Causal relationship between immune cells and bladder cancer: a bi-directional Mendelian randomization study

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Abstract

**Background and Objectives:** Previous studies have explored the role of immune cells on bladder cancer (BCa). This bi-directional Mendelian randomization (MR) study further assessed 731 immunocyte phenotypes on whether BCa a causal relationship exists and provides some evidence of causality.

**Methods:** The summary data for immune cell phenotypes was derived from a study cohort, including 3,757 individuals from Sardinia with data on 731 immune cell phenotypes. The summary data for BCa were obtained from their respective genome-wide association studies (GWAS). We used inverse variable weighting (IVW) as the primary analysis method. In addition, we simultaneously employed multiple analytical methods, including MR-Egger, weighted mode, simple mode, and weighted median, to strengthen the final results. Finally, sensitivity analyses were conducted to verify the stability and feasibility of the data.

**Results:** 16 immune cell traits involving the four immunological signal types (MFI, RC, AC, and MP) were shown to be significantly correlated with the risk of BCa in our investigation. BCa was positively correlated with 5 immune cell traits (P < 0.05, OR > 1); However, the remainder BCa incidence decreases by 11 traits (OR<1, P<0.05). Furthermore, none of them revealed reverse causality, heterogeneity, or horizontal pleiotropy (P > 0.05).

**Conclusions:** Through a comprehensive bi-directional MR analysis, we were able to reveal a complicated causal link between several immunological phenotypes and BCa, focusing on the intricate network of interactions between the immune system and BCa. The results of this study provide fresh viewpoints and resources for investigating immunotherapeutic targets and BCa preventive tactics.

Introduction

Globally, bladder cancer (BCa) is a major public health problem due to its complicated epidemiology, which is influenced by a combination of lifestyle, environmental, and genetic variables. This disease ranks as the second most prevalent urological cancer worldwide, responsible for 549,000 new cases and around 200,000 deaths annually[1]. BCa incidence varies geographically for a variety of reasons, such as differences in industrialization, pollution in the environment, smoking rates, food preferences, and access to treatment. Industrialized areas are more likely to be exposed to occupational carcinogens including industrial chemicals, polycyclic aromatic hydrocarbons (PAHs), and aromatic amines[2]. Developed countries tend to report greater incidence rates than emerging regions. There are two types of BCa: muscle invasive bladder cancer (MIBC) and non-muscle invasive bladder cancer (NMIBC). This classification is based on whether the bladder’s muscular layer has been affected by the tumor. Research has indicated that whereas the remaining instances of BCa are MIBC, over 75% are NMIBC[3]. Histological subtypes of BCa consist of urothelial carcinoma, squamous cell carcinoma and adenocarcinoma[4].
There is growing evidence that immune cells had a role in the development of BCa [5-10]. Nowadays, treatment for BCa involves a number of immunotherapies that target different immune cells within the immune system, including immune checkpoint inhibitors or direct targeting of the tumor immunological microenvironment[11]. There are several cell types that have been demonstrated to be involved in the control of BCa. Important players in anti-tumor immunity that successfully eradicate cancer cells include CD4+ T, CD8+ T lymphocytes and NK cells[12, 13]. On the other hand, anti-tumor immunity is inhibited by tumor-associated macrophages and other cells, and tumor development and progression may be linked to their overactivation[14]. The particular roles of different immune cell subtypes and whether there is a causal connection between these cells and tumor growth are still unknown, despite a basic understanding of the roles played by some immune cell types in the pathogenesis of prostate cancer.

Mendelian randomization (MR) is a method to evaluate observed causal linkages by using genetic differences as instrumental variables (IVs) [15]. This strategy aims to replicate a randomized controlled trial, reducing the impact of any confounding variables in observational research[16]. Conventional observational studies look at the correlation between exposure and results to identify illness risk factors. However, due to confounding variables or reverse causality, these studies may not be able to make reliable causal results. Because genetic variants are randomly allocated at conception, usually unrelated to confounding variables, and unaffected by reverse causation, MR is useful for examining causal links between risk factors and clinical disorders in comparison to standard observational research[17].

**Methods**

**Data sources**

A population-based immune profile analysis published in the journal Nature Genetics was used in this investigation. 3757 Sardinian citizens comprised the group used in the study. A broad spectrum of 731 immunophenotypes [ The GWAS catalog (GCST90001391 to GCST90002121)] (7 groups) were covered by the comprehensive investigation, involving relative cell counts (n = 92), morphological characteristics (n = 32), median fluorescence intensities (n = 389), and absolute cell counts (n = 118)[18].

The BCa data used in this study were sourced from the Integrative Epidemiology Unit Open GWAS database (https://gwas.mrcieu.ac.uk/). The study included 1,279 European BCa patients as the study group and 372,016 European people without BCa as the control group. A total of 9,904,926 SNPs were screened for their impact on prostate cancer. Figure 1 illustrates the study’s specific research approach, and Table 1 provides specific details on data sources and features. The established standards in endoscopy, imaging, and histology pathology determine the diagnostic criteria for bladder cancer.

**Study design**

In order to figure out which immune cell morphologies may be causally related to the risk of BCa, we first use these phenotypes as the exposure. We then evaluate the possible reverse causal links with immune cell morphologies using BCa as the exposure. In the study, single nucleotide polymorphisms, or SNPs,
are used as IVs. Three essential presumptions are met by the chosen IVs: (1) IVs are linked to risk exposure. (2) There is no connection between IVs and any confounding variables that affect the exposure-outcome link. (3) IVs cannot influence the result in any other manner than through the exposure.[19] IVs that don't adhere to the three main presumptions won't be allowed.

**Selection of IVs**

First, the criteria of P value < 1×10^{-5} was fulfilled by filtering the GWAS data to include associated SNPs[20]. In addition, the parameter r threshold was set to 0.001 and the SNPs' distance was adjusted to 10,000 Kb for the study in order to prevent linkage disequilibrium (LD) of SNPs from influencing the results. Second, the PhenoScanner V2 database was utilized to confirm if any other confounding factors were connected to the previously indicated included SNP sites. Lastly, the F statistic (defined as $F = \frac{\beta^2}{SE^2}$, where $\beta$ is the allelic effect value and SE is the standard error) was used to eliminate F values with a value larger than 10 in order to evaluate whether the included SNPs were affected by weak IVs. The SNPs were eliminated to prevent any influence on the results if their F statistic was less than 10, which suggested that they may have weak instrumental variable bias. Following that, the IEU OpenGWAS database or the FinnGen database was used to extract the outcome information, from which the correlations between SNPs that supported the hypothesis were derived. The datasets that were exposed and the outcome were combined, and the palindromic sequences were eliminated. Those last IVs for the exposure were the SNPs that remained.

**Statistical analysis**

"TwoSampleMR (v.0.6.2)",”ieugwasr1.0.0″,”ggplot2.3.5.1.9″ and “MR-PRESSO1.0″ packages in R (v.4.3.2) were used to carry out the MR analysis in this work. MR first used the TwoSampleMR software to analyze the screened IVs after they were retrieved from the ending factors. There were five popular approaches for MR analysis that were used: MR-Egger regression test, simple mode, weighted mode, inverse variance weighted (IVW), and weighted median. IVW was the primary analytical method and was supported by other ones. Because it employs the quadratic of se, which is the inverse of the ending variance, as the weight for the fit, the IVW methodology is distinguished by its refusal to consider the existence of an intercept term[21]. A number of sensitivity studies were carried out to better account for potential pleiotropy. The findings of the MR analysis were then exposed to sensitivity analyses, including the horizontal multiple validity examination and the heterogeneity test. Weighted linear regression with intercepts, as proposed by MR Egger[22], was utilized to evaluate the presence of horizontal multiplication among the IVs, and Cochran's Q test[23] was performed to quantify the heterogeneity of the IVs, with $P < 0.05$ showing the presence of heterogeneity. Furthermore, the leave-one-out sensitivity test was employed to evaluate the potential substantial effects of a single SNP on the causative effect. The study's dependability and rigor are further improved by the application of many statistical approaches, which also contribute to clarifying the complex interaction between immune cells and BCa. $P <0.05$ have been considered statistically significant. All data are displayed as odds ratios (OR) and 95% confidence intervals (CI).
The STROBE-MR statement[24] and Mendelian randomization investigations Guidelines[25] were followed in the conduct of this Mendelian randomization study. Supplementary word 1 lists STROBE-MR checklist.

**Results**

**Immunophenotypes’ causative relationship to bladder cancer**

The primary findings of the investigation into the relationship between the risk of BCa and 731 immune cell types. 16 immune cell traits involving the four immunological signal types (MFI, RC, AC, and MP) were shown to be significantly correlated with the risk of BCa in our investigation. Supplementary Table 1, 2 lists the IVs used for immunological characteristics.

The outcomes from the genetically predicted IVW and weighted median methods for 6 immune cell groups against BCa are illustrated in Figure 2, demonstrating a favorable correlation between the trait of the subsequent 4 immune cells and the onset of BCa (OR>1, P<0.05). Myeloid cell team: CD33br HLA DR+ CD14- %CD33br HLA DR+; Maturation stages of T cell team: CM CD4+ AC; TBNK team: CD4/CD8br; B cell team: CD38 on IgD+ CD24-; Treg team: CD25 on CD39+ resting Treg. However, the remainder BCa incidence decreases by 11 traits (OR<1, P<0.05). B cell team: CD19 on IgD- CD24- and CD19 on IgD- CD27-; cDC team: CD62L- monocyte AC, CD62L- HLA DR+++ monocyte AC and SSC-A on plasmacytoid DC; TBNK team: CD8br % T cell, HLA DR+ T cell% T cell and HLA DR+ CD4+ AC; Treg team: CD28 on CD28+ CD4+ and CD28 on CD39+ resting Treg; Myeloid cell team: CD11b on CD33br HLA DR+ CD14dim.

The outcomes of the five MR analysis methods are presented in Supplementary Table 3.

Sensitivity analyses revealed that none of the top 16 immunocyte phenotypes for MR analysis of BCa were horizontally pleiotropic (P>0.05 for MR-Egger’s intercept method) or heterogeneous (P>0.05 for Q-test), demonstrating the credibility of causally robust results. Additionally leave-one-out and scatter plots approach both showed trustworthy data. (Supplementary Figures. 1 and 2). The outcomes of the MR sensitivity analysis methods are presented in Table 2. While some cell types have the ability to prevent BCa from starting, others may encourage the development of BCa. These results offer fresh perspectives on the etiology and management of BCa.

**Bladder cancer’ causative relationship to immunophenotypes**

We found some encouraging results in the reverse MR analysis. Similarly, no significant findings were seen after adjustment.

**Discussion**

We revealed 16 immune cell types involving the four immunological signal types (MFI, RC, AC, and MP) in our MR study that are associated with BCa risk, mainly B cell, TBNK cell, Myeloid cell, Treg cell, Maturation stages of T cell, cDC cell.
Our results demonstrated a favorable correlation between the development of BCa and 6 immunocyte morphologies. Among them, CD33br HLA DR+ CD14- %CD33br HLA DR+, CD8br %T cell, HLA DR+ T cell% T cell, HLA DR+ CD4+ AC, CD28 on CD28+ CD4+, CD28 on CD39+ resting Treg, CD8br %T cell, HLA DR+ T cell% T cell, HLA DR+ CD4+ AC on CD33br HLA DR+ CD14dim. have also not been studied directly on BCa. However, CD25 is a part of the interleukin-2 (IL-2) receptor and was discovered on the surface of both immune and non-immune cells[26]. CD25 is extensively expressed on regulatory T cells (Tregs), which are known to accelerate the growth of tumors. Research has demonstrated that anti-CD25 antibodies can reduce Treg counts, which can have an anti-tumor immunological impact. Wojciech et al. demonstrated that Regulatory T cells (Tregs; CD4+CD25+FoxP3+) promote immunological tolerance and contribute in the development of BCa, and discovered Treg frequencies throughout the latter stages of tumor growth, which are linked to a reduced anti-tumor response, are a novel and significant prognostic factor in BCa[27]. IgD+ B cells are a subpopulation of B cells that express IgD on their surface to initiate immunological responses. IgD and other immunoglobulins can coexist on B cells and together control immunological responses[28, 29]. Not only may CD4+ T cells transform myeloid cells into IFNγ-induced antigen-presenting cells, but they can also reprogramme them into tumoricidal effectors that express iNOS and can eliminate tumors that evade the immune system[30]. Exhaustion of cytotoxic CD4+ T cells and CD8+ T cells is one of the mechanisms behind tumor immune evasion[31]. Therefore, the findings of the research that point to a mechanistically plausible positive link among the development of BCa and CM CD4+ AC and CD4/CD8br. Following Rituximab treatment of patients with B-cell non-Hodgkin's lymphoma, peripheral blood CD19+ and CD20+ B cell counts significantly dropped. Peripheral blood samples revealed a few CD19+ B cells six months after the treatment's conclusion. These cells displayed high amounts of CD38 and CD24 and had a naive B cell phenotype (IgD+, CD27-), indicating that IgD+ B cells may influence the pathophysiology of B-cell non-Hodgkin's lymphoma via specific pathways and constitute a risk factor for B-cell non-Hodgkin's lymphoma. regardless of the lack of research on the impact on BCa[32].

Leonie et al. demonstrated IgD-CD11c+ CD21low and IgD-CD24+ CD21 high B cells were demonstrated to be significantly reduced in a subset of patients who had autoantibody-positive Neurologic immune-related adverse events (irAE-n). indicating that IgD- B cells may influence the pathophysiology of irAE-n via specific pathways and constitute a protective factor for irAE-n. And, the findings of the research that point to a mechanistically plausible negative link among the development of BCa and IgD-CD24- %B cell, CD19 on IgD- CD24- and CD19 on IgD- CD27-[33]. DCs are essential when it comes to inducing the adaptive immune response. They change the direction of T helper responses' polarization and initiation. It is not surprising that changed Dendritic cells (DC) profiles, a subset of antigen-presenting cells (APCs), play a part in the development of autoimmunity. These profiles include migration, tissue distribution, phagocytosis, antigen presentation, and cytokine release[34, 35]. Treatment for vaccines have emerged as a promising treatment strategy for cancer immunotherapy in recent times. Zhang et al. described a vaccination approach based on dendritic cells that might inhibit the development of bladder tumors in vivo and boost the effectiveness of chemotherapy. Moreover, they also examined the in vivo anticancer effects of mature DCs elicited by antigen loading in bladder tumors[36]. This MR analysis also offers
evidence that CD62L- monocyte AC, CD62L- HLA DR++ monocyte AC and SSC-A on plasmacytoid DC of cDC team is negatively correlated with the development of BCa. A cell adhesion molecule called CD62L, sometimes referred to as L-selectin, binds to ligands on endothelial cells to engage in leukocyte rolling, adhesion, and migration[37]. Increased CD62L expression enhances the anti-tumor inflammatory response by increasing dendritic cells' capacity to chemoattract. The therapeutic importance of plasmacytoid dendritic cells(pDC), which are multifunctional immune cells, in the tumor microenvironment (TIME) is yet unknown[20]. While, Masanori et al. discovered that high pDC triple negative breast cancer (TNBC) were linked to high expression levels of all immunological check point markers analyzed as well as a large proportion of anti-cancer immune cells. In a word, there was a stronger correlation between pDC levels and immune cell infiltration as well as patient survival in TNBC compared to conventional dendritic cells(cDC)[38]. Previous studies have investigated the impact of CD11b on CD33br HLA DR+ CD14dim. on the risk of PD. While, the idea that CD11b on CD33br HLA DR+ CD14dim. and BCa are correlated is not yet supported by experimental evidence. This discovery opens up new experimental possibilities for studying the connection between BCa and Myeloid cell.

Our study's strengths are that it is the first to use MR methods to look into the connection between immune cell morphologies and BCa. Our findings were obtained by closely examining horizontal pleiotropy, which lessened the influence of reverse causality and confounding variables on the outcomes. Furthermore, immune cell morphologies that have not been as well studied in the past but are strongly linked to BCa were also found in our investigation. This might offer fresh perspectives on possible targets for immunotherapy in BCa. There are several further restrictions on our investigation. Despite the fact that our study includes 731 immune cell morphologies, data restrictions prevented us from analyzing some immune cell phenotypes. Moreover, the fact that the data sources are adult-only, primarily of European heritage, and do not allow for gender or age-based stratification may have an effect on how accurate and broadly applicable the findings are. Using a low threshold value \( p < 1.0 \times 10^{-5} \) when selecting tool variables might result in false positives or miss significant genetic variants associated with immune cell characteristics. There is insufficient experimental data to go deeper into and identify the underlying processes behind the limited link that our research has shown between immune cells and the development of prostate cancer. Important issue is the dearth of impartial cohort studies to support the study's conclusions. To further explore our results and look into possible processes, we want to carry out biological tests in the future.

**Conclusion**

Through a comprehensive bi-directional MR analysis, we were able to reveal a complicated causal link between several immunological phenotypes and BCa, focusing on the intricate network of interactions between the immune system and BCa. The results of this study provide fresh viewpoints and resources for investigating immunotherapeutic targets and BCa preventive tactics.

**Declarations**
Data availability statement

The original contributions presented in the study are included in the article /Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants’ legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tables

Table 1 comprehensive details on the data analysis.

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Table 2 MR sensitivity analysis
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**Figures**
### Supplementary Files

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