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Mendelian randomization analysis of the causal association between immune cells and pancreatic cancer

Pengkhun Nov, Socheat Touch, Syphanna Sou, Samnang Kouy, Duanyu Wang, Jiqiang Li

ABSTRACT

Aims: Pancreatic cancer (PC) is a highly lethal malignancy with limited treatment options. Tumor-infiltrating immune cells have been implicated in the progression and prognosis of PC. However, the causal role of immune cell populations in pancreatic cancer development and progression remains unclear. This study aims to elucidate the causal relationships between specific immune cell populations and the risk of pancreatic cancer, addressing gaps in current understanding.

Method: We conducted an extensive two-sample Mendelian randomization (MR) analysis. Using publicly available genetic data, we investigated the causal relationship between 731 immune cells and PC. We used inverse variance weighting (IVW) and weighted medians for MR analyses and used sensitivity analyses to assess heterogeneity and pleiotropy.

Results: In terms of the association between immune cells and PC, we found that CD62L– HLA DR++ monocyte % monocytes (OR = 1.1081, 95% CI = 1.0175–1.2068, p = 0.0184), SSC–A on HLA DR+ CD8br (OR = 1.1068, 95% CI = 1.0024–1.2221, p = 0.0448), CD64 on monocytes (OR = 0.8594, 95% CI = 0.8021–0.9207, p < 0.001), double-negative (DN) (CD4– CD8–) NKT %T cells (OR = 0.8712, 95% CI = 0.7802–0.9728, p = 0.0143), and SSC–A on HLA DR+ CD4+ cells (OR = 0.8902, 95% CI = 0.8028–0.9870, p = 0.0272) were strongly associated with PC. Among them, CD62L– HLA DR++ monocyte % monocytes and SSC–A on HLA DR+ CD8br are the risk factors, while CD64 on monocytes, DN (CD4–CD8–) NKT %T cells, and SSC–A on HLA DR+ CD4+ cells are protective factors for PC.

Conclusion: Our analysis provides evidence for a causal relationship between specific immune cell populations and PC. Targeting immune cell populations with therapeutic interventions such as immunotherapies may hold promise for improving outcomes in PC patients. Further studies are warranted to validate these findings and explore the underlying mechanisms involved in the immune response to PC.

Keywords: Genome wide association study (GWAS), Immune cells, Mendelian randomization (MR), Pancreatic cancer

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INTRODUCTION

Pancreatic cancer (PC), a leading cause of cancer-related deaths, is anticipated to become the second leading cause of cancer mortality in the United States in the coming decades due to its high fatality rate. The 5-year survival rate is approximately 10%, as most patients (80–85%) present with either advanced or metastatic disease at diagnosis [1, 2]. In 2019, the American

Cancer Society reported about 56,000 new cases and an estimated 45,000 deaths from PC, placing it behind lung and colorectal cancer [1]. Globally, it ranks seventh in cancer deaths, with GLOBOCAN 2018 estimating about 459,000 new cases and 432,000 deaths [3]. Pancreatic cancer is projected to surpass breast cancer as the third leading cause of cancer death in the European Union (EU) [3]. The high lethality is primarily due to late detection, often post-distant metastasis, and the absence of a single risk factor in most cases not associated with known factors or genetic mutations. Several factors have been suggested as reasons behind the observed increase in incidence rates. These include high rates of tobacco smoking, obesity, diabetes mellitus, lack of physical activity, and the consumption of high-fat/high-calorie diets, particularly prevalent in specific countries [4–7]. Alongside these lifestyle factors, enhancements in the medical identification and diagnosis of PC, as well as a rising average life expectancy worldwide, also contribute to the increased incidence [8, 9].

Pancreatic cancer (PC) is frequently described as a "cold tumor," [10, 11] characterized by a scarcity of neoantigens that can be recognized by immune cells. This classification underscores its low immunogenicity and high immunosuppressive characteristics, which are likely influenced by immunodeficiency and immunosuppression in the tumor microenvironment (TME). Recent comprehensive studies of the TME have provided valuable insights into the pathogenesis of PC and potential targeted therapies. Therefore, understanding the role of each immunophenotype within the PC TME is essential for developing effective therapeutic strategies.

Immune checkpoint inhibition has transformed cancer treatment over the past decade, with nearly 70 different FDA-approved indications spanning more than 18 histologies. However, these therapies have not demonstrated significant clinical benefits in pancreatic cancer (PC), except for a small subset of patients (less than 1%) who exhibit microsatellite instability (MSI) in their tumors [12]. Single-agent therapies and combinations of PD-1, PD-L1, or CTLA-4 inhibitors have proven ineffective in patients with advanced pancreatic cancer, with objective response rates (ORRs) of less than 5% [13–15], including patients with positive PD-L1 expression, a biomarker that has been associated with improved responses in other cancers. Proposed mechanisms of resistance to immunotherapy in pancreatic cancer (PC) encompass poor T cell infiltration, low tumor mutational burden, and a highly immunosuppressive tumor microenvironment (TME). However, recent comprehensive profiling of PC tumors suggests that 20–30% of patients may exhibit moderate T cell content, and in certain contexts, the presence of tumor immunogenic neo-epitopes and T cell immunity can correlate with overall survival (OS) [16–18].

The close link between cancer and inflammation, keenly observed by Virchow [19] in the 19th century, foreshadowed contemporary concerns about a possible immunological role in neoplastic pathogenesis. As Harold Dvorak [20] has observed, inflammation bears a striking resemblance to a wound that cannot heal. Currently, an estimated 20% of global cancer mortalities relate to unhealed infections and/or inflammation, and a high proportion of this burden of disease is attributable to gastrointestinal malignancies [21]. Despite these instances of the immune system contributing to tumorigenesis and progression, there is also a wealth of data supporting the protective role of immunity in tumor suppression. While numerous cross-sectional and cohort studies have investigated the relationship between immune cells and PC cancer [22, 23], their observational nature limits them to establishing correlations rather than causations [23]. Although randomized controlled trials (RCTs) could infer causation, interventions to manipulate immune cells are neither feasible nor ethical, thus constraining our ability to draw causal inferences. Given the limited evidence from observational and interventional studies, the Mendelian randomization (MR) approach in human genetics presents a unique opportunity to robustly explore the potential causal links between increased immune cells and PC cancer [24]. This approach leverages the random allocation of genetic variation at conception, well before the onset of disease, making MR a valuable tool for establishing causality and mitigating the risk of reverse causality, independent of confounders typically present in study designs [25–27].

Here, we utilized MR to investigate the histophysiology and pathophysiological involvement of the immune cells in the development of PC cancer, achieved by a recent statistical summary from a genome-wide association study (GWAS) focused on immune cells [28]. Our study is dedicated to exploring the causal relationship between 731 immune cells and PC cancer, with a special focus on those in tumor initiation, progression, and treatment resistance. We present an extensive MR study that not only identifies specific immune cells associated with PC cancer but also addresses the constraints in current research. Our goal is to provide valuable insights that could refine future immune cell methodologies and advance etiological research. This work is intended to support precision prevention, control, and the development of innovative therapeutic approaches.

MATERIALS AND METHODS

Study design

The cause-and-effect relationship between 731 immune cells and PC was assessed using two-sample MR analyses. Mendelian randomization leverages genetic variations as proxies for risk factors. To ensure reliable causal inference, instrumental variables (IVs) used in MR, three key assumptions must be satisfied: (1) The genetic variation must be associated with the exposure directly; (2) The genetic variant is not linked with potential confounding factors between the exposure

and outcome; and (3) The genetic variation influences the outcome exclusively through the exposure, not via alternative pathways (Figure 1).

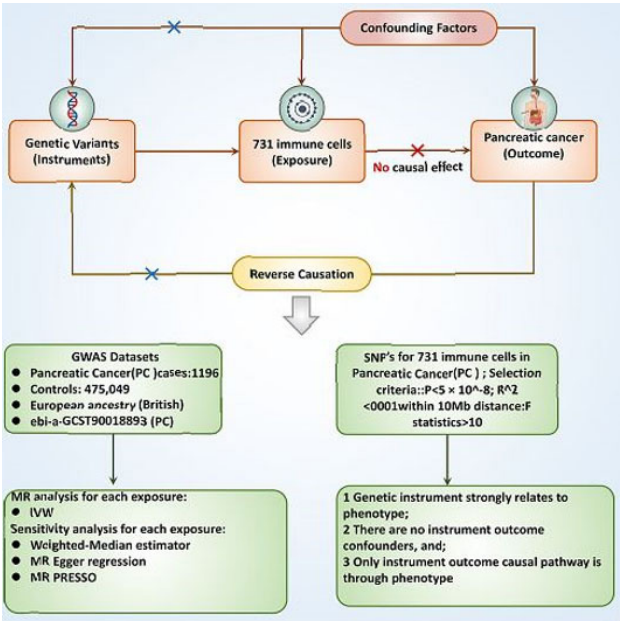

Figure 1: Study design flowchart. The initial assumption is that the instrument variables have a strong correlation with the exposure. The second assumption states that the instrument variables are not linked to any confounding factors. The third assumption asserts that the instrument variables affect the outcome exclusively through the exposure.

Data sources for exposure and outcome

A summary of Genome-Wide Association Study (GWAS) statistics for each immune trait is publicly accessible from the GWAS catalog (accession numbers: GCST0001391 to GCST0002121) [29]. We used cancer's keywords to find the immune traits from (https://gwas.mrcieu.ac.uk/). The immune traits included: ebi-a-GCST90018893 (PC). A total of 731 immunophenotypes, including absolute cell (AC) counts, median fluorescence intensities (MFI, which reflect surface antigen levels), morphological parameters (MP) and relative cell (RC) counts, were included. The MFI, AC, and RC features contain B cells, CDCs, mature T cells, monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells) cells, and Treg panels, while the MP feature contains CDC and TBNK panels. The GWAS database is a comprehensive collection of genetic variation and its association with various traits or diseases. It provides a valuable resource for researchers and clinicians interested in understanding the genetic basis of complex traits and diseases. Based on the inhibitor of differentiation (ID) of cancer, we used online data from GWAS including 476,245 European individuals (n = 1,196 case patients and 475,049 control participants) for PC to analyze the relationship between immune cells and PC according to IDs (immune traits) (https://www.ebi.ac.uk/gwas/).

Instrument selection

Considering that the single-nucleotide polymorphisms (SNPs) number demonstrating genome-wide significance ($p < 5 \times 10^{-8}$) for immune cells traits is extensive, we implemented more stringent correlation thresholds ($p < 5 \times 10^{-9}$) for Genetic Instrumental Variables (IVs) selection [28]. These IVs were identified by grouping them according to the reference panel of the Linkage Disequilibrium (LD) from the 1000 Genomes Project, with a threshold of $R^2 < 0.001$ at a distance of 1,000 kilobases (kb). Given the relatively limited size of the GWAS data for immune cells, we employed a p-value cutoff of 5×10^{-5} and a less significant clustering threshold ($R^2 < 0.001$ at a distance of 1000 kb) [29]. To ensure the reliability of our tools, we selected IVs with F-statistics exceeding 10, identifying them as strong instruments for subsequent analyses. We then extracted these IVs from the summary statistics pertaining to PC outcomes, excluding any that showed potential pleiotropic effects ($p < 10^{-5}$) on PC, in line with methodologies from previous studies [30]. To maintain consistency in our analysis, we synchronized the SNPs between the exposure and outcome datasets, ensuring uniform effect estimates for the same effect allele. Any alleles with mid-range effect the frequencies of allele (EAFs > 0.42) or SNPs incompatible with the allele were excluded from our analysis [29].

Statistical analysis

In our study, we employed a range of genetic variants as instrumental variables rather than relying solely on an allele score. This approach was chosen to thoroughly examine key assumptions, uncover potential pleiotropy, and facilitate more effective sensitivity and multivariable Mendelian randomization (MR) analyses [25]. To assess the consistency of our findings under different assumptions about heterogeneity and pleiotropy, we utilized four distinct MR methodologies: the inverse variance weighting (IVW; random-effects model), weighted median, MR-Egger, and MR-PRESSO. The IVW method, employing a random-effects model, served as the primary analysis framework for all four sets of instrumental variables. We quantified heterogeneity using Cochran's Q statistic.

Our study also included analyses with more stringent conditions. The IVW method, under the assumption that all genetic variants are valid, can be biased if many SNPs are influenced by horizontal pleiotropy [31]. Conversely, the weighted median approach, effective when less than 50% of variants exhibit horizontal pleiotropy, presumes most genetic variants are valid [32]. In cases where over 50% of variants are affected by horizontal pleiotropy, we evaluated the strength of our genetic instruments through F-statistics, considering a mean F-statistic of less than 10 indicative of weak instrumental variables [33].

Furthermore, the MR-Egger method was applied to check for potential directional pleiotropy. Here, a significant intercept would indicate a violation

of instrumental variable assumptions, suggesting directional pleiotropy [34]. We also implemented the MR pleiotropy residual sum and outlier (PRESSO) method, designed to minimize heterogeneity in causal effect estimates by excluding disproportionately influential SNPs (NbDistribution = 1,500) [35]. Additionally, Steiger-filtering analyses were conducted to detect and eliminate genetic variants more strongly associated with the outcome than the exposure, indicating possible reverse causality [36].

All statistical analyses were performed using R version 4.3.1 (R Foundation) and specific R packages ("TwoSampleMR" and "Mendelian Randomization") tailored for MR analysis [37, 38]. The TwoSampleMR package provided causal estimates from the four MR models (IVW, weighted median, MR-Egger, and MR-PRESSO), and the MendelianRandomization package was utilized for multivariable MR. Detailed methodologies are further provided in the online Supporting Information Methods.

RESULTS

The causal associations between immune cells and PC are summarized in Figure 2 and Table 1. Inverse variance weighting results showed a strong correlation between PC risk and CD62L– HLA DR++ monocyte % monocytes (OR = 1.1081, 95% CI = 1.0175–1.2068, p = 0.0184), SSC–A on HLA DR+ CD8br (OR = 1.1068, 95% CI = 1.0024–1.2221, p = 0.0448), CD64 on monocytes (OR = 0.8594, 95% CI = 0.8021–0.9207, p < 0.001), DN (CD4–CD8–) NKT %T cells (OR = 0.8712, 95% CI = 0.7802–0.9728, p = 0.0143), and SSC–A on HLA DR+ CD4+ cells (OR = 0.8902, 95% CI = 0.8028–0.9870, p = 0.0272). There was a weaker association between PC and CD33dim HLA DR+ CD11b+ % CD33dim HLA DR+ (OR = 0.9526, 95% CI = 0.9102–0.9969, p = 0.0364), CD11b on CD33dim HLA DR– (OR = 0.9196, 95% CI = 0.8518–0.9928, p = 0.0320), CD11b on CD33br HLA DR+ CD14dim (OR = 0.9042, 95% CI = 0.8373–0.9765, p = 0.0103), CD80 on CD62L+ plasmacytoid DCs (OR = 1.0807, 95% CI = 1.0045–1.1626, p = 0.0375), CD64 on CD14+ CD16– monocytes (OR = 0.9635, 95% CI = 0.9299–0.9983, p = 0.0402), CD28– CD127– CD25++ CD8br AC (OR = 0.9166, 95% CI = 0.8591–0.9779, p = 0.0084), CM CD4+ AC (OR = 0.9266, 95% CI = 0.8682–0.9889, p = 0.0216), CD33dim HLA DR+ CD11b– %CD33dim HLA DR+ (OR = 1.0470, 95% CI = 0.8591–0.9779, p = 0.0324), CD33br HLA DR+ CD14dim % CD33br HLA DR+ (OR = 1.0876, 95% CI = 1.0106–1.1706, p = 0.0251), and gD+ CD38– %lymphocytes (OR = 0.9043, 95% CI = 0.8320–0.9829, p = 0.0180). Neither the MR-Egger intercept test nor Cochran's Q test revealed pleiotropy or heterogeneity (Tables 2 and 3).

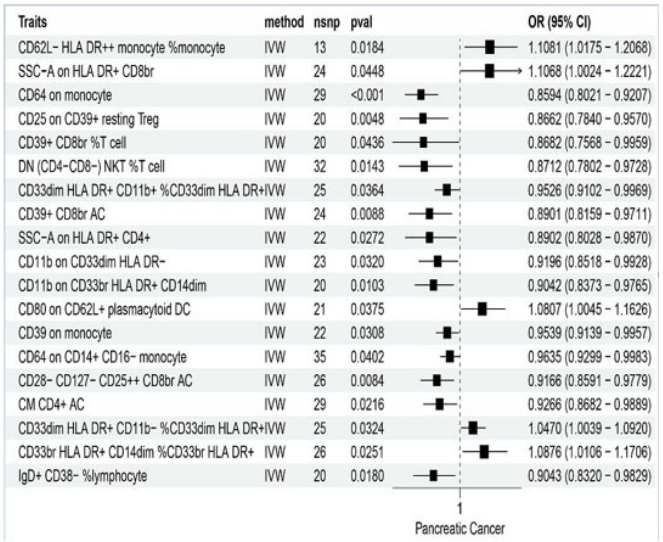

Figure 2: The causal estimation between immune cells and pancreatic cancer. We selected inverse variance weighting (IVW) as a primary method p < 0.05 showed statistically significant; OR value >1 indicated a risk factor, while OR value <1 indicated a protective factor.

Table 1: To summarize the odds ratios and confidence intervals for the causal relationship between each immune cell and PC

Trait (immune cell)	Odds ratios (OR)	Confidence intervals (CI)
CD62L– HLA DR++ monocyte %monocyte	1.1081	1.0175–1.2068
SSC–A on HLA DR+ CD8br	1.1068	1.0024–1.2221
CD64 on monocyte	0.8594	0.8021–0.9207
CD25 on CD39+ resting Treg	0.8662	0.7840–0.9570
CD39+ CD8br %T cell	0.8682	0.7568–0.9959
DN (CD4–CD8–) NKT %T cell	0.8712	0.7802–0.9728
CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+	0.9526	0.9102–0.9969
CD39+ CD8br AC	0.8901	0.8159–0.9711
SSC–A on HLA DR+ CD4+	0.8902	0.8028–0.9870
CD11b on CD33dim HLA DR–	0.9196	0.8518–0.9928
CD11b on CD33br HLA DR+ CD14dim	0.9042	0.8373–0.9765
CD80 on CD62L+ plasmacytoid DC	1.0807	1.0045–1.1626

Table 1: *(Continued)*

Trait (immune cell)	Odds ratios (OR)	Confidence intervals (CI)
CD39 on monocyte	0.9539	0.9139–0.9957
CD64 on CD14+ CD16– monocyte	0.9635	0.9299–0.9983
CD28– CD127– CD25++ CD8br AC	0.9166	0.8591–0.9779
CM CD4+ AC	0.9266	0.8682–0.9889
CD33dim HLA DR+ CD11b– %CD33dim HLA DR+	1.047	1.0039–1.0920
CD33br HLA DR+ CD14dim %CD33br HLA DR+	1.0876	1.0106–1.1706
IgD+ CD38– %lymphocyte	0.9043	0.8320–0.9829

Table 2: The pleiotropy of causal relationship between immune cells and pancreatic cancer

Triats	Egger_intercept	Se	p value
IgD+ CD38– %lymphocyte	0.004790713	0.021845732	0.828885915
CD62L- HLA DR++ monocyte %monocyte	0.019452798	0.021991486	0.395317282
CD33br HLA DR+ CD14dim %CD33br HLA DR+	−0.004533836	0.017308442	0.79560001
CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+	−0.000760484	0.016712846	0.964099166
CD33dim HLA DR+ CD11b- %CD33dim HLA DR+	−2.22E-05	0.016361062	0.998928175
CM CD4+ AC	0.012029053	0.016737606	0.478509473
DN (CD4-CD8-) NKT %T cell	−0.011124595	0.026159208	0.673678601
CD39+ CD8br %T cell	−0.058805707	0.027699124	0.047877516
CD39+ CD8br AC	−0.00384144	0.022368201	0.86521448
CD28- CD127- CD25++ CD8br AC	−0.009889837	0.017142134	0.569358344
CD25 on resting Treg	0.007609454	0.018087621	0.678954543
CD64 on CD14+ CD16- monocyte	−0.013683215	0.012519073	0.282313366
CD64 on monocyte	0.006948434	0.01783445	0.699883155
CD39 on monocyte	−0.002511929	0.016065081	0.877317176
CD80 on CD62L+ plasmacytoid DC	−0.003155661	0.015960104	0.845362723
SSC-A on HLA DR+ CD4+	−0.018642363	0.021108112	0.387629514
SSC-A on HLA DR+ CD8br	−0.006598285	0.018392262	0.723199575
CD11b on CD33dim HLA DR–	0.005248562	0.024788536	0.834356435
CD11b on CD33br HLA DR+ CD14dim	−0.009414844	0.027924681	0.739902277

Table 3: The heterogeneity of causal relationship between immune cells and pancreatic cancer

Method id	Traits	Q	Q_df	Q value
1		23.85943594	18	0.159687043
2	IgD+ CD38– %lymphocyte	23.92318219	19	0.199115512
1		9.437510073	11	0.581575963
2	CD62L– HLA DR++ monocyte %monocyte	10.21995721	12	0.596670691
1		25.87162124	24	0.359711034
2	CD33br HLA DR+ CD14dim %CD33br HLA DR+	25.94558656	25	0.41050707
1		19.55750608	23	0.66843534
2	CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+	19.5595766	24	0.721526344
1		17.43263489	23	0.787496067
2	CD33dim HLA DR+ CD11b %CD33dim HLA DR+	17.43263674	24	0.829633109
1		17.22079871	27	0.925534633
2	CM CD4+ AC	17.73730571	28	0.932573579

Table 2B: *(Continued)*

Method id	Traits	Q	Q_df	Q value
1		35.09799426	30	0.239092243
2	DN (CD4– CD8–) NKT %T cell	35.30957702	31	0.271696215
1		24.67078477	18	0.134256422
2	CD39+ CD8br %T cell	30.8483498	19	0.041946442
1		24.71762533	22	0.310767923
2	CD39+ CD8br AC	24.7507621	23	0.363262894
1		28.42157747	24	0.242660761
2	CD28– CD127– CD25++ CD8br AC	28.81574914	25	0.271691527
1		21.01854635	18	0.278478395
2	CD25 on resting Treg	21.22521485	19	0.324491081
1		32.89356445	33	0.472461476
2	CD64 on CD14+ CD16 monocyte	34.08819038	34	0.463498172
1		23.04375442	27	0.682659544
2	CD64 on monocyte	23.19554836	28	0.723192699
1		12.52096281	20	0.896979352
2	CD39 on monocyte	12.54541111	21	0.923710907
1		15.99480519	19	0.657624576
2	CD80 on CD62L+ plasmacytoid DC	16.0338991	20	0.714518995
1		18.66474635	20	0.543704016
2	SSC-A on HLA DR+ CD4+	19.44476168	21	0.556632194
1		21.72326657	22	0.476506554
2	SSC-A on HLA DR+ CD8br	21.8519705	23	0.529202799
1		9.137337895	21	0.988091694
2	CD11b on CD33dim HLA DR	9.182168945	22	0.992327688
1		13.34701685	18	0.770559197
2	CD11b on CD33br HLA DR+ CD14dim	13.46068789	19	0.814174845

DISCUSSION

Mendelian randomization analysis has been frequently employed to illustrate possible causality between risk factors and diseases. In the present study, we used MR to generate proof of an inverse causal relationship between immune cells and PC. In our study, we found a total of 19 immune cells associated with PC include CD62L– HLA DR++ monocyte % monocytes, SSC–A on HLA DR+ CD8br, CD64 on monocytes, DN (CD4–CD8–) NKT %T cells, SSC–A on HLA DR+ CD4+ cells, CD33dim HLA DR+ CD11b+ % CD33dim HLA DR+, CD11b on CD33dim HLA DR, CD11b on CD33br HLA DR+ CD14dim, CD80 on CD62L+ plasmacytoid DCs, CD64 on CD14+ CD16– monocytes, CD28– CD127– CD25++ CD8br AC, CM CD4+ AC, CD33dim HLA DR+ CD11b– % CD33dim HLA DR+, CD33br HLA DR+ CD14dim % CD33br HLA DR+, and gD+ CD38– %lymphocytes. Among them, CD62L– HLA DR++ monocyte % monocytes, SSC–A on HLA DR+ CD8br, CD80 on CD62L+ plasmacytoid DCs, CD33dim HLA DR+ CD11b– % CD33dim HLA DR+, CD33br HLA DR+ CD14dim % CD33br HLA DR+ are risk factors for PC, while the rest of immune cells are protective factors for PC. We have highlighted some of the strongest associations in the following discussion sections.

We found that CD62L–HLA DR++ monocyte infiltration was closely related to PC, and was a negative factor for PC prognosis. These monocytes express HLA-DR strongly but do not express CD62L. HLA-DR is a major histocompatibility complex (MHC) class II cell surface marker that is regulated by the human leukocyte antigen (HLA) complex located on chromosome 6 (region 6P21), and it contains two subunits with molecular weights of 36 and 27 kD, respectively (α Subunit and β Subunits). It is expressed mainly on antigen-presenting cells. Previous research has suggested that tumor-specific MHC-II expression is associated with good outcomes. For example, upregulated HLA-DR/MHC-II genes in tumors serve as predictive factors for neoadjuvant chemotherapy responses and good clinical outcomes in locally advanced rectal cancer [39]. In addition, increased MHC-II expression in tumor cells is associated with improved melanoma treatment responses, progression-free survival (PFS), and OS [40]. It can also be used as a positive

predictor of good outcomes in Hodgkin lymphoma after PD-1 blockade [41]. Interestingly, HLA-DR expression in non-tumor cells cannot predict treatment responses. CD62L is a gene encoding L-selectin protein, which belongs to the selectin family. Selectin is a cellular adhesion molecule related to leukocyte adhesion and migration. In some tumors, the expression level of tumor cell surface selectin significantly increases. In melanoma, increases in tumor-infiltrating CD62L+T cells promote growth, suggesting that CD62L is associated with poor prognosis, consistent with our present findings. We found that increases in CD62L–HLA DR++ monocyte infiltration were a risk factor for the development of PC.

SSC–A on HLA DR+ CD8br is a late-activated CD8+T cell. Overexpression of HLA DR+CD8br cells in acute myeloid leukemia may weaken the anti-tumor efficacy and benefits of relapsed refractory acute myeloid leukemia, which may be related to T cell depletion [42]. Interestingly, we found that activation of SSC–A on HLA DR+ CD8br was beneficial to PC prognosis, but the specific mechanism still needs further research. Another immune cell CD64 on monocytes, in humans, CD64 on monocytes are IgG Fc receptors (Fc γ R). There are three main categories of these receptors. Polymorpho nuclear phagocytes typically express the low-affinity receptor category (Fc γ RH [CD32] and FC γ RIII [CD16]). Studies have shown that, in malignant tumors, neutrophils overexpress high-affinity CD64 Fc receptors after granulocyte colony-stimulating factor (G-CSF) treatment γ RI [43]. In addition, CD64 is closely related to cancer immunotherapy [44]. We observed that monocyte CD64 overexpression was a protective factor for PC, but the mechanism underlying this interaction remains unclear.

Double-negative (CD4– CD8–) NKT %T cells account for a small proportion of circulating T lymphocytes, with phenotypic features such as loss of CD4 and CD8 co-receptors and γδ or αβ. T cell receptors (TCR) have complex roles, and the exclusion of skin and cardiac allotransplantation by exclusively suppressing anti-transplant-specific CD8+ T cell functions can have negative impacts [45]. Inhibiting DN (CD4– CD8) T cell activation can reduce IFN-γ-mediated inflammatory responses. However, a previous study found increases in the proportion of DN (CD4– CD8–) T cells in thyroid cancer, indicating that tumor growth may be related to DN (CD4– CD8–) T cell infiltration. DN T cells can express perforin and granzymes, which kill NK cells in the tumor microenvironment [46]. Double-negative T cells may also block NK-mediate pro-inflammatory immune environments and promote cancer cell survival [47]. Interestingly, recent studies have shown that DN (CD4– CD8–) tumor-infiltrating lymphocytes derived from solid tumor tissue inhibit tumor cell proliferation in an MHC-independent manner after expansion in vitro. Our study showed that DN (CD4– CD8–) NKT %T cells activation was a protective factor for PC.

Amplification of myeloid-derived suppressor cells (MDSCs) is associated with tumorigenesis in colorectal cancer (CRC). Here, we identified a strong correlation 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 correlated with the amplification of tumor-related myeloid-derived suppressor cells and decreased CRC survival [48]. Granulocyte MDSCs express CD33, CD11b, IL-4Rα, and low levels of CD15 and denote elevated levels of arginase. Myeloid-derived suppressor cell levels were notably increased in esophageal cancer (EC), gastric cancer (GC), and PC in our study, and increasing MDSC percentages were significantly correlated with an increased risk of mortality in previous work [49]. Another study demonstrated that high Birc5 expression and high MDSC infiltration within tumors were associated with HCC patients' prognosis. In vitro, hepatocyte Birc5 overexpression facilitated the expansion of immunosuppressive CD11b+CD33+HLA-DR-MDSCs in human peripheral blood mononuclear cells. Transgenic animal models of HCC showed that Birc5 deficiency upregulated genes were implicated in lymphocyte-mediated immunity, natural killer cell-mediated immunity, gamma-interferon production, T-cell activation, and T-cell-mediated cell cytotoxicity, which is in line with our finding of a correlation between CD33 and HCC risk [50]. These findings were consistent with ours in that CD33+ MDSC levels were associated with poor prognosis in PC. We also found that there were two CD33 subtypes: CD33dim HLA DR+ CD11b– %CD33dim HLA DR+ and CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+. We found that CD33dim HLA DR+ CD11b– %CD33dim HLA DR+ was more commonly a risk factor for cancer development, whereas CD33dim HLA DR+ CD11b+ %CD33dim HLA DR+ was a protective factor.

Interestingly, CM CD4+ AC typically represents a distinct subpopulation of immune cells characterized by the presence of CD4 co-receptors. CD4 is a co-receptor primarily found on helper T cells and is important for the interaction between T cells and antigen-presenting cells. Analysis of CD4+ cells may be important for both research and clinical purposes. CD4+ T cells are key coordinators of the immune system, as they produce several cytokines after activation and differentiation. CD4+ T helper cell subtypes (including T helper 1, T helper 2, T helper 17, T helper 9, and regulatory T cells) have different immune functions after differentiation from naïve T cells. Different types of CD4+ T cells require different cytokines and master transcription factors for activation [51]. Previous research has demonstrated that CD4+ T cells can be found in the tumor microenvironments of lung cancer, melanoma, CRC, lymphomas, cervical cancer, and ovarian cancer, but the role of CD4+ T cells in EC is relatively understudied [52–57]. Interestingly, our findings indicated that the presence of CM CD4+ T cells was a protective factor in PC. More functional research is needed to confirm these findings.

Strength and limitations

This study employed published GWAS data to perform a two-sample Mendelian randomization (MR) analysis, benefiting from a large sample size and strong statistical power. The findings are based on genetic instrumental variables, with causal inferences supported by various MR methodologies. The results are robust, demonstrating resilience against confounding factors, including horizontal pleiotropy. This approach addresses limitations of traditional observational studies by minimizing the impact of confounding variables and reverse causality. Additionally, MR helps overcome issues of representativeness and feasibility that are often associated with randomized controlled trials (RCTs). However, it is important to note a limitation of the study: its reliance on GWAS databases raises concerns about the applicability of the findings to the immune cell profile in pancreatic cancer (PC). Further research and validation across diverse populations are warranted, as the results are primarily generalizable to European populations due to the demographic focus of the original GWAS. Additional studies are needed to confirm these findings in non-European populations.

CONCLUSION

Our comprehensive two-sample Mendelian randomization (MR) analysis has revealed a causal connection between 19 different immune cell phenotypes and pancreatic cancer (PC), including specific immune cells such as monocytes, T cells, lymphocytes, and dendritic cells (DCs). This finding highlights the complex relationship between the immune system and PC, paving the way for further exploration of the underlying biological mechanisms and potential immunotherapeutic strategies for this cancer.

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Authors declare no conflict of interest.

Data Availability

All relevant data are within the paper and its Supporting Information files.