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Causal association of breast cancer with immune cells: new evidence from bi-directional Mendelian randomization using GWAS summary statistics

Zhixuan Wu[2†], Rongrong Zhang[2†], Xue Wu[2†], Xinyu Meng[2†], Haodong Wu[2], Xiaowu Wang[2], Danni Zheng[3*] and Yanyan Shen[1*]

Abstract

Background The tumor microenvironment of breast cancer encompasses a broad spectrum of immune cell populations. These cell populations are biologically/clinically relevant to varying degrees. The causal relationship between these immune cells and breast cancer remains uncertain despite their relevance.

Methods Bi-directional two-sample Mendelian randomization (MR) analyses were conducted to investigate the causal relationship between 731 immune cell phenotypes and breast cancer, utilizing genome-wide association study (GWAS) statistics. The primary analytical methods employed were the weighted median (WM) and random effects inverse variance weighting (IVW). The MR-Egger method, MR-PRESSO and Cochran's Q-statistic were utilized to evaluate heterogeneity and pleiotropy among the instrumental variables.

Results The study found a causal relationship between 27 immune cell traits and the onset of breast cancer using instrumental variables derived from GWAS data. Elevated levels of 13 immune cell populations and reduced levels of 14 immune cell populations were involved in triggering the development of breast cancer. Furthermore, the study revealed a causal relationship where breast cancer development had a causal effect on immune cell levels. Specifically, the onset of breast cancer may lead to elevated levels of 7 immune cell populations and reduced levels of 10 immune cell populations.

Conclusion This study utilized genetic approaches to establish a causal relationship between immune cell traits and breast cancer. These findings offer potential novel targets for diagnosing and treating breast cancer.

Keywords Breast cancer, Mendelian randomization, Immune cells, Genome wide association study, Causal inference

†Zhixuan Wu, Rongrong Zhang, Xue Wu and Xinyu Meng contributed equally to this work.

*Correspondence:
Danni Zheng
843649928@qq.com
Yanyan Shen
15888277002@163.com

[1]Department of Breast Surgery, The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou 325015, Zhejiang, People's Republic of China
[2]Wenzhou Medical University, Wenzhou 325200, Zhejiang, People's Republic of China
[3]Department of Thyroid and Breast Surgery, Taizhou Central Hospital (Taizhou University Hospital), Taizhou 318001, Zhejiang, China

Introduction

According to the International Agency for Research on Cancer of the World Health Organization, the year 2020 witnessed a significant rise in the incidence of breast cancer, making it the most widespread form of cancer worldwide. The data revealed a staggering 2.26 million newly reported cases of breast cancer globally [1]. Furthermore, breast cancer stands as the second-highest cause of cancer-related fatalities among women [2–4]. Notwithstanding the advent of various treatment modalities, including immunotherapy, surgery, chemotherapy, targeted therapy, and radiotherapy, the five-year survival rate for individuals afflicted with metastatic breast cancer remains below 30% [5–7]. The progression of breast cancer involves a complex sequence of stages that are influenced by a combination of genetic and immuno-environmental factors. However, the exact mechanisms underlying this process remain unknown [2, 8].

In recent years, mounting evidence indicates that the breast cancer tumor immune microenvironment (TIME) comprises diverse cell populations from both the innate and adaptive immune systems [9, 10]. These cell populations exhibit differing levels of biological and clinical significance. Among these cell populations, tumor-infiltrating lymphocytes (TILs) have emerged as a significant biomarker within various cell populations, demonstrating level 1b evidence of clinical validity, especially in the context of early-stage triple-negative breast cancer [11–13]. The findings of a meta-analysis indicate a noteworthy association between the density of tumor-associated macrophages (TAMs) and decreased survival rates in individuals diagnosed with breast cancer [14]. Preliminary data suggest a correlation between the presence of tumor-infiltrating dendritic cells (DCs) and adverse clinicopathological characteristics [15, 16]. Furthermore, immune cells like natural killer (NK) cells, TAMs, and cancer-associated fibroblasts (CAFs) exhibit severe phenotypic and functional defects in patients with breast cancer individuals [17–20]. Recent research has demonstrated the significant involvement of circulating immune cells in the pathogenesis and advancement of breast cancer. Specifically, studies have shown that heightened levels of circulating lymphocytes and monocytes serve as favorable prognostic markers in individuals with breast cancer [21, 22]. Conversely, increased levels of circulating myeloid-derived suppressor cells (MDSCs) have been strongly linked to advanced stages of breast cancer and positive lymph node involvement [23]. However, accurately measuring immune cell levels poses a challenge, and potential confounding factors could undermine the casual interpretation of this association.

Advancements in large-scale genome-wide association studies (GWAS) and Mendelian randomization (MR) techniques have provided the opportunity to assess the immune system's association with disease, enabling the investigation of potential causal relationships [24–26]. The MR method mitigates the influence of confounding variables by leveraging the inherent random assortment of genetic variants, thereby enhancing the robustness of bidirectional causal inference. To establish the causal connection between breast cancer and 731 immune cell characteristics, the research conducted a bidirectional MR analysis. This causal relationship may help to reveal new therapeutic strategies and interventions, thereby improving the prognosis and survival of breast cancer individuals.

Materials and methods

Causal analysis framework

Using a two-sample MR method, the analysis investigated a bidirectional causal association between 731 immune cell characteristics and breast cancer within seven immune categories. This investigation utilized genetic variants within the MR framework to represent risk factors. The three fundamental assumptions are crucial for valid instrumental variables in causal inference [27]. Firstly, it is imperative that genetic variation be linked to the exposure under investigation. Secondly, the selected instrument must be free from any potential confounding factors. Finally, genetic variation should not introduce bias into the results through alternative pathways unrelated to the exposure of interest. It is worth noting that all studies included in the dataset received approval from their respective institutional review boards.

Data sources for GWAS

The GWAS summary statistics pertaining to 731 immune cell traits were sourced from the research conducted by Orrù V et al. [28]. The evaluation in this extensive research covered 731 immune characteristics, which consisted of 118 absolute cell (AC) representing absolute cell counts, 389 median fluorescence intensities (MFI) indicating surface antigen levels, 32 morphological parameter (MP) describing morphologic parameters, and 192 relative cell (RC) representing relative cell counts. A total of seven panels were employed, covering B cells, cDCs, various stages of T cell maturation, monocytes, myeloid cells, TBNK, and Regulatory T cells (Tregs). Genotyping of samples was conducted using four Illumina arrays. Furthermore, a genome-wide estimation was executed based on data from 3514 individuals within the Sardinian sequence. After adjusting for covariates (namely, gender, age, and age^2), around 22 million single nucleotide polymorphisms (SNPs) were preserved for association examination and evaluated for correlation. Breast cancer GWAS data were obtained from the UK Biobank's UKB-B-16,890 dataset, available at https://gwas.mrcieu.ac.uk/datasets/ukb-b-16890/. This dataset comprised 10,303

exposure	nsnp	method	pval	OR(95% CI)
CD127 on CD28+ CD45RA− CD8br	8	Weighted median	**0.045**	1.002 (1.000 to 1.004)
	8	Inverse variance weighted	**0.011**	1.002 (1.000 to 1.003)
CD127 on CD45RA+ CD4+	17	Weighted median	0.146	1.001 (1.000 to 1.002)
	17	Inverse variance weighted	**0.022**	1.001 (1.000 to 1.002)
CD127 on CD8br	18	Weighted median	0.061	1.001 (1.000 to 1.003)
	18	Inverse variance weighted	**0.015**	1.001 (1.000 to 1.002)
CD127 on granulocyte	18	Weighted median	**0.020**	0.998 (0.997 to 1.000)
	18	Inverse variance weighted	**0.048**	0.999 (0.998 to 1.000)
CD19 on IgD+ CD24−	17	Weighted median	0.186	1.001 (1.000 to 1.002)
	17	Inverse variance weighted	**0.016**	1.001 (1.000 to 1.002)
CD19 on naive−mature B cell	16	Weighted median	0.259	1.001 (0.999 to 1.003)
	16	Inverse variance weighted	**0.023**	1.001 (1.000 to 1.003)
CD24 on IgD+ CD38br	17	Weighted median	0.050	0.998 (0.997 to 1.000)
	17	Inverse variance weighted	**0.003**	0.998 (0.997 to 0.999)
CD24+ CD27+ AC	13	Weighted median	**0.033**	0.998 (0.996 to 1.000)
	13	Inverse variance weighted	**0.001**	0.998 (0.996 to 0.999)
CD28− DN (CD4−CD8−) %DN	19	Weighted median	0.065	0.999 (0.997 to 1.000)
	19	Inverse variance weighted	**0.024**	0.999 (0.998 to 1.000)
CD28+ DN (CD4−CD8−) %DN	19	Weighted median	0.063	1.001 (1.000 to 1.003)
	19	Inverse variance weighted	**0.024**	1.001 (1.000 to 1.002)
CD4 on secreting Treg	15	Weighted median	0.441	1.000 (0.998 to 1.001)
	15	Inverse variance weighted	0.050	0.999 (0.998 to 1.000)
CD4+ AC	17	Weighted median	0.118	0.999 (0.997 to 1.000)
	17	Inverse variance weighted	**0.013**	0.998 (0.997 to 1.000)
CD45 on CD33br HLA DR+	11	Weighted median	0.106	0.999 (0.997 to 1.000)
	11	Inverse variance weighted	**0.037**	0.999 (0.998 to 1.000)
FSC−A on granulocyte	14	Weighted median	0.291	0.999 (0.998 to 1.001)
	14	Inverse variance weighted	0.050	0.999 (0.998 to 1.000)
HLA DR on B cell	11	Weighted median	0.092	0.999 (0.998 to 1.000)
	11	Inverse variance weighted	0.050	0.999 (0.999 to 1.000)
HLA DR on CD14+ CD16+ monocyte	13	Weighted median	0.055	1.001 (1.000 to 1.001)
	13	Inverse variance weighted	**0.005**	1.001 (1.000 to 1.001)
IgD− CD38br %lymphocyte	17	Weighted median	**0.040**	1.002 (1.000 to 1.004)
	17	Inverse variance weighted	**0.012**	1.002 (1.000 to 1.003)
IgD− CD38br AC	12	Weighted median	**0.002**	1.003 (1.001 to 1.005)
	12	Inverse variance weighted	**0.005**	1.002 (1.001 to 1.004)
IgD on unsw mem	15	Weighted median	0.116	0.999 (0.998 to 1.000)
	15	Inverse variance weighted	**0.013**	0.999 (0.998 to 1.000)
IgD+ %Lymphocyte	11	Weighted median	0.546	1.000 (0.998 to 1.001)
	11	Inverse variance weighted	**0.049**	0.999 (0.998 to 1.000)
IgD+ CD24+ %lymphocyte	9	Weighted median	**0.028**	0.997 (0.995 to 1.000)
	9	Inverse variance weighted	**0.029**	0.998 (0.996 to 1.000)
Lymphocyte AC	10	Weighted median	0.378	0.999 (0.997 to 1.001)
	10	Inverse variance weighted	**0.030**	0.998 (0.997 to 1.000)
Naive−mature B cell AC	16	Weighted median	0.176	0.999 (0.997 to 1.001)
	16	Inverse variance weighted	**0.021**	0.999 (0.997 to 1.000)
Naive CD8br AC	18	Weighted median	0.165	1.002 (0.999 to 1.004)
	18	Inverse variance weighted	**0.013**	1.002 (1.000 to 1.003)
TD DN (CD4−CD8−) %DN	19	Weighted median	0.197	1.001 (1.000 to 1.002)
	19	Inverse variance weighted	**0.013**	1.001 (1.000 to 1.002)
TD DN (CD4−CD8−) %T cell	18	Weighted median	0.309	1.001 (0.999 to 1.002)
	18	Inverse variance weighted	**0.025**	1.001 (1.000 to 1.002)
TD DN (CD4−CD8−) AC	15	Weighted median	0.163	1.001 (1.000 to 1.003)
	15	Inverse variance weighted	**0.042**	1.001 (1.000 to 1.002)

Fig. 1 (See legend on next page.)

(See figure on previous page.)

Fig. 1 Forest plots showed positive results for the causal effect of immune cell phenotypes on breast cancer risk by using weighted median and inverse variance weighted methods. Note: CD127 on CD45RA + CD4 + refers to the expression of the CD127 molecule on CD45RA+/CD4 + cells. CD24 + CD27 + AC refers to the absolute cell counts of CD24+/CD27 + cell. CD28- DN (CD4- CD8-) %DN represents the percentage of CD28-/CD4-/CD8- cells within the CD4-/CD8- double-negative (DN) cell population. CD45 on CD33br HLA DR + represents the expression of the CD45 molecule on CD33br /HLA DR + cells. IgD on unsw mem refers to the expression of the IgD molecule on University of New South Wales Memory T Cells (unsw mem)

breast cancer cases and 452,630 controls of European descent.

Selection of Instrumental Variables (IVs)

The significance threshold for IVs associated with each immune trait was established based on prior research at 1×10^{-5} [28, 29]. SNPs were obtained to identify independent IVs using a method that considered both independence and significance. SNPs were selected if they were both significant and independent (linkage disequilibrium [LD] r^2 threshold < 0.1 within 500 kb distance) for each immune trait. This process utilized the clustering procedure within the PLINK software (version v1.90). LD r2 values were calculated according to the 1000 Genomes Projects reference panel [30]. Moreover, F-statistics were calculated for each IV. IVs with F-statistics > 10 were considered strong instruments and retained for the following analyses. This step aimed to mitigate the potential bias associated with weak instrumentation. IVs were extracted from the breast cancer outcome summary statistics. Previously reported, the ones that have the potential to cause multiple effects on breast cancer were excluded [31].

Statistical analysis

This article adheres to the guidelines outlined in the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR) guidelines. The statistical analyses were conducted utilizing R 3.5.3 software. Immune cell levels were analyzed in their original percentage units, and ORs reflect the odds of breast cancer per 1% absolute increase. To assess the causal relationship between 731 immune cell phenotypes and breast cancer, the "MendelianRandomization" software (version 0.4.3) was utilized [32]. The primary analytical approaches employed in this study were the weighted median (WM) method and random effects inverse variance weighting (IVW). Furthermore, estimates regarding the effect of exposure on outcomes were provided under the conditions where MR assumptions were considered valid. The assessment of residual heterogeneity among the chosen IVs involved using Cochran's Q statistic along with corresponding *P*-value, with a *P*-value less than 0.05 indicating the presence of heterogeneity. Additionally, horizontal pleiotropy outliers that could significantly affect the results were excluded using the MR-Egger method. A significant intercept term implied the presence of horizontal pleiotropy [33]. The MR-PRESSO test offers a thorough evaluation of heterogeneity in order to detect potential outliers within SNP data. Odds ratios (ORs) and their associated 95% confidence intervals (CIs) were used to quantify the causal relationship between variables. In order to mitigate the potential impact of horizontal pleiotropy stemming from a singular SNP, a "leave-one-out" analysis was conducted.

Results

Causal effect of immune cell phenotypes on breast cancer risk identified by MR analysis

The investigation into the potential causal involvement of immune cells in breast cancer development was initiated through a MR analysis. The WM and IVW methods were the primary analytical approaches (Fig. 1, Supplementary Table 1). The causal association of 27 immune cells with breast cancer development was assessed at the nominal significance level. Specifically, elevated levels of 13 immune cells and reduced levels of 14 immune cells were associated with the potential induction of breast cancer development (Supplementary Table 2). Specifically, high levels of CD127 on CD28 + CD45RA – CD8br, CD127 on CD45RA + CD4+, CD127 on CD8br, CD19 on IgD + CD24–, CD19 on naive – mature B cell, CD28 + DN (CD4 – CD8–) %DN, HLA DR on CD14 + CD16 + monocyte, IgD – CD38br %lymphocyte, IgD – CD38br AC, Naive CD8br AC, TD DN (CD4 – CD8–) %DN, TD DN (CD4 – CD8–) %T cell, and TD DN (CD4 – CD8–) AC predicted higher breast cancer risk. The OR of CD127 on CD28 + CD45RA- CD8br to breast cancer risk observed using a WM approach was estimated to be 1.002 (95% CI = 1.000 ~ 1.004, $P = 0.045$). These consistent findings were replicated using IVW methods: OR = 1.002, 95% CI = 1.000 ~ 1.003, $P = 0.011$. The genetic predisposition toward lower levels of certain immune cell markers, such as CD127 on granulocytes, CD24 on IgD + CD38br, CD24 + CD27 + AC, CD28 – DN (CD4 – CD8–) %DN, CD4 on secreting T reg, CD4 + AC, CD45 on CD33br HLA DR+, FSC – A on granulocyte, HLA DR on B cell, IgD on unsw mem, IgD+ %Lymphocyte, IgD + CD24+ %lymphocyte, Lymphocyte AC, and Naive – mature B cell AC, might be correlated with an elevated risk of breast cancer, as depicted in Fig. 1. An example of a potentially protective effect was identified for CD28- DN (CD4-CD8-) %DN against breast cancer employing the IVW approach, showing an OR = 0.999 (95% CI = 0.998 ~ 1.000, $P = 0.024$). However, the WM method ($P = 0.065$) did not support this observed association. Additional

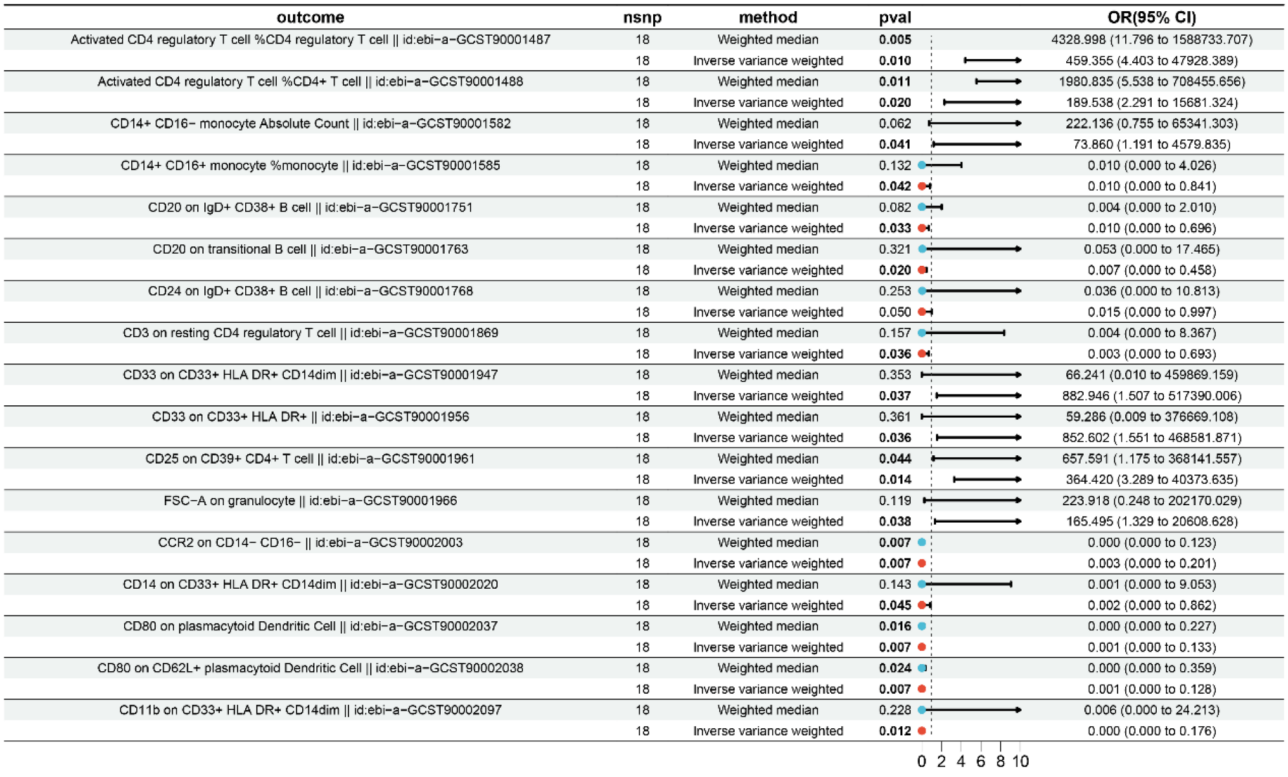

Fig. 2 Forest plots showed the causal effect of breast cancer development on immune cell phenotypes (ORs correspond to absolute percentage changes in immune cell levels). Note: Activated CD4 regulatory T cell % CD4 regulatory T cell represents the percentage of activated CD4 regulatory T cell within the CD4 regulatory T cell population. CD14 + CD16- monocyte Absolute Count refers to the absolute cell counts of CD14+/CD16- monocyte. CD20 on IgD + CD38 + B cell represents the expression of the CD20 molecule on IgD+/CD38 + B cell

examination using MR-Egger intercept analysis, MR-PRESSO analysis and Cochran's Q test did not reveal any evidence of pleiotropy or heterogeneity (Supplementary Tables 3–5). The leave-one-out analysis results were presented in Supplementary Fig. 1. The incremental removal of each SNP had little impact on the error bar, providing further evidence for the robustness of our findings.

Causal effect of breast cancer development on immune cell phenotypes

In order to explore the impact of breast cancer progression on the immune mechanisms within the body, this study conducted an examination of the causal effects of breast cancer on immune cells (Fig. 2). The primary analytical tools employed were the WM and IVW methods. By means of a two-sample MR analysis, the study successfully identified the causal effects of breast cancer on the levels of 17 immune cells. The development of breast cancer appears to potentially elevate the levels of seven immune cell populations, including Activated CD4 regulatory T cell %CD4 regulatory T cell, Activated CD4 regulatory T cell %CD4 + T cell, CD14 + CD16 – monocyte Absolute Count, CD33 on CD33 + HLA DR + CD14dim, CD33 on CD33 + HLA DR+, CD25 on CD39 + CD4 + T cell, and FSC – A on granulocyte. Conversely, it decreased the levels of 10 other immune cell populations. According to the WM approach, breast cancer development was found to potentially increase activated CD4 regulatory T cell %CD4 regulatory T cell levels (OR = 4328.998 per 1% increase, 95% CI = 11.796 ~ 1588733.707, $P = 0.005$). Similarly, other assessment methods, such as IVW (OR = 459.355, 95% CI = 4.403 ~ 47928.389, and $P = 0.010$); weighted mode (OR = 7345.265, 95% CI = 13.506 ~ 3994622.925, and $P = 0.013$) yielded similar results (Supplementary Table 6). According to the WM approach, activated CD4 regulatory T cell %CD4 + T cell levels were increased in breast cancer individuals (OR = 1980.835, 95% CI = 5.538 ~ 708455.656, and $P = 0.011$). Similarly, the IVW assessment method yielded similar results (OR = 189.538, 95% CI = 2.291 ~ 15681.324, and $P = 0.020$). For CD14 + CD16- monocyte AC, a positive correlation was observed utilizing the IVW method (OR = 73.860, 95% CI = 1.191 ~ 4579.835, and $P = 0.041$). In addition, a similar association was observed for CD33 on CD33 + HLA DR + CD14dim cells (OR = 882.946, 95% CI = 1.507 ~ 517390.006, and $P = 0.037$) and CD33 on CD33 + HLA DR + cells (OR = 852.602, 95% CI = 1.551 ~ 468581.871, and $P = 0.036$). No heterogeneity was observed via Cochran's Q-test (Supplementary Table 7). Furthermore, the intercept test of MR-Egger

and MR-PRESSO analysis ruled out horizontal pleiotropy (Supplementary Tables 8–9). The leave-one-out analysis offered additional support for the reliability of our results (Supplementary Fig. 2).

Discussion

This study employed bidirectional Mendelian randomization analysis to provide evidence indicating a plausible causal association between breast cancer and genetic immune cell traits. Based on available information, using a GWAS-based genetic approach, this study appears to represent the first MR analysis to uncover the causal links between 731 immune cell traits and breast cancer. Among the four categories of immune traits (MFI, RC, AC, and MP), 27 immune phenotypes were identified to exert a causal effect on breast cancer. This study offers insights into the significant causal effect exerted by the development of breast cancer on 17 immune cell populations.

The elevated levels of CD4- CD8- DN (double-negative lymphocytes) were correlated with an elevated risk of breast cancer. The CD4-CD8- DN subset denotes a population of immune cells characterized by the absence of CD4 and CD8 co-receptor expression, which are crucial markers for two major subtypes of T-cells [34]. CD4-CD8- DN T cells are commonly located in the thymus, lymph nodes, and peripheral blood, and have been strongly associated with inflammatory and autoimmune diseases as well as tumorigenesis [35]. Infiltration of CD4-CD8- DN T cells has been observed in various solid tumors, including lung cancer, liver cancer, gliomas, and pancreatic cancers [36–39]. Studies have demonstrated the immunosuppressive properties of DN T cells in neuroglioma and melanoma models, promoting tumor metastasis aligning with the present research findings [40, 41]. DN TILs express activation markers such as CD150, CD69, and CD137 in hepatocellular carcinoma, showcasing anti-hepatocellular carcinoma effects [37]. The contradictory results suggest that DN T cells may exhibit either tumor-promoting or tumor-suppressive effects, contingent upon the particular tumor microenvironment and tumor type. It is worth mentioning that DN T cells operate as autonomous anti-tumor agents, particularly in hematological malignancies, lung cancer, and pancreatic cancer [42, 43]. Additionally, DN T cells may demonstrate synergistic anti-tumor effects against leukemia and solid tumors in combination with chemotherapy and immunotherapy [36, 44, 45]. However, the precise involvement of CD4-CD8- DN cells in breast cancer remains unclear. Specific immunotherapies, such as CAR-T cell therapy and immune checkpoint inhibitors, have been extensively employed in the treatment of breast cancer within the medical field [46, 47]. The potential influence of DN cells on the efficacy of therapies and patient outcomes in breast cancer warrants further investigation to elucidate the specific role of CD4-CD8- DN cells. Progress in this field will deepen understanding of the mechanisms underlying breast cancer development and support the development of innovative therapeutic approaches and targeted drugs.

CD127 also recognized as interleukin 7 receptor alpha (IL-7Rα), functions as the receptor for IL-7. The IL-7R is primarily located in lymphocytes and plays a crucial role in the maturation, survival, and function of various immune cells, including T cells, B cells, and NK cells [48, 49]. Dysregulation of wild-type IL-7R expression has been implicated in the initiation of diseases and even carcinogenesis [50]. Increased expression of IL-7R has been shown to enhance thymocyte renewal and induce thymic hyperplasia through the promotion of T-cell precursor proliferation in a dose-dependent fashion, ultimately playing a role in the pathogenesis of leukemia [51]. However, IL-7/IL-7Rα has potent immunomodulatory and anti-tumor effects [52]. The research conducted by Wang X et al. has provided evidence supporting the idea that IL-7R may act as a positive prognostic factor for individuals with lung adenocarcinoma [53]. Nevertheless, these studies only confirmed the substantial role of CD127 in tumor progression, without establishing definitive causation. The current study observed associations between CD127 levels on CD28 + CD45RA- CD8br, CD45RA + CD4+, and CD8br with increased breast cancer risk. The presented results align with the research discoveries of Wang Z et al., demonstrating a connection between variations in the IL-7R gene and the vulnerability to breast cancer in Chinese Han females [54]. These collective findings suggest a potential role for CD127 in breast cancer, emphasizing its potential as a promising therapeutic target.

Breast cancer development has a pronounced causal impact on the levels of Activated CD4 regulatory T cells and CD14 + CD16- monocyte cells, which are noteworthy. Tregs are part of the immunosuppressive subset within the CD4 + T cell lineage and play a crucial role in suppressing immune responses to self-antigens and excessive immune-mediated inflammation [55]. An increased frequency of Treg cells within the TIME was observed across numerous cancers, comprising pancreatic cancer, melanoma, and breast cancer, further validating current research outcomes [56]. Tregs impede anti-tumor immune responses in tumor immunity through diverse mechanisms [57]. The inhibitory TIME triggered by Treg cells poses a significant challenge in effectively responding to immunotherapy [56]. Therefore, a comprehensive examination of the frequency, functionality, and dispersion of Treg cells holds immense importance in exploring the cancer microenvironment across diverse cancer types, notably in breast cancer.

CD14 + CD16- Monocytes, a class of immune cells, are often considered a subpopulation of classical monocytes. Extensive research has investigated the involvement of classical monocytes in autoimmune diseases [58]. Monocytes are pivotal in regulating the onset and progression of cancer. While numerous studies have focused on the relationship between classical monocytes and tumors, the association between CD14 + CD16- monocytes and breast cancer remains unclear. Additionally, achieving consistency has proven challenging across various studies, frequently resulting in contradictory findings. Previous research has indicated that classical monocytes play a role in promoting tumorigenesis through their differentiation into pro-tumor-associated macrophages, recruitment of Treg cells, facilitation of angiogenesis, and involvement in extracellular matrix remodeling [59]. Classical monocytes have been reported to exhibit tumor cytotoxicity [60]. However, the nature of tumors is highly heterogeneous. As a result, the disease pathogenesis in different cancer types among various patients may not exhibit consistency. Additionally, distinct therapeutic approaches might influence the quantity or functionality of monocytes. Hence, further investigations are crucial to elucidate the substantial involvement of CD14 + CD16- monocytes in breast cancer.

The results of this research demonstrated the possible influence of immune cell characteristics on breast cancer and offered guidance for predicting clinical disease prognosis and developing new drugs. However, it is essential to recognize that this research has specific constraints. First, the data on immune cells and GWAS of breast cancer were obtained from separate studies. Hence, there might be variations in the size of samples, approaches to quality control, and ethnic backgrounds. Upon evaluation, it was determined that setting the *P*-value threshold at $5e^{-8}$ would yield an insufficient number of SNPs to support our subsequent research endeavors. Consequently, the threshold was adjusted to 10^{-5}, a modification that may introduce potential bias into the results. Furthermore, the MR method necessitates a substantial sample size and adequate genetic diversity to yield robust evidence; otherwise, it may result in result instability and inadequate statistical power. Consequently, further validation of the findings in additional cohorts is warranted. Additionally, it is important to note that this study was exclusively conducted within a European population. Given that MR typically relies on genomic data from a specific population, the generalizability of the results may be constrained. Hence, the findings of this study may not be applicable to other racial populations and necessitate additional research to achieve broader generalizability across various racial groups.

Conclusion

In conclusion, this bidirectional MR analysis demonstrated the potential causal role played by several immune cell phenotypes in breast cancer, enhancing the current understanding of the role of the immune system in breast cancer. Further research should focus on exploring the potential mechanisms of immune cell subpopulation dysregulation in the pathogenesis of breast cancer. Such investigation could provide theoretical directions for new therapeutic strategies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13875-w.

Supplementary Table 1: Causal effects of immune cells on breast cancer using different methods. b: effect size (β). Se: the standard deviation of effect size. pval: *P*-value. lo_ci/ up_ci: the confidence interval of effect size. OR: odds ratio. or_lci95/ or_uci95: the confidence interval of odds ratio

Supplementary Table 2. The causal association of 27 immune cells with breast cancer development identified by the WM and IVW methods

Supplementary Table 3: Cochran's Q test results of causal effects of immune cells on breast cancer

Supplementary Table 4: MR-Egger analysis results of causal effects of immune cells on breast cancer

Supplementary Table 5: MR-PRESSO analysis results of causal effects of immune cells on breast cancer

Supplementary Table 6: Causal effects of breast cancer on immune cells

Supplementary Table 7. Cochran's Q test results of breast cancer on immune cells

Supplementary Table 8. MR-Egger analysis results of breast cancer on immune cells

Supplementary Table 9: MR-PRESSO analysis results of breast cancer on immune cells

Supplementary Fig. 1: The leave-one-out analysis results of causal effects of immune cells on breast cancer

Supplementary Fig. 2: The leave-one-out analysis results of breast cancer on immune cells

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Author contributions

Zhixuan Wu: Writing– original draft, Investigation. Rongrong Zhang: Validation, Writing– original draft. Xue Wu: Investigation. Xinyu Meng: Methodology, Software. Haodong Wu: Investigation. Xiaowu Wang: Conceptualization, Project administration. Danni Zheng: Writing– review & editing, Project administration. Yanyan Shen: Writing– review & editing, Project administration. All authors reviewed the manuscript.

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Data availability

The datasets supporting the conclusions of this article are included within the article.

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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