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Gut microbiota, immune cell, colorectal cancer association mediators: a Mendelian randomization study

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Abstract

Background There have been previously reported associations between the gut microbiota, immune cells, and colorectal cancer; however, the specific mechanisms underlying these relationships remain largely unexplored and require further research. Therefore, in this study, we aimed to unravel the interactions between the gut microbiota, immune cells, and colorectal cancer.

Methods The analysis used genome-wide association study (GWAS) data encompassing 207 microbial taxa and 205 functional pathways and data on 731 immune cell phenotypes. Colorectal cancer data on 6 581 cases and 463 421 controls were sourced from the Integrative Epidemiology Unit Open GWAS Project. Univariate inverse-variance weighted Mendelian randomization analysis was used to identify gut microbial taxa associated with colorectal cancer. Mediation analysis was used to identify the mediating role of specific immune cells in the link between gut bacteria and colorectal cancer.

Results Univariate inverse-variance weighted Mendelian randomization analysis revealed that several microbial taxa from the Actinobacteria and Firmicutes phyla were significantly associated with colorectal cancer. Coriobacteriaceae (odds ratio [OR]: 0.84, 95% confidence interval [CI]: 0.72–0.97), Sutterellaceae (OR: 0.88, 95% CI: 0.78–0.99), Eggerthella (OR: 0.91, 95% CI: 0.84–0.99), Coriobacteriales (OR: 0.84, 95% CI: 0.72–0.97), *Collinsella aerofaciens* (OR: 0.85, 95% CI: 0.74–0.99), and *Ruminococcus bromii* (OR: 0.91, 95% CI: 0.83–0.99) were negatively associated with colorectal cancer, whereas Lactobacillales (OR: 1.11, 95% CI: 1.03–1.20), Veillonella (OR: 1.08, 95% CI: 1.01–1.15), and *Bifidobacterium bifidum* (OR: 1.05, 95% CI: 1.00–1.09) were positively associated with colorectal cancer. Mediation analysis revealed that in the causal pathway from *Collinsella aerofaciens* to colorectal cancer, CD127 on $CD28^{+}$ $CD45RA^{-}$ CD8br and human leukocyte antigen (HLA) DR on $CD33^{-}$ HLA DR^{+}, mediated 11.30% and −6.52% of the effect, respectively, and that in the causal pathway from *Ruminococcus bromii* to colorectal cancer, IgD^{-} CD38dim %lymphocyte mediated − 14.80% of the effect.

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Conclusions These results highlight the potential of gut microbiota and immune cell phenotypes as novel treatment strategies for colorectal cancer.

Keywords Gut microbiota, Colorectal cancer, Mendelian randomization, Genome-wide association study, Mediation analysis

Background

Colorectal cancer (CRC) is one of the most common malignant tumors in humans, and the incidence and mortality rates are increasing annually. Globally, CRC stands as the third most common type of cancer and the second most common cause of cancer death among all cancer types [1]. The gut microbiota plays a pivotal role in the onset and progression of CRC. Beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, can generate anticancer substances that curtail the proliferation of cancer cells. Furthermore, they can bolster the body's ability to eradicate cancer cells by modulating the immune system [2, 3]. Conversely, certain detrimental bacteria, including *Escherichia coli* and *Proteus*, can synthesize carcinogens such as polycyclic aromatic hydrocarbons and nitrosamines, which can inflict direct damage on the DNA of intestinal cells, triggering carcinogenesis. Additionally, these harmful bacteria can induce chronic inflammatory responses, and sustained inflammation is a risk factor for cancer [4].

Certain specific components of the gut microbiota can induce the differentiation of T cells into specific subgroups. For example, segmented filamentous bacteria (SFB) can enhance the generation of Th17 cells, whereas the *Clostridium* genus can stimulate the differentiation of gut Treg cells [5, 6]. The gut microbiota can also influence the level of immune response in the body, and the immune status of the body can also cause changes in the gut microbiota [7, 8]. The gut microbiota can mediate the host's urea cycle metabolic pathway, forming an immune-microbiota metabolic axis with the host, thereby regulating the host's immune metabolism and function and affecting the occurrence of CRC [9]. Despite these known associations, the specific mechanisms underlying these relationships remain largely unexplored and require further research.

Mendelian randomization (MR) is a method that uses genetic variants as instrumental variables (IVs) and is considered an effective approach for controlling confounding factors [10, 11]. IVs are genetic variants associated with the exposure but not with the confounders. The MR method estimates the causal effect of the exposure on the outcome through these IVs, thereby helping to circumvent the issue of reverse causation bias. This approach strengthens the causal inference regarding the relationship between exposures and clinical outcomes. We conducted a bidirectional MR investigation and a pair of mediation analyses using summary statistics derived from the most comprehensive and current genome-wide association study (GWAS) data on the gut microbiota, immune cells, and CRC, with the aim of unravelling the interconnections among them.

Methods

Study design

This research was conducted using summary association data derived from prior studies. Because pre-collected, deidentified, aggregated data were used in this study, the requirement for institutional review board approval was waived. All original studies were conducted with ethical approval. Figure 1 depicts the study design. The causal interpretation of MR estimates the IVs must satisfy three fundamental assumptions: (i) The IV has a connection with the gut microbiota; (ii) the IV is not dependent on the confounding factors in the exposure-outcome relationship; (iii) the IV is mutually independent of the confounding factors. This observational study followed the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization reporting guidelines [12].

Data sources

The characteristics of the GWAS data sources are described in Additional File 1: Table S1 [13, 14].

The gut microbiota data originated from a Dutch GWAS study on the gut microbiome, encompassing 207 microbial taxa (five phyla, 10 classes, 13 orders, 26 families, 48 genera, and 105 species) and 205 functional pathways. After stringent quality control procedures, the final sample size was 7 738, incorporating 558 468 single-nucleotide polymorphisms (SNPs). Publicly accessible GWAS summary statistics for each immune trait can be found in the GWAS Catalog (accession numbers GCST0001391 to GCST0002121). The study included a total of 731 immunophenotypes, comprising absolute cell (AC) counts ($n = 118$), median fluorescence intensity (MFI) indicating surface antigen levels ($n = 389$), morphological parameters (MP) ($n = 32$), and relative cell (RC) counts ($n = 192$). Specifically, the MFI, AC, and RC features include B cells, CDCs, mature T cell stages, monocytes, myeloid cells; T cells, B cells, natural killer (TBNK) cells; and Treg panels, whereas the MP feature includes CDC and TBNK panels.

The CRC data were derived from the Integrative Epidemiology Unit Open GWAS Project, involving 470 002 participants of European ancestry, including 6,581 cases

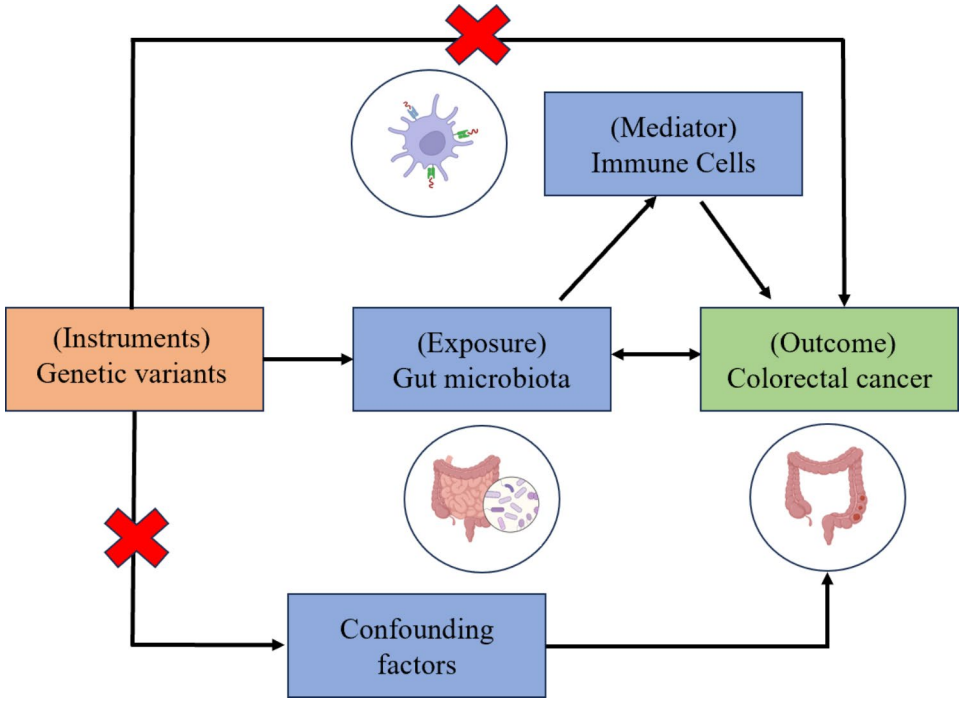

Fig. 1 Assumptions and design of the bidirectional and mediation Mendelian randomization (MR) analyses. Firstly, a two-sample bidirectional MR was performed to investigate the causal relationships between gut microbiota (exposure) and colorectal cancer (outcome). Secondly, immune cells (mediator) were selected for subsequent mediation analyses. Finally, a two-step MR analysis was conducted to detect potential mediating immune cells (Step 1, the effect of gut microbiota on immune cells; Step 2, the effect of immune cells on colorectal cancer), followed by a validation analysis using multivariable MR

and 463,421 controls. The total number included in the analysis was 377 6732, and this dataset covered 24 182 361 SNPs.

Instrumental variable selection

Strictly selected SNPs are included as IVs in MR. Within a 10,000 kb window, genetic variations with genome-wide significance ($P < 5 \times 10^{-8}$) and linkage disequilibrium ($r^2 < 0.001$) are screened to ensure the independence of each IV and exclude the influence of gene pleiotropy [15]. To avoid the bias of weak IVs, the degree of correlation between the chosen IVs and exposure was assessed. F values exceeding 10 were considered evidence of a robust association with the IV and confirmed the lack of weak bias [16].

Statistical analysis

In this study, the inverse-variance weighting (IVW) method was used for single-variable MR analysis, obtaining the overall effect estimate by averaging the effect estimates of each SNP locus [17]. In addition, the weighted median, simple model, weighted model, and MR-Egger regression methods were used as supplements [18–20].

Sensitivity analysis included MR-pleiotropy, MR-Egger, Cochran Q, and one-by-one exclusion test methods to ensure the robustness of the results [21–23]. Finally, this study verified the exclusion assumption through a two-step mediation analysis. First, the two-sample MR method was used to assess the effect of the gut microbiota on the mediator (immune cells), and then the effect of immune cells on the outcome (CRC) was assessed. Finally, the direct and indirect effects were calculated [24].

All statistical analyses were performed using R4.3.0 software and "TwoSampleMR" and "Mendelian Randomization" packages. P values < 0.05 were considered statistically significant.

Results

Instrumental variable selection

Initially, after removing weak IVs (F > 10) and under the premise of $P < 1 \times 10^{-5}$, we found 97, 253, 433, 114, 56, and 950 SNPs related to 201 gut microbes at the levels of order, family, genus, class, phylum, and species, respectively. These 1903 SNPs were selected as IVs for the 201 gut microbe taxa (Additional File 1: Table S2). We then identified 14,696 SNPs related to 721 types of immune cells at the level of F > 10 and $P < 5 \times 10^{-5}$ (Additional File 1: Table S3).

Table 1 Gut microbiota associated with colorectal cancer

Exposure	Outcome	Method	nSNP	Beta	SE	*P*-value	OR
f__Coriobacteriaceae	CRC	IVW	10	-0.176	0.076	0.021	0.838
f__Sutterellaceae	CRC	IVW	8	-0.131	0.061	0.032	0.877
g__Eggerthella	CRC	IVW	6	-0.093	0.041	0.023	0.911
o__Coriobacteriales	CRC	IVW	10	-0.176	0.076	0.021	0.838
o__Lactobacillales	CRC	IVW	12	0.106	0.040	0.008	1.112
s__Bifidobacterium_bifidum	CRC	IVW	16	0.044	0.022	0.045	1.045
s__Collinsella_aerofaciens	CRC	IVW	9	-0.157	0.075	0.037	0.855
s__Ruminococcus_bromii	CRC	IVW	7	-0.099	0.048	0.038	0.906
s__Veillonella_unclassified	CRC	IVW	10	0.075	0.033	0.024	1.077

SNP: single nucleotide polymorphisms; SE: standard error; OR: Odds ratios; CRC: colorectal cancer; IVW: inverse-variance-weighted

Table 2 The reverse mendelian between CRC and gut microbiota

Exposure	Outcome	Method	nSNP	Beta	SE	*P*-value	OR
CRC	f__Coriobacteriaceae	IVW	10	-0.176	0.076	0.021	0.838
CRC	f__Sutterellaceae	IVW	8	-0.131	0.061	0.032	0.877
CRC	g__Eggerthella	IVW	6	-0.093	0.041	0.023	0.911
CRC	o__Coriobacteriales	IVW	10	-0.176	0.076	0.021	0.838
CRC	o__Lactobacillales	IVW	12	0.106	0.040	0.008	1.112
CRC	s__Collinsella_aerofaciens	IVW	9	-0.157	0.075	0.037	0.855
CRC	s__Ruminococcus_bromii	IVW	7	-0.099	0.048	0.038	0.906
CRC	s__Veillonella_unclassified	IVW	10	0.075	0.033	0.024	1.077

SNP: single nucleotide polymorphisms; SE: standard error; OR: Odds ratios; CRC: colorectal cancer; IVW: inverse-variance-weighted

Genetic causality and association between the gut microbiota and CRC

In assessing the causal effects of the gut microbiota on CRC, IVW analysis showed that Coriobacteriaceae (OR: 0.84, 95% CI: 0.72–0.97), Sutterellaceae (OR: 0.88, 95% CI: 0.78–0.99), Eggerthella (OR: 0.91, 95% CI: 0.84–0.99), Coriobacteriales (OR: 0.84, 95% CI: 0.72–0.97) from the Actinobacteria phylum, and *Collinsella aerofaciens* (OR: 0.85; 95% CI, 0.74–0.99) and *Ruminococcus bromii* (OR: 0.91, 95% CI: 0.83–0.99) from the Firmicutes phylum were negatively associated with CRC. Conversely, Lactobacillales (OR: 1.11, 95% CI: 1.03–1.20), *Veillonella* (OR: 1.08, 95% CI: 1.01–1.15) from the Firmicutes phylum, and *Bifidobacterium bifidum* (OR: 1.05, 95% CI: 1.00–1.09) from the Actinobacteria phylum were directly associated with CRC (Table 1). When assessing the causal effect of CRC on the gut microbiota, the reverse MR analysis did not identify any statistically significant causal associations (Table 2).

Mediation analysis of potential immune cells in the two-sample MR

In the two-sample MR, the IVW analysis showed that 35 types of immune cells had a causal relationship with CRC, of which 17 were positively associated with CRC and 18 were negatively associated with CRC (Additional File 1, Table S4). In the previous positive MR analysis, among the nine gut microbes that appeared to have a causal relationship with CRC, two types of microbes were significantly related to three types of immune cells (CD127 on $CD28^+$ $CD45RA^-$ CD8br, human leukocyte antigen (HLA) DR on $CD33^-$ HLA DR^+, and IgD^- CD38dim %lymphocyte) (Additional File 1: Table S5).

The results of the IVW analysis showed that *Collinsella aerofaciens* protected against CRC. It exerted its effect by reducing the level of CD127 on $CD28^+$ $CD45RA^-$ CD8br (OR: 0.74, 95% CI: 0.58–0.93, $P = 0.0091$) and increasing the level of HLA DR on $CD33^-$ HLA DR^+ (OR: 1.37, 95% CI: 1.01–1.87, $P = 0.0446$) (Fig. 2a and b). *Ruminococcus bromii* had a protective effect against CRC (OR: 0.91, 95% CI: 0.83–0.995, $P = 0.0382$); however, it was associated with an increased level of IgD^- CD38dim %lymphocyte, which was a risk factor for CRC (OR: 1.08, 95% CI: 1.03–1.13, $P = 0.0025$) (Fig. 2c).

By calculating the indirect effects and proportions mediated by these immune cells, the mediation analysis showed that in the causal relationship between *Collinsella aerofaciens* and CRC, CD127 on $CD28^+$ $CD45RA^-$ CD8br accounted for 11.30% of the mediating effect, and HLA DR on $CD33^-$ HLA DR^+ accounted for −6.52% of the mediating effect. In the causal relationship between *Ruminococcus bromii* and CRC, IgD^- CD38dim %lymphocyte accounted for −14.80% of the mediating effect. The negative mediation proportions, such as those observed for HLA DR on CD33 − HLA DR + and IgD − CD38dim % lymphocytes, suggest that these immune cells act as negative regulators in the relationship between the microbiota and CRC, potentially

Figure 2A

exposure	outcome	nsnp	method	pval	OR(95% CI)
g__Collinsella.s__Collinsella_aerofaciens	CD127 on CD28+ CD45RA− CD8br	9	MR Egger	0.6413	0.769 (0.267 to 2.216)
		9	Weighted median	0.1051	0.771 (0.563 to 1.056)
		9	Inverse variance weighted	**0.0091**	0.735 (0.583 to 0.926)
		9	Simple mode	0.4238	0.818 (0.512 to 1.306)
		9	Weighted mode	0.4240	0.828 (0.533 to 1.285)
CD127 on CD28+ CD45RA− CD8br	Colorectal cancer	15	MR Egger	0.9488	1.004 (0.879 to 1.148)
		15	Weighted median	0.7490	1.012 (0.940 to 1.089)
		15	Inverse variance weighted	**0.0415**	1.059 (1.002 to 1.119)
		15	Simple mode	0.8682	1.008 (0.919 to 1.106)
		15	Weighted mode	0.8834	1.006 (0.935 to 1.082)
g__Collinsella.s__Collinsella_aerofaciens	Colorectal cancer	9	MR Egger	0.6881	0.846 (0.388 to 1.848)
		9	Weighted median	0.3261	0.924 (0.789 to 1.082)
		9	Inverse variance weighted	**0.0368**	0.855 (0.738 to 0.990)
		9	Simple mode	0.8787	0.981 (0.777 to 1.240)
		9	Weighted mode	0.8224	0.973 (0.775 to 1.222)

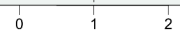

Figure 2B

exposure	outcome	nsnp	method	pval	OR(95% CI)
g__Collinsella.s__Collinsella_aerofaciens	HLA DR on CD33− HLA DR+	9	MR Egger	0.3024	2.227 (0.544 to 9.115)
		9	Weighted median	0.2044	1.320 (0.860 to 2.026)
		9	Inverse variance weighted	**0.0446**	1.374 (1.008 to 1.873)
		9	Simple mode	0.3322	1.388 (0.745 to 2.586)
		9	Weighted mode	0.2810	1.435 (0.778 to 2.648)
HLA DR on CD33− HLA DR+	Colorectal cancer	13	MR Egger	**0.0453**	1.042 (1.005 to 1.080)
		13	Weighted median	**0.0047**	1.040 (1.012 to 1.068)
		13	Inverse variance weighted	**0.0037**	1.033 (1.011 to 1.055)
		13	Simple mode	0.1189	1.043 (0.993 to 1.096)
		13	Weighted mode	**0.0161**	1.040 (1.012 to 1.069)
g__Collinsella.s__Collinsella_aerofaciens	Colorectal cancer	9	MR Egger	0.6881	0.846 (0.388 to 1.848)
		9	Weighted median	0.2971	0.924 (0.796 to 1.072)
		9	Inverse variance weighted	**0.0368**	0.855 (0.738 to 0.990)
		9	Simple mode	0.8811	0.981 (0.773 to 1.245)
		9	Weighted mode	0.8219	0.973 (0.776 to 1.221)

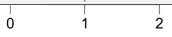

Figure 2C

exposure	outcome	nsnp	method	pval	OR(95% CI)
g__Ruminococcus.s__Ruminococcus_bromii	IgD− CD38dim %lymphocyte	7	MR Egger	0.9256	1.060 (0.330 to 3.403)
		7	Weighted median	0.2867	1.146 (0.892 to 1.472)
		7	Inverse variance weighted	**0.0408**	1.217 (1.008 to 1.468)
		7	Simple mode	0.6883	1.078 (0.760 to 1.529)
		7	Weighted mode	0.7704	1.059 (0.732 to 1.533)
IgD− CD38dim %lymphocyte	Colorectal cancer	22	MR Egger	0.2283	1.071 (0.961 to 1.195)
		22	Weighted median	0.0561	1.072 (0.998 to 1.151)
		22	Inverse variance weighted	**0.0025**	1.077 (1.027 to 1.131)
		22	Simple mode	**0.0368**	1.157 (1.018 to 1.316)
		22	Weighted mode	0.2187	1.069 (0.964 to 1.184)
g__Ruminococcus.s__Ruminococcus_bromii	Colorectal cancer	7	MR Egger	0.2869	0.684 (0.366 to 1.278)
		7	Weighted median	0.1565	0.916 (0.811 to 1.034)
		7	Inverse variance weighted	**0.0382**	0.906 (0.825 to 0.995)
		7	Simple mode	0.4070	0.926 (0.782 to 1.097)
		7	Weighted mode	0.3558	0.923 (0.788 to 1.080)

Fig. 2 Causal relationships between microorganisms and colorectal cancer mediated by immune cells. (**a**) Causal relationship between *Collinsella aerofaciens* and colorectal cancer, and the negative regulation of CD127 on $CD28^+$ $CD45RA^-$ CD8br; (**b**) causal relationship between *Collinsella aerofaciens* and colorectal cancer, and positive regulation of $CD33^-$ $HLA\ DR^+$ cells; (**c**) causal relationship between *Ruminococcus bromii* and colorectal cancer, and positive regulation of IgD^- CD38dim %Lymphocyte

reducing the risk of CRC by inhibiting certain pro-carcinogenic processes (Additional File 1: Table S6).

Mendelian randomization sensitivity analysis results

The scatter plot (Additional File 1: Figures S2 and S5) and funnel plot (Additional File 1: Figures S3 and S6) indicated that the sample selection was relatively balanced with no obvious bias. In addition, Cochran's Q test showed that, except for the heterogeneity between the IV of *Collinsella aerofaciens* and CRC analysis (IVW $P = 0.036643$), the rest of the exposure data and outcome data using the IVW test and the MR-Egger regression Q statistic exhibited no significant heterogeneity ($P = 0.130$ to 0.778). The MR-pleiotropy test result showed that all P values of the MR-Egger intercept were > 0.05 ($P = 0.408$ to 0.981), suggesting that there was no pleiotropy (Table 3). Finally, the leave-one-out test result showed that if any SNP was removed, the results of the remaining SNPs were all on the same side of the null line; that is, removing any SNP did not have a significant effect on the

Table 3 Mendel randomization sensitivity analysis results

Exposure	Outcome	Cochran's Q heterogeneity test				Pleiotropy test		
		MR-Egger Q	MR-Egger *P*	IVW Q	IVW *P*	MR-Egger intercept	MR-Egger SE	MR-Egger *P*
Collinsella_aerofaciens	CRC	16.427	0.021	16.428	0.037	0.001	0.043	0.981
Ruminococcus_bromii	CRC	3.068	0.690	3.865	0.695	0.037	0.041	0.413
CD127 on CD28 + CD45RA- CD8br	CRC	18.767	0.130	19.821	0.136	0.015	0.017	0.408
HLA DR on CD33- HLA DR+	CRC	8.080	0.706	8.456	0.749	-0.005	0.008	0.552
IgDCD38dim %lymphocyte	CRC	22.612	0.308	22.626	0.364	0.001	0.010	0.912
Collinsella_aerofaciens	CD127 on CD28 + CD45RA- CD8br	6.907	0.439	6.915	0.546	-0.005	0.060	0.933
Collinsella_aerofaciens	HLA DR on CD33- HLA DR+	5.518	0.597	5.992	0.648	-0.055	0.080	0.513
Ruminococcus_bromii	IgD- CD38dim %lymphocyte	3.183	0.672	3.238	0.778	0.018	0.077	0.824

CRC: colorectal cancer; IVW: inverse-variance-weighted

results, which further verified the robustness of the MR results (Additional File 1: Figure S1 and S4).

Discussion

This study revealed a causal link between the gut microbiota and CRC, potentially influenced by immune cells. Nine gut microbiota taxa exhibited a causal relationship with CRC; however, the impact of CRC on the gut microbiota was insignificant. Additionally, 35 types of immune cells exhibited a causal relationship with CRC; 17 were positively associated with CRC, whereas 18 were negatively associated with CRC. Notably, some taxa protect against CRC by altering specific immune cell levels. For example, *Collinsella aerofaciens* lowers CD127 levels and raises HLA DR levels, thereby reducing CRC risk. However, *Ruminococcus bromii*, although protective against CRC, was associated with increased levels of IgD^- CD38dim %lymphocyte, which is a CRC risk factor. This underscores the complex interaction between the gut microbiota, immune cells, and CRC. Mediation analysis further quantified the indirect effects of these immune cells and their mediation proportions in the causal relationship between certain taxa of the gut microbiota and CRC.

These results indicate that *Collinsella aerofaciens* and *Ruminococcus bromii* from the Firmicutes phylum were negatively associated with CRC. To our knowledge, no previous studies have directly analyzed the relationship between these two gut microbes and CRC. One study analyzed the structure of the gut microbiota in patients with CRC and healthy controls [25] and found that the abundance of bacteria such as *Collinsella aerofaciens* differed significantly between healthy individuals and patients with CRC. Healthy individuals had a significantly higher gut microbial diversity than patients with CRC, and gut microbial diversity decreased as the stage of CRC increased [25]. Another study found that *Collinsella aerofaciens*, a bacterium from the Actinobacteria phylum, was more abundant in patients with bloody stools [26]. *Ruminococcus bromii* decomposes resistant starch in the gut, forming short-chain fatty acids. These molecules can promote the proliferation and mucosal growth of colonic epithelial cells, inhibit the proliferation of colonic tumor cells, induce apoptosis of differentiated tumor cells, and mediate oncogene expression [27, 28]. A direct relationship between these two gut microbes and CRC has not been identified; however, this study provides genetic evidence of a causal relationship between them and CRC.

Immune cells play a crucial role in the occurrence and development of tumors, with their phenotype and function changing over time, giving the immune microenvironment "tumor-suppressive" and "tumor-promoting" characteristics. An MR study showed that peripheral blood immune cells, especially eosinophils and lymphocytes, are related to the development of CRC [29]. Another MR analysis revealed a genetic causal relationship between immune cells and telomere length. Among 16 types of immune cells, some were positively associated with telomere length, such as $CD28^+CD45RA^+CD8br$ %CD8br, $CD33^+$ HLA DR^-, although the article did not directly mention CRC [30]. However, the relationship between immune cells and telomere length may have important implications for understanding and treating various diseases, including cancer. We identified 35 types of immune cells that have a causal relationship with CRC. Further research is needed to validate these results and explore the specific mechanisms involved.

The emergence of programmed cell death protein 1 (PD-1) inhibitors has led to the recognition that tumors can be controlled, turned into chronic diseases, and possibly cured. However, many patients currently do not respond to PD-1 inhibitors. The gut microbiota provides an alternative option to explore. MR analysis provides

genetic evidence that three types of immune cells mediate the causal effect of the gut microbiota on CRC. In a mouse model of CRC in which the intestines of mice were colonized by *Helicobacter pylori*, the *Helicobacter pylori* did not change the microbial landscape [31]. However, *Helicobacter pylori* increased the tumor infiltration of cytotoxic lymphocytes and inhibited tumor growth, and the infiltration of helper T cells, B cells, and natural killer cells at the tumor site increased [31]. In addition, Chen et al. [9] discovered that gut bacteria such as *Fusobacterium nucleatum* and enterotoxigenic *Bacteroides fragilis* promote the occurrence and development of CRC by promoting inflammation, disrupting the gut microenvironment, and exacerbating metabolic disorders.

Our study also highlights the potential therapeutic value of targeting gut microbiota and immune cells in the management of CRC. By identifying specific gut microbial taxa associated with CRC, we provide a basis for developing targeted probiotic treatments, immunotherapies, and dietary modifications. These interventions can increase the abundance of beneficial bacteria such as Collinsella aerofaciens and Ruminococcus bromii, or modulate the levels of specific immune cells, such as increasing CD127 levels on CD28 + CD45RA – CD8br cells and HLA DR levels on CD33 – HLA DR + cells. Future research should further explore the potential of these intervention strategies in the prevention and treatment of CRC.

Compared with observational studies, this study used MR analysis methods to reduce confounding factors and reverse-causality bias and verified the robustness of the results through sensitivity analysis. Further, mediation analysis quantified the mediating effect of immune cells.

However, this study also has some limitations. As all the data were from European populations, this limits the generalizability of the results. Variations in gut microbiota composition and immune cell interactions across different demographics could significantly influence CRC risk. Second, the single-factor MR analysis assumed that the relationship between gut bacteria, immune cells, and CRC is linear. Therefore, it is necessary to further use individual-level data to investigate potential nonlinear causal relationships between these three factors, and further functional research is needed to explain the specific mechanisms of action of immune cells in this process. Specifically, future studies could explore these complex relationships using nonlinear models or individual-level data. Finally, although the Mendelian randomization framework controls for confounding, the possibility of residual confounding or pleiotropy cannot be entirely excluded. Future research should employ more robust genetic instruments, perform sensitivity analyses (e.g., MR-Egger regression), and integrate additional data sources to mitigate these limitations and validate our findings.

Conclusions

This study provides evidence of possible causal relationships between the gut microbiota, immune cells, and CRC using MR analysis. We identified several gut microbes and immune cells significantly associated with CRC. Notably, certain gut microbes appear to exert their effects on CRC through their influence on specific immune cells. However, these findings need to be confirmed. This research enhances understanding of the complex interplay between the gut microbiota, immune response, and CRC and provides new insights on potential targets for the prevention and treatment of CRC.

Abbreviations

AC	Absolute cell
CI	Confidence interval
CRC	Colorectal cancer
GWAS	Genome-wide association study
HLA	Human leukocyte antigen
IV	Instrumental variable
MFI	Median fluorescence intensity
MP	Morphological parameter
MR	Mendelian randomization
OR	Odds ratio
PD 1	Programmed cell death protein 1
RC	Relative cell
SFB	Segmented filamentous bacteria
SNP	Single-nucleotide polymorphism
TBNK	T cells, B cells, and natural killer cells

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13574-6.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Supplementary Material 11

Supplementary Material 12

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Author contributions

Y.L. and M.Z. contributed equally to this work. They designed the study, performed the experiments, analyzed the data, and wrote the manuscript. S.M. and G.H. performed the experiments and analyzed the data. J.Z. and W.Q. contributed to data analysis and interpretation. X.W. and J.T. supervised the project, contributed to the design of the study, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Data availability
All data used in the present study were obtained from genome-wide association study summary statistics, which were publicly released by genetic consortia.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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