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Causal role of immune cells in primary liver cancer: a mendelian randomization study

Jia Liu[1†], Tongyuan Zhang[2†], Yang Gao[1†], Dong Ji[1] and Lijian Chen[1*]

Abstract

Background Primary liver cancer is one of the most common fatal malignancies worldwide. Observational studies have shown that immune cells are closely linked to primary liver cancer, however, due to issues like reverse causality and confounding variables, the causal direction and extent of this association remain unclear. Thus, this study aimed to explore the potential causal association between immune cells and primary liver cancer, encompassing hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC).

Methods A two-sample mendelian randomization (MR) analysis was performed using summary statistics from genome-wide association studies (GWAS) of the 731 immune traits and primary liver cancer. The primary liver cancer dataset consisted of a total of 456,348 subjects, with 123 cases of HCC and 456,225 controls, as well as 104 cases of ICC and 456,244 controls, all of European ancestry. The primary method for assessing causality was inverse variance weighting (IVW), with sensitivity analysis utilized for testing heterogeneity and pleiotropy.

Results Two immunophenotypes were significantly associated with HCC risk: CD3 on CD45RA + CD4+ (OR [95% CI]: 1.334 [1.077 to 1.651], $p = 0.008$), CD80 on monocyte (OR [95% CI]: 0.578 [0.397 to 0.844], $p = 0.004$). Additionally, six immunophenotypes were identified to be significantly associated with the risk of ICC: SSC-A on NK (OR [95% CI]: 1.685 [1.166 to 2.436], $p = 0.006$); CD3 on CD28- CD8br: (OR [95% CI]: 1.826 [1.206 to 2.766], $p = 0.004$); CD45RA on naive CD4+: (OR [95% CI]: 1.391 [1.119 to 1.729], $p = 0.003$); Resting Treg %CD4: (OR [95% CI]: 1.290 [1.069 to 1.558], $p = 0.008$); HLA DR on HSC: (OR [95% CI]: 0.539 [0.343 to 0.846], $p = 0.007$); Plasmacytoid DC %DC: (OR [95% CI]: 0.610 [0.462 to 0.806], $p < 0.001$). And sensitivity analyses confirmed the robustness of the main findings.

Conclusions MR analysis has revealed the causal relationship between immune cells and primary liver cancer through genetic methods. These findings could assist in clinical decision-making and provide new directions for the treatment and research of primary liver cancer.

Keywords Immune cells, Primary liver cancer, Mendelian randomization, Hepatocellular carcinoma, Intrahepatic cholangiocarcinoma

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Introduction

Primary liver cancer is one of the most common and deadliest malignancies worldwide [1]. According to the World Health Organization, liver cancer-related deaths are expected to increase annually, surpassing one million by 2030 [2]. Hepatocellular carcinoma (HCC) comprises 80–90% of all primary liver cancer cases worldwide [3, 4]. Intrahepatic cholangiocarcinoma (ICC) follows HCC as the second most prevalent form of primary liver cancer [5]. And ICC is recognized for its substantial variability as a primary epithelial carcinoma of the liver [6, 7]. With liver cancers adding to the global disease burden, it is crucial to implement effective preventive strategies to tackle the escalating incidence rates each year.

Primary liver cancer is a multifactorial disease, and its occurrence and development are related to the interaction between genetics and the environment [8]. Studies have shown that primary liver cancer usually occurs under conditions of chronic inflammation [9]. The main risk factors related to HCC include viral hepatitis (B and/or C), metabolic alterations (alcoholic steatohepatitis and non-alcoholic steatohepatitis), and aflatoxin. While the risk factors of ICC include primary sclerosing cholangitis, hepatolithiasis and parasitic biliary infestation with flukes [10].

In addition, the immunological and genetic factors of the liver must be considered in the pathogenic mechanisms of primary liver cancer. The liver is an important immune regulatory organ and observational studies have shown that the dysregulation of the liver immunological network is a hallmark of primary liver cancer [11, 12]. Dendritic cells (DCs) are highly potent professional antigen-presenting cells (APCs). Evidence indicates that DCs could inhibit HCC and liver cancer stem cells (LCSCs) growths in vitro and in vivo, and DCs could suppress the growth of HCC cells by loading LCSCs antigens [13]. T cell subsets, such as regulatory T cells (Tregs), CD8 + and CD4 + T cells, can infiltrate liver tumors [14–16]. Tregs are essential for maintaining immunological homeostasis and employ various mechanisms to suppress anti-tumor immune responses, such as inhibiting APCs maturation, secreting inhibitory cytokines, and producing cytotoxic granzyme and perforin [17].

However, traditional studies, like cross-sectional, case-control, or cohort studies, may have limitations such as confounding and reverse causation, potentially introducing bias in effect estimates. Mendelian randomization (MR) is a genetic epidemiology approach that uses genetic variants as instrumental variables (IVs) to determine causal relationships between observed exposures and outcomes, avoiding the effects of confounding factors (such as gender and comorbid diseases) and reverse causation (such as lifestyle changes) in observational studies. Previous studies employing MR have provided important insights into the susceptibility factors for primary liver cancer. It is notable to discover the important roles of metabolic diseases and liver function markers in the pathogenesis of primary liver cancer [18, 19]. Observational studies support the correlation between the characteristics of immune cells and primary liver cancer, and immunotherapies are feasible treatment options for primary liver cancer [20, 21]. However, few studies have systematically assessed the causal relationship between immune cell phenotypes and primary liver cancer (HCC and ICC). Therefore, in this study, a comprehensive two-sample MR analysis was conducted to establish the causal relationship between immune cell traits and primary liver cancer.

Materials and methods

Study design

We employed a two-sample MR analysis to assess the causal influence of immune cells on the risk of developing primary liver cancer. The IVs used in MR analysis must satisfy three key assumptions to ensure the validity of the results: Assumption 1, the single nucleotide polymorphisms (SNPs) are robustly associated with exposure; Assumption 2, the SNPs are not associated with any confounding factors that could bias the results; Assumption 3, the SNPs are only associated with primary liver cancer through immune cells. An overview of the analytical approach can be visualized in Fig. 1.

Data sources

The genome-wide association studies (GWAS) summary statistics for immune traits are available on the GWAS Catalog, with accession numbers ranging from GCST90001391 to GCST90002121 [22]. A total of 731 immunophenotypes were analyzed, comprising absolute cell counts ($n = 118$), relative cell counts ($n = 192$), morphological parameters ($n = 32$) and median fluorescence intensities representing surface antigen levels ($n = 389$). These features encompassed a wide range of cell types such as B cells, mature stages of T cells, myeloid cells, monocytes, classical DCs (cDCs), TBNK (T cells, B cells, natural killer cells), and Treg panels. These data were derived from 3,757 Sardinian individuals. Approximately 22 million SNPs were analyzed utilizing high-density arrays and sequence-based insertion techniques, with adjustments for covariates such as sex and age to enhance the statistical robustness of the findings.

The GWAS statistics for primary liver cancer, including HCC and ICC, were sourced from UK Biobank, which includes a cohort of 456,348 individuals. And the study adjusted for age, age square, and study-specific covariates [23]. The diagnostic criteria for HCC and ICC were based on ICD-10 standards (ICD10 C22.0: Liver cell carcinoma and ICD10 C22.1: Intrahepatic bile duct carcinoma). The

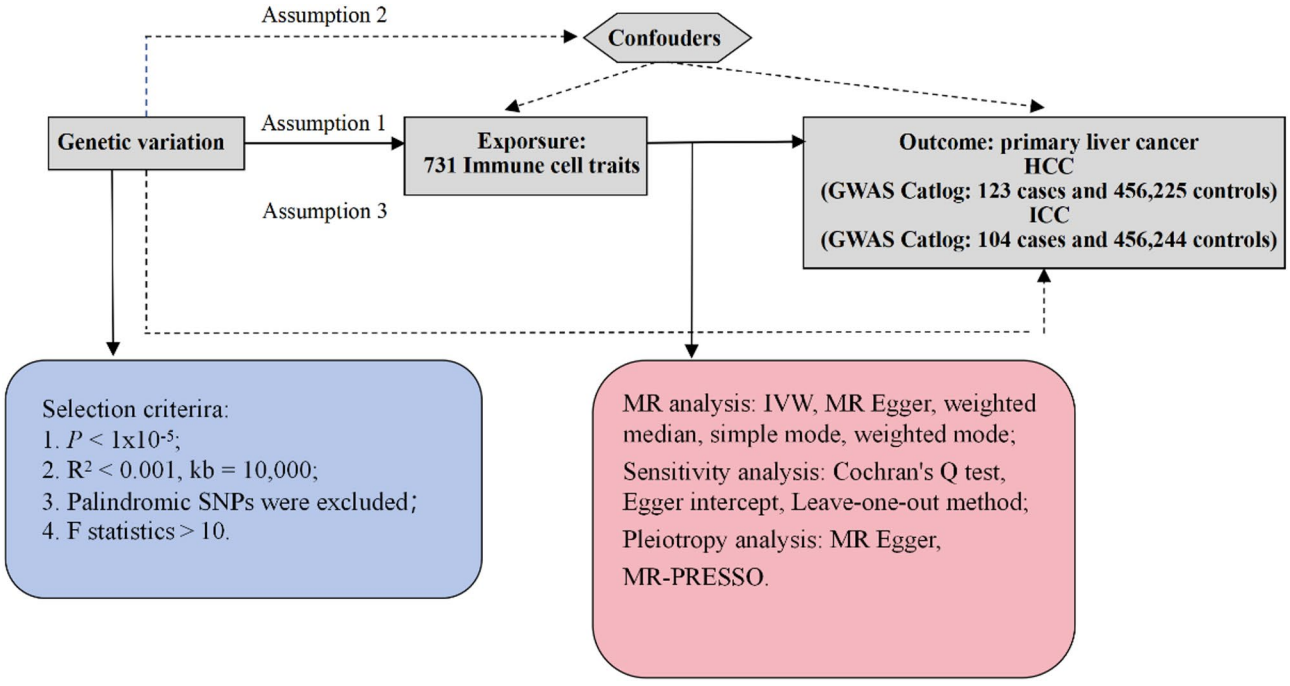

Fig. 1 The design of MR analysis between immune cells and primary liver cancer

GWAS data involved 123 cases of HCC with 456,225 controls, as well as 104 cases of ICC with 456,244 controls.

Genetic data processing

Selection criteria for IVs were as follows: (1) Significant single nucleotide polymorphisms (SNPs) were selected based on $p < 1 \times 10^{-5}$; (2) SNPs should satisfy the criteria for chain imbalance ($r^2 < 0.001$, kb = 10,000); (3) Palindromic SNPs were excluded; (4) For statistical strength, IVs with F statistics > 10 were retained, while those with F < 10 were removed according to their correlation strength.

Statistical analysis

We performed various MR analyses, including inverse-variance weighted (IVW), MR Egger, weighted median, weighted mode and simple mode approaches. Among these, the IVW method is widely utilized in MR analysis for its superior statistical efficiency and greater reliability in producing results compared to other methods [24]. Cochrane's Q value was used to assess the genetic instrument variability, while the presence of horizontal pleiotropy and outliers was detected through the MR Egger regression equation. If outliers were identified, we would remove them and then reanalyzed using the IVW method.

All analyses were conducted in R version 4.3.2 using the "TwoSampleMR" and "MR-PRESSO" packages for MR analysis, as well as for data visualization.

Results

Exploration of the causal relation of immunophenotypes on HCC and ICC

At the significance of 0.001 [25], we observed a significant association between CD3 on CD45RA + CD4 + cells and an elevated risk of HCC, whereas CD80 on monocytes exhibited a strong association with a reduced risk of HCC. Furthermore, 4 immunophenotypes were identified as risk factors for ICC: SSC – A on NK, CD3 on CD28- CD8br, CD45RA on naive CD4 + and Resting Treg %CD4. On the other hand, 2 immunophenotypes were found to be protective factors: HLA DR on HSC and Plasmacytoid DC %DC.

The odds ratio (OR) of CD3 on CD45RA + CD4+ (Treg panel) on HCC risk was calculated as 1.334 (95% CI = 1.077 to 1.651, $p = 0.008$) using the IVW method. Genetically predicted CD80 on monocytes (cDC panel) showed a significant protective effect against HCC, with an OR of 0.578 (95% CI = 0.397 to 0.844, $p = 0.004$).

The genetically prediction of SSC-A on natural killer (NK, TBNK panel) was positively correlated with the risk of ICC according to the IVW method (OR [95%]: 1.685 [1.166 to 2.436], $p = 0.006$). The OR for CD3 on CD28- CD8br (Treg panel) in relation to ICC risk was calculated as 1.826 (95% CI = 1.206 to 2.766, $p = 0.004$) using the IVW method. The OR of CD45RA on naive CD4+ (Maturation stages of T cell panel) compared to ICC calculated to be 1.391 (95% CI = 1.119 to 1.729, $p = 0.003$). The risk ratio of Resting Treg %CD4 (Treg panel) to ICC was estimated to be 1.290 (95% CI = 1.069 to 1.558, $p = 0.008$).

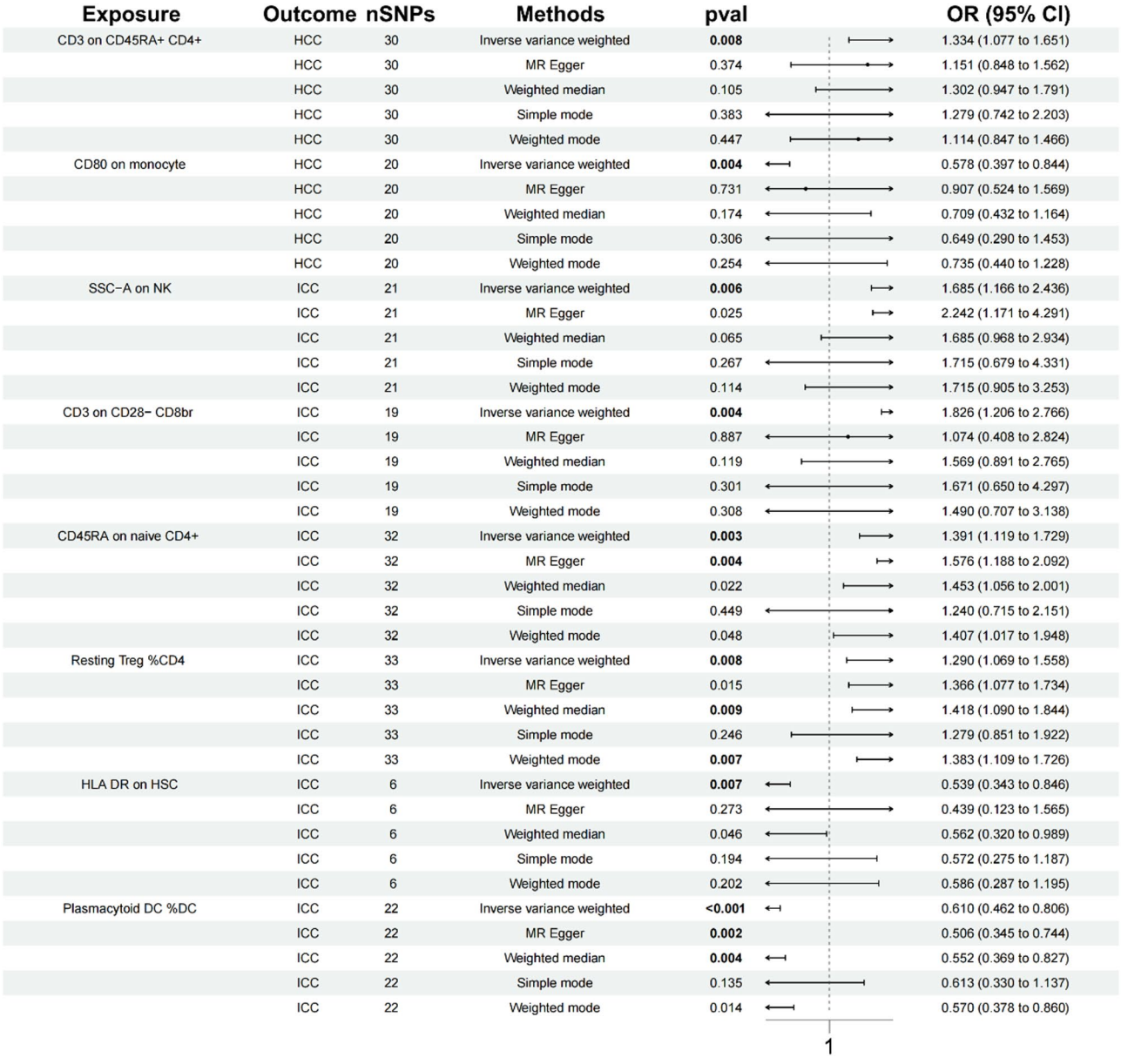

Fig. 2 Forest plots showed the causal relations between primary liver cancer and immunophenotypes

Additionally, the OR for HLA DR on HSC (Myeloid cell panel) in relation to ICC risk was estimated to be 0.539 (95% CI = 0.343 to 0.846, $p = 0.007$) by using the IVW method. The genetically prediction of Plasmacytoid DC %DC (cDC panel) was significantly associated with a protective effect against ICC, with an OR of 0.610 (95% CI = 0.462 to 0.806, $p < 0.001$, Fig. 2).

Sensitivity analysis

The results of the MR-Egger intercept test and MR-PRESSO global test suggested no signs of heterogeneity or horizontal pleiotropy in the relationships between immunophenotypes and primary liver cancer. These findings are summarized in additional Table 2. Additionally, the leave-one-out analysis validated the stability of the MR findings, as removing any individual SNP associated with immunophenotypes and primary liver cancer did not substantially alter the overall conclusions (refer to Supplementary Figures).

Discussion

Our study systematically investigated the causal association between 731 immunophenotypes and primary liver cancer using publicly available GWAS datasets. We finally identified 2 immunophenotypes, including CD3 on CD45RA + CD4 + and CD80 on monocyte, were significantly linked to the risk of HCC. Moreover, six immunophenotypes were notably linked to the risk of

ICC, comprising SSC – A on NK, CD3 on CD28- CD8br, CD45RA on naive CD4+, Resting Treg %CD4, HLA DR on HSC and plasmacytoid DC %DC.

For HCC, CD4 + Treg cells are a specific type of lymphocytes that are crucial for maintaining immune homeostasis and tolerance. It is widely recognized that CD4 + Treg cells exert suppressive effects on the functions of T cells, B cells, NK cells, and other immune cells [26]. Research in the tumor microenvironment had revealed that tumor cells utilize the immunosuppressive abilities of CD4 + Tregs present in the tumor microenvironment to escape immune surveillance and facilitate tumor advancement [27]. Sakaguchi et al. introduced a categorization of CD4 + Treg cells depending on the expression of CD45RA, delineating them into three distinct functional subsets: subset I comprises CD45RA + Foxp3^{lo}/CD25^{lo} resting Treg cells (rTregs); subset II comprises CD45RA-Foxp3^{hi}/CD25^{hi} effector Treg cells (eTregs), also known as activated Treg cells (aTregs); and subset III involves CD45RA-Foxp3^{lo}/CD25^{lo} non-Treg [28]. In terms of function, rTregs display a degree of immunosuppressive activity and express markers typical of naive cells like CCR7 and CD62L. Although most rTreg cells are in a resting state. Once rTreg cells are stimulated, they may produce high levels of chemokines, such as CXCL8, which mediates neutrophil migration to the tumor and promote tumor growth [29]. In addition, rTregs could upregulate FoxP3 expression, differentiate into aTreg cells and proliferate, demonstrating enhanced proliferation and stronger immunosuppressive capabilities.

Conversely, the CD80 molecule is a 44/54 kDa glycoprotein present on activated B cells, macrophages, and DCs. It plays a vital role in stimulating T cell-mediated antitumor immune responses. Research has found that CD80-transfected HCC cells can effectively generate primary cytolytic activity against HCC cells, indicating that strong expression of CD80 is sufficient to induce antitumor immunity, which may relate to CD80 enhanced the immunogenicity of the genetically-modified HCC cells, thereby activating T cells to target HCC cell lines in an HLA-restricted manner [30, 31]. Additionally, the CD80 may also inhibit IL-12-induced IFN-γ production [32].

As for ICC, it is featured by a highly desmoplastic tumor microenvironment with excessively infiltrating immune and stromal cells, including inhibitory subsets such as CD4 + Tregs and tumor-associated myeloid-derived suppressor cells. These cells can dampen immune responses and facilitate tumor progression [33]. The research conducted by Giorgia Alvisi et al. offers a comprehensive analysis of T-cell and myeloid cell infiltration in ICC, highlighting the substantial presence of hyperactivated CD4 + Treg cells in ICC alongside diminished effector functions of CD8 + T cells. Additionally, cholangiocarcinoma shows a varied presence of tumor-infiltrating lymphocytes, predominantly comprising CD3 + T cells, with a significant presence of CD8 + cells within the tumor tissue and CD4 + cells in the interface region [34]. Though adaptive immunity promotes immune surveillance, certain adaptive immune cells like CD8 + T cells, Th17 cells, and NKT cells can also trigger the progression of HCC [35]. Retrospective studies have indicated that an increased presence of CD8 + T lymphocytes in both the tumor and stromal areas correlates with an unfavorable prognosis [36]. CD45RA, an immune marker for naïve T cells, displayed decreased susceptibility to cell death caused by oxidative stress while retaining their suppressive capabilities [37]. And clinical prediction models have linked CD45RA to the prognosis of surgically resectable ICC [38].

NK cells play important parts in innate immunity and regulatory immunity. SSC-A can be used to assess the granularity or granularity-related properties of NK cells, which can provide information about the activation status or functional characteristics of these cells. However, the immunoregulatory role of NK cells is complex as they can inhibit or enhance the magnitude of inflammation, contribute to hepatocyte injury [39]. Some phenotypes of NK cells are highly infiltrated in tumor tissues and are associated with tumor progression and poor prognosis [40, 41]. Our results indicate that SSC-A on NK cells may promote intrahepatic cholangiocarcinoma. It is similar to other studies that have found NK cells may potentially contribute to the development of malignancies through inflammatory responses, while specific mechanisms require further exploration [42, 43].

In the context of protective factors for ICC, HLA-DR, a class II molecule of the major histocompatibility complex (MHC) predominantly expressed on professional antigen-presenting cells, has been identified as significant. Research indicates that transgenic expression of HLA-DR molecules can enhance the maturation and function of human T cells and B cells [44]. In patients with intrahepatic cholangiocarcinoma, tumors exhibiting positive HLA-DR staining have demonstrated a higher 5-year survival rate compared to those with negative staining [45].

DCs are a crucial component of the immune system and are divided into two main subsets: classical DCs (cDCs) and plasmacytoid DCs (pDCs). cDCs are responsible for detecting and interacting with foreign antigens in peripheral tissues. They then migrate to secondary lymphoid organs where they present these antigens to CD4 + T cells on MHC-II molecules and to CD8 + T cells on MHC-I molecules, triggering the activation of adaptive immune responses. Upon activation, pDCs release IFN-γ to support antiviral immunity. DCs, including both cDCs and pDCs, play a significant role in promoting

antitumor responses by presenting tumor antigens to T cells and activating immune responses against cancer cells.

To date, this is the first MR analysis that aimed to investigate the causal relationship between various immunophenotypes and primary liver cancer. However, there are several limitations to consider. Firstly, this research focused predominantly on a European cohort, necessitating a thorough examination of whether similar findings apply to non-European populations in future MR analyses. Secondly, the available data lack detailed demographic information such as age and gender, preventing further subgroup analysis. Thirdly, while we employed MR analysis to explore the potential causal connection between immune cells and primary liver cancer, further clinical studies to verify and explore the biological mechanisms are necessary. Finally, it is important to note that a looser threshold was applied in this study to evaluate the results, potentially leading to an increased number of false positives. However, this approach allowed for a more comprehensive assessment of the robust association between immune cells and primary liver cancer.

Conclusions

In conclusion, the MR studies indicate a causal relationship between immunophenotypes and primary liver cancer. However, the pathogenesis of primary liver cancer is multifaceted, and further studies are needed to investigate the interactions between innate immune cells and environmental, immune and inflammatory factors in patients with primary liver cancer.

Abbreviations

APCs	Antigen-presenting cells
DCs	Dendritic cells
GWAS	Genome-wide association studies
HCC	Hepatocellular carcinoma
ICC	Intrahepatic cholangiocarcinoma
IVs	Instrumental variables
IVW	Inverse variance weighted
LCSCs	Liver cancer stem cells
MHC	Major histocompatibility complex
MR	Mendelian randomization
NK	Natural killer
OR	Odds ratio
SNPs	Single nucleotide polymorphisms
Tregs	Regulatory T cells

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-14327-1.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Not applicable.

Author contributions

JL and LJC designed this research plan. JL and TYZ completed the manuscript writing. YG and DJ completed the image processing and data statistical analysis. All the authors approved the final version of the manuscript. Jia Liu, Tongyuan Zhang, and Yang Gao made equal contributions to this article.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

No additional ethical approval was necessary for this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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