HUMAN BIOLOGY
2024, VOL. 94, ISSUE 3
ORIGINAL ARTICLE

OPEN ACCESS

Investigating the Causal Relationship Between Immune Cell Phenotypes and Pancreatic Cancer Risk: A Mendelian Randomization Study

Weihao Chai[1], Kairui Huang[1]*, Jiajia Li[2], Fei Guo[3], Yizhan Wu[1], Jiangwei Liu[4]*

ABSTRACT

Background: Pancreatic cancer (PC) is a highly lethal malignancy with poor clinical prognosis, whose development is associated with genetic and environmental factors. The immunophenotype, referring to the immune-related molecular markers on the surface of tumor cells, significantly impacts the progression, development, and treatment response of PC.

Objective: This study employed a two-sample Mendelian randomization (MR) bidirectional analysis to explore the association between immunophenotype and the risk of PC.

Methods: We collected datasets on 731 immune cells associated with PC from the Finngen database and GWAScatalog database. Reverse-variance weighted (RVW), weighted median (WM), and MR-Egger methods were applied to analyze the causal relationship between immune cells and PC. Cochran's Q test, MR-Egger regression, MR-PRESSO, and Leave-one-out methods were used to evaluate the stability and reliability of the study results.

Results: After bidirectional FDR correction, we found no statistically significant impact of PC on the immunophenotype. However, when investigating the causal effect of immunophenotype on PC, we found that among four immunophenotype categories (MFI, RC, AC, MP), 26 immune phenotypes were causally associated with PC ($P < 0.05$). Among these, seven immune phenotypes could significantly inhibit the occurrence and development of PC (IVW ≥ 0.05, OR ≥ 1), while another 19 immune phenotypes might lead to the incidence of PC (IVW < 0.05, OR < 1).

Conclusion: This study preliminarily reveals the causal relationship between immune cells and pancreatic cancer from a genetic perspective, providing an important theoretical basis for precision medicine and individualized treatment in the future.

INTRODUCTION

Pancreatic cancer, a highly malignant tumor, originates from the abnormal proliferation of pancreatic cells. Its development involves numerous gene mutations and environmental factors, presenting complexity and diversity Mizrahi et al. (2020), McGuigan et al. (2018). The biological characteristics of this cancer are characterized by high invasiveness and early metastatic potential, leading to late-stage diagnosis and low treatment success rates. Additionally, pancreatic cancer exhibits strong resistance to existing treatment modalities, posing a significant challenge for treatment Traub et al. (2021). Currently, the only potentially curative treatment is surgical resection followed by postoperative chemotherapy; however, the five-year survival rate for patients undergoing surgery is still less than 10% Lambert et al. (2019), Robatel et al. (2022). This situation makes the treatment of pancreatic cancer a major challenge for clinicians, patients, and researchers.

Globally, the incidence and mortality rates of pancreatic cancer have been continuously rising, doubling over the past two decades, making it one of the important causes of cancer-related deaths worldwide Lambert et al. (2019), Robatel et al. (2022). Therefore, we should strengthen prevention and screening efforts in the face of this fatal disease Khalaf et al. (2022).

The relationship between immunophenotype and pancreatic cancer is a highly complex and multidimensional field of research. It not only involves interactions among various types of immune cells but also includes their roles and functions within the tumor microenvironment, presenting challenges for the screening and monitoring of pancreatic cancer. In-depth research is needed to develop more precise screening guidelines and sensitive detection technologies Khalaf et al. (2022), Wood et al. (2022). Studies have found characteristic immune/inflammatory cell infiltration in

[1]Graduate School, Xinjiang Medical University, Urumqi, China
[2]Key Laboratory of Special Environmental Medicine of Xinjiang, General Hospital of Xinjiang Military Command, Urumqi, China
[3]The First Affiliated Hospital of Xinjiang Medical University, Urumqi, China
[4]Key Laboratory of Special Environmental Medicine of Xinjiang, General Hospital of Xinjiang Military Command, Urumqi, China

Correspondence to: Jiangwei Liu, Key Laboratory of Special Environmental Medicine of Xinjiang, General Hospital of Xinjiang Military Command, Urumqi 830000, China. Email: ljw273273@163.com.

Keywords: Pancreatic cancer, immunocytes, Mendelian randomization analysis, causal analysis, sensitivity analysis.

the pancreatic cancer microenvironment, providing a new direction for the development of predictive biomarkers. These markers can assess characteristics of the tumor immune microenvironment and help determine which patients may benefit from specific treatments Ino et al. (2013). In pancreatic cancer, certain immune cells such as dendritic cells, natural killer cells, and CD8 and CD4 T cells are activated to inhibit tumor growth and progression Elebo et al. (2020). However, not all immune cells possess antitumor effects, for example, myeloid-derived suppressor cells can weaken antitumor immune responses through various mechanisms Huber et al. (2020). Additionally, certain T cell subsets may promote tumor formation Zhang et al. (2014). Research has shown that changes in the distribution of immune cell subsets in peripheral blood are closely related to the progression of pancreatic cancer, such as patients with a higher proportion of lymphocytes having a longer overall survival, suggesting that circulating immune cell subsets may serve as biomarkers of pancreatic cancer prognosis Xu et al. (2022). Despite the potential utility of certain immunophenotypes in the diagnosis and prognosis assessment of pancreatic cancer, current studies are limited by insufficient sample sizes and the lack of independent external validation cohorts, necessitating further confirmation through subsequent research Xu et al. (2020), Hyung et al. (2022). Mendelian Randomization (MR) studies, as a statistical analysis method, have significant application value in analyzing the causal relationship between risk factors and disease outcomes, particularly in the field of etiological inference in epidemiology Lu et al. (2020), Smith et al. (2014). Although previous studies have revealed the association between immune cells and the development of pancreatic cancer and validated the hypotheses of this association, there has been no research using MR methods to explore the relationship between immunophenotype and pancreatic cancer Elebo et al. (2020), Huber et al. (2020), Zhang et al. (2014).

In this study, we employed a two-sample MR approach to assess the causal relationship between immunophenotype and pancreatic cancer, aiming to provide new theoretical and experimental evidence for uncovering the mechanisms of occurrence and development of pancreatic cancer. By using genetic variants as instrumental variables, we were able to conduct causal inference in observational data, offering important scientific clues for the biological research of pancreatic cancer.

MATERIALS AND METHODS

Study Design

Utilizing a two-sample Mendelian Randomization (MR) analysis, we assessed the causal relationship between 731 immune cell traits (grouped into 7 categories) and pancreatic cancer. MR employs genetic variants to proxy for risk factors, necessitating that effective instrumental variables (IVs) in causal inference satisfy three key assumptions: (1) genetic variants are directly associated with the exposure; (2) genetic variants are unrelated to potential confounders of the exposure and outcome; and (3) genetic variants do not affect the outcome through routes other than the exposure. Studies included in our analysis were all approved by relevant institutional review boards, and participants provided informed consent.

Genome-Wide Association Study (GWAS) Data Source for Pancreatic Cancer

The GWAS summary statistics for pancreatic cancer were obtained from the European Bioinformatics Institute (EMBL) Sakaue et al. (2021). The study conducted GWAS analysis on 47,6245 European individuals (Ncase = 1,196, non-cases = 475,049), after quality control and imputation, analyzing approximately 24,195,229 SNPs.

Source of Whole-Immune GWAS Data

The summary statistics for each immune trait GWAS can be publicly accessed from the GWAS Catalog (accession numbers from GCST0001391 to GCST0002121) Orru et al. (2020). A total of 731 immune phenotypes are included, consisting of absolute cell (AC) counts (n=118), median fluorescence intensity (MFI) reflecting levels of surface antigens (n=389), morphological parameters (MP) (n=32), and relative cell (RC) counts (n=192). Specifically, MFI, AC, and RC traits include B cells, CDCs, maturation stages of T cells, monocytes, bone marrow cells, TBNK (T cells, B cells, natural killer cells), and Treg cells, while MP traits include CDC and TBNK panels. The initial immune trait GWAS was conducted using data from 3757 European individuals with no overlapping cohorts. Using a reference panel based on Sardinian sequences Sidore et al. (2015), approximately 22 million SNPs genotyped using high-density arrays were estimated and tested for association after adjusting for covariates (i.e., sex, age, and age squared).

Selection of Instrumental Variables (IVs)

Based on recent studies Ripke et al. (2014), Yu et al. (2021), the threshold for significance for IVs of each immune trait was set at $1\times10-5$. Clustering procedures within the PLINK (version v1.90) software were used to prune these SNPs (linkage disequilibrium (LD) r2 threshold < 0.1, distance < 500kb), with the LD r2 calculated based on a reference panel of 1000 Genomes Project as the standard. For pancreatic cancer disease, the significance level was adjusted to $5\times10-8$, and the proportion of phenotypic variance explained (PVE) and the F-statistic was calculated for each IV to assess IV strength and to avoid weak instrument bias.

Statistic Analysis

The statistical analysis was conducted in R version 4.3.1 （http://www.Rproject.org）.

To assess the causal relationship between 731 immunophenotypes and pancreatic cancer, the inverse variance weighted (IVW) method from the "Mendelian Randomization" package (version 0.4.3) Sidore et al. (2015) was used as the primary analysis technique, complemented by the weighted median Bowden et al. (2016) and model-based methods for validation Bowden et al. (2016).

The Cochran Q statistic and corresponding P values were employed to test for heterogeneity between the selected instrumental variables (IVs).

If the null hypothesis was rejected, the random-effects IVW was used instead of the fixed-effects IVW Hartwig et al. (2017).

To address the issue of horizontal pleiotropy, a commonly used method (MR-Egger) was applied, which meant that the intercept term for horizontal pleiotropy was significant Burgess et al. (2017).

Furthermore, a robust method, MR pleiotropy residual and outlier (MR-PRESO) analysis, was conducted to eliminate horizontal pleiotropic outliers that could potentially have a significant impact on the estimation results in the MR-PRESO package Verbanck et al. (2018).

In addition to these methods, scatter plots and funnel plots were also used. The scatter plots demonstrated that the results were not affected by outliers, while the funnel plots confirmed the robustness of the correlations and the absence of heterogeneity.

RESULTS

Exploring the Causal Relationship between Pancreatic Cancer and Immunophenotypes

To investigate the causal impact of pancreatic cancer on immunophenotypes, we employed a two-sample Mendelian Randomization (MR) analysis, with the Inverse Variance Weighted (IVW) method as the primary analysis technique.

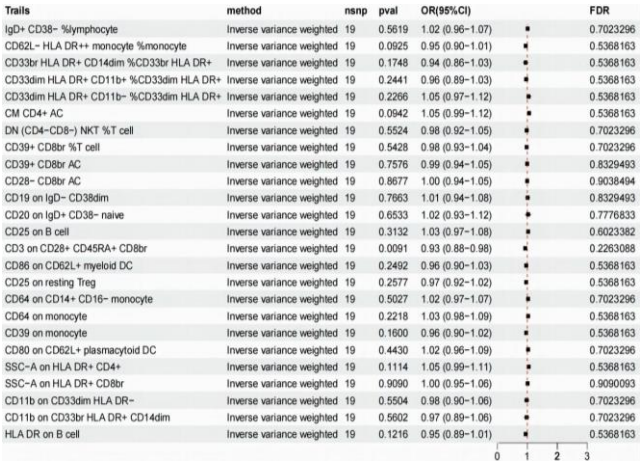
After multiple adjustments for results using the False Discovery Rate (FDR) method, no statistically significant differences in immunological traits were found (0.05).

Ultimately, at a significance level of 0.2, no significant associations were found between any immunological traits and pancreatic cancer (Figures 1).

It is important to note that the False Discovery Rate (FDR) correction is designed to control the overall proportion of false positives across a range of tests.

Even when individual P-values are less than 0.05, an FDR value above 0.2 suggests that the result may not be sufficiently supported by the evidence when considering the context of multiple comparisons.

Figure 1: Forest plots depict the causal relationship between pancreatic cancer and various immune cell traits.



IVW indicates the inverse variance weighting, and CI denotes the confidence interval. Trails: immunological phenotypes; Methods: IVW (inverse variance weighting); nsnp: number of single nucleotide polymorphisms (SNPs); pval: P value; OR (95% CI): odds ratio and 95% confidence interval; FDR: false discovery rate.

Exploring the Causal Relationship between Immunophenotypes and Pancreatic Cancer

After FDR adjustment (PFDR < 0.05), we identified 26 immunological cell phenotypes out of the four immune features that are causally associated with pancreatic cancer (Figures 2).

Among them, seven immunophenotypes were found to significantly inhibit the incidence and progression of pancreatic cancer (PC) (IVW ≥ 0.05, OR ≥ 1), including CD62L− HLA DR++ monocyte %monocyte IVW (OR=1.11,95%CI=1.02~1.21, P=0.0184), CD33br HLA DR+ CD14dim %CD33br HLA DR+ IVW (OR=1.09,95%CI=1.01~1.17, P=0.0251), CD33dim HLA DR+ CD11b−%CD33dim HLA DR+ IVW (OR=1.05,95%CI=1.00~1.09, P=0.0324), CD86 on CD62L+ myeloid DC IVW (OR=1.09,95%CI=1.00~1.19, P=0.0476), CD80 on CD62L+ plasmacytoid DC IVW (OR=1.08,95%CI=1.00~1.16, P=0.0375),

SSC−A on HLA DR+ CD8br, HLA DR on B cell IVW (OR=1.13,95%CI=1.06~1.21, P<0.001). Conversely, 19 immunophenotypes were found to potentially contribute to the onset of PC (IVW < 0.05, OR < 1), including: IgD+ CD38− %lymphocyte IVW (OR=0.90,95%CI=0.83~0.98, P=0.0180), CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+ IVW (OR=0.95,95%CI=0.91~1.00, P=0.0364), CM CD4+ AC IVW (OR=0.93,95%CI=0.87~0.99, P=0.0216), DN (CD4−CD8−) NKT %T cell IVW (OR=0, 87,95%CI=0.78~0.97, P=0.0143), CD39+ CD8br %T cell IVW (OR=0.87,95%CI=0.76~1.00, P=0.0436), CD39+ CD8br AC IVW (OR=0.89,95%CI=0.82~0.97, P=0.0088), CD28− CD8br AC IVW (OR=0.84,95%CI=0.72~0.97, P=0.0153), CD19 on IgD− CD38dim IVW (OR=0.90,95%CI=0.82~1.00, P=0.0493), CD20 on IgD+ CD38− naive IVW (OR=0.85,95%CI=0.76~0.95, P=0.0034), CD24 on unsw mem IVW (OR=0.92,95%CI=0.86~0.98, P=0.0060), CD25 on B cell IVW (OR=0.92,95%CI=0.86~0.98, P=0.0084), CD3 on CD28+ CD45RA+ CD8br IVW (OR=0.92,95%CI=0.85~1.00, P=0.0383), CD25 on resting Treg IVW (OR=0.87,95%CI=0.78~0.96, P=0.0048), CD64 on CD14+ CD16− monocyte IVW (OR=0.96,95%CI=0.93~1.00, P=0.0402), CD64 on monocyte IVW (OR=0.86,95%CI=0.80~0.92, P< 0.001), CD39 on monocyte IVW (OR=0.95,95%CI=0.91~1.00, P=0.0308), SSC−A on HLA DR+ CD4+ IVW (OR=0.89,95%CI=0.80~0.99, P=0.0272), CD11b on CD33dim HLA DR− IVW (OR=0.92,95%CI=0.85~0.99, P=0.0320), CD11b on CD33br HLA DR+ CD14dim IVW (OR=1.13,95%CI=1.06~1.21, P=0.0103).

Moreover, the MR–Egger regression intercept and the global test from MR-PRESSO did not indicate horizontal pleiotropy for the four significant associations, thus reinforcing the validity of our findings.

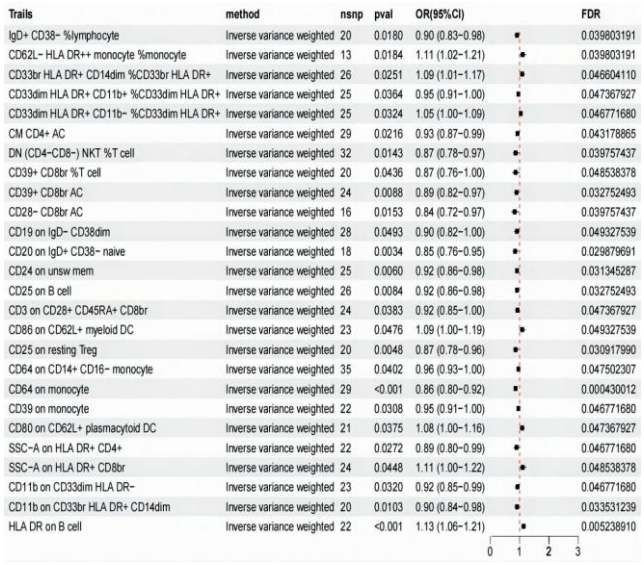
The consistency analysis provided further evidence of the robustness of the observed causal relationships, as detailed in Supplementary Figure 3.

The scatter plot indicated a relative consistency in the effects observed across different analytical methods, and the odds ratios (ORs) that were computed under various models were also in close agreement.

This consistency across methods reaffirms the stability of our findings, as depicted in Supplementary Figure 4 for a more comprehensive view.

Figure 2: Forest plots depicting the causal relationship between immune cell traits and pancreatic cancer.

Trails	method	nsnp	pval	OR(95%CI)	FDR
IgD+ CD38− %lymphocyte	Inverse variance weighted	20	0.0180	0.90 (0.83−0.98)	0.039803191
CD62L− HLA DR++ monocyte %monocyte	Inverse variance weighted	13	0.0184	1.11 (1.02−1.21)	0.039803191
CD33br HLA DR+ CD14dim %CD33br HLA DR+	Inverse variance weighted	26	0.0251	1.09 (1.01−1.17)	0.046604110
CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+	Inverse variance weighted	25	0.0364	0.95 (0.91−1.00)	0.047367927
CD33dim HLA DR+ CD11b− %CD33dim HLA DR+	Inverse variance weighted	25	0.0324	1.05 (1.00−1.09)	0.046771680
CM CD4+ AC	Inverse variance weighted	29	0.0216	0.93 (0.87−0.99)	0.043178865
DN (CD4−CD8−) NKT %T cell	Inverse variance weighted	32	0.0143	0.87 (0.78−0.97)	0.039757437
CD39+ CD8br %T cell	Inverse variance weighted	20	0.0436	0.87 (0.76−1.00)	0.048538378
CD39+ CD8br AC	Inverse variance weighted	24	0.0088	0.89 (0.82−0.97)	0.032752493
CD28− CD8br AC	Inverse variance weighted	16	0.0153	0.84 (0.72−0.97)	0.039757437
CD19 on IgD− CD38dim	Inverse variance weighted	28	0.0493	0.90 (0.82−1.00)	0.049327539
CD20 on IgD+ CD38− naive	Inverse variance weighted	18	0.0034	0.85 (0.76−0.95)	0.029879691
CD24 on unsw mem	Inverse variance weighted	25	0.0060	0.92 (0.86−0.98)	0.031345287
CD25 on B cell	Inverse variance weighted	26	0.0084	0.92 (0.86−0.98)	0.032752493
CD3 on CD28+ CD45RA+ CD8br	Inverse variance weighted	24	0.0383	0.92 (0.85−1.00)	0.047367927
CD86 on CD62L+ myeloid DC	Inverse variance weighted	23	0.0476	1.09 (1.00−1.19)	0.049327539
CD25 on resting Treg	Inverse variance weighted	20	0.0048	0.87 (0.78−0.96)	0.030917990
CD64 on CD14+ CD16− monocyte	Inverse variance weighted	35	0.0402	0.96 (0.93−1.00)	0.047502307
CD64 on monocyte	Inverse variance weighted	29	<0.001	0.86 (0.80−0.92)	0.000430012
CD39 on monocyte	Inverse variance weighted	22	0.0308	0.95 (0.91−1.00)	0.046771680
CD80 on CD62L+ plasmacytoid DC	Inverse variance weighted	21	0.0375	1.08 (1.00−1.16)	0.047367927
SSC−A on HLA DR+ CD4+	Inverse variance weighted	22	0.0272	0.89 (0.80−0.99)	0.046771680
SSC−A on HLA DR+ CD8br	Inverse variance weighted	24	0.0448	1.11 (1.00−1.22)	0.048538378
CD11b on CD33dim HLA DR−	Inverse variance weighted	23	0.0320	0.92 (0.85−0.99)	0.046771680
CD11b on CD33br HLA DR+ CD14dim	Inverse variance weighted	20	0.0103	0.90 (0.84−0.98)	0.033531239
HLA DR on B cell	Inverse variance weighted	22	<0.001	1.13 (1.06−1.21)	0.005238910



IVW indicates the inverse variance weighting, and CI denotes the confidence interval. Trails: immunological phenotypes; Methods: IVW (inverse variance weighting); nsnp: number of single nucleotide polymorphisms (SNPs); pval: P value; OR (95% CI): odds ratio and 95% confidence interval; FDR: false discovery rate.

Figure 3: Funnel plot analysis for the association between CD39+ CD8br AC absolute cell counts (ACs) and pancreatic cancer.

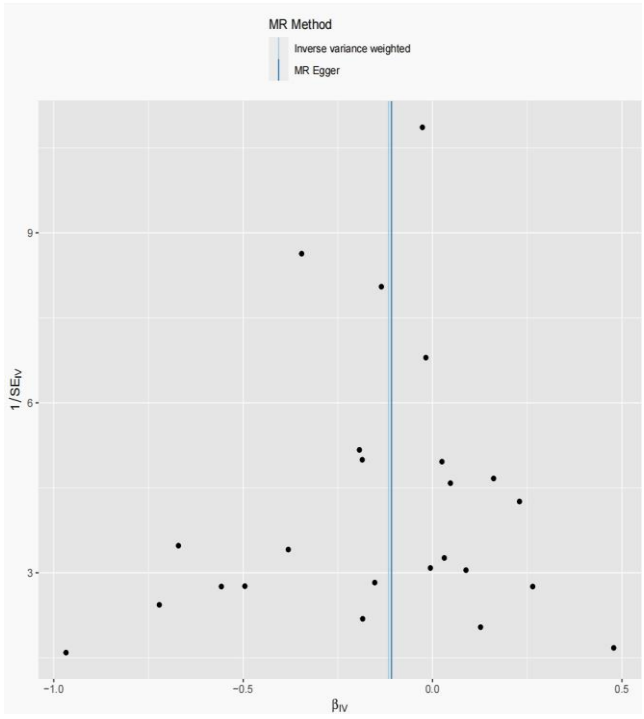
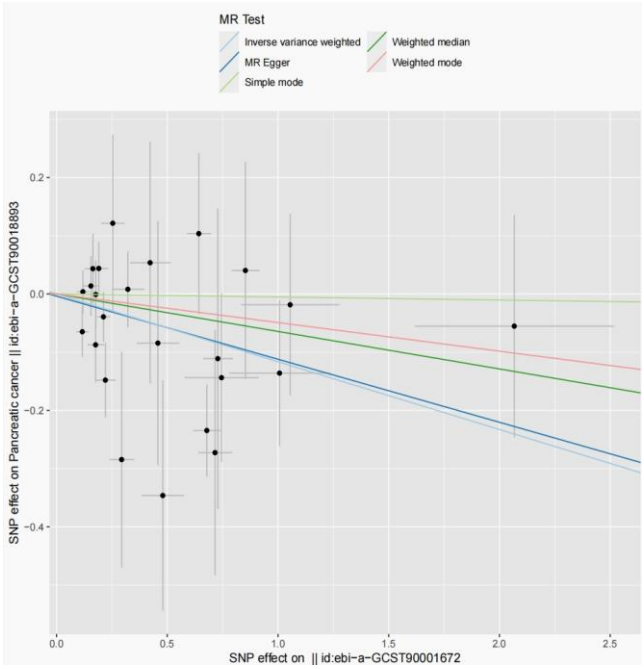


Figure 4: Scatter plot depicting the relationship between CD39+ CD8br AC absolute cell counts (ACs) and pancreatic cancer.



DISCUSSION

This study analyzed the association between 731 immune cell-related traits and pancreatic cancer using widely available genetic data. It is, to the best of our knowledge, the first to systematically explore the potential causal relationship between immunophenotypes and pancreatic cancer using multivariate regression analysis (MR analysis). The results indicate that out of the four immune traits (MFI, RC, AC, and MP) examined, there were 14, 7, 3, and 2 statistically significant interactions (FDR < 0.05) respectively. Based on these findings, this study plans to further investigate the relationship between immune cells and the occurrence and progression of pancreatic cancer, with the aim of providing scientific theoretical support for the prevention and detection of pancreatic cancer.

Monocytes are a crucial component of the innate immune system. Our study found that three types of monocytes (i.e., CD64 on CD14+ CD16- monocyte, CD64 on monocyte, CD39 on monocyte) were significantly inhibited in the occurrence and development of pancreatic cancer (IVW < 0.05, OR < 1). Additionally, one immunophenotype (CD62L- HLA DR++ monocyte %monocyte) was found to promote the progression of pancreatic cancer (IVW < 0.05, OR > 1). A population-based study found that the count of peripheral blood monocytes increased in the pre-diagnosis period of pancreatic cancer Verbanck et al. (2018).

Furthermore, related studies have discovered that monocytes can recruit monocytes and reduce the infiltration of CD8 T cells in pancreatic tumors through the targeting of the CCL2/CCR2 axis, thereby decreasing the survival rate of pancreatic cancer patients, indicating a significant inverse relationship between the count of monocytes in peripheral blood and patient survival Sanford et al. (2013), Li et al. (2022). This phenomenon suggests that the reduction of monocytes may have a positive independent predictive value for patient survival after certain tumor resection surgeries. Additionally, Christian Benzing et al. (2019) revealed that the level of TIMP2 (tissue inhibitor of metalloproteinase 2) in the tumor microenvironment of pancreatic ductal adenocarcinoma (PDAC) patients may have significant prognostic implications. In primary tumors, activated monocytes expressing TIMP2 may represent a potential therapeutic strategy to inhibit the invasive ability of tumor cells in PDAC. These research results highlight the complexity and heterogeneity of monocyte populations in the pancreatic tumor microenvironment.

Dendritic cells (cDCs) play a central role in regulating adaptive immune responses to tumor antigens, and the precise balance of their quantity and functional state is crucial for determining whether the immune response is protective or detrimental Bordon et al. (2020). Our study found that two immunophenotypes of dendritic cells (i.e., CD86 on CD62L+ myeloid DC and CD80 on CD62L+ plasmacytoid DC) promoted the progression of pancreatic cancer (IVW < 0.05, OR > 1). Related evidence also suggests that dendritic cells (PDCs, i.e., Plasmacytoid dendritic cells) may play a key role in tolerogenic functions within the tumor microenvironment, effectively suppressing host T cell responses against tumors. This effect may be achieved by activating regulatory T cells (Tregs) Bordon et al. (2020). Tregs are a class of T cells with immunomodulatory functions that can inhibit the activation and function of effector T cells, thereby helping tumor cells evade immune surveillance and clearance. PDCs can promote the proliferation and activation of Tregs by producing cytokines and presenting antigens, thereby influencing the immune balance in the tumor microenvironment and favoring the sustained growth and spread of tumors. However, the differences in T cell responses at different stages of pancreatic cancer (PDAC) reflect the heterogeneity of invasive traditional dendritic cells (cDCs) functions. In early PDAC, correcting cDC defects can effectively inhibit disease progression. In advanced PDAC, restoring cDC function not only reactivates tumor-suppressing immune responses but also enhances sensitivity to radiotherapy Hegde et al. (2020). Therefore, cDCs play a critical role in the progression and clinical outcomes of pancreatic cancer in patients. To further understand the specific role of human cDCs in pancreatic cancer, new research is needed.

Myeloid-derived suppressor cells (MDSCs) represent a class of activated neutrophils and monocytes that are characterized by significant immunosuppressive functions. These cells play a role in immune regulation under various pathological conditions and are closely associated with poor prognosis in cancer patients Veglia et al. (2021). Our study found that during the progression of pancreatic cancer, two immunophenotypes (i.e., CD33br HLA DR+ CD14dim %CD33br HLA DR+ and CD33dim HLA DR+ CD11b- %CD33dim HLA DR+) were promoting (IVW < 0.05, OR > 1), while another three immunophenotypes (i.e., CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+, CD11b on CD33dim HLA DR- and CD11b on CD33br HLA DR+ CD14dim) had the opposite effect (IVW < 0.05, OR < 1). Previous studies have confirmed that myeloid suppressor cells (MDSCs) not only promote the development of pancreatic ductal adenocarcinoma (PDAC) but also effectively suppress antitumor immune responses triggered by therapeutic agents. Within the microenvironment of pancreatic cancer, these cells reduce the proliferation of cytotoxic T cells through various non-specific and cell-dependent immunosuppressive mechanisms, while promoting the transition of macrophages to M2 phenotype and recruiting regulatory T cells, thereby providing immune protection for the sustained growth of tumors Pergamo et al. (2017), Thyagarajan et al. (2019). However, our findings challenge the existing theories and may provide new perspectives and methods for the immunotherapy of pancreatic cancer. These discoveries urgently require further experimental validation and elucidation.

Tumor-infiltrating lymphocytes (TILs) encompass several distinct lymphocyte subpopulations, primarily divided into B cells, T cells, and natural killer (NK) cells. Our study identified 11 immunophenotypes of TILs (i.e., IgD+ CD38- % lymphocyte, CM CD4+ AC, DN(CD4- CD8-) NKT % T cell, CD39+ CD8br AC, CD28- CD8br AC, CD19 on IgD- CD38dim, CD20 on IgD+ CD38- naive, CD24 on unsw mem, CD25 on B cell, CD3 on CD28+ CD45RA+ CD8br, SSC-A on HLA DR+ CD4+) that inhibit the progression of pancreatic cancer (IVW < 0.05, OR < 1), while two other immunophenotypes of TILs (SSC-A on HLA DR+ CD8br and HLA DR on B cell) promote the progression of pancreatic cancer (IVW < 0.05, OR > 1). B lymphocytes play a complex dual role in the tumor microenvironment (TME); they can both exert antitumor effects and promote tumor growth. When tumor-infiltrating B cells mature with the assistance of follicular helper T cells (Tfh), they can differentiate into plasma cells producing IgG1 and memory B cells in ectopic lymph nodes, such as tertiary lymphoid structures (TLS).

These cells respond to tumor-associated antigens by producing IgG1 and IgE antibodies, activating the the complement system of natural killer (NK) cells and macrophages, phagocytosis, or antibody-dependent cell-mediated cytotoxicity (ADCC). In TLS, B cells can also present tumor-associated antigens to T cells, further enhancing the immune response. However, on the other hand, pro-tumor B cells assist in tumor growth and progression by producing circulating immune complexes (CIC), inhibiting antitumor immune responses, and promoting angiogenesis Pergamo et al. (2017), Thyagarajan et al. (2019). Therefore, the role of B cells in the tumor microenvironment is not constant, but rather exhibits antitumor or pro-tumor dual properties depending on different microenvironment conditions and immune regulation. This complexity highlights the potential strategy of regulating and utilizing B cell functions in tumor therapy, which may play a crucial role in improving patient prognosis. Additionally, Sun G et al. have reported Sun et al. (2023) that there are significant changes in the composition of T cells in pancreatic cancer (PC) tissue compared to adjacent non-tumor tissue. In PC tissue, the proportions of total T cells, CD4+ T cells, and CD8+ cytotoxic T lymphocytes (CTLs) are significantly decreased, while the proportions of regulatory T cells (Tregs) and PD-L1-positive T cells are significantly increased. Furthermore, the infiltration levels of CD4+ T cells and CD8+ CTLs are inversely correlated with the degree of tumor differentiation, such that tumors with lower differentiation levels often accompany fewer immune cell infiltrations. The higher infiltration rates of Tregs and PD-L1+ T cells are closely associated with the advanced N stage and TNM stage of the disease, indicating that these T cells may be related to the invasiveness and progression of tumors. Daley D et al. have reported Daley et al. (2016) that approximately 40% of the tumor-infiltrating T cells in human pancreatic ductal adenocarcinoma (PDA) tumor microenvironments belong to a unique γδT cell subset. The recruitment and activation of these γδT cells are strictly dependent on a series of different chemokine signaling pathways.The study found that ablation, depletion, or blocking of these γδT cells can provide tumor protection in PDA models. This protection is accompanied by increased infiltration, activation, and Th1 polarization of αβT cells. Additionally, some studies have confirmed Lu et al. (2017), Paijens et al. (2021) that the CD8 TCRαβ subset—cytotoxic T lymphocytes (CTLs), different phenotypes of T cells can suppress immune responses through the expression of inhibitory receptors, such as PD-1, which is usually associated with immune tolerance and tumor escape. However, in some cases, they can also be used to activate antitumor immune responses. Or by activating the STAT (signal transduction and transcription activation) pathway, the activation and expansion of CD8+ T cells can be enhanced, thereby improving their killing ability against tumor cells. Or by enhancing the maturation and

function of DCs, their capture and presentation of tumor-associated antigens can be increased, thereby activating T cells and initiating an immune response. Or by regulating the expression of CXCR5 and BCL-6, the activation, proliferation, and differentiation of B cells into plasma cells can be promoted. CXCR5 is a chemokine receptor whose ligand CXCL13 can attract B cells to specific tissue microenvironments, while BCL-6 is a transcription factor crucial for the differentiation and function of B cells. By regulating these pathways, the humoral immune response can be enhanced, thereby playing an important role in antitumor immunity. However, Paijens ST et al. believe Paijens et al. (2021) that tumor-infiltrating lymphocytes play a crucial role in the tumor immune microenvironment. There is no single lymphocyte subset that can independently take on the task of tumor immune surveillance; instead, the positioning, aggregation, interaction, and co-stimulatory signal transmission of various lymphocyte subsets are necessary conditions for an effective antitumor immune response. Our current study has only analyzed the association between a single immune cell phenotype and pancreatic cancer; subsequent experiments can further investigate the impact of the interaction between various lymphocyte subsets on the progression of pancreatic cancer.

It is necessary to note separately that Regulatory T cells (Tregs) are an important subpopulation of CD4+ T cells and play a crucial role in maintaining the stability and balance of the immune system. Treg cells ensure the normal functioning of the immune system and the homeostasis of the body by inhibiting autoimmune reactions, regulating excessive inflammation, and maintaining tolerance between maternal and fetal tissues. This immunoregulatory property of Tregs can sometimes promote the occurrence and development of various types of cancer. This cell subset can create a microenvironment favorable for tumor growth by suppressing immune system responses Glasner et al. (2021). However, we found that an immunophenotype (CD25 on resting Treg) inhibits the progression of pancreatic cancer (IVW < 0.05, OR < 1). Simultaneously, studies have found Zhang et al. (2020) that regulatory T cells (Tregs) are present in higher amounts in human and mouse pancreatic cancer. To investigate the role of Tregs in the immunosuppressive microenvironment of pancreatic cancer, researchers have implemented strategies to deplete Tregs in pancreatic cancer mouse models. However, the results were surprising, as the depletion of Tregs did not alleviate the immunosuppressive state but instead accelerated tumor growth. This finding suggests that a deeper understanding of the role of Tregs in the treatment of pancreatic cancer is needed. Further experiments are required to uncover the relevant mechanisms.

This study relies on a large-scale GWAS cohort dataset and conducts an in-depth two-sample Mendelian randomization (MR) analysis on this rich genetic information. Thanks to the vast sample size of approximately 476,245 individuals, we achieve higher statistical power, ensuring the significance and reliability of the results. The conclusions of this study are based on the careful use of genetic instrumental variables, and through the comprehensive application of various Mendelian randomization (MR) analysis methods, we achieve detailed reasoning and precise inference of causal relationships. Our research results demonstrate significant robustness, successfully resisting the influence of horizontal pleiotropy and other potential interfering factors. Nevertheless, there are certain limitations to our study. Firstly, although we have implemented detailed multiple sensitivity analyses, the challenge of fully assessing and quantifying horizontal pleiotropy remains. Secondly, due to the availability of individual data, we were unable to conduct in-depth population stratification analysis to explore differences between different subgroups. Thirdly, considering that the foundation of our study is the European database, our findings cannot be universally applied to different racial groups around the world, which to some extent limits the generalizability of the conclusions. Lastly, we adopted a relatively lenient statistical threshold in evaluating the results, which may lead to an increase in false positive results and also potentially affect our comprehensive assessment of the association between the immune profile and pancreatic cancer.

We thoroughly investigated the application of four immunological metrics—Mean Fluorescence Intensity (MFI), Relative Count (RC), Absolute Count (AC), and Maturity Probability (MP)—in depicting the fundamental characteristics of the immune system. The study found that these metrics reveal the complexity of the immune system from different perspectives, particularly the causal relationship between MFI and pancreatic cancer, which exhibits significant findings that deserve high attention. Additionally, the difference in value between RC and AC and the proportional relationship they represent between immune cells and their progenitors provide us with deeper insights. This proportional relationship may more accurately reflect the subtle balance between immune cells and immune factors, which may have a significant impact on the occurrence and progression of pancreatic cancer.

Based on this, we can propose a hypothesis that during the interactive process between pancreatic cancer and the immune system, these proportional relationships may uncover factors related to immune molecular changes, and these immune molecules play a key role in maintaining the feedback mechanisms of cellular balance.

To verify this hypothesis, further functional studies are essential. By delving into the specific impact of changes in the proportion of cell types on pancreatic cancer, we hope to uncover new mechanisms of the disease's occurrence and progression, providing a new perspective and strategies for clinical prevention and monitoring.

Specifically, functional studies can be initiated in the following aspects: firstly, investigating the dynamic changes of different cell types during the development of pancreatic cancer to reveal their roles in the tumor microenvironment; secondly, exploring the interactions between immune cells and tumor cells, as well as the critical role of immune molecules in regulating these interactions; and lastly, monitoring and tracking the changes in the balance state of immune cells during the treatment process of pancreatic cancer, with the aim of providing a basis for the adjustment of clinical treatment strategies.

In summary, our research results have provided new insights and methods for the immunological study of pancreatic cancer, which is expected to bring about revolutionary changes in the prevention and monitoring of the disease. In future studies, we will continue to delve into the role of changes in the proportion of immune cells in the occurrence and progression of pancreatic cancer, providing theoretical evidence and practical guidance for clinical practice.

DECLARATIONS

Funding

Funding information National Natural Science Foundation of China.

REFERENCES

1.Mizrahi JD, Surana R, Valle JW, et al. 2020 Jun 27. Pancreatic cancer. Lancet. 395(10242):2008-2020.

2.McGuigan A, Kelly P, Turkington RC, et al. 2018 Nov 21. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. World J Gastroenterol. 24(43):4846-4861.

3.Traub B, Link KH, Kornmann M. 2021 Nov. Curing pancreatic cancer. Semin Cancer Biol. 76:232-246.

4.Lambert A, Schwarz L, Borbath I, et al. 2019 Sep 25. An update on treatment options for pancreatic adenocarcinoma. Ther Adv Med Oncol. 11:1758835919875568.

5.Robatel S, Schenk M. 2022 Feb 16. Current Limitations and Novel Perspectives in Pancreatic Cancer Treatment. Cancers (Basel). 14(4):985.

6.Klein AP. 2021 Jul. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. Nat Rev Gastroenterol Hepatol. 18(7):493-502.

7.Khalaf N, Ali B. 2022 Jun. Challenges and Opportunities in Pancreatic Cancer Screening Among High-Risk Individuals. Gastroenterology. 162(7):2113-2120.

8.Wood LD, Canto MI, Jaffee EM, et al. 2022 Aug. Pancreatic Cancer: Pathogenesis, Screening, Diagnosis, and Treatment. Gastroenterology. 163(2):386-402.

9.Ino Y, Yamazaki-Itoh R, Shimada K, et al. 2013 Mar 5. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. Br J Cancer. 108(4):914-23.

10.Elebo N, Fru P, Omoshoro-Jones J, et al. 2020 Dec. Role of different immune cells and metabolic pathways in modulating the immune response in pancreatic cancer (Review). Mol Med Rep. 22(6):4981-4991.

11.Huber M, Brehm CU, Gress TM, et al. 2020 Oct 3. The Immune Microenvironment in Pancreatic Cancer. Int J Mol Sci. 21(19):7307.

12.Zhang Y, McAllister F, Magliano MPD. 2014 Jun 5. Immune cells in pancreatic cancer: Joining the dark side. Oncoimmunology. 3:e29125.

13.Xu Y, Li Z, Shi H, et al. 2022 Oct. Clinicopathological and prognostic significance of circulating immune cells in the patients with pancreatic cancer. Int Immunopharmacol. 111:109157.

14.Xu C, Sui S, Shang Y, et al. 2020 Mar 29. The landscape of immune cell infiltration and its clinical implications of pancreatic ductal adenocarcinoma. J Adv Res. 24:139-148.

15.Hyung J, Lee H, Jin H, et al. 2022 Jun. Tumor immune-gene expression profiles and peripheral immune phenotypes associated with clinical outcomes of locally advanced pancreatic cancer following FOLFIRINOX. ESMO Open. 7(3):100484.

16.Lu Y, Gentiluomo M, Lorenzo-Bermejo J, et al. 2020 Dec. Mendelian randomisation study of the effects of known and putative risk factors on pancreatic cancer. J Med Genet. 57(12):820-828.

17.Smith GD, Hemani G. 2014 Sep 15. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet. 23(R1): R89-98.

18.Sakaue S, Kanai M, Tanigawa Y, et al. 2021 Oct. A cross-population atlas of genetic associations for 220 human phenotypes. Nat Genet. 53(10):1415-1424

19.Orru V, Steri M, Sidore C, et al. 2020 Oct. Complex genetic signatures in immune cells underlie autoimmunity and inform therapy. 52(10):1036-1045.

20.Sidore C, Busonero F, Maschio A, et al. 2015 Nov. Genome sequencing elucidates Sardinian genetic architecture and augments association analyses for lipid and blood inflammatory markers. Nat Genet. 47(11):1272-1281.

21.Ripke S, Neale BM, Corvin A, et al. 2014 Jul 24. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 511(7510):421-7.

22.Yu X-H, Yang Y-Q, Cao R-R, et al. 2021 Dec. The Causal Role of Gut Microbiota in Development of osteoarthritis. Osteoarthritis Cartilage. 29(12):1741-1750.

23.Bowden J, Smith GD, Haycock PC, et al. 2016 May. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet Epidemiol. 40(4):304-14.

24.Hartwig FP, Smith GD, Bowden J. 2017 Dec 1. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. Int J Epidemiol.

25.Burgess S, Thompson SG. 2017 May. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol. 32(5):377-389.

26.Verbanck M, Chen CY, Neale B, et al. 2018 May. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet. 50(5):693-698.

27.Fuente JDL, Sharma A, Chari S, et al. 2019 Dec. Peripheral blood monocyte counts are elevated in the pre-diagnostic phase of pancreatic cancer: A population-based study. Pancreatology. 19(8):1043-1048.

28.Sanford DE, Belt BA, Panni RZ, et al. 2013 Jul 1. Inflammatory monocyte mobilization decreases patient survival in pancreatic cancer: a role for targeting the CCL2/CCR2 axis. Clin Cancer Res. 19(13):3404-15.

29.Li X, He G, Liu J, et al. 2022 May. CCL2-mediated monocytes regulate immune checkpoint blockade resistance in pancreatic cancer. Int Immunopharmacol. 106:108598.

30.Benzing C, Lam H, Tsang CM, et al. 2019 Dec 13. TIMP-2 secreted by monocyte-like cells is a potent suppressor of invadopodia formation in pancreatic cancer cells. BMC Cancer. 19(1):1214.

31.Bordon Y. 2020 May. DC deployment in pancreatic cancer. Nat Rev Immunol. 20(5):276-277.

32.Jegalian AG, Facchetti F, Jaffe ES. 2009 Nov. Plasmacytoid dendritic cells: physiologic roles and pathologic states. Adv Anat Pathol. 16(6):392-404.

33.Hegde S, Krisnawan VE, Herzog BH, et al. 2020 Mar 16. Dendritic Cell Paucity Leads to Dysfunctional Immune Surveillance in Pancreatic Cancer. Cancer Cell. 37(3):289-307.

34.Veglia F, Sanseviero E, Gabrilovich DI. 2021 Aug. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat Rev Immunol. 21(8):485-498.

35.Pergamo M, Miller G. 2017 Mar. Myeloid-derived suppressor cells and their role in pancreatic cancer. Cancer Gene Ther. 24(3):100-105.

36.Thyagarajan A, Alshehri MSA, Miller KLR, et al. 2019 Oct 24. Myeloid-Derived Suppressor Cells and Pancreatic Cancer: Implications in Novel Therapeutic Approaches. Cancers (Basel). 11(11):1627.

37.Senturk ZN, Akdag I, Deniz B, et al. 2023 Mar 23. Pancreatic cancer: Emerging field of regulatory B-cell-targeted immunotherapies. Front Immunol. 14:1152551.

38.Sun G, Yang Z, Fang K, et al. 2023 May 2. Distribution characteristics and clinical significance of infiltrating T cells in the tumor microenvironment of pancreatic cancer. Oncol Lett. 25(6):261.

39.Daley D, Zambirinis CP, Seifert L, et al. 2016 Sep 8. γδ T Cells Support Pancreatic Oncogenesis by Restraining αβ T Cell Activation. Cell. 166(6):1485-1499.

40.Lu C, Talukder A, Savage NM, et al. 2017 Feb 10. JAK-STAT-mediated chronic inflammation impairs cytotoxic T lymphocyte activation to decrease anti-PD-1 immunotherapy efficacy in pancreatic cancer. Oncoimmunology. 6(3):e1291106.

41.Paijens ST, Vledder A, Bruyn MD, et al. 2021 Apr. Tumor-infiltrating lymphocytes in the immunotherapy era. Cell Mol Immunol. 18(4):842-859.

42.Glasner A, Plitas G. 2021 Feb. Tumor resident regulatory T cells. Semin Immunol. 52:101476.

43.Zhang Y, Lazarus J, Steele NG, et al. 2020 Mar. Regulatory T-cell Depletion Alters the Tumor Microenvironment and Accelerates Pancreatic Carcinogenesis. Cancer Discov. 10(3):422-439.

