Current Science
CS 5 (2), 1765-1780 (2025)
Received 23 **Jan** 2025 | **Revised 19 Feb** 2025 | **Accepted 13 March**. 2025 | **Online Available 08 April** 2025
https://doi.org/ 10.52845/CS/2025-5-2-40

Current Science

Current Science

OPEN ACCESS

ISSN(O)2795-8639

ORIGINAL ARTICLE

Casual Association between Immune Cells and Colorectal Cancer: A Mendelian Randomization Study

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Abstract

Colorectal cancer (CRC) is a multifactorial disease influenced by genetic, environmental, and immunological factors. This study employs Mendelian randomization (MR) to investigate the causal associations between immune cell types and the risk of developing colorectal cancer. Utilizing large-scale genomic data, we identified genetic variants associated with various immune cell populations, including T cells, B cells, and macrophages. By leveraging these variants as instrumental variables, we assessed their impact on CRC risk through two-sample MR analysis.

Our examination identified a total of 29 immune cells linked to colorectal cancer (CRC). The risk of CRC was correlated with CCR2 on monocytes (OR = 0.9384, 95% CI = 0.8890– 0.9905, p = 0.0212), Basophil Absolute Count (OR = 1.0466, 95% CI = 1.0027–1.0925, p = 0.0370), CD64 on CD14+ CD16+ monocytes (OR = 0.7853, 95% CI = 0.6616– 0.9321, p = 0.0057), Naive CD4+ T cell Absolute Count (OR = 0.8826, 95% CI = 0.7961– 0.9786, p=0.0177), Immature Myeloid-Derived Suppressor Cells %CD33dim HLA DR- CD66b- (OR = 0.9204, 95% CI = 0.8516– 0.9948, p = 0.0366), CD33dim HLA DR+ CD11b- %CD33dim HLA DR+ (OR = 0.9504, 95% CI = 0.9072– 0.9956, p = 0.0319), and CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+ (OR = 1.0509, 95% CI = 1.0021– 1.1021, p = 0.0403). Notably, while the absolute count of basophils, and CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+ served as a risk factor, CCR2 on monocytes, CD64 on CD14+ CD16+ monocytes, Naive CD4+ T cell Absolute Count, Immature Myeloid-Derived Suppressor Cells %CD33dim HLA DR- CD66b-, and CD33dim HLA DR+ CD11b- %CD33dim HLA DR+ acted as protective factors. These results underline the importance of immune modulation in colorectal carcinogenesis and offer insights into potential therapeutic targets for prevention and treatment. Further research is warranted to elucidate the underlying mechanisms by which immune cells influence CRC development.

Keyword: Colorectal cancer, Immune cell, Mendelian Randomization, GWAS

Introduction

Colorectal cancer (CRC) has stood out as one of the most prevalent solid tumors in the digestive system, contributing to hundreds of thousands of deaths annually. Although recent studies have shown promising advances in CRC treatment, curing this widespread cancer remains a significant challenge [1]. However, growing evidence suggests that molecular profiling to identify mutated genes, followed by targeted therapies, represents an auspicious approach in the field [2]. This precision strategy targets oncogenes or immune cells within tumor tissues and has demonstrated strong therapeutic efficacy and notable advantages [3]. Notably, this approach's significance and therapeutic potential extend beyond immune cells within the tissue [4]. Numerous research have shown the critical role of immune cells in circulating blood, which serve as valuable sources of potential biomarkers [5], influence treatment outcomes [6], and impact several aspects of cancer biology. This trend keeps gaining momentum in colorectal cancer therapy [7-8].

The significant role of the tumor microenvironment (TME) in propelling the evolvement of diverse cancers is increasingly acknowledged, with immune cells within the TME directly modulating the onset of cancer and dissemination [9]. Recent evidence highlights that the TME not only contributes to colorectal cancer (CRC) pathogenesis but also shapes its metastatic behavior and response to immunotherapy [10-14]. Single-cell analyses have delineated the immune landscape of CRC, revealing stark contrasts in immune cell composition between tumor tissue, healthy tissue, and other malignancies [10]. Interestingly, colorectal cancer (CRC) shows higher levels of cytotoxic T cells and regulatory T cells (Tregs), while the numbers of helper T cells are lower [15-16]. Additionally, B cells, natural killer (NK) cells, dendritic cells (DCs), and macrophages have been associated with the progression of CRC and its clinical outcomes [10]. Despite these associations, the causal contribution of immune cell-specific alterations to CRC development remains unresolved.

Mendelian randomization (MR) is an effective epidemiological method used to determine causal relationships by using genetic variants as instrumental variables, which helps reduce biases and the issue of reverse causation [17]. This approach takes advantage of the random distribution genetic variations at conception, ensuring that the associations between exposure-related alleles and results are less susceptible to residual confounding or timing uncertainty [18, 19]. Moreover, given that genetic variants are stable characteristics, Mendelian randomization (MR) analyses successfully distinguish the causal relationships between exposures and outcomes [17]. MR has been shown to be helpful in uncovering disease mechanisms, repurposing existing therapies, and identifying new drug targets [18-20]. In this research, we employed two-sample MR using genome-wide association study (GWAS) data that includes 731 immune cell phenotypes from peripheral blood and the risk associated with colorectal cancer to clarify the cause-related influence of immune cell dynamics in the development of colorectal cancer.

2. Materials and Methods

2.1 Study design

The relationship between colorectal cancer (CRC) and 731 immune cells was examined through two-sample Mendelian randomization (MR) analyses. MR utilized genetic variants as indicators for risk factors. To ensure accurate causal inference, the instrumental variables (IVs) employed in MR had to meet the following three essential criteria: (1) The genetic variant must have a direct association with the exposure; (2) The genetic variant should not be linked with any confounding factors that might exist between the exposure and the outcome; (3) The genetic variant must affect the outcome solely through the exposure, without influencing it through other pathways **(Figure 1)**.

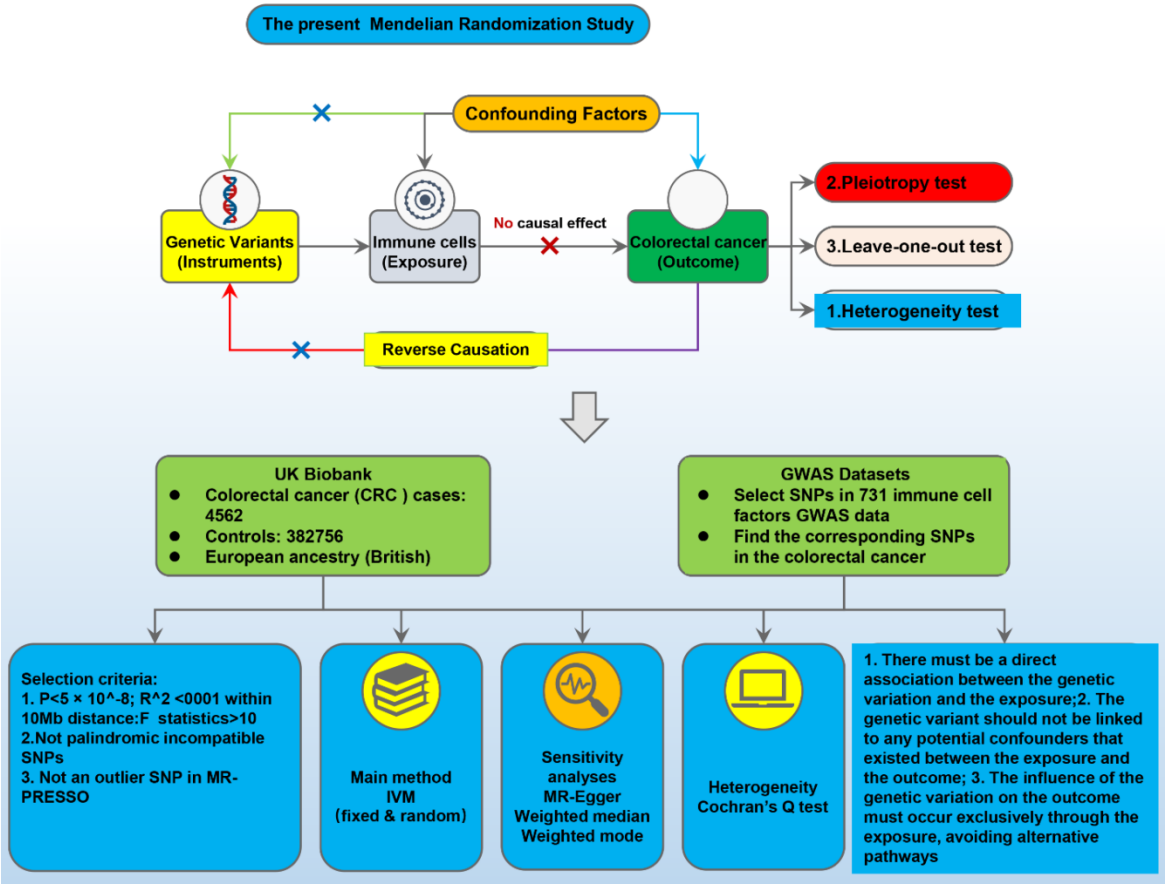

Figure 1. Study Design Flowchart.

2.2 Data Sources for Exposure and Outcome

A summary of GWAS statistics for various immune traits is available to the public through the "GWAS catalog (accession numbers: GCST0001391 to GCST0002121)" [21]. The immune characteristics were identified using keywords related to each type of cancer "(https://gwas.mrcieu.ac.uk/)". In total, 731 immune phenotypes were examined, which included absolute cell (AC) counts, morphological characteristics (MP), median fluorescence intensities (MFI, indicating surface antigen levels), and relative cell (RC) counts. The MFI, RC, and AC aspects included B cells, classical dendritic cells (DCs), mature T cells, monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells), and Treg panels. In contrast, the MP aspect focused on classical DC and TBNK panels. Referring to the ID of colorectal cancer, 387318 European individuals (n = 4562 case patients and 382756 control participants) for CRC-related data were downloaded from the UK Biobank pheweb database (https://pheweb.org/UKB-SAIGE/).

2.3 Instrument Selection

To ensure robust causal inference, we established the following stringent criteria for selecting instrumental variables (IVs): (1) Genetic variants were required to exhibit genome-wide significant associations between all immune cell phenotype ($p < 5 \times 10{-}8$) (2) SNPs with an F-statistic < 10 were removed to ensure adequate instrument strength and reduce weak instrument bias ; (3) Linkage disequilibrium (LD) was reduced by grouping variants at r² < 0.001 within a 10,000 kb window; (4) Potential pleiotropic SNPs—those influencing colorectal cancer risk through pathways independent of immune cell traits—were rigorously excluded using MR-Egger intercept tests and sensitivity analyses. These steps collectively enhanced the validity of the Mendelian randomization assumptions and the reliability of causal estimates.

2.4 Statistical Analysis

2.4.1 Forward MR

Mendelian Randomization (MR) was used with the "TwoSampleMR" package to focus on 731 immune cell phenotypes as our main factor and

looked at colorectal cancer as the outcome. We primarily used inverse variance weighting (IVW) because it gives accurate causal estimates, though it can be influenced by pleiotropy and issues with invalid instruments. We also applied several alternative methods to strengthen our findings, such as the weighted median, MR Egger, weighted mode, and simple mode approaches. The MR Egger method relies on the "InSIDE (INstrument Strength Independent of Direct Effect)" hypothesis, which helps identify and adjust for potential pleiotropy. The weighted median technique is particularly valuable for addressing invalid instrument bias, as it provides reliable causal estimates when more than half of the instrumental variables (IVs) are valid. While the weighted mode methods generally show less bias and lower rates of false positives, they may be less potent for detecting causal relationships.

To visualize our results, we created scatter plots and used the MR PRESSO method to identify any potentially problematic single nucleotide polymorphisms (SNPs) linked to horizontal pleiotropy. We also computed the Cochrane Q statistic to assess the variability within the IVW estimators. Furthermore, we conducted a sensitivity analysis employing the "leave-one-out" approach to determine if excluding each SNP from the analysis affected the overall estimates. Our findings are reported as odds ratios (OR) accompanied by their respective 95% confidence intervals (CI). To validate externally, a Mendelian randomization (MR) analysis was performed with a significance level established at $P < 0.05$. Ultimately, we merged results from two distinct colorectal cancer datasets through a meta-analysis utilizing a random effects model to unify our results. R software (version 4.3.2) was used to perform all analyses.

4. Results

To investigate the causal relationship between colorectal cancer (CRC) and immune profiling, we conducted a two-sample Mendelian randomization analysis using inverse variance weighting (IVW) as our primary method. Our results indicated a link between 29 different immune cell types and CRC (**Figure 2**).

Exposure	Method	No.of SNP	OR(95% CI)	P
CCR2 on monocyte	Inverse variance weighted	12	0.938 (0.889 to 0.991)	0.021
Basophil Absolute Count	Inverse variance weighted	3	1.047 (1.003 to 1.093)	0.037
CD64 on CD14+ CD16+ monocyte	Inverse variance weighted	6	0.785 (0.662 to 0.932)	0.006
Naive CD4+ T cell Absolute Count	Inverse variance weighted	10	0.883 (0.796 to 0.979)	0.018
FSC−A on myeloid Dendritic Cell	Inverse variance weighted	3	0.894 (0.803 to 0.995)	0.041
Immature Myeloid−Derived Suppressor Cells %CD33dim HLA DR− CD66b−	Inverse variance weighted	9	0.920 (0.852 to 0.995)	0.037
Plasmacytoid Dendritic Cell Absolute Count	Inverse variance weighted	2	0.772 (0.646 to 0.923)	0.005
CD33dim HLA DR+ CD11b− %CD33dim HLA DR+	Inverse variance weighted	12	0.950 (0.907 to 0.996)	0.032
CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+	Inverse variance weighted	13	1.051 (1.002 to 1.102)	0.040
CD62L− HLA DR++ monocyte Absolute Count	Inverse variance weighted	6	1.186 (1.031 to 1.363)	0.017
CD3 on HLA DR+ T cell	Inverse variance weighted	17	1.107 (1.039 to 1.179)	0.002
CD3 on CD8+ T cell	Inverse variance weighted	13	1.056 (1.005 to 1.109)	0.030
CD19 on IgD+ CD38− naive B cell	Inverse variance weighted	9	0.941 (0.901 to 0.983)	0.006
CD19 on IgD+ CD24− B cell	Inverse variance weighted	18	0.960 (0.923 to 0.999)	0.046
CD19 on naive−mature B cell	Inverse variance weighted	18	0.957 (0.916 to 1.000)	0.049
CD19 on IgD+ B cell	Inverse variance weighted	15	0.960 (0.922 to 1.000)	0.050
CD20 on unswitched memory B cell	Inverse variance weighted	16	1.069 (1.003 to 1.139)	0.039
HLA DR+ CD4+ T cell Absolute Count	Inverse variance weighted	11	1.123 (1.001 to 1.259)	0.048
CD45RA− CD4+ T cell Absolute Count	Inverse variance weighted	11	0.858 (0.778 to 0.946)	0.002
Lymphocyte %leukocyte	Inverse variance weighted	13	1.102 (1.021 to 1.189)	0.012
CD25++ CD8+ T cell %T cell	Inverse variance weighted	11	0.912 (0.848 to 0.980)	0.013
Activated & resting CD4 regulatory T cell %CD4+ T cell	Inverse variance weighted	19	1.073 (1.015 to 1.134)	0.013
SSC−A on HLA DR+ T cell	Inverse variance weighted	11	0.886 (0.801 to 0.979)	0.018
IgD− CD38− B cell %lymphocyte	Inverse variance weighted	14	0.906 (0.833 to 0.984)	0.019
CD28 on CD39+ secreting CD4 regulatory T cell	Inverse variance weighted	12	0.926 (0.865 to 0.991)	0.026
Unswitched memory B cell %B cell	Inverse variance weighted	19	0.948 (0.900 to 0.998)	0.040
CD25++ CD45RA− CD4 not regulatory T cell %T cell	Inverse variance weighted	14	0.953 (0.910 to 0.998)	0.042
IgD− CD38+ B cell Absolute Count	Inverse variance weighted	12	0.958 (0.918 to 0.999)	0.046
CD127 on CD28+ CD45RA− CD8+ T cell	Inverse variance weighted	9	1.091 (1.001 to 1.190)	0.048

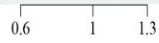

Figure 2. The causal association between immune cell and colorectal cancer. We selected Inverse

variance weighted (IVW) as a primary method p<0.05 showed statistically significant; OR value >1 indicated a risk factor; OR value <1 indicated a protective factor.

The common immune cell associations were subsequently emphasized. In summary, the risk of CRC was linked to CCR2 on monocytes (OR = 0.9384, 95% CI = 0.8890– 0.9905, p = 0.0212), Basophil Absolute Count (OR = 1.0466, 95% CI = 1.0027–1.0925, p = 0.0370), CD64 on CD14+ CD16+ monocytes (OR = 0.7853, 95% CI = 0.6616– 0.9321, p = 0.0057), Naive CD4+ T cell Absolute Count (OR = 0.8826, 95% CI = 0.7961–0.9786, p=0.0177), Immature Myeloid-Derived Suppressor Cells %CD33dim HLA DR- CD66b- (OR = 0.9204, 95% CI = 0.8516– 0.9948, p = 0.0366), CD33dim HLA DR+ CD11b- %CD33dim HLA DR+ (OR = 0.9504, 95% CI = 0.9072– 0.9956, p = 0.0319), and CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+ (OR = 1.0509, 95% CI = 1.0021– 1.1021, p = 0.0403) (**Figure 2**). Additionally, neither the Cochran's Q test nor the MR-Egger intercept indicated pleiotropy or heterogeneity.

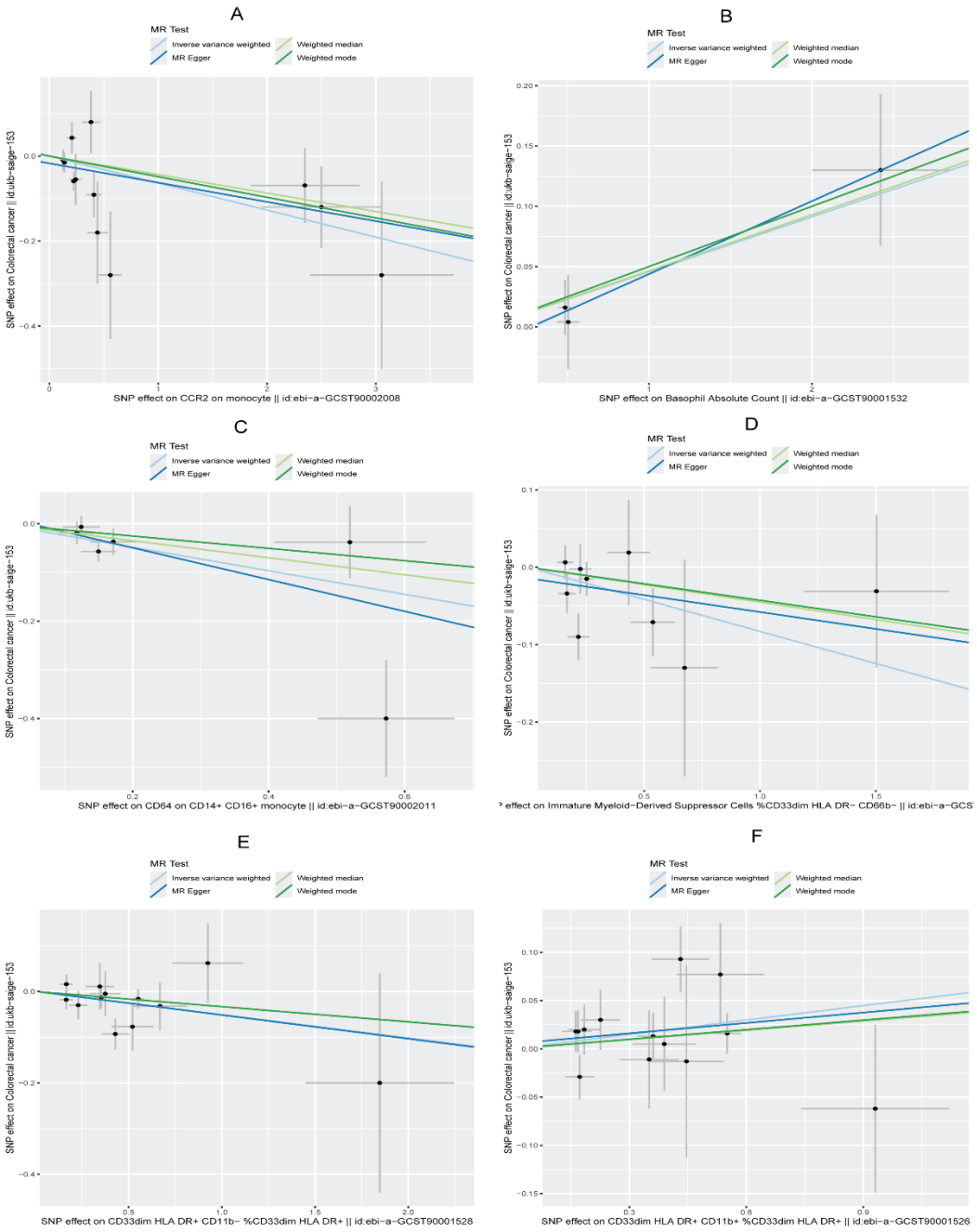

Figure 3 Scatter plot showing the relationship of immune cells with the risk of colorectal cancer (CRC). (A) CCR2**; (B)** Basophils**; (C) CD64 on CD14+ CD16+ monocyte; (D) Immature Myeloid-**

Derived Suppressor Cells %CD33dim HLA DR- CD66b-; (E) CD33dim HLA DR+ CD11b- %CD33dim HLA DR+; (F) CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+.

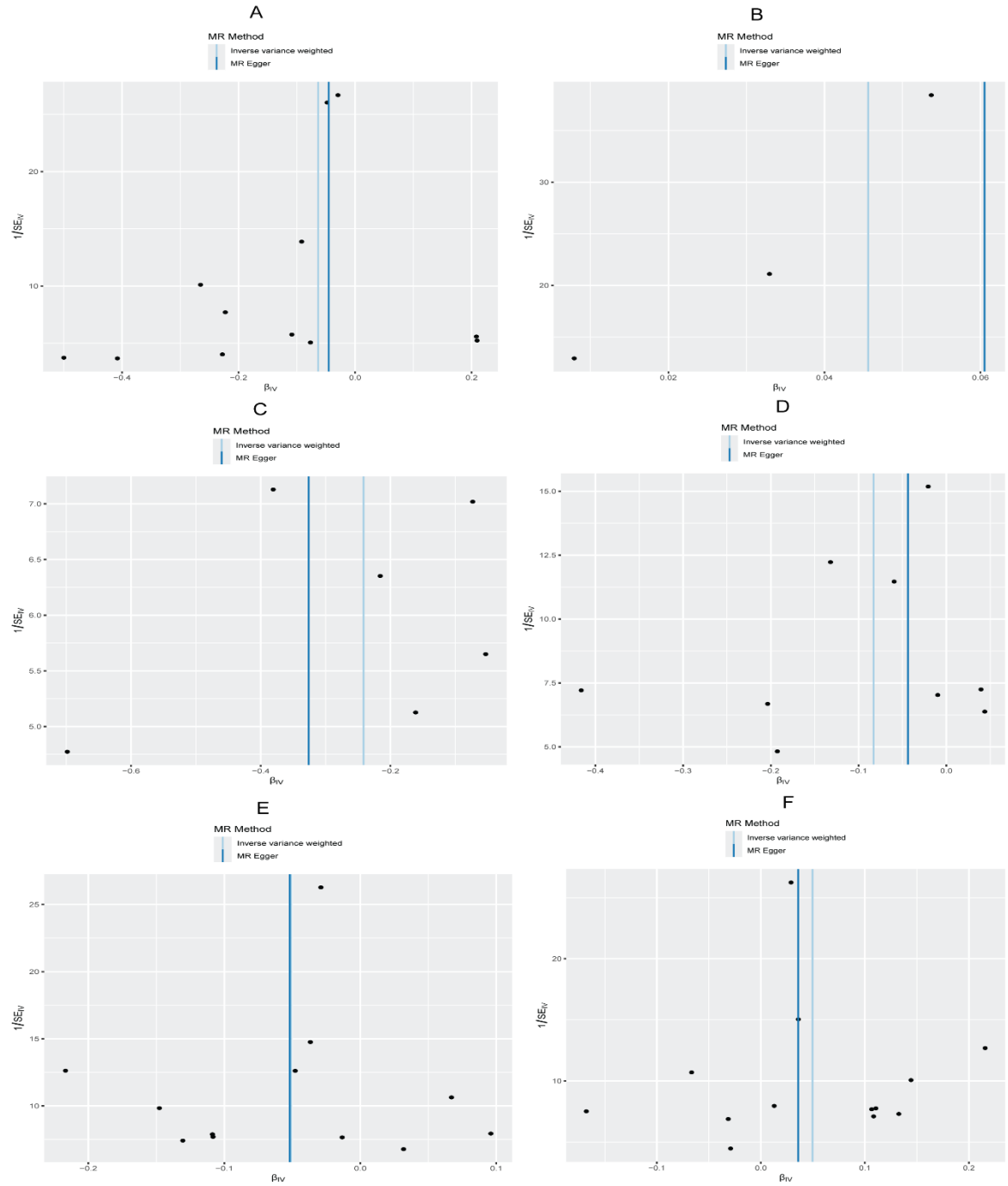

Figure 4 Funnel plot showing the relationship of immune cells with the risk of colorectal cancer (CRC). (A) CCR2; (B) Basophils; (C) CD64 on CD14+ CD16+ monocyte; (D) Immature Myeloid-Derived Suppressor Cells %CD33dim HLA DR- CD66b-; (E) CD33dim HLA DR+ CD11b- %CD33dim HLA DR+; (F) CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+.

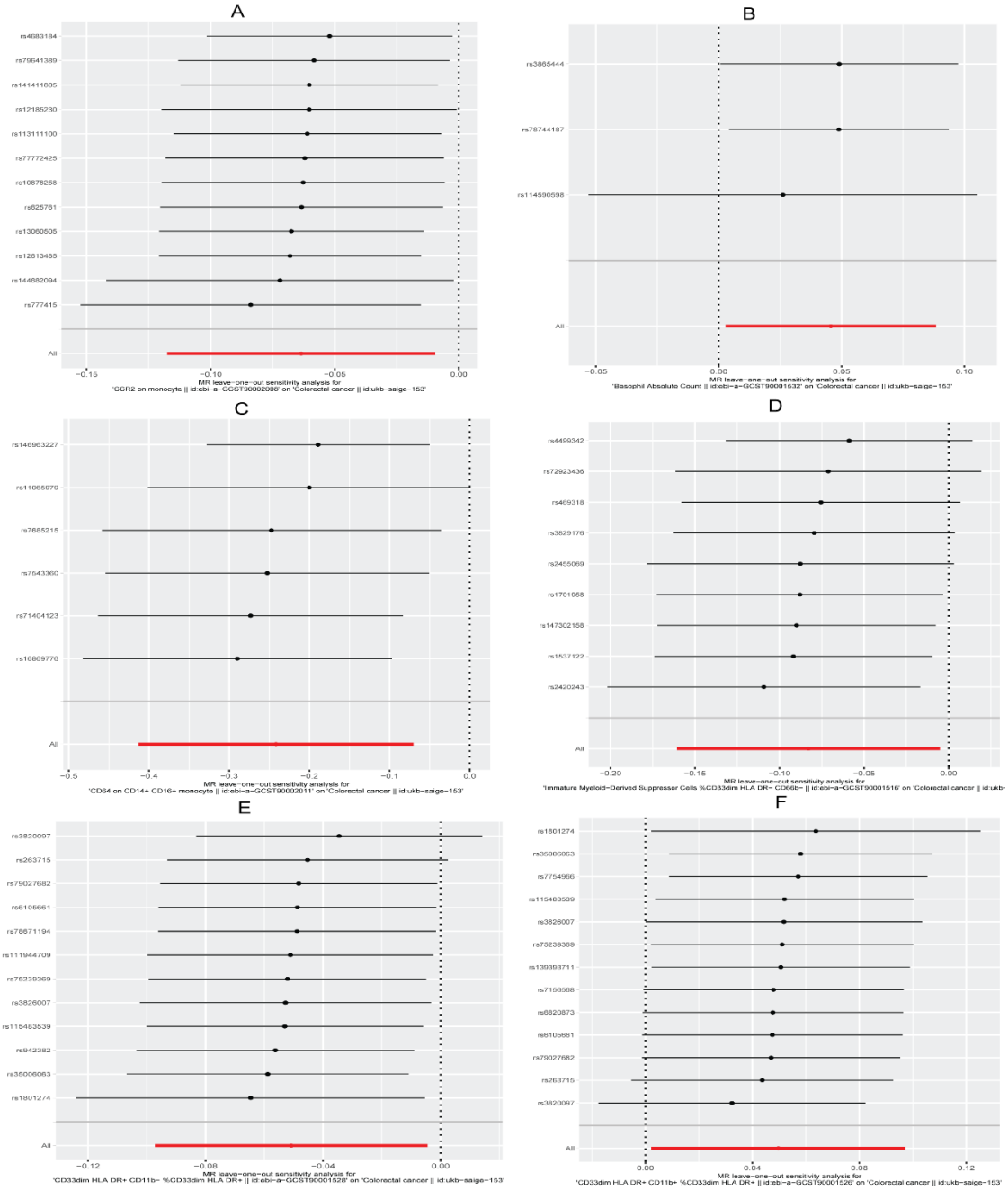

Figure 5 Leave one out showing the relationship of immune cells with the risk of colorectal cancer (CRC). (A) CCR2; (B) Basophils; (C) CD64 on CD14+ CD16+ monocyte; (D) Immature Myeloid-Derived Suppressor Cells %CD33dim HLA DR- CD66b-; (E) CD33dim HLA DR+ CD11b- %CD33dim HLA DR+; (F) CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+.

5. Discussion

MR analysis has frequently revealed possible causal links between risk factors and diseases. This research used MR to support an inverse association between immune cells and colorectal cancer (CRC), with some of the most significant connections explored in the subsequent sections.

CCR2 (C-C chemokine receptor type 2) is predominantly found on the surface of monocytes, macrophages, and lymphocytes. It serves as a regulatory receptor for chemotaxis. It plays a significant role in various diseases by influencing the migration of monocytes from the bone marrow into the bloodstream and their movement to areas of inflammation [22-23]. In the liver, CCR2 is involved in a range of hepato-pathological conditions, including acute liver damage, chronic hepatitis, liver fibrosis, and cirrhosis, as well as tumor development [24-26]. CCR2 may contribute to liver fibrosis by facilitating circulating monocyte movement to injured liver

cells and activating hepatic stellate cells (HSCs) [25-26]. "CCR2 serves as a high-affinity receptor for members of the monocyte chemotactic protein (MCP) family, such as C-C motif chemokine ligand 2 (CCL2), CCL7, CCL8, CCL12 (specific to mice), and CCL13 (specific to humans)" [27-31]. Given its crucial involvement in liver homeostasis and related diseases, research has focused significantly on adjusting CCR2 expression over the past decade. Various inflammatory cytokines, including TNF, IL-6, IL-1β, and IFN-γ, are noted as individual upstream regulators [32]. Previous research indicates that the CCL2/CCR2 pathway promotes the invasion of hepatocellular carcinoma (HCC), which aligns with our current results.

Basophils develop from stem cells and are primarily located in the bone marrow [32-34]. In both humans and mice, interleukin-3 (IL-3) is the main growth factor required for the maturation of basophils [35-38]. Research has shown that it's possible to culture basophils from these species in the lab. This is typically done by incubating bone marrow cells or, in the case of humans, CD34+ precursors with IL-3 for about 10 to 14 days" [35, 39-41]. While IL-3 is vital for the initial development of basophils, other growth factors also play a role in their growth and function. For example, combining FMS-like tyrosine kinase 3 ligand (Flt3L) with IL-3 has been shown to boost basophil production in culture [42]. Additionally, work by Siracusa and colleagues demonstrated that basophils in mice can be generated using thymic stromal lymphopoietin (TSLP). This process involves a receptor called the heterodimeric TSLP receptor (TSLPR/IL-7Rα) [43]. Their findings suggest that IL-3 and TSLP not only foster the development of basophils but also lead to the polarization of two distinct basophil types in mice, each with its own unique gene expression patterns and functions [44].

Basophils have received limited attention due to their sparse distribution and relatively unknown functions. They are crucial in hypersensitivity disorders and type 1 hypersensitivity reactions [45]. Moreover, basophils are attracted to tissues after infections caused by parasites, bacteria, and viruses. The recent development of antibodies that target basophils and the creation of basophil-deficient mouse models have improved the understanding of basophil biology beyond just allergic responses [46]. As a result, there may also be a potential link between basophils and immune responses to tumors [46].

Basophils boost humoral immune function along with the release of intracellular agents that promote anti-tumor immunity [47-49], including the chemokines CCL3 and CCL4, which facilitate CD8+ T-cell infiltration [47] and the chemokines TNF-α and IL-6, which boost inflammatory anti-tumor responses [50-51]. Some studies have indicated that basophils are associated with favorable survival outcomes in melanoma [52-53], ovarian cancer [54], endometrial [55], sarcoma cancers [56], Non-small cell lung cancer (NSCLC) [57], and glioblastoma [58]. While basopenia is linked to a more unfavorable prognosis for CRC (colorectal cancer) [59-61]. Conversely, basophilia has been associated with improved outcomes in patients with melanoma undergoing immunotherapy treatment [53]. These findings are at odds with our results, indicating that basophils were strongly connected to CRC and that increased basophil counts may leads to increased survival.

Ann M. Dvorak's groundbreaking discovery of piecemeal degranulation in basophils related to human pancreatic cancer (PC) laid the groundwork for further studies [62]. Many studies have focused on the role of basophils in human pancreatic ductal adenocarcinoma (PDAC) [63]. In patients diagnosed with PDAC, basophils that express interleukin-4 (IL-4) have been recognized in tumor-draining lymph nodes (TDLNs), and their presence has been linked with a decreased prognosis for patient survival. Further exploration of basophils' roles in PC was carried out using "basophil-deficient Mcpt8-Cre mice" [64] alongside wild-type (WT) mice. Following the implantation of pancreatic cancer (PC), studies revealed that a striking 80% of wild-type (WT) mice developed detectable tumors. In contrast, the Mcpt8-Cre mice exhibited a notable absence of cancerous growth, highlighting a significant difference between these two genetic backgrounds. An in-depth analysis of basophils in the tumor-draining lymph nodes (TDLNs) was conducted, revealing critical insights into the role of cancer-associated fibroblasts (CAFs). These specialized fibroblasts were found to be instrumental in the secretion of thymic stromal lymphopoietin (TSLP), a cytokine that plays a

vital role in the immune response [65]. The released TSLP subsequently stimulated dendritic cells (DCs), prompting them to produce interleukin-3 (IL-3) from CD4+ T cells, enhancing the immune milieu within the TDLNs. Both the activated DCs and CD14+ monocytes, which are key players in the immune response, were observed to secrete CCL7, a chemokine that acts as a beacon, attracting basophils to the TDLNs. Once in the lymph nodes, the basophils were activated by IL-3, which shifted their functional profile to a pro-tumorigenic state. In this activated state, the basophils began to secrete interleukin-4 (IL-4), a cytokine that fosters the polarization of T-helper cells towards the Th2 subtype and promotes the generation of M2 macrophages. In a separate intriguing study, during the advanced phase of chronic myeloid leukemia (CML), an accumulation of basophils was also noted, along with a significant decrease in the transcription factor IKAROS in the bone marrow of these patients" [66]. Basophils derived from CML patients were found to express HIGF, encouraging the proliferation of CML cells [67]. Also, a mouse model of chronic myeloid leukemia (CML) suggested that CCL3 derived from basophils plays a role in advancing CML [68]. "Elevated basophil levels have been identified as a standalone predictor for acute myeloid leukemia that develops from myelodysplastic syndrome" [69-70]. These findings align with our results, indicating that basophils are risk factors in colorectal cancer (CRC), where increased basophil numbers may be linked to reduced survival rates.

In other types of solid tumors, like gastric cancers [71] and prostate cancers [72], there have been reports of adverse effects linked to tissue infiltrating or circulating basophils. Interestingly, in bladder cancer patients, the initial count of basophils has been recognized as a predictor for the likelihood of recurrence after tumor removal and bacillus Calmette-Guérin (BCG) treatment [73]. On the other hand, a study conducted with a mouse model of breast cancer found that having a low basophil count, known as basopenia, was associated with a higher number of pulmonary metastases [74]. Nonetheless, no substantial relationship has been found between basophils and outcomes in patients with breast cancer [75]. This discrepancy in results indicates that the local tumor microenvironment may influence whether basophils exhibit pro-tumor or anti-tumor properties, which could clarify their variable effects on survival across various cancer types [76]. Basophils enhance humoral immunity primarily by releasing substances that regulate B-cell activity. Once activated, basophils can express IL-4 and IL-6, CD40L, crucial for maintaining B-cell proliferation and promoting the production of IgM and IgG1. Research conducted by Gomez et al. has shown that basophils are key in facilitating plasma cell survival, both in vitro and in vivo [49, 59]. Our study is consistent with these observations, specifically underscoring a strong link between basophils and colorectal cancer (CRC), highlighting the broader roles of basophils in cancer interactions.

CD64 (FcgRI) is an Fc IgG receptor that is constantly present in monocytes and macrophages. It is a high-affinity receptor for individual IgG or Ig in immune complexes, and it triggers immune and inflammatory responses in immunocompetent cells (i.e., monocytes and syncytial macrophages) [77-79]. In the human body, monocytes represent a diverse population of cells with three distinct subpopulations based on CD14 and CD16 expression levels [80]. The CD14++ CD16- classical subset represents the majority of circulating monocytes, whereas the CD14- CD16+ cells are typically classified as non-classical or mesenchymal monocytes. This group of monocytes is distinct from the classical ones and possesses unique functional traits. Additionally, there is a second subset of monocytes that express both CD14 and CD16 (CD14+ CD16++), known as intermediate monocytes. Finally, the third subset includes atypical monocytes, which display low levels of CD14 and high levels of CD16 (CD14+ CD16++ expression) [81]. In rheumatoid arthritis (RA), CD64 is assessed by the overexpression of CD14++CD16- and CD14++CD16+ monocytes and correlates with the likelihood of disease activation [82]. CD64 has also been found to increase in individuals with chronic HBV infection and to fluctuate with the duration of the disease. Treatment with interferon-α normalized these elevations, indicating that CD64 levels could act as biomarkers for both chronic HBV infection and the effectiveness of interferon-α therapy [83]. Our research also indicated that

higher proportions of CD64 were linked with better prognoses for CRC.

CD4+ T cells usually represent a specific group of immune cells that feature CD4 co-receptors. CD4 is a co-receptor predominantly located on helper T cells and plays a crucial role in the interactions between T cells and antigen-presenting cells. Examining CD4+ cells can be significant for both scientific studies and clinical applications.CD4+ T cells play a crucial role in orchestrating the immune response, as they secrete various cytokines following their activation and differentiation. The different subtypes of CD4+ T helper cells (such as T helper 1, T helper 2, T helper 17, T helper 9, and regulatory T cells) perform distinct immune functions after maturing from naïve T cells. Each subtype of CD4+ T cells necessitates specific cytokines and key transcription factors for their activation [84].Prior studies have shown that CD4+ T cells can be located within the tumor microenvironments of lung cancer, melanoma, colorectal cancer (CRC), lymphomas, cervical cancer, and ovarian cancer; however, the function of CD4+ T cells in endometrial cancer (EC) has been less explored [85-89]. Notably, our results suggested that the presence of CD4+ T cells acted as a protective factor in CRC. Additional functional investigations are necessary to validate these findings.

The percentage of myeloid dendritic cells (%DC) refers to the proportion of these specific immune cells within a sample or population. Myeloid dendritic cells are a subgroup of dendritic cells and play a crucial role as antigen-presenting cells, which are essential for triggering immune responses. Tumors recruit endogenous myeloid cells, which can transform into tumor-associated macrophages (TAMs), dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs), or neutrophils (TANs), thereby fostering an environment that suppresses immune activity. The overproduction of immune mediators driven by cancer cells, such as granulocyte-macrophage colony-stimulating factor and vascular endothelial growth factor, stimulates myeloid cell creation in the bone marrow [90]. Recent research [91] indicates that Myeloid DC is associated with a decreased risk of CRC.

Dendritic cells, a type of antigen-presenting cell (APC), are vital for triggering and activating T cells, improving the immune regulation of natural killer cells, and demonstrating cytotoxic functions [92]. Recent studies have shown promising results in employing dendritic cell-based immunotherapy for treating prostate cancer [93]. This Mendelian randomization analysis provides the first evidence that the absolute count of CD62L− dendritic cells is positively associated with the onset of prostate cancer, while the Forward Scatter Area (FSC-A) measurement on plasmacytoid dendritic cells indicates an inverse relationship [94]. CD62L, or L-selectin, is a cell adhesion molecule involved in the processes of leukocyte rolling, adhesion, and migration through its binding to specific ligands on endothelial cells. FSC-A is an important metric utilized in flow cytometry to assess forward scatter signals and gauge cell size. Plasmacytoid dendritic cells are some of the most effective modulators of the body's antiviral immune responses, generating high levels of type I interferons, including IFN-α. However, there is a lack of empirical data that supports the negative correlation between FSC-A levels in plasmacytoid dendritic cells and prostate cancer. This observation opens up new experimental pathways for investigating the connection between dendritic cells and prostate cancer.

In comparison to the other groups, it was observed that immune characteristics from myeloid cells exhibited the most significant correlations with PE or PB. Myeloid-derived suppressor cells (MDSCs) are dual-functioning and can either encourage immune tolerance between the mother and fetus or inhibit the immune response to aid in the advancement of disease [95]. CD33 is often recognized as a marker for myeloid-derived suppressor cells (MDSCs), which have been observed to accumulate in the maternal bloodstream and at the interface between the mother and fetus during pregnancy. Abnormal variations in the quantity or functionality of MDSCs can lead to several pregnancy-related issues, including preeclampsia, premature labor, and miscarriage [96-99]. Enhancement of myeloid-derived suppressor cells (MDSCs) is linked to tumor development in colorectal cancer (CRC). In this study, we observed a significant relationship between CD33+ MDSC levels and the levels of Yes-associated protein 1 (YAP1) and phosphatase and tensin homolog (PTEN) in CRC patients. Tumor expression of YAP1 and PTEN is associated with the increase of tumor-related

myeloid-derived suppressor cells and reduced survival rates in CRC patients [100]. Granulocyte MDSCs are characterized by the presence of CD33, CD11b, IL-4Rα, and minimal amounts of CD15, along with heightened levels of arginase. In our research, we observed a significant rise in MDSC levels in CRC, and higher percentages of MDSCs were closely associated with an decrease risk of mortality in earlier studies [101]. Another investigation revealed that elevated Birc5 expression and increased MDSC infiltration within tumors were correlated with the prognosis of HCC patients. In vitro, hepatocyte Birc5 overexpression promoted the proliferation of immunosuppressive CD11b+CD33+HLA-DR- MDSCs in human peripheral blood mononuclear cells. Transgenic animal models of HCC indicated that Birc5 deficiency led to the upregulation of genes associated with lymphocyte-mediated immunity, natural killer cell-mediated immunity, gamma interferon production, T-cell activation, and T-cell-mediated cytotoxic activity, which aligns with our observation of a link between CD33 and HCC risk [102]. The results aligned with our observations in that levels of CD33+ MDSCs were linked to favorable outcomes in CRC. Additional functional experimental research is necessary to validate these results. Moreover, we identified two subtypes of CD33: CD33dim HLA DR+ CD11b− %CD33dim HLA DR+ and CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+. Our findings revealed that CD33dim HLA DR+ CD11b− %CD33dim HLA DR+ was more frequently a protective factor for cancer progression, while CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+ acted as a risk factor.

6. Limitations

Our two-sample Mendelian randomization (MR) analysis aimed to explore the causal relationship between immune cells and colorectal cancer (CRC) using data from large-scale genome-wide association studies (GWAS). This approach helped overcome some of the limitations of traditional observational studies by reducing the effects of confounding factors and the risk of reverse causality. Moreover, MR addressed certain representativeness and feasibility challenges that are often associated with randomized controlled trials (RCTs).

However, there are some drawbacks to this study. Firstly, it relied on publicly available GWAS data, which limited our ability to investigate additional relevant factors influencing CRC, like gender, age, and body mass index. Secondly, the results can mainly be applied to European populations, as that was the demographic covered in the original GWAS, indicating a need for further research in other ethnic groups. Thirdly, despite performing multiple sensitivity analyses, we were unable to fully assess horizontal pleiotropy. Lastly, we used a broader threshold for evaluating the results, which might have led to more false positives but also allowed for a more comprehensive analysis of the links between immune profiles in circulation and CRC.

7. Conclusions

To sum up, our analysis of immune cells in colorectal cancer (CRC) offers valuable insights into the immune system's behavior in this context. By systematically examining the immune cells present in CRC, we can gain a clearer picture of the immune landscape, evaluate how well current checkpoint inhibitors are working, and, crucially, support the creation of new immunotherapy treatments.

Acknowledgements

The authors extend their gratitude to the medical engineering research team for their guidance and for supplying the appropriate coding framework for the current MR analysis.

Funding

The study was supported by the Natural Science Foundation of Guangdong Province (Grant No. 2023A1515012548) and the Science and Technology Program of Guangzhou (Grant No. 2024A04J4802).

Availability of data and materials

All the data for the present article can be found on the GWAS (http://www.ebi.ac.uk/gwas/) and yunshang Gwas (https://gwas.medicineitlab.Com /).

Authors' Contributions

Pengkhun Nov, and Arzoo Prasai collected, analyzed, and interpreted the data. Ying Li wrote the manuscript. Other authors, and JL designed, revised, and supervised the study. All authors had reviewed and approved the final manuscript.

Ethics approval and consent to participate.

Not applicable.

Patient Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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