-9
- Cheng, W., Xu, F., Gao, L., and Liu, J. (2020). The correlation between the determination of vaginal micro-ecological composition and the outcome of HPV infection by high-throughput metagenome sequencing information technology on the illumina platform. *J. Infect. Public Health* 13, 1961–1966. doi: 10.1016/j.jiph.2020.05.024
- Crow, J. M. (2012). HPV: The global burden. *Nature.* 488, S2–S3. doi: 10.1038/488S2a
- de Sanjosé, S., Diaz, M., Castellsagué, X., Clifford, G., Bruni, L., Muñoz, N., et al. (2007). Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect. Dis.* 7, 453–459. doi: 10.1016/S1473-3099(07)70158-5
- Fall, N. S., Lo, C. I., Fournier, P. E., Sokhna, C., Raoult, D., Fenollar, F., et al. (2019). *Arcanobacterium ihumii* sp. nov., *Varibaculum vaginae* sp. nov. and *Tessaracoccus timonensis* sp. nov., isolated from vaginal swabs from healthy Senegalese women. *New Microbes New Infect.* 31, 100585. doi: 10.1016/j.nmni.2019.100585
- Hadzega, D., Minarik, G., Karaba, M., Kalavaska, K., Benca, J., Ciernikova, S., et al. (2021). Uncovering microbial composition in human breast cancer primary tumour tissue using transcriptomic RNA-seq. *Int. J. Mol. Sci.* 22, 9058. doi: 10.3390/ijms22169058
- Harrandah, A. M., Chukkappalli, S. S., Bhattacharyya, I., Progulsk-Fox, A., and Chan, E. (2020). *Fusobacteria* modulate oral carcinogenesis and promote cancer progression. *J. Oral. Microbiol.* 13, 1849493. doi: 10.1080/20002297.2020.1849493
- Huang, X., Li, C., Li, F., Zhao, J., Wan, X., and Wang, K. (2018). Cervicovaginal microbiota composition correlates with the acquisition of high-risk human papillomavirus types. *Int. J. Cancer.* 143, 621–634. doi: 10.1002/ijc.31342
- Integrative HMP (iHMP) Research Network Consortium (2019). The integrative human microbiome project. *Nature* 569, 641–648. doi: 10.1038/s41586-019-1238-8
- Kovachev, S. M. (2020). Cervical cancer and vaginal microbiota changes. *Arch. Microbiol.* 202, 323–327. doi: 10.1007/s00203-019-01747-4
- Lee, J. G., Lee, Y. R., Lee, A. R., Park, C. H., Han, D. S., and Eun, C. S. (2021). Role of the global gut microbial community in the development of colitis-associated cancer in a murine model. *BioMed. Pharmacother.* 135, 111206. doi: 10.1016/j.biopha.2020.111206
- Lee, Y. H., Kang, G. U., Jeon, S. Y., Tägele, S. B., Pham, H. Q., Kim, M. S., et al. (2020). Vaginal microbiome-based bacterial signatures for predicting the severity of cervical intraepithelial neoplasia. *Diagnostics (Basel).* 10, 1013. doi: 10.3390/diagnostics10121013
- Lefèvre, J. C., Jean, M., Averous, S., Viraben, R., Blanc, C., Bauriaud, R., et al. (1985). [Etiology of bacterial vaginosis (non-specific vaginitis)]. *J. Gynecol Obstet Biol. Reprod. (Paris).* 14, 703–708.
- Liang, Y., Chen, M., Qin, L., Wan, B., and Wang, H. (2019). A meta-analysis of the relationship between vaginal microecology, human papillomavirus infection and cervical intraepithelial neoplasia. *Infect. Agent Cancer.* 14, 29. doi: 10.1186/s13027-019-0243-8
- Mitra, A., MacIntyre, D. A., Marchesi, J. R., Lee, Y. S., Bennett, P. R., and Kyrgiou, M. (2016). The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next. *Microbiome.* 4, 58. doi: 10.1186/s40168-016-0203-0
- Mortaki, D., Gkegkes, I. D., Psomiadou, V., Blontzos, N., Prodromidou, A., Lefkopoulou, F., et al. (2020). Vaginal microbiota and human papillomavirus: a systematic review. *J. Turk Ger Gynecol Assoc.* 21, 193–200. doi: 10.4274/jtgga.
- Norenhaag, J., Du, J., Olovsson, M., Verstraelen, H., Engstrand, L., and Brusselsaers, N. (2020). The vaginal microbiota, human papillomavirus and cervical dysplasia: a systematic review and network meta-analysis. *BJOG.* 127, 171–180. doi: 10.1111/1471-0528.15854
- Pan, H., Cheng, H., Wang, H., Ge, W., Yuan, M., Jiang, S., et al. (2021). Molecular profiling and identification of prognostic factors in Chinese patients with small bowel adenocarcinoma. *Cancer Sci.* 112 (11), 4758–4771. doi: 10.1111/cas.15119
- Patterson, J. L., Stull-Lane, A., Girerd, P. H., and Jefferson, K. K. (2010). Analysis of adherence, biofilm formation and cytotoxicity suggests a greater virulence potential of *Gardnerella vaginalis* relative to other bacterial-vaginosis-associated anaerobes. *Microbiol. (Reading).* 156, 392–399. doi: 10.1099/mic.0.034280-0
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks1219
- Ravel, J., Gajer, P., Abdo, Z., Schneider, G. M., Koenig, S. S., McCulle, S. L., et al. (2011). Vaginal microbiome of reproductive-age women. *Proc. Natl. Acad. Sci. U S A.* 108 Suppl 1, 4680–4687. doi: 10.1073/pnas.1002611107
- Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ.* 4, e2584. doi: 10.7717/peerj.2584

Conflict of interest

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- 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
- So, K. A., Yang, E. J., Kim, N. R., Hong, S. R., Lee, J. H., Hwang, C. S., et al. (2020). Changes of vaginal microbiota during cervical carcinogenesis in women with human papillomavirus infection. *PLoS One* 15, e0238705. doi: 10.1371/journal.pone.0238705
- Teka, B., Yoshida, K., Firdawoke, E., Chanyalew, Z., Gizaw, M., Addissie, A., et al. (2023). Cervicovaginal microbiota profiles in precancerous lesions and cervical cancer among Ethiopian women. *Microorganisms* 11, 833. doi: 10.3390/microorganisms11040833
- Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet-Tieulent, J., and Jemal, A. (2015). Global cancer statistics, 2012. *CA Cancer J. Clin.* 65, 87–108. doi: 10.3322/caac.21262
- Usyk, M., Zolnik, C. P., Castle, P. E., Porras, C., Herrero, R., Gradissimo, A., et al. (2020). Cervicovaginal microbiome and natural history of HPV in a longitudinal study. *PLoS Pathog.* 16, e1008376. doi: 10.1371/journal.ppat.1008376
- Walther-Antônio, M. R., Chen, J., Multinu, F., Hokenstad, A., Distad, T. J., Cheek, E. H., et al. (2016). Potential contribution of the uterine microbiome in the development of endometrial cancer. *Genome Med.* 8, 122. doi: 10.1186/s13073-016-0368-y
- Wang, B., Xiao, B. B., Shang, C. G., Wang, K., Na, R. S., Nu, X. X., et al. (2014). Molecular analysis of the relationship between specific vaginal bacteria and bacterial vaginosis metronidazole therapy failure. *Eur. J. Clin. Microbiol. Infect. Dis.* 33, 1749–1756. doi: 10.1007/s10096-014-2128-5
- Wei, G., Liu, Q. Y., Wang, X. Y., Zhou, Z., Zhao, X., Zhou, W., et al. (2023). A probiotic nanozyme hydrogel regulates vaginal microenvironment for *Candida* vaginitis therapy. *Sci. Adv.* 9, eadg0949. doi: 10.1126/sciadv.adg0949
- Wu, M., Gao, J., Wu, Y., Li, Y., Chen, Y., Zhao, F., et al. (2020). Characterization of vaginal microbiota in Chinese women with cervical squamous intra-epithelial neoplasia. *Int. J. Gynecol. Cancer.* 30, 1500–1504. doi: 10.1136/ijgc-2020-001341
- Xia, S., Li, S., and Li, H. (2023). HPV-infection status and urinary incontinence: a population-based analysis of the NHANES 2005–2016. *World J. Urol.* 41, 1597–1603. doi: 10.1007/s00345-023-04425-9
- Xie, Y., Feng, Y., Li, W., Zhan, F., Huang, G., Hu, H., et al. (2020). Revealing the disturbed vaginal microbiota caused by cervical cancer using high-throughput sequencing technology. *Front. Cell Infect. Microbiol.* 10, 538336. doi: 10.3389/fcimb.2020.538336
- Yang, J., Wang, W., Wang, Z., Wang, Z., Wang, Y., Wang, J., et al. (2020). Prevalence, genotype distribution and risk factors of cervical HPV infection in Yangqu, China: a population-based survey of 10086 women. *Hum. Vaccin Immunother.* 16, 1645–1652. doi: 10.1080/21645515.2019.1689743
- Yang, Q., Wang, Y., Wei, X., Zhu, J., Wang, X., Xie, X., et al. (2020). The alterations of vaginal microbiome in HPV16 infection as identified by shotgun metagenomic sequencing. *Front. Cell Infect. Microbiol.* 10, 286. doi: 10.3389/fcimb.2020.00286
- Yu, G., Gail, M. H., Consonni, D., Carugno, M., Humphrys, M., Pesatori, A. C., et al. (2016). Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features. *Genome Biol.* 17, 163. doi: 10.1186/s13059-016-1021-1



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Revealing the association between East Asian oral microbiome and colorectal cancer through Mendelian randomization and multi-omics analysis

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Background: Colorectal cancer (CRC) poses a global health threat, with the oral microbiome increasingly implicated in its pathogenesis. This study leverages Mendelian Randomization (MR) to explore causal links between oral microbiota and CRC using data from the China National GeneBank and Biobank Japan. By integrating multi-omics approaches, we aim to uncover mechanisms by which the microbiome influences cellular metabolism and cancer development.

Methods: We analyzed microbiome profiles from 2017 tongue and 1915 saliva samples, and GWAS data for 6692 CRC cases and 27178 controls. Significant bacterial taxa were identified via MR analysis. Single-cell RNA sequencing and enrichment analyses elucidated underlying pathways, and drug predictions identified potential therapeutics.

Results: MR identified 19 bacterial taxa significantly associated with CRC. Protective effects were observed in taxa like RUG343 and *Streptococcus_umgs_2425*, while HOT-345_umgs_976 and W5053_sp000467935_mgs_712 increased CRC risk. Single-cell RNA sequencing revealed key pathways, including JAK-STAT signaling and tyrosine metabolism. Drug prediction highlighted potential therapeutics like Menadione Sodium Bisulfite and Raloxifene.

Conclusion: This study establishes the critical role of the oral microbiome in colorectal cancer development, identifying specific microbial taxa linked to CRC risk. Single-cell RNA sequencing and drug prediction analyses further elucidate key pathways and potential therapeutics, providing novel insights and personalized treatment strategies for CRC.

KEYWORDS

oral microbiome, colorectal cancer, Mendelian randomization, single-cell RNA sequencing, therapeutic targets

1 Introduction

Colorectal cancer (CRC) remains one of the most prevalent cancer types globally. According to the 2020 GLOBOCAN statistics, CRC is ranked as the third most commonly diagnosed cancer worldwide, accounting for 10% of cases, and the second leading cause of cancer death, responsible for 9.4% of mortality (Sung et al., 2021). Furthermore, projections suggest a significant increase of approximately 3.2 million new CRC cases by 2040, posing substantial challenges to global healthcare systems (Xi and Xu, 2021). The incidence of CRC is higher in highly developed nations and is on the rise in middle- to low-income countries due to Westernization (Kwong et al., 2018).

Over the past decade, dysbiosis in the oral microbiome has enhanced our understanding of the pathogenesis of oral cancers and other diseases in distant organs. *Porphyromonas gingivalis*, a primary pathogen in periodontal disease (Mysak et al., 2014), is also implicated in other cancers such as pancreatic cancer (Öğrendik, 2017). Additionally, *Candida albicans* infections can activate oncogenes, overexpress inflammatory signaling pathways, and induce DNA damage, contributing to the progression of oral cancer and the onset of gastric cancer (Zaura et al., 2014; Engku Nasrullah Satiman et al., 2020). Studies have also indicated a link between the oral microbiome and colorectal cancer (Collins et al., 2011; Warren et al., 2013; Davey Smith and Hemani, 2014).

Based on these literary evidences, the oral microbiome may exert a distal influence on the onset and progression of colorectal cancer. We will employ Mendelian Randomization (MR), a powerful tool for causal inference in epidemiology (Bowden and Holmes, 2019; Weith and Beyer, 2023). Unlike conventional observational studies, MR as a genetic variation-based method for causal inference, effectively addresses the limitations of observational studies. Traditional observational studies often struggle with confounding factors and reverse causation, making accurate causal inference challenging. MR uses genetic variants, such as single nucleotide polymorphisms (SNPs), as instrumental variables. These variants are randomly allocated according to Mendel's laws, ensuring that they are independent of confounding factors. This approach minimizes bias inherent in traditional observational studies, enabling more accurate

identification of causal relationships between exposures and diseases, thereby enhancing the reliability and validity of research finding (Davey Smith and Hemani, 2014; Li et al., 2024). MR leverages genetic variants as instrumental variables to assess the causal relationship between the oral microbiome and colorectal cancer. This approach will aid in determining the true role of the oral microbiome in the development and progression of colorectal cancer. Moreover, this study will integrate single-cell transcriptomics and bulk RNA sequencing technologies to elucidate the underlying mechanisms of the oral microbiome in CRC development comprehensively (Yazar et al., 2022). Single-cell transcriptomics offers high-resolution insights into cell types and functional characteristics, facilitating a better understanding of the interactions between the oral microbiome and colorectal cancer (Jiang et al., 2024). In contrast, bulk RNA sequencing provides an overview of gene expression, further validating and complementing the findings from single-cell transcriptomics. Additionally, our research will analyze and align potential therapeutic drugs to explore new treatment strategies for colorectal cancer. By combining the regulatory mechanisms of the oral microbiome with existing drug databases, we can identify potential therapeutic agents and further validate their efficacy and safety. Our aim is to more accurately assess the relationship between the oral microbiome and colorectal cancer, uncover the mechanisms involved, and provide new insights and strategies for personalized treatment of colorectal cancer.

2 Materials and methods

2.1 MR design

The interplay between the oral microbiome and colorectal cancer is an area of growing scientific inquiry. There is increasing evidence that suggests oral bacteria may influence the development of colorectal cancer through mechanisms such as microbial dysbiosis and systemic inflammation.

This study adheres to the STROBE-MR guidelines, which are part of the broader Strengthening the Reporting of Observational

Studies in Epidemiology (STROBE) initiative, to ensure high-quality reporting of observational data.

To ensure the integrity of the Mendelian Randomization analysis, the genetic variants serving as instrumental variables must satisfy three critical assumptions: (1) the variants must show strong associations with specific taxa of the oral microbiome, thereby clearly defining the exposure variable in our study; (2) the variants must be independent of unmeasured confounders that could potentially bias the results, ensuring that observed associations are not affected by factors such as lifestyle or genetic background; (3) the impact of these variants on the risk of colorectal cancer must occur exclusively through alterations in the oral microbiome, thus excluding any alternative mediating pathways and emphasizing the unique role of the microbiome (Emdin et al., 2017).

2.2 Data source

The exposure data for this study were sourced from CNGBdb and encompassed comprehensive microbiome profiles from an East Asian cohort. This dataset included 309 tongue dorsum microbiomes with a total of 2,017 samples and 285 salivary microbiomes comprising 1,915 samples (Liu et al., 2021). Notably, these samples represent the first large-scale collection of its kind, featuring high-depth whole genome sequencing. Rigorous criteria were employed to ensure data quality, including a variant calling rate of no less than 98%, a mean sequencing depth exceeding 20x, and the absence of population stratification as confirmed by principle component analysis (PCA). Additionally, related individuals were excluded based on pairwise identity by descent estimates. The study further implemented strict selection protocols, requiring a minimum mean depth of 8x, Hardy-Weinberg equilibrium (HWE) values greater than 10^{-5} , and a genotype calling rate above 98%. Following these stringent quality control measures, a robust cohort of 2,984 participants was established, comprised of 2,017 individuals with tongue dorsum samples and 1,915 with salivary samples. The dataset maintained for analysis included approximately 10 million genetic variants, both common and low-frequency, with a minor allele frequency (MAF) of at least 0.5%.

In contrast, the genome-wide association study (GWAS) data for colorectal cancer were obtained from the Biobank Japan (BBJ), which included a sample size of 33,870, encompassing 6,692

patients and 27,178 controls from the general population. This comprehensive data collection facilitated the exploration of genetic correlations and potential causative links between the oral microbiome and colorectal cancer within this population. Detailed information could be viewed in Table 1.

2.3 Genetic instruments selection

Prior to data analysis, several criteria were established to optimize the selection of instrumental variables. Firstly, a significance threshold was set with p-values greater than 1×10^{-5} , allowing a liberal inclusion of SNPs to enhance statistical power. Linkage disequilibrium (LD) was calculated using reference populations such as the 1000 Genomes European panel, selecting SNPs with a low LD threshold ($r^2 < 0.001$, 10,000 kb) and prioritizing those with lower p-values. Only SNPs with an effect allele frequency (EAF) greater than 0.01 were retained, ensuring the variants' prevalence. Specific SNPs, including palindromic SNPs and those with an F-statistic below 10, were excluded to avoid weak instrumental variables and reduce bias, where the F-statistic is calculated using the formula: $F = (\text{beta}/\text{se})^2$ (Lv et al., 2023). Finally, Steiger filtering was conducted to retain SNPs where the exposure's R-squared was greater than that of the outcome, ensuring the instrumental variables did not exhibit reverse causality (Hemani et al., 2017).

2.4 MR analysis

In our study, the inverse variance-weighted (IVW) method (Burgess and Thompson, 2015) served as the primary analytical technique for assessing the causal impacts of oral microbiome taxa on colorectal cancer. This approach aggregates the effects associated with all SNPs to produce a comprehensive estimate. To further explore the robustness and validity of the instrumental variables, additional Mendelian Randomization methods such as MR Egger (Bowden et al., 2015), weighted median (Bowden et al., 2016), and weighted mode (Hartwig et al., 2017) were implemented. To address the possibility of reverse causation, positive MR findings were subjected to the Steiger directionality test.

We applied multiple testing correction using the false discovery rate (FDR) method to adjust p-values. This was performed

TABLE 1 This table summarizes the dataset characteristics for the oral microbiome and colorectal cancer studies.

Exposures/ Outcomes	Consortium	Ethnicity	Sample sizes	N. SNPs	Year
Oral microbiome	CNGBdb	East Asian	2948	Tongue N = 8426	2021
			Tongue N = 2017	Saliva N = 8009	
			Saliva N = 1914		
Colorectal cancer	BioBank Japan	East Asian	33870	7492477	2019

For the oral microbiome, data were sourced from the China National GeneBank (CNGBdb) and included 2,948 samples from East Asian individuals, further divided into 2,017 tongue and 1,914 saliva samples, with respective SNP counts. The colorectal cancer data, obtained from Biobank Japan (BBJ), included 33,870 East Asian participants, comprising 6,692 patients and 27,178 controls, with a total of 7,492,477 SNPs collected in 2019.

separately for saliva and tongue microbiome data, and grouped by Phylum, Class, and Order to enhance the detection of significant associations while maintaining rigorous statistical standards. This stratified approach allows for more refined detection of significant associations, balancing rigorous statistical control with the exploratory nature of our study to identify biologically relevant signals.

To address the possibility of reverse causation, positive MR findings were subjected to the Steiger directionality test.

To evaluate the presence of horizontal pleiotropy, our analysis incorporated the MR-PRESSO and MR-Egger regression tests. Each SNP underwent the MR-PRESSO (Verbanck et al., 2018) outlier test to ascertain its significance concerning pleiotropic effects, with each test generating a distinct p-value. The overarching pleiotropy was then assessed using the MR-PRESSO global test, which recalibrated the global test p-value following the sequential removal of SNPs, starting with those displaying the lowest outlier test p-values. This iterative removal continued until the global test p-value surpassed the threshold of 0.05, suggesting an absence of significant pleiotropic influences. The resulting set of SNPs, cleared of pleiotropic biases, was utilized in the further stages of the MR analysis. The Cochran Q analysis (Shen et al., 2022) is used to assess heterogeneity among the instrumental variables (IVs) in a Mendelian Randomization (MR) study. If the p-value from the Cochran Q test is above 0.05, indicating no evidence of significant heterogeneity, a fixed-effects inverse variance-weighted (IVW) method is employed as the primary analytical approach. Conversely, if significant heterogeneity is detected ($p < 0.05$), a random-effects IVW method is utilized to accommodate the variability among the IVs. is used to assess heterogeneity among the instrumental variables (IVs) in a Mendelian Randomization (MR) study. If the p-value from the Cochran Q test is above 0.05, indicating no evidence of significant heterogeneity, a fixed-effects inverse variance-weighted (IVW) method is employed as the primary analytical approach. Conversely, if significant heterogeneity is detected ($p < 0.05$), a random-effects IVW method is utilized to accommodate the variability among the IVs.

In addressing the potential issue of reverse causation within our Mendelian Randomization study, we subjected all positive findings to rigorous scrutiny using the Steiger directionality test. This methodological step ensures that the observed associations are not a result of the outcome influencing the exposure, thereby reinforcing the credibility of our causal inferences.

All statistical computations were conducted using R software version 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria). The analyses employed the “TwoSampleMR” package version 0.5.8, designed specifically for MR investigations.

2.5 SNP annotation

For the annotation of SNPs, we utilized the VarNote database (Huang et al., 2020), which is distinguished by its innovative indexing system and a parallel random-sweep searching algorithm. This system enables VarNote to deliver substantial enhancements in performance, accelerating processing by two to

three orders of magnitude compared to existing solutions. VarNote supports both region-based and allele-specific annotations and offers advanced functionalities for the flexible retrieval of detailed annotations, making it well-suited for complex genomic analyses.

In our study, the parameters set for SNP annotation through VarNote were tailored to optimize the relevance and precision of the data. The annotations were specifically geared towards tissue/cell type-specific epigenomes, with a focus on E127 (NHEK-Epidermal Keratinocyte Primary Cells: CellLine). For the prioritization of variants, prediction scores such as FitCons2, FUNLDA, GenoNet, and GenoSkylinePlus were utilized. The population reference was set to European (EUR), ensuring the relevance of the data to the demographic of interest. Additionally, a linkage disequilibrium (LD) cutoff of 0.8 and an LD window of 100KB were applied, with gene annotations referencing the Ensembl database.

2.6 Gene function enrichment

To analyze the biological pathways and processes significantly associated with our identified genes, we conducted an enrichment analysis using the clusterProfiler R package (version 4.4.4) (Wu et al., 2021). First, gene identifiers were accurately mapped to human genes (*Homo sapiens*) using the org.Hs.eg.db package. This step ensured the precision of our molecular data before proceeding with the enrichment analysis. The clusterProfiler facilitated a detailed exploration into the biological pathways that were significantly enriched, focusing on those related to fatty acid metabolism and their influence on blood glucose levels.

For a clear and intuitive presentation of these results, we utilized the sangerbox tool to create enrichment circle plots, as detailed by Shen et al. (2022) (Shen et al., 2022). These plots provided a visual summary of the key pathways enriched in our study, offering a user-friendly way to interpret the complex interactions and implications of the identified genes. This method of visualization helped emphasize the most pertinent biological processes and pathways involved in our analysis.

2.7 Clustering and annotation of single-cell RNA sequencing data

Single-cell analysis employed nine cancer samples from dataset GSE205506 and four normal control samples from GSE231993, all of which were downloaded from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo).

To process a dataset of single-cell RNA sequencing from colorectal cancer, the Read10X function is utilized to import the data into an R environment, where it is subsequently converted into a Seurat object using the “Seurat” package (version 4.3.0.1). Quality control metrics are calculated by assessing the proportion of mitochondrial and ribosomal genes, as well as erythrocyte content within each cell. Cells with gene counts below 4000 or above 200, and those with a mitochondrial gene proportion exceeding the allowable threshold of 20% (pctMT = 20), are excluded to remove low-quality

cells from the dataset. Subsequently, the `NormalizeData` function is applied to standardize the merged dataset. For the analysis of single-cell RNA sequencing (scRNA-seq) data, Principal Component Analysis (PCA) is employed for dimensionality reduction and clustering. Initially, the `FindVariableFeatures` function identifies the top 2000 highly variable genes. Principal component scores from 1 to 8 are assigned for the dimensionality reduction step. Finally, t-distributed Stochastic Neighbor Embedding (t-SNE) is generated to visualize the resulting unsupervised cell clusters. To annotate the cell type of each cluster, marker genes from previous studies are used. We employed the 'ggplot2' package (version 3.4.3) in R for heatmap generation, a robust tool for data visualization. The 'ggplot2' package, based on The Grammar of Graphics, allows for complex, layered visualizations.

2.8 Drug accessibility analysis using DSigDB

The Drug Signatures Database (DSigDB) is an extensive repository (Freshour et al., 2021) that contains 22,527 gene sets and 17,389 different compounds, covering 19,531 genes. It plays a crucial role in bridging drugs and other chemical entities with their target genes. By inputting genes relevant to specific diseases or those that show significant expression changes under certain biological conditions, DSigDB facilitates the prediction of potential small molecule drugs. This prediction is based on the connections between the inputted genes

and known drug target genes as well as drug sensitivity genes, providing a valuable tool for understanding drug-gene interactions and enhancing drug discovery processes.

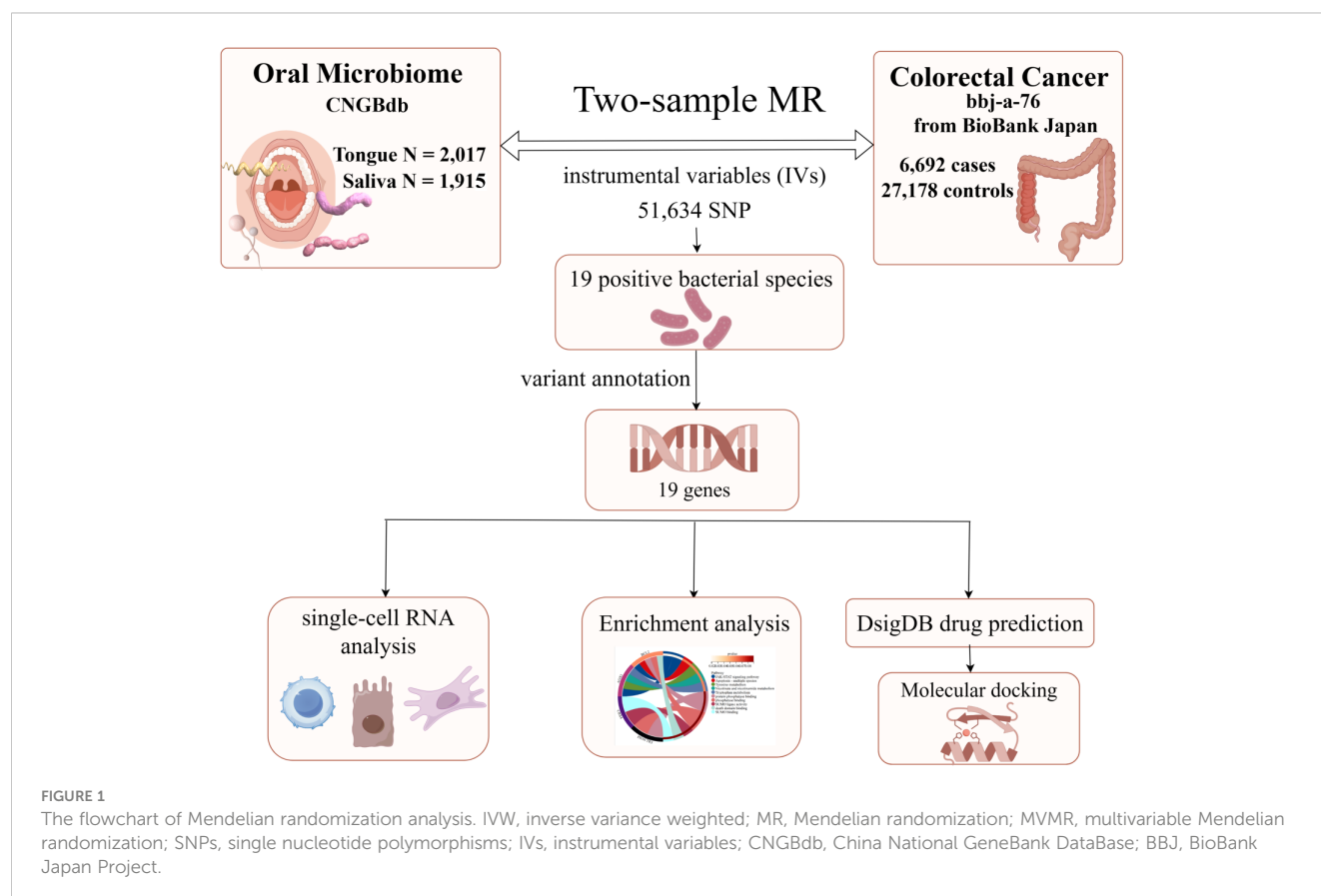
2.9 Molecular docking

Obtain the three-dimensional crystal structure of the target protein in PDB format through the RCSB PDB database (<https://www.rcsb.org/>) and the two-dimensional structure of the active component in SDF format through the Pubchem database (<https://pubchem.ncbi.nlm.nih.gov/>), saving the small molecule in mol2 format. Use PyMOL software to preprocess the target protein by removing solvents and ligands, etc. Use AutoDockTools-1.5.7 software to preprocess the target protein by removing water, adding hydrogens, and calculating charges, and preprocess the active component by adding hydrogens and setting torsion angles, etc., before performing molecular docking and calculating the binding energy (affinity). Visualization software: Rymol 2.6.0.

3 Results

3.1 Research workflow

Firstly, Figure 1 (By Figdraw) illustrates the steps of our study, which commenced with data acquisition from the China National



GeneBank (CNCBdb). The data comprises oral microbiomes from tongue samples (2,017 instances) and saliva samples (1,915 instances). Utilizing this data, we conducted two-sample Mendelian Randomization (MR) analyses, identifying instrumental variables (IVs) for colorectal cancer through 58,904 SNPs. This analysis confirmed 19 bacterial species positively associated with colorectal cancer and further annotated 19 genes related to these bacterial species. Subsequently, single-cell RNA analysis and enrichment analyses were employed to investigate the roles and interactions of these genes in the disease context. In the final phase, potential therapeutics associated with these genes were predicted using the Drug Signatures Database (DsigDB), and their interactions with target proteins were examined through molecular docking techniques, aiming to discover new methods for treating colorectal cancer. Additionally, our study incorporates data on 6,692 colorectal cancer cases and 27,178 controls from the BioBank Japan, enhancing the universality and accuracy of our findings.

3.2 Causal impact of the oral microbiome on colorectal cancer development

Figure 1 provides a comprehensive overview of the entire MR analysis process. Prior to further analysis, SNPs affected by linkage disequilibrium and those indicative of weak instrumental variables were removed. Ultimately, 25,488 SNPs associated with the salivary microbiome and 26,146 SNPs linked to the tongue microbiome were retained for analysis—with a significance threshold of $p < 1 \times 10^{-5}$. The F-statistic ranged from 19.601 to 56.906, with all SNPs exceeding the threshold of 10, thus indicating no evidence of weak instrument bias (see [Supplementary Table 1](#)). Based on the inverse variance-weighted (IVW) MR analysis with a significance threshold of $p < 0.05$, a total of 161 taxa were initially identified as having a causal association with colorectal cancer. A comprehensive overview of these results is visually represented in the volcano plot (see [Figure 2](#); [Supplementary Table 2](#)). Following FDR correction for

multiple testing, 19 taxa were ultimately determined to exhibit a causal impact on colorectal cancer, including 11 taxa from tongue samples and 9 from saliva samples. In saliva samples, the taxa RUG343 and *Streptococcus_umgs_2425* demonstrated protective effects against colorectal cancer, with odds ratios (ORs) of 0.817 (95% confidence interval [CI]: 0.704–0.949; $p = 0.008$) and 0.797 (95% CI: 0.700–0.909; $p = 0.001$), respectively. Conversely, taxa such as HOT-345_umgs_976 and W5053_sp000467935_umgs_712 were associated with an increased likelihood of negative health outcomes, with ORs of 1.210 (95% CI: 1.066–1.373; $p = 0.003$) and 1.183 (95% CI: 1.007–1.391; $p = 0.041$), respectively. Scatter plots illustrating the associations between individual SNPs and the outcomes for these taxa can be found in [Figure 3](#), while a forest plot detailing the MR analysis results for colorectal cancer risk across different taxa is presented in [Figure 4](#). In tongue samples, taxa such as *Campylobacter_A_umgs_3358* and HOT-345_umgs_3064 showed significant negative associations with beneficial health outcomes, with ORs of 1.614 (95% CI: 1.217–2.141; $p = 0.001$) and 1.242 (95% CI: 1.063–1.451; $p = 0.006$), respectively. In contrast, increased abundance of the *Anaerovoracaceae* family was negatively correlated with health, exhibiting an OR of 0.759 (95% CI: 0.625–0.922; $p = 0.005$). Corresponding scatter plots showing the relationships between each SNP and the outcome for these taxa are displayed in [Figure 5](#), with a forest plot summarizing the MR results and ORs for colorectal cancer across different taxa in [Figure 6](#). For each positive result, the corresponding funnel plots and forest plots can be found in [Supplementary Files 1, 2](#), respectively. Except for *s:unclassified_mgs_2717*, *s:Capnocytophaga_sputigena_mgs_3567*, and *s:unclassified_mgs_389*, the MR-Egger regression intercepts demonstrated no significant deviations from zero, indicating a lack of horizontal pleiotropy across most taxa (all intercepts $> p 0.05$), as detailed in [Supplementary Table 3](#). The MR-PRESSO analysis further supported these findings, with all examined taxa showing no evidence of outliers, affirming the robustness of the data (refer to [Supplementary Table 4](#)). Moreover, Cochran's Q test highlighted some heterogeneity specifically for *s:mgs_389*, *s:mgs_2717*, and *s:Capnocytophaga_sputigena_mgs_3567*, with Q-values falling below

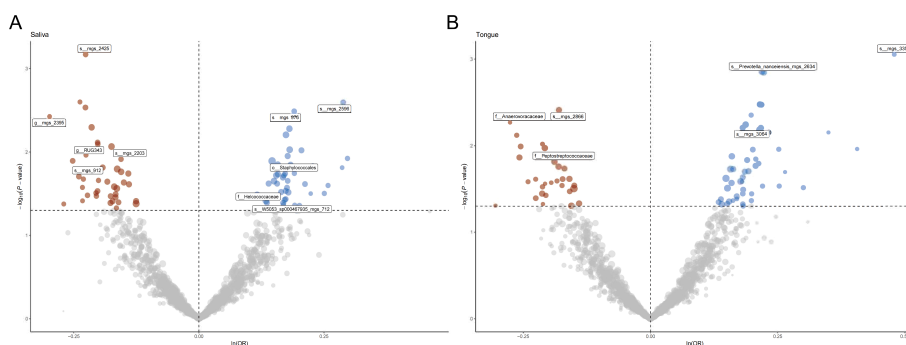


FIGURE 2

(A) Volcano plot illustrating the effect of single nucleotide polymorphisms (SNPs) on various taxa in saliva samples. Points are color-coded to indicate statistical significance: significant positive associations are shown in blue, significant negative associations in red, and non-significant associations in gray. Labeled taxa represent those with the most significant associations. (B) Volcano plot illustrating the effect of single nucleotide polymorphisms (SNPs) on various taxa in tongue samples. Points are color-coded to indicate statistical significance: significant positive associations are shown in blue, significant negative associations in red, and non-significant associations in gray. Labeled taxa represent those with the most significant associations.

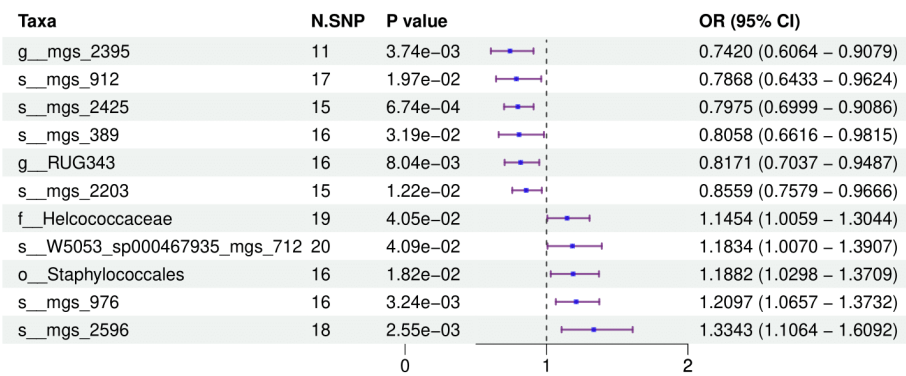


FIGURE 3 This forest plot illustrates the odds ratios (ORs) and 95% confidence intervals (CIs) for the impact of single nucleotide polymorphisms (SNPs) on various taxa within saliva samples, highlighting the statistically significant associations that affect health outcomes.

0.05, suggesting variability in the effects across these taxa (see [Supplementary Table 5](#)). The leave-one-out analysis revealed consistent causal estimates across the different oral microbiome taxa, with no single SNP disproportionately influencing the overall results related to colorectal cancer (details in [Supplementary Table 6](#); [Supplementary File 1](#)). The Steiger directionality test also found no evidence of a causal relationship between these diseases and the specific oral microbial taxa, as documented in [Supplementary Table 7](#).

3.3 Genes and functionality

The correspondence between SNPs and genes, along with their functions, is detailed in [Supplementary Table 8](#). [Figure 7B](#) presents a circular plot derived from the enrichment analysis of gene interactions with biological pathways. This figure highlights the associations of key genes (e.g., BCL2, AOX1, CBX4, PHACTR3) with multiple biological pathways. Each color in the plot represents a different biological pathway, and the connections between genes and pathways illustrate the involvement of these genes in specific pathways (details in [Supplementary Table 9](#)). The JAK-STAT signaling pathway is linked to all four genes, suggesting its central role in the biological processes regulated by these genes. Notably,

the connections between BCL2 and AOX1 with the JAK-STAT pathway are particularly significant, indicating that these genes might play roles in cellular signaling via this pathway. The apoptosis pathways are primarily associated with BCL2 and PHACTR3, consistent with BCL2’s function as a major anti-apoptotic protein, while the involvement of PHACTR3 might suggest novel regulatory mechanisms. Tyrosine metabolism shows a strong association with AOX1, reflecting its role in amino acid metabolism. Additionally, both CBX4 and PHACTR3 also exhibit associations with the tyrosine metabolism pathway, albeit to a lesser extent. Niacin and nicotinamide metabolism, as well as tryptophan metabolism, are enriched in AOX1 and PHACTR3, indicating that these metabolic pathways might play significant roles in regulating the functions of these genes. Furthermore, the plot reveals enrichment for pathways such as protein phosphatase binding, phosphatase binding, SUMO ligase activity, death domain binding, and SUMO binding. Notably, CBX4 is significantly enriched in SUMO-related pathways, potentially elucidating its role in protein modification and signal transduction. Overall, this enrichment diagram effectively reveals the participation of oral microbiome-associated genes in multiple key biological pathways, providing clues for further research into the roles of these genes in biological processes. These results emphasize the complex interplay of intracellular signaling and metabolic pathways in cellular functionality.

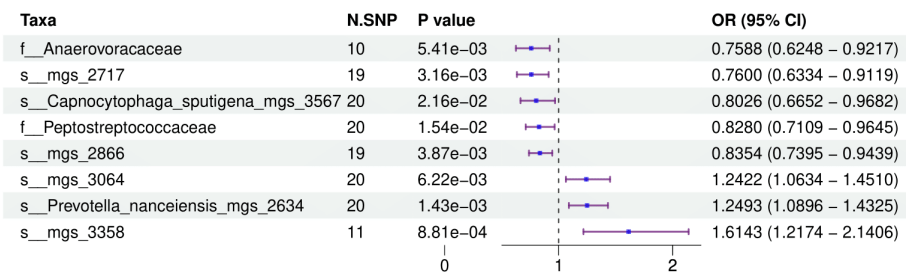


FIGURE 4 This forest plot displays the effects of SNPs on taxa within tongue samples, showing detailed ORs and 95% CIs. It identifies both positive and negative associations that these genetic variations have with health outcomes.

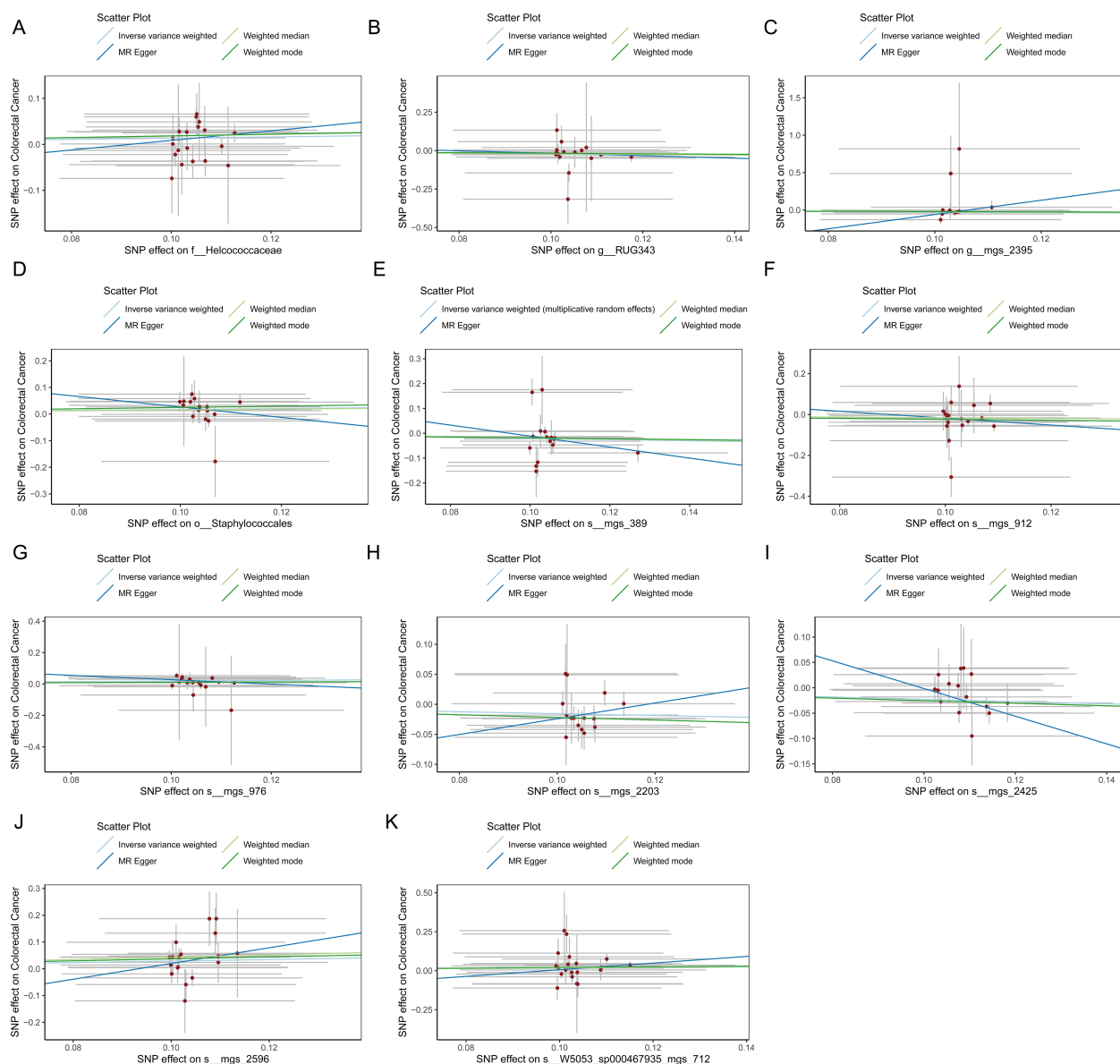


FIGURE 5

(A) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the family Helcococcaceae and their corresponding effect on colorectal cancer. (B) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the genus RUG343 and their corresponding effect on colorectal cancer. (C) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the genus *g__Lachnospiraceae* and their corresponding effect on colorectal cancer. (D) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the order Staphylococcales and their corresponding effect on colorectal cancer. (E) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species *mgs_389* and their corresponding effect on colorectal cancer. (F) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species *mgs_912* and their corresponding effect on colorectal cancer. (G) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species *mgs_976* and their corresponding effect on colorectal cancer. (H) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species *mgs_2203* and their corresponding effect on colorectal cancer. (I) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species *mgs_2425* and their corresponding effect on colorectal cancer. (J) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species *mgs_2596* and their corresponding effect on colorectal cancer. (K) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species *W5053_sp000467935_mgs_712* and their corresponding effect on colorectal cancer.

3.4 Single-cell global analysis results

Initially, quality control was performed on the single-cell data, with Figure 7A depicting the distribution of cells across several QC parameters, including RNA feature numbers (Feature_RNA), ribonucleic acid molecule counts (nCount_RNA), mitochondrial

gene expression ratio (percent.mt), and hemoglobin gene expression ratio (percent.HB). Violin plots illustrate significant differences among various cell types in these parameters, revealing heterogeneity and cell-specific characteristics within the samples. Subsequently, a t-SNE technique provided a two-dimensional spatial distribution of cell samples, differentiating between tumor

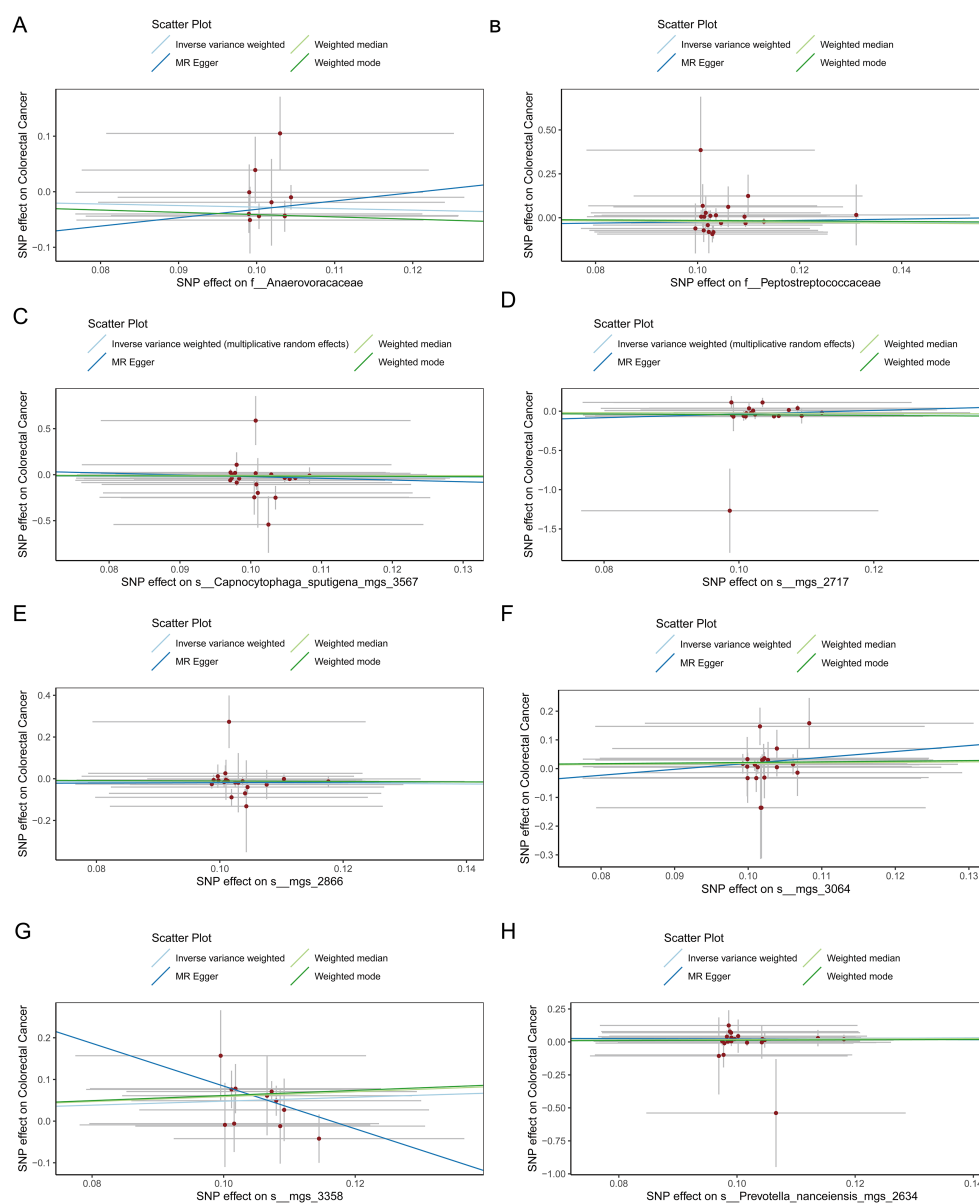
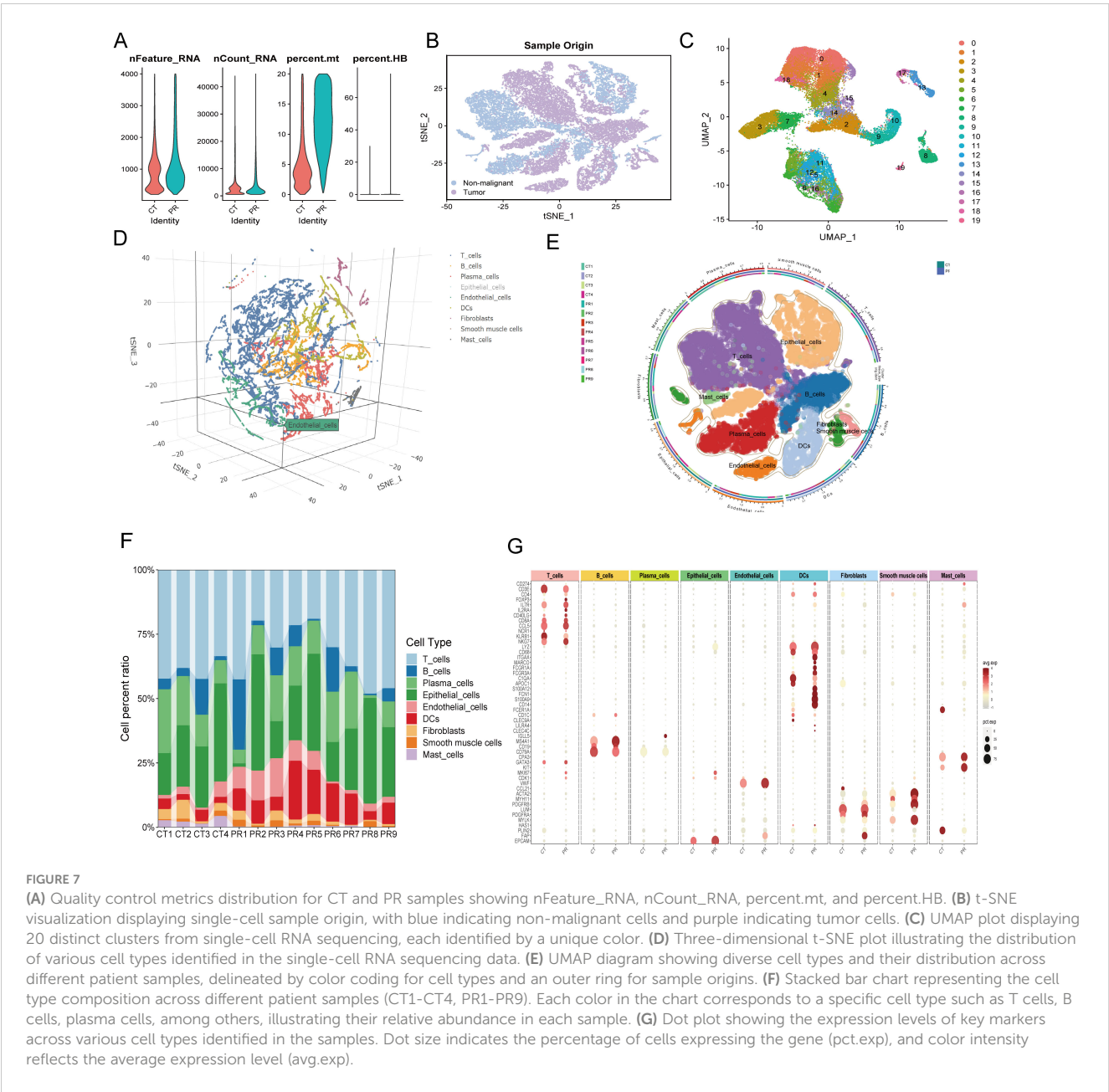


FIGURE 6

(A) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the family Anaerovoracaceae and their corresponding effect on colorectal cancer. (B) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the family Peptostreptococcaceae and their corresponding effect on colorectal cancer. (C) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species Capnocytophaga sputigena_mgs_3567 and their corresponding effect on colorectal cancer. (D) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species mgs_2717 and their corresponding effect on colorectal cancer. (E) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species mgs_2866 and their corresponding effect on colorectal cancer. (F) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species mgs_3064 and their corresponding effect on colorectal cancer. (G) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species mgs_3358 and their corresponding effect on colorectal cancer. (H) Scatter plot depicting the relationship between single nucleotide polymorphisms (SNPs) effect on the species Prevotella nanceiensis_mgs_2634 and their corresponding effect on colorectal cancer.

and non-tumor cells. Figure 7B offers an intuitive view of the cellular composition within the tumor microenvironment, displaying fundamental differences in cell components between tumors and surrounding tissues. Clustering of colorectal cancer single-cell data using the UMAP algorithm identified eight cell populations (Figure 7C), including T cells, B cells, plasma cells, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells, and mast cells. This detailed clustering underscores the diversity of complex cell types and

states at the single-cell level. Figure 7D presents a three-dimensional t-SNE scatter plot labeling various cell types, such as T cells, B cells, plasma cells, epithelial cells, and endothelial cells. This plot demonstrates the spatial relationships among cell types within the samples, providing a foundation for further analysis of intercellular interactions. A pie chart (Figure 7E) further subdivides the cell distribution in UMAP, where each segment represents a specific cell subtype, aiding in the identification of cellular compositions and



functional regions within the microenvironment. A stacked bar graph (Figure 7F) shows the proportions of cell types across different patient samples, revealing the relative abundance of cell types within each sample and comparing cell composition under different samples or treatment conditions. Figure 7G, through a scatter plot, displays the expression of key genes across cell types. The size and color depth of the dots reflect the abundance and statistical significance of gene expression, providing molecular-level evidence for understanding the functional states and interactions of various cell types. A critical heatmap (Figure 8A) illustrates the expression of selected genes across different cell types. This heatmap depicts the variance in gene expression levels among cell groups, highlighting specific genes like NFIB that are highly expressed in endothelial cells. In summary, these results not only reveal cellular heterogeneity within the tumor microenvironment but also provide valuable data

resources for further studying the cellular dynamics and interactions during tumor progression. These findings are significant for deepening the understanding of tumor biological mechanisms and developing targeted therapeutic strategies.

3.5 Single-cell analysis of endothelial cell populations

We utilized Mendelian Randomization (MR) to infer specific gene sets from SNP data, subsequently assessing their expression at the single-cell level through various scoring metrics, including AUCell, UCell, singscore, ssgea, and Add Scoring. Figure 8C illustrates the gene set scoring across various cell types—including mast cells, smooth muscle cells, fibroblasts, dendritic cells, endothelial

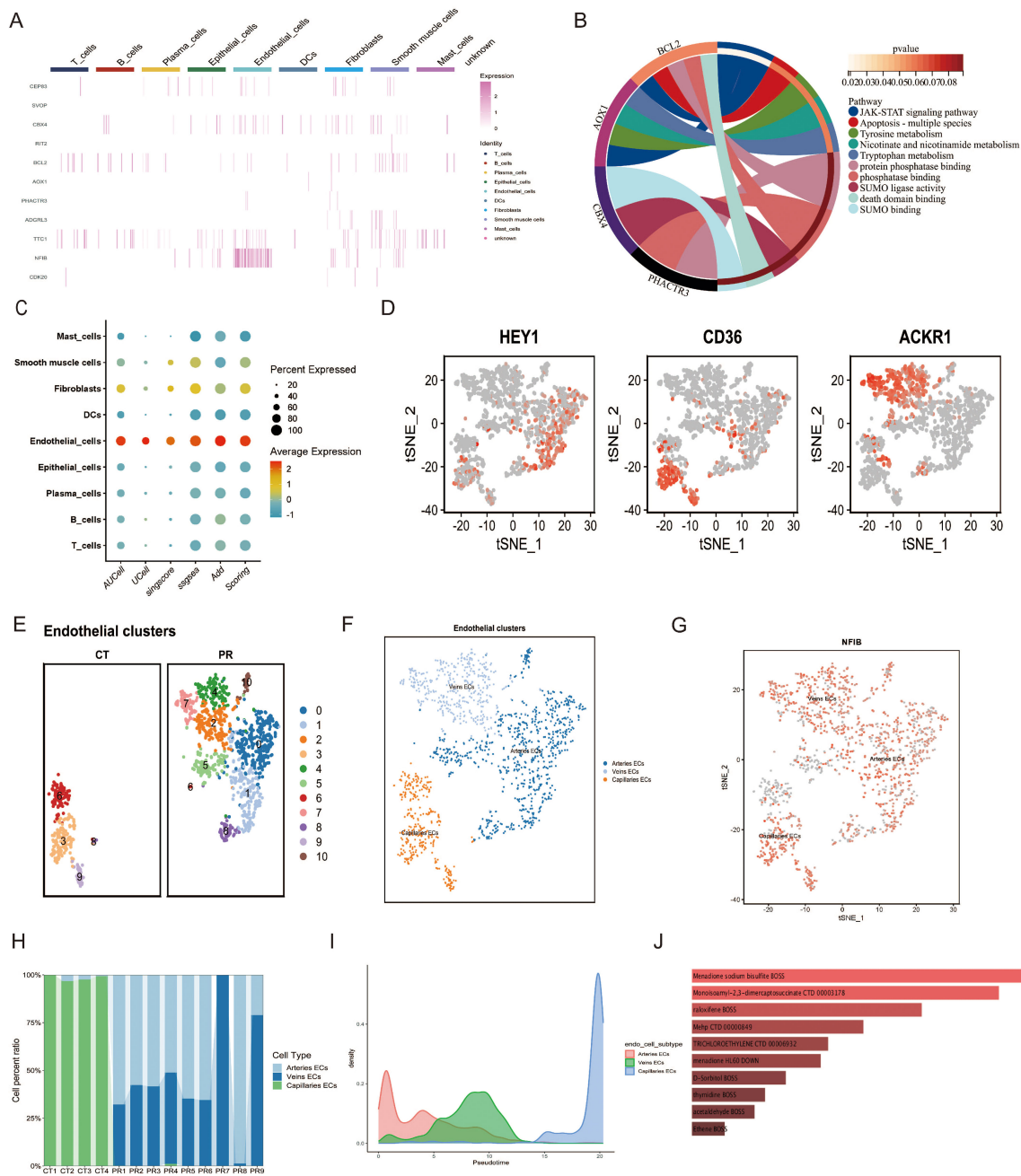


FIGURE 8 (A) Heatmap displaying the expression levels of selected genes across various cell types. (B) Chord diagram illustrating the functional pathways associated with selected genes. (C) Dot plot illustrating the scoring of a gene set across various cell types, based on different scoring metrics such as AUCell, UCell, singscore, ssgsea, and Add Scoring. Dot size indicates the percent of cells expressing the gene, and color scale reflects the scoring intensity. (D) t-SNE plots highlighting the expression of marker genes (HEY1, CD36, ACKR1) used for subtyping endothelial cells in single-cell data. Cells expressing each gene are highlighted in red, showing their distribution within the endothelial cell subpopulations. (E) UMAP plot displaying epithelial cell clusters across two sample groups, CT and PR. Each cluster is distinguished by a unique color, representing 11 distinct cell populations. (F) UMAP visualization of endothelial cell subtypes, categorized into arteries ECs (blue), veins ECs (light blue), and capillaries ECs (orange), demonstrating the distinct spatial distribution of these subtypes. (G) Feature plot of the NFIB gene expression across the endothelial cell subtypes in a t-SNE plot. Each dot represents a cell, with color intensity indicating levels of NFIB expression in arteries ECs, veins ECs, and capillaries ECs. (H) Stacked bar chart showing the distribution of endothelial cell subtypes (arteries ECs, veins ECs, capillaries ECs) across different patient samples, displayed as CT1 to CT4 and PR1 to PR9. (I) Density plot representing the pseudotime trajectory analysis of endothelial cell subtypes, illustrating the developmental progression of arteries ECs, veins ECs, and capillaries ECs. (J) Bar chart displaying drugs predicted to target specific genes identified through Mendelian randomization analysis from endothelial cell subtypes, with the bar length indicating the strength of the predicted association based on the analysis.

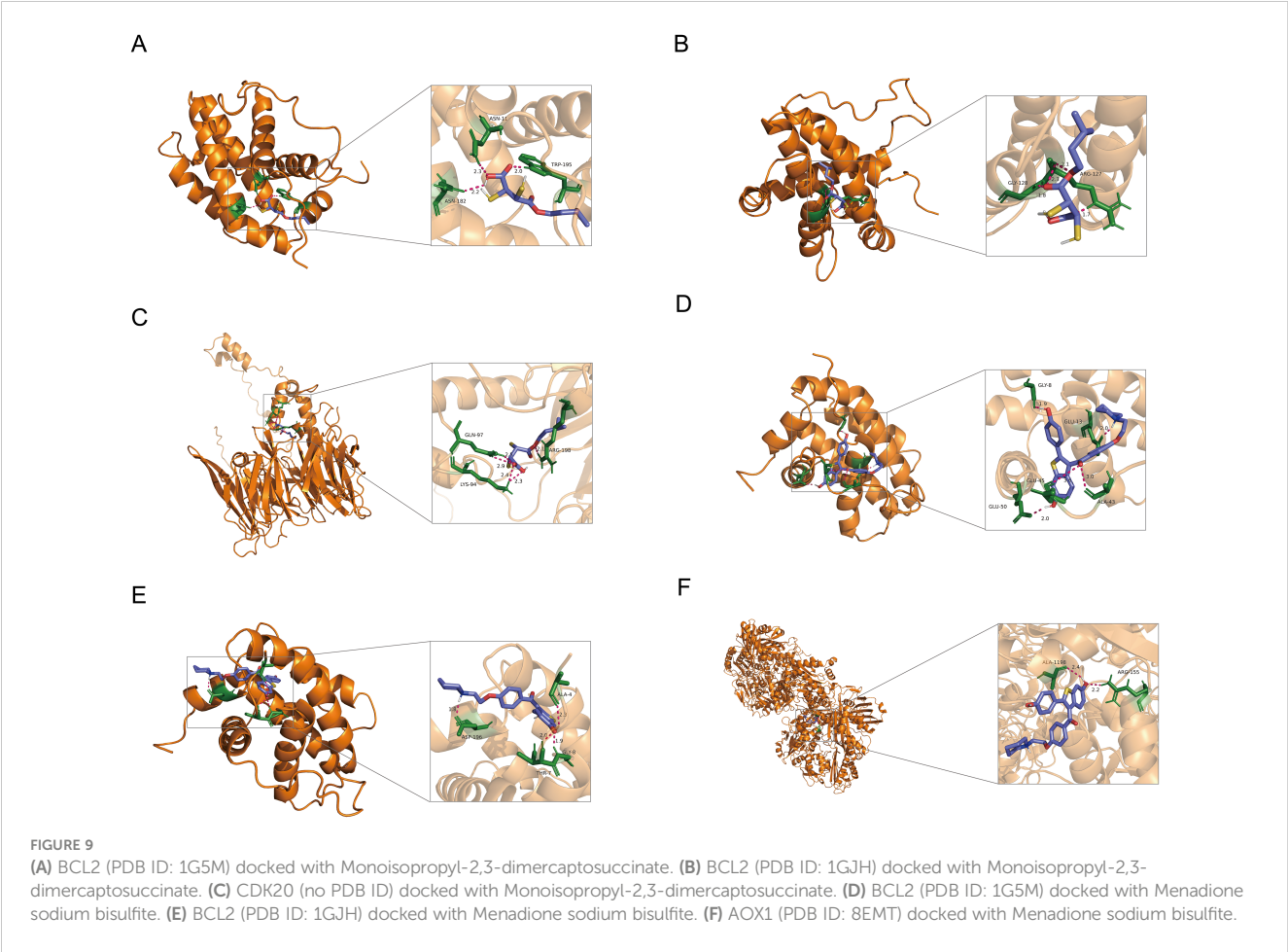
TABLE 2 This table presents molecular docking results showing the interaction of various drugs with specific targets.

Target	PDB ID	Pubchem iD	Binding energy	Drug
BCL2	1G5M	164448	−3.56	Monoisoamyl-2,3-dimercaptosuccinate
BCL2	1GJH	164448	−3.26	Monoisoamyl-2,3-dimercaptosuccinate
CDK20	–	164448	−2.17	Monoisoamyl-2,3-dimercaptosuccinate
BCL2	1G5M	5035	−7.91	Menadione sodium bisulfite
BCL2	1GJH	5035	−10.11	Menadione sodium bisulfite
AOX1	8EMT	5035	−4.08	Menadione sodium bisulfite

It includes the Protein Data Bank (PDB) ID, PubChem ID of the drugs, and their binding energy values indicating the strength of interaction. The table lists multiple interactions for BCL2, CDK20, and AOX1 targets, with corresponding drugs such as Monoisamyl-2,3-dimercaptosuccinate and Menadione sodium bisulfite, highlighting potential therapeutic implications.

cells, epithelial cells, plasma cells, B cells, and T cells—demonstrating the percentage and average intensity of gene expression within different cell types. Notably, endothelial cells exhibited significant gene expression activity, underscoring their importance in our study. Further subclassification of endothelial cells distinguished between arterial endothelial cells (Arteries ECs), venous endothelial cells (Veins ECs), and capillary endothelial cells (Capillaries ECs). Figures 8E, F display the UMAP analysis utilized to differentiate these endothelial subpopulations. These diagrams, through color coding, provide an in-depth view of the spatial distribution of various subgroups, offering insights into the diversity of cell types and the complexity of the microenvironment. Classification was

based on spatial distributions derived from t-SNE methodology, where differential expression of specific genes such as HEY1, CD36, and ACKR1 among the endothelial subtypes served as the basis for classification (Figure 8D). A feature plot of the NFIB gene was employed to elucidate its expression patterns, providing clues to its functional roles across different cell types. Figure 8H presents a stacked bar graph showing the proportions of endothelial cell subtypes across different patient samples, facilitating comparisons of cellular composition among samples. A density plot (Figure 8I) illustrates the pseudotemporal analysis results of the endothelial cell subgroups, displaying the distribution of arterial endothelial cells, venous endothelial cells, and capillary endothelial cells along the



predicted developmental timeline. Arterial endothelial cells displayed a density peak at earlier hypothetical time points, while capillary endothelial cells showed peaks at later time points, suggesting that these cell types may undergo different developmental paths and timings. The distribution of venous endothelial cells was relatively uniform, indicating a steady presence throughout the time series. Through such analyses, we observed the dynamic changes of different endothelial cell types during tumor progression, revealing their potential developmental trajectories and functional evolution. This provides vital insights into how endothelial cells adapt and evolve within the tumor microenvironment.

3.6 Drug prediction

In our study, Mendelian Randomization was applied in conjunction with the Drug Signatures Database (DsigDB) to predict potential therapeutics targeting genes identified from top SNPs. The following drugs demonstrated significant associations with specific genes: Menadione Sodium Bisulfite (p-value = 3.91×10^{-4} , OR = 80.94), predicted by genes BCL2 and AOX1; Monosomy1-2-3-dimercaptosuccinate (p-value = 1.74×10^{-4} , OR = 71.12), associated with genes BCL2 and CDK20; Raloxifene (p-value = 1.01×10^{-4} , OR = 47.86), also predicted by BCL2 and AOX1; MehP (p-value = 0.00187, OR = 35.45) and D-Sorbitol (p-value = 0.00400, OR = 23.87), both linked to BCL2 and AOX1; Trichloroethylene (p-value = 0.00265, OR = 29.64), affecting NFIB and BCL2; Menadione (p-value = 0.00285, OR = 28.55), impacting BCL2 and CPEB3; Thymidine (p-value = 0.00491, OR = 21.45), associated with BCL2 and CDK20; Acetaldehyde (p-value = 0.00544, OR = 20.32) and Ethene (p-value = 0.00727, OR = 17.42), the former related to BCL2 and AOX1, and the latter to RIT2 and BCL2. These findings provide robust molecular evidence for drug development targeting specific cell subtypes within the tumor microenvironment, emphasizing the importance of gene-targeted drug screening.

3.7 Molecular docking

In this study, we conducted a detailed analysis of the binding affinities and interactions between six different small molecules and their target proteins using molecular docking methods (Figures 9A–F). We observed that the binding affinities of BCL2 protein with two different ligands (PDB IDs: 1G5M and 1GJH) were -7.91 kcal/mol and -10.11 kcal/mol, respectively, indicating strong binding capabilities. This robust affinity is attributed to multipoint hydrogen bonding and van der Waals interactions with key residues such as Gly181, Ser184, Arg143, and Thr141, ensuring efficient and stable ligand binding at the active site. In contrast, the binding affinity with CDK20 protein was lower (-2.17 kcal/mol), primarily due to relatively weaker interactions with residues Lys33 and Ala35. Additionally, the binding affinity of AOX1 protein with its ligand was -4.08 kcal/mol, characterized by moderate binding strength through hydrogen bonds with the residue Arg400. Detailed analyses of these binding characteristics and interactions have been visualized using Pymol 2.6.0 software and are thoroughly

documented in the [Supplementary Files](#) of the study ([Supplementary File 4; Table 2](#)), providing a crucial molecular basis for future drug development and optimization.

4 Discussion

The investigation into the interrelationship between the oral microbiome and colorectal cancer reveals significant gaps in existing research. Compared to the extensively studied gut microbiome, research on the oral microbiome is relatively scarce. This disparity in research not only limits our comprehensive understanding of the relationship but also hinders the discovery of potential preventative and therapeutic approaches. Traditional microbiome studies, reliant on sequencing technologies, face limitations due to technological and sampling constraints, resulting in significant heterogeneity in findings. Our research aims to explore the causal relationship between the oral microbiome and colorectal cancer, emphasizing the role of oral microbes in the oncogenic process. Moreover, our study delves deeper into colorectal cancer at the single-cell level, identifying significant expression of oral microbiome-related genes in colorectal cancer cells, which led to drug prediction and molecular docking analysis. This innovative approach enhances the robustness of our results and advances precise inference of the causal relationship between microbiomes and colorectal cancer.

Utilizing summary statistics from GWAS meta-analyses of oral microbiomes and colorectal cancer provided by the MiBioGen Consortium, we conducted a two-sample MR analysis to evaluate their causal relationship. The bidirectional MR analysis aims to comprehensively understand the complex interactions between the oral microbiome and colorectal cancer. This method not only assesses how the microbiome influences the onset of colorectal cancer but also systematically considers reverse causality, thereby revealing potential changes in the oral microbiome induced by colorectal cancer. The study highlights that controlling for confounders through common genetic factors ensures reliable causal inference.

In our study, we identified various oral microbial taxa and analyzed their correlation with colorectal cancer (CRC). By examining saliva and tongue samples, we found that the enrichment of Absconditabacterales (in saliva), Campylobacter_A (in tongue), Prevotella (in tongue), and Catonella (in saliva) is associated with an increased risk of CRC, suggesting these microbes may act as risk factors. Conversely, the presence of Capnocytophaga, Gemella (in both saliva and tongue samples), Anaerovoracaceae (in tongue), Peptostreptococcaceae (in tongue), Streptococcus, Centipeda, and Lachnospiraceae (in saliva) appears to exert a protective effect, potentially inhibiting cancer development. These findings not only enhance our understanding of the role of oral microbial diversity in the onset of CRC but may also provide a theoretical basis for developing microbiome-based preventive or therapeutic strategies. Campylobacter_A is a principal cause of bacterial colon infections globally and has shown a robust capacity to survive under various stressful conditions by interacting with certain intestinal pathogens (Kim et al., 2020). Studies indicate that

Campylobacter jejuni promotes the occurrence of colorectal tumors through multiple mechanisms. Notably, it produces a genotoxin known as cytolethal distending toxin (CDT), which has been proven to cause DNA damage. Animal model studies suggest that introducing *C. jejuni* into a germ-free environment can significantly alter the gut microbiota and enhance tumor formation, indicating complex interactions between this bacterium, the gut microbiome, and the carcinogenic process (He et al., 2019). Further human clinical data and animal studies reveal higher abundance of *Campylobacter* species, including *C. jejuni*, in CRC tissues compared to normal tissues, suggesting that the presence of *Campylobacter* may influence the development of CRC by altering microbial community structures (Costa et al., 2022). Additionally, a comprehensive review of the mucosal microbiota of CRC patients found that *Campylobacter* and several other bacteria are more common in cancerous tissues compared to healthy controls, supporting the hypothesis that *Campylobacter* and specific bacteria are related to CRC pathology (Butzler, 1982). In a pioneering study conducted in Iran, researchers analyzed the overall microbiome of saliva and fecal samples from CRC patients and healthy controls. The study identified significant changes in the abundance of certain bacterial genera, including *Catonella*, potentially linking these bacteria to CRC development. Furthermore, differences in microbial diversity between the saliva samples of healthy controls and CRC patients suggest that oral microbiota may be relevant for the early detection and prevention of CRC (Rezasoltani et al., 2022). Research has also highlighted that *Prevotella* abundance is significantly higher in Indian populations compared to Western populations, suggesting a relationship with dietary habits and gut health status (Vishnu Prasoodanan et al., 2021). Moreover, the presence of *Prevotella* in CRC patients is linked to prognosis, with specific species' relative abundance in pre-operative fecal samples correlating with clinical outcomes, serving as potential prognostic biomarkers (Huh et al., 2022). Further, *Prevotella* plays a role in modulating immune responses, with studies showing a positive correlation between *Prevotella* and the expression of intestinal inflammatory markers like IL-9, which may promote the pathological process of CRC (Niccolai et al., 2020). Despite these microbial groups showing potential to increase the risk of CRC, our research has also revealed other taxa that may inhibit the progression of colorectal cancer. The *Anaerovoracaceae* family, for instance, may influence the response to cancer immunotherapy by affecting T-cell function (Blumenberg et al., 2021). These preliminary findings suggest that the gut microbiome, including the *Anaerovoracaceae*, could play a role in the tumor immune environment, thereby impacting the efficacy of immunotherapies, consistent with our results. *Lachnospiraceae* may play a significant role in the development of CRC. The negative correlation of this family with CRC risk offers critical insights into preventing and treating CRC by regulating the gut microbiota and controlling inflammatory factors (Ma et al., 2024). Specifically, bacteria from the *Lachnospiraceae* family are thought to reduce colorectal tumor formation by altering the tumor immune microenvironment. For instance, studies suggest that fiber-rich *Lachnospiraceae* may reduce CRC incidence by modulating immune responses (Almeida et al., 2021). Additionally, the metabolic products of *Lachnospiraceae* bacteria, particularly short-chain fatty acids (SCFAs) like butyrate, play roles in gut

health and cancer prevention. SCFAs promote the health of colon epithelial cells and have anti-inflammatory effects, which are crucial in the anticancer process (Coker et al., 2022). Existing research also shows that the *Streptococcus* genus is associated with the development and progression of CRC (Wang et al., 2021; Quaglio et al., 2022). Findings reveal geographical and racial differences in the association rates between *S. bovis/gallolyticus* and colorectal tumors. Additionally, the link between *S. bovis/gallolyticus*-related colonic lesions and bloodstream infections (bacteremia/endocarditis) suggests a unique pathway for these bacteria to enter the bloodstream via the portal venous system (Alozie et al., 2015). In a prospective study targeting low-income and African-American populations, analyzing the oral microbiome and subsequent CRC risk revealed that several bacterial taxa, including the *Streptococcus* genus, were associated with reduced CRC risk, although these associations were not significant after multiple testing corrections (de Almeida et al., 2018).

To better understand the biological functions of these genes in disease, we conducted GO and KEGG analyses. We discovered that the JAK-STAT signaling pathway, an essential intracellular signaling system, is ubiquitous across a variety of organisms, from humans to fruit flies. This pathway is primarily initiated by extracellular signals such as cytokines and growth factors, which transmit signals through receptors on the cell membrane to the intracellular Janus kinase (JAK), subsequently triggering the phosphorylation of signal transducer and activator of transcription (STAT). Phosphorylated STAT proteins form dimers that regulate the expression of specific genes by entering the nucleus (Hu et al., 2023). The JAK-STAT pathway plays a crucial role in cellular processes such as growth, differentiation, apoptosis, and immune regulation. For instance, it is vital in determining the fate of T-helper cells, influencing the differentiation of various cell types such as Th1, Th2, Th17, and regulatory T cells (Seif et al., 2017). However, aberrant activation of this pathway is often associated with various diseases, particularly in cancer. For example, the activation of STAT3 is closely linked to the occurrence of multiple tumors, the formation of drug resistance, and the maintenance of cancer stem cells (Rah et al., 2022). Therefore, the JAK-STAT signaling pathway is a significant target in cancer therapy, and a deeper understanding of it could lead to the development of more effective treatment methods.

In mammals, the intrinsic pathway of apoptosis primarily revolves around mitochondria, involving several key proteins such as members of the Bcl-2 family, which maintain cell survival by directly or indirectly inhibiting pro-apoptotic proteins (like BAK and BAX). When cells are overwhelmed by stress or developmental signals, this survival signal is overridden, triggering the initiation of apoptosis (Cavalcante et al., 2019). Additionally, studies have indicated that miRNAs also play a crucial role in regulating apoptosis-related genes, and any imbalance in these mechanisms could lead to the development of various diseases (Cavalcante et al., 2019). Apoptosis is not just a form of cell death; it involves numerous finely regulated molecular mechanisms, significantly impacting the understanding and treatment of various diseases.

Tyrosine metabolism is a complex biochemical process primarily occurring in the liver, involving multiple enzymes and metabolic

pathways. In diseases like hepatocellular carcinoma, abnormalities in tyrosine metabolism can affect the regulation of the cell cycle and cellular proliferation. Studies have shown that the expression of tyrosine metabolism enzymes is decreased in hepatocellular carcinoma, closely associated with poor prognosis. Furthermore, abnormalities in tyrosine metabolism can also activate the cell cycle, promoting cell proliferation (Wang et al., 2022). Niacin and nicotinamide metabolism are crucial biochemical processes related to cellular energy production and repair. Studies have found that niacin and nicotinamide metabolism, through their metabolites such as NAD⁺ and 1-methyl nicotinamide, play a significant role in treating various diseases. For instance, nicotinamide mononucleotide (NMN), a vital precursor of NAD⁺, has been shown to combat aging and enhance cellular metabolic status (Song et al., 2023; Malik et al., 2024). Moreover, niacin and nicotinamide metabolism are also closely linked to the development of many diseases, such as diabetic peripheral neuropathy and the inflammatory processes in rheumatoid arthritis. Regulating this metabolic pathway can influence the progression and treatment outcomes of diseases (Malik et al., 2024; Ye et al., 2024). Tryptophan metabolism plays a key role in various physiological and pathological processes, particularly in digestive system tumors, including gastric and colorectal cancers. The expression levels of tryptophan and its metabolites are closely associated with the clinical characteristics of tumors. For example, in gastric cancer, increased expression of the tryptophan-metabolizing enzyme TDO2 is negatively correlated with tumor aggressiveness and overall patient survival (Ye et al., 2021; Yu et al., 2024). Additionally, tryptophan metabolism pathways, such as the kynurenine pathway, also play roles in regulating gut inflammation and brain health, demonstrating potential therapeutic applications (Chen et al., 2021; Roth et al., 2021; Michaudel et al., 2023).

In our study, we utilized the DsigDB database to predict potential drug candidates targeting genes identified through Mendelian randomization from top SNP data. Among these, the vitamin K derivative Menadione sodium bisulfite (MSB) has demonstrated potential value in cancer therapy. Existing research indicates that MSB can inhibit tumor cell growth by depleting the pool of acid-soluble thiols such as glutathione, particularly evident in mouse leukemia L1210 cells (Akman et al., 1985). Additionally, when used in combination with vitamin C, MSB significantly enhances the cytotoxicity against prostate cancer cells and can synergize with anticancer drugs like bortezomib to reduce toxicity and enhance antitumor effects (sodium ascorbate study). These studies suggest that MSB impacts cancer cell survival and proliferation through multiple mechanisms, warranting further exploration and validation of its application in cancer treatment (Astakhova et al., 2018). Other potential drug candidates include the heavy metal chelator Monoisomy1-2-3-dimercaptosuccinate, which may facilitate the removal of heavy metals from cells; Raloxifene, a selective estrogen receptor modulator that may influence endothelial cells via the estrogen receptor pathway; and MEHP, which could affect cellular behavior through endocrine disruption. Additionally, the industrial solvent Trichloroethylene may induce oxidative stress and cytotoxicity affecting cellular physiology, while Menadione acts by influencing redox reactions

and pathways related to cellular apoptosis. D-Sorbitol, as an osmotic diuretic, may regulate intracellular osmotic pressure and carbohydrate metabolism; Thymidine affects cell cycles by impacting DNA synthesis; Acetaldehyde may induce DNA damage and stress responses; and Ethene could influence hormonal signaling and cellular response mechanisms (Janakiram et al., 2009; McGuire et al., 2012; Shih et al., 2023). These analytical results not only highlight the potential of drug screening based on single-cell data but also provide valuable insights for developing treatment strategies targeting specific cell subtypes within the tumor microenvironment. This study presents several notable advantages. Firstly, we utilized the latest genome-wide association study (GWAS) data related to the oral microbiome and employed Mendelian Randomization (MR) as a methodological approach to establish causative relationships. This method allows us to assess potential links between the components of the oral microbiome and colorectal cancer from a genetic perspective. Secondly, we innovatively integrated single-cell transcriptome analysis, precisely identifying endothelial cell populations through cell-specific scoring techniques. Enrichment analysis further revealed the functional characteristics of the gene sets we identified. Lastly, through drug prediction and molecular docking techniques, we explored several genes as potential therapeutic targets and predicted potential therapeutic drugs associated with these targets. This series of studies provides new insights into the development of personalized treatment strategies for colorectal cancer and offers preliminary candidate targets and drugs for future drug development.

However, several limitations of this study must be acknowledged. A significant concern in Mendelian Randomization studies is the possibility of horizontal pleiotropy, which could affect the accuracy of selecting instrumental variables. The composition of the oral microbiome can be influenced by multiple factors, including genetic background, lifestyle choices, dietary habits, and environmental factors, all of which could impact the outcomes of the study. Additionally, the instrumental variables used may only explain a small portion of the observed variability, necessitating further research to fully understand the complex changes in the oral microbiome. Moreover, our MR analysis focused predominantly on populations of Asian descent, meaning our results may not be generalizable to other ethnicities, such as those of European descent. Further research is needed to validate and extend our findings to other populations. Single-cell RNA sequencing (scRNA-seq) and drug prediction analyses offer tremendous potential for providing high-resolution cellular characteristics and identifying potential therapeutic strategies. However, several limitations of these methods must be acknowledged. Firstly, the quality and resolution of scRNA-seq data are highly dependent on sampling and technical factors, such as cell capture efficiency and sequencing depth. This dependency may result in underrepresentation of certain cell types or subpopulations. Additionally, the complexity of single-cell data analysis increases the difficulty of interpreting results, particularly in distinguishing between technical noise and biological significance. In the realm of drug prediction, although databases like DsigDB offer extensive gene-drug association information,

these predictions require further experimental validation to confirm their efficacy and safety. The accuracy of prediction models is also limited by the coverage of existing datasets and the assumptions made by algorithms. Furthermore, the effects of drugs observed at the cellular level may not directly translate to *in vivo* outcomes. Hence, more *in vivo* studies and clinical trials are necessary to verify their practical application value.

Future research should expand to populations of different ethnic and geographical backgrounds to validate and generalize current findings, and collect larger sample sizes to improve statistical accuracy and result robustness. Longitudinal studies will reveal the temporal dynamics between the oral microbiome and colorectal cancer development. In-depth functional genomics and metabolomics studies will elucidate the specific mechanisms of the microbiome. Future studies should integrate multi-omics data and apply single-cell multi-omics and spatial transcriptomics technologies to depict interactions between the microbiome and host cells at higher resolution. These directions will deepen our understanding of the oral microbiome's relationship with colorectal cancer and advance personalized medicine and precision therapy.

5 Conclusion

This study establishes the significant role of the oral microbiome in colorectal cancer (CRC) development through Mendelian Randomization (MR) analysis. We identified 19 bacterial taxa associated with CRC risk, with specific microbes showing both protective and harmful effects. Single-cell RNA sequencing highlighted key pathways, including JAK-STAT signaling, involved in CRC progression. Drug prediction and molecular docking identified potential therapeutics like Menadione Sodium Bisulfite and Raloxifene. These findings offer new insights and therapeutic targets for personalized CRC treatment strategies.

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/s.

Author contributions

YG: Conceptualization, Visualization, Writing – original draft. LJ: Data curation, Software, Writing – original draft. MS: Writing – original draft. HoL: Writing – original draft. XZ: Writing – original draft. SZ: Writing – original draft. CJ: Writing – original draft. JH: Writing – original draft. HC (9TH Author): Writing – original draft. JT: Writing – original draft. YF: Writing – original draft. HuL: Writing – original draft. GY: Writing – original draft. KX: Writing –

original draft, Writing – review & editing. HC (15th Author): Funding acquisition, Visualization, Writing – original draft. JL: Funding acquisition, Writing – original draft, Writing – review & editing. SH: Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that this 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/fcimb.2024.1452392/full#supplementary-material>

References

- Akman, S. A., Dietrich, M., Chlebowski, R., Limberg, P., and Block, J. B. (1985). Modulation of cytotoxicity of menadiene sodium bisulfite versus leukemia L1210 by the acid-soluble thiol pool. *Cancer Res.* 45, 5257–5262.
- Almeida, A. S., Tran, T. T. T., Ghosh, T. S., Ribiere, C., Foley, C., Azevedo, L. A., et al. (2021). Fiber-associated Lachnospiraceae reduce colon tumorigenesis by modulation of the tumor-immune microenvironment. doi: 10.1101/2021.02.24.432654
- Alozie, A., Köller, K., Pose, L., Raftis, M., Steinhoff, G., Westphal, B., et al. (2015). *Streptococcus bovis* infectious endocarditis and occult gastrointestinal neoplasia: experience with 25 consecutive patients treated surgically. *Gut Pathog.* 7, 27. doi: 10.1186/s13099-015-0074-0
- Astakhova, T. M., Morozov, A. V., Erokhov, P. A., Mikhailovskaya, M. I., Akopov, S. B., Chupikova, N. I., et al. (2018). Combined effect of bortezomib and menadiene sodium bisulfite on proteasomes of tumor cells: the dramatic decrease of bortezomib toxicity in a preclinical trial. *Cancers* 10, 351. doi: 10.3390/cancers10100351
- Blumenberg, V., Busch, G., Baumann, S., Schmidt, S., Schuhmacher, H., Winkelmann, M., et al. (2021). High bacterial abundances of dorea and pediococcus in the gut microbiome linked to expansion, immune checkpoint expression and efficacy of CD19-directed CAR T-cells in patients with r/r DLBCL. *Blood* 138 (Supplement 1), 2792.
- 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., Davey Smith, G., Haycock, P. C., and Burgess, S. (2016). Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet. Epidemiol.* 40, 304–314. doi: 10.1002/gepi.21965
- Bowden, J., and Holmes, M. V. (2019). Meta-analysis and Mendelian randomization: A review. *Res. synthesis Methods* 10, 486–496. doi: 10.1002/jrsm.1346
- Burgess, S., and Thompson, S. G. (2015). Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. *Am. J. Epidemiol.* 181, 251–260. doi: 10.1093/aje/kwu283
- Butzler, J. P. (1982). *Campylobacter enteritis*. *Infection* 10 Suppl 2, S67–S69. doi: 10.1007/BF01640857
- Cavalcante, G. C., Schaan, A. P., Cabral, G. F., Santana-da-Silva, M. N., Pinto, P., Vidal, A. F., et al. (2019). A cell's fate: an overview of the molecular biology and genetics of apoptosis. *Int. J. Mol. Sci.* 20, 4133. doi: 10.3390/ijms20174133
- Chen, L.-M., Bao, C.-H., Wu, Y., Liang, S.-H., Wang, D., Wu, L.-Y., et al. (2021). Tryptophan-kynurenine metabolism: a link between the gut and brain for depression in inflammatory bowel disease. *J. Neuroinflamm.* 18, 135. doi: 10.1186/s12974-021-02175-2
- Coker, O. O., Liu, C., Wu, W. K. K., Wong, S. H., Jia, W., Sung, J. J. Y., et al. (2022). Altered gut metabolites and microbiota interactions are implicated in colorectal carcinogenesis and can be non-invasive diagnostic biomarkers. *Microbiome* 10, 35. doi: 10.1186/s40168-021-01208-5
- Collins, D., Hogan, A. M., and Winter, D. C. (2011). Microbial and viral pathogens in colorectal cancer. *Lancet Oncol.* 12, 504–512. doi: 10.1016/S1470-2045(10)70186-8
- 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
- Davey Smith, G., and Hemani, G. (2014). Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum. Mol. Genet.* 23, R89–R98. doi: 10.1093/hmg/ddu328
- de Almeida, C. V., Taddei, A., and Amedei, A. (2018). The controversial role of *Enterococcus faecalis* in colorectal cancer. *Ther. Adv. Gastroenterol.* 11, 175624818783606. doi: 10.1177/175624818783606
- Emdin, C. A., Khera, A. V., and Kathiresan, S. (2017). Mendelian randomization. *JAMA* 318, 1925–1926. doi: 10.1001/jama.2017.17219
- Engku Nasrullah Satiman, E. A. F., Ahmad, H., Ramzi, A. B., Abdul Wahab, R., Kaderi, M. A., Harun, W. H. A. W., et al. (2020). The role of *Candida albicans* candidalysin ECE1 gene in oral carcinogenesis. *J. Oral. Pathol. Medicine: Off. Publ. Int. Assoc. Oral. Pathologists Am. Acad. Oral. Pathol.* 49, 835–841. doi: 10.1111/jop.13014
- Freshour, S. L., Kiwala, S., Cotto, K. C., Coffman, A. C., McMichael, J. F., Song, J. J., et al. (2021). Integration of the Drug-Gene Interaction Database (DGIdb 4.0) with open crowdsourcing efforts. *Nucleic Acids Res.* 49, D1144–D1151. doi: 10.1093/nar/gkaa1084
- Hartwig, F. P., Davey Smith, G., and Bowden, J. (2017). Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int. J. Epidemiol.* 46, 1985–1998. doi: 10.1093/ije/dyx102
- He, Z., Gharaibeh, R. Z., Newsome, R. C., Pope, J. L., Dougherty, M. W., Tomkovich, S., et al. (2019). *Campylobacter jejuni* promotes colorectal tumorigenesis through the action of cytolethal distending toxin. *Gut* 68, 289–300. doi: 10.1136/gutjnl-2018-317200
- Hemani, G., Tilling, K., and Davey Smith, G. (2017). Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Genet.* 13, e1007081. doi: 10.1371/journal.pgen.1007081
- Hu, Q., Bian, Q., Rong, D., Wang, L., Song, J., Huang, H.-S., et al. (2023). JAK/STAT pathway: Extracellular signals, diseases, immunity, and therapeutic regimens. *Front. Bioengineering Biotechnol.* 11, 1110765. doi: 10.3389/fbioe.2023.1110765
- Huang, D., Yi, X., Zhou, Y., Yao, H., Xu, H., Wang, J., et al. (2020). Ultrafast and scalable variant annotation and prioritization with big functional genomics data. *Genome Res.* 30, 1789–1801. doi: 10.1101/gr.267997.120
- Huh, J.-W., Kim, M. J., Kim, J., Lee, H. G., Ryoo, S.-B., Ku, J.-L., et al. (2022). Enterotypic Prevotella and three novel bacterial biomarkers in preoperative stool predict the clinical outcome of colorectal cancer. *Microbiome* 10, 203. doi: 10.1186/s40168-022-01388-8
- Janakiram, N. B., Steele, V. E., and Rao, C. V. (2009). Estrogen receptor-beta as a potential target for colon cancer prevention: chemoprevention of azoxymethane-induced colon carcinogenesis by raloxifene in F344 rats. *Cancer Prev. Res.* 2, 52–59. doi: 10.1158/1940-6207.CAPR-08-0140
- Jiang, L., Ren, X., Yang, J., Chen, H., Zhang, S., Zhou, X., et al. (2024). Mitophagy and clear cell renal cell carcinoma: insights from single-cell and spatial transcriptomics analysis. *Front. Immunol.* 15, 1400431. doi: 10.3389/fimmu.2024.1400431
- Kim, S.-H., Chelliah, R., Ramakrishnan, S. R., Perumal, A. S., Bang, W.-S., Rubab, M., et al. (2020). Review on stress tolerance in campylobacter jejuni. *Front. Cell. Infection Microbiol.* 10, 596570. doi: 10.3389/fcimb.2020.596570
- Kwong, T. N. Y., Wang, X., Nakatsu, G., Chow, T. C., Tipoe, T., Dai, R. Z. W., et al. (2018). Association between bacteremia from specific microbes and subsequent diagnosis of colorectal cancer. *Gastroenterology* 155, 383–390.e8. doi: 10.1053/j.gastro.2018.04.028
- Li, Z., Wen, J., Wu, W., Dai, Z., Liang, X., Zhang, N., et al. (2024). Causal relationship and shared genes between air pollutants and amyotrophic lateral sclerosis: A large-scale genetic analysis. *CNS Neurosci. Ther.* 30, e14812. doi: 10.1111/cns.14812
- Liu, X., Tong, X., Zhu, J., Tian, L., Jie, Z., Zou, Y., et al. (2021). Metagenome-genome-wide association studies reveal human genetic impact on the oral microbiome. *Cell Discovery* 7, 117. doi: 10.1038/s41421-021-00356-0
- Lv, X., Hu, Z., Liang, F., Liu, S., Gong, H., Du, J., et al. (2023). Causal relationship between ischemic stroke and its subtypes and frozen shoulder: a two-sample Mendelian randomization analysis. *Front. Neurol.* 14, 1178051. doi: 10.3389/fneur.2023.1178051
- Ma, M., Zheng, Z., Li, J., He, Y., Kang, W., and Ye, X. (2024). Association between the gut microbiota, inflammatory factors, and colorectal cancer: evidence from Mendelian randomization analysis. *Front. Microbiol.* 15, 1309111. doi: 10.3389/fmicb.2024.1309111
- Malik, S., Chakraborty, D., Agnihotri, P., Kumar, V., and Biswas, S. (2024). Unveiling the nexus: cellular metabolomics unravels the impact of estrogen on nicotinamide metabolism in mitigating rheumatoid arthritis pathogenesis. *Metabolites* 14, 214. doi: 10.3390/metabo14040214
- McGuire, K., Neal, D., Fraizer, G. C., Gilloteaux, J., Taper, H., and Jamison, J. (2012). Abstract 2076: Oxidative stress induced by sodium ascorbate:menadiene sodium-bisulfate combination results in lysosomal and mitochondrial changes that lead to prostate cancer cell death. *Cancer Res.* 72, 2076. doi: 10.1158/1538-7445.AM2012-2076
- Michaudel, C., Danne, C., Agus, A., Magniez, A., Aucouturier, A., Spatz, M., et al. (2023). Rewiring the altered tryptophan metabolism as a novel therapeutic strategy in inflammatory bowel diseases. *Gut* 72, 1296–1307. doi: 10.1136/gutjnl-2022-327337
- Mysak, J., Podzimek, S., Sommerova, P., Lyuya-Mi, Y., Bartova, J., Janatova, T., et al. (2014). Porphyromonas gingivalis: major periodontopathic pathogen overview. *J. Immunol. Res.* 2014, 476068. doi: 10.1155/2014/476068
- Niccolai, E., Russo, E., Baldi, S., Ricci, F., Nannini, G., Pedone, M., et al. (2020). Significant and conflicting correlation of IL-9 with prevotella and bacteroides in human colorectal cancer. *Front. Immunol.* 11, 573158. doi: 10.3389/fimmu.2020.573158
- Ögrendik, M. (2017). Periodontal pathogens in the etiology of pancreatic cancer. *Gastrointestinal Tumors* 3, 125–127. doi: 10.1159/000452708
- Quaglio, A. E. V., Grillo, T. G., Oliveira, E. C. S. De, Stasi, L. C. Di, and Sasaki, L. Y. (2022). Gut microbiota, inflammatory bowel disease and colorectal cancer. *World J. Gastroenterol.* 28, 4053–4060. doi: 10.3748/wjg.v28.i30.4053
- Rah, B., Rather, R. A., Bhat, G. R., Baba, A. B., Mushtaq, I., Farooq, M., et al. (2022). JAK/STAT signaling: molecular targets, therapeutic opportunities, and limitations of targeted inhibitions in solid Malignancies. *Front. Pharmacol.* 13, 821344. doi: 10.3389/fphar.2022.821344
- Rezasoltani, S., Aghdaei, H. A., Jasemi, S., Gazouli, M., Dovrolis, N., Sadeghi, A., et al. (2022). Oral microbiota as novel biomarkers for colorectal cancer screening. *Cancers* 15, 192. doi: 10.3390/cancers15010192
- Roth, W., Zadeh, K., Vekariya, R., Ge, Y., and Mohamadzadeh, M. (2021). Tryptophan metabolism and gut-brain homeostasis. *Int. J. Mol. Sci.* 22, 2973. doi: 10.3390/ijms22062973
- Seif, F., Khoshmirsafa, M., Aazami, H., Mohsenzadegan, M., Sedighi, G., and Bahar, M. (2017). The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. *Cell communication signaling: CCS* 15, 23. doi: 10.1186/s12964-017-0177-y
- Shen, W., Song, Z., Zhong, X., Huang, M., Shen, D., Gao, P., et al. (2022). Sangerbox: A comprehensive, interaction-friendly clinical bioinformatics analysis platform. *iMeta* 1, e36. doi: 10.1002/imt2.36
- Shih, P.-C., Chen, H.-P., Hsu, C.-C., Lin, C.-H., Ko, C.-Y., Hsueh, C.-W., et al. (2023). Long-term DEHP/MEHP exposure promotes colorectal cancer stemness associated

with glycosylation alterations. *Environ. pollut.* 327, 121476. doi: 10.1016/j.envpol.2023.121476

Song, M., Yin, C., Xu, Q., Liu, Y., Zhang, H., Liu, X., et al. (2023). Enhanced production of β -nicotinamide mononucleotide with exogenous nicotinamide addition in *saccharomyces boulardii*-YS01. *Foods* 12, 2897. doi: 10.3390/foods12152897

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

Verbanck, M., Chen, C.-Y., Neale, B., and Do, R. (2018). Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat. Genet.* 50, 693–698. doi: 10.1038/s41588-018-0099-7

Vishnu Prasoodanan, P. K., Sharma, A. K., Mahajan, S., Dhakan, D. B., Maji, A., Scaria, J., et al. (2021). Western and non-western gut microbiomes reveal new roles of *Prevotella* in carbohydrate metabolism and mouth-gut axis. *NPJ biofilms microbiomes* 7, 77. doi: 10.1038/s41522-021-00248-x

Wang, J., Qiao, Y., Sun, H., Chang, H., Zhao, H., Zhang, S., et al. (2022). Decreased SLC27A5 suppresses lipid synthesis and tyrosine metabolism to activate the cell cycle in hepatocellular carcinoma. *Biomedicines* 10, 234. doi: 10.3390/biomedicines10020234

Wang, Y., Zhang, Y., Qian, Y., Xie, Y.-H., Jiang, S.-S., Kang, Z.-R., et al. (2021). Alterations in the oral and gut microbiome of colorectal cancer patients and association with host clinical factors. *Int. J. Cancer.* doi: 10.1002/ijc.33596

Warren, R. L., Freeman, D. J., Pleasance, S., Watson, P., Moore, R. A., Cochrane, K., et al. (2013). Co-occurrence of anaerobic bacteria in colorectal carcinomas. *Microbiome* 1, 16. doi: 10.1186/2049-2618-1-16

Weith, M., and Beyer, A. (2023). The next step in Mendelian randomization. *eLife* 12, e86416. doi: 10.7554/eLife.86416

Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., et al. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Cambridge (Mass.))* 2, 100141. doi: 10.1016/j.xinn.2021.100141

Xi, Y., and Xu, P. (2021). Global colorectal cancer burden in 2020 and projections to 2040. *Trans. Oncol.* 14, 101174. doi: 10.1016/j.tranon.2021.101174

Yazar, S., Alquicira-Hernandez, J., Wing, K., Senabouth, A., Gordon, M. G., Andersen, S., et al. (2022). Single-cell eQTL mapping identifies cell type-specific genetic control of autoimmune disease. *Science* 376 (6589), eabf3041. doi: 10.1126/science.abf3041

Ye, C., Fu, Y., Zhou, X., Zhou, F., Zhu, X., and Chen, Y. (2024). Identification and validation of NAD⁺ metabolism-related biomarkers in patients with diabetic peripheral neuropathy. *Front. Endocrinol.* 15, 1309917. doi: 10.3389/fendo.2024.1309917

Ye, D., Xu, H., Xia, H., Zhang, C., Tang, Q., and Bi, F. (2021). Targeting SERT promotes tryptophan metabolism: mechanisms and implications in colon cancer treatment. *J. Exp. Clin. Cancer research: CR* 40, 173. doi: 10.1186/s13046-021-01971-1

Yu, L., Lu, J., and Du, W. (2024). Tryptophan metabolism in digestive system tumors: unraveling the pathways and implications. *Cell communication signaling: CCS* 22, 174. doi: 10.1186/s12964-024-01552-7

Zaura, E., Nicu, E. A., Krom, B. P., and Keijser, B. J. F. (2014). Acquiring and maintaining a normal oral microbiome: current perspective. *Front. Cell. Infection Microbiol.* 4, 85. doi: 10.3389/fcimb.2014.00085



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Genetically predicted blood metabolites mediate relationships between gut microbiota and ovarian cancer: a Mendelian randomization study

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Background and purpose: While there is evidence that gut microbiota (GM) and blood metabolites are associated with ovarian cancer (OC), the precise mechanisms underlying this relationship are still unclear. This study used Mendelian randomization (MR) to elucidate the causal connections between GM, blood metabolite biomarkers, and OC.

Methods: In this study, we leveraged summary data for GM (5,959 individuals with genotype-matched GM), blood metabolites (233 circulating metabolic traits with 136,016 participants), and OC (63,702 participants with 23,564 cases and 40,138 controls) from genome-wide association studies (GWASs). We performed MR analysis to explore the causal relationship between GM and OC. Further, we harnessed univariable MR (UVMR) analysis to evaluate the causal associations between GM and circulating metabolites. Finally, we employed a two-step approach based on multivariable MR (MVMR) to evaluate the total genetic prediction effect of metabolites mediating the GM on the risk of OC to discover a potential causal relationship.

Results: In the MR analysis, 24 gut bacteria were causally associated with the pathogenesis of OC, including 10 gut bacteria (*Dorea phocaeense*, *Succinivibrionaceae*, *Raoultella*, *Phascolarctobacterium* sp003150755, *Paenibacillus* J, NK4A144, K10, UCG-010 sp003150215, *Pseudomonas aeruginosa*, and *Planococcaceae*) that were risk factors, and 14 gut bacteria (CAG-177 sp002438685, GCA-900066135 sp900066135, *Enorma massiliensis*, *Odoribacter laneus*, *Ruminococcus* E sp003521625, *Streptococcus sanguinis*, *Turicibacter* sp001543345, *Bacillus velezensis*, CAG-977, *Cyanobacteria* *Staphylococcus* A fleurettii, *Caloranaerobacteraceae*, RUG472 sp900319345, and CAG-269 sp001915995) that were protective factors. The UVMR analysis showed that these 24 positive gut bacteria were causally related to lipoproteins, lipids, and amino acids. According to the MVMR analysis, *Enorma massiliensis* could reduce the risk of OC by raising the total cholesterol to total lipids ratio in large low-density lipoprotein (LDL) and cholesteryl esters to total lipids ratio in intermediate-density lipoprotein (IDL). *Turicibacter* sp001543345, however, could reduce the risk of OC by lowering free

cholesterol in small high-density lipoprotein (HDL) and increasing the ratios of saturated fatty acids to total fatty acids, total cholesterol to total lipids ratio in very small very-low-density lipoprotein (VLDL), and cholesteryl esters to total lipids ratio in very small VLDL.

Conclusion: The current MR study provides evidence that genetically predicted blood metabolites can mediate relationships between GM and OC.

KEYWORDS

gut microbiota (GM), ovarian cancer (OC), metabolites, Mendelian randomization (MR) analysis, biomarker

1 Introduction

Ovarian cancer (OC) is a prevalent type of cancer affecting women worldwide and with a high incidence and the lowest survival rate in all gynecological malignancies, is seriously endangering women's health (Salehi et al., 2019; Sung et al., 2021; Yang et al., 2022; Zara et al., 2022). The early symptoms of OC are imperceptible, and most of them are already advanced when diagnosed (Torre et al., 2018). It is very significant to clarify the incidence factors for the treatment and prevention of OC; however, the pathogenesis of OC is still unknown.

Studies have shown that the risk factors for OC mainly include family history of OC or endometriosis, environmental pollution, and bad living habits (Mallen et al., 2018). Observational and experimental studies have recently shown an association between gut microbial dysbiosis and the occurrence of various tumors, including gastric, breast, and intestinal cancer (Banerjee et al., 2017; Łaniewski et al., 2020). Patients with OC are sensitive to the gut microbiota (GM), often showing obvious intestinal symptoms in the early stage of the onset, including abdominal pain and distension, indigestion, constipation, and early satiety. Moreover, the gastrointestinal symptoms of patients with OC are more prominent in the treatment process than those of patients with cervical cancer or endometrial cancer. Some scholars have compared and analyzed the GM in high-grade serous OC (HGSOC) and benign tumors by 16S rRNA sequencing and confirmed that gut microbial dysbiosis played an important role in OC with animal models (Zhou et al., 2019; Hu et al., 2023). Furthermore, GM are associated with chemotherapy sensitivity, and the regulation of GM can alleviate cisplatin resistance in OC (Chambers et al., 2022), while fecal microbiota transplantation of *Akkermansia muciniphila* plays an important role in inhibiting OC progression through T cell activation (Wang et al., 2022). In addition, the proportion of Proteobacteria and Firmicutes was significantly higher in cancer samples than in controls. In a 2019 study, Nené et al. reported that the number of *Lactobacillus* was significantly reduced in women with ovarian cancer compared with controls. This change was particularly pronounced in patients with

BRCA (1/2) mutations; these mutations seemed to promote the growth of microbial communities dominated by non-*Lactobacillus* bacteria. *Lactobacilli* produce lactic acid through glycogen metabolism, and high estrogen levels cause glycogen secretion by vaginal epithelial cells. Widschwendter et al. found that BRCA mutation carriers had higher progesterone levels throughout the menstrual cycle, especially during the luteal phase. High concentrations of progesterone lead to a decrease in vaginal glycogen levels, making the environment unfavorable for the growth of *Lactobacilli*. However, no clinical studies have confirmed a causal relationship between GM and the risk of OC.

Currently, molecular biology studies have shown that disorders of glycerophospholipid metabolism, sphingolipid metabolism, and glyceride lipid metabolism are important metabolic pathways in the progression of OC. Animal-level studies through liquid chromatography-mass spectrometry (LC-MS) testing of serum from mice with early and advanced HGSOC, respectively, have shown that lipid metabolism disorders such as glycerophospholipid metabolism and sphingolipid metabolism often occur with altered levels of 29 metabolites in the early stages of OC (Huang et al., 2019).

As mentioned above, the specific relevance of GM to the onset of OC has not been fully elucidated, and the mechanism of metabolites between OC and GM is still unknown. Therefore, a thorough study of the relationship between the GM, metabolites, and OC is urgently needed. This study may expand our understanding of the pathogenesis of OC and may provide new biomarkers and therapeutic targets based on multi-omic studies on OC.

Mendelian randomization (MR) utilizes genetic variation as an instrumental variable (IV) for risk factors or exposures and disease occurrence as a clinical outcome to analyze the causal relationship between clinical outcomes and risk factors (Lawlor et al., 2008; Zheng et al., 2017). This approach can avoid confounders and reverse causality in observational studies and allow for more robust causal inferences between exposures and clinical outcomes. In addition, growing evidence has illustrated the value of clinical studies using human genetic information for gut microbial traits,

allowing us to employ MR to infer a causal relationship between GM and OC. Herein, employing summary data from genome-wide association studies (GWASs), we conducted MR analysis to explore the causal relationship between GM and OC. Furthermore, we conducted mediation analysis with MR, applying a two-step approach to investigate the total genetic prediction effect of metabolites mediating the GM on the risk of OC, thus guiding the prevention, diagnosis, and treatment of OC.

2 Methods

2.1 Study design

Single nucleotide polymorphisms (SNPs) were used in this study as IVs to explore the causal relationship between GM and OC. The three criteria listed in the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomisation (STROBE-MR) checklist (Skrivankova et al., 2021) must be fulfilled. 1) There is a significant association between exposure and each IV. 2) The exposure alone influences each IV's result. 3) Linkage disequilibrium (LD) reduces the bias by ensuring no confounding factors impact any IV (van Kippersluis and Rietveld, 2018).

We evaluated the causal associations between GM, circulating metabolites, and OC using univariable MR (UVMR) and multivariable MR (MVMR) analysis.

Figure 1 shows the flowchart for the UVMR study. First, the positive UVMR analysis was investigated between GM as the exposure and OC as the outcome; subsequently, the robustness of the causal association between GM and the development of OC was verified using Inverse Variance Weighted (IVW), Robust IVW, Penalized IVW, and Penalized Robust IVW. MR-Egger Intercept, Penalized MR-Egger Intercept, Robust MR-Egger Intercept, and

Penalized Robust MR-Egger Intercept were employed to lessen the influence of horizontal pleiotropy. Finally, the MR-PRESSO test was employed to eliminate the abnormal IVs, resulting in the most robust GM following the detection and correction of outliers.

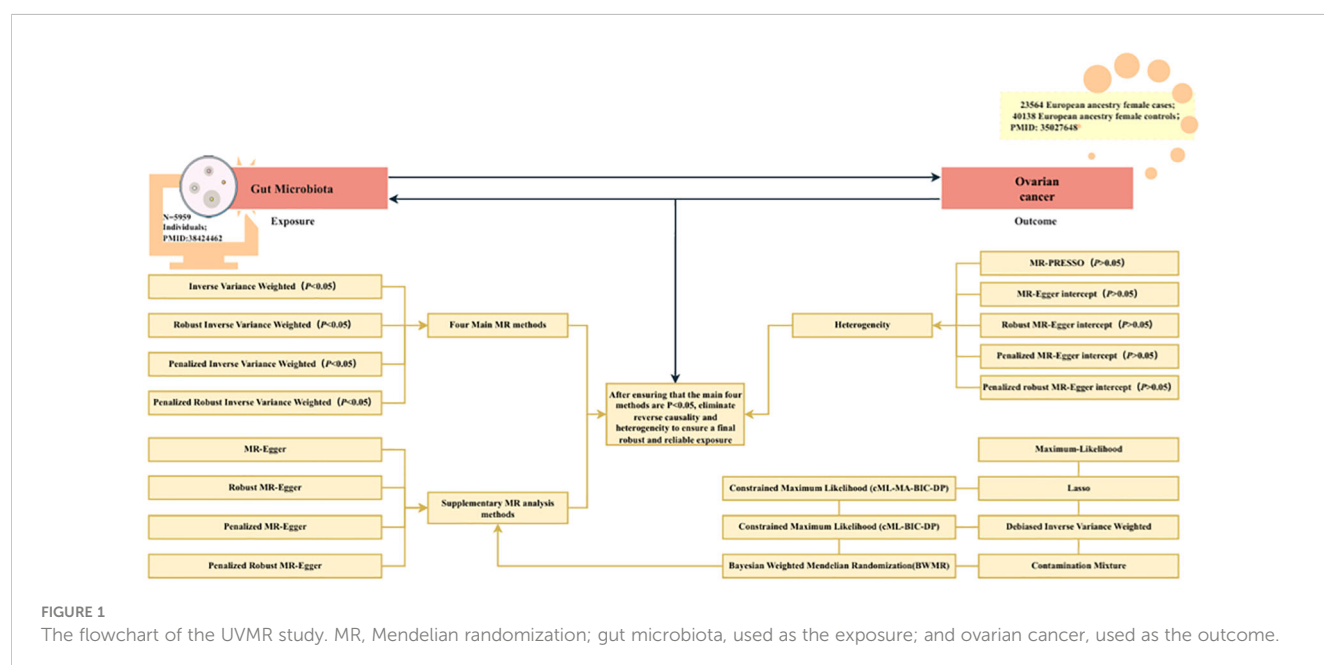
We further employed a two-step approach based on MVMR to evaluate the total genetic prediction effect of metabolites mediating GM on the risk of OC. The flowchart of mediation analysis based on MVMR is shown in Figure 2, with GM as the exposure, metabolites as the mediator, and OC as the outcome. In the first step of the two-step method, routine UVMR analysis of gut microbes and metabolites was performed to obtain β_1 ($P < 0.05$). In the second step, MVMR analysis of the positive metabolites, GM, and OC was carried out to yield β_2 ($P < 0.05$). In this way, with the UVMR analysis of GM and OC, the direct effect was $\beta_1 * \beta_2$, the mediation effect was $\beta_1 * \beta_2/\beta$, and the total effect was β .

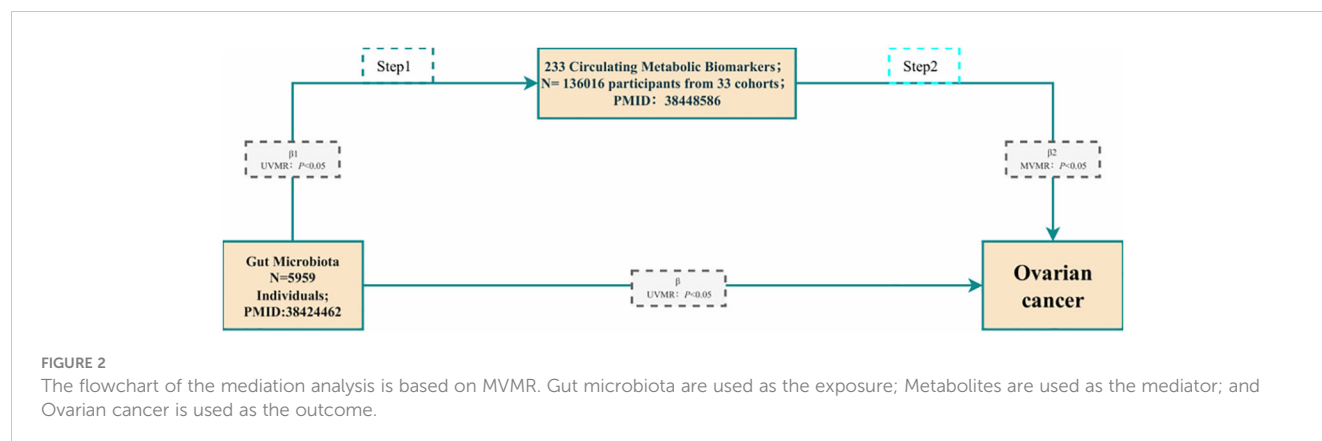
2.2 Data sources

GWAS data for OC were obtained from the Catalog GWAS database with GCST GCST90016665 (<https://www.ebi.ac.uk/gwas/studies/GCST90016665>) (Dareng et al., 2022), which included 63,702 participants (23,564 cases and 40,138 controls).

GWAS data for GM were obtained from the study by Qin et al. in 2022 (Qin et al., 2022). This study examined the impact of human genetic variation on the abundance of GM by analyzing data from 5,959 individuals with genotype-matched GM, diet, and health records and identified 567 independent SNP-taxon associations.

The GWAS data for the metabolites were derived from a study published by Karjalainen et al. in 2024 (Karjalainen et al., 2024). With 136,016 participants, a GWAS analysis of 233 circulating metabolic traits revealed over 400 independent loci, of which two-thirds were likely causal genes. This highlighted the significant impact of sample and participant characteristics on genetic





associations, revealed the genetic basis of circulating metabolic traits and their impact on complex diseases, and provided an ample data resource for metabolism-disease relationships.

2.3 Instrument selection

To ensure the stability of the study data and the accuracy of the results when GM was used as an exposure factor and OC was used as the outcome, we proposed the following requirements for IVs: (a) the significance threshold of $P < 1 \times 10^{-5}$ is applied to IVs related to the GM to ensure genome-wide significance (Cheng et al., 2020); (b) to satisfy the conditions for the MR analysis, we performed an LD analysis based on the European Thousand Person Genome Project, which required $R^2 < 0.001$ of IVs, LD = 10000 kb; (c) we assessed the statistical strength of genetic variation as the IVs using the F-statistic to prevent the effect of the allele on the causal relationship between the GM and OC. IVs with an F-statistic of variation ≤ 10 are treated as weak IVs and may bias the analysis results, while an F-statistic > 10 indicates that the IVs are strong. Thus, we excluded IVs with an F-statistic of variation ≤ 10 (Burgess et al., 2017).

Moreover, in the two-step mediation analysis based on MVMR, the IVs of metabolites should meet the following requirements: $P < 1 \times 10^{-5}$, $R^2 < 0.001$, LD = 10000 kb. Similarly, IVs with an F-statistic of variation ≤ 10 were excluded.

2.4 Statistical analysis

After obtaining the required data from the Catalog GWAS, we conducted an MR analysis to explore the causal relationship between GM and OC. Finally, we applied a two-step mediation analysis with MR to investigate the total genetic prediction effect of metabolite-mediated GM on the risk of OC.

During the MR analysis, we mainly used R (version 4.3.1) with the “Two Sample MR” R package (version 0.5.7) (Mounier and Kutalik, 2023), “Mendelian Randomization” R package (version 0.9.0) and “Bayesian Weighted Mendelian Randomization (BWMR)” R package (version 0.1.1) (Zhao et al., 2020). The R^2 was used to represent the proportion of the phenotypic variants explained by the SNPs and was calculated by the equation $R^2 =$

$\frac{2 \times \beta \times \text{EAF} \times (1 - \text{EAF})}{2 \times \beta^2 \times \text{EAF} \times (1 - \text{EAF}) + \text{SE}^2 \times 2 \times \text{Sample size} \times \text{EAF} \times (1 - \text{EAF})}$. To assess the strength of the IVs; we calculated the F-statistic with the formula $F = \frac{R^2 \times (\text{Sample size} - 1 - k)}{(1 - R^2) \times k}$, where R^2 was the proportion of phenotypic variation explained by SNPs and k was the number of SNPs included in the tool (Lai et al., 2018). Thresholds with an F-statistic > 10 were generally considered statistically significant, indicating that bias did not affect causal links (Zuber et al., 2020).

In the UVMR study, we first verified the validity of all IVs using the IVW method and obtained the weighted total effect according to the P-value (Brion et al., 2013). To verify the robustness of the conclusions, we used the following three methods to reduce the bias of the causal analysis: (a) using the Robust IVW to reduce the sensitivity of IVs to outliers and strong pleiotropy; (b) using Penalized IVW to adjust for effect estimates of outliers or inconsistency in the data, thus to gain more reliable causal estimates; (c) using Penalized Robust IVW to adjust for outliers in the data and for inconsistent effect estimates, and to reduce the effects of IVs with pleiotropy, thus providing the most rigorous and robust causal estimates. Secondly, the P-value of the MR-Egger intercept was introduced to detect the presence of directional pleiotropy (Burgess et al., 2024). If $P > 0.05$, no obvious directional pleiotropy was considered, which increased the reliability of the causal effect estimate. Meanwhile, to verify the reliability of causal effects in the conclusions, we used four methods to exclude the interference of horizontal pleiotropy: (a) using MR-Egger Intercept to detect the directional pleiotropy of IVs, indicating whether the average effect of pleiotropy differed from zero; (b) using Penalized MR-Egger Intercept, with a penalty term introduced into the MR-Egger Intercept method to reduce the impact of pleiotropic IVs; (c) using the Robust MR-Egger Intercept to adjust the robustness of the MR-Egger Intercept method, thus reducing the effects of outliers and strong pleiotropic IVs; (d) using Penalized Robust MR-Egger Intercept, with both robustness and pleiotropy penalties considered and acting together to improve the accuracy and robustness of causal estimates. Finally, outliers were detected and corrected by removing abnormal IVs using the MR-PRESSO test (Fan et al., 2023), and the results were more reliable when the effect size of IVW was consistent with the sensitivity analysis and $P < 0.05$. We also performed a variety of supplementary MR analyses, including

Contamination mixture, maximum likelihood, Debiased IVW, MR-Egger, and BWMR. Although Contamination mixture MR analysis did not remove abnormal IVs, based on the assumption that valid IVs were the largest subset of all IVs, the analysis method would have a more precise causal effect than IVW results (Burgess et al., 2020). The MR analysis method of maximum likelihood is applied to unrelated and related genetic variants. In IVW, if the fixed effect model was incorrect and there was great heterogeneity in the causal effect of different variables, the MR analysis method of maximum likelihood analyzed the existing heterogeneity by random effect model (Burgess et al., 2013). If there were unavoidable weak IVs, we performed MR analysis using the method of Debiased IVW. This approach was robust to many weak IVs and did not require screening (Ye et al., 2021). MR-Egger evaluated whether genetic variation was pleiotropic for results that, on average, differ from zero by targeted pleiotropy tests, causal effect tests, and causal effect estimates, and provided a consistent estimate of causal effect under weaker assumptions (Burgess and Thompson, 2017). BWMR considered the uncertainty of weak effects due to polygenes and detected their outliers through Bayesian Weighted, thus solving the violation of MR assumptions due to polygenes.

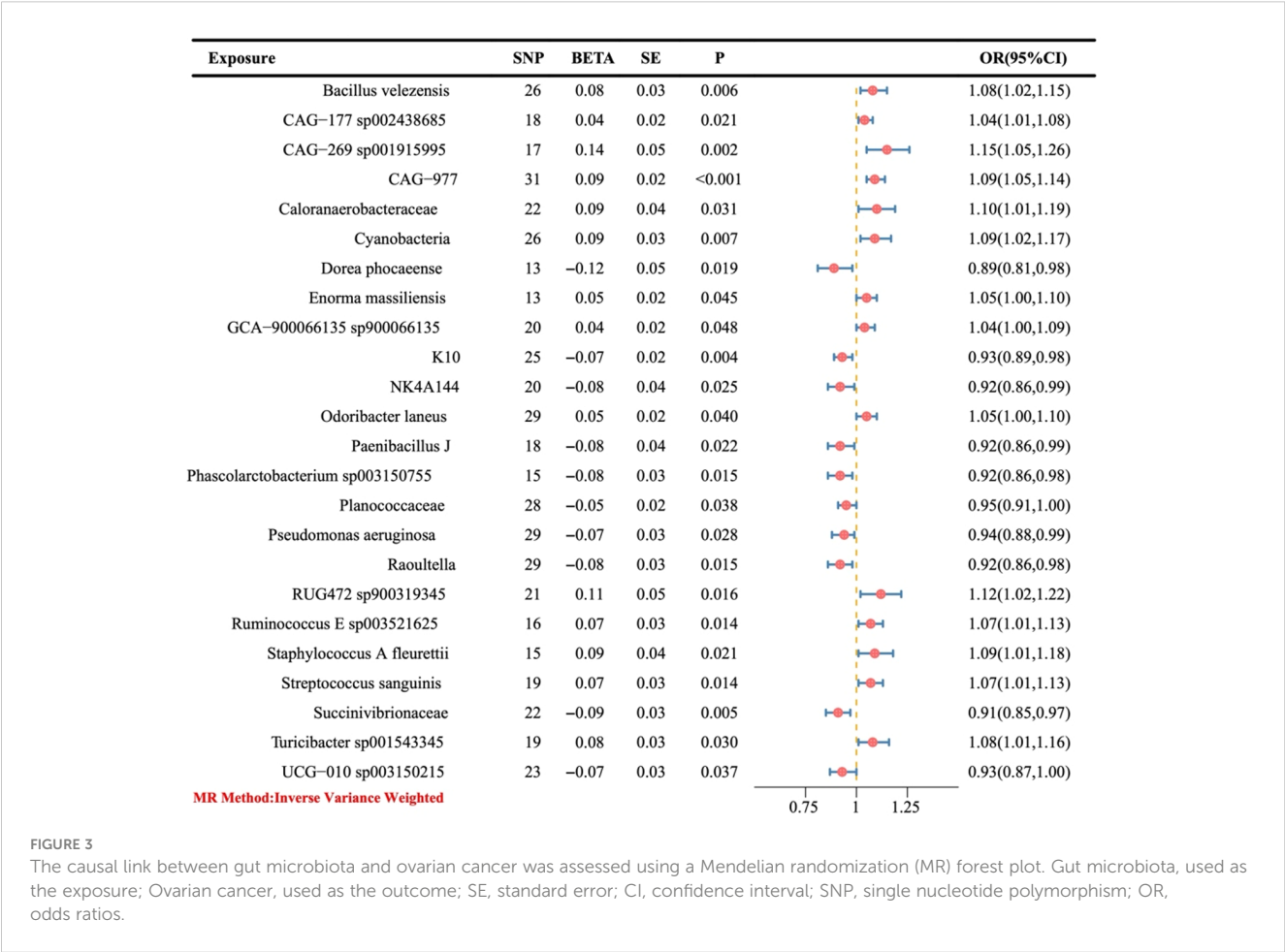
In the two-step mediation MR, first, the most robust GM and metabolites were used to perform UVMR analysis to obtain β_1 ; then, the positive mediator (metabolites) determined by the first step was combined with the most robust GM to obtain β_2 . At this

time, with the UVMR analysis of GM and OC, the total effect was β , the mediation effect was $\beta_1 * \beta_2$, the direct effect was $\beta - \beta_1 * \beta_2$, and the mediation effect was $\beta_1 * \beta_2 / \beta$. In the second step of the two-step MR for MVMR, we used Multivariable IVW to verify the validity of all IVs and generate weighted total effects by judging the magnitude of the P-value.

3 Results

3.1 Causal effect of the gut microbiota on ovarian cancer

The preliminary IVW results showed that a total of 24 gut bacteria played a causal role in OC (Figure 3), of which the bacteria with a positive correlation were *Dorea phocaense*, *Succinivibrionaceae*, *Raoultella*, *Phascolarctobacterium* sp003150755, *Paenibacillus* J, *NK4A144*, *K10*, *UCG-010* sp003150215, *Pseudomonas aeruginosa* and *Planococcaceae* (OR > 1), and the bacteria with a negative correlation were *CAG-177* sp002438685, *GCA-900066135* sp900066135, *Enorma massiliensis*, *Odoribacter laneus*, *Ruminococcus* E sp003521625, *Streptococcus sanguinis*, *Turicibacter* sp001543345, and *Bacillus velezensis*, *CAG-977*, *Cyanobacteria* *Staphylococcus* A *fleurettii*, *Caloranaerobacteraceae*, *RUG472* sp900319345, and *CAG-269* sp001915995 (OR < 1).



Moreover, we conducted sensitivity analysis, horizontal pleiotropy, and removal of abnormal IVs on the above 24 positive gut bacteria (Figure 4), which also confirmed the robustness of the causal association between GM and OC.

3.2 Causal effect of the gut microbiota on metabolites

In MR analysis, we analyzed the above 24 positive gut bacteria with 233 metabolites. As shown in Figure 5, 242 positive results were presented as a heatmap (part had been overlapped), which suggested that the above 24 positive gut bacteria may be causally related to the following categories of metabolites. The first category included lipoproteins, such as the ratio of free cholesterol to total lipids in very large high-density lipoprotein (HDL), the ratio of phospholipids in medium HDL, and the total lipids in medium HDL; the second category included lipids, such as levels of linoleic acid (18:2), the ratio of 18:2 linoleic acid to total fatty acids, and the ratio of saturated fatty acids to total fatty acids; the third category included amino acids, such as citrate, glycine, and histidine levels.

3.3 Causal effect of metabolites on ovarian cancer

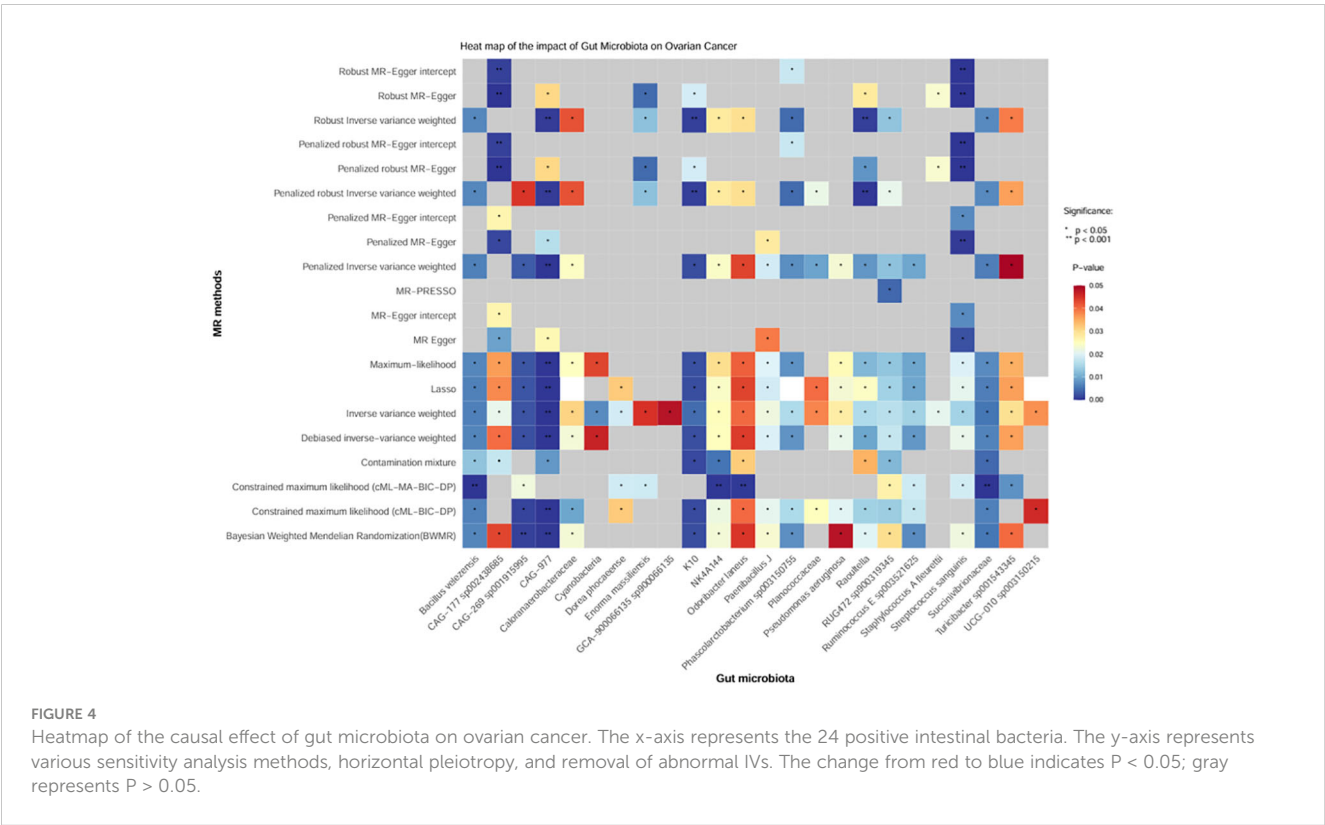
According to the MVMR analysis of the positive gut bacteria and their corresponding positive metabolites together with the outcomes, the metabolites with a causal relationship with OC were obtained. Finally, the mediation effect ratio was calculated.

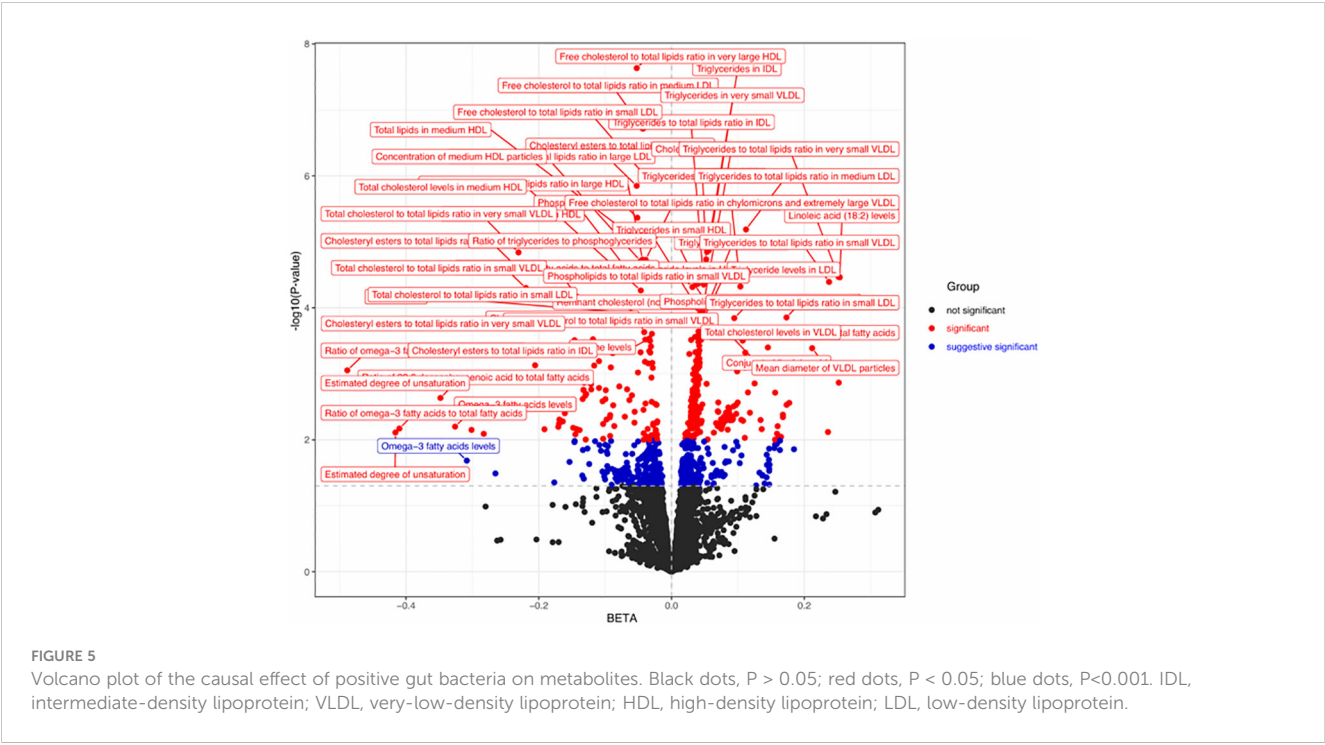
After excluding the mediation effect ratio, which was negative and of $P > 0.05$, six metabolites that had a causal relationship with OC were gained.

Since $OR > 1$ is a positive correlation, it indicates that the corresponding metabolite is a risk factor for the outcome. At the same time, $OR < 1$ is a negative correlation, which indicates that the corresponding metabolite is a protective factor. As illustrated in Figure 6, an increase in free cholesterol in small HDL ($OR = 1.09$, 95% CI 1.02-1.18; $P = 0.015$) was associated with higher odds of developing OC. Conversely, five other metabolites, including the total cholesterol to total lipids ratio in large low-density lipoprotein (LDL) ($OR = 0.97$, 95% CI 0.95-0.99; $P = 0.009$), cholesteryl esters to total lipids ratio in intermediate-density lipoprotein (IDL) ($OR = 0.97$, 95% CI 0.95-0.99; $P = 0.011$), the ratio of saturated fatty acids to total fatty acids ($OR = 0.69$, 95% CI 0.60-0.79; $P < 0.001$), the total cholesterol to total lipids ratio in very small very-low-density lipoprotein (VLDL) ($OR = 0.85$, 95% CI 0.79-0.92; $P < 0.001$), and the cholesteryl esters to total lipids ratio in very small VLDL ($OR = 0.87$, 95% CI 0.80-0.95; $P = 0.001$) were linked to decreased odds of developing OC.

3.4 Mediation effect of metabolite-mediating genetic predictions of the gut microbiota on ovarian cancer

Based on the causal effect of positive gut bacteria and positive metabolites on the risk of OC, we analyzed the mediation effect of metabolite-mediating genetic predictions of the gut microbiota on the risk of OC (Table 1).





In the process of *Enorma massiliensis* acting as a risk factor for OC, the total cholesterol to total lipids ratio in large LDL (mediation effect ratio = 1.82%) and the cholesteryl esters to total lipids ratio in IDL (mediation effect ratio = 2.60%) both mediated the total genetic prediction impact of *Enorma massiliensis* on the risk of OC. This suggests that *Enorma massiliensis* could lower the risk of OC by increasing the total cholesterol to total lipids ratio in large LDL and cholesteryl esters to total lipids ratio in IDL.

Turicibacter sp001543345 was found to be a risk factor for OC in the following ways: free cholesterol in small HDL (mediation effect ratio = 11.64%), ratio of saturated fatty acids to total fatty acids (69.09%), total cholesterol to total lipids in very small VLDL (mediation effect ratio = 49.36%), and cholesteryl esters to total lipids in very small VLDL (mediation effect ratio = 35.92%). These factors, in turn, mediated the overall genetic prediction impact of *Turicibacter sp001543345* on the risk of OC. These findings suggest

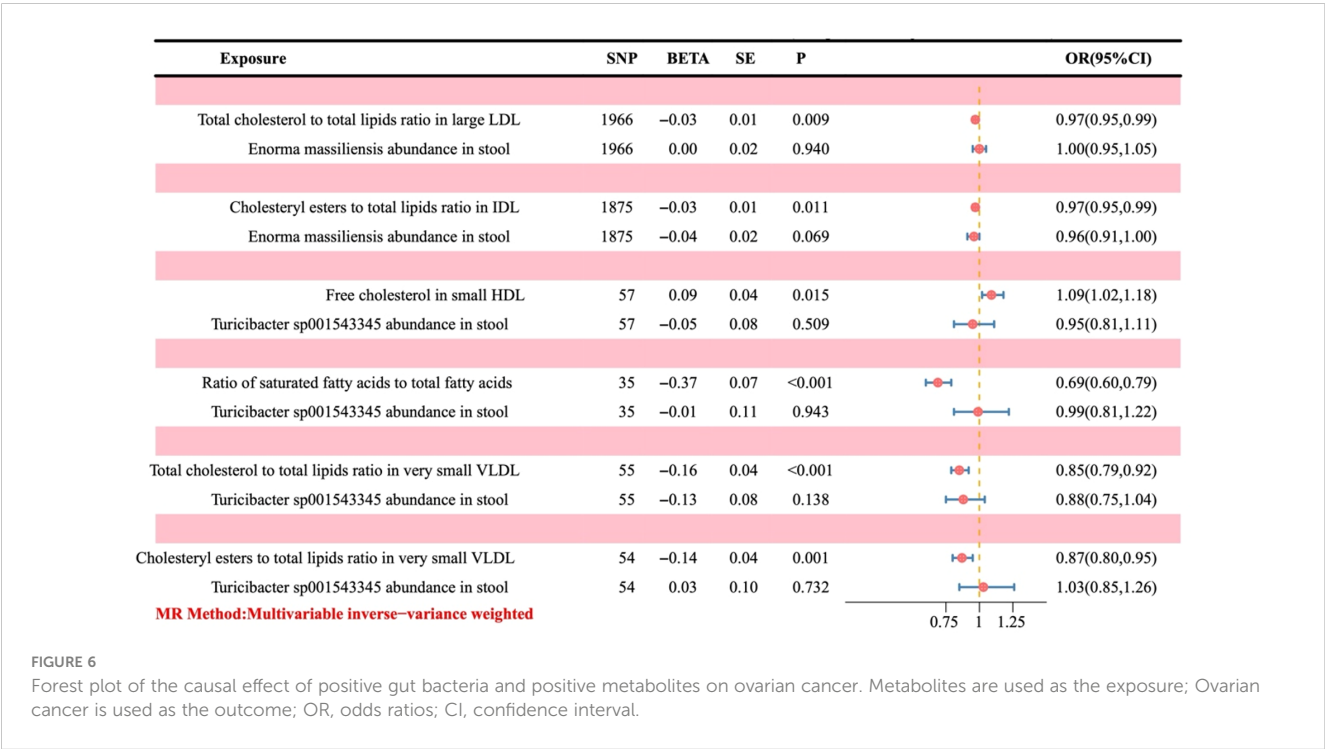


TABLE 1 Mediation effect of metabolite-mediating genetic predictions of GM on OC.

Exposure	Mediator	Outcome	Mediation effect ratio	LCI ratio	UCI ratio	Z	P
Enorma massiliensis	Total cholesterol to total lipids ratio in large LDL	Ovarian cancer	1.82%	0.001	0.035	2.13	0.03
Enorma massiliensis	Cholesteryl esters to total lipids ratio in IDL	Ovarian cancer	2.60%	0.001	0.051	2.05	0.04
Turicibacter sp001543345	Free cholesterol in small HDL	Ovarian cancer	11.64%	0.003	0.230	2.00	0.05
Turicibacter sp001543345	Ratio of saturated fatty acids to total fatty acids	Ovarian cancer	69.09%	0.019	1.363	2.02	0.04
Turicibacter sp001543345	Total cholesterol to total lipids ratio in very small VLDL	Ovarian cancer	49.36%	0.139	0.848	2.73	0.01
Turicibacter sp001543345	Cholesteryl esters to total lipids ratio in very small VLDL	Ovarian cancer	35.92%	0.050	0.668	2.28	0.02

that *Turicibacter sp001543345* may lower the risk of OC by reducing free cholesterol in small HDL and increasing the ratio of saturated fatty acids to total fatty acids, total cholesterol to total lipids in very small VLDL, and cholesteryl esters to total lipids in very small VLDL.

4 Discussion

In this study, MR analysis found that GM (24 gut bacteria) had a causal relationship with the pathogenesis of OC, among which 10 gut bacteria including *Dorea phocaeeense*, *Succinivibrionaceae*, *Raoultella*, *Phascolarctobacterium sp003150755*, *Paenibacillus J*, *NK4A144*, *K10*, *UCG-010 sp003150215*, *Pseudomonas aeruginosa*, and *Planococcaceae* (OR > 1) were risk factors and 14 gut bacteria including *CAG-177 sp002438685*, *GCA-900066135 sp900066135*, *Enorma massiliensis*, *Odoribacter laneus*, *Ruminococcus E sp003521625*, *Streptococcus sanguinis*, *Turicibacter sp001543345*, and *Bacillus velezensis*, *CAG-977*, *CyanobacteriaStaphylococcus A fleurettii*, *Caloranaerobacteraceae*, *RUG472 sp900319345*, and *CAG-269 sp001915995* (OR < 1) were protective factors. Moreover, the robustness of the causal association between GM and OC was verified by IVW, Robust IVW, Penalized IVW, and Penalized Robust IVW.

The UVMR analysis suggested that the above 24 positive gut bacteria were causally related to three categories of metabolites, including lipoproteins, lipids, and amino acids. Furthermore, the MVMR analysis indicated that six metabolites had a causal relationship with OC. The mediation effect of metabolite-mediating genetic predictions of the gut microbiota on the risk of OC suggested that two protective factors (*Enorma massiliensis* and *Turicibacter sp001543345*, OR < 1) could reduce the risk of OC by increasing or decreasing the mediation effect ratio. Specifically, *Enorma massiliensis* can potentially lower the risk of OC by increasing the ratio of total cholesterol to total lipids in large LDL and cholesteryl esters to total lipids in IDL. *Turicibacter sp001543345* can potentially lower the risk of OC by lowering free cholesterol in small HDL and increasing the ratio

of saturated fatty acids to total fatty acids and total cholesterol to total lipids ratio in very small VLDL.

Our research results suggested that *Enorma massiliensis* and *Turicibacter sp001543345* may act as protective agents against OC. Notably, *Enorma massiliensis* is a new genus within the family *Coriobacteriaceae*, *Enorma gen. nov.*, and was found in the stool of a 26-year-old woman who had morbid obesity as part of a culturomics study that attempted to cultivate every species of bacteria found in human feces individually (Mishra et al., 2013). This is a rod-shaped, anaerobic, non-endospore-forming, indole-negative bacterium that is Gram-positive. In addition, a study on the gut microbiome of breast cancer patients in Vietnam revealed that patients who experienced a significant delay in diagnosis had higher abundances of *Enorma massiliensis* (Nguyen et al., 2024). There is very little literature on the study of *Enorma massiliensis*, which also indicates that this genus *Enorma massiliensis* deserves further exploration as a protective factor for the pathogenesis of OC. *Turicibacter sp001543345* is a member of the family genus *Turicibacter*, which can reach relative abundances of 0.5% in the human fecal microbiota (Martínez et al., 2015; Browne et al., 2016). In numerous microbiota community profiling studies, correlations between *Turicibacter* and features of host fat metabolism, including adiposity and dietary lipids, have been revealed (Liu et al., 2016; Jiao et al., 2018; Li et al., 2019; Petersen et al., 2019; Velázquez et al., 2019; Dhakal et al., 2020). A recent study identified genes that allow different strains of *Turicibacter bacteria* to alter host bile acids and lipid metabolism, demonstrating how these bacteria affect host metabolites, including lipids and bile acids. These findings position *Turicibacter bacteria* as modulators of host fat biology (Desorcy-Scherer et al., 2024).

Blood metabolites may be linked to the pathogenesis of OC and can predict survival outcomes, but little is known about the genetics of these metabolites. Previous studies have shown that patients with ovarian tumors have higher cholesterol levels in the ascites. An early report by Helzlsouer et al. indicated a positive correlation between blood cholesterol concentration and ovarian cancer risk. In addition, LDL, as the main transporter of cholesterol, is associated with the aggressiveness of ovarian cancer and poor survival prognosis. In a

mouse ID8 ovarian cancer model, mice fed a high-cholesterol diet had accelerated tumor growth compared with the control group. Studies have shown that cholesterol homeostasis disorders may enhance the resistance of ovarian cancer to platinum drugs. At the same time, elevated cholesterol levels in invasive ascites activate LXR α/β nuclear receptors, upregulating multidrug resistance protein 1 (MDR1) and causing ovarian tumor cells to become resistant to cisplatin. Liver cancer cells become resistant to chemotherapy when their mitochondria have a high cholesterol load. It interferes with mitochondrial function, inhibits membrane permeability, and reduces the pro-apoptotic signal cytochrome c release. In addition, cholesterol affects energy metabolism, thereby promoting tumor development. Exogenous cholesterol can alter metabolic pathways, enhance cell proliferation in a manner dependent on the estrogen-related receptor α , elevate oxidative phosphorylation, and activate the tricarboxylic acid cycle (TCA cycle) in breast cancer cells. Studies have found that exogenous cholesterol can enhance aerobic glycolysis in triple-negative breast cancer cell lines. In addition, elevated mitochondrial cholesterol load promotes hexokinase transfer to mitochondria and may augment aerobic glycolysis in cancer cells. In this study, we analyzed the mediation effect of metabolite-mediating genetic predictions of the gut microbiota on the risk of OC based on the causal effect of 24 positive gut bacteria and 6 positive metabolites on the risk of OC, providing insights into the positive gut bacteria could affect the risk of OC by decreasing or increasing the mediation effect ratio of corresponding metabolites. This study evaluated the causal relationship between GM and the pathogenesis of OC based on MR analysis and confirmed the association of GM with the pathogenesis of OC. The advantages of this study mainly included the following aspects: first, MR could infer the causal association of exposure to the outcome and not be affected by confounding factors; second, GWAS data acquisition based on large population samples in this study improved the reliability of the results; finally, multiple methods were applied to reduce the bias of the causal analysis, thus ensuring the confidence and robustness of the results. However, the present study also had some limitations. On the one hand, the data set used in the study may have some unknown confounders that would impact the results. On the other hand, the GM GWAS data contained multiple populations, but mainly European populations, and the OC GWAS data was also a European population, so the universality of the conclusion needs to be further confirmed.

In conclusion, this study explored the causality of GM, metabolites, and risk of OC and revealed the mediation effect of metabolite-mediating genetic predictions of GM on OC. Therefore, this study could reference GM-based control measures against OC. On this basis, in the future, it is necessary to explore further the

mechanism of causality of specific gut bacteria, specific metabolites, and the risk of OC and find new strategies for preventing and treating OC with specific gut bacteria and metabolites.

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.

Author contributions

LZ: Data curation, Writing – review & editing, Formal analysis. TC: Methodology, Writing – review & editing. KL: Conceptualization, Data curation, Writing – original draft. PS: Data curation, Writing – original draft. WW: Formal analysis, Funding acquisition, Writing – original draft. JG: Data curation, Writing – original draft, 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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References

- Banerjee, S., Tian, T., Wei, Z., Shih, N., Feldman, M. D., Alwine, J. C., et al. (2017). The ovarian cancer microbiome. *Oncotarget* 8, 36225–36245. doi: 10.18632/oncotarget.v8i22
- Brion, M. J., Shakhbazov, K., and Visscher, P. M. (2013). Calculating statistical power in Mendelian randomization studies. *Int. J. Epidemiol.* 42, 1497–1501. doi: 10.1093/ije/dyt179
- Browne, H. P., Forster, S. C., Anonye, B. O., Kumar, N., Neville, B. A., Stares, M. D., et al. (2016). Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature* 533, 543–546. doi: 10.1038/nature17645
- 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

- Burgess, S., Foley, C. N., Allara, E., Staley, J. R., and Howson, J. M. M. (2020). A robust and efficient method for Mendelian randomization with hundreds of genetic variants. *Nat. Commun.* 11, 376. doi: 10.1038/s41467-019-14156-4
- Burgess, S., Small, D. S., and Thompson, S. G. (2017). A review of instrumental variable estimators for Mendelian randomization. *Stat. Methods Med. Res.* 26, 2333–2355. doi: 10.1177/0962280215597579
- Burgess, S., Sun, Y. Q., Zhou, A., Buck, C., Mason, A. M., and Mai, X. M. (2024). Body mass index and all-cause mortality in HUNT and UK biobank studies: revised non-linear Mendelian randomisation analyses. *BMJ Open* 14, e081399. doi: 10.1136/bmjopen-2023-081399
- 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
- Chambers, L. M., Esakov Rhoades, E. L., Bharti, R., Braley, C., Tewari, S., Trestan, L., et al. (2022). Disruption of the gut microbiota confers cisplatin resistance in epithelial ovarian cancer. *Cancer Res.* 82, 4654–4669. doi: 10.1158/0008-5472.CAN-22-0455
- Cheng, Q., Yang, Y., Shi, X., Yeung, K. F., Yang, C., Peng, H., et al. (2020). MR-LDP: a two-sample Mendelian randomization for GWAS summary statistics accounting for linkage disequilibrium and horizontal pleiotropy. *NAR. Genom. Bioinform.* 2, lqaa028. doi: 10.1093/nargab/lqaa028
- Dareng, E. O., Tyrer, J. P., Barnes, D. R., Jones, M. R., Yang, X., Aben, K. K. H., et al. (2022). Polygenic risk modeling for prediction of epithelial ovarian cancer risk. *Eur. J. Hum. Genet.* 30, 349–362. doi: 10.1038/s41431-021-00987-7
- Desorcy-Scherer, K., Zuniga-Chaves, I., Reisner, M. A., Suen, G., and Hernandez, L. L. (2024). Investigating the influence of perinatal fluoxetine exposure on murine gut microbial communities during pregnancy and lactation. *Sci. Rep.* 14, 13762. doi: 10.1038/s41598-024-62224-7
- Dhakal, S., McCormack, L., and Dey, M. (2020). Association of the gut microbiota with weight-loss response within a retail weight-management program. *Microorganisms* 8, 1246. doi: 10.3390/microorganisms8081246
- Fan, Y., Huang, H., Chen, X., Chen, Y., Zeng, X., Lin, F., et al. (2023). Causal effect of vitamin D on myasthenia gravis: a two-sample Mendelian randomization study. *Front. Nutr.* 10, 1171830. doi: 10.3389/fnut.2023.1171830
- Hu, X., Xu, X., Zeng, X., Jin, R., Wang, S., Jiang, H., et al. (2023). Gut microbiota dysbiosis promotes the development of epithelial ovarian cancer via regulating Hedgehog signaling pathway. *Gut. Microbes* 15, 2221093. doi: 10.1080/19490976.2023.2221093
- Huang, D., Gaul, D. A., Nan, H., Kim, J., and Fernández, F. M. (2019). Deep metabolomics of a high-grade serous ovarian cancer triple-knockout mouse model. *J. Proteome Res.* 18, 3184–3194. doi: 10.1021/acs.jproteome.9b00263
- Jiao, N., Baker, S. S., Nugent, C. A., Tsompana, M., Cai, L., Wang, Y., et al. (2018). Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. *Physiol. Genomics* 50, 244–254. doi: 10.1152/physiolgenomics.00114.2017
- Karjalainen, M. K., Karthikeyan, S., Oliver-Williams, C., Sliz, E., Allara, E., Fung, W. T., et al. (2024). Genome-wide characterization of circulating metabolic biomarkers. *Nature* 628, 130–138. doi: 10.1038/s41586-024-07148-y
- Lai, F. Y., Nath, M., Hamby, S. E., Thompson, J. R., Nelson, C. P., and Samani, N. J. (2018). Adult height and risk of 50 diseases: a combined epidemiological and genetic analysis. *BMC Med.* 16, 187. doi: 10.1186/s12916-018-1175-7
- Łaniewski, P., İlhan, Z. E., and Herbst-Kralovetz, M. M. (2020). The microbiome and gynaecological cancer development, prevention and therapy. *Nat. Rev. Urol.* 17, 232–250. doi: 10.1038/s41585-020-0286-z
- Lawlor, D. A., Harbord, R. M., Sterne, J. A., Timpson, N., and Davey Smith, G. (2008). Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat. Med.* 27, 1133–1163. doi: 10.1002/sim.3034
- Li, T. T., Tong, A. J., Liu, Y. Y., Huang, Z. R., Wan, X. Z., Pan, Y. Y., et al. (2019). Polyunsaturated fatty acids from microalgae *Spirulina platensis* modulates lipid metabolism disorders and gut microbiota in high-fat diet rats. *Food Chem. Toxicol.* 131, 110558. doi: 10.1016/j.fct.2019.06.005
- Liu, W., Crott, J. W., Lyu, L., Pfalzer, A. C., Li, J., Choi, S. W., et al. (2016). Diet- and genetically-induced obesity produces alterations in the microbiome, inflammation and wnt pathway in the intestine of *apc(+/-1638N)* mice: comparisons and contrasts. *J. Cancer* 7, 1780–1790. doi: 10.7150/jca.15792
- Mallen, A. R., Townsend, M. K., and Tworoger, S. S. (2018). Risk factors for ovarian carcinoma. *Hematol. Oncol. Clin. North Am.* 32, 891–902. doi: 10.1016/j.hoc.2018.07.002
- Martínez, I., Stegen, J. C., Maldonado-Gómez, M. X., Eren, A. M., Siba, P. M., Greenhill, A. R., et al. (2015). The gut microbiota of rural Papua New Guineans: composition, diversity patterns, and ecological processes. *Cell Rep.* 11, 527–538. doi: 10.1016/j.celrep.2015.03.049
- Mishra, A. K., Hugon, P., Lagier, J. C., Nguyen, T. T., Couderc, C., Raoult, D., et al. (2013). Noncontiguous-finished genome sequence and description of *Enorma massiliensis* gen. nov., sp. nov., a new member of the Family Coriobacteriaceae. *Stand. Genomic. Sci.* 8, 290–305. doi: 10.4056/signs.3426906
- Mounier, N., and Kutalik, Z. (2023). Bias correction for inverse variance weighting Mendelian randomization. *Genet. Epidemiol.* 47, 314–331. doi: 10.1002/gepi.22522
- Nguyen, S. M., Tran, H. T. T., Long, J., Shrubsole, M. J., Cai, H., Yang, Y., et al. (2024). Gut microbiome of patients with breast cancer in Vietnam. *JCO. Glob. Oncol.* 10, e2300234. doi: 10.1200/GO.23.00234
- Petersen, C., Bell, R., Klag, K. A., Lee, S. H., Soto, R., Ghazaryan, A., et al. (2019). T cell-mediated regulation of the microbiota protects against obesity. *Science* 365, 6451. doi: 10.1126/science.aat9351
- Qin, Y., Havulinna, A. S., Liu, Y., Jousilahti, P., Ritchie, S. C., Tokolyi, A., et al. (2022). Combined effects of host genetics and diet on human gut microbiota and incident disease in a single population cohort. *Nat. Genet.* 54, 134–142. doi: 10.1038/s41588-021-00991-z
- Salehi, B., Selamoglu, Z., Mileski, K. S., Pezzani, R., Redaelli, M., Cho, W. C., et al. (2019). Liposomal cytarabine as cancer therapy: from chemistry to medicine. *Biomolecules* 9, 773. doi: 10.3390/biom9120773
- Skrivankova, V. W., Richmond, R. C., Woolf, B. A. R., Yarmolinsky, J., Davies, N. M., Swanson, S. A., et al. (2021). Strengthening the reporting of observational studies in epidemiology using mendelian randomization: the STROBE-MR statement. *Jama* 326, 1614–1621. doi: 10.1001/jama.2021.18236
- 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
- Torre, L. A., Trabert, B., DeSantis, C. E., Miller, K. D., Samimi, G., Runowicz, C. D., et al. (2018). Ovarian cancer statistics, 2018. *CA: A. Cancer J. Clin.* 68, 284–296. doi: 10.3322/caac.21456
- van Kippersluis, H., and Rietveld, C. A. (2018). Pleiotropy-robust mendelian randomization. *Int. J. Epidemiol.* 47, 1279–1288. doi: 10.1093/ije/dyx002
- Velázquez, K. T., Enos, R. T., Bader, J. E., Sougiannis, A. T., Carson, M. S., Chatzistamou, I., et al. (2019). Prolonged high-fat-diet feeding promotes non-alcoholic fatty liver disease and alters gut microbiota in mice. *World J. Hepatol.* 11, 619–637. doi: 10.4254/wjh.v11.i8.619
- Wang, Z., Qin, X., Hu, D., Huang, J., Guo, E., Xiao, R., et al. (2022). Akkermansia supplementation reverses the tumor-promoting effect of the fecal microbiota transplantation in ovarian cancer. *Cell Rep.* 41, 111890. doi: 10.1016/j.celrep.2022.111890
- Yang, Y., Li, X., Qian, H., Di, G., Zhou, R., Dong, Y., et al. (2022). C-reactive protein as a prognostic biomarker for gynecologic cancers: A meta-analysis. *Comput. Intell. Neurosci.* 2022, 6833078. doi: 10.1155/2022/6833078
- Ye, T., Shao, J., and Kang, H. (2021). Debiased inverse-variance weighted estimator in two-sample summary-data Mendelian randomization. *Ann. Statist.* 49, 2079–2100. doi: 10.1214/20-AOS2027
- Zara, R., Rasul, A., Sultana, T., Jabeen, F., and Selamoglu, Z. (2022). Identification of *Macropleiota procera* extract as a novel G6PD inhibitor for the treatment of lung cancer. *Saudi. J. Biol. Sci.* 29, 3372–3379. doi: 10.1016/j.sjbs.2022.02.018
- Zhao, J., Ming, J., Hu, X., Chen, G., Liu, J., and Yang, C. (2020). Bayesian weighted Mendelian randomization for causal inference based on summary statistics. *Bioinformatics* 36, 1501–1508. doi: 10.1093/bioinformatics/btz749
- Zheng, J., Baird, D., Borges, M. C., Bowden, J., Hemani, G., Haycock, P., et al. (2017). Recent developments in mendelian randomization studies. *Curr. Epidemiol. Rep.* 4, 330–345. doi: 10.1007/s40471-017-0128-6
- Zhou, B., Sun, C., Huang, J., Xia, M., Guo, E., Li, N., et al. (2019). The biodiversity composition of microbiome in ovarian carcinoma patients. *Sci. Rep.* 9, 1691. doi: 10.1038/s41598-018-38031-2
- Zuber, V., Colijn, J. M., Klaver, C., and Burgess, S. (2020). Selecting likely causal risk factors from high-throughput experiments using multivariable Mendelian randomization. *Nat. Commun.* 11, 29. doi: 10.1038/s41467-019-13870-3



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Multidimensional analysis of the impact of Gemmatimonas, Rhodothermus, and Sutterella on drug and treatment response in colorectal cancer

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Background: Colorectal cancer is the third most prevalent cancer across the globe. Despite a diversity of treatment methods, the recurrence and mortality rates of the disease remain high. Recent studies have revealed a close association of the gut microbiota with the occurrence, development, treatment response, and prognosis of colorectal cancer.

Objective: This study aims to integrate transcriptome and microbiome data to identify colorectal cancer subtypes associated with different gut microbiota and evaluate their roles in patient survival prognosis, tumor microenvironment (TME), and drug treatment response.

Methods: An integrated analysis of microbiome data was conducted on samples of colorectal cancer from public databases. Based on this, two tumor subtypes (C1 and C2) closely associated with patient survival prognosis were identified and a risk score model was constructed. The survival status, clinical parameters, immune scores, and other features were analyzed in-depth, and the sensitivity of various potential drugs was examined.

Results: A thorough examination of microbiome information obtained from colorectal cancer patients led to the identification of two primary tumor clusters (C1 and C2), exhibiting notable variations in survival outcomes. Patients with the C1 subtype were closely associated with better prognosis, while those with the C2 subtype had higher gut microbial richness and poorer survival prognosis. A predictive model utilizing the microbiome data was developed to accurately forecast the survival outcome of patients with colorectal cancer. The TME scores provided a biological basis for risk assessment in high-risk (similar to the C2 subtype) patient cohorts. Evaluation of the sensitivity of different subtypes to various potential drugs, indicated the critical importance of personalized treatment. Further analysis showed good potential of the developed risk-scoring model in predicting immune checkpoint functions and treatment response of patients, which may be crucial in guiding the selection of immunotherapy strategies for patients with colorectal cancer.

Conclusion: This study, through a comprehensive analysis of colorectal cancer microbiome, immune microenvironment, and drug sensitivity, enhances the current understanding of the multidimensional interactions of colorectal cancer and provides important clinical indications for improving future treatment strategies. The findings offer a new perspective on improving treatment response and long-term prognosis of patients with CRC through the regulation of microbiota or the utilization of biomarkers provided by it.

KEYWORDS

colorectal cancer, microbiome, immunotherapy, drug sensitivity, risk score model NKG7, GNLy, KLRD1

Introduction

Colorectal cancer is a prevalent form of cancer worldwide, presenting a significant risk to humans. According to reports from the World Health Organization, colorectal cancer ranks among the top in terms of incidence as well as mortality rates of cancers worldwide (Sung et al., 2021). The etiology of colorectal cancer is still not elucidated completely, but it is known to involve several genetic and environmental factors, including family history, intestinal inflammation, and dietary habits, which considerably increase the risk of its occurrence (Venugopal and Carethers, 2022). Emerging studies suggest that lifestyle factors such as sedentary behavior and obesity also play critical roles in the modulation of this risk, further complicating the interaction between genetics and environmental influences. Recent advancements in high-throughput sequencing technology and extensive research have provided mounting evidence linking the human gut microbiota to the development of colorectal cancer (Qu et al., 2023). This burgeoning field of research has begun to decode the complex dialogues between gut microbial communities and host cellular pathways that may contribute to carcinogenic processes. However, despite significant progress, the specific microbial species and their mechanisms of action in colorectal carcinogenesis remain poorly understood, highlighting the need for more targeted studies in this area.

The gut microbiota refers to the microbiome present in the human or animal intestines, and includes bacteria, fungi, viruses, and other microorganisms (Adak and Khan, 2019). The impact of the human gut microbiota on health and disease states is increasingly being recognized (Illiano et al., 2020). These microorganisms are essential for maintaining the health of the host by engaging in a range of bodily functions like digesting food, absorbing nutrients, and regulating the immune system (Zhao et al., 2019; Zhou et al., 2021). This symbiotic relationship underscores a critical balance, where disruptions can lead to significant health issues, including metabolic and autoimmune diseases. Recent research has shown a strong association between gut bacteria and the onset and progression of different illnesses. Moreover, studies have begun to illustrate how variations in microbiota composition

can influence systemic inflammation and immune tolerance, which are pivotal in the context of oncogenesis. Research indicates that particular intestinal bacteria could contribute to the onset and progression of colorectal cancer, potentially influencing the response of patients to cancer treatments like chemotherapy and immunotherapy (Yi et al., 2021; Wong and Yu, 2023). For instance, certain gut bacteria can produce pro-inflammatory factors and trigger chronic inflammatory responses, leading to cancer development (Bishehsari et al., 2020). These responses are often mediated by specific bacterial metabolites that interact with immune cell receptors and signaling pathways involved in inflammation and tumorigenesis. Certain bile acid metabolites or toxins produced by the gut can directly damage the host DNA, increase mutation rates, and promote carcinogenesis. These microbial byproducts could function as cancer-causing agents, impacting the growth and death of cells lining the intestine, altering the biology of intestinal cells (Fang et al., 2021). Research has also shown that gut microbiota dysbiosis can weaken intestinal barrier function, facilitating the entry of bacterial strains or their products through the intestinal wall into the bloodstream, affecting distant cellular populations (Gasaly et al., 2021).

The microbiome in the gut has the ability to influence the behavior of immune cells within the tumor environment, including influencing the roles of macrophages and dendritic cells associated with tumors, consequently interfering with tumor growth and spread (Zhou and Li, 2023). The structure and properties of the tumor microenvironment (TME) have been extensively studied to understand its impact on cancer treatment. The TME plays a vital role in the progression of colorectal cancer, involving a diverse range of immune cells like T cells, B cells, and macrophages. These cells are essential for triggering and maintaining immune responses and suppressing immune activity (Bu et al., 2022). Additionally, the features of TME are considered potential biomarkers for predicting the responses of patients to immunotherapies such as immune checkpoint inhibitors (ICIs). Distinct compositions of gut microbiota have been linked to the response of patients to ICI medications, with variations in gut microbiota potentially impacting patients' receptiveness to immunotherapy (Zhou et al., 2021).

Although the association between the gut microbiome and colorectal cancer is increasingly becoming evident, comprehensive studies on the categorization of colorectal cancer microbiota and its impact on patient survival predictions are, nonetheless, lacking. A thorough understanding of the classification of microbiota and its relationship with immune infiltration may reveal new methods for the treatment and prognosis assessment, which are crucial for personalized and precision medicine. Therefore, this study aimed to identify microbiota classifications associated with survival prognosis through comprehensive utilization of transcriptomic and microbiome data from patients with colorectal cancer. This study additionally aimed to investigate the relationships among these categorizations and immune features of tumors, and how patients react to chemotherapy and immunotherapy, providing more insight into the influence and function of the intestinal microbiome in colorectal cancer.

Methods

Acquisition and processing of transcriptomic and microbiome data

RNA expression profiles and clinical data were obtained from The Cancer Genome Atlas Rectum Adenocarcinoma (TCGA-READ) database. The RNA sequencing data were in Transcripts Per Million (TPM) format, and a log2 transformation was performed for subsequent analysis. Data for the microbiome were obtained from the website https://ftp.microbio.me/pub/cancer_microbiome_analysis/TCGA/. The dataset Kraken-TCGA-Voom-SNM-All-Putative-Contaminants-Removed-Data.csv was chosen, along with the sample details in Metadata-TCGA-Kraken-17625-Samples.csv. After filtering samples from TCGA-READ, a total of 99 samples containing survival information were used for model construction. The data was split into training and validation sets in a 5:5 ratio, with the training set containing 49 samples and the validation set containing 50 samples. The validation set was used to assess the stability and accuracy of the model.

Acquisition and analysis of scRNA-seq data

We obtained a single-cell dataset containing colorectal cancer samples from the GSE166555 dataset in the Gene Expression Omnibus (GEO) database (Uhlitz et al., 2021). Data were analyzed utilizing the Seurat package in R software (version 4.3.3) (Stuart et al., 2019). When assessing cell quality, we specified that the number of mitochondria should not exceed 10%, and established boundaries for Unique Molecular Identifier counts and gene counts to fall between 200 and 20,000 and 200 and 5,000, respectively. Subsequently, data were normalized, and 2000 highly variable genes were selected. To reduce the impact of the cell cycle, we employed the NormalizeData, FindVariableFeatures, and ScaleData functions in the Seurat package, specifying the vars.to.regress parameter as c('S.Score', 'G2M.Score').

Batch effects were addressed using the harmony method. The uniform manifold approximation and projection (UMAP), t-distributed stochastic neighbor embedding (t-SNE), and Louvain clustering algorithms to reduce dimensionality and clustering in further analysis. Utilizing the FindAllMarkers function, differential genes were identified across various clusters or cell types based on specified criteria, including $p\text{-value} < 0.05$, $\log_2\text{FC} > 0.25$, and expression proportion > 0.1 .

Cell annotation

We utilized a series of cell markers to identify different cell types, and then filtered immune cells for further analysis. The remaining cells were subjected to re-clustering analysis. The mast cells were identified and Sc-Type cell annotation software was employed to annotate the remaining cells. Finally, t-SNE plots and bubble plots of marker genes were generated to visualize the annotation results. The cell markers utilized herein included epithelial cells (EPCAM, KRT18, KRT19, CDH1), natural killer (NK) cells (NCAM1, NKG7, GNLY, KLRD1), fibroblasts (DCN, THY1, COL1A1, COL1A2), T cells (CD3D, CD3E, TRAC, CD3G), endothelial cells (FLT1, CLDN5, RAMP2, PECAM1), myeloid cells (LYZ, MARCO, CD68, FCGR3A), B cells (CD79A, IGHM, IGHG3, IGHA2), and mast cells (MS4A2, KIT, GATA2).

Identifying microbiome-related tumor subtypes

First, univariate Cox analysis was performed to identify genes linked to patient survival within the microbiome-related gene set ($P < 0.05$). Afterward, the gene expression matrix was used to conduct consensus clustering on the microbiome data using the ConsensusClusterPlus package (Wilkerson and Hayes, 2010). We selected the k-means method and utilized Euclidean distance as the similarity measure for the clustering algorithm. Then, 100 bootstrap samplings were conducted, each comprising 80% of the samples. The clustering numbers ranging from 2 to 6 were tested and the optimal classification was determined using the Proportion of Ambiguous Clustering (PAC) and consistency matrix.

Construction and validation of the prognostic model

The limma package was utilized to calculate the differences in microbiome composition between varying tumor subtypes (Ritchie et al., 2015). Next, we employed univariate Cox analysis to identify prognosis-linked microbiota ($P < 0.05$). To decrease the amount of microbiota, we utilized the Glmnet package to conduct the Least Absolute Shrinkage and Selection Operator (LASSO)-Cox regression analysis (Engelbrechtsen and Bohlin, 2019). We further reduced the number of microbiota using Stepwise Cox regression analysis (StepCox). Ultimately, we derived a formula to determine

the risk score, $\text{RiskScore} = \beta_1 * \text{exp1} + \beta_2 * \text{exp2} + \dots + \beta_i * \text{expi}$. In this context, β symbolizes the microbiota coefficient, and 'exp' signifies the microbiota level. Using this equation, the risk scores in the TCGA training set was calculated. Following that, we carried out receiver operating characteristic (ROC) assessment using the timeROC package (Blanche et al., 2013), illustrating ROC curves for 1, 2, and 3 years, and executing survival examination with the survminer package to establish the threshold value (Kassambara et al., 2021). Finally, the stability of the prognostic model was validated using the TCGA test set and the entire TCGA dataset.

Gene set enrichment analysis and functional annotation

The clusterProfiler package (Wu et al., 2021) was used to perform GSEA on the upregulated genes of various tumor subtypes to evaluate their functional characteristics. We used gene sets from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database as enrichment signatures, considering a functional enrichment significance when the adjusted p-value after the Benjamini-Hochberg correction was <0.05 . We utilized the enrichplot package for visualization and the Single Sample Gene Set Enrichment Analysis (ssGSEA) algorithm from the Immuno-Oncology Biological Research package to compute functional scores for individual samples. We performed differential analysis between tumor subtypes/risk groups using the Hallmark gene set. For this assessment, we employed the Wilcoxon test, deeming a p-value < 0.05 to be statistically significant.

Tumor immune infiltration analysis

Metrics related to immune infiltration in the The Cancer Genome Atlas (TCGA)-READ dataset, including StromalScore, ImmuneScore, ESTIMATEScore, and TumorPurity, were computed using the ESTIMATE algorithm. The immune cell composition in TCGA-READ was assessed using Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORTx). Using single-cell data on immune cell composition as a reference, we predicted the immune composition of bulk samples. Comparisons between different groups were conducted using the Kruskal-Wallis test. We utilized the pheatmap package to visualize Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data (ESTIMATE) score distribution and immune cells across tumor subtypes, as well as the distribution of ssGSEA scores for signatures such as Hallmark between risk groups (Kolde, 2019).

Prediction of the response to immunotherapy/chemotherapy

The oncoPredict package in R was used to determine the IC50 of standard chemotherapy medications for evaluating the effectiveness of chemotherapy. The T-cell-inflamed gene expression profile score was utilized to forecast the reaction to immunotherapy in various

tumor subtypes and risk groups. Next, the cytolytic activity (CYT) score and type 1 T helper/interferon gamma (Th1/IFN γ) gene signature score was computed utilizing the single-sample GSEA (ssGSEA) algorithm. Additionally, the Tumor Immune Dysfunction and Exclusion (TIDE) web analysis tool from <http://tide.dfci.harvard.edu/> was utilized to forecast the immune reaction and ratings in the TCGA dataset. Immune checkpoint genes were used to analyze differentially using the Kruskal-Wallis test, with a $\text{padj} < 0.05$ deemed as statistically significant. Subsequently, a correlation analysis was conducted between immune checkpoint genes and risk scores upon utilizing the ggcorrplot package (Kassambara and Patil, 2023).

Somatic Nucleotide Variant (SNV) analysis.

The TCGA database provided us with data on SNV mutations, and then the maftools package (Mayakonda et al., 2018) was used to analyze and determine tumor mutation burden (TMB), mutant-allele tumor heterogeneity (MATH), and homologous recombination defects (HRD) for every sample. Furthermore, a comparative analysis among tumor subtypes/risk groups was performed utilizing the Wilcoxon test; a p-value < 0.05 was deemed as statistically significant. Additionally, a correlation analysis was conducted between risk scores and immune cells, as well as the Hallmark signature, utilizing the ggcorrplot tool.

Results

Consensus clustering analysis of microbiota-associated tumor subtypes

Microbial genera associated with survival were identified through univariate Cox analysis of the microbiome data ($P < 0.05$) (Figure 1A). Subsequently, consensus clustering on the TCGA data was performed utilizing the abundance matrix of genera and the ConsensusClusterPlus package. Through PAC analysis, we found that the best grouping effect occurred with a value of $k=2$ (Figure 1B). Principal component analysis revealed the distribution of samples from the two subtypes (Figure 1C). Survival analysis results showed poorer prognosis of subtype C2 ($p=0.00046$) (Figure 1D). Furthermore, significant differences were observed between the two subtypes in terms of the abundance expression of most genera (Figure 1E). The abundance heatmap of genera revealed that *Robiginitomaculum*, *Myxococcus*, *Terriglobus*, *Clavibacter*, *Chitinimonas*, *Alpharetrovirus*, *Paenarthrobacter*, and *Xenococcus* had higher abundance in C1, while the remaining genera (Cytomegalovirus, *Zymomonas*, *Lentimicrobium*, *Flaviumibacter*, *Emticicia*, *Sutterella*, *Fimbriimonas*, *Segetibacter*, *Gemmatispora* and *Zavarzinella*) had higher abundance in C2 (Figure 1F).

Variations in clinical markers and single nucleotide variations across tumor subtypes

We combined the subtype outcomes with various clinical factors, including age, tumor stage, survival status, and of T, N,

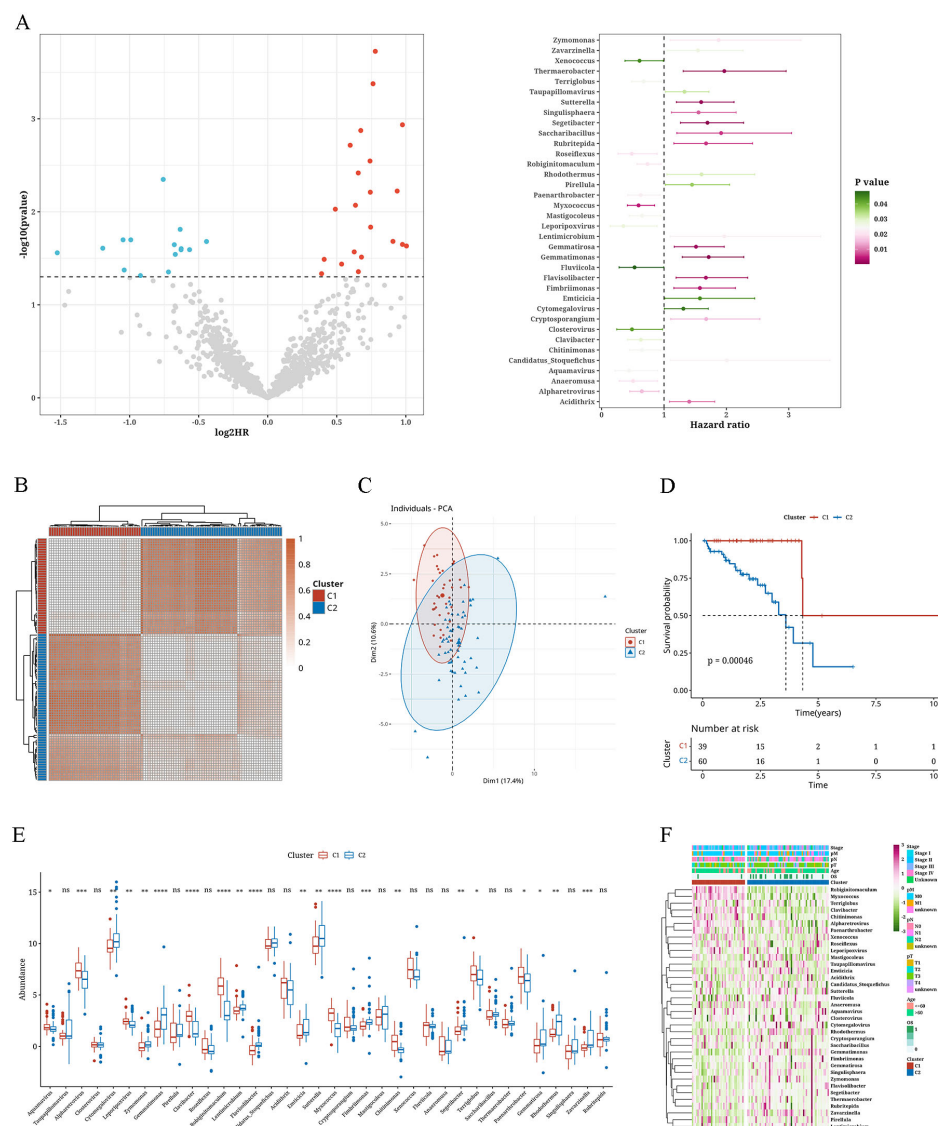


FIGURE 1

Consensus clustering analysis of microbiota-associated tumor subtypes. (A) Volcano plot and error bar graph representing survival-associated microbiota. (B) Consistency clustering plot of microbial abundance matrix. (C) Principal Component Analysis plot with $K=2$. (D) Survival analysis curves of two clusters. (E) Box plot showing differences in abundance of microbiota between two clusters. (F) Heatmap depicting the abundance of microbiota between two clusters. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

and M stages. Chi-square tests revealed that the survival status distribution differed significantly between C1 and C2 through ($P < 0.05$), and other factors showed near significance (Figures 2A–F). The SNV mutations in these two subtypes were calculated in terms of tumor mutation burden (TMB), mutant-allele tumor heterogeneity (MATH), and homologous recombination defects (HRD) values. No notable variances were observed between C1 and C2 in (Figures 2G–I). The waterfall chart displays the mutation status of the most frequently mutated top 30 genes in both subtypes. We observed that the genes Adenomatous Polyposis Coli (*APC*) (87.5% mutation rate), *TP53* (70% mutation rate), and *KRAS* (50% mutation rate) exhibited relatively high mutation rates (Figure 2J).

Functional differences in tumor subtypes

After comparing gene expression differences between the two subtypes, we utilized the marker genes identified for GSEA to assess their functional importance through KEGG analysis. According to this enrichment analysis, subtype C1 was enriched in the KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION pathway, whereas subtype C2 showed enrichment in the KEGG_RNA_DEGRADATION, KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450, and KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY pathways (Figures 3A, B). Further evaluation of key pathways revealed that ADIPOGENESIS, ANDROGEN_RESPONSE,

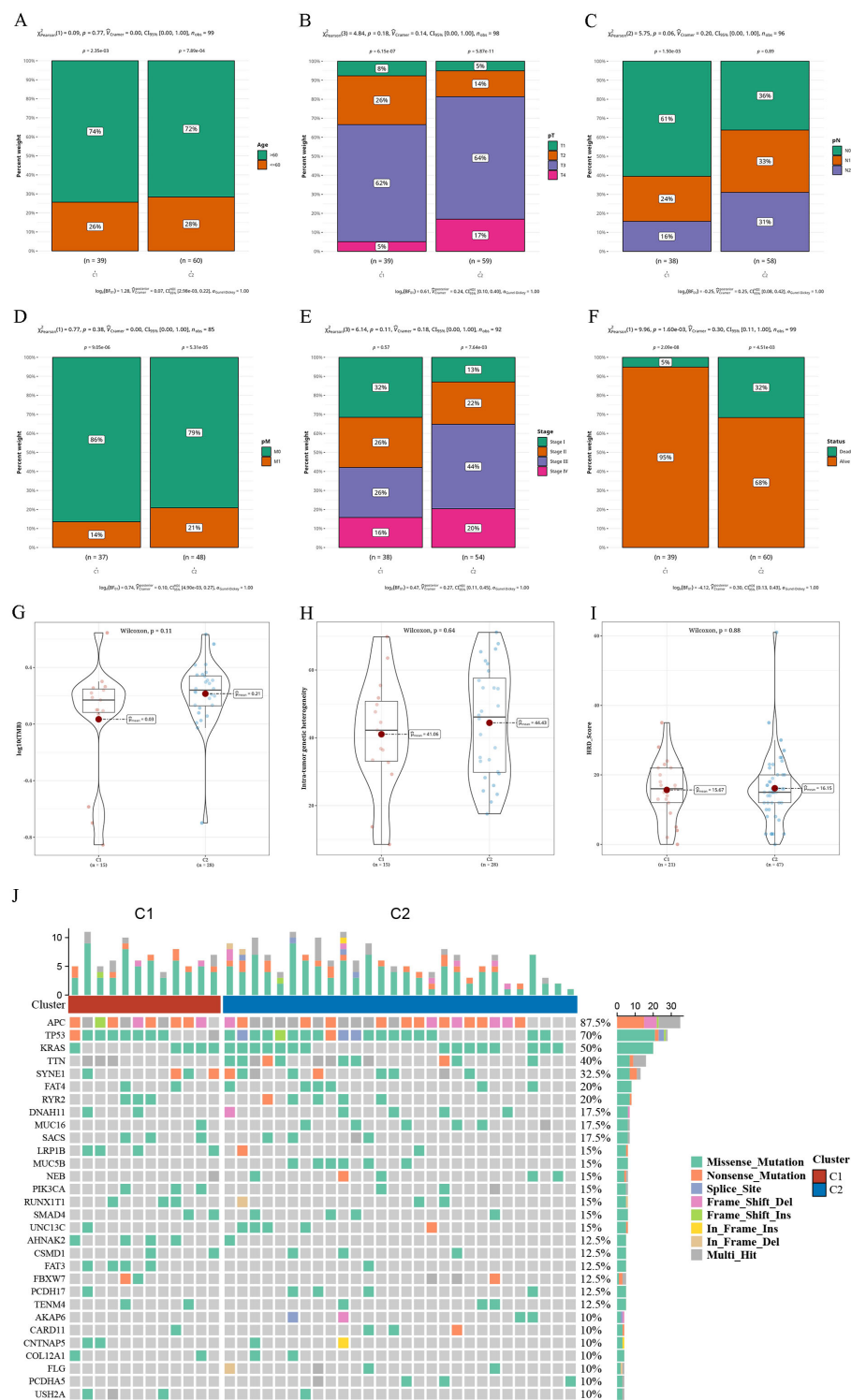


FIGURE 2

Differential clinical indicators and somatic nucleotide variants mutations in tumor subtypes (A–F) Bar charts illustrating the proportion of various clinical indicators (age, pT, pN, pM, Tumor Stage, survival status) in two clusters. (G–I) Violin plots displaying variances in tumor mutation burden (TMB), mutant-allele tumor heterogeneity (MATH), and homologous recombination defects (HRD) between two clusters. (J) Waterfall plot depicting the top 30 gene mutations in two clusters.

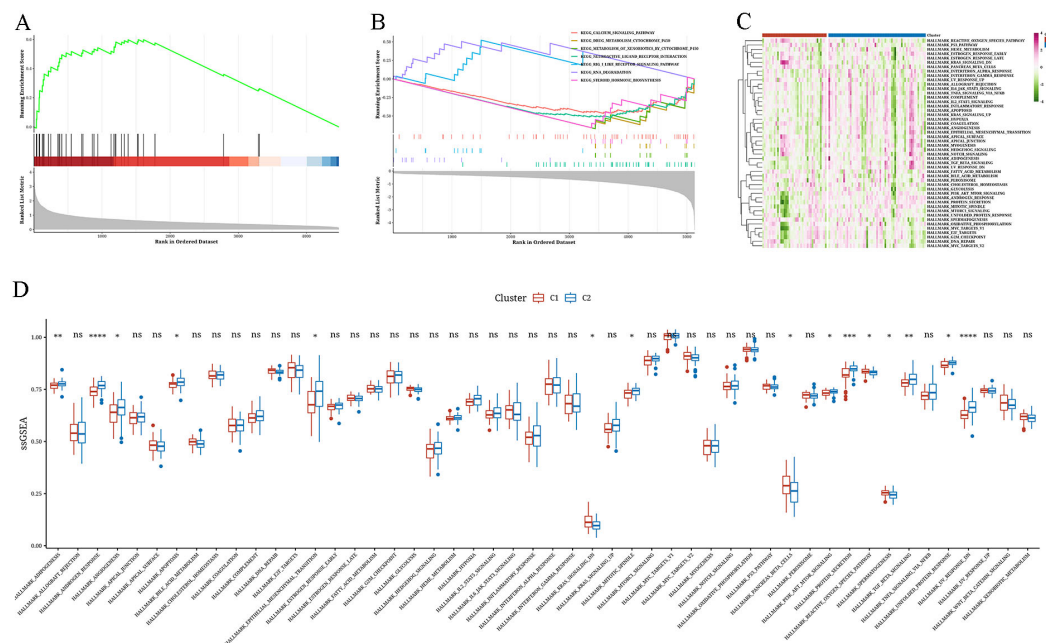


FIGURE 3

The results of functional differential analysis of tumor subtypes (A) Gene Set Enrichment Analysis (GSEA) results of upregulated genes in cluster 1 (C1). (B) GSEA results of upregulated genes in cluster 2 (C2). (C) Heatmap displaying single-sample GSEA (ssGSEA) scores for samples categorized into two clusters using the Hallmark gene set. (D) Boxplot illustrating the variation in ssGSEA scores between samples in two clusters using the Hallmark gene set. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

ANGIOGENESIS, APOPTOSIS, and EMT were more prevalent in subtype C2, whereas REACTIVE_OXYGEN_SPECIES_PATHWAY, PANCREAS_BETA_CELLS, and KRAS_SIGNALING_DN were more prevalent in subtype C1 (Figures 3C, D).

Immune infiltration differences among tumor subtypes

analysis of single-cell data of READ, particularly focusing on isolating immune cells, resulted in a total of 18 clusters. Based on the distribution of cell sources, immune cells were derived primarily from tumor samples. Employing distinct markers and the Sc-Type program, we could categorize immune cells and acquire cell annotation outcomes for every cluster. Subsequently, bubble expression plots of markers for various cell types were displayed (Figures 4A–D). The ESTIMATE algorithm produced four indices that showed no significant differences between the two subtypes. However, ImmuneScore and Tumor-Purity were higher in C1, while StromalScore and ESTIMATEScore were higher in C2 (Figures 4E–H). Employing the CIBERSORTx algorithm and leveraging single-cell data as a reference to predict bulk data, we found higher levels of macrophages in C1, whereas myeloid dendritic cells (mDCs) were more abundant in C2 (Figures 4I, J).

Examining anticipated reaction to immunotherapy and chemotherapy in different types of tumors

For every subtype, the IC50 values of traditional chemotherapy medications were determined. In C2, RO-3306_1052, Tozasertib_1096, Doramapimod_1042, and NU7441_1038 exhibited higher sensitivity levels (Figures 5A–D). Assessment of the reaction to immunotherapy through the T-cell-inflamed Gene Expression Profile (GEP) score, CYT score, and Th1/IFN γ gene signature showed that C1 had a greater T-cell-inflamed gene expression profile score than C2, whereas C2 had a higher cytolytic activity score compared to C1. Both subtypes exhibited comparable levels of Th1/IFN γ gene signature scores (Figures 5E–G). We then examined the levels of 28 immune checkpoint genes in every subtype and observed significant variations in the levels of CD200, TNFRSF4, and CD86 immune checkpoint genes between the two subtypes, and all three were more abundant in C2 (Figures 5H, I).

Construction and validation of risk score models

Based on differential analysis between the two subtypes ($|\log_2FC| > 0.5$, $P < 0.05$), we identified 133 distinct genes. Univariate Cox analysis

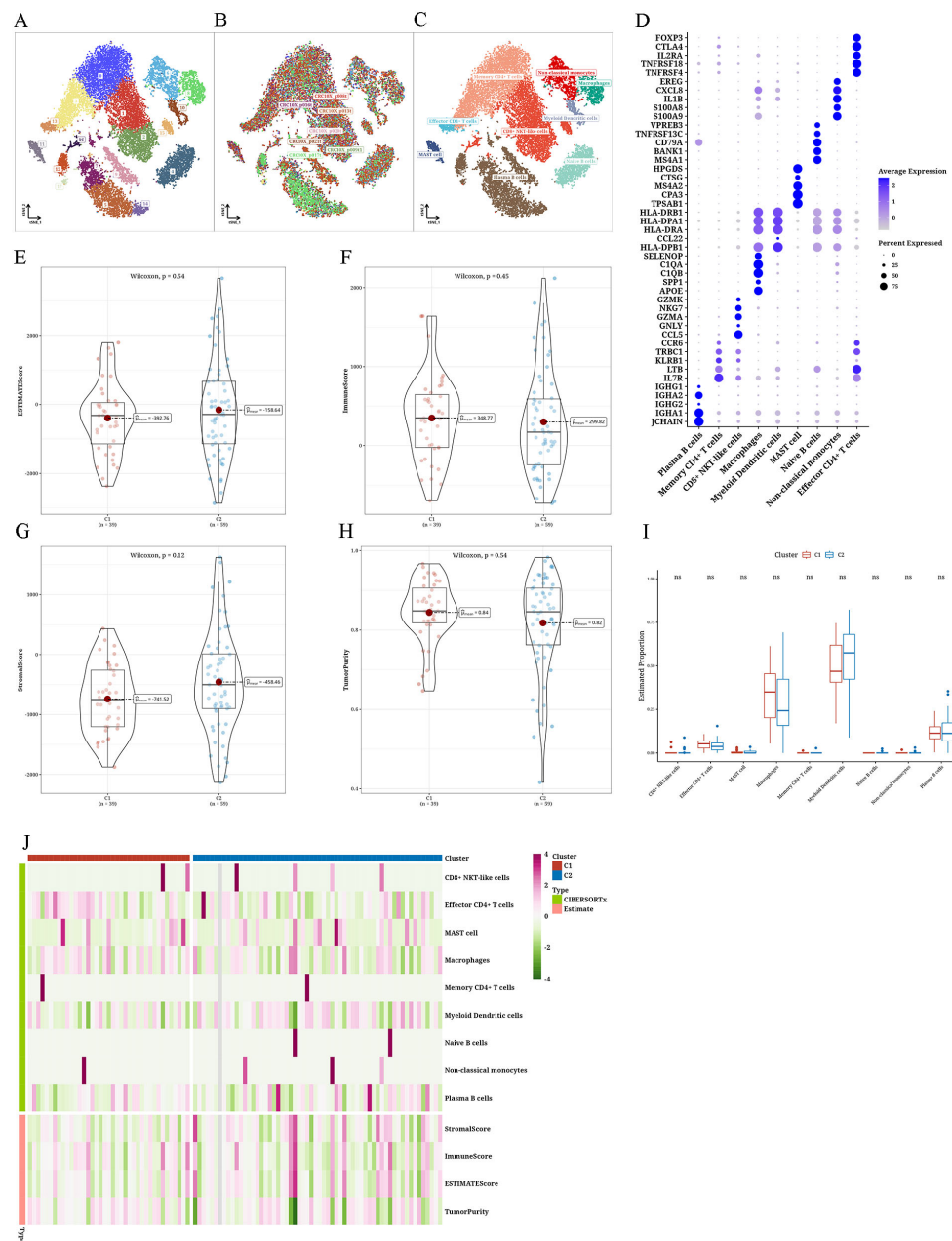


FIGURE 4

Differential immune infiltration results of tumor subtypes (A–C) The t-distributed stochastic neighbor embedding plots of single-cell data from The Cancer Genome Atlas Rectum Adenocarcinoma (TCGA-READ). (D) Bubble plot showing marker annotations for various cell types. (E) Violin plot depicting the differential Estimation of STromal and Immune cells in Malignant Tumor tissues using Expression data (ESTIMATE) score between two clusters. Violin plot illustrating the differential (F) ImmuneScore between two clusters, (G) StromalScore between two clusters, and (H) TumorPurity between two clusters. (I) Boxplot showing the differential CIBERSORTx scores between two clusters. (J) Heatmap presenting the differential analysis results of ESTIMATE and CIBERSORTx scores between two clusters. ns: No statistical significance.

identified 16 genera exhibiting significant associations with survival ($P < 0.05$), with 9 genera linked to risk and 7 linked to protection. Following this, a model was created using the LASSO-Cox regression technique, leading to a decrease in the number of genera. With the increase in the λ coefficient, the number of genera approached zero (Figure 6A). Ten-fold cross-validation yielded confidence intervals for each λ value (Figure 6B). Ultimately, nine prognostic-related genera could be identified. StepCox was used to enhance the genera selection,

leading to the following equation for RiskScore calculation: $\text{RiskScore} = 0.44 * \text{Gemmatimonas} + 0.378 * \text{Rhodothermus} + 0.351 * \text{Sutterella} - 0.288 * \text{Myxococcus} - 0.402 * \text{Paenarthrobacter}$ (Figure 6C). Applying this formula, we determined the risk levels of the TCGA training set, TCGA test set, and the complete TCGA dataset, uncovering a worse outlook for the high-risk category. Furthermore, the estimated Area Under the Curve (AUC) values for 1, 2, and 3 years, were all greater than 0.8 for every dataset (Figures 6D–I).

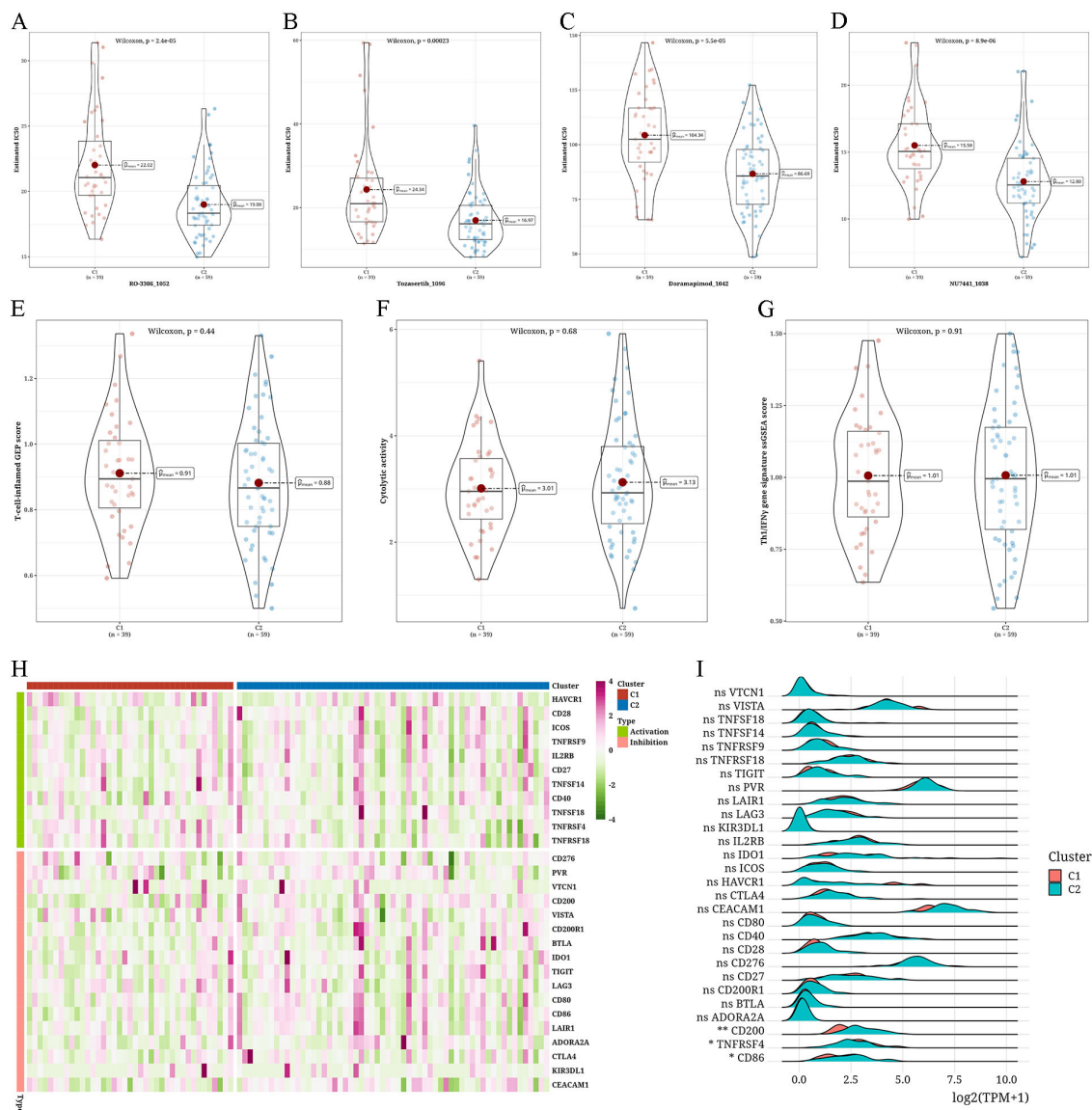


FIGURE 5 Assessment of anticipated reaction to immunotherapy and chemotherapy in different types of tumors (A–D) Violin plots depicting the differential IC50 values of RO-3306_1052, Tazemetib_1096, Doramapimod_1042, and NU7441_1038 between two clusters (C1 and C2). (E–G) Violin plots showing the differences in T-cell-inflamed gene expression profile (GEP) score, cytolytic score (CYT) score, and type 1 T helper/interferon-gamma (Th1/IFN γ) gene signature between two clusters. (H) Heatmap showing the expression levels of 28 immune checkpoint genes across different subtypes. (I) A Ridge plot illustrating the levels of expression for 28 immune checkpoint genes among various subtypes (* $p < 0.05$; ** $p < 0.01$; ns, not significant).

Variations in clinical markers within different risk groups

A combined analysis of the risk groups with various clinical indicators revealed that with the increase in age, T stage, N stage, M stage, pathological grading, and tumor staging, the risk value also increased (Figure 7A). In particular, the risk assessment for C2 was notably greater compared to C1 (Figure 7B). Figure 7C presents a Sankey diagram for the allocation of tumor subtypes and risk categories. Subsequently, for survival analysis, we segregated samples based on pathological T stage, age, and tumor stage in three groups. Figure 7D shows a worse outcome of individuals in the high-risk category.

Differences in immune infiltration among the risk groups

The three matrices computed through the ESTIMATE algorithm exhibited notable variances among the risk categories ($P < 0.05$), and no significant variance was detected in the ImmuneScore. In particular, the values for StromalScore, ImmuneScore, and ESTIMATEScore were elevated in the high-risk category, whereas the Tumor-Purity score was increased in the low-risk category (Figure 8A). Applying the CIBERSORTx algorithm to predict bulk information employing single-cell data as a guide, we discovered a greater prevalence of macrophages in the

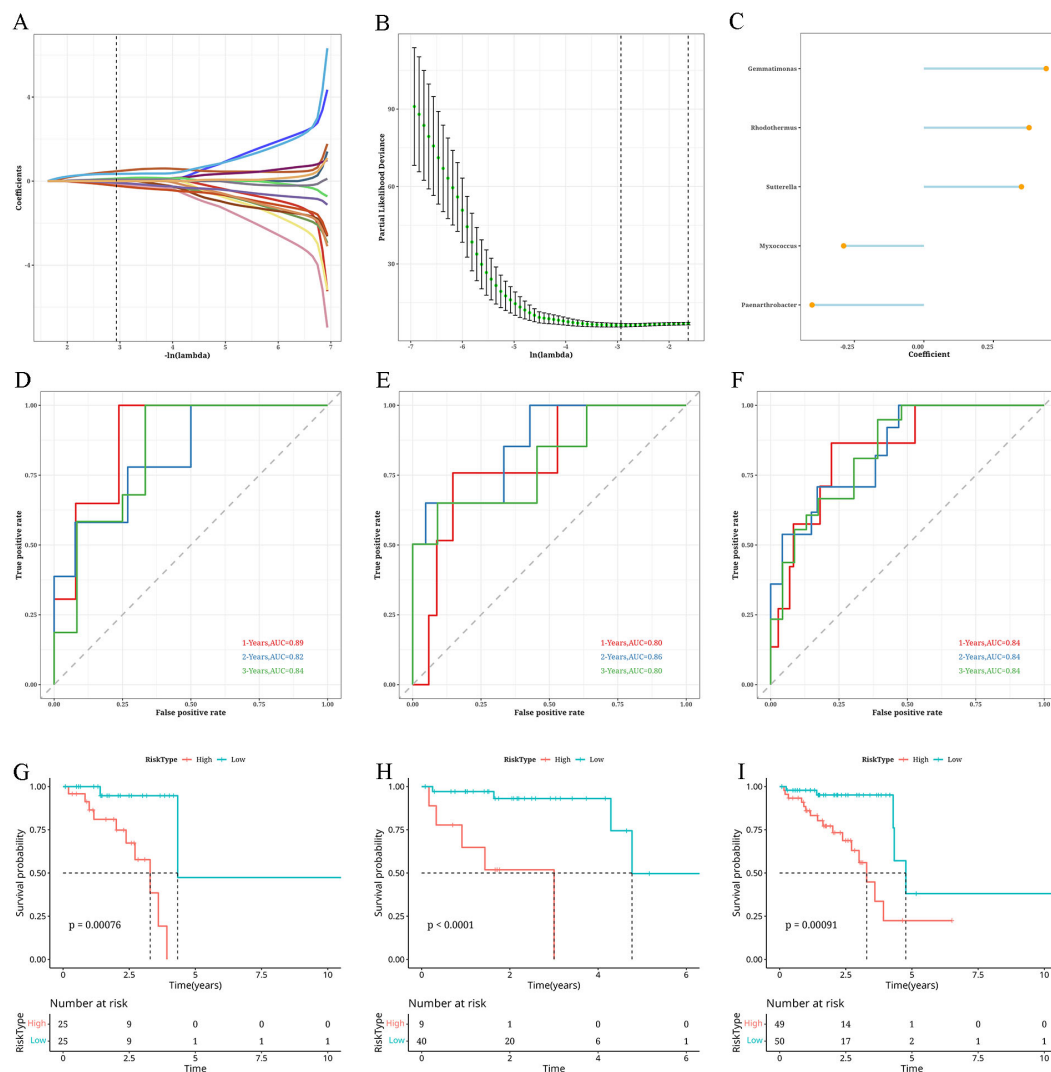


FIGURE 6
Analysis of building and validation of risk score models (A) A Curve plot showing the coefficient of prognostic genera with respect to lambda values. (B) An error bar graph showing the partial likelihood deviance for the LassoCox model at different lambda values. (C) A bubble plot showing the coefficient values of genera in the risk score model. (D) Survival analysis findings from the TCGA validation dataset. (E) Survival analysis findings from the TCGA validation dataset. (F) Findings from the survival analysis of the complete TCGA database. (G) The timeROC analysis results for the 1, 2, and 3-year intervals in the TCGA testing set. (H) The timeROC analysis results for the 1, 2, and 3-year intervals in the TCGA testing set. (I) The timeROC analysis results for the 1, 2, and 3-year intervals in the entire TCGA dataset.

high-risk category, whereas mDCs and plasma B cells were more prevalent in the low-risk category (Figure 8B). The pathway enrichment analysis revealed enrichment of pathways like MYC_TARGETS_V2 and BILE_ACID_METABOLISM in the low-risk group, whereas pathways like ANDROGEN_RESPONSE and ANGIOGENESIS were enriched in the high-risk group (Figures 8C, E). Following this, the Spearman correlation analysis showed a negative correlation of the risk-score with plasma B cells, BILE_ACID_METABOLISM, and other factors, concurrently showing a positive correlation with CD8+ NKT-like cells, naive B cells, ADIPOGENESIS, and other factors. As shown in Figure 8D, a positive correlation is indicated by the dashed line, while a negative correlation is represented by the solid line.

Evaluation of anticipated reaction to immunotherapy/chemotherapy within the risk groups

In the high-risk group, enhanced sensitivity was observed for BMS-754807_2171, ZM447439_1050, JQ1_2172, and NU7441_1038 (Figure 9A). The mutation analysis exhibited elevated levels of TMB, MATH, and HRD scores in the high-risk group in comparison to the low-risk group. Furthermore, all three metrics had a positive association with the risk factor, although this relationship was not statistically significant, as shown in Figures 9B–D. Investigation of immunotherapy revealed elevated levels of T-cell-inflamed GEP score, Th1/IFN γ gene signature, and CYT score in the high-risk group compared to

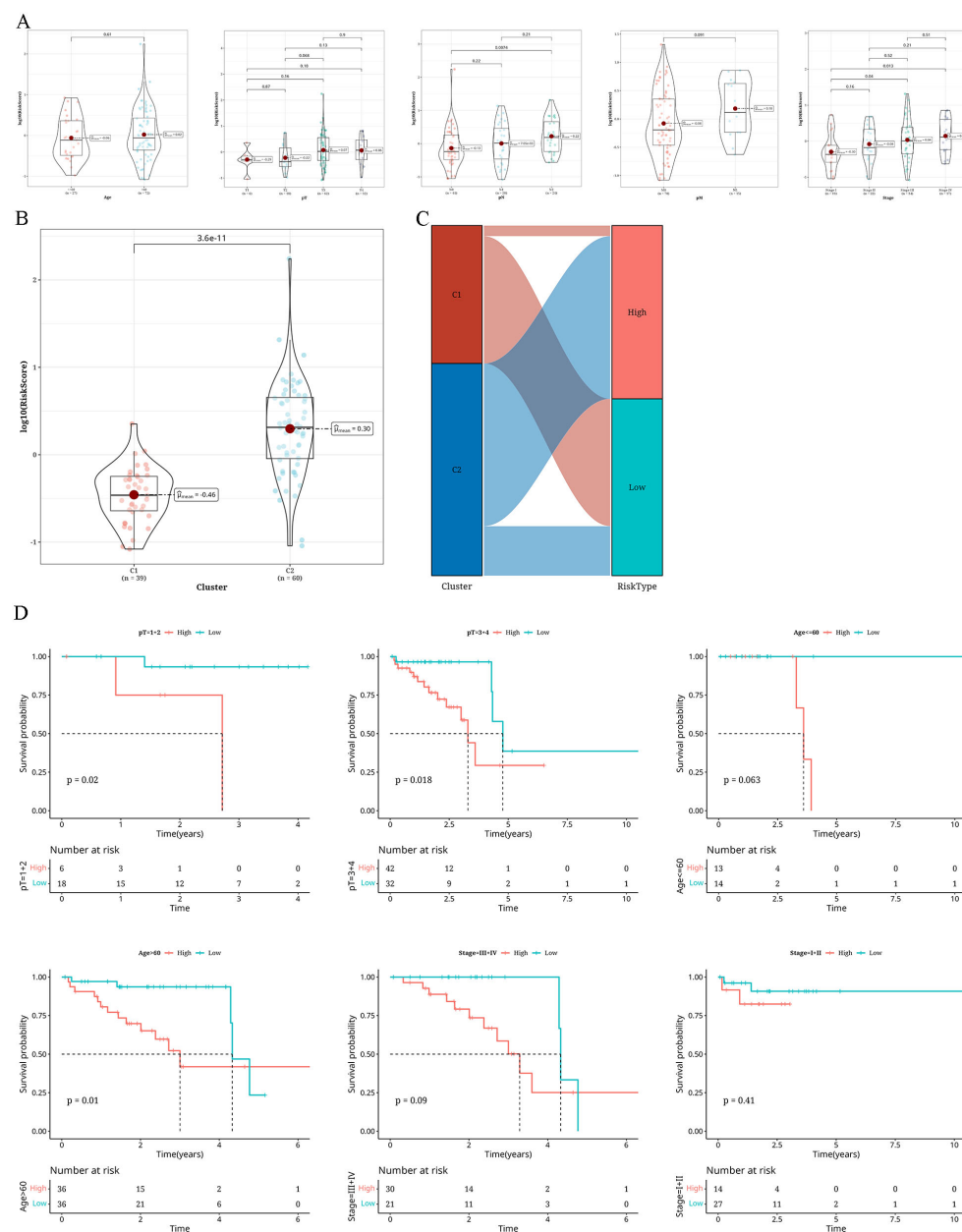


FIGURE 7

Findings from the examination of distinct clinical markers in various risk categories **(A)** A Violin plot showing the variations in risk levels of different clinical markers (age, pT, pN, pM, Tumor_Stage) within The Cancer Genome Atlas database cohort. **(B)** A violin plot illustrating the variations in risk levels across two distinct clusters (C1 and C2). **(C)** A sankey diagram showing the compositional differences between two clusters and risk groups. **(D)** Survival analysis results classified according to pT, age, and Tumor_Stage.

the low-risk group. These three factors were all linked to the risk value, although the association was not statistically significant (Figures 9E–G). Using TIDE online analysis, we discovered significantly higher TIDE value in the high-risk group compared to the low-risk group, with results nearing statistical significance (Figure 9H). Survival analysis showed a worse prognosis for the high-risk non-responding group (Figure 9I). During the examination of immune checkpoints, the level of risk showed a strong association with the majority of immune checkpoints, and the high-risk group had a notably higher presence of immune checkpoints (Figures 9J, K).

Discussion

Colorectal cancer is a globally prevalent type of cancer; it is the third most frequently detected cancer, according to the World Cancer Research Fund. Traditional treatment methods for colorectal cancer primarily include surgery, radiotherapy, and chemotherapy. Personalized treatment options are also now available for certain patients with the development of targeted therapies and immunotherapy (Benson et al., 2022). However, despite the increasing diversity of treatment modalities, the recurrence and mortality rates of colorectal cancer remain high. A

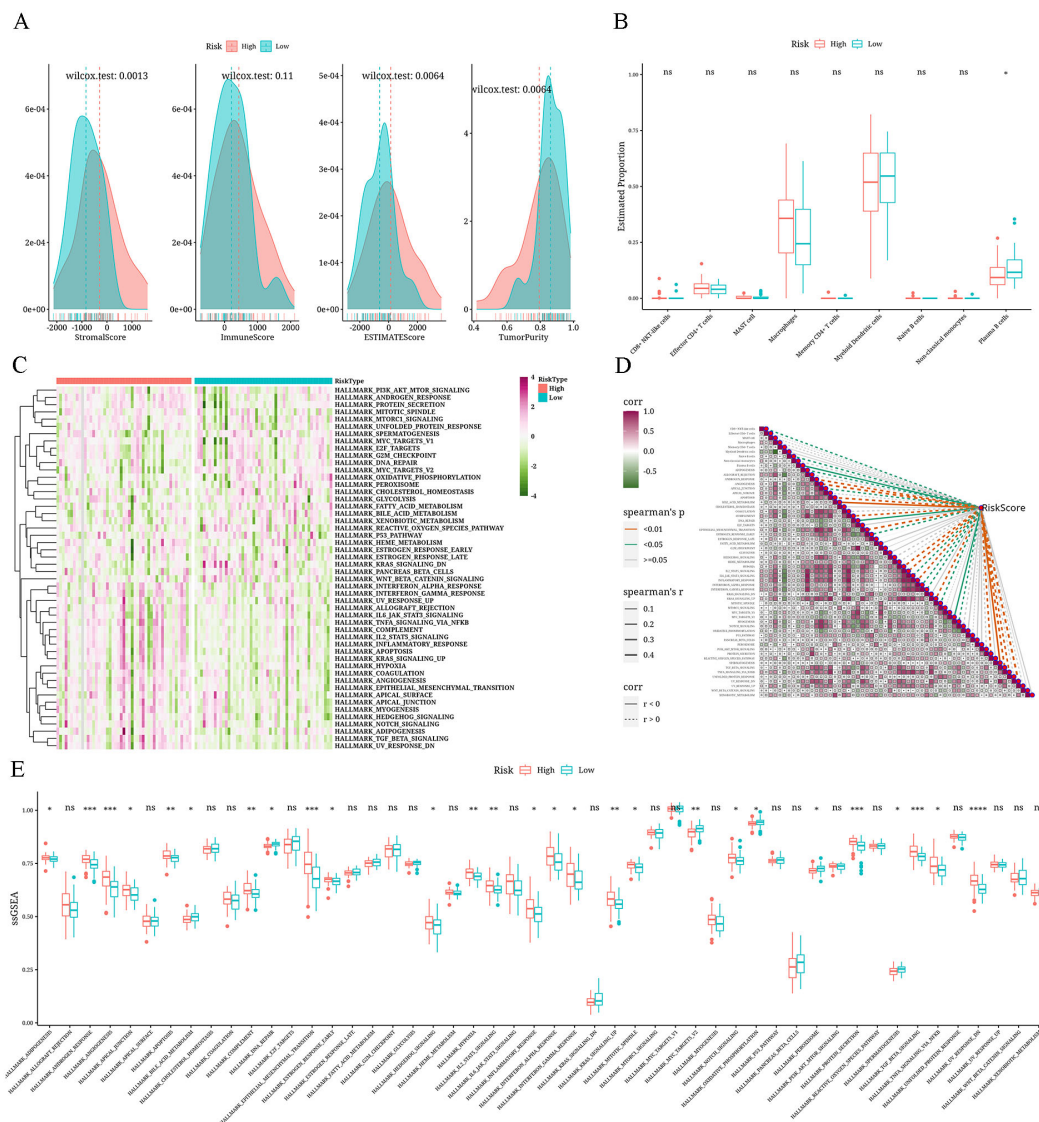


FIGURE 8

Analysis results of differential immune microenvironment in risk groups. (A) Density distribution plots depicting StromaScore, ImmuneScore, ESTIMATEscore, and TumorPurity across different risk groups. (B) Boxplot showing the differential CIBERSORTx scores between risk groups. (C) Heatmap displaying single-sample Gene Set Enrichment Analysis (ssGSEA) scores using the Hallmark gene set for different risk groups. (D) Correlation heatmap illustrating the relationship between risk values, immune cell composition, and Hallmark gene set (ssGSEA scores) (positive correlation is shown as a dashed line and negative correlation, as a solid line). (E) A boxplot displaying the differences among different risk groups in terms of ssGSEA scores for the Hallmark gene set. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

strong link has been shown between the intestinal flora of individuals with colorectal cancer and the onset, progression, response to treatment, and outlook of the disease, suggesting a significant impact of the intestinal flora on the treatment of colorectal cancer.

By combining transcriptomic and microbiota data in individuals with colorectal cancer, we could discover two unique tumor subcategories (C1 and C2) linked to varying microbiota compositions that significantly influence patient survival predictions. Individuals classified as the C1 subtype exhibited a more favorable outlook. Individuals with the C2 subtype showed elevated abundance of various genera of gut bacteria, which is potentially linked to tumor development (Lee et al., 2018). In line

with earlier research, this discovery implies a strong association between intestinal flora and tumor development, suggesting that gut bacteria could contribute to the occurrence of inflammatory conditions that support tumor growth, influence immune system avoidance, and aid in tumor spread and growth (Dzutsev et al., 2015; Fu et al., 2024). Clinical parameters too, showed differences in survival status between C1 and C2. Nonetheless, the lack of significance in the variances of TMB, MATH, and HRD values between the two subtypes indicates the presence of alternative mechanisms contributing to the differences in subtypes. Additionally, the APC gene was identified with a high mutation rate. APC, a key gene involved in tumor suppression, is essential in regulating intestinal mucosal epithelial cells by regulating cell

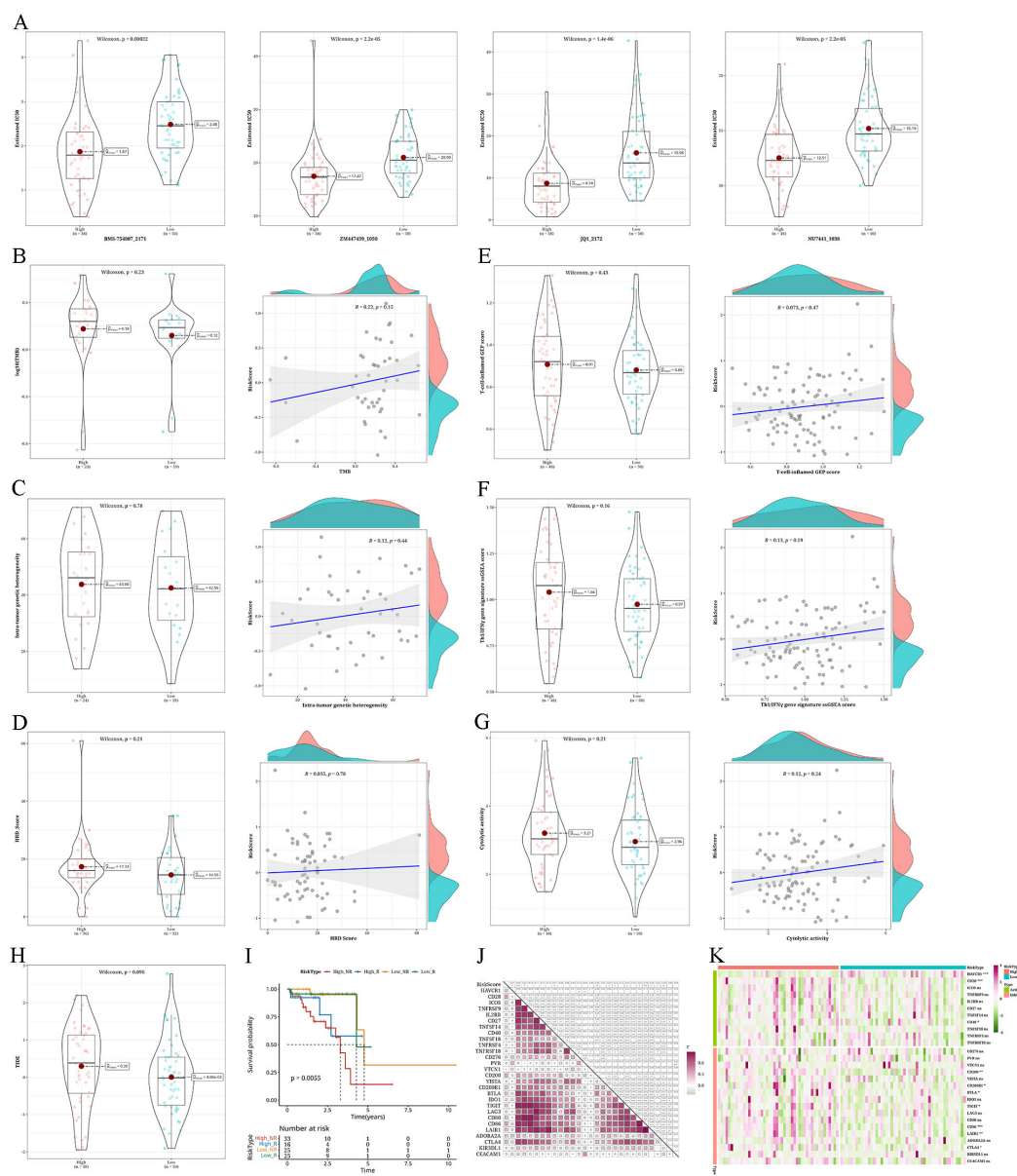


FIGURE 9

Analysis of anticipated reaction to immunotherapy/chemotherapy and variations in somatic nucleotide variants (SNV) mutations among different risk categories **(A)** Violin plots depicting the differential IC50 of BMS-754807_2171, ZM447439_1050, JQ1_2172, and NU7441_1038 between risk groups. **(B–D)** Violin plots showing the differences in tumor mutation burden (TMB), mutant-allele tumor heterogeneity (MATH), and homologous recombination defects (HRD) between risk groups, along with corresponding correlation scatter plots. **(E–G)** Violin plots demonstrating variations in T-cell-inflamed gene expression profile (GEP) score, type 1 T helper/interferon gamma (Th1/IFN γ) gene signature, and cytolytic score (CYT) score among different risk groups, accompanied by correlation scatter plots. **(H)** Violin plot displaying the differences in TIDE values between high- and low-risk groups. **(I)** Survival analysis results of risk groups categorized as Response and NonResponse predicted by combining risk groups with TIDE analysis. **(J)** A heatmap displaying the association between risk values and the expression levels of immune checkpoint genes. **(K)** Heatmap illustrating the expression levels of immune checkpoint genes in patients categorized by risk groups (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant).

proliferation, differentiation, apoptosis, and cell cycle. Mutation or loss of the *APC* gene can lead to dysregulated cell cycle, thereby promoting the occurrence of colorectal tumors (Goss and Groden, 2000; Malki et al., 2020).

Tumors with a poor prognosis often have a higher mutational burden, meaning they present more neoantigens on their surface. These neoantigens make the tumor cells more recognizable to the immune system. ICIs work by blocking immune checkpoint

molecules (like PD-1 and CTLA-4), which removes the brakes on T cells, enabling them to attack these highly mutated tumors more effectively. Poor prognosis tumors may have developed mechanisms to escape immune surveillance, often involving overexpression of immune checkpoint molecules. ICIs can reverse this immune escape by blocking these checkpoints, leading to reactivation of the immune response against the tumor. The heterogeneity within tumor cells might mean that certain subgroups within a poor

prognosis tumor are particularly sensitive to ICIs. Patients with a poor prognosis might have a higher proportion of these sensitive subgroups, resulting in a better response to ICIs. Poor prognosis tumors that do not respond well to conventional therapies (like chemotherapy or radiation) might still undergo immunogenic cell death. This form of cell death releases tumor antigens, which can further activate the immune system, thereby enhancing the effectiveness of ICIs. Studies have shown that combining ICIs with other treatments (such as chemotherapy, radiation, or targeted therapies) can lead to better outcomes, particularly in patients with poor prognosis. These combination therapies might improve the tumor microenvironment or expose more tumor antigens, making ICIs more effective.

Interestingly, by combining microbiota and single-cell data, we uncovered unique immune cell infiltration patterns displayed by tumors categorized into various gut microbiota subgroups. The presence of these patterns might have a direct impact on the advancement of the illness and the outlook for the patient. Our examination showed that while there was no notable variation in immune scores among the two subtypes, the C1 subtype potentially harbored a greater number of macrophages, thus culminating in an enhanced anti-cancer immune reaction; whereas, the C2 subtype showed a higher proportion of Dendritic Cells (DCs), which may suppress anti-tumor responses, thus explaining its association with poorer prognosis.

Response prediction to chemotherapy and immunotherapy shows a close association between drug sensitivity and treatment response in different tumor subtypes. For example, medications like RO-3306, Tozasertib, Doramapimod, and NU7441 have been thoroughly studied and shown to inhibit cancer cells, control cellular stress responses, trigger cancer cell death, and consequently slow down tumor progression (Moon et al., 2015; Matsumoto, 2022; Yang et al., 2022; Huang et al., 2023). The C2 subtype appears to be more sensitive to the above-mentioned chemotherapy drugs, suggesting better treatment outcomes for patients with this subtype upon treatment with these drugs. Additionally, immune checkpoint molecules CD200, TNFRSF4, and CD86 are all enriched in the C2 subtype, while the C1 subtype exhibits enrichment of T-cell inflamed gene expression patterns. These results indicate that patients with the C2 subtype respond better to immunotherapy.

Poor prognosis was confirmed by the AUC values, validating the effectiveness of the model. Combined with clinical parameters, patients with higher-risk clinical-pathological parameters tended to have higher scores. Additionally, the risk scores were higher in the C2 group, consistent with its poorer prognosis. Additionally, within the high-risk category, the levels of StromalScore, ImmuneScore, and ESTIMATEScore were elevated, while BMS-754807, ZM447439, JQ1, and NU7441 displayed increased sensitivity (Carboni et al., 2009; Kaestner et al., 2009; Ding et al., 2020). In experimental studies, these potential drugs have shown inhibitory effects on colorectal cancer, but their clinical safety and efficacy still need further validation. This study further confirmed the effectiveness of the scoring model in terms of immune infiltration and checkpoints; irrespective of microbiome-related tumor subtypes or microbiome-based risk scoring models, they exhibit

excellent predictive value in the prognosis, immunotherapy, and drug sensitivity response of patients with colorectal cancer. This research further highlights the diverse functions and efficacy of the gut microbiome in colorectal cancer, such as its ability to predict patient outcomes, control immune responses in the intestines, affect the metabolism of carcinogens, influence the efficacy of immunotherapy, and regulate the environment surrounding tumors. Our findings align with the conclusions reported in various other studies (Hanus et al., 2021; Kim and Lee, 2021; Dougherty and Jobin, 2023; Yu et al., 2023; Zheng et al., 2023).

In this study, we explored the association between the gut microbiome and colorectal cancer, unveiling significant variations in microbiome compositions that correlate with distinct tumor subtypes. Our findings underscore the potential clinical applications of microbial biomarkers, as exemplified by genera such as *Robiginitomaculum* and *Myxococcus*, which are prevalent in the subtype associated with favorable prognosis, and *Sutterella* and *Zymomonas*, which dominate in the subtype linked to poor outcomes. This differential abundance not only highlights the pivotal role of microbial markers in influencing tumor progression and patient survival but also sets the stage for developing targeted therapeutic strategies that harness specific microbial profiles. Moreover, the identification and functional analysis of these biomarkers are crucial for refining existing treatment modalities and crafting novel personalized therapies. By analyzing the expression patterns of these markers across tumor subtypes, we can enhance the precision of patient-specific treatment predictions, thereby optimizing therapeutic outcomes. Additionally, these insights provide a new perspective on the complex interactions between the microbiome and the tumor immune microenvironment.

Although our research presents convincing proof of the intricate connection between colorectal cancer, the microbiome, and the immune environment, some constraints still persisted. The microbiome information was collected from openly accessible repositories, with a restricted number of samples. Moreover, the source and collection techniques of microbiome data may have a confounding effect on the analysis results. Additionally, although the sample information provided by the dataset used in this study is rich, it lacks information on directly isolated microbial samples. Future research should consider obtaining more accurate microbiome data from *in situ* tumor tissues. Additional research is also required to assess the impact of the identified microbial populations and microbiome communities on tumor advancement in terms of influencing immune responses, drug processing, and direct interactions with host cells.

In summary, our comprehensive analysis of the colorectal cancer microbiome, immune microenvironment, drug sensitivity, and other factors enhances our understanding of the multidimensional interactions in colorectal cancer biology as well as provides important clinical indications for the future improvements in the treatment of colorectal cancer. These findings offer a new perspective on treating colorectal cancer and suggest the possibility of improving patient treatment responses and long-term prognosis by modulating the microbiome composition or utilizing biomarkers presented by the microbiome.

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

SJ: Data curation, Writing – original draft. WZ: Formal analysis, Writing – original draft. BL: Conceptualization, Writing – review & editing. KW: Methodology, Writing – review & editing. DL: 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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References

- Adak, A., and Khan, M. R. (2019). An insight into gut microbiota and its functionalities. *Cell Mol. Life Sci. CMLS*. 76, 473–493. doi: 10.1007/s00018-018-2943-4
- Benson, A. B., Venook, A. P., Al-Hawary, M. M., Azad, N., Chen, Y. J., Ciombor, K. K., et al. (2022). Rectal cancer, version 2.2022, NCCN clinical practice guidelines in oncology. *J. Natl. Compr. Cancer Netw. JNCCN*. 20, 1139–1167. doi: 10.6004/jnccn.2022.0051
- Bishehsari, F., Voigt, R. M., and Keshavarzian, A. (2020). Circadian rhythms and the gut microbiota: from the metabolic syndrome to cancer. *Nat. Rev. Endocrinol.* 16, 731–739. doi: 10.1038/s41574-020-00427-4
- Blanche, P., Dartigues, J. F., and Jacqmin-Gadda, H. (2013). Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks. *Stat. Med.* 32, 5381–5397. doi: 10.1002/sim.5958
- Bu, F., Zhao, Y., Zhao, Y., Yang, X., Sun, L., Chen, Y., et al. (2022). Distinct tumor microenvironment landscapes of rectal cancer for prognosis and prediction of immunotherapy response. *Cell Oncol. Dordr.* 45, 1363–1381. doi: 10.1007/s13402-022-00725-1
- Carboni, J. M., Wittman, M., Yang, Z., Lee, F., Greer, A., Hurlburt, W., et al. (2009). BMS-754807, a small molecule inhibitor of insulin-like growth factor-1R/IR. *Mol. Cancer Ther.* 8, 3341–3349. doi: 10.1158/1535-7163.MCT-09-0499
- Ding, W., Zhang, H., and Mei, G. (2020). Synergistic antitumor activity of DHA and JQ1 in colorectal carcinoma. *Eur. J. Pharmacol.* 885, 173500. doi: 10.1016/j.ejphar.2020.173500
- Dougherty, M. W., and Jobin, C. (2023). Intestinal bacteria and colorectal cancer: etiology and treatment. *Gut Microbes* 15, 2185028. doi: 10.1080/19490976.2023.2185028
- Dzutsev, A., Goldszmid, R. S., Viaud, S., Zitvogel, L., and Trinchieri, G. (2015). The role of the microbiota in inflammation, carcinogenesis, and cancer therapy. *Eur. J. Immunol.* 45, 17–31. doi: 10.1002/eji.201444972
- Engelbrechtsen, S., and Bohlin, J. (2019). Statistical predictions with glmnet. *Clin. Epigenetics*. 11, 123. doi: 10.1186/s13148-019-0730-1
- Fang, Y., Yan, C., Zhao, Q., Xu, J., Liu, Z., Gao, J., et al. (2021). The roles of microbial products in the development of colorectal cancer: a review. *Bioengineered*. 12, 720–735. doi: 10.1080/21655979.2021.1889109
- Fu, Y., Li, J., Cai, W., Huang, Y., Liu, X., Ma, Z., et al. (2024). The emerging tumor microbe microenvironment: From delineation to multidisciplinary approach-based interventions. *Acta Pharm. Sin. B*. 14, 1560–1591. doi: 10.1016/j.apsb.2023.11.018
- Gasaly, N., de Vos, P., and Hermoso, M. A. (2021). Impact of bacterial metabolites on gut barrier function and host immunity: A focus on bacterial metabolism and its relevance for intestinal inflammation. *Front. Immunol.* 12. doi: 10.3389/fimmu.2021.658354
- Goss, K. H., and Groden, J. (2000). Biology of the adenomatous polyposis coli tumor suppressor. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* 18, 1967–1979. doi: 10.1200/JCO.2000.18.9.1967
- Hanus, M., Parada-Venegas, D., Landskron, G., Wielandt, A. M., Hurtado, C., Alvarez, K., et al. (2021). Immune system, microbiota, and microbial metabolites: the unresolved triad in colorectal cancer microenvironment. *Front. Immunol.* 12. doi: 10.3389/fimmu.2021.612826
- Huang, Y., Fan, Y., Zhao, Z., Zhang, X., Tucker, K., Staley, A., et al. (2023). Inhibition of CDK1 by RO-3306 exhibits anti-tumorigenic effects in ovarian cancer cells and a transgenic mouse model of ovarian cancer. *Int. J. Mol. Sci.* 24, 12375. doi: 10.3390/ijms241512375
- Illiano, P., Brambilla, R., and Parolini, C. (2020). The mutual interplay of gut microbiota, diet and human disease. *FEBS J.* 287, 833–855. doi: 10.1111/febs.15217
- Kaestner, P., Stolz, A., and Bastians, H. (2009). Determinants for the efficiency of anticancer drugs targeting either Aurora-A or Aurora-B kinases in human colon carcinoma cells. *Mol. Cancer Ther.* 8, 2046–2056. doi: 10.1158/1535-7163.MCT-09-0323
- Kassambara, A., Kosinski, M., Biecek, P., and Fabian, S. (2021). *survminer: Drawing Survival Curves using “ggplot2”*. Available online at: <https://cran.r-project.org/web/packages/survminer/index.html> (Accessed April 6, 2024).
- Kassambara, A., and Patil, I. (2023). *ggcorrplot: Visualization of a Correlation Matrix using “ggplot2”*. Available online at: <https://cran.r-project.org/web/packages/ggcorrplot/index.html> (Accessed April 6, 2024).
- Kim, J., and Lee, H. K. (2021). Potential role of the gut microbiome in colorectal cancer progression. *Front. Immunol.* 12. doi: 10.3389/fimmu.2021.807648
- Kolde, R. (2019). *heatmap: pretty heatmaps*. Available online at: <https://cran.r-project.org/web/packages/heatmap/index.html> (Accessed April 6, 2024).
- Lee, C., Park, S., Ayush, I., Cho, K., Kim, S. S., and Kang, I. (2018). Effects of myxococcus fulvus KYC4048 metabolites on breast cancer cell death. *J. Microbiol. Biotechnol.* 28, 765–775. doi: 10.4014/jmb.1711.11003
- Malki, A., ElRuz, R. A., Gupta, I., Allouch, A., Vranic, S., and Al Moustafa, A. E. (2020). Molecular mechanisms of colon cancer progression and metastasis: recent insights and advancements. *Int. J. Mol. Sci.* 22, 130. doi: 10.3390/ijms22010130
- Matsumoto, Y. (2022). Development and evolution of DNA-dependent protein kinase inhibitors toward cancer therapy. *Int. J. Mol. Sci.* 23, 4264. doi: 10.3390/ijms23084264
- Mayakonda, A., Lin, D. C., Assenov, Y., Plass, C., and Koeffler, H. P. (2018). Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome Res.* 28, 1747–1756. doi: 10.1101/gr.239244.118

- Moon, S. H., Choi, S. W., and Kim, S. H. (2015). *In vitro* anti-osteoclastogenic activity of p38 inhibitor doramapimod via inhibiting migration of pre-osteoclasts and NFATc1 activity. *J. Pharmacol. Sci.* 129, 135–142. doi: 10.1016/j.jphs.2015.06.008
- Qu, R., Zhang, Y., Ma, Y., Zhou, X., Sun, L., Jiang, C., et al. (2023). Role of the gut microbiota and its metabolites in tumorigenesis or development of colorectal cancer. *Adv. Sci. Weinh Baden-Wuert Ger.* 10, e2205563. doi: 10.1002/adv.202205563
- Ritchie, M. E., Phipson, B., Wu, D. I., Hu, Y., Law, C. W., Shi, W., et al. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* 43, e47. doi: 10.1093/nar/gkv007
- Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W. M., et al. (2019). Comprehensive integration of single-cell data. *Cell.* 177, 1888–1902.e21. doi: 10.1016/j.cell.2019.05.031
- 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
- Uhrlitz, F., Bischoff, P., Peidli, S., Sieber, A., Trinks, A., Lüthen, M., et al. (2021). Mitogen-activated protein kinase activity drives cell trajectories in colorectal cancer. *EMBO Mol. Med.* 13, e14123. doi: 10.15252/emmm.202114123
- Venugopal, A., and Carethers, J. M. (2022). Epidemiology and biology of early onset colorectal cancer. *EXCLI J.* 21, 162–182. doi: 10.17179/excli2021-4456
- Wilkerson, M. D., and Hayes, D. N. (2010). ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. *Bioinforma Oxf Engl.* 26, 1572–1573. doi: 10.1093/bioinformatics/btq170
- 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
- Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., et al. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innov. Camb Mass.* 2, 100141. doi: 10.1016/j.xinn.2021.100141
- Yang, J., Zhou, X., Dong, J., Wang, W., Lu, Y., Gao, Y., et al. (2022). Single-cell profiling reveals molecular basis of Malignant phenotypes and tumor microenvironments in small bowel adenocarcinomas. *Cell Discovery* 8, 92. doi: 10.1038/s41421-022-00434-x
- Yi, Y., Shen, L., Shi, W., Xia, F., Zhang, H., Wang, Y., et al. (2021). Gut microbiome components predict response to neoadjuvant chemoradiotherapy in patients with locally advanced rectal cancer: A prospective, longitudinal study. *Clin. Cancer Res. Off J. Am. Assoc. Cancer Res.* 27, 1329–1340. doi: 10.1158/1078-0432.CCR-20-3445
- Yu, S., Wang, S., Xiong, B., and Peng, C. (2023). Gut microbiota: key facilitator in metastasis of colorectal cancer. *Front. Oncol.* 13. doi: 10.3389/fonc.2023.1270991
- Zhao, J., Zhang, X., Liu, H., Brown, M. A., and Qiao, S. (2019). Dietary protein and gut microbiota composition and function. *Curr. Protein Pept. Sci.* 20, 145–154. doi: 10.2174/1389203719666180514145437
- Zheng, Z., Hou, X., Bian, Z., Jia, W., and Zhao, L. (2023). Gut microbiota and colorectal cancer metastasis. *Cancer Lett.* 555, 216039. doi: 10.1016/j.canlet.2022.216039
- 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
- Zhou, D., and Li, Y. (2023). Gut microbiota and tumor-associated macrophages: potential in tumor diagnosis and treatment. *Gut Microbes* 15, 2276314. doi: 10.1080/19490976.2023.2276314

Glossary

APC	Adenomatous Polyposis Coli
AUC	Area Under the Curve
CIBERSORTx	Cell-type Identification By Estimating Relative Subsets of RNA Transcripts
DC	Dendritic Cells
GEP	Gene Expression Profile
GSEA	Gene Set Enrichment Analysis
HRD	Homologous Recombination Defects
ICIs	Immune Checkpoint Inhibitors
KEGG	Kyoto Encyclopedia of Genes and Genomes
LASSO	Least Absolute Shrinkage and Selection Operator
MATH	Mutant-Allele Tumor Heterogeneity
PAC	Proportion of Ambiguous Clustering
PCA	Principal Component Analysis
ROC	Receiver Operating Characteristic
ssGSEA	Single Sample Gene Set Enrichment Analysis
TCGA	The Cancer Genome Atlas
TIDE	Tumor Immune Dysfunction and Exclusion
TMB	Tumor Mutation Burden
TME	Tumor Microenvironment
TPM	Transcripts Per Million
UMAP	Uniform Manifold Approximation and Projection



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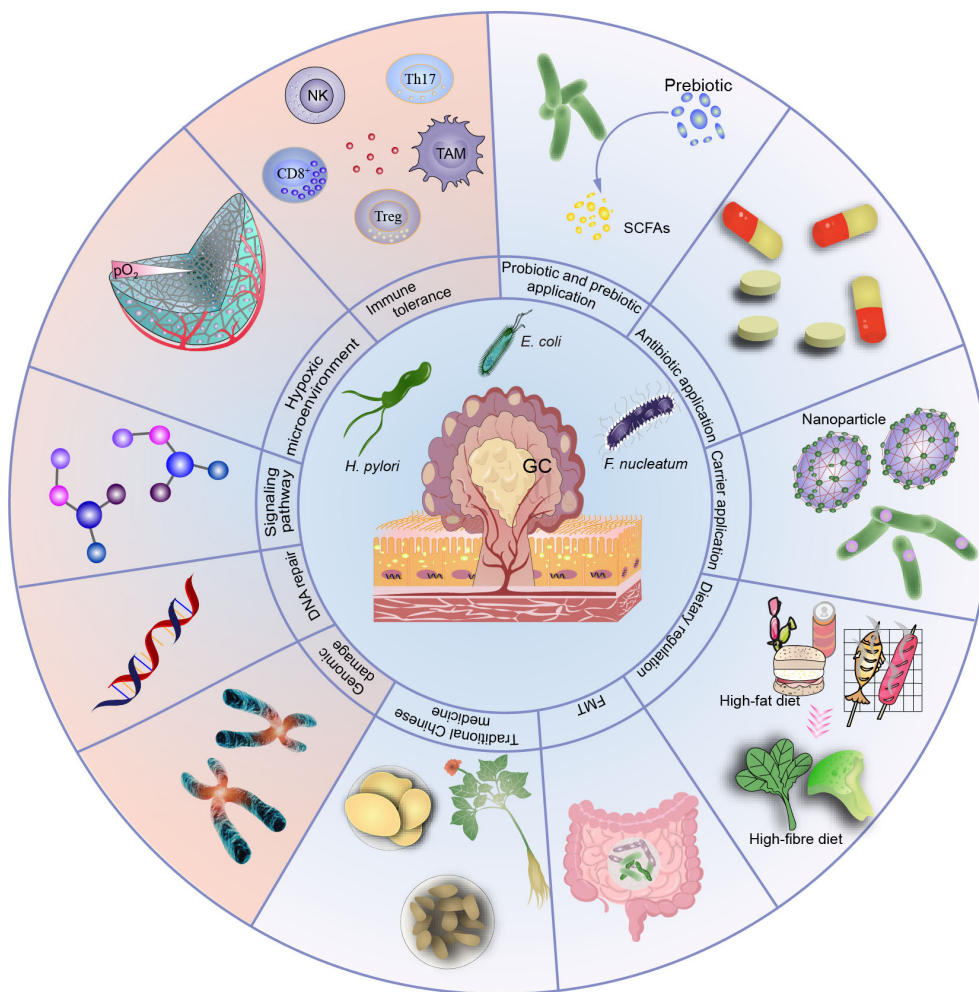
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Gastric cancer, a prevalent malignancy globally, is influenced by various factors. The imbalance in the gut microbiome and the existence of particular intratumoural microbiota could have a strong connection with the onset and progression of gastric cancer. High-throughput sequencing technology and bioinformatics analysis have revealed a close correlation between abnormal abundance of specific microbial communities and the risk of gastric cancer. These microbial communities contribute to gastric cancer progression through mechanisms including increasing cellular genomic damage, inhibiting DNA repair, activating abnormal signaling pathways, exacerbating tumor hypoxia, and shaping a tumor immune-suppressive microenvironment. This significantly impacts the efficacy of gastric cancer treatments, including chemotherapy and immunotherapy. Probiotic, prebiotic, antibiotic, carrier-based, dietary interventions, fecal microbiota transplantation, and traditional Chinese medicine show potential applications in gastric cancer treatment. However, the molecular mechanisms regarding dysbiosis of microbiota, including gut microbiota, and intra-tumoral microbiota during the progression of gastric cancer, as well as the therapeutic efficacy of microbiota-related applications, still require extensive exploration through experiments.

KEYWORDS

gastric cancer, microbiota, molecular mechanisms, tumor microenvironment, targeted therapy



GRAPHICAL ABSTRACT

This article presented the role of microbiota in gastric cancer from multiple aspects, including from molecular mechanisms to therapeutic interventions, in order to provide some suggestions for future research on tumor mechanism and clinical treatment.

1 Introduction

The association between microorganisms and tumor genesis can be traced back to the 13th century (Pack, 1967). In the late 19th century, William Coley pioneered the use of a vaccine named “Coley’s toxins,” composed of two killed bacteria, *Streptococcus pyogenes* and *Serratia marcescens*, to treat various malignant tumors, resulting in promising therapeutic outcomes (Starnes, 1992). In the mid to late 20th century, some oncogenic viruses such as human herpesvirus 4 (HHV-4) and hepatitis B virus (HBV) were discovered (Epstein et al., 1964; Dane et al., 1970). Subsequently, the connection between microbiomics and tumor occurrence and development has aroused widespread interest among scholars. In particular, the advent of the first-generation sequencing method, the chain-termination approach, opened the door to deciphering the genetic code of life (Sanger et al., 1977). With advancements in sequencing technologies, such as next-generation

sequencing, also known as high-throughput sequencing, and single-molecule, long-read sequencing, direct sequencing is possible. This has addressed issues of information loss and base mispairing, allowing us to better understand the structural composition of microbial communities (Radelof et al., 1998; Eid et al., 2009; Metzker, 2010). The microbial community, as an emerging field of research, has been found to exist in various types of tumors, including breast cancer, lung cancer, ovarian cancer, pancreatic cancer, and melanoma (Nejman et al., 2020). However, research on the microbial community in gastric cancer (GC) has only received widespread attention in the past decade. Dysbiosis of the microbial community may participate in the occurrence and development of GC through pathways such as activating inflammatory responses, influencing host immune systems, and interfering with cell signaling. Moreover, the structure of the microbial community is closely related to the efficacy of treatments such as chemotherapy and radiotherapy, as certain microbes may affect drug metabolism, absorption, and resistance.

Therefore, some studies are attempting to target the microbial community for GC treatment, aiming to improve the tumor microenvironment (TME), enhance immune suppression, and increase drug efficacy by modulating the microbial community. Overall, research on the microbial community in GC is in a stage of vigorous development, requiring further in-depth studies and clinical validation, which are crucial for elucidating the pathogenesis of GC, identifying new therapeutic targets, and formulating personalized treatment strategies.

2 GC and alterations in the microbiota structure

GC is closely associated with alterations in the microbial community structure. Current research focuses on dysbiosis in the oral, gastric, and colonic microbiota (Table 1). These studies suggest that changes in the microbial community may play a significant role in the occurrence and development of GC, offering new insights into its prevention and treatment.

TABLE 1 Studies of microbiota related to GC.

Specimen	Sample size (tumor vs non-tumor)	Sequencing methods	Elevated microbiota	Decreased microbiota	Ref
Gastric mucosa	54, 81	16S rRNA gene sequencing	Actinobacteria, Bacillota	<i>Bacteroidota</i> , <i>Fusobacteria</i> spp.	(Federici et al., 2022)
Gastric juice	50, 45	16S rRNA gene sequencing	<i>Neisseria sicca</i> , <i>Prevotella melaninogenica</i> , <i>Veillonella parvula</i> , <i>Prevotella jejuni</i> , <i>Veillonella atypica</i>	–	(Wang et al., 2022)
Gastric cancer and adjacent noncancerous tissues	45, 45	Internal transcribed spacer rDNA gene analysis	<i>Candida</i> , <i>Alternaria</i> , <i>Fusicolla acetilerea</i> ,	<i>Saitozyma</i> , <i>Thermomyces</i>	(Zhong et al., 2021)
Gastric cancer and gastric mucosal tissue	53, 30	16S rRNA gene sequencing	<i>Prevotella</i> spp., <i>Oceanobacter</i> , <i>Methylobacterium</i> , <i>Syntrophomonas</i> , <i>Acinetobacter</i> , <i>Deiftia</i>	<i>Helicobacter</i> , <i>norank furibaculaceae</i> , <i>Escherichia-Shigella</i> , <i>Bifidobacterium</i>	(Peng et al., 2022)
Saliva	99, 194	16S rRNA gene sequencing	<i>Corynebacterium</i> , <i>Streptococcus</i> spp.	<i>Bulleidia</i> , <i>Fusobacterium</i> , <i>Peptostreptococcus</i> , <i>Lachnoanaerobaculum</i> , <i>Haemophilus</i> , <i>Neisseria</i> spp., <i>Parvimonas</i> , <i>Porphyromonas</i> , <i>Prevotella</i> spp.	(Huang et al., 2021)
Tongue coating	57, 80	16S rRNA gene sequencing	Bacillota	<i>Bacteroidota</i>	(Wu et al., 2018)
Mouthwash	165, 323	Shotgun metagenomic sequencing	<i>Betaproteobacteria</i> , <i>Neisseriales</i> , <i>Neisseriaceae</i> , <i>Neisseria mucocosa</i> , <i>Prevotella pleuritidis</i>	<i>Mycoplasma orale</i> , <i>Tenericutes</i> , <i>Eubacterium yurii</i> , <i>Cutibacterium</i>	(Yang et al., 2022b)
Saliva	10, 118	16S rRNA gene sequencing	<i>Neisseria</i> spp., <i>Porphyromonas gingivalis</i>	–	(Kageyama et al., 2019)
Tongue coating	11, 27	16S rDNA sequencing	<i>Neisseria</i> spp., <i>Prevotella</i> spp., <i>Veillonella</i> , <i>Porphyromonas</i> , <i>Leptotrichia</i>	<i>Sphingomonas</i> , <i>Rudaea</i> , <i>Phyllobacterium</i> , <i>Helicobacter</i> , <i>Bradyrhizobium</i> , <i>Arthrobacter</i>	(Wu et al., 2021)
Saliva and tongue coating	26, 18	2b-RAD sequencing	<i>Malassezia globosa</i>	<i>Saccharomyces cerevisiae</i>	(He et al., 2023)
Fecal samples	49, 49	16S rRNA gene sequencing	<i>Lactobacillus</i> , <i>Fusobacterium</i> , <i>Streptococcus</i> spp.	<i>Lachnospiraceae</i> , <i>Clostridia</i> , <i>Roseburia</i> , <i>Blautia</i> , <i>Actinobacteria</i>	(Yu et al., 2021)
Fecal samples	50, 56	Shotgun metagenomics sequencing	<i>Streptococcus</i> spp., <i>Prevotella</i> spp., <i>Veillonella</i> , <i>Lactobacillus</i>	–	(Erawijantari et al., 2020)

2.1 Intratumoural microbiota

The gastric microbiota plays a crucial role in maintaining endocrine balance, immune modulation, and promoting digestion and absorption. Significant alterations in the microbial composition and abundance occur in GC tumor tissues, leading to a state of microbial dysbiosis. This imbalance is widely considered to be a result of decreased microbial diversity and increased pathogenic microorganisms, among which *Helicobacter pylori* (*H. pylori*) infection is closely related. *H. pylori* is widely recognized as one of the most dangerous infection factors associated with GC, with a global infection rate exceeding 50%, and 1% to 3% of *H. pylori*-infected individuals developing GC (Noto et al., 2019; Xiao and Ma, 2022). *H. pylori* infection can result in the enrichment of other bacterial phyla, such as *Proteobacteria*, *Bacillota*, and *Bacteroidota* (Gao et al., 2018). On one hand, *H. pylori* has been demonstrated to activate NF- κ B and induce the production of β -defensin in gastric epithelial cells through the cytotoxin-associated gene A protein (CagA), potentially influencing the microbiota composition (Hamanaka et al., 2001; Wada et al., 2001; Brandt et al., 2005). On the other hand, *H. pylori* directly inhibits acid secretion in epithelial cells through T4SS, CagA, and NF- κ B-dependent mechanisms, leading to an increase in gastric pH. This less acidic environment promotes microbial diversification in the ecological niche, potentially enhancing diversity and reshaping the community structure of the gastric microbiota (Gööz et al., 2000; Saha et al., 2008; Smolka and Backert, 2012). Additionally, studies by Noto et al. have found that changes in the gastric microbiota are dependent on CagA and are not related to inflammatory responses, suggesting that CagA itself directly influences the microbial community structure (Noto et al., 2019). Moreover, in *Drosophila* intestinal stem cells, the expression of CagA fosters excessive cell proliferation and triggers the expression of innate immune components, such as Dipteracin and Duox, which have the potential to modify the host microbiota (Jones et al., 2017). *H. pylori* also expresses the duodenal ulcer-promoting gene A (DupA), closely linked to peptic ulcer disease but exerting minimal impact on the microbiota, thereby preserving the relative abundance of the gastric microbial community. However, DupA(-) *H. pylori* is abundant in precancerous lesions (Chen et al., 2023a). Other studies suggest that although microbial diversity decreases in GC tissue, this result appears unrelated to *H. pylori*. In GC tissue, the abundance of *H. pylori* decreases, while other bacterial genera, such as *Citrobacter*, *Achromobacter*, *Clostridium*, *Prevotella* spp., *Rhodococcus*, *Propionibacterium acnes* (*P. acnes*), *Clostridium*, *Slackia exigua*, *Fusobacterium*, *Parvimonas micra*, *Streptococcus* spp., and *Dialister pneumosintes*, increase in TME, with most of these genera representing intestinal symbionts (Coker et al., 2018; Ferreira et al., 2018; Hsieh et al., 2018; Liu et al., 2019; Dai et al., 2021; Png et al., 2022; Zhou et al., 2022). Moreover, in patients with favorable prognosis, the presence of *H. pylori* in TME is significantly increased (Yang et al., 2023b). Another study indicates that *H. pylori* infection may diminish the efficacy of immune checkpoint inhibitor therapy, resulting in markedly shorter median progression-free survival (PFS) and overall

survival (OS) among *H. pylori*-positive patients. Consequently, long-term dynamic monitoring becomes essential for individuals with *H. pylori* infection (Magahis et al., 2023). Certain bacterial genera such as *Bacteroidota* and *Fusobacteria* spp., *Prevotella* spp., show variable abundance in tumor tissue (Ferreira et al., 2018; Liu et al., 2019). *Fusobacteria* spp. and *Prevotella* spp. are significantly associated with poorer overall survival in GC patients (Lehr et al., 2023). Interestingly, *Lactobacillus species* (spp.), a probiotic, are significantly enriched in GC tissue, especially in the absence of *H. pylori* (Ferreira et al., 2018; Hsieh et al., 2018; Gantuya et al., 2020; Dai et al., 2021). This may be related to the elevated expression of IL-1 β , mucin 4, and mucin 13 in gastric mucosa (Ferreira et al., 2018; Breugelmans et al., 2022; Kim et al., 2022). However, the exact role of probiotics in the TME of GC tissue warrants further investigation.

In GC patients with concomitant bile reflux, the gastric microbiota is significantly altered. These patients exhibit an enrichment of bacterial genera such as *Comamonas*, *Pseudomonas*, *Halomonas*, *Arthrobacter*, *Bradymonas*, *Shewanella*, and *Marinobacter* (Huang et al., 2022). This phenomenon is likely attributed to the presence of bile acids (BAs), which include free and conjugated forms. The presence of BAs in gastric fluid, such as deoxycholic acid (DCA), reduces microbial diversity and leads to significant enrichment of *Limosilactobacillus*, *Burkholderia-Caballeronia-Paraburkholderia*, and *Rikenellaceae* RC9 (Xu et al., 2023b). Conjugated BAs elevate gastric pH, promoting the proliferation of bacteria producing lipopolysaccharide (LPS) in the stomach. As a result, the relative abundance of bacteria such as *Neisseria sicca*, *Veillonella parvula*, *Veillonella atypica*, *Prevotella melaninogenica*, and *Parvimonas pallens* significantly increases in gastric fluid (Wang et al., 2022). This has a profound impact on patients who have undergone gastrointestinal reconstruction surgery.

Some bacterial genera previously unreported in the gastric microbiota have been identified, such as *Keratinibaculum* spp., an anaerobic thermophilic bacterium isolated from soil (Mannion et al., 2023).

In GC tissues, fungal dysbiosis is observed, with *Candida albicans* serving as a biomarker for GC. The abundance of *Candida albicans* significantly increases in GC, reshaping the microbial composition. This is characterized by an elevated presence of filamentous fungi such as *Fusicolla acetilerea*, *Fusicolla aquaeductuum*, and *Arcopilus aureus*, while *Candida glabrata*, *Saitozyma podzolica*, *Penicillium arenicola*, and *Aspergillus montevidensis* exhibit markedly reduced abundance (Zhong et al., 2021). These alterations in microbial composition, predominantly featuring certain pathogenic bacteria, impact the prognosis of GC. For example, the heightened presence of *Methylobacterium* in GC tissues is significantly linked to an unfavorable prognosis in GC patients (Peng et al., 2022). A correlation analysis of the gastric mucosal microbiome in 170 GC tumor tissues and matched non-tumor tissues with immune-activated related transcripts revealed that *Akkermansia muciniphila* may play a role in modulating the expression of Granzyme B in the gastric cancer mucosal microenvironment. However, this requires further exploration (Lu et al., 2024). In addition, the GC microbiome was classified into three distinct subtypes (MS1, MS2, and MS3): MS1

exhibited high immune activity and enrichment of microbiota associated with immunotherapy and butyrate production, suggesting a potential sensitivity to immunotherapy; MS2 showed the highest α -diversity and activation of the TFF signaling pathway; MS3 was characterized by epithelial-mesenchymal transition (EMT), associated with poor prognosis and reduced responsiveness to chemotherapy. These findings provide novel insights into the relationship between GC microbiome characteristics, prognosis, and treatment efficacy, contributing to the development of personalized therapeutic strategies (Wang et al., 2024).

2.2 Oral microbiota

The functions of oral microbiota in oral health include maintaining the health of oral mucosa, participating in food digestion, regulating oral pH balance, and resisting invasion by external pathogenic microorganisms. When the oral microbial community becomes imbalanced, it not only leads to the occurrence of oral diseases but also correlates with the risk of GC. In fact, abundant oral bacteria, such as *Peptostreptococcus*, *Streptococcus* spp., *Fusobacterium*, and *Campylobacter concisus*, can be detected in GC samples and may serve as potential non-invasive biomarkers (Chen et al., 2019; Cui et al., 2019; Feng et al., 2023). Oral-associated microbial communities, including *Veillonella parvula* and *Streptococcus oralis*, are enriched in gastric cancer tissues and are associated with overall survival (Lei et al., 2024). The changes in microbial composition are characterized by the accumulation of pro-inflammatory bacteria such as *Corynebacterium* and *Streptococcus* spp., and a reduction in bacteria metabolizing carcinogenic substances like *Haemophilus* and *Neisseria* spp (Wu et al., 2018; Huang et al., 2021). However, in other studies, *Neisseria* spp. and *Prevotella* spp. are significantly enriched, while *Mycoplasma* and *Eubacterium* are reduced (Kageyama et al., 2019; Yang et al., 2022b). The ectopic colonization of oral microbiota may drive dysbiosis in the microbial ecology of GC tissue infected with *H. pylori* (Wu et al., 2021). Furthermore, fungal dysbiosis has been observed in the oral microbiome. For instance, samples of saliva and tongue coating collected from GC patients are enriched with *Malassezia globosa*, while *Saccharomyces cerevisiae* is reduced (He et al., 2023). Tongue coating displays varying colors and thicknesses, each harboring distinct microbial communities. Bacteria such as *Capnocytophaga leadbetteri*, fungus *Ampelomyces_sp_IRAN_1* could potentially serve as biomarkers for the white thin coating, while *Megasphaera micronuciformis*, *Prevotella maculosa*, *Acinetobacter ursingii*, and *Selenomonas sputigena* ATCC 35185 may serve as biomarkers for the white thick coating (Xu et al., 2019). This provides a novel approach to tongue coating diagnosis.

2.3 Fecal microbiota

The occurrence of GC is intricately linked to the composition and dynamics of the gut microbiota. Under normal circumstances, the gut microbiota plays a critical role in maintaining intestinal homeostasis and overall host health. It contributes to various physiological

processes, including nutrient metabolism, immune system regulation, and protection against pathogenic invaders. However, when the gut microbiota is imbalanced, it may trigger chronic inflammation, affect the host's immune system, and thereby increase the risk of GC. For example, fecal *Streptococcus* spp. alterations are closely linked to GC incidence and liver metastasis, suggesting their potential as biomarkers for GC prediction. These findings offer valuable insights into early diagnosis and treatment strategies for GC (Yu et al., 2021; Chen et al., 2022a). In animal models, the abundance of the phyla *Actinobacteria* and *Bacillota* is highest in the GC group (Yu et al., 2020). The intestinal microbiota composition of invasive GC patients infected with *H. pylori* has changed, characterized by a significant reduction in protective bacterial genera such as *Lactobacillus* (Devi et al., 2021). Additionally, post GC surgery patients exhibit higher species diversity and richness in their intestinal microbiota, along with increased abundance of aerobic, facultative anaerobic bacteria, and oral microbiota, indicating an association with postoperative complications such as the occurrence of metachronous colorectal cancer after gastric resection (Erawijantari et al., 2020). Certain intestinal microbial communities can differentiate between surgical and non-surgical GC patients, including *Enterococcus*, *Corynebacterium*, *Megasphaera*, *Roseburia*, and *Lachnospira*. GC patients with lymph node metastasis show no significant differences compared to those receiving chemotherapy. Furthermore, the abundance of *Blautia*, *Oscillospira*, and *Ruminococcus* is associated with Ki67 expression, while the abundance of *Prevotella* spp., *Lachnospira*, *Eubacterium*, and *Desulfovibrio* correlates with HER2 expression (Chen et al., 2022a). The dysbiosis of microbial communities in GC patients involves the enrichment or reduction of multiple microbial taxa, and the identification of representative microbes remains challenging. In an *in vivo* GC model, the colonization of *Enterotoxin Bacteroides fragilis* in the mouse intestines significantly accelerated chemotherapy-induced muscle and adipose tissue depletion, and promoted the development of GC cachexia by disrupting cell junctions and attracting M1 macrophages, thereby damaging the intestinal mucosal barrier (Wu et al., 2024a).

3 The pro-carcinogenic mechanisms of microbiota in GC

The dysregulation of the microbial community contributes to the complex mechanisms underlying the initiation and progression of GC. Current research indicate that dysbiotic microbiota can drive tumorigenesis and progression by enhancing host genomic damage, impeding cellular DNA repair, activating aberrant cellular signaling pathways, influencing tumor cell metabolism, and reshaping the tumor immune microenvironment.

3.1 Microbiota dysbiosis and gastric epithelial cell genomic damage

In its quest for long-term residence in the host stomach, *H. pylori* employs a diverse array of outer membrane adhesins to

optimize its binding to the gastric mucosa. These adhesins facilitate a strong and persistent interaction with the host epithelial cells, promoting the bacterium's survival and persistence in the gastric environment (Figure 1). *H. pylori* attaches to gastric epithelial cells using adhesins like HopQ and carcinoembryonic antigen-related cell adhesion molecules (Odenbreit et al., 2000; Javaheri et al., 2016; Hamway et al., 2020). Key adhesins in *H. pylori*, such as AlpA/B and BabA/B, are glycosylated, enhancing their binding ability. Loss of glycosylation severely impairs adhesin resistance to proteases, stability, and binding capacity (Teng et al., 2022). Upon binding to host epithelial cells, the *cag* pathogenicity island encodes a bacterial type IV secretion system (T4SS) that delivers a potent virulence protein, CagA, directly into epithelial cells. This event affects multiple pathways in host cells, stimulating epithelial cell proliferation and contributing to gastric carcinogenesis (Odenbreit et al., 2000; Ohnishi et al., 2008; Javaheri et al., 2016; Noto et al., 2019; Hamway et al., 2020). Mechanistically, CagA leads to aberrant β -catenin activation, promoting GC cell proliferation (Franco et al., 2005). Treatment of *H. pylori*-infected mice with the β -catenin inhibitor (KYA1797A) could significantly alleviate gastric epithelial DNA damage (Li et al., 2023). *Escherichia coli* (*E. coli*) and *Fusobacterium nucleatum* (*F. nucleatum*) possess a unique bacterial adhesin/invasin called FadA, which presents in two distinct states: pre-FadA and mature FadA (mFadA). Initially, pre-FadA is embedded within the inner membrane and remains soluble under neutral pH conditions. Upon maturation, mFadA becomes insoluble and is subsequently secreted outside the bacterium. When fluorescently labeled mFadA is introduced to epithelial cells alone, no binding is detected. However, when combined with unlabeled pre-FadA, binding and invasion of epithelial cells by mFadA occur. The Pre-FadA-mFadA complex

could anchor within the inner membrane and extend through the outer membrane, facilitating bacterial invasion of host cells (Xu et al., 2007). Once internalized by host cells, *E. coli* secretes the genotoxin colibactin, leading to crosslinking between induced DNA strand and double-strand DNA breaks (Cullin et al., 2021). *F. nucleatum* utilizes lectin-like adhesins and a “zipping” mechanism to adhere to and invade human gingival epithelial cells (Han et al., 2000), or interacts with the Gal-GalNAc carbohydrate moiety on cell surfaces through its Fap2 galactose-binding lectin, specifically colonizing colorectal cancer and breast cancer (Abed et al., 2016; Parhi et al., 2020). This interaction may induce EMT, a critical process associated with cancer cell invasion, metastasis, stemness, and therapeutic resistance (Zhang et al., 2020). *F. nucleatum* can generate significant quantities of hydrogen sulfide (H_2S) from L-cysteine via the enzymatic activity of L-cysteine desulfhydrase, leading to increased DNA damage (Fukamachi et al., 2002; Basudhar et al., 2016). Evidence suggests that the production of H_2S contributes to DNA damage, partly through the generation of reactive oxygen species (ROS) (Attene-Ramos et al., 2010). The invasion of oral epithelial cells by *Prevotella intermedia* requires type C fimbriae, which are highly enriched in GC tissue (Dorn et al., 1998). Further research is needed to determine if *Prevotella intermedia* invades GC cells in the same manner and to elucidate the specific molecular mechanisms involved.

Pathogenic bacteria can also generate carcinogens through the metabolism of dietary components. Chronic *H. pylori* infection reduces gastric acid secretion, potentially fostering the growth of diverse gastric bacterial communities. The alteration in the microbiota could enhance aggression towards the gastric mucosa, potentially culminating in malignant tumor formation. The

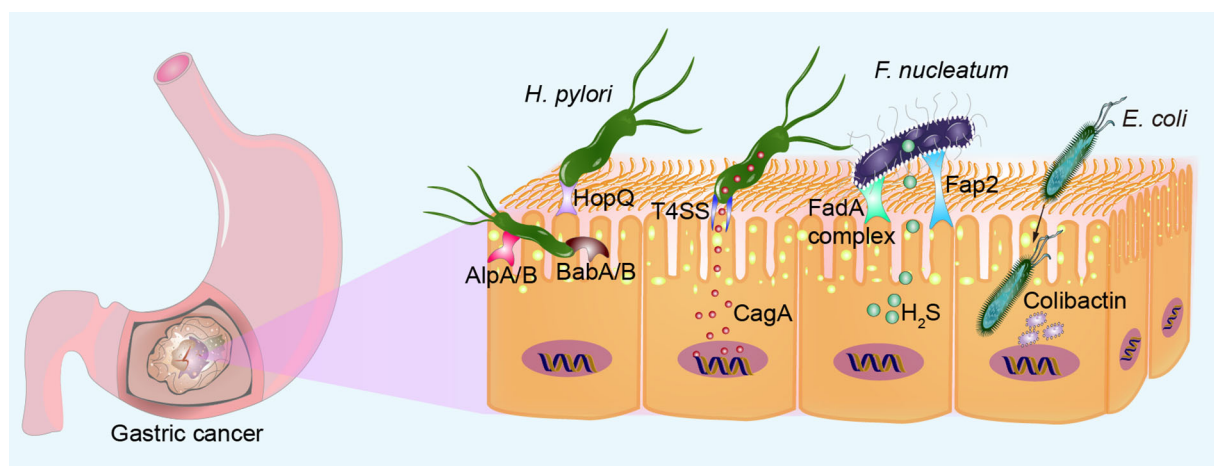


FIGURE 1

Microbial adhesion and invasion of gastric epithelial cells. *H. pylori* can bind to gastric epithelial cells via adhesin HopQ, glycan-modified proteins AlpA/B and BabA/B. *H. pylori* directly injects a potent virulence protein CagA into epithelial cells via the T4SS. *F. nucleatum* adheres to gastric epithelial cells through the pre-FadA-mFadA complex and Fap2 galactose-binding lectin, ensuring bacterial invasion of host cells. *F. nucleatum* produces high levels of H_2S , increasing DNA damage. Once internalized by host cells, *E. coli* secretes genotoxin colibactin, inducing DNA double-strand breaks.

microbiota sustains inflammation and converts nitrate to N-nitroso compounds, thereby promoting malignancy. The functional composition of the overall GC microbiota demonstrates an augmented presence of enzymes such as nitrate reductase, which catalyzes the reduction of nitrate to nitrite, and nitrite reductase, facilitating the conversion of nitrite to nitric oxide (Ferreira et al., 2018). This increased enzymatic activity suggests a potential mechanism through which the microbiota contributes to the pathogenesis of GC. Elevated protein intake can result in heightened levels of protein within the colon. In this environment, various bacteria, including certain *Bacillota* and *Bacteroidota*, metabolize amino acids into N-nitrosyl compounds. These compounds can induce DNA alkylation and host mutations, potentially contributing to carcinogenesis (Gill and Rowland, 2002). Colonic bacteria metabolize carcinogens, generating compounds that damage DNA, such as ethanol and heterocyclic amines, or directly producing carcinogens like non-hexane (Huycke and Gaskins, 2004). Primary bile acids are converted into secondary deoxycholic acid (DCA) by certain bacteria, including *Clostridium scindens*. DCA disrupts cell membranes, releasing arachidonic acid as a tumor promoter. Arachidonic acid, when metabolized by COX-2 and lipoxygenase, undergoes conversion into prostaglandins and ROS. These compounds play a crucial role in triggering inflammatory responses and causing DNA damage, contributing to various pathological conditions. Furthermore, taurocholic acid serves as a tumor promoter by fostering the production of genotoxic hydrogen sulfide and fueling the expansion of specific inflammatory bacteria, such as *Bilophila wadsworthia*, contributing to carcinogenesis (Ridlon et al., 2016). Under conditions of iron deficiency, *H. pylori* exacerbates gastric injury in insulin-gastrin mice, highlighting the interplay between bacterial infection and nutrient status in gastrointestinal health. While the observed phenotypes are not mechanistically driven by changes in the gastric microbiota, targeted metabolomics studies unveiled substantial alterations in bile acids among iron-deficient mice infected with *H. pylori*. Notably, the carcinogenic bile acid DCA showed significant upregulation. Treatment with DCA worsened the severity of gastric injury in *H. pylori*-infected mice. *In vitro* experiments demonstrated that DCA enhances the translocation of the *H. pylori* oncogenic protein CagA into host cells (Noto et al., 2022). TDCA and LPS drive gastric carcinogenesis by triggering activation of the IL-6/JAK1/STAT3 pathway in gastric epithelial cells, implicating inflammation in the development of GC (Wang et al., 2022). Conversely, DCA induces alterations in the gastric environment, characterized by abnormal bile acid metabolism and microbial dysbiosis. Specifically, there is a notable enrichment of *Gemmobacter* and *Lactobacillus*, suggesting a complex interplay between bile acids and the gastric microbiota in gastric pathophysiology (Jin et al., 2022). Bile acids, shown to function as endogenous antagonists of leukemia inhibitory factor (LIF), bind to a heterodimeric receptor during tumor initiation. Tissue analysis of bile acid content in both non-cancerous and GC biopsies demonstrates an accumulation of bile acids within cancer tissues. Specifically, glycodeoxycholic acid acts as a negative regulator of LIFR expression (Di Giorgio et al., 2024).

3.2 Microbiota dysbiosis and gastric epithelial cell genome repair

Cells undergo a series of complex biological processes to repair DNA when subjected to external damage or internal errors resulting in DNA breaks, base damage, and other situations, thereby preserving the genome's integrity and stability. These processes primarily include direct damage repair mechanisms such as mismatch repair (MMR), single-strand break repair (SSBR), and double-strand break repair (DSBR). Additionally, they encompass indirect damage repair mechanisms like nucleotide excision repair (NER), base excision repair (BER), cross-link repair. MMR is a highly conserved biological pathway crucial for maintaining genome stability. This pathway specifically targets base mispairs and insertion/deletion mispairs that arise during DNA replication and recombination processes (Li, 2008). Co-culturing GC cells with various strains of *H. pylori* results in a dose-dependent decrease in the levels of MMR proteins, including MutS (MSH2 and MSH6) and MutL (MLH1, PMS2, and PMS1) (Kim et al., 2002). This may be attributed to CagA EPIYA motifs and vacuolating cytotoxin A (vacA) genotypes (Mi et al., 2020). *H. pylori* suppresses the expression of MMR proteins by upregulating miR-155-5p, miR-3163, and miR-150-5p (Santos et al., 2017). *F. nucleatum* triggers the expression of miR-205-5p by activating the Toll-like receptor 4 (TLR4) and MyD88-dependent innate immune signaling pathway. This upregulation, in turn, suppresses the expression of key MMR proteins (MLH1, MSH2, and MSH6). The resulting MMR deficiency leads to increased DNA damage and enhanced cell proliferation, contributing to the progression of squamous cell carcinoma of the head and neck (Hsueh et al., 2022). Microsatellite instability (MSI) refers to the alteration in the length of microsatellite sequences, which are DNA sequences consisting of short repetitive motifs, during cellular replication. MSI is typically caused by defects in the DNA MMR system, including mutations in MMR genes, epigenetic changes, or other mechanisms. Therefore, the detection of MSI has become an important indicator for assessing tumor risk, diagnosis, and treatment strategy selection. In patients with GC, oral microbiota of oral origin is associated with immune gene expression and tumor mutation burden (Byrd et al., 2023). There is a lack of foundational experimental evidence regarding whether microbiota in GC affects other DNA repair deficiencies. Investigating the disruption of microbiota on DNA repair may hold significant implications for understanding the molecular mechanisms underlying the onset and progression of GC. Microbiota increase host cell genome damage and inhibit genome repair as shown in Figure 2.

3.3 Microbiota dysbiosis and aberrant signaling pathways in GC

The interaction between dysbiosis and cancer cell aberrations involves multiple signaling pathways that can influence tumor initiation, progression, and therapeutic response (Figure 3). Infection with *H. pylori* drives the nuclear accumulation and

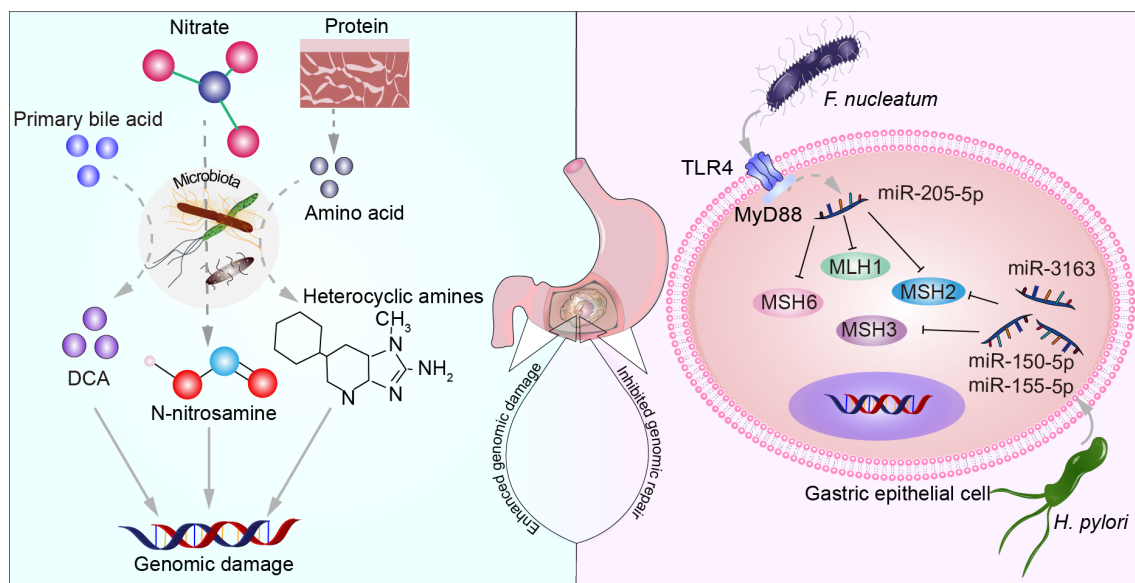


FIGURE 2

The microbiota increases host cell genomic damage and suppresses genome repair. Primary bile acids, nitrate, and proteins metabolized by certain microbial communities produce substances such as DCA, N-nitrosamines, and heterocyclic amines, leading to DNA damage. *F. nucleatum* increases the expression of miR-205-5p through the TLR4 and MyD88-dependent innate immune signaling pathway, suppressing the expression of MLH1, MSH2, and MSH6. *H. pylori* upregulates miR-150-5p, miR-155-5p, and miR-3163 to suppress the expression of MSH2 and MSH3 proteins.

transcriptional activity of yes-associated protein 1 (YAP) and β -catenin in gastric epithelial cells and transgenic insulin-gastrin mice. This interaction between YAP and β -catenin promotes their nuclear activation. Consequently, the activation of target genes such as CDX2, LGR5, and RUVBL1 is initiated, fostering cell proliferation, and contribute to the pathogenesis of GC (Li et al., 2023). *H. pylori* infection not only triggers the expression of IL-11 but also upregulates cancer-related genes such as PTGER4 and TGF- β in insulin-gastrin mice. These molecular changes further expedite the progression of gastric cancer (Lertpiriyapong et al., 2014). *F. nucleatum* induces the activation of actin and genes regulating cell motility, promoting the invasiveness of GC cells (Hsieh et al., 2021). In addition, the microbiota can induce sustained inflammatory responses, generating ROS and causing DNA fragmentation, membrane breakdown, and protein misfolding through modifications of key substrates such as nucleic acids, lipids, and proteins (Chen et al., 2022c). These processes may lead to cellular senescence (Pérez-Mancera et al., 2014). Senescent cells stand apart from quiescent and apoptotic cells by maintaining high cellular viability and efficient metabolic function (Campisi and D'adda Di Fagagna, 2007). Senescent cells collectively produce a range of cytokines, chemokines, growth factors, proteases, and other secretory signaling factors, forming what is known as the senescence-associated secretory phenotype (SASP) (Coppé et al., 2010). Senescent cells have a dual role through autocrine or paracrine signaling: they play a physiological role in tissue development, prevent proliferation of damaged cells, aid in tissue repair, and contribute to tumor suppression. However, they

also promote the onset of age-related diseases, including cancer (Chen et al., 2022b). Mounting evidence indicates that dysregulated SASP sustains an inflammatory environment, promoting cancer cell proliferation, migration, invasion, and EMT, thereby accelerating the growth of xenograft tumors (Chen et al., 2022b). Research on the mechanisms of microbial dysbiosis in GC cells remains unclear, presenting a highly promising avenue for investigation. In addition, certain viruses can also trigger the abnormal activation of signaling pathways. For example, Epstein-Barr virus (EBV) infection can activate the cGAS-STING pathway and upregulate the expression of olfactomedin 4 (OLFM4), which binds to the extracellular cadherin domain of FAT1, thereby disrupting its intracellular interaction with MST1 and subsequently activating YAP in recipient cells (Wen et al., 2024). Naturally occurring or genetically engineered viruses, such as the CF33 oncolytic virus, are capable of delivering functional proteins (e.g., hNIS-antiPDL1) and exhibit significant antitumor activity in peritoneal metastasis gastric cancer models following intraperitoneal injection (Yang et al., 2023a). The expression and/or integration of human papillomavirus oncogenes in gastric cancer may play a potential etiological role, but the underlying mechanisms remain to be further explored (Xu et al., 2023a).

The imbalance of the microbial community may lead to abnormal accumulation or deficiency of metabolites, thereby affecting host metabolic health. These metabolites can influence host cell function and metabolic status through different signaling pathways. For example, in atrophic gastritis induced by *H. pylori*, there is an elevated expression of glucose-6-phosphate 1-dehydrogenase

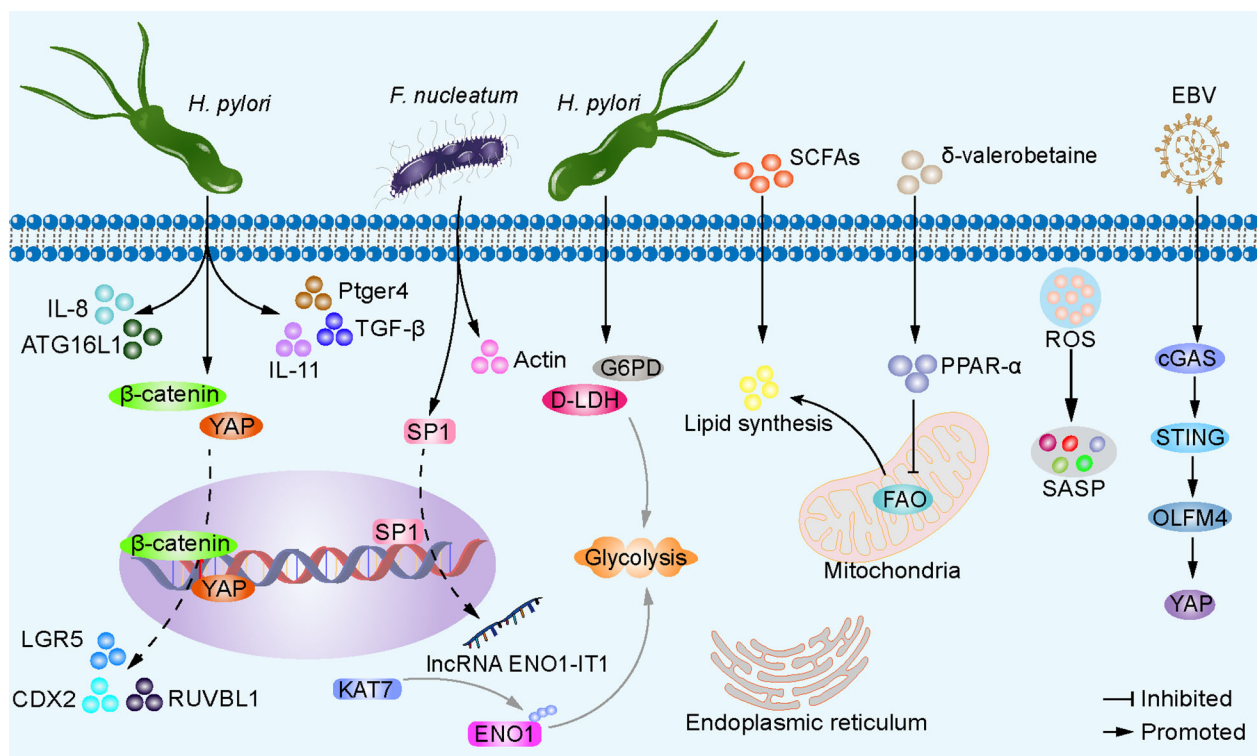


FIGURE 3

Microbiota dysbiosis alters gastric epithelial cell signaling pathways. *H. pylori* promotes nuclear accumulation and transcriptional activity of YAP and β -catenin in gastric epithelial cells, leading to activation of target genes CDX2, LGR5, and RUVBL1, facilitating cell proliferation and expansion, ultimately resulting in GC development. *H. pylori* also induces the expression of IL-11 and cancer-related genes Ptger4 and TGF- β . *H. pylori* enhances autophagy gene ATG16L1, increasing IL-8 production, driving carcinogenesis. *H. pylori* induces the expression of G6PD and D-LDH in host cells, facilitating glycolysis, and energy production. *F. nucleatum* upregulates transcription factor SP1, activates lncRNA ENO1-IT1 transcription, guides KAT7 histone acetyltransferase to modify target gene ENO1, increasing host cell glycolysis. δ -valerobetaine produced by various bacteria inhibits mitochondrial FAO and increases lipid accumulation via transcription factor PPAR- α . SCFAs serve as substrates for lipid synthesis. Additionally, the microbiota can induce sustained inflammatory responses, generate ROS, causing DNA fragmentation, membrane disintegration, and protein misfolding through modification of key substrates such as nucleic acids, lipids, and proteins, leading to cellular senescence, secretion of SASPs, and accelerated tumor growth. EBV infection can activate the cGAS-STING pathway and upregulate the expression of OLFM4, thereby leading to the activation of YAP in recipient cells.

(G6PD) and D-lactate dehydrogenase (D-LDH) (Parsons et al., 2017). This contributes to inducing anaerobic metabolic shift, thereby generating energy (Chen et al., 2023c). Some bacteria enriched in GC tissues, although not yet reported in GC, have been shown to alter the glycolipid metabolism of other tumor cells. For example, *F. nucleatum* activates the transcription of long non-coding RNA ENO1-IT1 by enhancing the binding efficiency of transcription factor SP1 to the promoter region of ENO1-IT1. The increased expression of ENO1-IT1 acts as a guiding module for KAT7 histone acetyltransferase, directing its histone modification pattern on target genes, including ENO1, a key glycolytic enzyme, thereby altering glycolysis in colorectal cancer cells (Hong et al., 2021). The microbiota provides lipid synthesis precursors or stimulates host cell lipid synthesis through its own metabolic products. For example, short-chain fatty acids (SCFAs) can serve as substrates for energy production, lipid synthesis, gluconeogenesis, and cholesterol synthesis (Bergman, 1990). δ -Valerobetaine, generated by diverse bacterial strains, activates the transcription factor PPAR- α , thereby driving transcriptional regulation of lipid processing and

mitochondrial energy metabolism in the liver of mice. As a result, there is a reduction in mitochondrial fatty acid oxidation (FAO) and an increase in lipid accumulation (Liu et al., 2021). The biologically active components derived from the small bowel microbiota *Clostridium bifermentans* selectively induce the expression of diacylglycerol O-acyltransferase 2 (DGAT2), which participates in triacylglycerol synthesis. The exact mechanism behind this induction remains to be explored (Liu et al., 2021). *H. pylori* can decrease endoplasmic reticulum stress levels in gastric epithelial cells while enhancing the autophagy gene ATG16L1 (rs2241880, G-allele) expression, thereby promoting increased IL-8 production and driving the carcinogenesis process. This may be associated with the role of IL-8 recruitment of granulocytes in the development of intestinal metaplasia and GC (Fu et al., 2016; Mommersteeg et al., 2022). Investigating dysregulated microbiota and abnormal signaling pathways in GC enhances our comprehension of tumorigenesis mechanisms. This exploration sheds light on the microbiota's involvement in GC development, offering novel insights and strategies for GC prevention, diagnosis, and treatment.

3.4 Microbiota and GC hypoxia

Hypoxia is considered a hallmark of cancer, with most solid tumors, including GC, exhibiting oxygen deficiency (Ye et al., 2019). The intratumoural microbiota is intricately associated with hypoxia in TME. This linkage is evident through several avenues: Microbial colonization within tumor tissues elicits inflammatory responses, leading to endothelial cell injury, dysfunctional endothelial cell activity, and compromised vascular function, ultimately culminating in hypoxia due to impaired blood perfusion. Certain microbes, such as *H. pylori* and *Streptococcus* spp., which are facultative anaerobes or aerobes, further contribute to hypoxia by consuming oxygen (Cullin et al., 2021; Chen et al., 2022c). Mechanistically, bacteria can utilize high-affinity terminal oxidases to scavenge O₂ at low concentrations, even at nanomolar levels, exacerbating the degree of hypoxia in tumor tissues (Morris and Schmidt, 2013; Kelly et al., 2015). Furthermore, microbial derivatives such as SCFAs increase oxygen consumption by pathways including β -oxidation of butyrate and oxidative phosphorylation-dependent epithelial O₂ consumption (Hamer et al., 2008; Kelly et al., 2015; Zheng et al., 2017). Additionally, microbial communities recruit innate and adaptive immune cell infiltrates, most notably neutrophils and eosinophils, which consume local oxygen via the NADPH oxidase-2 (NOX-2) during oxidative bursts (Campbell et al., 2014; Masterson et al., 2019). Therefore, the microbial community in TME is one of the factors contributing to the formation and maintenance of chronic hypoxia, driving alterations in tumor cell signaling pathways, primarily associated with increased expression of the hypoxia-inducible factor (HIF). This is associated with tumor size, lymph node involvement, vascular invasion, and pathological staging (Zhang et al., 2010). In mice infected with *H. pylori*, levels of HIF-1 α significantly increase, enhancing the toxicity of CagA, promoting IL-8 secretion, and exacerbating host pro-inflammatory responses (Noto et al., 2023). Conversely, the massive production of ROS generated by inflammatory responses not only stimulates the expression of HIF-1 α but also contributes to its stabilization under hypoxic conditions (Leung and Chan, 2009). TLRs are a highly conserved class of pattern recognition receptors that detect pathogen-associated molecular patterns and play a crucial role in the immune system, protecting the body from infections by initiating immune responses (Akira and Takeda, 2004). LPS activates the tumor cell TLR4 signaling pathway and NF- κ B, thereby upregulating HIF-1 α , promoting the progression of pancreatic adenocarcinoma (Zhang et al., 2010). Activation of HIF reprograms metabolism, protein synthesis, and cell cycle processes (Chen et al., 2023c).

3.5 Microbiota and tumor immune microenvironment

A wealth of evidence suggests that dysbiosis of the gastric microbiota and immune system dysfunction, particularly immune evasion, are critical for the onset and progression of GC. Changes in

the recruitment and function of innate and adaptive immune cells predominantly drive the progression and prognosis of GC. *H. pylori* induces the expression of natural killer group 2, member D (NKG2D) ligands on gastric epithelial cells through vacA, which are released from the cell surface via protein hydrolysis or extracellular vesicles (EVs). This leads to downregulation of the NKG2D receptor expression on NK cells and cytotoxic granule release, thereby contributing to immune evasion by tumor cells (Anthofer et al., 2024). *P. acnes* significantly increases in GC tissues infected with *H. pylori*, activating the TLR4/PI3K/Akt signaling pathway, inducing polarization of M2-type tumor-associated macrophages (TAMs), and promoting the secretion of immunosuppressive factors IL-10 and CCR-2 (Li et al., 2021c). M2 TAMs maintain an inflammatory environment in TME, creating an immunosuppressive microenvironment that promotes tumor cell proliferation and survival, fosters cancer stem cells, supports metastasis, and contributes to the progression and metastasis of GC (Mantovani et al., 2017; Long et al., 2019; Piao et al., 2022). Butyrate derivatives from probiotics negatively regulate the NLRP3-mediated inflammatory signaling pathway, inhibit the activation of associated macrophages, and reduce their expression levels of PD-L1 and IL-10, thereby suppressing tumor growth in mice (Yao et al., 2022; Lee et al., 2024). *H. pylori* and *Methylobacterium* can decrease the TGF- β expression and infiltration of CD8⁺ T cells in GC mouse models, but their mechanisms remain to be elucidated (Oster et al., 2022; Peng et al., 2022). Some less abundant bacterial genera in GC tissues, such as *Selenomonas* and *Brevundimonas*, are positively correlated with regulatory T cells (Tregs) (Ling et al., 2019; Yang et al., 2022a). Mechanistically, *H. pylori* activates the TLR2/NLRP3/caspase-1/IL-18 axis in dendritic cell to induce Tregs, shaping an immunosuppressive microenvironment (Koch and Müller, 2015). *H. pylori* drives the activation of pro-inflammatory T cells, secretes IL-21, induces STAT3 phosphorylation, and promotes ROR γ -t expression, facilitating the differentiation of T helper 17 (Th17) cells and the secretion of IL-17 (Carbo et al., 2014). Additionally, *H. pylori* activates TLR9, promotes the expression of the negative regulatory factor TRIM family protein TRIM30a, thereby downregulating the activation of transcription factor interferon regulatory factor 3 (IRF3) and inhibiting the stimulator of interferon genes (STING) signaling pathway. These mechanisms contribute to the induction of Th17 cell inflammatory response and tumor-promoting effects *in vivo* (Dooyema et al., 2022). *Candida* is positively correlated with pro-inflammatory immune factors IL1A, IL1B, IL6, IL8, CXCL1, CXCL2, and IL17C, which are associated with neutrophil and Th17 cell infiltration (Dohman et al., 2022; Li et al., 2022). These possible mechanisms are summarised in Figure 4.

Furthermore, dysbiosis of the microbiota may affect other stromal cells in the tumor microenvironment, such as endothelial cells. Dysbiosis of the microbiota can disrupt the balance between pro-angiogenic and anti-angiogenic factors, crucial for angiogenesis. This imbalance may accelerate tumor angiogenesis, leading to rapid but abnormal blood vessel formation (Carmeliet and Jain, 2000; Jain, 2005; 2014). *In vitro*, low concentrations of probiotic metabolite butyrate promote angiogenesis via G-protein-coupled receptor 43 (GPR43, also known as FFAR2) (Castro et al., 2021). LPS stimulation of NOD-like

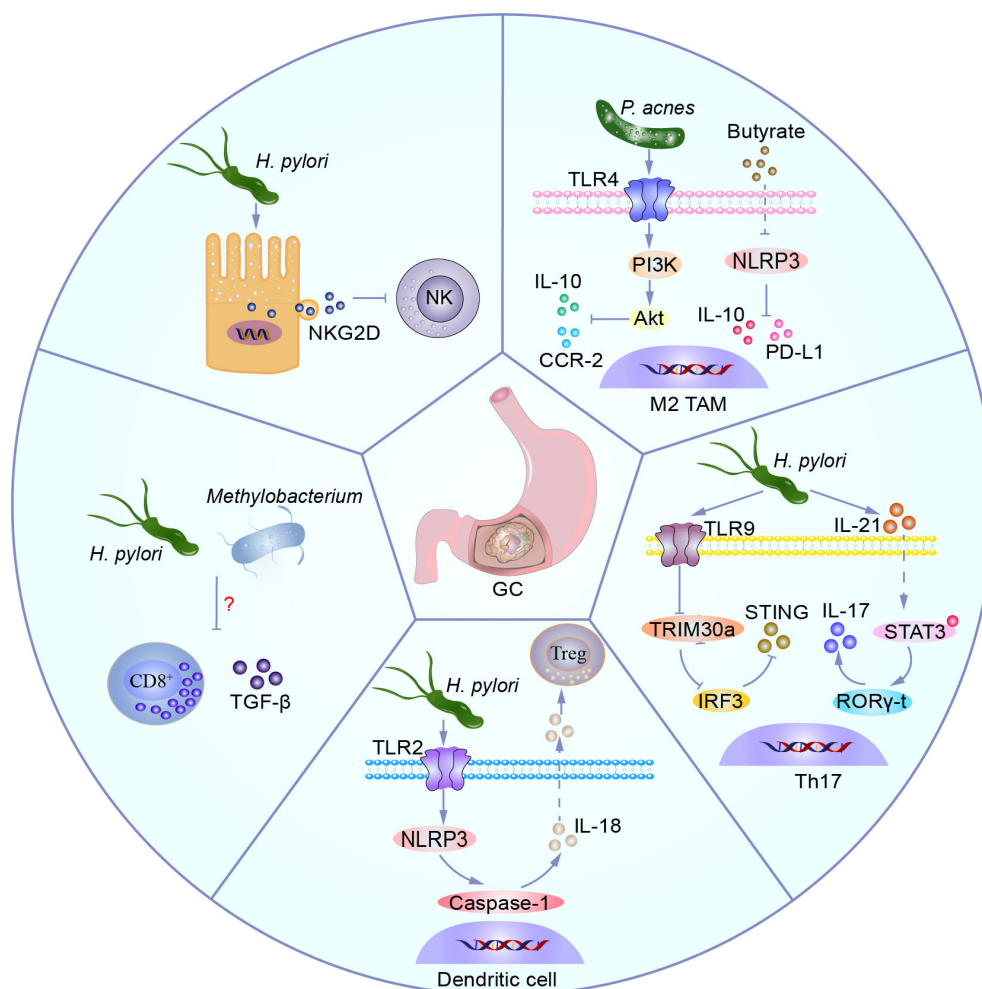


FIGURE 4

Microbiota shapes the suppressive immune microenvironment. *H. pylori* induces expression of the NKG2D ligand in gastric epithelial cells, which is released from the cell surface via protein hydrolysis or extracellular vesicles, leading to decreased expression of the NKG2D receptor on NK cells and cytotoxic granule degranulation, thereby facilitating immune evasion by tumor cells. *P. acnes* activates the TLR4/PI3K/Akt signaling pathway, inducing M2 TAM polarization, promoting secretion of immunosuppressive factors IL-10 and CCR-2. Butyrate, a derivative of probiotics, negatively regulates the NLRP3-mediated inflammatory signaling pathway, inhibits related macrophage activation, and decreases levels of PD-L1 and IL-10 expression, thereby suppressing tumor growth in mice. *H. pylori* activates TLR9, promotes expression of negative feedback regulator TRIM30a, downregulates activation of transcription factor IRF3, inhibits the STING signaling pathway, and promotes Th17 inflammatory responses and anti-tumor responses *in vivo*. *H. pylori* drives activation of pro-inflammatory T cells, secretes IL-21, induces phosphorylation of STAT3, and induces expression of RORγ-t, promoting Th17 differentiation and IL-17 secretion. *H. pylori* activates dendritic cells via the TLR2/NLRP3/caspase-1/IL-18 axis to induce Tregs, shaping the immune suppressive microenvironment. *H. pylori* and *Methylobacterium* can reduce expression of TGF-β and CD8⁺ T cell infiltration in a GC mouse model, but their mechanisms remain to be elucidated.

receptor (NLR) and TLR increases microvascular formation, inducing human intestinal microvascular endothelial cell migration and proliferation (Peng et al., 2004). Nevertheless, dysbiosis of the microbiota plays a vital role in various critical aspects of GC development. Particularly, the enrichment of intratumoral probiotics and their metabolites in GC warrants further exploration. The σC protein from avian reovirus or UV-inactivated avian reovirus can bind to TLR3 on the surface of CD8⁺ tumor-infiltrating lymphocytes, activating the TLR3/NF-κB/IFN-γ/TRAIL signaling pathway in immune cells. This induces the production of TRAIL, thereby initiating immunogenic apoptosis targeting cancer cells (Wu et al., 2024b). Based on these mechanisms, microbial dysbiosis affects GC

chemotherapy and immunotherapy (Li et al., 2021b; Kim et al., 2023; Magahis et al., 2023). Exploring the microbial mechanisms of carcinogenesis helps deepen our understanding of the interplay between microbiota and GC development, providing new research perspectives and strategies for both preventing and treating GC.

Although this study systematically explores several key molecular mechanisms (such as CagA, short-chain fatty acids, DNA repair pathways, and the IL-6/JAK/STAT3 signaling axis), it lacks in-depth analysis linking these mechanisms to clinical practice. Their potential value in diagnostic biomarker development, prognostic assessment, therapeutic target identification, and resistance mechanisms has not been fully demonstrated. Future research could further investigate the

clinical applicability of these mechanisms, such as their roles in biomarker screening, patient stratification, and prediction of treatment response, in order to enhance the translational relevance and clinical impact of the study.

4 Microbiota-related therapeutic application

The application of microbiota-related therapy in GC is gradually becoming a focus of research. These therapeutic approaches include probiotic therapy, prebiotic therapy, antimicrobial therapy, carrier application, dietary adjustments, fecal microbiota transplantation (FMT), and traditional Chinese medicine treatment, aiming to regulate gut microbiota balance, improve intestinal health in GC patients, and enhance immune system function (Figure 5). Although these therapeutic methods are still in the research and exploration stages, they offer new insights and hope for the treatment of GC.

4.1 Probiotic application

The application of probiotics in GC treatment is an area of great interest. Research indicates that probiotics and their derivatives can impact the onset and progression of GC by altering the gut microbiota, influencing the host's immune status, and regulating inflammation levels (Cao et al., 2022). On one hand, probiotics can improve the structure of the microbiota, particularly in the post-GC surgery gut microbiota, enhancing host immunity (Zheng et al., 2019, 2021a; He et al., 2022). This helps to ameliorate intestinal dysbiosis caused by mechanical bowel preparation, thereby reducing the incidence of postoperative delirium (Yang et al., 2022c). Furthermore, probiotics and their derivatives have been found to alleviate intestinal damage induced by chemotherapy drugs like oxaliplatin in both mice and human patients. They also enhance the response to anti-programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) immunotherapy (Yuan et al., 2022; Han et al., 2023). On the other hand, probiotic derivatives such as butyrate salts enhance the cytotoxic function

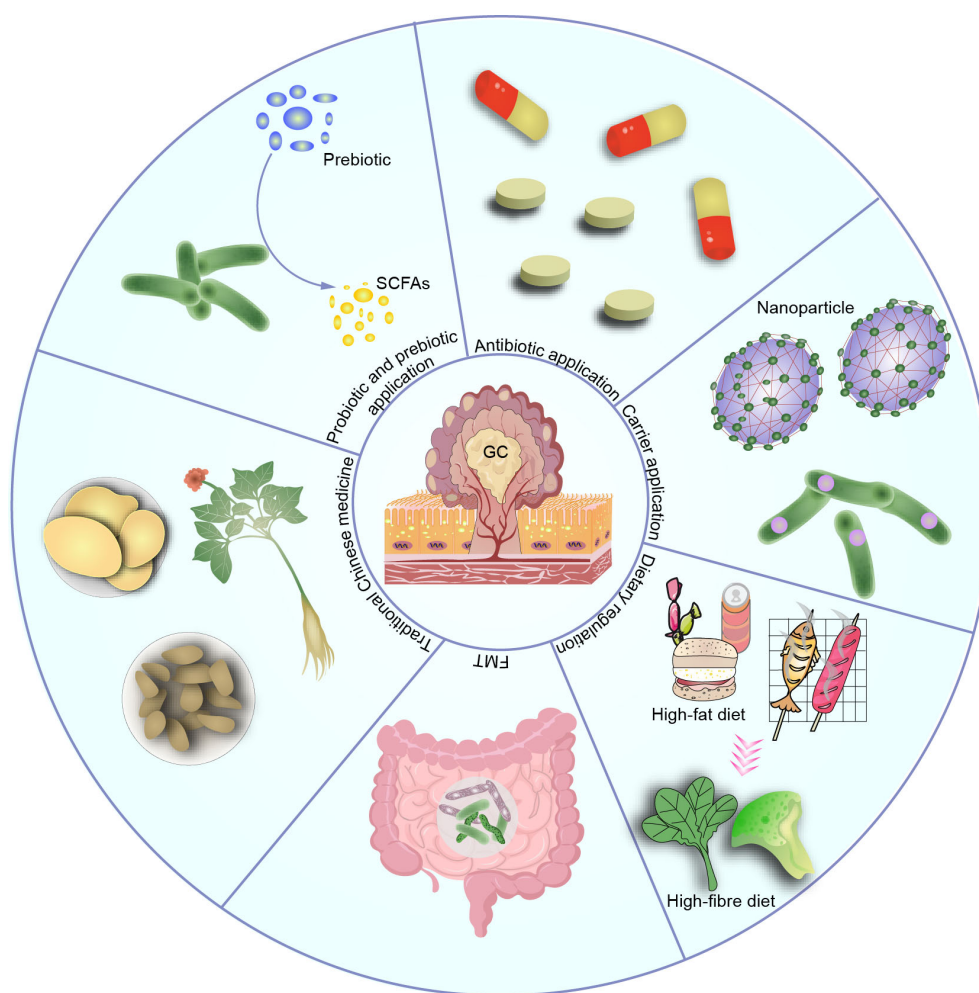


FIGURE 5

Therapeutic applications based on the microbiota, such as probiotic, prebiotic, antibiotic use, carrier application, dietary modulation, and traditional Chinese medicine, have shown promising efficacy. However, most of these applications are still in the preclinical stage, and their clinical efficacy and potential complications remain to be determined.

of CD8⁺ T cells or CAR-Claudin 18.2 CD8⁺ T cells against GC cells via GPR109A and homeodomain-only protein X (HOPX) (Yu et al., 2024). A combination of probiotics (*Lactobacillus acidophilus* NCFM and *Lactobacillus plantarum* Lp-115) effectively recruits more lymphocytes, plasma cells, and neutrophils (Shen et al., 2023; Ye et al., 2024). Furthermore, probiotics such as *Lactobacillus* significantly reduce inflammatory cytokines, preventing host macrophages from producing pro-inflammatory cytokines IL-1 β , IL-6, IL-8, TNF- α , and IFN- γ (Gebremariam et al., 2019; Zhao et al., 2022; Wu et al., 2023). *Lactobacillus rhamnosus* GG induces FPR1, a tumor suppressor, to maintain inflammation resolution with anti-angiogenic potential (Liotti et al., 2022). However, the heightened presence of *lactobacillus* during cancer progression challenges the notion of their predominantly protective role in GC. *Lactobacillus* contributes to carcinogenesis by promoting factors such as ROS, N-nitroso compounds, lactate production, as well as inducing EMT and immune tolerance (Yang et al., 2021; Nabavi-Rad et al., 2022). Therefore, due to the unclear roles and functionalities of some probiotics enriched in GC tissues, the application of probiotics in GC patients needs to be approached with caution.

4.2 Prebiotic application

Prebiotics, indigestible substances metabolized by probiotic bacteria into SCFAs like acetate, propionate, and butyrate, play a vital role in promoting human health. They enhance resistance to pathogenic colonization, maintain mucosal barrier integrity, regulate intestinal pH, and boost anti-tumor immunity, thereby enhancing anti-cancer activity (Verspreet et al., 2016). The combination of sodium butyrate and dexamethasone significantly downregulates the oncogene TNS4 in GC cells, exhibiting a notable anti-proliferative effect (Eladwy et al., 2024). Raffinose is a polysaccharide composed of one molecule of glucose, one molecule of galactose, and one molecule of fructose. It is particularly abundant in foods such as beans, onions, beets, and carrots. Human gastrointestinal tract cannot directly digest and absorb raffinose, but it is fermented by the microbes in the intestine, producing SCFAs, which can lower the risk of GC (Turati et al., 2023). Ellagic acid is a bioactive phytochemical known for its high antioxidant and anticancer effects. However, its absorption rate in the intestine is low, and it is easily excreted. When encapsulated with low methoxylated and high methoxylated pectin films at a 1:4 molar ratio, ellagic acid lysine salt not only increases the water solubility of ellagic acid but also preserves its biological activity. After fermentation by gut microbiota, it produces SCFAs, demonstrating good prebiotic activity (Ortenzi et al., 2021). Some prebiotics, such as mushroom polysaccharides, can stimulate the growth of beneficial bacteria in the colon (Nowak et al., 2018). Combination formulations containing both probiotics and prebiotics are promising for promoting intestinal health, enhancing immune function, and improving nutrient utilization. More research is necessary to fully comprehend the role and functionality of prebiotics in mitigating the risk of digestive tract tumors (Enache et al., 2022). In addition, some

postbiotics, such as Urolithin A, not only exert their anti-tumor effects by activating autophagy and further activating the downstream Hippo pathway, inhibiting the Warburg effect, and promoting cell apoptosis, but also by modulating the composition of the gut microbiota, resulting in an increase in probiotics and a decrease in pathogenic bacteria (Qiao et al., 2024).

4.3 Antibiotic application

Antibiotics have the potential to impact tumors through altering the microbiota, modulating immune responses, and affecting their own drug metabolism. Co-administration of antibiotics with probiotics can reduce the changes and imbalance in the intestinal microbiota induced by antibiotics and improve the success rate of eradicating *H. pylori* (Oh et al., 2016). However, long-term antibiotic use may increase the risk of cancer development (Boursi et al., 2015; Hao et al., 2022; Chen et al., 2023b), or lead to complications such as anemia, gastrointestinal bleeding, and mortality (Quinn et al., 2020). For example, in multiple cohorts of patients with advanced GC undergoing PD-1 inhibitor therapy, the use of antibiotics has consistently been associated with poorer PFS and OS (Kim et al., 2023). Additionally, existing antibiotic-based traditional approaches lack targeted effects, resulting not only in failure in approximately 20% of patients but also in severe bacterial resistance and disruption of gut microbiota. This may be associated with upregulation of multidrug resistance proteins, methicillin-resistant regulator proteins, vancomycin-resistant sensor histidine kinases, chloramphenicol resistance proteins, and tetracycline resistance proteins (Guo et al., 2020). Therefore, the development of alternative or antibacterial agents is crucial for treating GC. Nanostructured lipid carriers (NLC), even when not loaded with any drugs, show bactericidal effects against *H. pylori* at low concentrations. Mechanistically, NLC can rapidly bind to and penetrate the membrane of *H. pylori*, causing destabilization and disruption. This leads to the leakage of cytoplasmic contents and ultimately results in bacterial death (Seabra et al., 2018; Chitas et al., 2022). A pH-responsive metal-organic framework hydrogen-generation nanoparticle (Pd(H)@ZIF-8) encapsulated in an ascorbate palmitate (AP) hydrogel can target and adhere to inflammatory sites through electrostatic interactions. Subsequently, it undergoes hydrolysis by matrix metalloproteinases. The released Pd(H)@ZIF-8 nanoparticles are further decomposed by gastric acid, producing zinc ions (Zn²⁺) and hydrogen gas. This process effectively kills *H. pylori*, alleviates inflammation, and helps restore damaged gastric mucosa. Additionally, this approach helps to avoid dysbiosis of the intestinal microbiota (Zhang et al., 2022). It's interesting that cancer risk, including GC, is reduced in diabetic patients treated with metformin. Metformin exhibits direct antibacterial activity against *H. pylori*, but its widespread applicability and mechanism require further elucidation (Jauvain et al., 2021). Engineering common dairy probiotics like *Lactobacillus* into complexes that secrete *H. pylori*-binding guide peptide (MM1) and broad-spectrum antimicrobial peptides can offer high selectivity against *H. pylori* while avoiding the development of pathogen resistance (Choudhury et al., 2023).

4.4 Carrier application

Some microbiota can metabolize chemotherapy drugs, greatly reducing their bioavailability. Therefore, carriers act as a medium, delivering drugs to specific areas through their own specific biological functions or by carrying substances with biological functions. Common carriers include NPs and biological carriers. Encapsulating 5-fluorouracil (5-FU) in chitosan NPs (CS NPs) and incorporating them into retrograde starch and pectin (RS/P) microparticles can prevent premature degradation or release of the NPs as they pass through the stomach and upper digestive tract, ensuring that 5-FU reaches the colon (Dos Santos et al., 2021). Rhamnogalacturonan-I is a type of natural pectic polysaccharide. When passing through gastric and intestinal fluids, capsules exhibit minimal *in vitro* release, degrading only through the action of colonic microbiota. Leveraging this property, the substance can serve as an excellent carrier for drug delivery (Svagan et al., 2016). Probiotics coated with silk fibroin NPs or mineralized coatings can prevent damage in the stomach, enhance survival rates, reach the intestine, regulate the gut microbiota, and synergistically enhance therapeutic effects in a mouse model of intestinal mucosal inflammation (Hou et al., 2021; Geng et al., 2023). Conversely, probiotics can also serve as oral drug carriers, transporting medications (such as metal NPs) to the intestines. This not only enhances the gut microbiota but can also be utilized for magnetic hyperthermia and photothermal therapy (Garcés et al., 2022). Furthermore, some rare elements such as selenium have beneficial effects on intestinal inflammation after trace intake. Constructed Se@Albumin complex NPs significantly ameliorate chemotherapy-induced complications of intestinal mucositis in a mouse model by reducing intestinal oxidative stress levels, lowering intestinal permeability, and alleviating gastric motility disorders (Deng et al., 2021). A pH-responsive ROS nanogenerator (Fe-HMME@DHA@MPN) consists of an acid-responsive metal phenolic network (MPN) shell and a mesoporous metal-organic nanostructure core [Fe-HMME (hematoporphyrin monomethyl ether, a sonosensitizer)]. Encapsulating dihydroartemisinin (DHA), these NPs generate more ROS singlet oxygen under ultrasound than the sonosensitizer HMME alone. The sonochemical process is driven by the Fenton/Fenton-like reaction between the degradation product Fe (II) in gastric acid and hydrogen peroxide (H₂O₂) in the infected microenvironment, producing oxygen. Encapsulated DHA acts as a hydrogen peroxide source, enhancing the peroxidase-like activity of Fe-HMME@DHA@MPN, thereby generating ROS hydroxyl radicals to kill multidrug-resistant *Helicobacter pylori* and eradicate biofilms, with minimal effects on the normal gut microbiota (Yu et al., 2023).

4.5 Dietary regulation

Dietary habits can influence the occurrence and development of GC by modulating the microbiota. Diet represents an economical, non-invasive, natural, and sustainable therapeutic approach. On one

hand, a Western diet, characterized by high fat, high sugar, and low fiber intake, can disrupt the balance of gut microbiota, a condition known as dysbiosis. This dysbiosis contributes to an increased risk of GC (Rinninella et al., 2020). The high-fat diet leads to severe dysbiosis in the stomach. Changes in the microbiota are accompanied by an increase in gastric leptin, leading to the development of intestinal metaplasia (Arita and Inagaki-Ohara, 2019; Arita et al., 2019). Typically, high-temperature (150–300°C) cooking and nitrite-curing of meats result in the formation of toxic compounds like heterocyclic amines. These compounds have a high mutagenic potential and are implicated in the development of colon cancer and GC. *Lactobacillus casei* DN 114001 reduces the genotoxicity of heterocyclic amines, suggesting that bacteria may metabolize or adsorb heterocyclic amines (Nowak and Libudzisz, 2009; Van Hecke et al., 2015). Recent studies suggest that a diet rich in capsaicin, the primary pungent compound in chili peppers, might promote gastric cancer metastasis. This effect could occur through the regulation of transient receptor potential vanilloid 1 (TRPV1) expression and alterations in the gut microbiota composition. This suggests the importance of controlling chili consumption for GC patients (Deng et al., 2023). On the other hand, a high-fiber diet, foods rich in probiotics and prebiotics, and other similar dietary choices can foster the growth of beneficial bacteria and help maintain the balance of intestinal microbiota, thereby reducing the risk of developing GC (Rinninella et al., 2020). Some foods contribute to the growth of probiotics. For example, spinach rich in cobalamin is positively correlated with genera of *Bacteroides*, propionates, and butyrates (Zheng et al., 2021b). Vegetable and seafood patterns may interact with dysbiosis to mitigate the risk of male GC, while dairy patterns may interact with dysbiosis to reduce the risk of GC in females (Gunathilake et al., 2021). Based on exogenous metabolites, adenosylcobalamin, soybean, common wheat, dates, and barley are considered potential candidates for the treatment of atrophic gastritis without *H. pylori* infection, while gallate from gallnuts is considered a candidate for the treatment of atrophic gastritis with *H. pylori* infection (Gao et al., 2023). Dairy products containing baicalin and baicalein can inhibit the expression of the *vacA* gene in *H. pylori*, interfere with its adhesion and invasion capabilities to human GC cells, and reduce the levels of *H. pylori*-specific serum IgM and IgA as well as IL-8 expression (Chen et al., 2018). Taken together, adjusting the dietary habits of GC patients to modulate the microbiota offers multiple advantages, including safety, comprehensiveness, naturalness, sustainability, and comprehensiveness. This approach represents an effective means of preventing and supporting the treatment of GC.

4.6 FMT

FMT not only alters the composition of bacteria but also establishes a cross-domain balance between intestinal fungi, viruses, and bacteria to promote the restoration of microbial homeostasis. Prior to first-line chemotherapy, FMT from healthy obese donors may improve the chemotherapy response (to

capecitabine and oxaliplatin) and survival rates of patients with metastatic esophagogastric cancer (De Clercq et al., 2021). After radical gastrectomy, patients undergoing FMT exhibit immunomodulatory effects by adjusting the intestinal microbiota structure, characterized by an increase in the relative abundance of certain bacteria producing SCFAs. Mechanistically, butyrate downregulates the NLRP3-mediated inflammatory signaling pathway, inhibits macrophage activation, and suppresses the secretion of pro-inflammatory mediators such as cysteine aspartate-specific protease-1 and IL-1 β , thereby reducing intestinal inflammation levels and promoting nutrient absorption (Yao et al., 2022). FMT is an effective treatment for recurrent *Clostridium difficile* infection, with its effectiveness in preventing recurrence reaching approximately 90% (Konturek et al., 2015). However, FMT may lead to complications that should not be overlooked, including the possibility of pathogen transmission to the recipient. Therefore, FMT is not a one-size-fits-all approach, and research is needed to determine the microbial composition that has specific effects on patients with different diseases. *Akkermansia muciniphila* can enhance the anticancer effect of oxaliplatin by producing pentadecanoic acid, which inhibits the activity of the glycolysis regulator far upstream element binding protein 1, thereby blocking aerobic glycolysis in cancer cells (Xu et al., 2024). This suggests that FMT can alter the gut microbiota structure, thereby enhancing the potential efficacy of chemotherapeutic agents, such as oxaliplatin, in GC.

4.7 Traditional Chinese medicine

Traditional Chinese medicine can influence the progression of cancer by regulating the gut microbiota. It alters the composition

and structure of the gut microbiota and modifies the levels of endogenous metabolites. These changes enhance intestinal barrier function, bolster the immune system, and improve overall body metabolism, contributing to the significant anti-tumor properties of Chinese herbal medicine (Wei et al., 2024). Jianpi Yangzheng helps regulate the structure of the gut microbiota and reduces the proportion of myeloid-derived suppressor cells, along with their production of inflammatory factors (Zhu et al., 2024). Gexia-Zhuyu Tang inhibits GC growth, reduces the expression levels of proteins associated with metastasis and invasion, including CD147, vascular endothelial growth factor (VEGF), and matrix metalloproteinase-9 (MMP-9). Additionally, modified Gexia-Zhuyu Tang significantly enhances caspase-1-dependent pyroptosis. This is supported by a dose-dependent rise in TNF- α , IL-1 β , IL-18, and lactate dehydrogenase (LDH) levels, accompanied by increased protein expression of NLRP3, apoptosis-associated speck-like protein (ASC), and caspase-1 (Zhao and Yu, 2024). Cordycepin has antibacterial and anti-inflammatory effects on mice infected with *H. pylori*. Compared to the control group treated with the carrier alone, cordycepin treatment results in approximately 50% reduction in the production of inflammatory cytokines, including IL-6 and IL-1 β , and about 60% reduction in the infiltration of immune cells such as Th17 cells (Kong et al., 2022). Therefore, oral traditional Chinese medicine exhibits multiple effects such as anti-tumor properties, immune modulation, alleviation of side effects, and improvement of overall health. Although the efficacy of traditional Chinese medicine in treating GC requires further scientific validation and clinical research, its role as an adjunctive therapy holds promise in enhancing patient quality of life and mitigating treatment side effects. The mechanisms, outcomes, and limitations of microbiota-related therapeutic applications are summarized in Table 2.

TABLE 2 Mechanisms, outcomes, and limitations of Microbiota-related therapeutic applications.

Microbiota-related therapeutic application	Mechanisms	Outcomes	Limitations
Probiotic application	Regulate gut microbiota structure and restore balance after GC surgery. Enhance host immunity and CD8 ⁺ T/ CAR-T cell cytotoxicity. Reduce inflammation by lowering IL-1 β , IL-6, TNF- α , and mitigating chemo-induced gut damage. Improve response to PD-1/PD-L1 immunotherapy. Recruit immune cells via specific probiotic combinations.	Promote postoperative recovery and reduce delirium by enhancing gut barrier and immunity. Alleviate chemotherapy-induced gut toxicity. Boost PD-1/PD-L1 immunotherapy response. Improve tumor immune microenvironment by enhancing anti-tumor immunity and reducing inflammation.	Some probiotics like <i>Lactobacillus</i> may promote GC by inducing ROS, EMT, and immune tolerance. Their roles remain unclear, and effects may be double-edged. Probiotic use in GC should be cautious and strain-specific.
Prebiotic application	Prebiotics boost SCFA production, lower gut pH, suppress harmful bacteria, strengthen the barrier, and enhance anti-tumor immunity. They also promote beneficial bacteria and microbiota stability. Encapsulation improves the stability and efficacy of anti-cancer compounds.	Sodium butyrate with dexamethasone enhances anti-cancer effects in GC. Prebiotics like raffinose and tannic acid boost SCFA production and reduce GC risk. Modified tannic acid shows strong prebiotic activity. Synbiotics offer synergistic benefits for gut and immune health.	Individual gut microbiota differences lead to variable prebiotic effects. Some compounds have low bioavailability before encapsulation. Anti-cancer mechanisms remain under-researched, with limited clinical validation. Prebiotics may also interfere with cancer treatments.

(Continued)

TABLE 2 Continued

Microbiota-related therapeutic application	Mechanisms	Outcomes	Limitations
Antibiotic application	Antibiotics combined with probiotics can reduce dysbiosis and improve <i>H. pylori</i> eradication. New strategies include NLCs that disrupt bacterial membranes, Pd(H) @ZIF-8 nanoparticles that release Zn ²⁺ and hydrogen to kill <i>H. pylori</i> and reduce inflammation, and engineered probiotics that selectively target <i>H. pylori</i> without promoting resistance.	Antibiotics can effectively eradicate <i>H. pylori</i> and combining with probiotics improves success rates. However, long-term use may increase GC risk and reduce immunotherapy efficacy. New approaches like nanocarriers and engineered probiotics offer better targeting and lower resistance.	Long-term use can lead to dysbiosis, an increase in resistant strains, and poor targeting. When combined with immunotherapy (e.g., PD-1 inhibitors), it may reduce efficacy.
Carrier application	Nanoparticles encapsulating chemotherapy drugs can prevent premature release of the drugs in the stomach and upper digestive tract, ensuring they reach the colon to exert their effects. Protecting probiotics through gastric acid increases their colonization rate and improves their therapeutic effect on intestinal mucosal inflammation. Mechanisms such as reducing intestinal permeability help improve chemotherapy-induced intestinal mucosal damage.	The nanodelivery system delays drug release, improves targeting, and enhances therapeutic efficacy. Alleviates chemotherapy-related intestinal toxicity and promotes recovery. Effectively kills resistant bacteria and maintains the stability of the normal gut microbiota.	Primarily animal models, lacking clinical research validation. Complex formulation technology, high production costs, and challenges in large-scale application. The long-term safety, metabolic process, and impact of nanomaterials on the microecosystem are not yet fully understood. Individual differences in gut microbiota may affect drug efficacy and response, requiring personalized strategies for support.
Dietary regulation	Western diets and high-temp cooking disrupt gut microbiota, increasing GC risk. Some compounds (e.g., capsaicin) may promote GC, while foods like spinach and dairy support beneficial bacteria. Veggie/seafood diets may lower GC risk in men; dairy diets may help women.	Western diets and processed meats increase GC risk, while fiber, probiotics, and prebiotics reduce it. Foods like spinach, soybeans, and dates promote beneficial bacteria, lowering GC risk. Dairy products with baicalin and baicalein inhibit <i>H. pylori</i> and reduce GC incidence.	Dietary responses vary across populations, influenced by genetics and lifestyle. Most studies are observational, lacking long-term trials to confirm dietary impacts on GC. Due to GC's multifactorial nature, diet alone may have limited effectiveness, requiring combination with other therapies.
FMT	FMT restores gut microbiota balance in GC patients by promoting SCFA-producing bacteria, which modulate the immune system. Butyrate downregulates the NLRP3 inflammatory pathway, inhibits macrophage activation, and reduces intestinal inflammation, improving nutrient absorption.	FMT improves chemotherapy responses and survival rates in some cancer patients, including those with metastatic esophagogastric cancer. It is highly effective in preventing recurrent <i>Clostridium difficile</i> infections (90% success rate) and helps regulate gut microbiota and function.	While FMT shows benefits, it carries risks, such as potential pathogen transmission. It is not suitable for all patients, and personalized treatment based on individual conditions is needed. Further research is required to identify effective microbial compositions for different diseases.
Traditional Chinese medicine	Traditional Chinese medicine impacts gut microbiota composition and metabolism, improving intestinal barrier function, immunity, and metabolism. Herbal formulas like Jianpi Yangzheng and Gexia-Zhuyu Tang inhibit gastric cancer growth by regulating microbiota and immune responses, enhancing pyroptosis. Ingredients like cordycepin reduce inflammation and immune cell infiltration in GC through antibacterial and anti-inflammatory effects.	Traditional Chinese medicine has anti-tumor properties, modulates the immune system, reduces tumor invasion and metastasis, and improves patients' quality of life. It serves as an adjunct therapy for GC, reducing inflammation, alleviating chemotherapy side effects, and regulating the gut microbiota to improve immune and intestinal function.	The efficacy of traditional Chinese medicine for GC requires more scientific research and clinical trials. Its mechanisms are not fully understood, and more studies are needed to explore its specific actions. Current data is insufficient to support its independent use, and more clinical evidence is necessary.

5 Conclusions and perspectives

Although these processes have been extensively studied for decades, the potential impact of the microbiome on cancer development, progression, and treatment response has remained elusive until recently. The microbiota is diverse, abundant, and

influenced by factors such as altitude, climate, diet, host immunity, GC heterogeneity, and surgical procedures (Ravegnini et al., 2020; Li et al., 2021a; Jang et al., 2022). This complexity complicates the use of the microbiome in precision therapeutics. Therefore, our primary goal is to identify differentially abundant taxa more accurately. Developing effective metatranscriptomic strategies is crucial for

accurately characterizing the microbiome in human tissues with lower microbial biomass, which plays a significant role in microbiome research (Pereira-Marques et al., 2024). Furthermore, we should delve deeper into the specific molecular mechanisms of differential taxa in TME, targeting both tumor and stromal cells. This will help us better understand the roles and functions of microbes in tumor progression, facilitating the development of drugs targeting these key points. Finally, microbial-related therapies await further development. Although some therapeutic applications, such as probiotics, prebiotics, antibiotic applications, carrier applications, dietary regulation, traditional Chinese medicine, and bacteriophages, have shown promising efficacy, most of these are in preclinical stages and come with some significant side effects that cannot be overlooked in GC patients (Federici et al., 2022). Currently, only a small number of clinical trials (such as NCT06250075, NCT05901779, NCT05544396) are underway regarding the clinical investigation of probiotics on the gut microbiota of GC patients, neoadjuvant chemotherapy, and the progression mechanisms of GC. These clinical trials are in either the “enrolling by invitation” or “recruiting” phase, and the therapeutic efficacy and potential complications remain to be determined. In conclusion, research on the microbiota in GC has not only deepened our understanding of this disease but also provided new hope and directions for future treatments.

Author contributions

ZC: Conceptualization, Funding acquisition, Visualization, Writing – original draft, Writing – review & editing. DJ: Writing – review & editing, Conceptualization, Writing – original draft. JH: Conceptualization, Writing – original draft. DG: Conceptualization, Writing – original draft. QB: Conceptualization, Writing – original draft, Writing – review & editing. YG: Conceptualization, Visualization, Writing – original draft, Writing – review & editing.

References

- Abed, J., Emgård, J. E. M., Zamir, G., Faroja, M., Almog, G., Grenov, A., et al. (2016). Fap2 mediates fusobacterium nucleatum colorectal adenocarcinoma enrichment by binding to tumor-expressed gal-galNAc. *Cell Host Microbe* 20, 215–225. doi: 10.1016/j.chom.2016.07.006
- Akira, S., and Takeda, K. (2004). Toll-like receptor signalling. *Nat. Rev. Immunol.* 4, 499–511. doi: 10.1038/nri1391
- Anthofer, M., Windisch, M., Haller, R., Ehmann, S., Wrighton, S., Miller, M., et al. (2024). Immune evasion by proteolytic shedding of natural killer group 2, member D ligands in *Helicobacter pylori* infection. *Front. In Immunol.* 15, 1282680. doi: 10.3389/fimmu.2024.1282680
- Arita, S., and Inagaki-Ohara, K. (2019). High-fat-diet-induced modulations of leptin signaling and gastric microbiota drive precancerous lesions in the stomach. *Nutr. (Burbank Los Angeles County Calif.)* 67–68, 110556. doi: 10.1016/j.nut.2019.110556
- Arita, S., Ogawa, T., Murakami, Y., Kinoshita, Y., Okazaki, M., and Inagaki-Ohara, K. (2019). Dietary fat-accelerating leptin signaling promotes protumorigenic gastric environment in mice. *Nutrients* 11, 2127. doi: 10.3390/nu11092127
- Attene-Ramos, M. S., Nava, G. M., Mueller, M. G., Wagner, E. D., Plewa, M. J., and Gaskins, H. R. (2010). DNA damage and toxicogenomic analyses of hydrogen sulfide in human intestinal epithelial FHs 74 Int cells. *Environ. Mol. Mutagen.* 51, 304–314. doi: 10.1002/em.20546
- Basudhar, D., Ridnour, L. A., Cheng, R., Kesarwala, A. H., Heinecke, J., and Wink, D. A. (2016). Biological signaling by small inorganic molecules. *Coord. Chem. Rev.* 306, 708–723. doi: 10.1016/j.ccr.2015.06.001
- Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* 70, 567–590. doi: 10.1152/physrev.1990.70.2.567
- Boursi, B., Mamtani, R., Haynes, K., and Yang, Y.-X. (2015). Recurrent antibiotic exposure may promote cancer formation—Another step in understanding the role of the human microbiota? *Eur. J. Cancer (Oxford England: 1990)* 51, 2655–2664. doi: 10.1016/j.ejca.2015.08.015
- Brandt, S., Kwok, T., Hartig, R., König, W., and Backert, S. (2005). NF-kappaB activation and potentiation of proinflammatory responses by the *Helicobacter pylori* CagA protein. *Proc. Natl. Acad. Sci. United States America* 102, 9300–9305. doi: 10.1073/pnas.0409873102
- Breugelmans, T., Oosterlinck, B., Arras, W., Ceuleers, H., De Man, J., Hold, G. L., et al. (2022). The role of mucins in gastrointestinal barrier function during health and disease. *Lancet Gastroenterol. Hepatol.* 7, 455–471. doi: 10.1016/S2468-1253(21)00431-3
- Byrd, D. A., Fan, W., Greathouse, K. L., Wu, M. C., Xie, H., and Wang, X. (2023). The intratumor microbiome is associated with microsatellite instability. *J. Natl. Cancer Instit.* 115, 989–993. doi: 10.1093/jnci/djad083

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- Campbell, E. L., Bruyninckx, W. J., Kelly, C. J., Glover, L. E., McNamee, E. N., Bowers, B. E., et al. (2014). Transmigrating neutrophils shape the mucosal microenvironment through localized oxygen depletion to influence resolution of inflammation. *Immunity* 40, 66–77. doi: 10.1016/j.immuni.2013.11.020
- Campisi, J., and D'adda Di Fagagna, F. (2007). Cellular senescence: when bad things happen to good cells. *Nat. Rev. Mol. Cell Biol.* 8, 729–740. doi: 10.1038/nrm2233
- 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
- Carbo, A., Olivares-Villagómez, D., Hontecillas, R., Bassaganya-Riera, J., Chaturvedi, R., Piazzuelo, M. B., et al. (2014). Systems modeling of the role of interleukin-21 in the maintenance of effector CD4⁺ T cell responses during chronic *Helicobacter pylori* infection. *MBio* 5, e01243–e01214. doi: 10.1128/mBio.01243-14
- Carmeliet, P., and Jain, R. K. (2000). Angiogenesis in cancer and other diseases. *Nature* 407, 249–257. doi: 10.1038/35025220
- Castro, P. R., Bittencourt, L. F. F., Larochelle, S., Andrade, S. P., Mackay, C. R., Slevin, M., et al. (2021). GPR43 regulates sodium butyrate-induced angiogenesis and matrix remodeling. *Am. J. Physiol. Heart Circulatory Physiol.* 320, H1066–H1079. doi: 10.1152/ajpheart.00515.2019
- Chen, Z., Guan, D., Wang, Z., Li, X., Dong, S., Huang, J., et al. (2023b). Microbiota in cancer: molecular mechanisms and therapeutic interventions. *MedComm* 4, e417. doi: 10.1002/mco2.v4.6
- Chen, Z., Han, F., Du, Y., Shi, H., and Zhou, W. (2023c). Hypoxic microenvironment in cancer: molecular mechanisms and therapeutic interventions. *Signal Trans. Target. Ther.* 8, 70. doi: 10.1038/s41392-023-01332-8
- Chen, R., Li, Y., Chen, X., Chen, J., Song, J., Yang, X., et al. (2023a). dupA + H. pylori reduces diversity of gastric microbiome and increases risk of erosive gastritis. *Front. Cell. Infect. Microbiol.* 13, 1103909. doi: 10.3389/fcimb.2023.1103909
- Chen, C., Shen, J., Du, Y., Shi, X., Niu, Y., Jin, G., et al. (2022a). Characteristics of gut microbiota in patients with gastric cancer by surgery, chemotherapy and lymph node metastasis. *Clin. Trans. Oncol.* 24, 2181–2190. doi: 10.1007/s12094-022-02875-y
- Chen, M.-E., Su, C.-H., Yang, J.-S., Lu, C.-C., Hou, Y.-C., Wu, J.-B., et al. (2018). Baicalin, baicalein, and lactobacillus rhamnosus JB3 alleviated *Helicobacter pylori* infections *in vitro* and *in vivo*. *J. Food Sci.* 83, 3118–3125. doi: 10.1111/jfds.2018.83.issue-12
- 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. In Microbiol.* 10, 1261. doi: 10.3389/fmicb.2019.01261
- Chen, Z., Wang, Z., Du, Y., Shi, H., and Zhou, W. (2022b). The microbiota and aging microenvironment in pancreatic cancer: Cell origin and fate. *Biochim. Et Biophys. Acta Rev. On Cancer* 1877, 188826. doi: 10.1016/j.bbcan.2022.188826
- Chen, Z., Zhang, S., Dong, S., Xu, H., and Zhou, W. (2022c). Association of the microbiota and pancreatic cancer: opportunities and limitations. *Front. In Immunol.* 13, 844401. doi: 10.3389/fimmu.2022.844401
- Chitas, R., Nunes, C., Reis, S., Parreira, P., and Martins, M. C. L. (2022). How Charge, Size and Protein Corona Modulate the Specific Activity of Nanostructured Lipid Carriers (NLC) against *Helicobacter pylori*. *Pharmaceutics* 14, 2745. doi: 10.3390/pharmaceutics14122745
- Choudhury, A., Ortiz, P. S., Young, M., Mahmud, M. T., Stoffel, R. T., Greathouse, K. L., et al. (2023). Control of *Helicobacter pylori* with engineered probiotics secreting selective guided antimicrobial peptides. *Microbiol. Spectr.* 11, e0201423. doi: 10.1128/spectrum.02014-23
- 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
- Coppé, J.-P., Desprez, P.-Y., Krtolica, A., and Campisi, J. (2010). The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu. Rev. Pathol.* 5, 99–118. doi: 10.1146/annurev-pathol-121808-102144
- Cui, J., Cui, H., Yang, M., Du, S., Li, J., Li, Y., et al. (2019). Tongue coating microbiome as a potential biomarker for gastritis including precancerous cascade. *Protein Cell* 10, 496–509. doi: 10.1007/s13238-018-0596-6
- Cullin, N., Azevedo Antunes, C., Straussman, R., Stein-Thoeringer, C. K., and Elinav, E. (2021). Microbiome and cancer. *Cancer Cell* 39, 1317–1341. doi: 10.1016/j.ccell.2021.08.006
- Dai, D., Yang, Y., Yu, J., Dang, T., Qin, W., Teng, L., et al. (2021). Interactions between gastric microbiota and metabolites in gastric cancer. *Cell Death Dis.* 12, 1104. doi: 10.1038/s41419-021-04396-y
- Dane, D. S., Cameron, C. H., and Briggs, M. (1970). Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. *Lancet (London England)* 1, 695–698. doi: 10.1016/S0140-6736(70)90926-8
- De Clercq, N. C., Van Den Ende, T., Prodan, A., Hemke, R., Davids, M., Pedersen, H. K., et al. (2021). Fecal microbiota transplantation from overweight or obese donors in cachectic patients with advanced gastroesophageal cancer: A randomized, double-blind, placebo-controlled, phase II study. *Clin. Cancer Res.: an Off. J. Am. Assoc. For Cancer Res.* 27, 3784–3792. doi: 10.1158/1078-0432.CCR-20-4918
- Deng, R., Yu, S., Ruan, X., Liu, H., Zong, G., Cheng, P., et al. (2023). Capsaicin orchestrates metastasis in gastric cancer via modulating expression of TRPV1 channels and driving gut microbiota disorder. *Cell Commun. Signal.: CCS* 21, 364. doi: 10.1186/s12964-023-01265-3
- Deng, L., Zeng, H., Hu, X., Xiao, M., He, D., Zhang, Y., et al. (2021). Se@Albumin nanoparticles ameliorate intestinal mucositis caused by cisplatin via gut microbiota-targeted regulation. *Nanoscale* 13, 11250–11261. doi: 10.1039/D0NR07981B
- Devi, T. B., Devadas, K., George, M., Gandhimathi, A., Chouhan, D., Retnakumar, R. J., et al. (2021). Low bifidobacterium abundance in the lower gut microbiota is associated with *Helicobacter pylori*-related gastric ulcer and gastric cancer. *Front. In Microbiol.* 12, 631140. doi: 10.3389/fmicb.2021.631140
- Di Giorgio, C., Morretta, E., Lupia, A., Bellini, R., Massa, C., Urbani, G., et al. (2024). Bile acids serve as endogenous antagonists of the Leukemia inhibitory factor (LIF) receptor in oncogenesis. *Biochem. Pharmacol.* 223, 116134. doi: 10.1016/j.bcp.2024.116134
- Dohlman, A. B., Klug, J., Mesko, M., Gao, I. H., Lipkin, S. M., Shen, X., et al. (2022). A pan-cancer mycobiome analysis reveals fungal involvement in gastrointestinal and lung tumors. *Cell* 185, 3807–3822.e12. doi: 10.1016/j.cell.2022.09.015
- Dooyema, S. D. R., Noto, J. M., Wroblewski, L. E., Piazzuelo, 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
- Dorn, B. R., Leung, K. L., and Progluske-Fox, A. (1998). Invasion of human oral epithelial cells by *Prevotella intermedia*. *Infect. Immun.* 66, 6054–6057. doi: 10.1128/IAI.66.12.6054-6057.1998
- Dos Santos, A. M., Meneguín, A. B., Akhter, D. T., Fletcher, N., Houston, Z. H., Bell, C., et al. (2021). Understanding the role of colon-specific microparticles based on retrograded starch/pectin in the delivery of chitosan nanoparticles along the gastrointestinal tract. *Eur. J. Pharma. Biopharma.* 158, 371–378. doi: 10.1016/j.ejpb.2020.12.004
- Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., et al. (2009). Real-time DNA sequencing from single polymerase molecules. *Sci. (New York N.Y.)* 323, 133–138. doi: 10.1126/science.1162986
- Eladwy, R. A., Alsherbiny, M. A., Chang, D., Fares, M., Li, C.-G., and Bhuyan, D. J. (2024). The postbiotic sodium butyrate synergizes the antiproliferative effects of dexamethasone against the AGS gastric adenocarcinoma cells. *Front. In Nutr.* 11, 1372982. doi: 10.3389/fnut.2024.1372982
- Enache, I. M., Vasile, M. A., Crăciunescu, O., Prelipcean, A. M., Oancea, A., Enachi, E., et al. (2022). Co-Microencapsulation of Anthocyanins from Cornelian Cherry (*Cornus mas* L.) Fruits and Lactic Acid Bacteria into Antioxidant and Anti-Proliferative Derivative Powders. *Nutrients* 14, 3458. doi: 10.3390/nu14173458
- Epstein, M. A., Achong, B. G., and Barr, Y. M. (1964). Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet (London England)* 1, 702–703. doi: 10.1016/S0140-6736(64)91524-7
- Erawijantari, P. P., Mizutani, S., Shiroma, H., Shiba, S., Nakajima, T., Sakamoto, T., et al. (2020). Influence of gastrectomy for gastric cancer treatment on faecal microbiome and metabolome profiles. *Gut* 69, 1404–1415. doi: 10.1136/gutjnl-2019-319188
- Federici, S., Kredon-Russo, S., Valdés-Mas, R., Kvietcovsky, D., Weinstock, E., Matiuhiu, Y., et al. (2022). Targeted suppression of human IBD-associated gut microbiota commensals by phage consortia for treatment of intestinal inflammation. *Cell* 185, 2879–2898.e24. doi: 10.1016/j.cell.2022.07.003
- Feng, K., Ren, F., and Wang, X. (2023). Association between oral microbiome and seven types of cancers in East Asian population: a two-sample Mendelian randomization analysis. *Front. In Mol. Biosci.* 10, 1327893. doi: 10.3389/fmolb.2023.1327893
- 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
- Franco, A. T., Israel, D. A., Washington, M. K., Krishna, U., Fox, J. G., Rogers, A. B., et al. (2005). Activation of beta-catenin by carcinogenic *Helicobacter pylori*. *Proc. Natl. Acad. Sci. United States America* 102, 10646–10651. doi: 10.1073/pnas.0504927102
- Fu, H., Ma, Y., Yang, M., Zhang, C., Huang, H., Xia, Y., et al. (2016). Persisting and increasing neutrophil infiltration associates with gastric carcinogenesis and E-cadherin downregulation. *Sci. Rep.* 6, 29762. doi: 10.1038/srep29762
- Fukamachi, H., Nakano, Y., Yoshimura, M., and Koga, T. (2002). Cloning and characterization of the L-cysteine desulphydrase gene of *Fusobacterium nucleatum*. *FEBS Microbiol. Lett.* 215, 75–80. doi: 10.1016/S0378-1097(02)00916-3
- 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
- Gao, X., Yin, P., Ren, Y., Yu, L., Tian, F., Zhao, J., et al. (2023). Predicting personalized diets based on microbial characteristics between patients with superficial gastritis and atrophic gastritis. *Nutrients* 15, 202. doi: 10.3390/nu15224738
- Gao, J.-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. In Cell. Infect. Microbiol.* 8, 202. doi: 10.3389/fcimb.2018.00202

- Garcés, V., González, A., Gálvez, N., Delgado-López, J. M., Calvino, J. J., Trasobares, S., et al. (2022). Magneto-optical hyperthermia agents based on probiotic bacteria loaded with magnetic and gold nanoparticles. *Nanoscale* 14, 5716–5724. doi: 10.1039/D1NR08513A
- Gebremariam, H. G., Qazi, K. R., Somiah, T., Pathak, S. K., Sjölander, H., Sverremark Ekström, E., et al. (2019). Lactobacillus gasseri Suppresses the Production of Proinflammatory Cytokines in Helicobacter pylori-Infected Macrophages by Inhibiting the Expression of ADAM17. *Front. In Immunol.* 10, 2326. doi: 10.3389/fimmu.2019.02326
- Geng, Z., Wang, X., Wu, F., Cao, Z., and Liu, J. (2023). Biointerface mineralization generates ultraresistant gut microbes as oral biotherapeutics. *Sci. Adv.* 9, eade0997. doi: 10.1126/sciadv.ade0997
- Gill, C. I. R., and Rowland, I. R. (2002). Diet and cancer: assessing the risk. *Br. J. Nutr.* 88 Suppl 1, S73–S87. doi: 10.1079/BJN2002632
- Gööz, M., Hammond, C. E., Larsen, K., Mukhin, Y. V., and Smolka, A. J. (2000). Inhibition of human gastric H(+)-K(+)-ATPase alpha-subunit gene expression by Helicobacter pylori. *Am. J. Physiol. Gastrointest. Liver Physiol.* 278, G981–G991. doi: 10.1152/ajpgi.2000.278.6.G981
- Gunathilake, M., Lee, J.-H., Choi, I.-J., Kim, Y.-I., and Kim, J.-S. (2021). Effect of the interaction between dietary patterns and the gastric microbiome on the risk of gastric cancer. *Nutrients* 13, 2692. doi: 10.3390/nu13082692
- Guo, Y., Zhang, Y., Gerhard, M., Gao, J.-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
- Hamanaka, Y., Nakashima, M., Wada, A., Ito, M., Kurazono, H., Hojo, H., et al. (2001). Expression of human beta-defensin 2 (hBD-2) in Helicobacter pylori induced gastritis: antibacterial effect of hBD-2 against Helicobacter pylori. *Gut* 49, 481–487. doi: 10.1136/gut.49.4.481
- Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., and Brummer, R. J. (2008). Review article: the role of butyrate on colonic function. *Aliment. Pharmacol. Ther.* 27, 104–119. doi: 10.1111/j.1365-2036.2007.03562.x
- Hamway, Y., Taxauer, K., Moonens, K., Neumeyer, V., Fischer, W., Schmitt, V., et al. (2020). Cysteine residues in helicobacter pylori adhesin hopQ are required for CEACAM-hopQ interaction and subsequent cagA translocation. *Microorganisms* 8, 465. doi: 10.3390/microorganisms8040465
- Han, Z., Cheng, S., Dai, D., Kou, Y., Zhang, X., Li, F., et al. (2023). The gut microbiome affects response of treatments in HER2-negative advanced gastric cancer. *Clin. Trans. Med.* 13, e1312. doi: 10.1002/ctm2.v13.7
- Han, Y. W., Shi, W., Huang, G. T., Kinder Haake, S., Park, N. H., Kuramitsu, H., et al. (2000). Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells. *Infect. Immun.* 68, 3140–3146. doi: 10.1128/IAI.68.6.3140-3146.2000
- Hao, Y., Karaöz, 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.v151.10
- 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, S., Sun, Y., Sun, W., Tang, M., Meng, B., Liu, Y., et al. (2023). Oral microbiota disorder in GC patients revealed by 2b-RAD-M. *J. Trans. Med.* 21, 831. doi: 10.1186/s12967-023-04599-1
- Hong, J., Guo, F., Lu, S.-Y., Shen, C., Ma, D., Zhang, X., et al. (2021). F. nucleatum targets lncRNA ENO1-IT1 to promote glycolysis and oncogenesis in colorectal cancer. *Gut* 70, 2123–2137. doi: 10.1136/gutjnl-2020-322780
- Hou, W., Li, J., Cao, Z., Lin, S., Pan, C., Pang, Y., et al. (2021). Decorating bacteria with a therapeutic nanocoating for synergistically enhanced biotherapy. *Small (Weinheim an Der Bergstrasse Germany)* 17, e2101810. doi: 10.1002/sml.202101810
- Hsieh, Y.-Y., Tung, S.-Y., Pan, H.-Y., Chang, T.-S., Wei, K.-L., Chen, W.-M., 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.-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
- Hsueh, C.-Y., Lau, H.-C., Huang, Q., Gong, H., Sun, J., Cao, P., et al. (2022). Fusobacterium nucleatum impairs DNA mismatch repair and stability in patients with squamous cell carcinoma of the head and neck. *Cancer* 128, 3170–3184. doi: 10.1002/cncr.v128.17
- Huang, K., Gao, X., Wu, L., Yan, B., Wang, Z., Zhang, X., et al. (2021). Salivary microbiota for gastric cancer prediction: an exploratory study. *Front. In Cell. Infect. Microbiol.* 11, 640309. doi: 10.3389/fcimb.2021.640309
- Huang, G., Wang, S., Wang, J., Tian, L., Yu, Y., Zuo, X., et al. (2022). Bile reflux alters the profile of the gastric mucosa microbiota. *Front. In Cell. Infect. Microbiol.* 12, 940687. doi: 10.3389/fcimb.2022.940687
- Huycke, M. M., and Gaskins, H. R. (2004). Commensal bacteria, redox stress, and colorectal cancer: mechanisms and models. *Exp. Biol. Med. (Maywood N.J.)* 229, 586–597. doi: 10.1177/153537020422900702
- Jain, R. K. (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Sci. (New York N.Y.)* 307, 58–62. doi: 10.1126/science.1104819
- Jain, R. K. (2014). Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia. *Cancer Cell* 26, 605–622. doi: 10.1016/j.ccell.2014.10.006
- Jang, S., Hansen, L. M., Su, H., Solnick, J. V., and Cha, J.-H. (2022). Host immune response mediates changes in cagA copy number and virulence potential of Helicobacter pylori. *Gut Microbes* 14, 2044721. doi: 10.1080/19490976.2022.2044721
- Jauvain, M., Courtois, S., Lehours, P., and Bessède, E. (2021). Metformin modifies the gut microbiota of mice infected with helicobacter pylori. *Pharma. (Basel Switzerland)* 14, 329. doi: 10.3390/ph14040329
- Javaheri, A., Kruse, T., Moonens, K., Mejias-Luque, R., Debraekeleer, A., Asche, C. I., et al. (2016). Helicobacter pylori adhesin HopQ engages in a virulence-enhancing interaction with human CEACAMs. *Nat. Microbiol.* 2, 16189. doi: 10.1038/nmicrobiol.2016.189
- Jin, D., Huang, K., Xu, M., Hua, H., Ye, F., Yan, J., et al. (2022). Deoxycholic acid induces gastric intestinal metaplasia by activating STAT3 signaling and disturbing gastric bile acids metabolism and microbiota. *Gut Microbes* 14, 2120744. doi: 10.1080/19490976.2022.2120744
- Jones, T. A., Hernandez, D. Z., Wong, Z. C., Wandler, A. M., and Guillemin, K. (2017). The bacterial virulence factor CagA induces microbial dysbiosis that contributes to excessive epithelial cell proliferation in the Drosophila gut. *PLoS Pathog.* 13, e1006631. doi: 10.1371/journal.ppat.1006631
- Kageyama, S., Takeshita, T., Takeuchi, K., Asakawa, M., Matsumi, R., Furuta, M., et al. (2019). Characteristics of the salivary microbiota in patients with various digestive tract cancers. *Front. In Microbiol.* 10, 1780. doi: 10.3389/fmicb.2019.01780
- Kelly, C. J., Zheng, L., Campbell, E. L., Saeedi, B., Scholz, C. C., Bayless, A. J., et al. (2015). Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* 17, 662–671. doi: 10.1016/j.chom.2015.03.005
- 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. K. doi: 10.3390/nu14234981
- Kim, C. G., Koh, J.-Y., Shin, S.-J., Shin, J.-H., Hong, M., Chung, H. C., et al. (2023). Prior antibiotic administration disrupts anti-PD-1 responses in advanced gastric cancer by altering the gut microbiome and systemic immune response. *Cell Reports. Med.* 4, 101251. doi: 10.1016/j.xcrm.2023.101251
- Kim, J. J., Tao, H., Carloni, E., Leung, W. K., Graham, D. Y., and Sepulveda, A. R. (2002). Helicobacter pylori impairs DNA mismatch repair in gastric epithelial cells. *Gastroenterology* 123, 542–553. doi: 10.1053/gast.2002.34751
- Koch, K. N., and Müller, A. (2015). Helicobacter pylori activates the TLR2/NLRP3/caspase-1/IL-18 axis to induce regulatory T-cells, establish persistent infection and promote tolerance to allergens. *Gut Microbes* 6, 382–387. doi: 10.1080/19490976.2015.1105427
- Kong, W., Liu, W., Wang, M., Hui, W., Feng, Y., Lu, J., et al. (2022). Cordycepin exhibits anti-bacterial and anti-inflammatory effects against gastritis in Helicobacter pylori-infected mice. *Pathog. Dis.* 80, ftac005. doi: 10.1093/femspd/ftac005
- Konturek, P. C., Haziri, D., Brzozowski, T., Hess, T., Heyman, S., Kwiecien, S., et al. (2015). Emerging role of fecal microbiota therapy in the treatment of gastrointestinal and extra-gastrointestinal diseases. *J. Physiol. Pharmacol.* 66, 483–491.
- 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
- 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, L., Zhao, L.-Y., Cheng, R., Zhang, H., Xia, M., Chen, X.-L., et al. (2024). Distinct oral-associated gastric microbiota and Helicobacter pylori communities for spatial microbial heterogeneity in gastric cancer. *MSystems* 9, e0008924. doi: 10.1128/mSystems.00089-24
- 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
- Leung, P. S., and Chan, Y. C. (2009). Role of oxidative stress in pancreatic inflammation. *Antioxid. Redox Signaling* 11, 135–165. doi: 10.1089/ars.2008.2109
- Li, G.-M. (2008). Mechanisms and functions of DNA mismatch repair. *Cell Res.* 18, 85–98. doi: 10.1038/cr.2007.115
- Li, J. V., Ashrafian, H., Sarafian, M., Homola, D., Rushton, L., Barker, G., et al. (2021a). Roux-en-Y gastric bypass-induced bacterial perturbation contributes to altered host-bacterial co-metabolic phenotype. *Microbiome* 9, 139. doi: 10.1186/s40168-021-01086-x

- Li, N., Bai, C., Zhao, L., Sun, Z., Ge, Y., and Li, X. (2021b). The relationship between gut microbiome features and chemotherapy response in gastrointestinal cancer. *Front. In Oncol.* 11, 781697. doi: 10.3389/fonc.2021.781697
- Li, X. V., Leonardi, I., Putzel, G. G., Semon, A., Fiers, W. D., Kusakabe, T., et al. (2022). Immune regulation by fungal strain diversity in inflammatory bowel disease. *Nature* 603, 672–678. doi: 10.1038/s41586-022-04502-w
- Li, Q., Wu, W., Gong, D., Shang, R., Wang, J., and Yu, H. (2021c). Propionibacterium acnes overabundance in gastric cancer promote M2 polarization of macrophages via a TLR4/PI3K/Akt signaling. *Gastric Cancer* 24, 1242–1253. doi: 10.1007/s10120-021-01202-8
- Li, N., Xu, X., Zhan, Y., Fei, X., Ouyang, Y., Zheng, P., et al. (2023). YAP and β -catenin cooperate to drive H. pylori-induced gastric tumorigenesis. *Gut Microbes* 15, 2192501. doi: 10.1080/19490976.2023.2192501
- Ling, Z., Shao, L., Liu, X., Cheng, Y., Yan, C., Mei, Y., et al. (2019). Regulatory T cells and plasmacytoid dendritic cells within the tumor microenvironment in gastric cancer are correlated with gastric microbiota dysbiosis: A preliminary study. *Front. In Immunol.* 10, 533. doi: 10.3389/fimmu.2019.00533
- Liotti, F., Marotta, M., Sorriento, D., Pagliuca, C., Caturano, V., Mantova, G., et al. (2022). Probiotic Lactobacillus rhamnosus GG (LGG) restrains the angiogenic potential of colorectal carcinoma cells by activating a proresolving program via formyl peptide receptor 1. *Mol. Oncol.* 16, 2959–2980. doi: 10.1002/1878-0261.13280
- Liu, K. H., Owens, J. A., Saeedi, B., Cohen, C. E., Bellissimo, M. P., Naudin, C., et al. (2021). Microbial metabolite delta-valerobetaine is a diet-dependent obesogen. *Nat. Metab.* 3, 1694–1705. doi: 10.1038/s42255-021-00502-8
- Liu, X., Shao, L., Liu, X., Ji, F., Mei, Y., Cheng, Y., et al. (2019). Alterations of gastric mucosal microbiota across different stomach microhabitats in a cohort of 276 patients with gastric cancer. *EBioMedicine* 40, 336–348. doi: 10.1016/j.ebiom.2018.12.034
- Long, X., Wong, C. C., Tong, L., Chu, E. S. H., Ho Szeto, C., Go, M. Y. Y., et al. (2019). Peptostreptococcus anaerobius promotes colorectal carcinogenesis and modulates tumour immunity. *Nat. Microbiol.* 4, 2319–2330. doi: 10.1038/s41564-019-0541-3
- Lu, Y.-Y., Fang, Y.-Y., Wang, S.-S., Guo, J., Song, J.-L., Zhu, L., et al. (2024). Cepharanthine sensitizes gastric cancer cells to chemotherapy by targeting TRIB3-FOXO3-FOXM1 axis to inhibit autophagy. *Phytomed.: Int. J. Phytother. Phytopharmacol.* 135, 156161. doi: 10.1016/j.phymed.2024.156161
- Magahis, P. T., Maron, S. B., Cowzer, D., King, S., Schattner, M., Janjigian, Y., et al. (2023). Impact of Helicobacter pylori infection status on outcomes among patients with advanced gastric cancer treated with immune checkpoint inhibitors. *J. For Immunother. Cancer* 11, e007699. doi: 10.1136/jitc-2023-007699
- Mannion, A., Sheh, A., Shen, Z., Dzink-Fox, J., Piazzuelo, M. B., Wilson, K. T., et al. (2023). Shotgun metagenomics of gastric biopsies reveals compositional and functional microbiome shifts in high- and low-gastric-cancer-risk populations from Colombia, South America. *Gut Microbes* 15, 2186677. doi: 10.1080/19490976.2023.2186677
- Mantovani, A., Marchesi, F., Malesci, A., Laghi, L., and Allavena, P. (2017). Tumour-associated macrophages as treatment targets in oncology. *Nat. Rev. Clin. Oncol.* 14, 399–416. doi: 10.1038/nrclinonc.2016.217
- Masterson, J. C., Biette, K. A., Hammer, J. A., Nguyen, N., Capocelli, K. E., Saeedi, B. J., et al. (2019). Epithelial HIF-1 α /claudin-1 axis regulates barrier dysfunction in eosinophilic esophagitis. *J. Clin. Invest.* 129, 3224–3235. doi: 10.1172/JCI126744
- Metzker, M. L. (2010). Sequencing technologies - the next generation. *Nat. Rev. Genet.* 11, 31–46. doi: 10.1038/nrg2626
- Mi, Y., Dong, H., Sun, X., Ren, F., Tang, Y., and Zheng, P. (2020). The association of Helicobacter pylori CagA EPIYA motifs and vacA genotypes with homologous recombination repair markers during the gastric precancerous cascade. *Int. J. Biol. Markers* 35, 49–55. doi: 10.1177/1724600820914935
- Mommersteeg, M. C., Simovic, I., Yu, B., Van Nieuwenburg, S., Bruno, I. M. J., Doukas, M., et al. (2022). Autophagy mediates ER stress and inflammation in Helicobacter pylori-related gastric cancer. *Gut Microbes* 14, 2015238. doi: 10.1080/19490976.2021.2015238
- Morris, R. L., and Schmidt, T. M. (2013). Shallow breathing: bacterial life at low O₂. *Nat. Rev. Microbiol.* 11, 205–212. doi: 10.1038/nrmicro2970
- Nabavi-Rad, A., Sadeghi, A., Asadzadeh Aghdaei, H., Yadegar, A., Smith, S. M., and Zali, M. R. (2022). The double-edged sword of probiotic supplementation on gut microbiota structure in Helicobacter pylori management. *Gut Microbes* 14, 2108655. doi: 10.1080/19490976.2022.2108655
- Nejman, D., Livyatan, I., Fuks, G., Gavert, N., Zwang, Y., Geller, L. T., et al. (2020). The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Sci. (New York N.Y.)* 368, 973–980. doi: 10.1126/science.aay9189
- Noto, J. M., Piazzuelo, M. B., Romero-Gallo, J., Delgado, A. G., Suarez, G., Akritidou, K., et al. (2023). Targeting hypoxia-inducible factor-1 α suppresses Helicobacter pylori-induced gastric injury via attenuation of both cag-mediated microbial virulence and proinflammatory host responses. *Gut Microbes* 15, 2263936. doi: 10.1080/19490976.2023.2263936
- Noto, J. M., Piazzuelo, M. B., Shah, S. C., Romero-Gallo, J., Hart, J. L., Di, C., et al. (2022). Iron deficiency linked to altered bile acid metabolism promotes Helicobacter pylori-induced inflammation-driven gastric carcinogenesis. *J. Clin. Invest.* 132, e147822. doi: 10.1172/JCI147822
- Noto, J. M., Zackular, J. P., Varga, M. G., Delgado, A., Romero-Gallo, J., Scholz, M. B., et al. (2019). Modification of the gastric mucosal microbiota by a strain-specific helicobacter pylori oncoprotein and carcinogenic histologic phenotype. *MBio* 10, e00955-19. doi: 10.1128/mBio.00955-19
- Nowak, A., and Libudzisz, Z. (2009). Ability of probiotic Lactobacillus casei DN 114001 to bind or/and metabolise heterocyclic aromatic amines *in vitro*. *Eur. J. Nutr.* 48, 419–427. doi: 10.1007/s00394-009-0030-1
- Nowak, R., Nowacka-Jechalke, N., Juda, M., and Malm, A. (2018). The preliminary study of prebiotic potential of Polish wild mushroom polysaccharides: the stimulation effect on Lactobacillus strains growth. *Eur. J. Nutr.* 57, 1511–1521. doi: 10.1007/s00394-017-1436-9
- Odenbreit, S., Püls, J., Sedlmaier, B., Gerland, E., Fischer, W., and Haas, R. (2000). Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. *Sci. (New York N.Y.)* 287, 1497–1500. doi: 10.1126/science.287.5457.1497
- Oh, B., Kim, B.-S., Kim, J. W., Kim, J. S., Koh, S.-J., Kim, B. G., et al. (2016). The Effect of Probiotics on Gut Microbiota during the Helicobacter pylori Eradication: Randomized Controlled Trial. *Helicobacter* 21, 165–174. doi: 10.1111/hel.2016.21.issue-3
- Ohnishi, N., Yuasa, H., Tanaka, S., Sawa, H., Miura, M., Matsui, A., et al. (2008). Transgenic expression of Helicobacter pylori CagA induces gastrointestinal and hematopoietic neoplasms in mouse. *Proc. Natl. Acad. Sci. United States America* 105, 1003–1008. doi: 10.1073/pnas.0711183105
- Ortenzi, P. M., Antenucci, S., Marzorati, S., Panzella, L., Molino, S., Rufián-Henares, J. Á., et al. (2021). Pectin-based formulations for controlled release of an ellagic acid salt with high solubility profile in physiological media. *Mol. (Basel Switzerland)* 26, 433. doi: 10.3390/molecules26020433
- Oster, P., Vaillant, L., Riva, E., Mcmillan, B., Begka, C., Truntzer, C., et al. (2022). Helicobacter pylori infection has a detrimental impact on the efficacy of cancer immunotherapies. *Gut* 71, 457–466. doi: 10.1136/gutjnl-2020-323392
- Pack, G. T. (1967). St. Peregrine, O.S.M.—the patron saint of cancer patients. *CA: A Cancer J. For Clin.* 17, 181–182. doi: 10.3322/canjclin.17.4.183
- Parhi, L., Alon-Maimon, T., Sol, A., Nejman, D., Shhadeh, A., Fainsod-Levi, T., et al. (2020). Breast cancer colonization by Fusobacterium nucleatum accelerates tumor growth and metastatic progression. *Nat. Commun.* 11, 3259. doi: 10.1038/s41467-020-16967-2
- Parsons, B. N., Ijaz, U. Z., D'amore, R., Burkitt, M. D., Eccles, R., Lenzi, L., et al. (2017). Comparison of the human gastric microbiota in hypochlorhydric states arising as a result of Helicobacter pylori-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use. *PLoS Pathog.* 13, e1006653. doi: 10.1371/journal.ppat.1006653
- Peng, R., Liu, S., You, W., Huang, Y., Hu, C., Gao, Y., et al. (2022). Gastric microbiome alterations are associated with decreased CD8⁺ Tissue-resident memory T cells in the tumor microenvironment of gastric cancer. *Cancer Immunol. Res.* 10, 1224–1240. doi: 10.1158/2326-6066.CIR-22-0107
- Peng, X., Ueda, H., Zhou, H., Stokol, T., Shen, T.-L., Alcaraz, A., et al. (2004). Overexpression of focal adhesion kinase in vascular endothelial cells promotes angiogenesis in transgenic mice. *Cardiovasc. Res.* 64, 421–430. doi: 10.1016/j.cardiores.2004.07.012
- Pereira-Marques, J., Ferreira, R. M., and Figueiredo, C. (2024). A metatranscriptomics strategy for efficient characterization of the microbiome in human tissues with low microbial biomass. *Gut Microbes* 16, 2323235. doi: 10.1080/19490976.2024.2323235
- Pérez-Mancera, P. A., Young, A. R. J., and Narita, M. (2014). Inside and out: the activities of senescence in cancer. *Nat. Rev. Cancer* 14, 547–558. doi: 10.1038/nrc3773
- Piao, H., Fu, L., Wang, Y., Liu, Y., Wang, Y., Meng, X., et al. (2022). A positive feedback loop between gastric cancer cells and tumor-associated macrophage induces Malignancy progression. *J. Exp. Clin. Cancer Res.: CR* 41, 174.
- Png, C. W., Lee, W. 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/tno.65302
- Qiao, Y., Xia, Q., Cao, X., Xu, J., Qiao, Z., Wu, L., et al. (2024). Urolithin A exerts anti-tumor effects on gastric cancer via activating autophagy-Hippo axis and modulating the gut microbiota. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 397, 6633–6645.
- Quinn, L., Sheh, A., Ellis, J. L., Smith, D. E., Booth, S. L., Fu, X., et al. (2020). Helicobacter pylori antibiotic eradication coupled with a chemically defined diet in INS-GAS mice triggers dysbiosis and vitamin K deficiency resulting in gastric hemorrhage. *Gut Microbes* 11, 820–841. doi: 10.1080/19490976.2019.1710092
- Radelof, U., Hennig, S., Seranski, P., Steinfath, M., Ramser, J., Reinhardt, R., et al. (1998). Preselection of shotgun clones by oligonucleotide fingerprinting: an efficient and high throughput strategy to reduce redundancy in large-scale sequencing projects. *Nucleic Acids Res.* 26, 5358–5364. doi: 10.1093/nar/26.23.5358
- Ravegnini, G., Fosso, B., Saverio, V. D., Sammarini, G., Zanotti, F., Rossi, G., et al. (2020). Gastric adenocarcinomas and signet-ring cell carcinoma: unraveling gastric cancer complexity through microbiome analysis-deepening heterogeneity for a personalized therapy. *Int. J. Mol. Sci.* 21, 9735. doi: 10.3390/ijms21249735
- Ridlon, J. M., Wolf, P. G., and Gaskins, H. R. (2016). Taurocholic acid metabolism by gut microbes and colon cancer. *Gut Microbes* 7, 201–215. doi: 10.1080/19490976.2016.1150414

- Rinninella, E., Mele, M. C., Cintoni, M., Raoul, P., Ianaro, G., Salerno, L., et al. (2020). The Facts about Food after Cancer Diagnosis: A Systematic Review of Prospective Cohort Studies. *Nutrients* 12, 2345. doi: 10.3390/nu12082345
- Saha, A., Hammond, C. E., Trojanowska, M., and Smolka, A. J. (2008). Helicobacter pylori-induced H,K-ATPase alpha-subunit gene repression is mediated by NF-kappaB p50 homodimer promoter binding. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294, G795–G807.
- Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. United States America* 74, 5463–5467. doi: 10.1073/pnas.74.12.5463
- Santos, J. C., Brianti, M. T., Almeida, V. R., Ortega, M. M., Fischer, W., Haas, R., et al. (2017). Helicobacter pylori infection modulates the expression of miRNAs associated with DNA mismatch repair pathway. *Mol. Carcinogen.* 56, 1372–1379. doi: 10.1002/mc.22590
- Seabra, C. L., Nunes, C., Brás, M., Gomez-Lazaro, M., Reis, C. A., Gonçalves, I. C., et al. (2018). Lipid nanoparticles to counteract gastric infection without affecting gut microbiota. *Eur. J. Pharma. Biopharma.* 127, 378–386. doi: 10.1016/j.ejpb.2018.02.030
- Shen, S., Ren, F., Qin, H., Bukhari, I., Yang, J., Gao, D., et al. (2023). Lactobacillus acidophilus NCFM and Lactiplantibacillus plantarum Lp-115 inhibit Helicobacter pylori colonization and gastric inflammation in a murine model. *Front. In Cell. Infect. Microbiol.* 13, 1196084. doi: 10.3389/fcimb.2023.1196084
- Smolka, A. J., and Backert, S. (2012). How Helicobacter pylori infection controls gastric acid secretion. *J. Gastroenterol.* 47, 609–618. doi: 10.1007/s00535-012-0592-1
- Starnes, C. O. (1992). Coley's toxins in perspective. *Nature* 357, 11–12. doi: 10.1038/357011a0
- Svagan, A. J., Kusic, A., De Gobba, C., Larsen, F. H., Sassene, P., Zhou, Q., et al. (2016). Rhamnogalacturonan-I based microcapsules for targeted drug release. *PloS One* 11, e0168050. doi: 10.1371/journal.pone.0168050
- Teng, K.-W., Hsieh, K.-S., Hung, J.-S., Wang, C.-J., Liao, E.-C., Chen, P.-C., et al. (2022). Helicobacter pylori employs a general protein glycosylation system for the modification of outer membrane adhesins. *Gut Microbes* 14, 2130650. doi: 10.1080/19490976.2022.2130650
- Turati, F., Concina, F., Bertuccio, P., Fiori, F., Parpinel, M., Garavello, W., et al. (2023). Prebiotics and the risk of upper digestive tract and stomach cancers: the prebiotiCa study. *J. Acad. Nutr. Diet.* 123, 1772–1780. doi: 10.1016/j.jand.2023.07.008
- Van Hecke, T., Vossen, E., Hemeryck, L. Y., Vanden Bussche, J., Vanhaecke, L., and De Smet, S. (2015). Increased oxidative and nitrosative reactions during digestion could contribute to the association between well-done red meat consumption and colorectal cancer. *Food Chem.* 187, 29–36. doi: 10.1016/j.foodchem.2015.04.029
- Verspreet, J., Damen, B., Broekaert, W. F., Verbeke, K., Delcort, J. A., and Courtin, C. M. (2016). A critical look at prebiotics within the dietary fiber concept. *Annu. Rev. Food Sci. Technol.* 7, 167–190. doi: 10.1146/annurev-food-081315-032749
- Wada, A., Ogushi, K., Kimura, T., Hojo, H., Mori, N., Suzuki, S., et al. (2001). Helicobacter pylori-mediated transcriptional regulation of the human beta-defensin 2 gene requires NF-kappaB. *Cell. Microbiol.* 3, 115–123. doi: 10.1046/j.1462-5822.2001.00096.x
- Wang, S., Kuang, J., Zhang, H., Chen, W., Zheng, X., Wang, J., et al. (2022). Bile acid-microbiome interaction promotes gastric carcinogenesis. *Adv. Sci. (Weinheim Baden-Wuerttemberg Germany)* 9, e2200263. doi: 10.1002/adv.202200263
- Wang, G., Wang, H., Ji, X., Wang, T., Zhang, Y., Jiang, W., et al. (2024). Intratumoral microbiome is associated with gastric cancer prognosis and therapy efficacy. *Gut Microbes* 16, 2369336. doi: 10.1080/19490976.2024.2369336
- Wei, X., Wang, F., Tan, P., Huang, H., Wang, Z., Xie, J., et al. (2024). The interactions between traditional Chinese medicine and gut microbiota in cancers: Current status and future perspectives. *Pharmacol. Res.* 203, 107148. doi: 10.1016/j.phrs.2024.107148
- Wen, F., Han, Y., Zhang, H., Zhao, Z., Wang, W., Chen, F., et al. (2024). Epstein-Barr virus infection upregulates extracellular OLFM4 to activate YAP signaling during gastric cancer progression. *Nat. Commun.* 15, 10543. doi: 10.1038/s41467-024-54850-6
- Wu, S., Cui, W., Zhou, Q., Liang, Y., Wu, D., Xu, Y., et al. (2023). Phenyl lactic acid alleviates Helicobacter pylori infection in C57BL/6 mice. *Food Funct.* 14, 5032–5047. doi: 10.1039/D3FO00099K
- Wu, Y.-Y., Wu, F.-H., Chen, I. C., Liao, T.-L., Munir, M., and Liu, H.-J. (2024b). Oncolytic avian reovirus-sensitized tumor infiltrating CD8+ T cells triggering immunogenic apoptosis in gastric cancer. *Cell Commun. Signal.: CCS* 22, 514. doi: 10.1186/s12964-024-01888-0
- Wu, J., Xu, S., Xiang, C., Cao, Q., Li, Q., Huang, J., et al. (2018). Tongue coating microbiota community and risk effect on gastric cancer. *J. Cancer* 9, 4039–4048. doi: 10.7150/jca.25280
- Wu, J., Zhang, R., Yin, Z., Chen, X., Mao, R., Zheng, X., et al. (2024a). Gut microbiota-driven metabolic alterations reveal the distinct pathogenicity of chemotherapy-induced cachexia in gastric cancer. *Pharmacol. Res.* 209, 107476. doi: 10.1016/j.phrs.2024.107476
- Wu, Z.-F., Zou, K., Xiang, C.-J., Jin, Z.-J., Ding, H.-H., Xu, S., et al. (2021). Helicobacter pylori infection is associated with the co-occurrence of bacteria in the oral cavity and the gastric mucosa. *Helicobacter* 26, e12786. doi: 10.1111/hel.12786
- Xiao, W., and Ma, Z. S. (2022). Influences of Helicobacter pylori infection on diversity, heterogeneity, and composition of human gastric microbiomes across stages of gastric cancer development. *Helicobacter* 27, e12899. doi: 10.1111/hel.12899
- Xu, Q., Dong, H., Wang, Z., Zhang, P., Albers, A. E., Kaufmann, A. M., et al. (2023a). Integration and viral oncogene expression of human papillomavirus type 16 in oropharyngeal squamous cell carcinoma and gastric cancer. *J. Med. Virol.* 95, e28761. doi: 10.1002/jmv.28761
- Xu, Q., Gao, J., Zhao, R., Li, H., Cui, H., Yuan, Z., et al. (2024). Akkermansia muciniphila-derived pentadecanoic acid enhances oxaliplatin sensitivity in gastric cancer by modulating glycolysis. *Pharmacol. Res.* 206, 107278. doi: 10.1016/j.phrs.2024.107278
- Xu, J., Xiang, C., Zhang, C., Xu, B., Wu, J., Wang, R., et al. (2019). Microbial biomarkers of common tongue coatings in patients with gastric cancer. *Microb. Pathogen.* 127, 97–105. doi: 10.1016/j.micpath.2018.11.051
- Xu, Z., Xiao, L., Wang, S., Cheng, Y., Wu, J., Meng, Y., et al. (2023b). Alteration of gastric microbiota and transcriptome in a rat with gastric intestinal metaplasia induced by deoxycholic acid. *Front. In Microbiol.* 14, 1160821. doi: 10.3389/fmicb.2023.1160821
- Xu, M., Yamada, M., Li, M., Liu, H., Chen, S. G., and Han, Y. W. (2007). FadA from Fusobacterium nucleatum utilizes both secreted and nonsecreted forms for functional oligomerization for attachment and invasion of host cells. *J. Biol. Chem.* 282, 25000–25009. doi: 10.1074/jbc.M611567200
- Yang, Y., Long, J., Wang, C., Blot, W. J., Pei, Z., Shu, X., et al. (2022b). Prospective study of oral microbiome and gastric cancer risk among Asian, African American and European American populations. *Int. J. Cancer* 150, 916–927. doi: 10.1002/ijc.v150.6
- Yang, Z., Tong, C., Qian, X., Wang, H., and Wang, Y. (2022c). Mechanical bowel preparation is a risk factor for postoperative delirium as it alters the gut microbiota composition: A prospective randomized single-center study. *Front. In Aging Neurosci.* 14, 847610. doi: 10.3389/fnagi.2022.847610
- Yang, J., Xu, J., Ling, Z., Zhou, X., Si, Y., Liu, X., et al. (2023b). Prognostic effects of the gastric mucosal microbiota in gastric cancer. *Cancer Sci.* 114, 1075–1085. doi: 10.1111/cas.v114.3
- Yang, A., Zhang, Z., Chaurasiya, S., Park, A. K., Jung, A., Lu, J., et al. (2023a). Development of the oncolytic virus, CF33, and its derivatives for peritoneal-directed treatment of gastric cancer peritoneal metastases. *J. For Immunother. Cancer* 11, e006280. doi: 10.1136/jitc-2022-006280
- Yang, W., Zhao, Y., Ge, Q., Wang, X., Jing, Y., Zhao, J., et al. (2022a). Genetic mutation and tumor microbiota determine heterogeneity of tumor immune signature: Evidence from gastric and colorectal synchronous cancers. *Front. In Immunol.* 13, 947080. doi: 10.3389/fimmu.2022.947080
- Yang, J., Zhou, X., Liu, X., Ling, Z., and Ji, F. (2021). Role of the gastric microbiome in gastric cancer: from carcinogenesis to treatment. *Front. In Microbiol.* 12, 641322. doi: 10.3389/fmicb.2021.641322
- Yao, Y., Sun, S., Gu, J., Ni, H., Zhong, K., Xu, Q., et al. (2022). Roux-en-Y reconstruction alleviates radical gastrectomy-induced colitis via down-regulation of the butyrate/NLRP3 signaling pathway. *EBioMedicine* 86, 104347. doi: 10.1016/j.ebiom.2022.104347
- Ye, Y., Hu, Q., Chen, H., Liang, K., Yuan, Y., Xiang, Y., et al. (2019). Characterization of hypoxia-associated molecular features to aid hypoxia-targeted therapy. *Nat. Metab.* 1, 431–444. doi: 10.1038/s42255-019-0045-8
- Ye, Z., Liang, L., Xu, Y., Yang, J., and Li, Y. (2024). Probiotics influence gut microbiota and tumor immune microenvironment to enhance anti-tumor efficacy of doxorubicin. *Probiot. Antimicrob. Proteins* 16, 606–622. doi: 10.1007/s12602-023-10073-7
- Yu, J., Guo, Z., Yan, J., Bu, C., Peng, C., Li, C., et al. (2023). Gastric Acid-Responsive ROS Nanogenerators for Effective Treatment of Helicobacter pylori Infection without Disrupting Homeostasis of Intestinal Flora. *Adv. Sci. (Weinheim Baden-Wuerttemberg Germany)* 10, e2206957. doi: 10.1002/adv.202206957
- Yu, X., Ou, J., Wang, L., Li, Z., Ren, Y., Xie, L., et al. (2024). Gut microbiota modulate CD8+ T cell immunity in gastric cancer through Butyrate/GPR109A/HOPX. *Gut Microbes* 16, 2307542. doi: 10.1080/19490976.2024.2307542
- Yu, C., Su, Z., Li, Y., Li, Y., Liu, K., Chu, F., et al. (2020). Dysbiosis of gut microbiota is associated with gastric carcinogenesis in rats. *Biomed. Pharmacother. = Biomed. Pharmacother.* 126, 110036. doi: 10.1016/j.biopha.2020.110036
- 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
- Yuan, W., Xiao, X., Yu, X., Xie, F., Feng, P., Malik, K., et al. (2022). Probiotic therapy (BIO-THREE) mitigates intestinal microbial imbalance and intestinal damage caused by oxaliplatin. *Probiot. Antimicrob. Proteins* 14, 60–71. doi: 10.1007/s12602-021-09795-3
- Zhang, S., Li, C., Liu, J., Geng, F., Shi, X., Li, Q., et al. (2020). Fusobacterium nucleatum promotes epithelial-mesenchymal transition through regulation of the lncRNA MIR4435-2HG/miR-296-5p/Akt2/SNAI1 signaling pathway. *FEBS J.* 287, 4032–4047. doi: 10.1111/febs.v287.18
- Zhang, J.-J., Wu, H.-S., Wang, L., Tian, Y., Zhang, J.-H., and Wu, H.-L. (2010). Expression and significance of TLR4 and HIF-1alpha in pancreatic ductal adenocarcinoma. *World J. Gastroenterol.* 16, 2881–2888. doi: 10.3748/wjg.v16.i23.2881

- Zhang, W., Zhou, Y., Fan, Y., Cao, R., Xu, Y., Weng, Z., et al. (2022). Metal-organic-framework-based hydrogen-release platform for multieffective helicobacter pylori targeting therapy and intestinal flora protective capabilities. *Adv. Mater. (Deerfield Beach Fla.)* 34, e2105738. doi: 10.1002/adma.202105738
- Zhao, Y., Li, Z., Zhao, L., Wang, J., Wang, F., Zhang, Q., et al. (2022). Two novel lactic acid bacteria, *Limosilactobacillus fermentum* MN-LF23 and *Lactobacillus gasseri* MN-LG80, inhibited *Helicobacter pylori* infection in C57BL/6 mice. *Food Funct.* 13, 11061–11069. doi: 10.1039/D2FO02034C
- Zhao, T., and Yu, Z. (2024). Modified Gexia-Zhuyu Tang inhibits gastric cancer progression by restoring gut microbiota and regulating pyroptosis. *Cancer Cell Int.* 24, 21. doi: 10.1186/s12935-024-03215-6
- Zheng, C., Chen, T., Lu, J., Wei, K., Tian, H., Liu, W., et al. (2021a). Adjuvant treatment and molecular mechanism of probiotic compounds in patients with gastric cancer after gastrectomy. *Food Funct.* 12, 6294–6308. doi: 10.1039/D1FO01375K
- Zheng, C., Chen, T., Wang, Y., Gao, Y., Kong, Y., Liu, Z., et al. (2019). A randomised trial of probiotics to reduce severity of physiological and microbial disorders induced by partial gastrectomy for patients with gastric cancer. *J. Cancer* 10, 568–576. doi: 10.7150/jca.29072
- Zheng, L., Kelly, C. J., Battista, K. D., Schaefer, R., Lanis, J. M., Alexeev, E. E., et al. (2017). Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor-dependent repression of claudin-2. *J. Immunol. (Baltimore Md.: 1950)* 199, 2976–2984. doi: 10.4049/jimmunol.1700105
- Zheng, Y., Xiang, S., Zhang, H., Ye, K., Zhang, Y., Ge, Y., et al. (2021b). Vitamin B12 enriched in spinach and its effects on gut microbiota. *J. Agric. Food Chem.* 69, 2204–2212. doi: 10.1021/acs.jafc.0c07597
- Zhong, M., Xiong, Y., Zhao, J., Gao, Z., Ma, J., Wu, Z., et al. (2021). *Candida albicans* disorder is associated with gastric carcinogenesis. *Theranostics* 11, 4945–4956. doi: 10.7150/thno.55209
- 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
- Zhu, X., Zhang, X., Shen, J., Zheng, S., Li, H., Han, B., et al. (2024). Gut microbiota-dependent modulation of pre-metastatic niches by Jianpi Yangzheng decoction in the prevention of lung metastasis of gastric cancer. *Phytomed.: Int. J. Phytother. Phytopharmacol.* 128, 155413. doi: 10.1016/j.phymed.2024.155413



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Anaerobic gut bacteria and their potential role in the initiation, exacerbation, and development of human colorectal cancer: a narrative review

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Colorectal cancer (CRC) is known as the second leading cause of cancer-related deaths around the world. Rectal bleeding, changes in bowel movements, weight loss, and fatigue are the main clinical presentations of CRC. While the exact etiology of the disease is unknown, CRC is considered a complex and multifactorial disease resulted from an intricate interplay of genetic and environmental factors. Moreover, CRC is known as a chronic inflammation-associated tumor, and patients with inflammatory bowel disease (IBD) or Irritable bowel syndrome (IBS) are susceptible groups to CRC development. The gut microbiota and its metabolites play a crucial role in the development and progression of CRC. CRC can be created after anaerobic bacterial infections such as Enterotoxigenic *Bacteroides fragilis* (ETBF), *Fusobacterium*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium septicum*, *Peptostreptococcus*, *Prevotella*, *Veillonella*, etc. Activation of Wnt signaling, loss of tissue architecture, proinflammatory signaling, and genotoxic cellular DNA damage are the primary mechanisms by which anaerobic bacteria induce carcinogenesis in CRC. Besides, spore germination and toxin production are done in hypoxic and acidic conditions. Therefore, according to the presence of this condition (anaerobic glycolysis) in tumor tissue, the tumor environment is suitable for the formation of anaerobic infections. The current review-based study aims to discuss the important aspects of these mechanisms and their possible roles in the initiation, development, and exacerbation of CRC.

KEYWORDS

colorectal cancer, CRC, anaerobic gut bacterial, gut bacterial, narrative review

Introduction

Colorectal cancer (CRC) is the third most common cancer around the world (Bray et al., 2024; Cao et al., 2024; Hong et al., 2024). Despite the existence of different treatment regimens, CRC ranks as the second leading cause of cancer-related deaths with an estimated 3.2 million new cases and 1.6 million deaths in 2024 (BGI Genomics, 2024). Rectal bleeding, changes in bowel movements, weight loss, and fatigue are the primary clinical presentations of CRC (Koliarakis et al., 2024). CRC is a multifactorial disease, and its etiology is not fully determined. It is presumed that genetic and environmental factors are linked to the initiation, progression, and exacerbation of CRC (Cheng et al., 2024). Indeed, CRC is known as a chronic inflammation-associated tumor, and patients with ulcerative colitis or Crohn's disease (inflammatory bowel disease) are susceptible groups to CRC development (Eskandari-Malayeri et al., 2024).

Globally, previously published studies have stated that CRC can be triggered by anaerobic bacterial infections, including Enterotoxigenic *Bacteroides fragilis* (ETBF), *Fusobacterium nucleatum*, *Clostridium difficile*, *C. perfringens*, *C. septicum*, *Peptostreptococcus*, *Prevotella*, and *Veillonella* (Kajihara et al., 2023; Conde-Pérez et al., 2024; Hong et al., 2024). The gut microbiota and its metabolites play a crucial role in the development and progression of CRC (Cheng et al., 2024). Disruptions in microbiota composition are termed dysbiosis (Shariati et al., 2019a; Azimi et al., 2020). Microbiota-related carcinogenesis is primarily caused by dysbiosis-related inflammation and carcinogen formation (Azimi et al., 2018; Shariati et al., 2019b). Moreover, secondary bile acids (BAs) and short-chain fatty acids (SCFAs), which are two main bacterial metabolites in the colon, play either protective or harmful roles in the initiation and development of CRC (Cheng et al., 2024). Activation of Wnt signaling, loss of tissue architecture, proinflammatory signaling, and genotoxic cellular DNA damage are the primary mechanisms by which anaerobic bacteria induce carcinogenesis in CRC (Hong et al., 2024). The role and possible mechanisms of anaerobic pathogenic bacteria are summarized in Table 1. According to what was mentioned earlier and with respect to possible roles of anaerobic bacteria in cancer development and progression, the current review-based study aims to discuss their potential roles and important aspects of mechanisms in the initiation, development, and exacerbation of CRC.

Fusobacterium nucleatum

Fusobacterium nucleatum (*F. nucleatum*) is a spindle-shaped, non-spore-forming Gram-negative anaerobe bacterium. It is identified as a member of the human normal microbiota in oral cavity [12]. However, prior studies have indicated that *F. nucleatum* is an invasive, pro-inflammatory pathogen associated with various human disease conditions (Koliarakis et al., 2024).

F. nucleatum plays a key role in initiating and developing various cancers, such as liver, lung, and CRC (Liu et al., 2021).

It has been discovered that different mechanisms of CRC are mediated by *F. nucleatum*. In most cases, the entrance of *F. nucleatum* into colorectal tissues has been facilitated in three main ways (Figure 1): 1) interaction of the effector *Fusobacterium* adhesin A (FadA) with E-cadherin, 2) fibroblast activation protein 2 (Fap2) interaction with D-galactose- β (1-3)-N-acetyl-D-galactosamine (Gal-GalNAc), and 3) interaction of Lipopolysaccharide (LPS) of *F. nucleatum* with Toll-like receptor 4 (TLR4) (Wang et al., 2023a; Martin-Gallausiaux et al., 2024).

Previously published studies have revealed overexpressed FadA gene levels in CRC patients' faecal samples (Martin-Gallausiaux et al., 2024; Wang et al., 2024). Annexin A1 is a 35–40 kDa molecule and is considered as a main part of FadA protein. It is proposed that Annexin A1 can enable the FadA protein to influence different biological processes such as apoptosis, cell differentiation, cell proliferation, cell migration, and tumor growth (Figure 2) (Liu et al., 2024). The binding of the FadA protein to E-cadherin upregulates the Annexin A1 expression and can stimulate carcinogenesis (Cheng et al., 2024). Moreover, by activating the Wnt/ β -catenin signalling pathway cyclin D1 (CycD1), it can induce and trigger oncogenic and inflammatory responses (Pignatelli et al., 2023). Interaction of Annexin A1 with epidermal growth factor receptor (EGFR) induces immune suppression in the tumor microenvironment (Araújo et al., 2021).

In addition, the interaction of *F. nucleatum* FadA protein with E-cadherin leads to DNSA damage (DNA Double-Strand breaks (DSBs)) and has a key role in cancer development (Guo et al., 2020).

F. nucleatum Fap2 protein plays a significant role in attachment and instability of intestinal barriers. Fap2 can promote the entrance of *F. nucleatum* to intestinal epithelial cells (Luo et al., 2024). Furthermore, by interacting with tumor-specific sugar residue Gal-GalNAc, FadA and Fap2 proteins promote the attachment of *F. nucleatum* to biofilm and facilitate its localization and enrichment in CRC (Figure 3) (Zeddou, 2023). Fap2 binds to human immune inhibitory receptor T-cell immunoglobulin and ITIM domain, inhibiting natural killer cell (NK) cytotoxicity and modulating the tumor-immune microenvironment. Therefore, Fap2 can inhibit antitumor immunity in tumor microenvironment (Gur et al., 2015).

F. nucleatum has numerous adhesins on its surface, including RadD, Aid1, and FomA, that help in formation of bacterial co-aggregation and biofilm. In CRC, *F. nucleatum* dominant biofilms are a common finding, particularly when the cancer is on the right side of the colon (Liu et al., 2024).

M2 macrophages have a crucial role in tissue healing and inflammation. A previously published study stated that the interaction of *F. nucleatum* with a TLR4-dependent mechanism promotes the M2 polarization of macrophages. The promotion of M2 polarization of macrophages by *F. nucleatum* leads to immunosuppression in the tumor microenvironment (Chen et al., 2018; Mohammadi et al., 2022).

Butyrate is the main energy source for colonic cells and has anti-inflammatory and immunomodulatory effects. It inhibits tumor cell proliferation and migration, downregulates the Wnt signalling pathway, and limits tumor angiogenesis (Martin-Gallausiaux et al., 2024). Therefore, butyrate can inhibit tumor development

TABLE 1 The possible mechanisms of anaerobic gut bacteria implicated in the initiation, exacerbation, and development of human CRC.

Gut pathogens		Possible mechanisms	Gut pathogens		Possible mechanisms
1	<i>Fusobacterium nucleatum</i>	• Interaction of the FadA with E-cadherin upregulates the Annexin A1 expression • Activation of the Wnt/β-catenin signalling pathway • Breaks of DNA Double-Strand • Fap2 interaction with D-galactose-β (1-3)-N-acetyl-D-galactosamine • Interaction of LPS with TLR-4 • Fap2 binds to the human immune inhibitory receptor T-cell immunoglobulin and ITIM domain • Inhibits NK cytotoxicity and modulating the tumor-immune microenvironment • Formation of bacterial co-aggregation and biofilm • Promotes the M2 polarization of macrophages and leads to immunosuppression in the tumor microenvironment • <i>F. nucleatum</i> can consume butyric acid, significantly decreasing the concentration of butyrate in CRC cells and promoting CRC development • <i>F. nucleatum</i> upregulates the expression levels of BIRC3 in CRC cells and promotes chemoresistance to chemotherapy • Ppplies the miR18α, miR4802, MYD88 TLR-4, and unc-51-like kinase 1/autophagy related 7 autophagy networks to create and control chemoresistance • Increase the expression of miR-21 • Activates the autophagy signals • inhibits the N6-adenosine-methyltransferase 70 kDa subunit enzyme • Induces the long non-coding RNA EVADR • Initiates the nuclear factor kappa-light-chain-enhancer of activated B cells • Binding of ADP-H to ALPK1 strongly activates NF-κB in intestinal epithelial cells and induces the expression of the inflammatory cytokine and anti-apoptotic genes • H₂S produced by <i>F. nucleatum</i> disrupts the colonic epithelial cell barrier and induces free radical-related DNA damage • Overproduction of H₂S promotes the proliferation of cancer cells in CRC • Activates the MAPK (JNK) - AP1 axis and up-regulates the MMP7 expression. • The overexpression of MMP7 on the cancer cell promotes metastasis in colon epithelial cells	6	<i>Clostridium perfringens</i>	• Long-term PPI use initiates and exacerbates the <i>C. perfringens</i> infection in the intestinal tract • <i>C. perfringens</i> enterotoxin could impair impairs Claudin-4, which forms tight junctions in colorectal cells and enhances cancer malignancy • Bacterium activates the YAP and promotes cancer progression
2	Enterotoxigenic <i>Bacteroides fragilis</i>	• BFT can induce the cleavage of the extracellular domain of the E-cadherin junction protein and lead to the disruption of E-cadherin and β-catenin linkage • Changes in the epithelial cell cytoskeletal structure and increases intestinal secretion and cell signalling via the β-catenin/Wnt pathway • Activates (NF-κB and promotes the production of inflammatory cytokines such as CXCL1 • Stimulate the secretion of IL-8 and c-myc expression • Activation of signal transducer and activator of transcription 3 • Produce the ROS and causes oxidative DNA damage	7	<i>Clostridium septicum</i>	• <i>C. septicum</i> via DNA damage and defects in cellular DNA repair processes can lead to carcinogenesis in the colon • Hypoxia and acidity conditions in the tumor environment are suitable for spore germination, active <i>C. septicum</i> infection, and toxin production • Hyaluronidase, fibrinolysin, deoxyribonuclease, and hemolysins enable bacteria to metastasize and invade tissues in the colon

(Continued)

TABLE 1 Continued

Gut pathogens		Possible mechanisms	Gut pathogens		Possible mechanisms
		- stimulates the high expression of IL-17 - ETBF down-regulates the miR-149-3p expression - ETBF can accelerate colitis and carcinogenesis related to DSS and azoxymethane.			
3	<i>Veillonella parvula</i>	<i>V. parvula</i> used nitrate respiration to colonize the intestine during the inflammatory response - Increases B lymphocyte stimulator (BLyS) expression level and B-cell infiltration <i>V. parvula</i> can exert an immunomodulatory effect in CRC cells	8	<i>Actinomyces israelii</i>	- This bacterium can disrupt the intestinal wall and enter the abdominal cavity - During the surgery for local recurrence, it's possible that <i>A. israelii</i> entered the abdominal cavity - Injuries to the oral cavity or gastrointestinal tract could have allowed organisms to enter the bloodstream
4	<i>Prevotella intermedia</i>	<i>P. intermedia</i> metabolize the glucose for formate formation <i>P. intermedia</i> induces colon cancer cell invasion and leads to metastatic dissemination in CRC <i>P. intermedia</i> secretes the AMF molecule and ultimately promotes the CRC cell motility and metastasis	9	<i>Actinomyces odontolyticus</i>	- This bacterium secretes MVs and induces mitochondrial dysfunction and excessive ROS production - MVs specifically bind the TLR2 receptor and activate the NF-κB signaling pathway
5	<i>Clostridioides difficile</i>	- TcdB is an important toxin and has more toxic effects on colon cells - TcdB induces the inflammatory response, deregulates Rho-GTPases, and increases the expression levels of proto-oncogenes in colon - Expression of <i>PLC-γ</i> is upregulated in CRC cells and breast carcinoma - TcdA can induce apoptosis in PLC-γ-transformed cells - TcdA can upregulate the expression levels of BIM and activation of caspase-3 in PLC-γ-transformed cells	10	<i>Peptostreptococcus anaerobius</i>	- The interaction of <i>P. anaerobius</i> with TLR-2 and TLR-4 elevates ROS production in colon cells and leads to CRC tumorigenesis <i>P. anaerobius</i> facilitates the activity of SREBP-2 <i>P. anaerobius</i> promotes cholesterol biosynthesis and enhances cell proliferation and tumorigenesis in CRC <i>P. anaerobius</i> colonization increases MDSCs abundances in the tumor microenvironment - Recruitment of MDSCs into tumor microenvironment increases the chemoresistance of colorectal cancer - The accumulation of MDSCs in the tumour microenvironment can antagonistically affect antitumor CD8+ and CD4+ T cells <i>P. anaerobius</i> stimulates the secretion of chemokine (C-X-C motif) ligand 1 - Interaction of PCWBR2 with epithelial cell receptor integrin α2/β1 leads to activation of NF-κB and ultimately promotes cell proliferation and pro-inflammatory immune responses in CRC cells
11	<i>Peptostreptococcus stomatis</i>	<i>P. stomatis</i> can activate the ERBB2-MAPK) and promotes colonic tumorigenesis - Bacterium used its surface protein FBA to attach to the CRC cells - Attachment of <i>P. stomatis</i> to CRC cells leads to the activation of ERBB2 and downstream Ras/Raf/MAPK (MEK)-ERK cascades <i>P. stomatis</i> can inactivate and inhibit the BRAF inhibitor and RTK inhibitor leading to the non-responsiveness to these drugs in CRC - Bacterium can suppress apoptosis and impair the gut barrier function		-	-

FadA, *Fusobacterium* adhesin A; Fap2, Fibroblast activation protein 2; LPS, Lipopolysaccharide; TLR-4, Toll-like receptor 4; NK cell, natural killer cell; BIRC3, Baculoviral IAP repeat-containing protein 3; EVADR, endogenous retroviral-associated adenocarcinoma lncRNA; ROS, reactive oxygen species; DSS, dextran sulfate sodium; YAP, Yes-associated protein; PPI, Proton pump inhibitors; MV, membrane vesicle; SREBP-2, *sterol regulatory element-binding protein* 2; MDSC, Myeloid-derived suppressor cell; ERBB-2, Erb-b2 receptor tyrosine kinase 2; MAPK, Mitogen-activated protein kinase; FBA, fructose-1,6-bisphosphate aldolase; ERK, extracellular signal-regulated kinase; RTK, receptor tyrosine kinase.

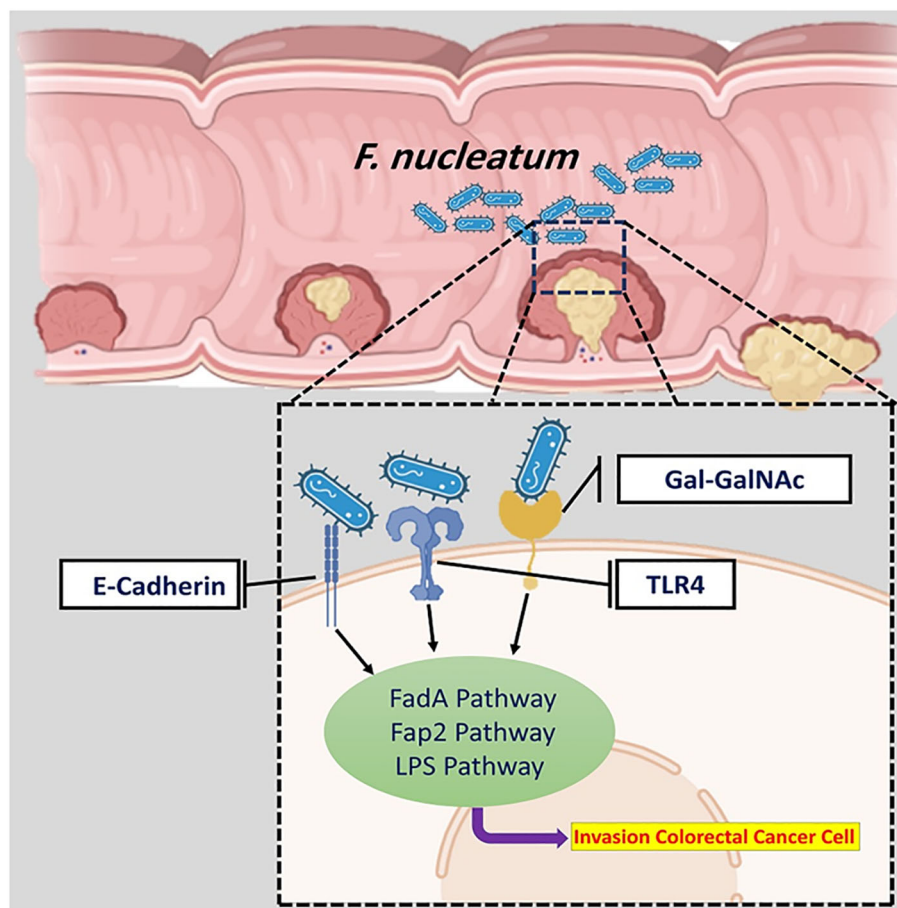


FIGURE 1

The overall mechanism of *F. nucleatum* in CRC. The entrance of *F. nucleatum* into colorectal tissues has been facilitated in three main ways.

in colonic cells. Wu et al. suggested that *F. nucleatum* can actively consume butyric acid, significantly reduce the concentration of butyrate in CRC cells, and promote CRC development. They presumed that *F. nucleatum* promotes the occurrence and development of CRC by reducing the population of butyrate-producing bacteria (Wu et al., 2024).

The combination of 5-Fluorouracil (5-Fu), irinotecan, or oxaliplatin is used as a standard treatment of advanced CRC (André et al., 2009). One of the primary causes of poor prognosis in CRC is chemoresistance. During the period of drug treatment, *F. nucleatum* upregulates the expression levels of Baculoviral IAP repeat-containing protein 3 (BIRC3) in CRC cells. An increase in expression levels of BIRC3 promotes chemoresistance to chemotherapy (Martin-Gallausiaux et al., 2024).

Moreover, *F. nucleatum* applies the miR18 α , miR4802, MYD88 TLR-4, and unc-51-like kinase 1/autophagy related 7 autophagy networks to create and control chemoresistance in CRC cells (Figure 4) (Bostanghadiri et al., 2023).

It is revealed that *F. nucleatum* infection can promote CRC cell metastasis by several mechanisms. This bacterium activates the autophagy signals, inhibits the N6-adenosine-methyltransferase 70 kDa subunit (METTL3) enzyme, induces the long non-coding RNA endogenous retroviral-associated adenocarcinoma lncRNA

(EVADR), and initiates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) (Du et al., 2019; Chen et al., 2022; Liu et al., 2024).

ADP-heptose (ADP-H) is known as the pathogen-associated molecular pattern (PAMP) produced by *F. nucleatum* (Chen et al., 2022). Alpha kinase 1 (ALPK1) is the new pattern recognition receptor (PRR) that senses ADP-H. The binding of ADP-H to ALPK1 strongly activates nuclear factor- κ B (NF- κ B) in intestinal epithelial cells and induces the expression of inflammatory cytokines and anti-apoptotic genes (Milivojevic et al., 2017; Martin-Gallausiaux et al., 2022). It is revealed that the binding of ADP-H to ALPK1 increases the expression levels of IL-6, IL-8, IL-17, TNF- α , TLR-2, and TLR-4 genes in CRC cells. Moreover, this process reduces the chemosensitivity of CRC cells (Martin-Gallausiaux et al., 2024).

Previously published studies have identified a significant relationship between miR-21 expression and high levels of *F. nucleatum* in CRC cells. The expression of miR-21 is upregulated by hyperactive NF- κ B binding to its promoter in CRC cells. This finding is partial support for the role of *F. nucleatum* in carcinogenesis by inducing miR-21. It is possible that *F. nucleatum* induces the expression of miR-21 and suppresses the Ras p21 protein activator 1 (RASA1) (a regulator of Ras GDP and GTP) (Bostanghadiri et al., 2023).

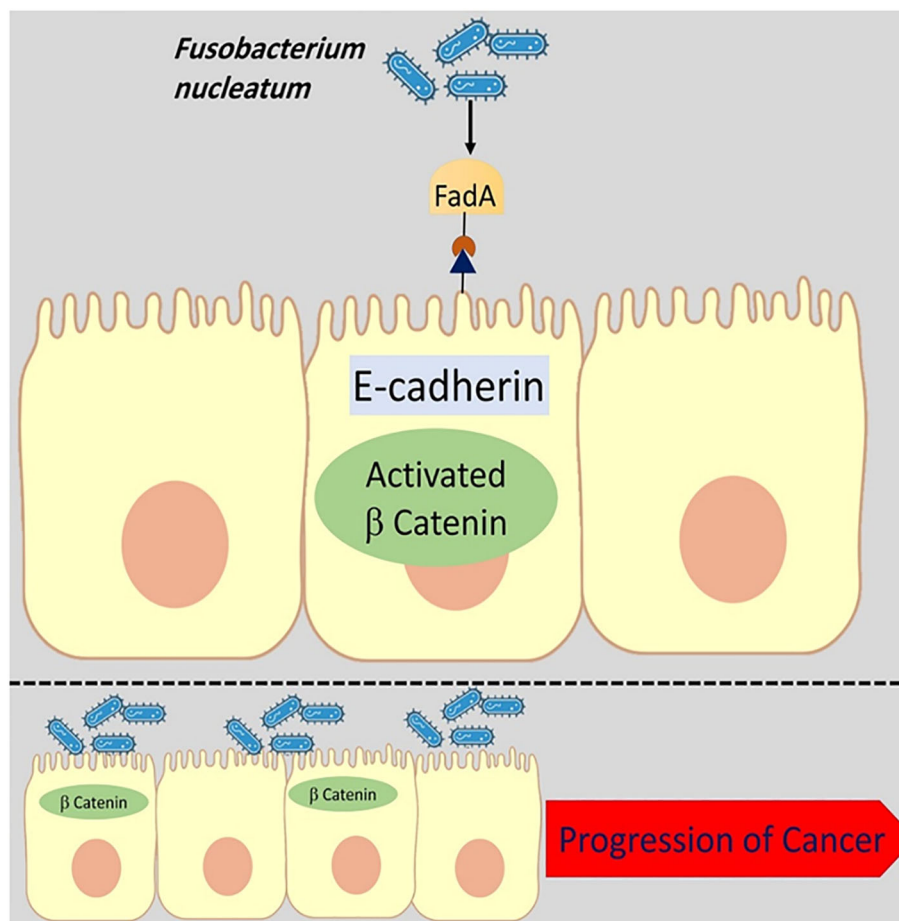


FIGURE 2

The binding of the FadA protein to E-cadherin upregulates the Annexin A1 expression and can stimulate carcinogenesis. The disruption of E-cadherin and β -catenin linkage leads to changes in the epithelial cell cytoskeletal structure and increases cancer progression.

Both hydrogen sulfide (H₂S) and sulfur-containing amino acids can initiate or promote the development of various cancers, such as CRC (Kim et al., 2016). *F. nucleatum* metabolizes sulfur-containing amino acids and produces H₂S. Moreover, this bacterium can produce H₂S from L-cysteine. H₂S produced by *F. nucleatum* disrupts the colonic epithelial cell barrier and induces free radical-related DNA damage. It is revealed that the overproduction of H₂S promotes the proliferation of cancer cells in CRC patients (Wang et al., 2023b).

Matrix metalloproteinase 7 (MMP7) is a significant member of the MMPs family and has a significant role in metastasis, tumor growth, and angiogenesis (Pezeshkian et al., 2021). MMP7 played a vital role in the activation of other MMPs, such as pro-MMP2 and pro-MMP9. It is revealed that *F. nucleatum* activates the MAPK (JNK) - AP1 axis (a transcription factor which targets MMP7) and upregulates the MMP7 expression. The overexpression of MMP7 in cancer cells promotes metastasis in colon epithelial cells (Ou et al., 2023).

Enterotoxigenic *Bacteroides fragilis*

Bacteroides fragilis (*B. fragilis*) is a gram-negative anaerobic bacterium that has been identified as a human colonic symbiont

(Jo et al., 2023). *B. fragilis* constitutes 1% of the total intestinal microbiome, and 80% of children and adults are tested positive for this bacterium in the intestines (Dadgar-Zankbar et al., 2023). This bacterium is divided into two types: 1) enterotoxigenic *B. fragilis* (ETBF) and 2) non-enterotoxigenic types of *B. fragilis*. *B. fragilis* toxin (*bft*) is a 20 kDa zinc metalloprotease toxin with three isotypes, including 1) *bft-1*, 2) *bft-2*, and 3) *bft-3* (Cheng et al., 2020). Published studies have shown that *bft-2* is more carcinogenic than *bft-1* (Dadgar-Zankbar et al., 2023). ETBF plays a main role in initiating and exacerbating gastrointestinal diseases such as CRC through multiple molecular processes (Qu et al., 2023).

E-cadherin is an important regulator of epithelial-mesenchymal transition (EMT) (Tabowei et al., 2022). Both *in vitro* and *in vivo* model studies have revealed that BFT can induce the cleavage of extracellular domain of the E-cadherin junction protein and lead to the disruption of E-cadherin and β -catenin linkage. The disruption of E-cadherin and β -catenin linkage leads to changes in epithelial cell cytoskeletal structure. It increases intestinal secretion and cell signalling via the β -catenin/Wnt pathway. Moreover, the cleavage of E-cadherin activates nuclear factor- κ B (NF- κ B) and promotes the production of inflammatory cytokines such as CXCL1 (C-X-C Motif Chemokine Ligand 1) (Figure 5) (Cheng et al., 2020;

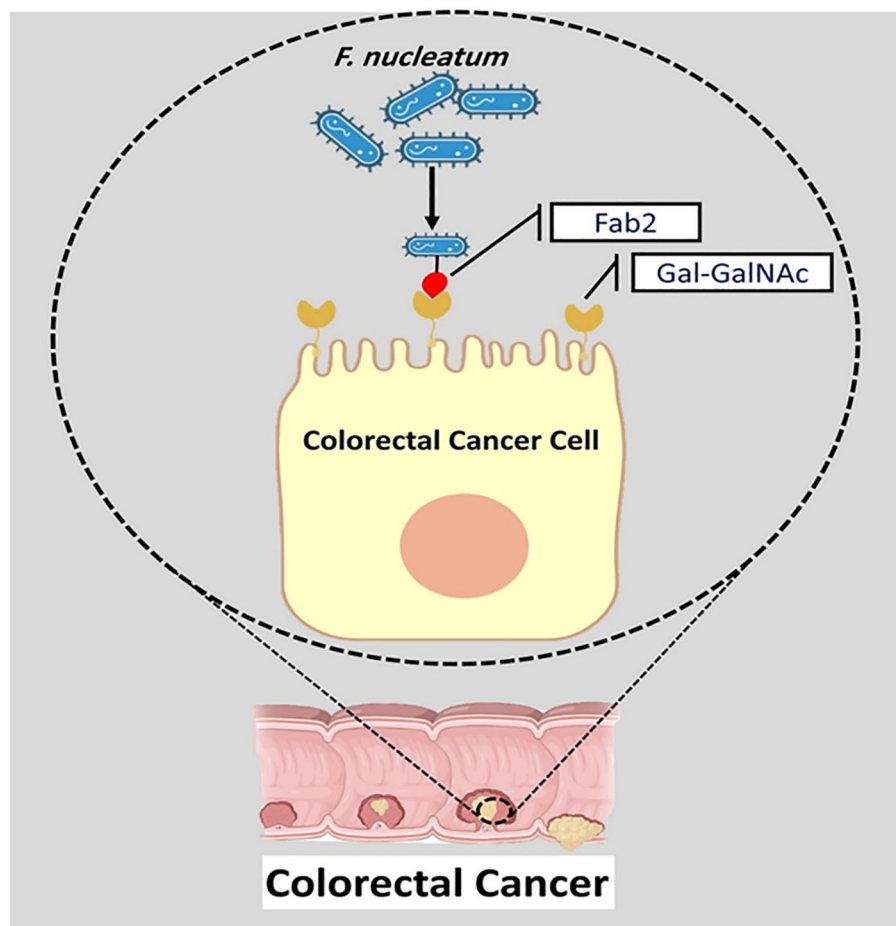


FIGURE 3

The interaction of Fap2 with tumor-specific sugar residue Gal-GalNAc promotes the attachment of *F. nucleatum* to biofilm and facilitates its localization and enrichment in CRC.

Khodaverdi et al., 2021). It is revealed that the disruption of E-cadherin and β -catenin linkage can stimulate the secretion of IL-8 and c-myc expression. C-myc is often persistently expressed in cancer cells. IL-8 can lead to activation of signal transducer and activator of transcription 3 (Stat3; regulators of various cell functions such as acute inflammation). Stat3 can act as an oncogene, constitutively activating it to promote chronic inflammation and tumorigenesis (Figure 6). Moreover, studies have stated that Stat3 can skew host immune responses towards tolerance in ETBF infections (Hwang et al., 2013; Purcell et al., 2022). BFT also produces reactive oxygen species (ROS) and causes oxidative DNA damage (Figure 5). Besides, ETBF stimulates the high expression of IL-17 and increases the risk of CRC (Khodaverdi et al., 2021). MicroRNAs (miRNAs) are noncoding and single-stranded RNAs with approximately 22 base pairs. Protein translational processes are regulated by miRNAs. Moreover, the molecular and cellular processes in cancer and inflammatory states are greatly influenced by miRNAs (Bartel, 2018; Slack and Chinnaiyan, 2019).

Another carcinogenic process related to ETBF is the downregulation of miR-149-3p. It is revealed that ETBF

downregulates the miR-149-3p and promotes intestinal inflammation and malignancy in CRC cells (Cao et al., 2021). Moreover, animal model studies have shown that ETBF can accelerate colitis and carcinogenesis related to dextran sulfate sodium (DSS) and azoxymethane (AOM). However, the molecular mechanism of this process remains unclear (Tabowei et al., 2022).

Veillonella parvula

Veillonella parvula (*V. parvula*) is a strictly gram-negative anaerobic coccus-shaped bacterium belonging to the genus *Veillonella*. This bacterium is a member of normal microbiota in human intestinal, oral, and respiratory tract (Qian et al., 2023). *V. parvula* uses nitrate respiration to colonize the intestine during the inflammatory response. Therefore, *V. parvula* could play a significant role in different digestive diseases such as CRC (Rojas-Tapias et al., 2022). This bacterium increases B lymphocyte stimulator (BLS) expression level and B-cell infiltration in CRC patients (Qian et al., 2023). Studies have indicated that an increase

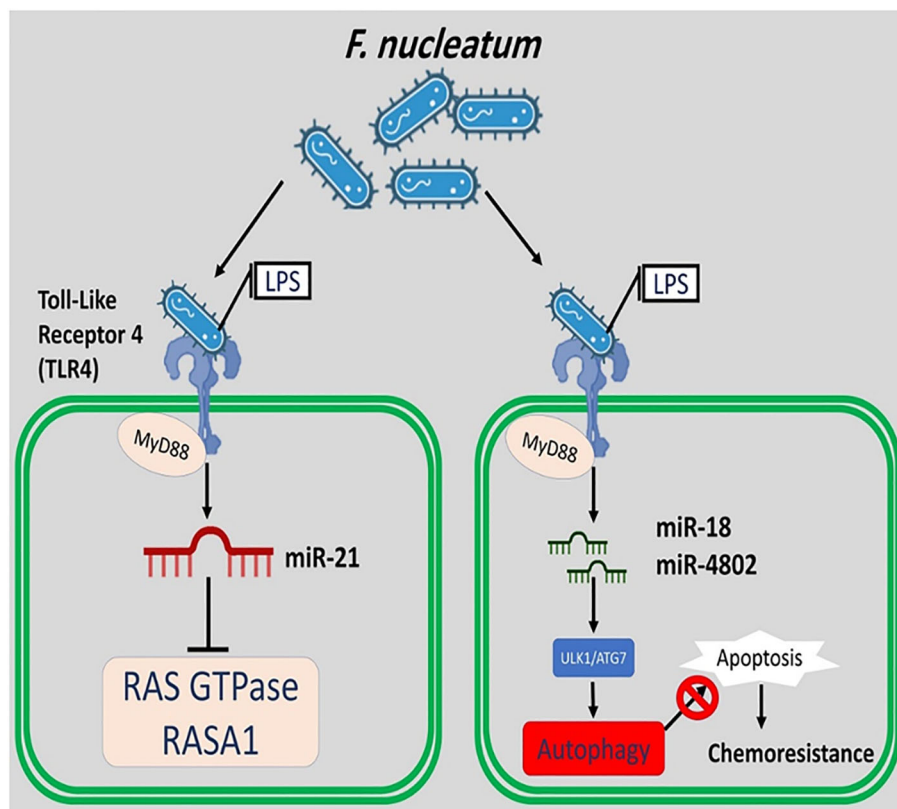


FIGURE 4

F. nucleatum applies the miR18, miR4802, MYD88, TLR-4, and unc-51-like kinase 1/autophagy related 7 autophagy networks to create and control chemoresistance in CRC cells. *F. nucleatum* induces the expression of miR-21 and suppresses the RAS GTPase and RASA1.

in BLYS expression level leads to an increase in the level of B cells in tumor tissues (Du et al., 2022; Qian et al., 2023). It is presumed that *V. parvula* can exert an immunomodulatory effect in CRC cells. CRC patients with high fecal *V. parvula* abundance are more likely to develop an advanced tumor stage, higher rates of lymph node metastasis, and a lower prognosis (Qian et al., 2023).

Prevotella

Colorectal adenomas (CRAs) are precancerous lesions present in 30–40% and <20% of individuals aged ≥ 70 years and ≤ 50 years, respectively [50]. *Prevotella intermedia* (*P. intermedia*) is an anaerobic bacterium that can transform CRAs into colorectal cancer (CRC). Previously published studies have revealed that this bacterium can increase cell migration and invasion in CRC cells [7, 50, 51]. The gut microbial metabolite formate plays a key role in activating aryl hydrocarbon receptor (AhR) signalling [52]. AhR is a ligand-activated transcription factor and a receptor for multiple physiological ligands [53]. Interestingly, *P. intermedia* utilizes several enzymes to produce formate. Furthermore, this bacterium metabolizes glucose for formate formation. The formate produced by *P. intermedia* induces colon cancer cell invasion and leads to metastatic dissemination in CRC [50, 54]. Autocrine motility factor (AMF), also known as glucose-6-phosphate isomerase (GPI),

secreted by tumor cells, drives epithelial-mesenchymal transition, and stimulates invasion and metastasis in various cancers, including CRC [55]. It is supposed that *P. intermedia* secretes the AMF molecule and ultimately promotes CRC cell motility and metastasis [56, 57].

Clostridioides difficile

Clostridioides difficile (*C. difficile*) is an anaerobic gram-positive spore-forming bacterium. 5–15% of the adult population is tested positive for this bacterium as a normal gut microbiota (Tirelli et al., 2023). Most patients infected with this bacterium are asymptomatic or have limited to mild diarrhea. However, *C. difficile* can cause different forms of infectious diseases, ranging from infectious diarrhea (antibiotic-associated diarrhea), pseudomembranous colitis, and fulminant colitis (toxic megacolon). Pseudomembranous colitis and fulminant colitis are seen in 25% and 1–3% of patients, respectively (Magat et al., 2020; Tirelli et al., 2023). Several studies have reported that infection by toxin-producing *C. difficile* has a potential role in disruption of gut microbiome and leads to tissue damage and malignant transformation of cells in the colon (Jahani-Sherafat et al., 2019; Kaźmierczak-Siedlecka et al., 2020). This bacterium can produce three toxins, including TcdA (*C. difficile* Toxin A), TcdB (*C. difficile* Toxin B), and cdtA/B (*C. difficile* binary toxin A and B). In

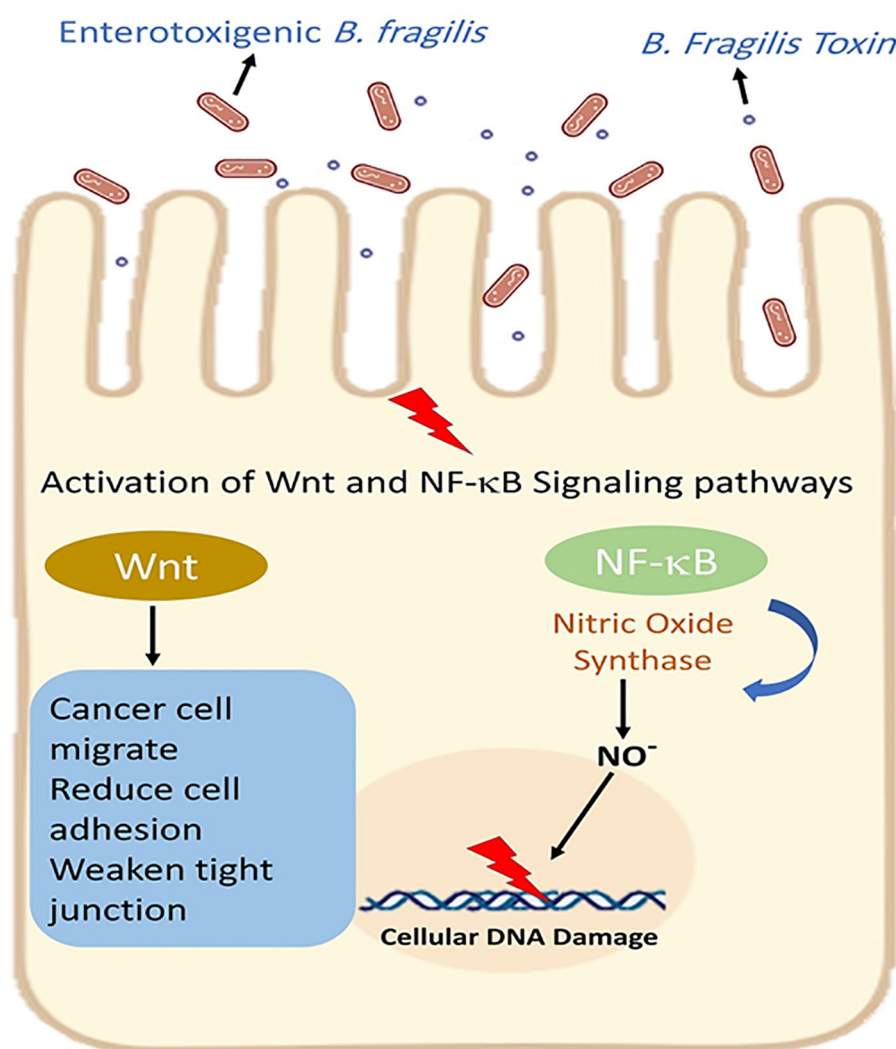


FIGURE 5

Enterotoxigenic *B. fragilis* increases intestinal secretion and cell signaling via the β -catenin/Wnt pathway. Moreover, BFT activates nuclear factor- κ B (NF- κ B) and promotes the production of inflammatory cytokines.

comparison to TcdA, TcdB is an important toxin and has more toxic effects on colon cells in *C. difficile* infection (CDI) (Dicks et al., 2019).

It is proven that TcdB toxin induces the inflammatory response, deregulates Rho-GTPases, and increases the expression levels of proto-oncogenes in colon (Jank et al., 2007). Briefly, TcdB binds to its specific receptors, and the toxin-receptor complex enters the host cells by endocytosis. At the next step, TcdB passes from the acidic endosomal membrane and is translocated into the cytosol. Finally, the active part of the TcdB toxin (glucosyltransferase) is released into the cytosol. Glucosyltransferase transfers glucose molecules into Rho proteases and prevents the normal binding of Guanosine-5'-triphosphate (GTP) to Guanosine diphosphate (GDP) bound form of Rho protein (Fettucciari et al., 2023). Therefore, the TcdB toxin can inactivate the Rho protein (Jena et al., 2013; Dicks et al., 2019). Inactivation of the Rho protein stimulates pro-inflammatory interleukin production, tumor necrosis factor (TNF) activation, increases vascular permeability, and finally can lead to the growth and proliferation of tumor cells in CRC cells (Dicks et al., 2019).

Phospholipase C- γ (PLC- γ) is an important signalling molecule, and overexpression of PLC- γ is related to cellular transformation (Nam et al., 2012). It is shown that the expression of PLC- γ is upregulated in CRC cells and breast carcinoma. TcdA can induce apoptosis in PLC- γ -transformed cells. Moreover, TcdA can upregulate the expression levels of BIM (a novel member of the BCL-2 family that promotes apoptosis) and activation of caspase-3 in PLC- γ -transformed cells. The high upregulation of PLC- γ by TcdA suggests that this toxin may have good potential as an anticancer agent against different malignancies such as CRC (Warny et al., 2000; Nam et al., 2012).

Clostridium perfringens

Clostridium perfringens (*C. perfringens*) is a gram-positive, anaerobic spore-forming bacterium belonging to the Clostridiaceae family (Huang and Wang, 2020). This bacterium

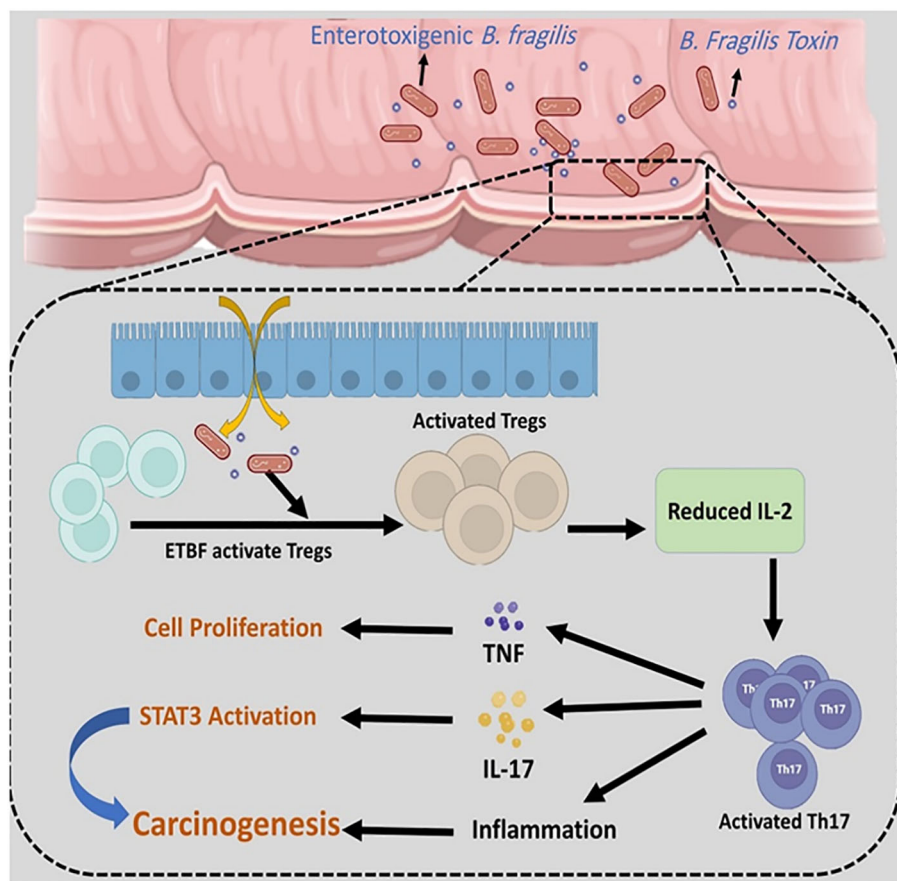


FIGURE 6

ETBF can activate regulatory T cells (Tregs) and stimulate the high expression of Th17. The overexpression of Th17 leads to an increase in TNF- α , IL-17, and inflammation. STAT3 can act as an oncogene, and constitutively activating it promotes chronic inflammation and tumorigenesis.

can be isolated from sewage, soil, human genital (reproductive) and urinary systems, and the intestinal tract (Kohya et al., 2022). *C. perfringens* is a causative agent of food poisoning after trauma and gas gangrene. This bacterium could lead to death in a short time in patients with cancer or immunocompromised individuals (Huang and Wang, 2020). *C. perfringens* has the highest frequency in patients with CRC, pancreatic cancer and gastric cancer, respectively (Kohya et al., 2022). First-line treatment for peptic gastrointestinal disorders involves proton pump inhibitors (PPIs). PPIs have been proven to inhibit colon cancer cell growth and carcinogenesis (Zeng et al., 2016; Sasaki et al., 2020). By suppressing the overexpressed protein kinase in T-LAK cells in various cancers, PPIs can inhibit the development and progression of cancer. Furthermore, membrane-bound ATP-binding cassette transporters are suppressed by PPIs, which can reduce drug resistance in cancer (Sasaki et al., 2020). It is revealed that long-term PPI use initiates and exacerbates the *C. perfringens* infection in intestinal tract. *C. perfringens* enterotoxin could impair Claudin-4 (CLDN4), which forms tight junctions in colorectal cells and enhances cancer malignancy (Sasaki et al., 2020). Moreover, this bacterium activates the Yes-associated protein (YAP) and promotes cancer progression (Fujiwara-Tani et al., 2018).

Clostridium septicum

Clostridium septicum (*C. septicum*) is an anaerobic spore-forming gram-positive rod-shaped bacterium belonging to the *Clostridium* family (Lintin et al., 2014). This bacterium is a highly virulent pathogen that produces different toxins and has been linked to hematological malignancy, colorectal malignancy, cyclical neutropenia, diabetes mellitus, and immunosuppression. (Nanjappa et al., 2015) Overall, 50 to 85% of *C. septicum* infections are associated with underlying malignancy (Kennedy et al., 2005). Anaerobic and spore-forming abilities allow *C. septicum* to flourish in the gut. It enters the bloodstream directly after passing through the mucosal layer (Lintin et al., 2014). Nevertheless, the pathophysiology behind how this bacterium promotes colonic malignancy is still unclear. A previously published study pointed out that *C. septicum* and UshA genotoxin in *Escherichia coli* may follow a similar pathogenesis. It is presumed that these bacteria, via DNA damage and defects in cellular DNA repair processes, can lead to carcinogenesis in the colon (Abraham and Padam, 2023). Spore germination, active *C. septicum* infection, and toxin production are done in hypoxic and acidic conditions. Therefore, according to the presence of this condition (anaerobic glycolysis) in tumor tissue, the

tumor environment is suitable for the formation of active infection (Jessamy et al., 2016; Justesen et al., 2022). Furthermore, blood supply is relatively low in tumor tissue and this condition could prepare the environment for *C. septicum* proliferation (Justesen et al., 2022). *C. septicum* has several toxins and enzymes such as hyaluronidase, fibrinolysin, deoxyribonuclease, and hemolysins, which enable bacteria to metastasize and invade tissues in the colon (Bickerton et al., 2022).

Actinomyces

Actinomycetes are a heterogeneous group of gram-positive bacteria commonly found in the human digestive and genital tract, and oral cavity (Fukunaga et al., 2024). The bacteria belonging to the Actinomycetes can cause severe infections in the peritoneal cavity, abdominal wall, face, neck and chest (Acquaro et al., 2010; Valour et al., 2014). *Actinomyces israelii* (*A. israelii*) is a rod-shaped anaerobic gram-positive bacterium and is considered as the most common pathogen among Actinomycetes (Fukunaga et al., 2024). Actinomycosis is a chronic suppurative and granulomatous disease that is caused by *A. israelii* (Valour et al., 2014). A few studies have presumed that *A. israelii* could have a role in initiation or exacerbation of CRC (Fukunaga et al., 2024). There are three conceivable reasons for inflammation and perforation of gastrointestinal tract caused by this bacterium; 1) this bacterium can disrupt the intestinal wall and enter the abdominal cavity; 2) during the surgery for local recurrence, it's possible that *A. israelii* enters the abdominal cavity; 3) injuries to the oral cavity or gastrointestinal tract could have allowed organisms to enter the bloodstream (Yang and Im, 2018; Fukunaga et al., 2024).

Actinomyces odontolyticus (*A. odontolyticus*) is another anaerobic bacterium in human oral cavity and gastrointestinal tract (Könönen and Wade, 2015). Previously published studies have reported that this bacterium is frequently isolated from stool samples of patients with early-stage CRC (Yachida et al., 2019; Breau, 2024). It is revealed that *A. odontolyticus* plays a main role in colorectal dysplasia. This bacterium secretes membrane vesicles (MVs) and induces mitochondrial dysfunction and excessive reactive oxygen species (ROS) production. ROS production can lead to DNA damage and cellular transformation in colonic epithelium (Miyakawa et al., 2024). Furthermore, MVs enriched for lipoteichoic acid (LTA) specifically binds with the TLR2 receptor and activates the NF- κ B signaling pathway (Breau, 2024).

Peptostreptococcus

Peptostreptococcus anaerobius (*P. anaerobius*) is an anaerobic gram-positive coccus and is considered as a member of the human oral and gut normal microbiota (Gu et al., 2023). This bacterium can cause several infectious diseases, including endocarditis, periodontal disease, genitourinary and gastrointestinal tract infections (Tsoi et al., 2017). Results of studies have shown that this bacterium was significantly present in stool and mucosal samples of patients with

CRC (Yu et al., 2017; Brennan and Garrett, 2019; Cheng et al., 2020). The interaction of *P. anaerobius* with TLR-2 and TLR-4 elevates ROS production in colon cells and leads to CRC tumorigenesis (Tsoi et al., 2017). The increases in the level of intracellular ROS enhance the biosynthesis of cholesterol (Tsoi et al., 2017). Briefly, *P. anaerobius* facilitates the activity of sterol regulatory element-binding protein 2 (SREBP-2). SREBP2 is a key regulator of cholesterol biosynthesis, and this molecule controls the expression levels of genes involved in the synthesis of cholesterol. Therefore, *P. anaerobius* promotes ROS production and cholesterol biosynthesis and enhances cell proliferation and tumorigenesis in CRC (Tsoi et al., 2017; Huang et al., 2024).

Myeloid-derived suppressor cells (MDSCs) are known as the main immune suppressant cells in tumor microenvironment and are linked to cancer progression (Liu et al., 2024). It is presumed that the intratumoral bacteria play a main role in accumulation of MDSCs in CRC cells (Gu et al., 2023). Results of a study performed on the mouse models revealed that *P. anaerobius* colonization increases MDSCs abundances in tumor microenvironment (Figure 7) (Liu et al., 2024). The presence of *P. anaerobius* in tumor microenvironment stimulates the secretion of chemokine (C-X-C motif) ligand 1 (CXCL1) (Long et al., 2019; Liu et al., 2024). CXCL1 is a main chemokine in the recruitment and activation of neutrophils for microbial killing. This chemokine plays a main role in host immune response. CXCL1 participates in the chemotaxis of CXCR2 (C-X-C Motif Chemokine Receptor 2) + MDSCs. The accumulation of MDSCs in tumor microenvironment can antagonistically affect antitumor CD8+ and CD4+ T cells (Long et al., 2019; Gu et al., 2023). Moreover, it is revealed that the recruitment of MDSCs into tumor microenvironment increases the chemoresistance of colorectal cancer to oxaliplatin and mediates anti-PD1 therapy resistance (Liu et al., 2024).

The putative cell wall binding repeat 2 (PCWBR2) is a surface protein of *P. anaerobius*. The direct interaction of PCWBR2 with epithelial cell receptor integrin $\alpha 2/\beta 1$ leads to the activation of B cells (NF- κ B) cascade and ultimately promotes cell proliferation and pro-inflammatory immune responses in CRC cells (Long et al., 2019).

Peptostreptococcus stomatis (*P. stomatis*) is another species of *Peptostreptococcus* spp. that is frequently isolated from patients with CRC (Osman et al., 2021). Results of a previously published animal model study indicated that *P. stomatis* can activate the erb-b2 receptor tyrosine kinase 2 (ERBB2)-mitogen-activated protein kinase (MAPK) and promote colonic tumorigenesis (Huang et al., 2024). This bacterium applies its surface protein fructose-1,6-bisphosphate aldolase (FBA) to become attached to CRC cells. The attachment of *P. stomatis* to CRC cells leads to the activation of ERBB2 and downstream of Ras/Raf/MAPK (MEK)- extracellular signal-regulated kinase (ERK) cascades (Figure 8) (Shen et al., 2021). Moreover, it is revealed that *P. stomatis* can inactivate and inhibit the BRAF inhibitor (a member of RAF serine/threonine kinases) (vemurafenib) and receptor tyrosine kinase (RTK) inhibitor (cetuximab, erlotinib), leading to the non-responsiveness to these drugs in CRC. This bacterium can suppress apoptosis and impair the gut barrier function in mouse models of CRC (Osman et al., 2021; Huang et al., 2024).

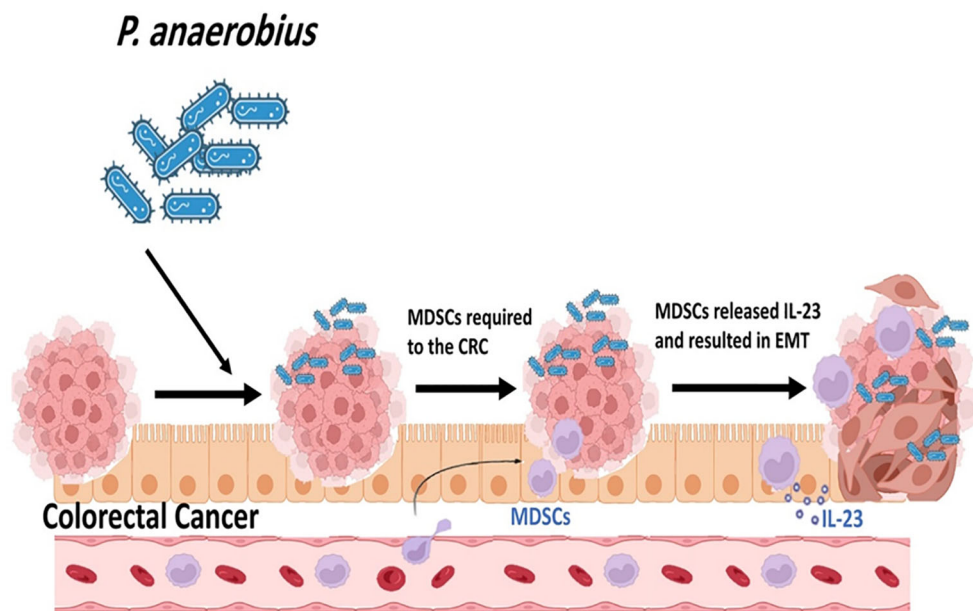


FIGURE 7

P. anaerobius colonization increases Myeloid-derived suppressor cells' (MDSCs) abundances in tumor microenvironment.

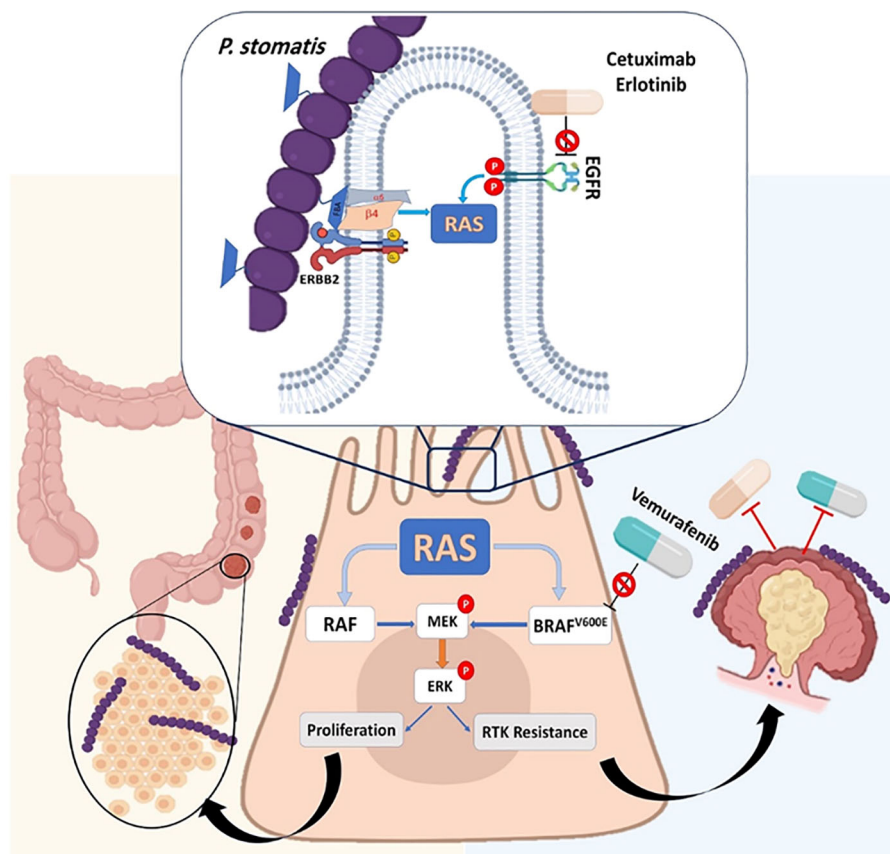


FIGURE 8

P. stomatis can activate the erb-b2 receptor tyrosine kinase 2 (ERBB2)-mitogen-activated protein kinase (MAPK) and promote colonic tumorigenesis. This bacterium applies its surface protein fructose-1,6-bisphosphate aldolase (FBA) to attachment on the CRC cells. The attachment of *P. stomatis* to CRC cells leads to the activation of ERBB2 and downstream Ras/Raf/MAPK (MEK)- extracellular signal-regulated kinase (ERK) cascades. *P. stomatis* can inactivate and inhibit the BRAF inhibitor (a member of RAF serine/threonine kinases) (vemurafenib) and receptor tyrosine kinase (RTK) inhibitor (cetuximab, erlotinib), leading to non-responsiveness to these drugs in CRC.

Conclusion

In general, the exact role of anaerobic gut bacteria in initiation, exacerbation and development of human CRC is not completely identified. In the present study, we searched various databases and gathered the results of previously published studies and clinical and epidemiological signs and the possible mechanisms of some anaerobic gut bacteria in CRC were shown. Different studies have revealed that many anaerobic gut bacteria including *Fusobacterium nucleatum*, *C. difficile*, Enterotoxigenic *B. fragilis*, *P. anaerobius*, *P. stomatis*, etc. could contribute to initiation, development, and exacerbation of CRC in animal models and human. However, more animal models and clinical trial studies are required for a precise conclusion regarding the role of anaerobic gut bacteria in CRC development.

Author contributions

SS: Conceptualization, Data curation, Investigation, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. HS: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. HG: Data curation, Investigation, Software, Writing – original draft. MA: Conceptualization, Data curation, Software, Writing – original draft. TA: Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

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References

- Abraham, A. T., and Padam, S. (2023). Clostridium septicum bacteremia as the presenting sign of colon cancer. *Cureus* 15, e45343. doi: 10.7759/cureus.45343
- Acquaro, P., Tagliabue, F., Confalonieri, G., Faccioli, P., and Costa, M. (2010). Abdominal wall actinomycosis simulating a Malignant neoplasm: Case report and review of the literature. *World J. Gastrointest Surg.* 2, 247–250. doi: 10.4240/wjgs.v2.i7.247
- André, T., Boni, C., Navarro, M., Tabernero, J., Hickish, T., Topham, C., et al. (2009). Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. *J. Clin. Oncol.* 27, 3109–3116. doi: 10.1200/JCO.2008.20.6771
- Araújo, T. G., Mota, S. T. S., Ferreira, H. S. V., Ribeiro, M. A., Goulart, L. R., and Vecchi, L. (2021). Annexin A1 as a regulator of immune response in cancer. *Cells* 10, 2245. doi: 10.3390/cells10092245
- Azimi, T., Nasiri, M. J., Chirani, A. S., Pouriran, R., and Dabiri, H. (2018). The role of bacteria in the inflammatory bowel disease development: a narrative review. *Apmis* 126, 275–283. doi: 10.1111/apm.12814
- Azimi, T., Nasser, A., Shariati, A., Shideh, S. M., Safari, H., Alizade-Sani, M., et al. (2020). The possible role of pathogenic and non-pathogenic bacteria in initiation and exacerbation of celiac disease; a comprehensive review. *Curr. Pharm. Biotechnol.* 21, 452–466. doi: 10.2174/1389201021666191219160729
- Bartel, D. P. (2018). Metazoan micrornas. *Cell* 173, 20–51. doi: 10.1016/j.cell.2018.03.006
- BGI Genomics (2024). *Global State of Colorectal Cancer Awareness Report* (BGI Center). Available online at: <https://www.bgi.com/global/news/bgi-genomics-2024-global-state-of-colorectal-cancer-awareness-report>.
- Bickerton, S., Awopetu, A., Abood, A., Lee, H., Lane, T., and Reid, A. (2022). A traumatic Clostridium septicum myonecrosis presenting as upper limb ischaemia in a patient with undiagnosed bowel cancer. *Ann. R. Coll. Surg. Engl.* 104, e95–e97. doi: 10.1308/rcsann.2021.0153
- Bostanghadiri, N., Razavi, S., Shariati, A., Talebi, M., Mirkalantari, S., Emami Razavi, A., et al. (2023). Exploring the interplay between *Fusobacterium nucleatum* with the expression of microRNA, and inflammatory mediators in colorectal cancer. *Front. Microbiol.* 14. doi: 10.3389/fmicb.2023.1302719
- Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., et al. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 74, 229–263. doi: 10.3322/caac.21834
- Breau, K. A. (2024). *Actinomyces odontolyticus*: from carries to colorectal cancer. *Cell Mol. Gastroenterol. Hepatol.* 17, 879–880. doi: 10.1016/j.jcmgh.2024.02.009
- Brennan, C. A., and Garrett, W. S. (2019). *Fusobacterium nucleatum*—symbiont, opportunist and oncobacterium. *Nat. Rev. Microbiol.* 17, 156–166. doi: 10.1038/s41579-018-0129-6
- Cao, W., Qin, K., Li, F., and Chen, W. (2024). Comparative study of cancer profiles between 2020 and 2022 using global cancer statistics (GLOBOCAN). *J. Natl. Cancer Cent.* 4, 128–134. doi: 10.1016/j.jncc.2024.05.001
- Cao, Y., Wang, Z., Yan, Y., Ji, L., He, J., Xuan, B., et al. (2021). Enterotoxigenic *Bacteroides fragilis* promotes intestinal inflammation and Malignancy by inhibiting exosome-packaged miR-149-3p. *Gastroenterology* 161, 1552–1566.e12. doi: 10.1053/j.gastro.2021.08.003

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- 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, S., Zhang, L., Li, M., Zhang, Y., Sun, M., Wang, L., et al. (2022). Fusobacterium nucleatum reduces METTL3-mediated m6A modification and contributes to colorectal cancer metastasis. *Nat. Commun.* 13, 1248. doi: 10.1038/s41467-022-28913-5
- Cheng, W. T., Kantilal, H. K., and Davamani, F. (2020). The mechanism of Bacteroides fragilis toxin contributes to colon cancer formation. *Malays J. Med. Sci.* 27, 9–21. doi: 10.21315/mjms2020.27.4.2
- Cheng, W., Li, F., and Yang, R. (2024). The roles of gut microbiota metabolites in the occurrence and development of colorectal cancer: Multiple insights for potential clinical applications. *Gastro Hep Adv.* 3, 855–870. doi: 10.1016/j.gastha.2024.05.012
- Cheng, Y., Ling, Z., and Li, L. (2020). The intestinal microbiota and colorectal cancer. *Front. Immunol.* 11. doi: 10.3389/fimmu.2020.615056
- Conde-Pérez, K., Aja-Macaya, P., Buettas, E., Trigo-Tasende, N., Nasser-Ali, M., Rumbo-Feal, S., et al. (2024). The multispecies microbial cluster of Fusobacterium, Parvimonas, Bacteroides and Faecalibacterium as a precision biomarker for colorectal cancer diagnosis. *Mol. Oncol.* 18, 1093–1122. doi: 10.1002/1878-0261.13604
- Dadgar-Zankbar, L., Shariati, A., Bostanghadiri, N., Elahi, Z., Mirkalantari, S., Razavi, S., et al. (2023). Evaluation of enterotoxigenic Bacteroides fragilis correlation with the expression of cellular signaling pathway genes in Iranian patients with colorectal cancer. *Infect. Agent Cancer* 18, 48. doi: 10.1186/s13027-023-00523-w
- Dicks, L. M., Mikkelsen, L. S., Brandsborg, E., and Marcotte, H. (2019). Clostridium difficile, the difficult “Kloster” fuelled by antibiotics. *Curr. Microbiol.* 76, 774–782. doi: 10.1007/s00284-018-1543-8
- Du, X., Li, Q., Tang, Z., Yan, L., Zhang, L., Zheng, Q., et al. (2022). Alterations of the gut microbiome and fecal metabolome in colorectal cancer: implication of intestinal metabolism for tumorigenesis. *Front. Physiol.* 13. doi: 10.3389/fphys.2022.854545
- Du, F., Qiao, C., Li, X., Chen, Z., Wu, S., Hu, S., et al. (2019). Forkhead box K2 promotes human colorectal cancer metastasis by upregulating ZEB1 and EGFR. *Theranostics* 9, 3879–3902. doi: 10.7150/thno.31716
- Eskandari-Malayeri, F., Rezaei, M., Narimani, T., Esmail, N., and Azizi, M. (2024). Investigating the effect of Fusobacterium nucleatum on the aggressive behavior of cancer-associated fibroblasts in colorectal cancer. *Discov. Oncol.* 15, 292. doi: 10.1007/s12672-024-01156-0
- Fettucciari, K., Fruganti, A., Stracci, F., Spaterna, A., Marconi, P., and Bassotti, G. (2023). Clostridioides difficile toxin B induced senescence: A new pathologic player for colorectal cancer? *Int. J. Mol. Sci.* 24, 8155. doi: 10.3390/ijms24098155
- Fujiwara-Tani, R., Sasaki, T., Luo, Y., Goto, K., Kawahara, I., Nishiguchi, Y., et al. (2018). Anti-claudin-4 extracellular domain antibody enhances the antitumoral effects of chemotherapeutic and antibody drugs in colorectal cancer. *Oncotarget*. 9, 37367–37378. doi: 10.18632/oncotarget.26427:37367-37378
- Fukunaga, Y., Maeda, H., Yamaguchi, S., Tsutsui, M., Okamoto, K., Tanaka, T., et al. (2024). An actinomycosis infection resembling peritoneal dissemination of rectal cancer: a case report. *Surg. Case Rep.* 10, 207. doi: 10.1186/s40792-024-02005-6
- Gu, J., Lv, X., Li, W., Li, G., He, X., Zhang, Y., et al. (2023). Deciphering the mechanism of Peptostreptococcus anaerobius-induced chemoresistance in colorectal cancer: The important roles of MDSC recruitment and EMT activation. *Front. Immunol.* 14. doi: 10.3389/fimmu.2023.1230681
- Guo, P., Tian, Z., Kong, X., Yang, L., Shan, X., Dong, B., et al. (2020). FadA promotes DNA damage and progression of Fusobacterium nucleatum-induced colorectal cancer through up-regulation of chk2. *J. Exp. Clin. Cancer Res.* 39, 202. doi: 10.1186/s13046-020-01677-w
- Gur, C., Ibrahim, Y., Isaacson, B., Yamin, R., Abed, J., Gamliel, M., et al. (2015). Binding of the Fap2 protein of Fusobacterium nucleatum to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* 42, 344–355. doi: 10.1016/j.immuni.2015.01.010
- Hong, B.-Y., Chhaya, A., Robles, A., Cervantes, J., and Tiwari, S. (2024). The role of Fusobacterium nucleatum in the pathogenesis of colon cancer. *J. Invest. Med.* 72, 819–827. doi: 10.1177/10815589241277829
- Huang, P., Ji, F., Cheung, A. H.-K., Fu, K., Zhou, Q., Ding, X., et al. (2024). Peptostreptococcus stomatis promotes colonic tumorigenesis and receptor tyrosine kinase inhibitor resistance by activating ERBB2-MAPK. *Cell Host Microbe* 32, 1365–1379.e10. doi: 10.1016/j.chom.2024.07.001
- Huang, C.-Y., and Wang, M.-C. (2020). Clostridium perfringens bacteremia associated with colorectal cancer in an elderly woman. *Turk J. Gastroenterol.* 31, 960–961. doi: 10.5152/tjg.2020.19987
- Hwang, S., Gwon, S.-Y., Kim, M. S., Lee, S., and Rhee, K.-J. (2013). Bacteroides fragilis toxin induces IL-8 secretion in HT29/C1 cells through disruption of E-cadherin junctions. *Immun. Netw.* 13, 213–217. doi: 10.4110/in.2013.13.5.213
- Jahani-Sherafat, S., Azimrad, M., Alebouyeh, M., Amoli, H. A., Hosseini, P., Ghasemian-Safaei, H., et al. (2019). The rate and importance of Clostridium difficile in colorectal cancer patients. *Gastroenterol. Hepatol. Bed Bench.* 12, 358–363.
- Jank, T., Giesemann, T., and Aktories, K. (2007). Rho-glucosylating Clostridium difficile toxins A and B: new insights into structure and function. *Glycobiology* 17, 15R–22R. doi: 10.1093/glycob/cwm004
- Jena, P. K., Trivedi, D., Chaudhary, H., Sahoo, T. K., and Seshadri, S. (2013). Bacteriocin PJ4 active against enteric pathogen produced by Lactobacillus helveticus PJ4 isolated from gut microflora of wistar rat (Rattus norvegicus): partial purification and characterization of bacteriocin. *Appl. Biochem. Biotechnol.* 169, 2088–2100. doi: 10.1007/s12010-012-0044-7
- Jessamy, K., Ojevve, F. O., Ubagharaji, E., Sharma, A., Anozie, O., Gilman, C. A., et al. (2016). Clostridium septicum: an unusual link to a lower gastrointestinal bleed. *Case Rep. Gastroenterol.* 10, 489–493. doi: 10.1159/000448881
- Jo, M., Hwang, S., Lee, C.-G., Hong, J.-E., Kang, D.-H., Yoo, S.-H., et al. (2023). Promotion of colitis in B cell-deficient C57BL/6 mice infected with enterotoxigenic bacteroides fragilis. *Int. J. Mol. Sci.* 25, 364. doi: 10.3390/ijms25010364
- Justesen, U. S., Nielsen, S. L., Jensen, T. G., Dessau, R. B., Møller, J. K., Coia, J. E., et al. (2022). Bacteremia with anaerobic bacteria and association with colorectal cancer: a population-based cohort study. *Clin. Infect. Dis.* 75, 1747–1753. doi: 10.1093/cid/ciac259
- Kajihara, T., Yahara, K., Kitamura, N., Hirabayashi, A., Hosaka, Y., and Sugai, M. (2023). Distribution, trends, and antimicrobial susceptibility of Bacteroides, Clostridium, Fusobacterium, and Prevotella species causing bacteremia in Japan during 2011–2020: a retrospective observational study based on national surveillance data. *Open Forum Infect. Dis.* 10, ofad334. doi: 10.1093/ofid/ofad334
- Każmierczak-Siedlecka, K., Dąca, A., Fic, M., van de Wetering, T., Folwarski, M., and Makarewicz, W. (2020). Therapeutic methods of gut microbiota modification in colorectal cancer management—fecal microbiota transplantation, prebiotics, probiotics, and synbiotics. *Gut Microbes* 11, 1518–1530. doi: 10.1080/19490976.2020.1764309
- Kennedy, C. L., Krejany, E. O., Young, L. F., O’Connor, J. R., Awad, M. M., Boyd, R. L., et al. (2005). The α -toxin of Clostridium septicum is essential for virulence. *Mol. Microbiol.* 57, 1357–1366. doi: 10.1111/j.1365-2958.2005.04774.x
- Khodaverdi, N., Zeighami, H., Jalilvand, A., Haghi, F., and Hesami, N. (2021). High frequency of enterotoxigenic Bacteroides fragilis and Enterococcus faecalis in the paraffin-embedded tissues of Iranian colorectal cancer patients. *BMC Cancer* 21, 1353. doi: 10.1186/s12885-021-09110-x
- Kim, M.-J., Yoon, J.-H., and Ryu, J.-H. (2016). Mitophagy: a balance regulator of NLRP3 inflammasome activation. *BMB Rep.* 49, 529–535. doi: 10.5483/BMBRep.2016.49.115
- Kohya, R., Murai, T., Taguchi, Y., Sawai, K., Takehara, M., Nagahama, M., et al. (2022). An autopsy case of rapidly aggravated clostridium perfringens septicemia with colorectal cancer. *Case Rep. Infect. Dis.* 2022, 1071582. doi: 10.1155/2022/1071582
- Koliarakis, I., Lagkouravos, I., Vogiatzoglou, K., Tsamandouras, I., Intze, E., Messaritakis, I., et al. (2024). Circulating bacterial DNA in colorectal cancer patients: the potential role of fusobacterium nucleatum. *Int. J. Mol. Sci.* 25, 9025. doi: 10.3390/ijms25169025
- Könönen, E., and Wade, W. G. (2015). Actinomyces and related organisms in human infections. *Clin. Microbiol. Rev.* 28, 419–442. doi: 10.1128/CMR.00100-14
- Lintin, L., Wheeler, R., Whiston, R., Gordon, A., Berry, D., and Torkington, J. (2014). Mycotic thoracic aortic arch aneurysm from haematogenous spread of Clostridium septicum due to metastatic colorectal cancer: a survival guide. *J. Surg. Case Rep.* 2014, rju117. doi: 10.1093/jscr/rju117
- Liu, Y., Wong, C. C., Ding, Y., Gao, M., Wen, J., Lau, H. C.-H., et al. (2024). Peptostreptococcus anaerobius mediates anti-PD1 therapy resistance and exacerbates colorectal cancer via myeloid-derived suppressor cells in mice. *Nat. Microbiol.* 9, 1467–1482. doi: 10.1038/s41564-024-01695-w
- Liu, K., Yang, X., Zeng, M., Yuan, Y., Sun, J., He, P., et al. (2021). The role of fecal Fusobacterium nucleatum and pks+ Escherichia coli as early diagnostic markers of colorectal cancer. *Dis. Markers*. 2021, 1171239. doi: 10.1155/2021/1171239
- Liu, H., Yu, Y., Dong, A., Elsbahy, M., Yang, Y. W., and Gao, H. (2024). Emerging strategies for combating Fusobacterium nucleatum in colorectal cancer treatment: Systematic review, improvements and future challenges. *Explor. (Beijing)*. 4, 20230092. doi: 10.1002/EXP.20230092
- Long, X., Wong, C., Tong, L., Chu, E., Ho Szeto, C., Go, M., et al. (2019). Peptostreptococcus anaerobius promotes colorectal carcinogenesis and modulates tumour immunity. *Nat. Microbiol.* 4, 2319–2330. doi: 10.1038/s41564-019
- Luo, M., Li, Q., Gu, Q., and Zhang, C. (2024). Fusobacterium nucleatum: a novel regulator of antitumor immune checkpoint blockade therapy in colorectal cancer. *Am. J. Cancer Res.* 14, 3962–3975. doi: 10.62347/MYZA2640
- Magat, E. M., Balanag, G. A., Cariño, A. M., Fellizar, A., Ortin, T. S., Guevarra, L. Jr., et al. (2020). Clostridioides difficile antibody response of colorectal cancer patients versus clinically healthy individuals. *Biosci. Microbiota Food Health* 39, 123–127. doi: 10.12938/bmfh.2020-010
- Martin-Gallausiaux, C., Garcia-Weber, D., Lashermes, A., Larraufie, P., Marinelli, L., Teixeira, V., et al. (2022). Akkermansia muciniphila upregulates genes involved in maintaining the intestinal barrier function via ADP-heptose-dependent activation of the ALPK1/TIFA pathway. *Gut Microbes* 14, 2110639. doi: 10.1080/19490976.2022.2110639
- Martin-Gallausiaux, C., Salesse, L., Garcia-Weber, D., Marinelli, L., Beguet-Crespel, F., Brochard, V., et al. (2024). Fusobacterium nucleatum promotes inflammatory and anti-apoptotic responses in colorectal cancer cells via ADP-heptose release and ALPK1/TIFA axis activation. *Gut Microbes* 16, 2295384. doi: 10.1080/19490976.2023.2295384

- Milivojevic, M., Dangeard, A.-S., Kasper, C. A., Tschon, T., Emmenlauer, M., Pique, C., et al. (2017). ALPK1 controls TIFA/TRAF6-dependent innate immunity against heptose-1, 7-bisphosphate of gram-negative bacteria. *PLoS Pathog.* 13, e1006224. doi: 10.1371/journal.ppat.1006224
- Miyakawa, Y., Otsuka, M., Shibata, C., Seimiya, T., Yamamoto, K., Ishibashi, R., et al. (2024). Gut bacteria-derived membrane vesicles induce colonic dysplasia by inducing DNA damage in colon epithelial cells. *Cell Mol. Gastroenterol. Hepatol.* 17, 745–767. doi: 10.1016/j.jcmgh.2024.01.010
- Mohammadi, M., Mirzaei, H., and Motalebi, M. (2022). The role of anaerobic bacteria in the development and prevention of colorectal cancer: A review study. *Anaerobe* 73, 102501. doi: 10.1016/j.anaerobe
- Nam, H.-J., Kang, J.-K., Chang, J.-S., Lee, M.-S., Nam, S.-T., Jung, H.-W., et al. (2012). Cells transformed by PLC- γ 1 overexpression are highly sensitive to Clostridium difficile toxin A-induced apoptosis and mitotic inhibition. *J. Microbiol. Biotechnol.* 22, 50–57. doi: 10.4014/jmb.1107.07018
- Nanjappa, S., Shah, S., and Pabbathi, S. (2015). Clostridium septicum gas gangrene in colon cancer: importance of early diagnosis. *Case Rep. Infect. Dis.* 2015, 694247. doi: 10.1155/2015/694247
- Osman, M. A., Neoh, H.-m., Ab Mutalib, N.-S., Chin, S.-F., Mazlan, L., Raja Ali, R.A., et al. (2021). Parvimonas micra, Peptostreptococcus stomatis, Fusobacterium nucleatum and Akkermansia muciniphila as a four-bacteria biomarker panel of colorectal cancer. *Sci. Rep.* 11, 2925. doi: 10.1038/s41598-021-82465-0
- Ou, S., Chen, H., Wang, H., Ye, J., Liu, H., Tao, Y., et al. (2023). Fusobacterium nucleatum upregulates MMP7 to promote metastasis-related characteristics of colorectal cancer cell via activating MAPK (JNK)-AP1 axis. *J. Transl. Med.* 21, 704. doi: 10.1186/s12967-023-04527-3
- Pezeshkian, Z., Nobili, S., Peyravian, N., Shojae, B., Nazari, H., Soleimani, H., et al. (2021). Insights into the role of matrix metalloproteinases in precancerous conditions and in colorectal cancer. *Cancers (Basel)* 13, 6226. doi: 10.3390/cancers13246226
- Pignatelli, P., Nuccio, F., Piattelli, A., and Curia, M. C. (2023). The role of Fusobacterium nucleatum in oral and colorectal carcinogenesis. *Microorganisms* 11, 2358. doi: 10.3390/microorganisms11092358
- Purcell, R. V., Permain, J., and Keenan, J. I. (2022). Enterotoxigenic Bacteroides fragilis activates IL-8 expression through Stat3 in colorectal cancer cells. *Gut Pathog.* 14, 16. doi: 10.1186/s13099-022-00489-x
- Qian, Y., Kang, Z., Zhao, L., Chen, H., Zhou, C., Gao, Q., et al. (2023). Berberine might block colorectal carcinogenesis by inhibiting the regulation of B-cell function by Veillonella parvula. *Chin. Med. J. (Engl.)* 136, 2722–2731. doi: 10.1097/CM9.0000000000002752
- Qu, R., Zhang, Y., Ma, Y., Zhou, X., Sun, L., Jiang, C., et al. (2023). Role of the gut microbiota and its metabolites in tumorigenesis or development of colorectal cancer. *Adv. Sci. (Weinh)* 10, e2205563. doi: 10.1002/adv.202205563
- Rojas-Tapias, D. F., Brown, E. M., Temple, E. R., Onyekaba, M. A., Mohamed, A. M., Duncan, K., et al. (2022). Inflammation-associated nitrate facilitates ectopic colonization of oral bacterium Veillonella parvula in the intestine. *Nat. Microbiol.* 7, 1673–1685. doi: 10.1038/s41564-022-01224-7
- Sasaki, T., Mori, S., Kishi, S., Fujiwara-Tani, R., Ohmori, H., Nishiguchi, Y., et al. (2020). Effect of proton pump inhibitors on colorectal cancer. *Int. J. Mol. Sci.* 21, 3877. doi: 10.3390/ijms21113877
- Shariati, A., Aslani, H. R., Shayesteh, M. R., Taghipour, A., Nasser, A., Safari, H., et al. (2019a). Are viruses and parasites linked to celiac disease? A question that still has no definite answer. *Curr. Pharm. Biotechnol.* 20, 1181–1193. doi: 10.2174/1389201020666190828124924
- Shariati, A., Fallah, F., Pormohammad, A., Taghipour, A., Safari, H., Chirani, A. S., et al. (2019b). The possible role of bacteria, viruses, and parasites in initiation and exacerbation of irritable bowel syndrome. *J. Cell Physiol.* 234, 8550–8569. doi: 10.1002/jcp.27828
- Shen, X., Li, J., Li, J., Zhang, Y., Li, X., Cui, Y., et al. (2021). Fecal enterotoxigenic Bacteroides fragilis-peptostreptococcus stomatis-parvimonas micra biomarker for noninvasive diagnosis and prognosis of colorectal laterally spreading tumor. *Front. Oncol.* 11. doi: 10.3389/fonc.2021.661048
- Slack, F. J., and Chinnaiyan, A. M. (2019). The role of non-coding RNAs in oncology. *Cell.* 179, 1033–1055. doi: 10.1016/j.cell.2019.10.017
- 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
- Tirelli, F., Lorenzon, L., Biondi, A., Langellotti, L., Santoro, G., Agnes, A., et al. (2023). Predictors of Clostridium difficile infection after stoma reversal following TaTME surgery. *Updates Surg.* 75, 1589–1596. doi: 10.1007/s13304-023-01614-4
- Tsoi, H., Chu, E. S., 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
- Valour, F., Sénéchal, A., Dupieux, C., Karsenty, J., Lustig, S., Breton, P., et al. (2014). Actinomycosis: etiology, clinical features, diagnosis, treatment, and management. *Infect. Drug Resist.* 7, 183–197. doi: 10.2147/IDR.S39601
- Wang, M., Li, Y., Yang, X., Liu, Z., Wang, K., Gong, D., et al. (2023a). Effects of metronidazole on colorectal cancer occurrence and colorectal cancer liver metastases by regulating Fusobacterium nucleatum in mice. *Immun. Inflammation Dis.* 11, e1067. doi: 10.1002/iid3.1067
- Wang, M., Wang, Z., Lessing, D. J., Guo, M., and Chu, W. (2023b). Fusobacterium nucleatum and its metabolite hydrogen sulfide alter gut microbiota composition and autophagy process and promote colorectal cancer progression. *Microbiol. Spectr.* 11, e0229223. doi: 10.1128/spectrum.02292-23
- Wang, N., Zhang, L., Leng, X.-X., Xie, Y.-L., Kang, Z.-R., Zhao, L.-C., et al. (2024). Fusobacterium nucleatum induces chemoresistance in colorectal cancer by inhibiting pyroptosis via the Hippo pathway. *Gut Microbes* 16, 2333790. doi: 10.1080/19490976.2024.2333790
- Warny, M., Keates, A. C., Keates, S., Castagliuolo, I., Zacks, J. K., Aboudola, S., et al. (2000). p38 MAP kinase activation by Clostridium difficile toxin A mediates monocyte necrosis, IL-8 production, and enteritis. *J. Clin. Invest.* 105, 1147–1156. doi: 10.1172/JCI7545
- Wu, Q.-L., Fang, X.-T., Wan, X.-X., Ding, Q.-Y., Zhang, Y.-J., Ji, L., et al. (2024). Fusobacterium nucleatum-induced imbalance in microbiome-derived butyric acid levels promotes the occurrence and development of colorectal cancer. *World J. Gastroenterol.* 30, 2018–2037. doi: 10.3748/wjg.v30.i14.2018
- 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, S. S., and Im, Y. C. (2018). Severe abdominopelvic actinomycosis with colon perforation and hepatic involvement mimicking advanced sigmoid colon cancer with hepatic metastasis: a case study. *BMC Surg.* 18, 51. doi: 10.1186/s12893-018-0386-3
- Yu, T., Guo, F., Yu, Y., Sun, T., Ma, D., Han, J., et al. (2017). Fusobacterium nucleatum promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell.* 170, 548–563.e16. doi: 10.1016/j.cell.2017.07.008
- Zeddou, M. (2023). Class I hla allele predicted restricted antigenic coverages for fap2 protein of Fusobacterium nucleatum are associated with colorectal cancer incidence. *Asian Pac J. Cancer Prev.* 24, 3629–3636. doi: 10.31557/APJCP.2023.24.10.3629
- Zeng, X., Liu, L., Zheng, M., Sun, H., Xiao, J., Lu, T., et al. (2016). Pantoprazole, an FDA-approved proton-pump inhibitor, suppresses colorectal cancer growth by targeting T-cell-originated protein kinase. *Oncotarget.* 7, 22460–22473. doi: 10.18632/oncotarget.7984

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