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Novel insights into immune-gut microbiota interactions in colorectal cancer: a Mendelian randomization study

Zenghui Liu[1,2], Xiaohui Zhou[2], Lu Kuang[1], Qijun Chen[1], Jiaxing Zhao[2], Huayu Yin[2], Zeyu Zhou[3], Xuehui Liu[1], Dabin Liu[1], Shaoguo Wu[1*†] and Limei Wu[1,2*†]

Abstract

Background The relationship between immune cells and colorectal cancer (CRC) development has been extensively studied; however, the mediating role of gut microbiota in this relationship remains poorly understood.

Methods We utilized summary data from genome-wide association studies (GWAS) to analyze 731 immune cell phenotypes, 473 gut microbiota, and CRC-related data. A two-step mediation analysis was employed to identify mediating gut microbiota. The primary analysis method was inverse variance weighting (IVW), supplemented by MR-Egger, simple mode, weighted median, and weighted mode analyses. Robustness of the results was ensured through systematic sensitivity analyses.

Results Our analysis identified 13 immune cell phenotypes significantly associated with CRC, including 10 protective factors and 3 risk factors. Additionally, 13 gut microbiota showed significant associations with CRC, comprising 8 protective factors and 5 risk factors. Mediation analysis revealed that 4-gut microbiota (1 order, 1 family, 1 genus, and 1 unclassified) mediated the relationship between immune cells and CRC. For instance, unclassified *CAG – 977* mediated the effects of FSC-A on NK and NKT %lymphocyte on CRC risk, with mediation proportions of 11% and 12.3%, respectively. Notably, 22.3% of the protective effect of EM CD8br %CD8br on CRC was mediated through order *Francisellales*.

Conclusion This study provides evidence for a potential causal relationship between immune cells, gut microbiota, and CRC, highlighting the mediating role of specific gut microbiota. These findings offer new insights into the pathogenesis of CRC and may inform future therapeutic strategies.

Keywords Immune cells, Gut microbiota, Colorectal cancer, Mendelian randomization, Mediation analysis

†Shaoguo Wu and Limei Wu contributed equally to this work.

*Correspondence:
Shaoguo Wu
wsg0930@163.com
Limei Wu
oldrabbit2007@163.com

[1]Department of Clinical Laboratory, The Affiliated Guangzhou Twelfth People's Hospital, Guangzhou Medical University, Guangzhou, Guangdong, China
[2]Department of Immunology, Mudanjiang Medical University, Mudanjiang, Heilongjiang, China
[3]Department of Clinical Laboratory, The Affiliated Hospital of Chengde Medical College, Cengde,, Hebei,, China

Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors of the digestive system, ranking third in incidence and second in cancer-related mortality worldwide, posing a significant public health burden [1]. The development of CRC is influenced by multiple factors. These include genetic predisposition (such as Lynch syndrome and Li-Fraumeni syndrome), environmental factors (such as high-fat diet, lack of physical activity, smoking, and alcohol consumption), and alterations in the gut microbiota [2–4]. Recently, the crucial roles of immune cells and gut microbiota in CRC initiation and progression have attracted increasing attention [5].

Immune cells are key components of immune surveillance and provide protective antitumor immunity. However, in the tumor microenvironment, immune cells may undergo remodeling, contributing to immune evasion by cancer cells [6]. Studies have shown that T cells, natural killer cells, macrophages, and dendritic cells play essential roles in tumor defense [7, 8]. However, these cells can also promote tumor growth and metastasis by secreting cytokines or modulating the immune microenvironment [9]. For instance, peripheral regulatory T cells promote an immunosuppressive microenvironment by secreting IL-10 and TGF-β, thereby inhibiting the activity of effector T cells [10]. Consequently, the status of peripheral immune cells has become an important factor in assessing patients' immune status and prognosis. However, research on the relationship between immune cells and CRC remains limited, with only a few immune cell types being studied.

The gut microbiota, often referred to as the "second genome," is essential for maintaining intestinal homeostasis and regulating immune function [11, 12]. Dysbiosis of the gut microbiota has been shown to significantly promote CRC development [13]. Pathogenic bacteria, such as adherent-invasive *Escherichia coli* and *Fusobacterium nucleatum*, promote carcinogenesis by activating pro-inflammatory pathways and suppressing antitumor immune responses [14]. In contrast, beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, produce metabolites like short-chain fatty acids, which inhibit inflammation and prevent tumor formation [15, 16]. Studies have shown that CD4 + T cells, particularly Th17 cells, can regulate the composition of the gut microbiota by secreting cytokines such as IL-17 and IL-22. IL-17 enhances intestinal barrier function, while IL-22 promotes the secretion of antimicrobial peptides, thereby influencing gut microbiota homeostasis [17, 18]. Moreover, immune system dysregulation, such as a reduction in Treg cells or an imbalance in the Th17/Treg ratio, can lead to changes in the abundance of specific gut microbiota, including *Bacteroides* and *Ruminococcus*, ultimately affecting CRC risk [19–21]. However, the mechanisms by which the gut microbiota modulates immune cell interactions in CRC remain unclear.

Mendelian randomization (MR) is a causal inference method based on genetic variation, enabling the assessment of causal relationships between exposures and disease outcomes [22]. Since genetic variations are determined early in life, MR analysis is less susceptible to confounding factors and reverse causality [23]. Two-sample MR integrates large-scale publicly available genome-wide association study (GWAS) data, providing greater statistical power to explore causal relationships between exposures and outcomes [24]. This approach offers deeper insights into the potential associations between immune cells, gut microbiota, and CRC and further investigates whether the gut microbiota mediates the effect of immune cells on CRC risk. Additionally, to further validate the directionality of the causal relationship, we performed reverse causality analysis to assess whether genetic susceptibility to CRC influences immune cell composition and gut microbiota abundance, thereby providing a more comprehensive causal inference.

Methods

Study design

This study consists of three main components: (1) analysis of the potential causal relationships between 731 immune cell phenotypes and CRC, (2) analysis of the potential causal relationships between 473 gut microbiota and CRC, and (3) mediation analysis of gut microbiota in the pathway from immune cells to CRC (Fig. 1). The inverse variance weighting (IVW) method was used as the primary analytical approach, supplemented by MR-Egger, simple mode, weighted median, and weighted mode analyses. Robustness was assessed using Cochran's Q test, MR-PRESSO test, MR-Egger intercept, and leave-one-out analysis.

MR assumptions

Mendelian randomization analysis is based on the following three core assumptions: (1) Relevance assumption: The selected genetic variants, serving as instrumental variables (IVs), must be significantly associated with the exposure. (2) Independence assumption: The genetic variants must be independent of confounders, ensuring that instrumental variables are not influenced by other factors affecting the exposure-outcome relationship. (3) Exclusion restriction assumption: The genetic variants should affect the outcome only through the exposure and not through any alternative pathways [25]. This study strictly adheres to the STROBE-MR guidelines [26]. Additionally, all GWAS data used in this study are publicly available summary-level datasets, and informed consent was obtained from participants by the respective institutional review boards of the original studies.

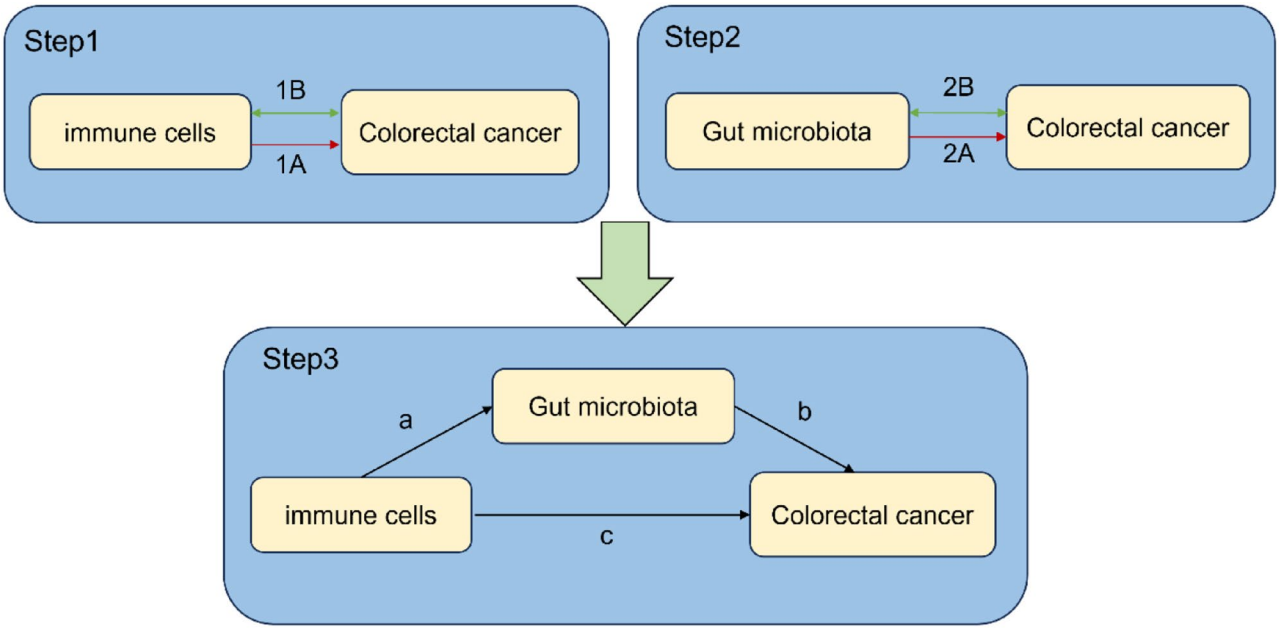


Fig. 1 Study flowchart. Step 1A represents the causal effects of immune cells on CRC. Step 1B represents the bidirectional causal effects between immune cells and CRC. Step 2A represents the causal effects of gut microbiota on CRC. Step 2B represents the bidirectional causal effects between gut microbiota and CRC. Step 3 represents the mediation analysis of gut microbiota in the pathway from immune cells to CRC: path a indicates the causal effect of immune cells on gut microbiota; path b indicates the causal effect of gut microbiota on CRC; and path c represents the total effect of immune cells on CRC

As this study is a secondary analysis of published data, no additional ethical approval is required. Notably, since the samples for CRC, immune cells, and gut microbiota originate from different study cohorts, there is no concern regarding sample overlap.

CRC GWAS data source

The GWAS summary data for CRC were derived from a large-scale European population study conducted by the Finnish FinnGen project. This study utilized the latest R11 CRC dataset, which includes 8,801 CRC cases and 345,118 control samples. The FinnGen database encompasses genetic information from over 400,000 Finnish individuals, aiming to elucidate the genetic basis of various diseases through GWAS and other genetic data [27]. For more details, please visit the FinnGen official website (https://www.finngen.fi/en/access_results).

Immune cell GWAS data source

The IEU OpenGWAS project database of the UK Biobank (https://gwas.mrcieu.ac.uk/) provides summary statistics for immune traits from genome-wide association studies (GWAS) published in 2020, with registered IDs ranging from GCST90001391 to GCST90002121. This immune cell cohort includes data from 3,757 European individuals and covers four characteristic types: absolute cell counts (AC, $n = 118$), relative cell counts (RCs, $n = 192$), median fluorescence intensity reflecting surface antigen levels (MFI and SAL, $n = 389$), and morphological parameters (MPs, $n = 32$). The MFI, AC, and RC features cover mature stages of various immune cells: B cells, cDCs, T cells, monocytes, myeloid cells, TBNK cells (T cells, B cells, and natural killer cells), and Treg cells. The MP features specifically include cDC and TBNK panels [28].

Gut microbiota GWAS data source

The GWAS data for gut microbiota were obtained from EBI database(https://www.ebi.ac.uk/gwas/), based on the analysis of fecal samples from 5,959 Finnish individuals [29]. This dataset provides the characteristics of each gut microbiota. A total of 473 taxa were included in the analysis, encompassing 10 phyla, 18 classes, 24 orders, 58 families, 143 genera, 213 species, and 7 unclassified taxa. The registered IDs range from GCST90032172 to GCST90032644.

Selection of instrumental variables

First, we selected SNPs significantly associated with immune cells and CRC ($P < 5 \times 10^{-8}$). For gut microbiota, the threshold was set at $P < 5 \times 10^{-6}$ to ensure that at least 3 relevant SNPs were retained, thereby maintaining representativeness and robustness. Next, SNPs in linkage disequilibrium were filtered based on the criteria of $r^2 < 0.001$ and distance > 10,000 kb [30]. The strength of the selected IVs was assessed using the F-statistic, where SNPs with an F-statistic < 10 were considered weak instruments and removed from the analysis. We

extracted SNPs outcome data from the IEU OpenGWAS and FinnGen databases. Then, exposure and outcome datasets were harmonized, and palindromic SNPs (SNPs with A/T or G/C alleles) were excluded. After these filtering steps, the remaining SNPs were determined to be valid instrumental variables (IVs). Additionally, we used the Bonferroni-corrected *P*-value as the threshold for statistical significance, which is 6.84×10^{-5} (0.05/731) and 1.05×10^{-4} (0.05/473). $P < 0.05$, but above the Bonferroni-corrected threshold, was considered suggestive of an association.

We extracted the relevant information: chromosome, effect allele (EA), other allele (OA), effect allele frequency (EAF), effect sizes (β), standard error (SE), and P-value. Last, we calculated the explained variance (R^2) and F-statistic parameters to determine whether the identified IVs were strongly associated with exposure. Generally, SNPs with F-statistic parameters < 10 are considered weak instruments [31]. The odds ratio (OR) was used to quantify the strength of the association between genetic IVs and a binary outcome. Specifically, the MR-estimated ORs represented the impact of the exposure factor on the risk of the outcome event. In this study, $R^2 = 2\times EAF\times(1\text{-}EAF)\times\beta^2 / (2\times EAF\times(1\text{-}EAF)\times\beta^2 + 2\times EAF\times(1\text{-}EAF)\times N\times SE^2)$, where N is the effective sample size, and $F = R^2\times(N\text{-}2)/(1\text{-}R^2)$ [32].

Statistical analysis

Data analysis was performed using R software (version 4.4.1) with the MRPRESSO (version 1.0) and TwoSampleMR (version 0.6.8) packages. We performed five distinct MR methods to assess the causal relationship between exposures and outcomes, with IVW being the primary analysis method. To ensure result robustness, sensitivity analyses were performed, considering various assumptions and pleiotropy correction methods. Cochran's Q test was used to identify heterogeneity, with a *P* value < 0.05 indicating significant heterogeneity. Horizontal pleiotropy was evaluated and corrected using the MR-PRESSO test and MR-Egger regression intercept, where a *P* value > 0.05 suggests no significant pleiotropic bias. Additionally, a leave-one-out analysis was conducted by sequentially excluding each SNP to assess its influence on the overall causal estimates. For the Two-step method, we first calculated the total effect of immune cells on CRC(βall), the effect of immune cells on gut microbiota (β1), and the effect of gut microbiota on CRC (β2). The mediation effect (β1*β2) was then calculated, and the direct effect was derived by subtracting the mediation effect from the total effect(βall-β1*β2). *P*-value of < 0.05 was indicative of significant mediation effects.

Results

Causal relationship between immune cell phenotypes and CRC

This study primarily employed the IVW method for MR analysis to systematically evaluate the potential causal relationships between 731 immune cell phenotypes and CRC. Heterogeneity and pleiotropy tests were incorporated to validate the robustness of the results. The analysis revealed 13 immune cell phenotypes significantly associated with CRC, of which 10 were protective factors and 3 were risk factors. Notably, protective phenotypes such as CD39 + CD8br %T cell, CD39 + CD8br AC, CD39 + CD8br %CD8br, EM CD8br %CD8br, and Mo MDSC AC were associated with reduced CRC risk, with odds ratios (ORs) of 0.923 (95% CI: 0.871–0.979), 0.931 (95% CI: 0.875–0.991), 0.932 (95% CI: 0.876–0.990), 0.939 (95% CI: 0.891–0.989), and 0.953 (95% CI: 0.913–0.995), respectively. These results suggest that these phenotypes may have a significant protective effect against CRC. In contrast, FSC – A on NK, CD4 on CD39 + activated Treg, and NKT %lymphocyte were revealed as risk factors for CRC, with ORs of 1.139 (95% CI: 1.026–1.263), 1.104 (95% CI: 1.008–1.209), and 1.098 (95% CI: 1.014–1.190), respectively. These immune phenotypes were positively associated with CRC risk, suggesting their potential involvement in disease onset or progression (Fig. 2A). Additionally, the potential causal relationships between these immune cell phenotypes and CRC were consistent across four other MR analysis methods, confirming the robustness of the IVW method results (Supplementary Fig. 1). Sensitivity analyses showed no significant heterogeneity and pleiotropy, further validating the reliability and robustness of the study findings (Supplementary Tables S1 and S2).

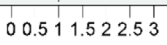
Furthermore, we assessed the reverse causal relationships between CRC and the 13 immune cell phenotypes. The results revealed that CRC had a significant causal effect only on BAFF – R on CD24 + CD27+, with no significant impact observed on the remaining 12 immune phenotypes (Fig. 2B).

Causal relationship between gut microbiota and CRC

Through a two-sample MR analysis, 13 gut microbiota (including 1 order, 1 class, 1 species, 2 families, 6 genera, and 2 unclassified) were revealed as significantly associated with CRC from a total of 473 gut microbiota (Fig. 3A). We revealed 8 gut microbiota that have a protective effect on CRC. Notably, order *Francisellales*, class *Peptococcia*, genus *Demequina*, genus *Halarcobacter*, and family *Thermococcaceae* exhibited significant protective effects, with ORs of 0.429 (95% CI: 0.206–0.892), 0.511 (95% CI: 0.322–0.813), 0.568 (95% CI: 0.328–0.983), 0.641 (95% CI: 0.467–0.880), and 0.648 (95% CI: 0.433–0.969), respectively. Meanwhile, we also revealed 5 gut

A

exposure	outcome	nsnp	method	pval	OR(95% CI)
CD39+ CD8br %T cell	Colorectal cancer	7	IVW	**0.0080**	0.923 (0.871 to 0.979)
CD39+ CD8br AC	Colorectal cancer	6	IVW	**0.0237**	0.931 (0.875 to 0.991)
CD39+ CD8br %CD8br	Colorectal cancer	7	IVW	**0.0231**	0.932 (0.876 to 0.990)
EM CD8br %CD8br	Colorectal cancer	4	IVW	**0.0173**	0.939 (0.891 to 0.989)
Mo MDSC AC	Colorectal cancer	5	IVW	**0.0274**	0.953 (0.913 to 0.995)
BAFF−R on IgD+ CD38− unsw mem	Colorectal cancer	7	IVW	**0.0355**	0.966 (0.935 to 0.998)
BAFF−R on IgD− CD24−	Colorectal cancer	9	IVW	**0.0496**	0.967 (0.935 to 1.000)
CD33 on Im MDSC	Colorectal cancer	6	IVW	**0.0384**	0.968 (0.940 to 0.998)
BAFF−R on CD24+ CD27+	Colorectal cancer	9	IVW	**0.0467**	0.969 (0.939 to 1.000)
BAFF−R on sw mem	Colorectal cancer	10	IVW	**0.0484**	0.969 (0.939 to 1.000)
NKT %lymphocyte	Colorectal cancer	7	IVW	**0.0215**	1.098 (1.014 to 1.190)
CD4 on CD39+ activated Treg	Colorectal cancer	3	IVW	**0.0328**	1.104 (1.008 to 1.209)
FSC−A on NK	Colorectal cancer	3	IVW	**0.0143**	1.139 (1.026 to 1.263)



B

exposure	outcome	nsnp	method	pval	OR(95% CI)
Colorectal cancer	CD39+ CD8br AC	14	IVW	0.2050	0.923 (0.816 to 1.045)
Colorectal cancer	CD4 on CD39+ activated Treg	14	IVW	0.2765	0.930 (0.815 to 1.060)
Colorectal cancer	CD39+ CD8br %T cell	14	IVW	0.3379	0.942 (0.835 to 1.064)
Colorectal cancer	NKT %lymphocyte	14	IVW	0.4110	0.950 (0.841 to 1.073)
Colorectal cancer	CD39+ CD8br %CD8br	14	IVW	0.6564	0.973 (0.861 to 1.098)
Colorectal cancer	Mo MDSC AC	14	IVW	0.7806	0.978 (0.833 to 1.147)
Colorectal cancer	CD33 on Im MDSC	14	IVW	0.9068	0.989 (0.827 to 1.184)
Colorectal cancer	EM CD8br %CD8br	14	IVW	0.6550	1.024 (0.923 to 1.137)
Colorectal cancer	FSC−A on NK	14	IVW	0.6133	1.034 (0.909 to 1.175)
Colorectal cancer	BAFF−R on IgD− CD24−	14	IVW	0.1092	1.110 (0.977 to 1.261)
Colorectal cancer	BAFF−R on sw mem	14	IVW	0.0722	1.117 (0.990 to 1.260)
Colorectal cancer	BAFF−R on CD24+ CD27+	14	IVW	**0.0395**	1.135 (1.006 to 1.280)
Colorectal cancer	BAFF−R on IgD+ CD38− unsw mem	14	IVW	0.0561	1.180 (0.996 to 1.399)

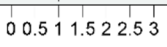


Fig. 2 MR results in forest plots. (**A**) MR forest plot of immune cell phenotypes and CRC. (**B**) Reverse outcome forest plot

microbiota considered risk factors for CRC, including unclassified *GCA – 900,066,135 sp900066135*, genus *Psychroserpens*, genus *Holdemania*, family *Succinivibrionaceae*, and genus *Megamonas*. Their ORs were 1.619 (95% CI: 1.123–2.336), 1.448 (95% CI: 1.093–1.918), 1.274 (95% CI: 1.053–1.541), 1.215 (95% CI: 1.050–1.406), and 1.119 (95% CI: 1.005–1.247), respectively. These findings reveal the complex relationship between specific gut microbiota and CRC and lay the foundation for further mediation analysis. Additionally, the potential causal relationships between these gut microbiota and CRC were consistent across four other MR analysis methods, confirming the robustness of the IVW method results (Supplementary Fig. 2). Furthermore, sensitivity analysis showed no significant heterogeneity and horizontal pleiotropy (Supplementary Tables S3 and S4).

We also assessed the reverse causal relationship between CRC and the 13-gut microbiota. The IVW analysis showed that all *p*-values were greater than 0.05, indicating that CRC did not have a significant impact on the considered gut microbiota (Fig. 3B).

Gut microbiota mediates the effect of immune cells on CRC

To ensure the scientific rigor of the analysis and the reliability of causal inferences, this study excluded cell phenotypes with reverse causal effects. Based on the selected immune cell phenotypes and gut microbiota, we employed a Two-step MR method to calculate mediation effects. Specifically, 12 immune cell phenotypes were used as exposure factors, and 13 gut microbiotas were treated as outcome variables to perform the MR analysis from immune cell phenotypes to gut microbiota (Fig. 4). The results indicated that 8 immune cell phenotypes were significantly associated with four gut microbiotas (including 1 order, 1 family, 1genus, and 1 unclassified). For example, a negative correlation was observed

A

exposure	outcome	nsnp	method	pval	OR(95% CI)
Order Francisellales	Colorectal cancer	3	IVW	**0.0233**	0.429 (0.206 to 0.892)
Class Peptococcia	Colorectal cancer	6	IVW	**0.0046**	0.511 (0.322 to 0.813)
Genus Demequina	Colorectal cancer	6	IVW	**0.0434**	0.568 (0.328 to 0.983)
Genus Halarcobacter	Colorectal cancer	9	IVW	**0.0059**	0.641 (0.467 to 0.880)
Family Thermococcaceae	Colorectal cancer	10	IVW	**0.0347**	0.648 (0.433 to 0.969)
Species Blautia A sp002159835	Colorectal cancer	9	IVW	**0.0081**	0.724 (0.570 to 0.919)
Unclassified CAG−977	Colorectal cancer	12	IVW	**0.0294**	0.740 (0.564 to 0.970)
Genus Alloprevotella	Colorectal cancer	8	IVW	**0.0266**	0.817 (0.684 to 0.977)
Genus Megamonas	Colorectal cancer	27	IVW	**0.0398**	1.119 (1.005 to 1.247)
Family Succinivibrionaceae	Colorectal cancer	25	IVW	**0.0090**	1.215 (1.050 to 1.406)
Genus Holdemania	Colorectal cancer	12	IVW	**0.0126**	1.274 (1.053 to 1.541)
Genus Psychroserpens	Colorectal cancer	12	IVW	**0.0099**	1.448 (1.093 to 1.918)
Unclassified GCA−900066135 sp900066135	Colorectal cancer	7	IVW	**0.0099**	1.619 (1.123 to 2.336)

0 0.5 1 1.5 2 2.5 3

B

exposure	outcome	nsnp	method	pval	OR(95% CI)
Colorectal cancer	Genus Holdemania	14	IVW	0.4062	0.962 (0.879 to 1.053)
Colorectal cancer	Class Peptococcia	14	IVW	0.4662	0.987 (0.952 to 1.023)
Colorectal cancer	Genus Psychroserpens	14	IVW	0.4839	0.988 (0.956 to 1.022)
Colorectal cancer	Species Blautia A sp002159835	14	IVW	0.6122	0.989 (0.946 to 1.033)
Colorectal cancer	Genus Alloprevotella	14	IVW	0.8305	0.994 (0.942 to 1.049)
Colorectal cancer	Unclassified GCA−900066135 sp900066135	14	IVW	0.8097	0.996 (0.965 to 1.029)
Colorectal cancer	Genus Halarcobacter	14	IVW	0.8501	0.997 (0.962 to 1.033)
Colorectal cancer	Genus Megamonas	14	IVW	0.9039	0.997 (0.950 to 1.047)
Colorectal cancer	Family Succinivibrionaceae	14	IVW	0.9344	0.998 (0.962 to 1.036)
Colorectal cancer	Unclassified CAG−977	14	IVW	0.9419	0.999 (0.960 to 1.039)
Colorectal cancer	Family Thermococcaceae	14	IVW	0.9279	0.999 (0.977 to 1.021)
Colorectal cancer	Order Francisellales	14	IVW	0.9003	1.001 (0.980 to 1.024)
Colorectal cancer	Genus Demequina	14	IVW	0.7742	1.003 (0.982 to 1.024)

0 0.5 1 1.5 2 2.5 3

Fig. 3 MR results in forest plots. (**A**) MR forest plot of gut microbiota and CRC. (**B**) Reverse outcome forest plot

exposure	outcome	nsnp	method	pval	OR(95% CI)
FSC−A on NK	Unclassified CAG−977	3	IVW	**0.0264**	0.954 (0.915 to 0.994)
NKT %lymphocyte	Unclassified CAG−977	7	IVW	**0.0185**	0.962 (0.932 to 0.994)
CD33 on Im MDSC	Order Francisellales	5	IVW	**0.0073**	0.989 (0.981 to 0.997)
EM CD8br %CD8br	Order Francisellales	3	IVW	**0.0481**	1.017 (1.000 to 1.034)
BAFF−R on sw mem	Genus Megamonas	8	IVW	**0.0215**	1.027 (1.004 to 1.050)
CD39+ CD8br AC	Family Succinivibrionaceae	4	IVW	**0.0264**	1.039 (1.005 to 1.075)
CD39+ CD8br %T cell	Family Succinivibrionaceae	4	IVW	**0.0271**	1.040 (1.004 to 1.077)
CD39+ CD8br %CD8br	Family Succinivibrionaceae	4	IVW	**0.0265**	1.041 (1.005 to 1.078)

0 0.5 1 1.5 2 2.5 3

Fig. 4 Mediation MR analysis results of gut microbiota

between FSC – A on NK and unclassified *CAG – 977* (OR = 0.954, 95% CI: 0.915–0.994), while a positive correlation was found between BAFF – R on sw mem and genus *Megamonas* (OR = 1.027, 95% CI: 1.004–1.050), among others.

Further analysis showed that a single gut microbiota may be regulated by multiple immune cell phenotypes. Notably, family *Succinivibrionaceae* was positively regulated by CD39 + CD8br AC (OR = 1.039, 95% CI: 1.005–1.075), CD39 + CD8br %T cell (OR = 1.040, 95% CI: 1.004–1.077), and CD39 + CD8br %CD8br (OR = 1.041, 95% CI: 1.005–1.078). Additionally, unclassified *CAG – 977* was negatively regulated by FSC – A on NK (OR = 0.954, 95% CI: 0.915–0.994) and NKT %lymphocyte (OR = 0.962, 95% CI: 0.932–0.994). Moreover, order *Francisellales* was negatively regulated by CD33 on Im MDSC (OR = 0.989, 95% CI: 0.981–0.997) and positively regulated by EM CD8br %CD8br (OR = 1.017, 95%

Table 1 Mediation effects and proportions of each gut microbiota

Immune cell	Gut microbiota	Outcome	Mediated effect	Mediated proportion	Direct effect	*P*-value
FSC-A on NK	Unclassified *CAG-977*	CRC	0.0142 (0.00152, 0.027)	11% (1.17%, 20.8%)	0.116	0.028
NKT %lymphocyte	Unclassified *CAG-977*	CRC	0.0116 (0.00186, 0.0213)	12.3% (1.99%, 22.6%)	0.082	0.019
CD33 on Im MDSC	Order *Francisellales*	CRC	0.00959 (0.00258, 0.0166)	-29.9% (-8.05%, -51.8%)	-0.042	0.007
EM CD8br %CD8br	Order *Francisellales*	CRC	-0.014 (-0.028, -0.000114)	22.3% (44.4%, 0.182%)	-0.049	0.048
BAFF-R on sw mem	Genus *Megamonas*	CRC	0.00296 (0.000369, 0.00555)	-9.37% (-1.17%, -17.6%)	-0.035	0.025
CD39 + CD8br AC	Family *Succinivibrionaceae*	CRC	0.00746 (0.000748, 0.0142)	-10.4% (-1.05%, -19.8%)	-0.079	0.029
CD39 + CD8br %T cell	Family *Succinivibrionaceae*	CRC	0.00766 (0.00073, 0.0146)	-9.6% (-0.915%, -18.3%)	-0.087	0.030
CD39 + CD8br %CD8br	Family *Succinivibrionaceae*	CRC	0.00777 (0.000764, 0.0148)	-11% (-1.08%, -20.9%)	-0.079	0.030

CI: 1.000–1.034). Heterogeneity and pleiotropy analyses further confirmed the robustness and reliability of these conclusions (Supplementary Tables S5 and S6).

Mediation analysis of immune cell phenotypes, gut microbiota, and CRC

After identifying the key mediators affecting CRC and the influence of immune cell phenotypes on these mediators, we conducted a mediation analysis. Specifically, we first assessed the effect of immune cell phenotypes on gut microbiota (β1) and then examined the mediating role of gut microbiota in CRC development using the same approach (β2). Ultimately, we identified four gut microbiotas (including 1 order, 1 family, 1genus, and 1 unclassified) that exert significant mediation effects in the relationship between immune cell phenotypes and CRC (Table 1). The results indicated that the effects of FSC-A on NK and NKT %lymphocyte on CRC risk were partially mediated by unclassified *CAG-977*. The mediation proportions were 11% and 12.3%, respectively. Notably, In the protective effect of EM CD8br %CD8br on CRC, 22.3% is mediated through order *Francisellales*.

Additionally, variations in the abundance of genus *Megamonas* exhibited a negative regulatory effect on the protective function of BAFF-R on sw mem, suggesting that an increased abundance of genus *Megamonas* may partially counteract the protective effect of BAFF-R on sw mem, with a mediation proportion of -9.37%. Moreover, an increased abundance of family *Succinivibrionaceae* was found to partially weaken the protective effects of CD39 + CD8br AC, CD39 + CD8br %T cell, and CD39 + CD8br %CD8br, with mediation proportions of -10.4%, -9.6%, and – 11%, respectively.

In summary, these findings highlight the complex mediating role of gut microbiota in the relationship between immune cell phenotypes and CRC, offering new insights into the mechanisms underlying CRC development.

Discussion

This study employed a robust MR approach to systematically investigate the potential causal relationships among immune cells, gut microbiota, and CRC. The findings indicate that immune cells and gut microbiota play crucial roles in modulating CRC risk, with certain immune cell phenotypes and gut microbiota exerting protective effects, while others may contribute to disease progression by enhancing immune responses or inducing immune dysregulation. Further mediation analysis revealed the complex interactions between immune cells and gut microbiota, highlighting the critical role of gut microbiota as a bridge in the relationship between immune cells and CRC. These findings provide novel insights into the pathogenesis of CRC and lay a theoretical foundation for developing CRC prevention and treatment strategies based on immune and microbiota modulation.

Our study reveals the dual role of various immune cell phenotypes in the development of CRC, which aligns closely with recent research on the dynamic changes in the tumor immune microenvironment [33, 34]. For instance, immune cell phenotypes such as CD39 + CD8br %T cells, CD39 + CD8br AC, and Mo MDSC AC may suppress the occurrence and progression of CRC by enhancing antitumor immune responses. Specifically, CD8 + T cells, as key players in antitumor immunity, may reduce CRC risk through mechanisms such as promoting tumor cell apoptosis and enhancing immune response efficiency [35]. CD39, a critical regulator of the adenosine pathway, may influence the antitumor activity of T cells by modulating adenosine levels in the tumor microenvironment, suggesting its potential as a therapeutic target

for CRC immunotherapy [36]. Furthermore, although myeloid-derived suppressor cells (MDSCs) are generally regarded as immunosuppressive, certain subsets, such as Mo MDSC AC, may exhibit pro-antitumor immune functions under specific conditions [37, 38]. This finding provides new insights for further investigating the complex roles of MDSCs in CRC. On the other hand, FSC – A on NK, CD4 on CD39 + activated Tregs, and NKT %lymphocyte was positively associated with CRC risk, indicating their potential roles in CRC initiation and progression. Previous studies have shown that CD4 + Treg cells play a crucial role in maintaining immune tolerance, and specific subtypes, such as CD4 on CD39 + activated Tregs, may promote CRC through immunosuppressive mechanisms [39]. For example, CD39 + Tregs can hydrolyze ATP to generate adenosine, thereby inhibiting effector T cell activity and facilitating tumor immune evasion [40]. Consequently, targeting CD39 + Tregs may represent a novel immunotherapeutic strategy for CRC. In addition, functional alterations in NK cells may be a critical factor in CRC development [41]. The positive association between FSC – A on NK and CRC suggests that the morphology and function of NK cells may be impaired, thereby reducing their ability to eliminate tumor cells [42]. Thus, restoring NK cell activity could be an important direction for CRC immunotherapy.

Among the protective gut microbiota, order *Francisellales*, class *Peptococcia*, genus *Demequina*, genus H*alarcobacter*, and family *Thermococcaceae* exhibited significant protective effects against CRC. Notably, order *Francisellales* and class *Peptococcia* have been previously linked to beneficial immune modulation, potentially enhancing anti-tumor immune responses through metabolic byproducts such as short-chain fatty acids (SCFAs), which have been shown to exert anti-inflammatory and tumor-suppressive effects in the gut [43, 44]. Similarly, genus *Demequina* and genus *Halarcobacter* may contribute to maintaining gut homeostasis by modulating local immune responses and reducing intestinal inflammation, which is a key driver of CRC development [45, 46]. These protective gut microbiotas could serve as potential candidates for probiotic-based CRC prevention strategies, highlighting the translational potential of microbiome-targeted interventions. Conversely, our study identified several gut microbiotas that were associated with increased CRC risk, including genus *Psychroserpens*, genus *Holdemania*, family *Succinivibrionaceae*, genus *Megamonas*, and an unclassified *GCA – 900,066,135 sp900066135*. Among them, genus *Psychroserpens* and genus *Holdemania* have been implicated in dysbiosis-related inflammatory processes, possibly contributing to CRC pathogenesis through the production of pro-inflammatory metabolites and the disruption of epithelial integrity [47]. These findings suggest that gut microbiota could serve as biomarkers for CRC risk stratification and potential targets for microbiota modulation therapies.

Our study reveals the potential role of the immune-microbiome axis in the pathogenesis of CRC. For instance, the unclassified *CAG-977* was identified as a partial mediator in the association between FSC – A on NK and NKT %lymphocyte with CRC risk, with mediation proportions of 11% and 12.3%, respectively. This finding suggests that the antitumor effects of NK and NKT cells may be mediated through the regulation of *CAG-977* abundance. Additionally, our results indicate that the order *Francisellales* may attenuate the protective effect of CD33 on Im MDSCs, while the protective role of EM CD8br %CD8br appears to be enhanced through changes in order *Francisellales* abundance, with mediation effects of -29.9% and 22.3%, respectively. These findings suggest that order *Francisellales* may serve as a crucial microbial regulator of the CRC immune microenvironment. Similarly, an increased abundance of genus *Megamonas* may partially counteract the protective effect of BAFF-R on sw mem, while the enrichment of family *Succinivibrionaceae* was found to weaken the protective effects of CD39 + CD8br AC, CD39 + CD8br %T cells, and CD39 + CD8br %CD8br. This may occur through immune suppression or changes in immune cell metabolism, reducing the ability of CD39 + CD8br cells to effectively combat CRC.

Although the analysis in this study is robust, there are still some limitations. First, the study relies on summary-level GWAS data, which may limit the capture of individual-level genetic and phenotypic data. Second, while MR helps establish causal relationships, it cannot completely rule out potential confounders or biases. Additionally, the genetic variants used in the study may not fully capture all aspects of the immune environment and gut microbiota. Lastly, the population and environmental factors of the study cohort may affect the generalizability of the results.

Conclusion

In summary, this study evaluates the potential causal relationships between immune cells, gut microbiota, and CRC, revealing significant disruptions in the immune-gut microbiota network linked to the disease. Our findings provide new insights into CRC pathogenesis, highlighting the essential role of gut microbiota in regulating immune-CRC interactions. By elucidating the complex interactions between immune cells and gut microbiota, our research offers novel approaches for the precision treatment and early intervention of CRC.

Abbreviations

CRC	Colorectal cancer
MR	Mendelian randomization
IVW	Inverse variance weighted

IVs	Instrumental variables
GWAS	Genome-wide association studies
EA	Effect allele
OA	Other allele
EAF	Effect allele frequency
β	Effect sizes
SE	Standard error
OR	Odds ratio
CI	Confidence interval
MDSCs	Myeloid derived suppressor cells
SCFAs	Short-chain fatty acids

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

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Author contributions
L.Z.wrote the main manuscript text, K.L.and Z.X. contributed to conceptualization, C. Q. and Z. Z.was responsible for software development and validation, Z.J. and Y.H. contributed to investigation and validation, L.X. and L.D.prepared figures 4, W. S. and W.L. contributed to conceptualization, project administration. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study did not require ethical approval as it exclusively utilized data from publicly accessible databases.

Consent for publication
All authors and their affiliated institutions have agreed to the publication of this article.

Competing interests
The authors declare no competing interests.

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