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Analysis

Genetically predicted immune cells mediates the association between hepatocellular carcinoma and inflammatory proteins: a Mendelian randomization study

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Received: 31 July 2024 / Accepted: 23 October 2024
Published online: 12 June 2025

Abstract

Background Hepatocellular carcinoma (HCC) currently poses a formidable threat to human life and health, and an observable increase in the number of deaths is evident year by year. Currently, surgical resection stands as the foremost treatment modality; however, recurrence remains a persistent challenge, posing a significant barrier to the long-term prognosis for individuals diagnosed with HCC. Studies indicated that the risk of HCC may be influenced by inflammatory proteins and immune cells, but the associations between inflammatory proteins, immune cells, and HCC remained unclear.

Methods The investigation integrated data from 731 types of circulating immune cells and 91 inflammatory proteins, alongside a cohort involving 456,348 participants (comprising 456,220 controls and 128 cases) sourced from genome-wide association studies (GWAS). The principal objective of our research was to assess the potential causal association between inflammatory proteins and HCC by bidirectional univariate MR (UVMR) analysis. Furthermore, the total genetic prediction effect of immune cells-mediating inflammatory proteins on the likelihood of developing HCC was investigated by a two-step multivariable MR (MVMR).

Results Our results indicated that 2-positive inflammatory proteins (IL-17A and TNF-β) suggest a potential causal relationship on HCC, and HCC could affect FGF-21 by bidirectional UVMR analysis. Additionally, four immune cell types (CD25 on IgD+ CD38dim B cells, CD4 on CD39+ secreting CD4 regulatory T cells, CD25 on B cells, and CD25 on IgD+ B cells) exhibited an inverse relationship with the risk of HCC. Moreover, two inflammatory proteins demonstrated dual effects on HCC risk through modulation—either decreasing or increasing—the aforementioned four immune cell types, each with varying proportions of mediation effects as analyzed through two-step mediation MR analysis.

Conclusion This study revealed the potential causality between inflammatory proteins, immune cells, and HCC risk by MR analyses, which may potentially offer a deeper comprehension for the risk of HCC and the interaction between inflammatory proteins, immune cells and HCC and may help to seek new biomarkers for predicting the likelihood of developing HCC.

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Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12672-025-02805-8.

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Keywords Immune cells · Hepatocellular carcinoma (HCC) · Inflammatory proteins · Mendelian randomization (MR) · Biomarkers

1 Introduction

Hepatocellular carcinoma (HCC) manifests as a leading malignancy of the liver characterized by an abundance of hepatocyte-derived cells exhibiting prominent cytoplasmic features and carrying a significant propensity for metastasis. Its annual increase in mortality rates poses a significant threat to human life and health [1]. The molecular pathogenesis of HCC is very complex and heterogeneous, the causes of HCC mainly including the infection of virus (hepatitis B and hepatitis C virus), aflatoxin, drinking water pollution, long-term alcohol consumption, cirrhosis, nitrite amine substances, and sex hormones [2]. Patients with HCC typically present with liver area pain, hepatomegaly, and jaundice. Some may exhibit residual signs of cirrhosis and systemic symptoms such as cachexia, fever, anorexia, fatigue, and severe malnutrition. The study found that more than 80% of patients with HCC experienced the inflammation-cancer transformation process of chronic hepatitis, liver injury, liver cirrhosis, liver fibrosis, and liver cancer [3]. Inflammation, especially uncontrollable inflammatory reaction, has a huge impact on inducing the HCC occurrence rate and promoting the progression or advancement of HCC [4, 5].

Inflammatory proteins (mainly including C-reactive protein (CRP), des-γ-carboxyprothrombin, alpha-fetoprotein, and cytokines) exert a crucial function in inflammation, and exhibit a strong correlation with the occurrence of HCC [6]. For example, the tumor necrosis factor (TNF) activates the signaling transduction pathway of Wnt/β-catenin and c-Jun N-terminal kinase (JNK) in oxidative stress, thus causing cancer; interleukin (IL)−6 has the close relationship with liver cancer caused by diethylnitrosamine, and is a biomarker of the transition from viral hepatitis to HCC [7]. In addition, abundant angiogenin, vascular endothelial growth factor (VEGF), C-X-C motif chemokine ligand 1 (CXCL1) and CXCL8 can stimulate angiogenesis in an inflammatory environment, which is an essential process for liver tumorigenesis [8]. Although the function and potential effects of certain inflammatory proteins in HCC are known, the potential causal relationship between them needs to be further explored and elucidated.

The liver stands as the largest organ among the internal viscera of the human body, and it contains a large number of non-substantial components including immune cells and interstitial cells, which have many functions such as establishing system defense and providing scaffold structure. Whether they exert immunostimulatory or immunosuppressive effects, these components play crucial roles in the immune microenvironment of HCC. They modulate tumor cell activity through interactions with a diverse range of cell surface ligands, either by generating substantial quantities of anti-inflammatory molecules or by directly inducing tumor cell apoptosis [9]. Studies have found that the immune cells of HCC tissues are different from those of healthy liver tissues [10]. For example, the T cell and B cell counts of HCC tissues and its peripheral tissues are higher than that of healthy tissues. However, no study has clarified the possible causality between inflammatory proteins, immune cells, and HCC.

Based on the principles established by Mendel's laws of inheritance, Mendelian randomization (MR) analysis employs the data information of single nucleotide polymorphisms (SNPs) sourced from public databases to delineate causal links between exposures and outcomes [11]. This analytical approach utilizes the benefits of the random assignment characteristics of the SNP, and which employs the SNP as an instrumental variable [12]. Because of the stochastic allocation of alleles during germ cell meiosis, Mendelian randomization (MR) analysis adequately mitigates impact from confounding factors, measurement inaccuracies, and reverse causation, thereby substantially bolstering the reliability of study outcomes [13]. In this investigation, we investigated potential causal relationships between 91 proteins involved in inflammation and the incidence of HCC using bidirectional univariate Mendelian randomization (UVMR) analyses. Furthermore, we investigated how immune cells potentially mediate relationship between these proteins involved in inflammation and the likelihood of developing HCC, employing mediation analysis.

2 Methods and materials

2.1 Structure design of this study

To perform MR analysis, it is essential to posit three key assumptions as follows: (1) relevance, which mandates a robust correlation between independent variables (IVs) and exposure factors; (2) autonomy, which necessitates that IVs remain independent of confounding variables; (3) exclusion of restriction, which dictates IVs can solely influence outcomes through exposure factors. Considering that all the data employed in our research were sourced

from publicly accessible and previously published datasets, no requests were made for informed consent or ethical approval.

We generated workflow plot to illustrates the study's methodology (Fig. 1). Initially, bidirectional univariate MR (UVMR) was employed to investigate the potential causal links between proteins involved in inflammation and HCC. Using a two-stage MR approach, we employed mediation analysis to investigate whether immune cells serve as intermediaries in the causal pathway connecting proteins involved in inflammation to the onset of HCC. Specifically, β_1 ($P < 0.05$) was obtained by UVMR analysis of positive inflammatory proteins and immune cells in the first step; β_2 ($P < 0.05$) was obtained by multivariate MR (MVMR) of the positive immune cells and HCC in the second step. Thus, mediation effect could be evaluated by the total effect (β_{all}), the mediation effect ($\beta_1 * \beta_2$), and the mediation effect proportion ($\beta_1 * \beta_2/\beta_{all}$).

2.2 Source of data

2.2.1 Data sources of hepatocellular carcinoma (HCC)

The data for GWAS on HCC originated from the website of GWAS Catalog Database (GCST90041812, https://www.ebi.ac.uk/gwas/studies/GCST90041812). Within this repository, the HCC cohort comprised a total of 456,348 individuals, consisting of 128 cases and 456,220 controls.

2.2.2 Data source for proteins involved in inflammation

An investigation by Zhao et al. in 2023 provided the foundational inflammatory protein GWAS data for this study [14]. This study analyzed quantitative trait loci of protein (pQTL) for 91 plasma proteins across participants (total 14,824) through the platform of Olink arget, identifying 180 pQTLs (comprising 59 cis and 121 trans). Integration of pQTL with expression

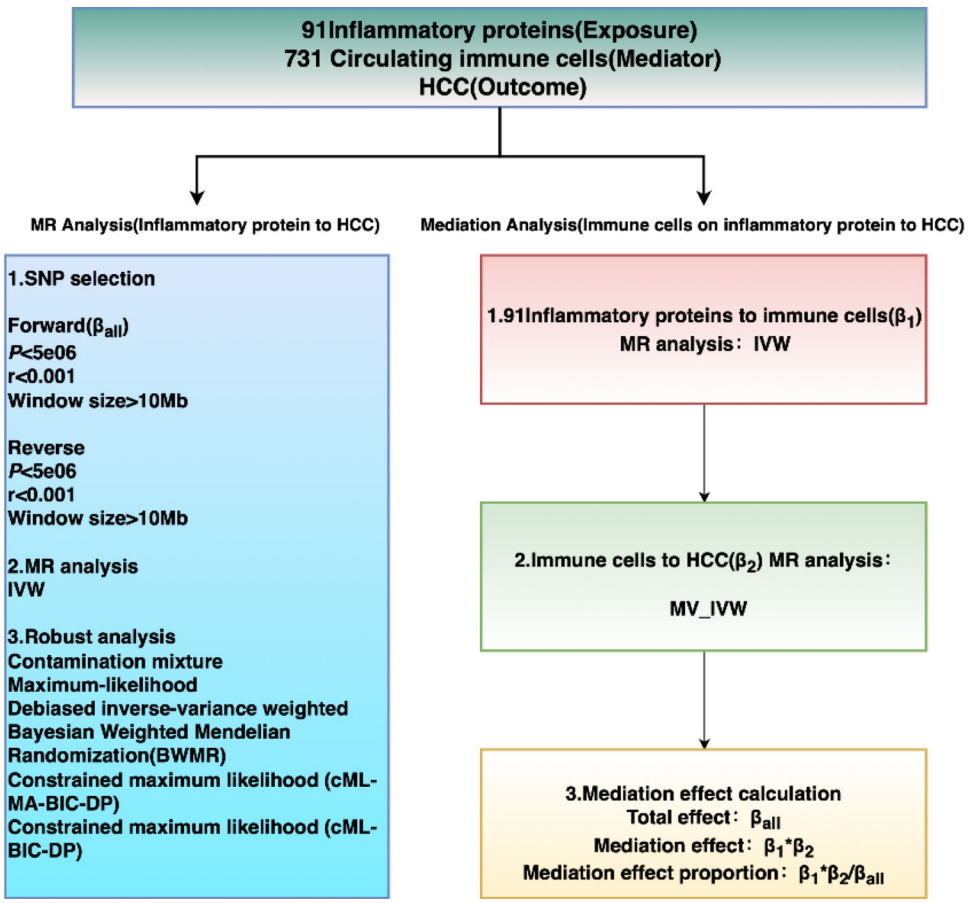

Fig. 1 Our design for the Mendelian randomization study workflow

quantitative trait loci (eQTL) and diseases data of GWAS elucidated associations linking inflammation-related proteins with disease pathology.

2.2.3 Data sources for immune cells

The study by Orrù et al. in 2020 provided GWAS data on immune cells [15], encompassing 731 distinct cell types derived from a cohort of 3757 individuals from Sardinia.

2.3 Opting for instrumental variables (IVs) in the analysis

For the purpose of ensure compliance with the relevance assumption of MR analysis, the SNPs having a strong association with exposure were selected using the threshold of $P < 5 \times 10^{-6}$ [16]. Implementing cluster analysis to mitigate linkage disequilibrium (LD) risk in the analysis of these SNPs ($R^2 < 0.001$, LD = 10,000 kb). In order to guarantee the instrumental variables (IVs) reliability, SNPs with an F-statistic < 10 were excluded to mitigate biases stemming from weak IVs [17]. In addition, when performing reverse MR analysis, the IVs for HCC should meet the following conditions: $R^2 < 0.001$, $P < 5 \times 10^{-6}$, LD = 10,000 kb, as well as F—statistic > 10.

2.4 Statistical analysis

The investigation leveraged data extracted from the Catalog Gwas and MiBioGen databases, facilitating subsequent MR analyses aimed at elucidating the causal link between proteins involved in inflammation and HCC developing likelihood. Moreover, these datasets facilitated the quantification of the extent to which immune cells mediate the association between HCC and inflammatory proteins. In MR analysis, R (v4.3.1) with R packages ("Two Sample MR" (v0.5.7) [18] and "Mendelian Randomization" (v0.9.0)) were mainly used. R^2 and the F—statistic were calculated as $R^2 = \frac{2\times\beta\times EAF\times(1-EAF)}{2\times\beta^2\times EAF\times(1-EAF)+SE^2\times 2\times Sample\ size\times EAF\times(1-EAF)}$ and $F = \frac{R^2\times(Samplesize-1-k)}{(1-R^2)\times k}$, respectively [19, 20].

In the UVMR analysis, the inverse variance weighted (IVW) approach was employed to assess the causal relationship between exposure and outcome [21]. This technique, however, assumed that all instrumental variables (IVs) were valid, a condition that may not consistently hold true in real-world scenarios. Therefore, we supplemented this method with alternative approaches such as Maximum-Likelihood, Debiased IVW, Contamination Mixture, and Bayesian Weighted Mendelian Randomization (BWMR), which accommodate errors in IVs, thereby enhancing the overall reliability of the findings. To evaluate horizontal pleiotropy, we utilized the MR-Egger intercept test, and the corresponding results can be found in Supplementary File 1.

For mediation analysis which employed the approach of two-step MR, the internal variables pertaining to immune cells must satisfy criteria: $P < 1 \times 10–5$, $R^2 < 0.1$, LD = 500 kb, and an F-statistic exceeding 10. In the MVMR, multivariable IVW was utilized to verify the effectiveness of all IVs, and thus generating a weighted total effect by P-value.

3 Results

3.1 Inflammatory proteins'causal impact on HCC

Our results of IVW identified 2 out of 91 inflammatory proteins suggest a potential causal relationship with the risk of HCC (Fig. 2). Concretely, the level of IL-17 A demonstrated a negative correlation with the likelihood of developing HCC ($P = 0.04$; OR 95% CI = 0.25 (0.07, 0.91)), suggesting that IL-17 A was the protective factor for HCC; while the level of TNF-β demonstrated a positive correlation with the likelihood of developing HCC ($P = 0.03$; OR 95% CI = 1.26 (1.03, 1.54)), suggesting that TNF-β was the risk factor for HCC.

Moreover, to enhance reliability, an additional set of 6 methods complemented the IVW approach in assessing the causal impact of inflammatory proteins on HCC. Additional MR methods, such as the weighted median approach and MR-Egger, were also employed, and the results of these analyses have been compiled in tabular form (Supplementary File 2). This supplementary analysis is depicted in Figs. 3 and 4, providing robust support for the observed causality.

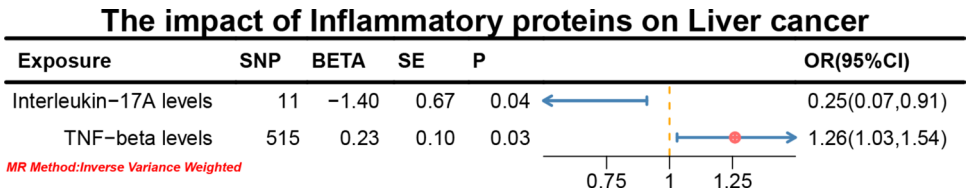

Fig. 2 Using MR analysis with the IVW method, forest plots are utilized to illustrate the effects of inflammatory proteins on HCC. In this framework, inflammatory proteins are treated as the exposure variable, while HCC serves as the outcome variable, presenting odds ratios (ORs) alongside their corresponding confidence intervals (CIs)

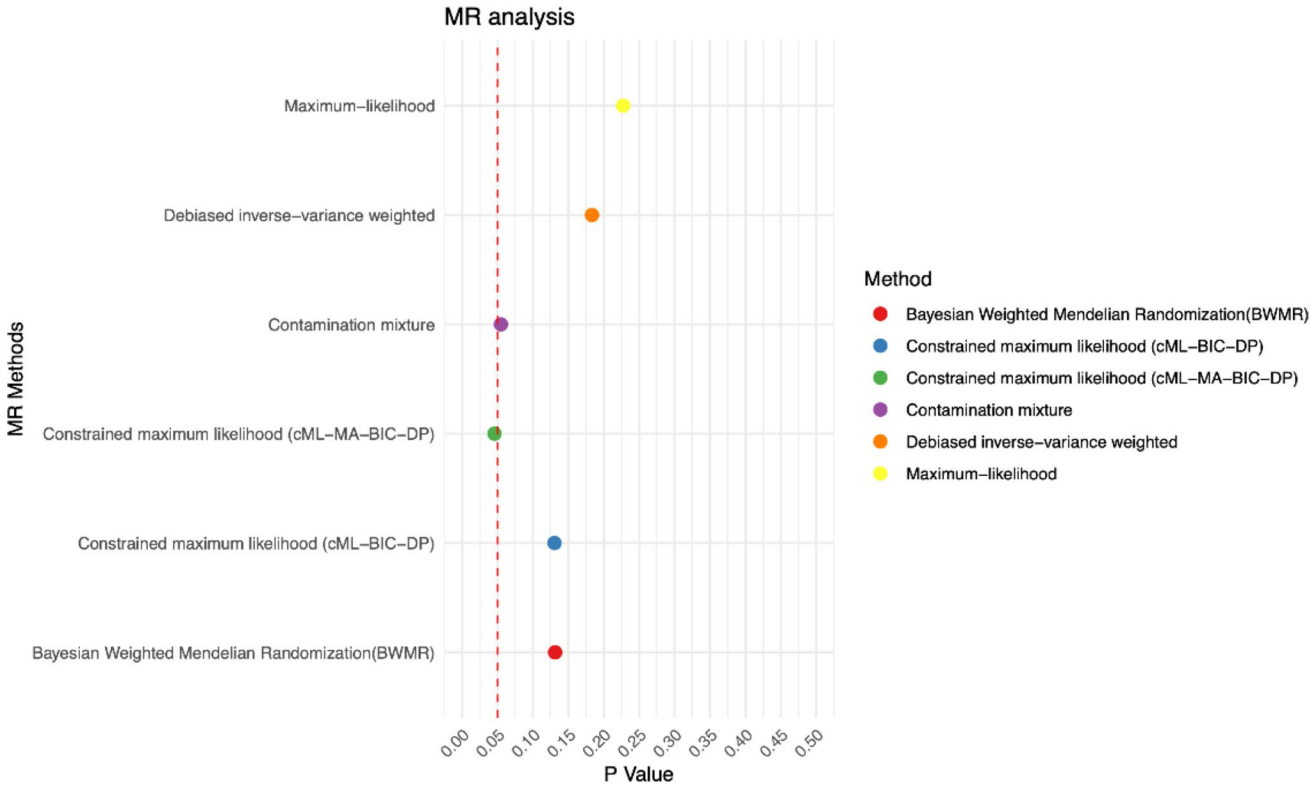

Fig. 3 A scatter plot demonstrating how inflammatory proteins, especially IL-17 A levels, influence HCC based on MR analysis that includes a variety of six additional methods

3.2 The causal influence of HCC on inflammatory proteins

As exhibited in Fig. 5, our IVW results suggested that fibroblast growth factor (FGF)−21 could be positively affected by HCC ($P = 0.03$; OR 95% CI = 0.02 (0.00, 0.04)), showing that HCC having a positive correlation with FGF-21.

Additionally, 6 other methods complementary for IVW method were employed to evaluate the potential causal relationship of proteins involved in inflammation on HCC to improve the reliability, and result (Fig. 6) also confirmed the causal relationship.

3.3 The causal influence of inflammatory proteins on immune cells

For the purpose of investigating the interplay between inflammatory proteins and immune cells, MR analyses employing the IVW method were utilized to assess and evaluate the influence of key inflammatory proteins (such as IL-17 A and

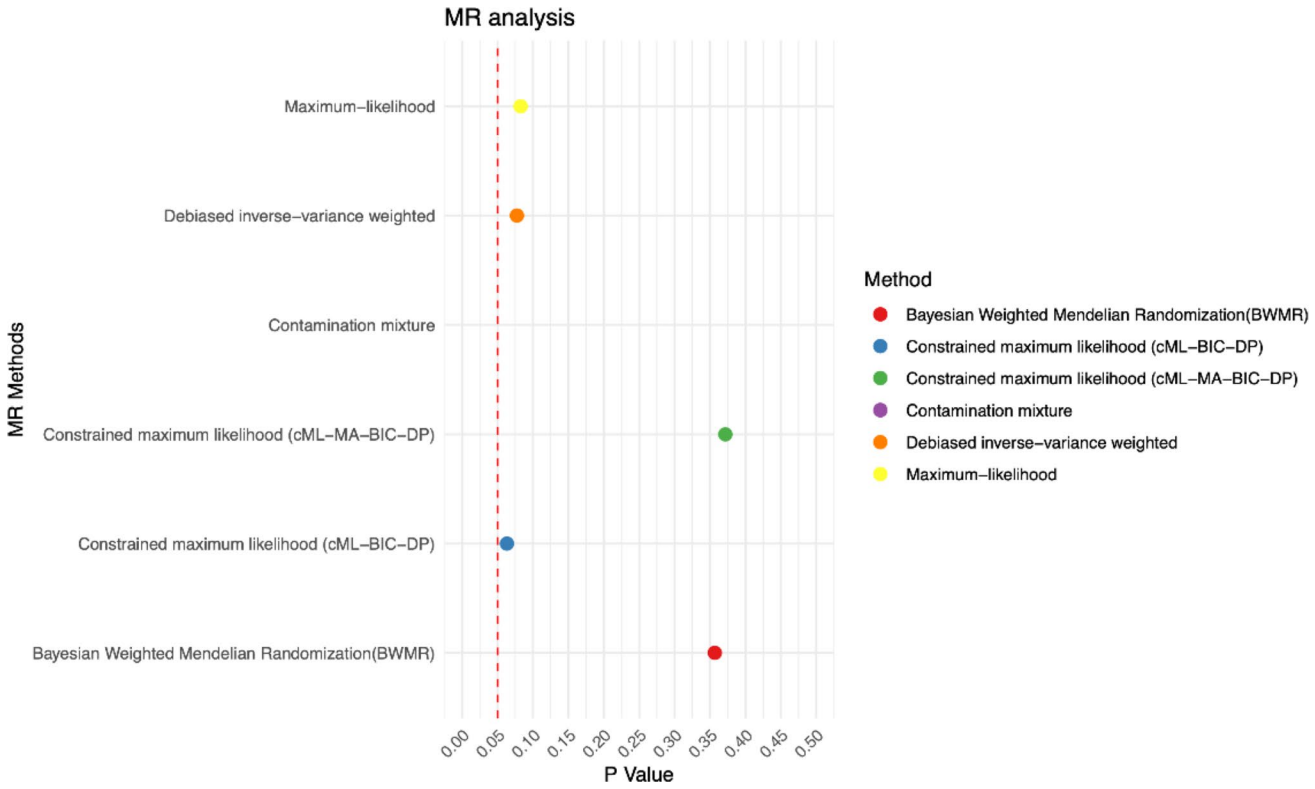

Fig. 4 Scatter plot demonstrating the causal influence of inflammatory proteins (levels of TNF-β) on HCC, utilizing MR analysis alongside six other techniques

TNF-β) on populations of immune cells. The results showed that IL-17 A suggests a potential causal relationship on 29 immune cells (Table 1) and TNF-β had causal effect on 34 immune cells (Table 2).

Concretely, IL-17 A may have a positive correlation with 6 immune cells ($CD28^+$ $CD45RA^+$ CD8 dim T cell % T cell, hematopoietic stem cell absolute count, CD38 on IgD^- $CD38^+$ B cell, CD24 on unswitched memory B cell, CD28 on $CD39^+$ $CD8^+$ T cell, and$CD16^-$ CD56 on HLA DR^+ natural killer) ($\beta > 0$); while may have a negative correlation with 23 immune cells ($CD11c^+$ monocyte % monocyte, $CD11c^+$ monocyte absolute count, $CD62L^-$ monocyte % monocyte, $CD11c^+$ $CD62L^-$ monocyte absolute count, $CD11c^+$ $CD11c^+$ HLA DR^{++} monocyte absolute count, activated CD4 regulatory T cell % $CD4^+$ T cell, activated CD4 regulatory T cell % CD4 regulatory T cell, CD25 on IgD^- $CD38^+$ B cell, CD45 on HLA DR^+ natural killer, CD4 on monocyte, CD19 on B cell, CD8 on central memory $CD8^+$ T cell, CD8 on naive $CD8^+$ T cell, CD8 on effector memory $CD8^+$ T cell, CD8 on $CD8^+$ T cell, CD8 on natural killer T, CD8 on HLA DR^+ $CD8^+$ T cell, CD4 on CD4 regulatory T cell, CD4 on activated CD4 regulatory T cell, CD4 on secreting CD4 regulatory T cell, CD4 on $CD39^+$ secreting CD4 regulatory T cell, CD4 on activated & secreting CD4 regulatory T cell, and CD8 on $CD28^+$ $CD45RA^+$ $CD8^+$ T cell) ($\beta < 0$). TNF-β may have a positive correlation with 18 immune cells (IgD^+ CD38 dim B cell % B cell, IgD^- $CD38^+$ B cell % lymphocyte, IgD^+ CD38 dim B cell % lymphocyte, naive-mature B cell % lymphocyte, IgD^+ $CD24^-$ B cell % lymphocyte, $CD39^+$ activated CD4 regulatory T cell absolute count, secreting CD4 regulatory T cell % $CD4^+$ T cell, activated & secreting CD4 regulatory T cell % $CD4^+$ T cell, $CD39^+$ $CD4^+$ T cell % $CD4^+$ T cell, $CD39^+$ $CD4^+$ T cell absolute count, $CD39^+$ $CD8^+$ T cell % T cell, $CD39^+$ $CD8^+$ T cell % $CD8^+$ T cell, $CD39^+$ $CD8^+$ T cell absolute count, CD39 on $CD39^+$ activated CD4 regulatory T cell, CD39 on $CD39^+$ $CD4^+$ T cell, CD11c on monocyte, HLA DR on HLA DR^+ T cell, HLA DR on HLA DR^+ $CD4^+$ T cell) ($\beta > 0$); while may

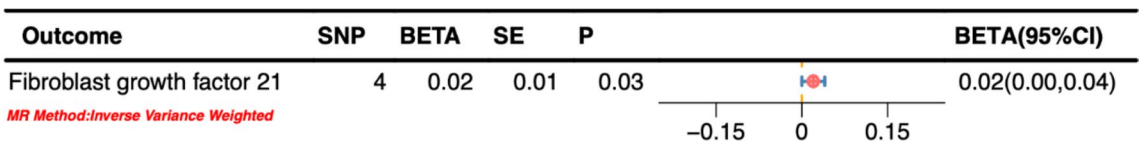

Fig. 5 Forest plot illustrating the causal impact of HCC on proteins associated with inflammation through MR analysis. Inflammatory proteins serve as the outcome variable, while HCC acts as the exposure variable; BETA refers to β, and CI denotes the confidence interval

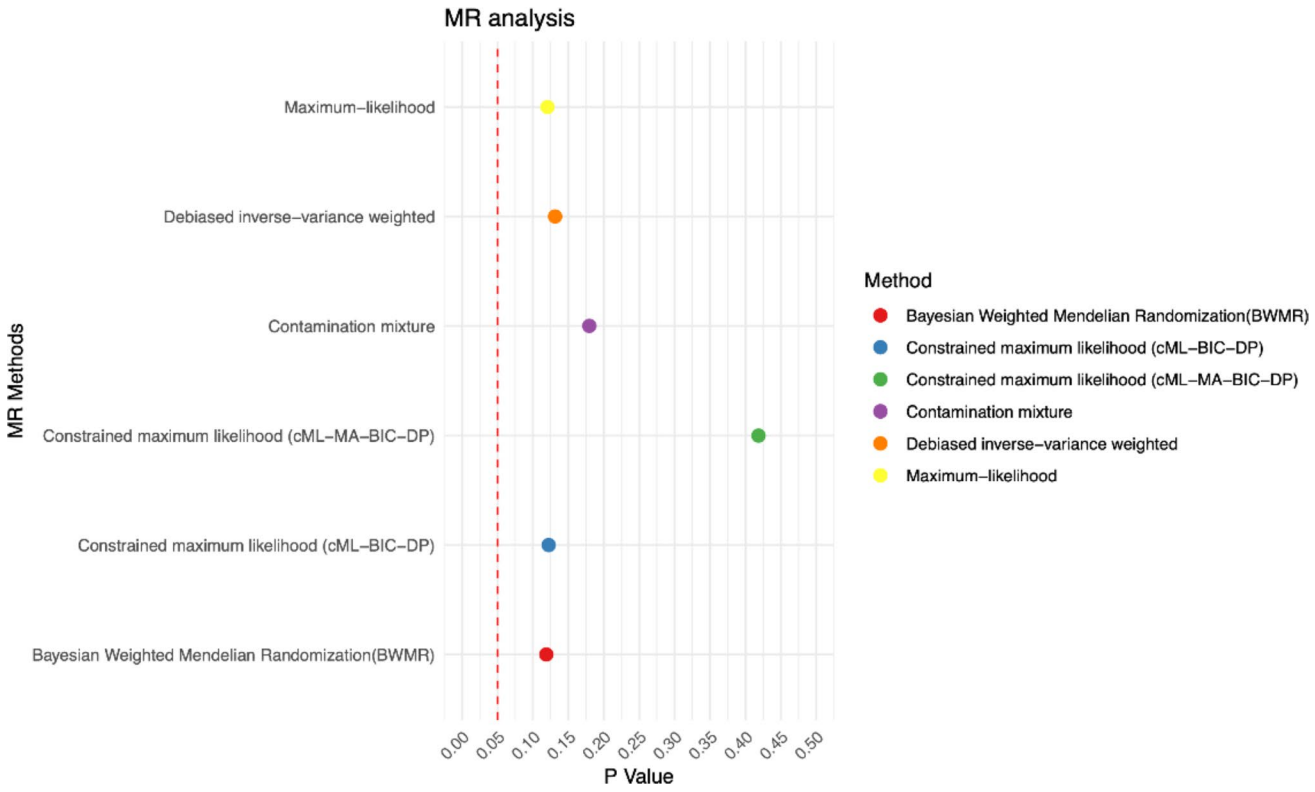

Fig. 6 The scatter plot presented the influence that HCC has on inflammatory proteins. This analysis utilizes MR as the primary methodology, while also incorporating six other complementary approaches. Through these various methodologies, the plot highlights the relationship between HCC and inflammatory markers

Table 1 The causal influence of IL-17 A on immune cells

Exposure	nSNP	Outcome	Method	BETA	SE	P	95%BETA
	11	CD11c+ monocyte Absolute Count		−0.412	0.165	0.012	−0.41(−0.74,−0.09)
	11	CD11c+ monocyte %monocyte		−0.364	0.176	0.038	−0.36(−0.71,−0.02)
	11	CD11c+ CD62L- monocyte Absolute Count		−0.399	0.159	0.012	−0.40(−0.71,−0.09)
	11	CD11c+ CD62L- monocyte %monocyte		−0.338	0.172	0.049	−0.34(−0.68,0.00)
	11	CD11c+ HLA DR++ monocyte Absolute Count		−0.348	0.141	0.014	−0.35(−0.62,−0.07)
	11	Activated CD4 regulatory T cell %CD4 regulatory T cell		−0.315	0.127	0.013	−0.31(−0.56,−0.07)
	11	Activated CD4 regulatory T cell %CD4+ T cell		−0.315	0.130	0.015	−0.32(−0.57,−0.06)
	11	Hematopoietic Stem Cell Absolute Count		0.459	0.166	0.006	0.46(0.13,0.78)
	11	CD28+ CD45RA+ CD8dim T cell %T cell		0.254	0.121	0.036	0.25(0.02,0.49)
	11	CD24 on unswitched memory B cell		0.256	0.128	0.046	0.26(0.00,0.51)
	11	CD25 on IgD- CD38+ B cell		−0.347	0.155	0.025	−0.35(−0.65,−0.04)
	11	CD38 on IgD- CD38+ B cell		0.313	0.134	0.019	0.31(0.05,0.58)
	11	CD16-CD56 on HLA DR+ Natural Killer		0.437	0.146	0.003	0.44(0.15,0.72)
	11	CD28 on CD39+ CD8+ T cell		0.279	0.141	0.048	0.28(0.00,0.56)
Interleukin-17A levels	11	CD45 on HLA DR+ Natural Killer	IVW	−0.385	0.141	0.006	−0.38(−0.66,−0.11)
	11	CD4 on monocyte		−0.364	0.140	0.010	−0.36(−0.64,−0.09)
	11	CD19 on B cell		−0.416	0.138	0.003	−0.42(−0.69,−0.14)
	11	CD8 on Central Memory CD8+ T cell		−0.516	0.153	0.001	−0.52(−0.82,−0.22)
	11	CD8 on naive CD8+ T cell		−0.524	0.155	0.001	−0.52(−0.83,−0.22)
	11	CD8 on Effector Memory CD8+ T cell		−0.342	0.143	0.017	−0.34(−0.62,−0.06)
	11	CD8 on CD8+ T cell		−0.346	0.139	0.013	−0.35(−0.62,−0.07)
	11	CD8 on Natural Killer T		−0.306	0.137	0.025	−0.31(−0.57,−0.04)
	11	CD8 on HLA DR+ CD8+ T cell		−0.306	0.146	0.036	−0.31(−0.59,−0.02)
	11	CD4 on CD4 regulatory T cell		−0.303	0.143	0.034	−0.30(−0.58,−0.02)
	11	CD4 on activated CD4 regulatory T cell		−0.368	0.143	0.010	−0.37(−0.65,−0.09)
	11	CD4 on secreting CD4 regulatory T cell		−0.300	0.143	0.036	−0.30(−0.58,−0.02)
	11	CD4 on CD39+ secreting CD4 regulatory T cell		−0.344	0.157	0.029	−0.34(−0.65,−0.04)
	11	CD4 on activated & secreting CD4 regulatory T cell		−0.330	0.143	0.021	−0.33(−0.61,−0.05)
	11	CD8 on CD28+ CD45RA+ CD8+ T cell		−0.343	0.140	0.014	−0.34(−0.62,−0.07)

have a negative correlation with 16 immune cells (IgD^+ $CD38^-$ B cell % B cell, CD19 on IgD^- CD38 dim B cell, CD24 on IgD^+ $CD24^+$ B cell, CD24 on IgD^+ $CD38^+$ B cell, CD24 on IgD^- $CD38^-$ B cell, CD24 on IgD^- CD38 dim B cell, CD24 on memory B cell, CD24 on switched memory B cell, CD25 on B cell, CD25 on IgD^+ $CD24^-$ B cell, CD25 on IgD^+ CD38 dim B cell, CD25

Table 2 The causal influence of TNF-β on immune cells

Exposure	nSNP	Outcome	Method	BETA	SE	P	95%BETA
TNF-beta levels	504	IgD+ CD38dim B cell %B cell	IVW	0.041	0.018	0.024	0.04(0.01,0.08)
	504	IgD+ CD38- B cell %B cell		−0.037	0.018	0.040	−0.04(−0.07,0.00)
	504	IgD- CD38+ B cell %lymphocyte		0.043	0.016	0.009	0.04(0.01,0.07)
	504	IgD+ CD38dim B cell %lymphocyte		0.053	0.018	0.003	0.05(0.02,0.09)
	504	Naive-mature B cell %lymphocyte		0.045	0.018	0.013	0.05(0.01,0.08)
	504	IgD+ CD24- B cell %lymphocyte		0.041	0.018	0.025	0.04(0.01,0.08)
	503	CD39+ activated CD4 regulatory T cell Absolute Count		0.041	0.019	0.028	0.04(0.00,0.08)
	503	Secreting CD4 regulatory T cell %CD4+ T cell		0.039	0.018	0.026	0.04(0.00,0.07)
	503	Activated & secreting CD4 regulatory T cell %CD4+ T cell		0.034	0.018	0.050	0.03(0.00,0.07)
	503	CD39+ CD4+ T cell %CD4+ T cell		0.036	0.017	0.035	0.04(0.00,0.07)
	503	CD39+ CD4+ T cell Absolute Count		0.039	0.018	0.036	0.04(0.00,0.07)
	503	CD39+ CD8+ T cell %T cell		0.044	0.018	0.014	0.04(0.01,0.08)
	503	CD39+ CD8+ T cell %CD8+ T cell		0.039	0.018	0.030	0.04(0.00,0.07)
	503	CD39+ CD8+ T cell Absolute Count		0.046	0.018	0.012	0.05(0.01,0.08)
	504	CD19 on IgD- CD38dim B cell		−0.038	0.018	0.032	−0.04(−0.07,0.00)
	504	CD24 on IgD+ CD24+ B cell		−0.035	0.017	0.042	−0.04(−0.07,0.00)
	504	CD24 on IgD+ CD38+ B cell		−0.037	0.017	0.033	−0.04(−0.07,0.00)
	504	CD24 on IgD- CD38- B cell		−0.048	0.017	0.006	−0.05(−0.08,−0.01)
	504	CD24 on IgD- CD38dim B cell		−0.045	0.017	0.010	−0.04(−0.08,−0.01)
	504	CD24 on memory B cell		−0.037	0.017	0.031	−0.04(−0.07,0.00)
	504	CD24 on switched memory B cell		−0.046	0.018	0.011	−0.05(−0.08,−0.01)
	504	CD25 on B cell		−0.040	0.018	0.029	−0.04(−0.08,0.00)
	504	CD25 on IgD+ CD24- B cell		−0.039	0.018	0.026	−0.04(−0.07,0.00)
	504	CD25 on IgD+ CD38dim B cell		−0.037	0.018	0.042	−0.04(−0.07,0.00)
	504	CD25 on IgD+ B cell		−0.042	0.018	0.019	−0.04(−0.08,−0.01)
	503	CD25 on CD39+ CD4+ T cell		−0.043	0.019	0.026	−0.04(−0.08,−0.01)
	504	CD40 on CD14+ CD16- monocyte		−0.038	0.018	0.032	−0.04(−0.07,0.00)
	501	CD14 on CD33+ HLA DR+ CD14dim		−0.061	0.026	0.017	−0.06(−0.11,−0.01)
	503	CD39 on CD39+ activated CD4 regulatory T cell		0.063	0.019	0.001	0.06(0.03,0.10)
	503	CD39 on CD39+ CD4+ T cell		0.042	0.019	0.031	0.04(0.00,0.08)
	503	CD11c on monocyte		0.045	0.019	0.020	0.05(0.01,0.08)
	503	HLA DR on plasmacytoid Dendritic Cell		−0.040	0.019	0.034	−0.04(−0.08,0.00)
	503	HLA DR on HLA DR+ T cell		0.038	0.018	0.035	0.04(0.00,0.07)
	503	HLA DR on HLA DR+ CD4+ T cell		0.044	0.018	0.014	0.04(0.01,0.08)

Table 3 The impact of immune cells on HCC development

Exposure	Outcome	nSNP	BETA	SE	P	95%OR
CD25 on B cell	Liver cancer	600	−0.18	0.08	0.03	0.83(0.71,0.98)
TNF-beta levels		600	0.22	0.10	0.02	1.25(1.04,1.51)
CD25 on IgD+ CD38dim B cell	Liver cancer	600	−0.18	0.08	0.03	0.83(0.71,0.98)
TNF-beta levels		600	0.21	0.10	0.03	1.23(1.02,1.49)
CD25 on IgD+ B cell	Liver cancer	603	−0.18	0.08	0.02	0.84(0.72,0.97)
TNF-beta levels		603	0.22	0.10	0.02	1.24(1.03,1.50)
CD4 on CD39+ secreting CD4 regulatory T cell	Liver cancer	60	−0.21	0.10	0.04	0.81(0.66,0.99)
Interleukin-17A levels		60	0.16	0.50	0.75	1.17(0.44,3.12)

on IgD^+ B cell, CD25 on $CD39^+$ $CD4^+$ T cell, CD40 on $CD14^+$ $CD16^-$ monocyte, CD14 on $CD33^+$ HLA DR^+ CD14 dim, and HLA DR on plasmacytoid dendritic cell) ($\beta < 0$).

3.4 The impact of immune cells on HCC development

The multivariate mediation and moderation analysis identified that four immune cell types (CD25 expression on B cells, CD25 expression on IgD+ CD38 dim B cells, CD25 expression on IgD+ B cells, and CD4 expression on CD39+ secreting CD4 regulatory T cells) exhibited a negative correlation with HCC risk (OR < 1, Table 3). Our results suggest these markers have the possibility to serve as protective factors against HCC.

3.5 Immune cell-driven inflammatory protein mediation in hepatocellular carcinoma

Examining the Role of Immune Cell-Mediated Inflammatory Proteins in Hepatocellular Carcinoma: Two-Step MR Approach and Presentation of Findings in Table 4. Based on the results of the level of IL-17 A having a negative correlation with the likelihood of developing HCC (OR < 1), the level of TNF-β having a positive correlation with the likelihood of developing HCC (OR > 1), and 4 immune cells (,CD4 on $CD39^+$ secreting CD4 regulatory T cell, CD25 on IgD^+ B cell, CD25 on B cell, and CD25 on IgD^+ CD38 dim B cell) exhibiting an inverse relationship with the risk of HCC (OR < 1), we could infer that IL-17 A demonstrated the capacity to mitigate the risk of HCC by decreasing CD4 on CD39+ secreting CD4 regulatory T cell with a mediation effect proportion of −5.14%, and TNF- β was able to reduce the risk of HCC by increasing CD25 on

IgD+ CD38 dim B cell, CD25 on B cell, and CD25 on IgD+ B cell with a mediation effect proportion of 3.19%, 2.98%, and 3.29% respectively.

4 Discussion

Within the context of this investigation, the causal associations among immune cells, HCC, and inflammatory proteins were dissected through MR analyses, with the results indicating that 2 inflammatory proteins (IL-17 A and TNF-β) exhibited a potential causal influence on the likelihood of HCC. No potential causal relationships were identified between other relevant scores of inflammatory proteins that were either close to or showed poor association with HCC. HCC could affect FGF-21, 4 immune cells (CD4 on $CD39^+$ secreting CD4 regulatory T cell, CD25 on IgD^+ B cell, CD25 on B cell, and CD25 on IgD^+ CD38 dim B cell) had an association demonstrating an inverse relationship with the risk of HCC, and the 2-positive inflammatory proteins could reduce the risk of HCC by decreasing or increasing the corresponding 4-positive immune cells with different mediation effect proportions.

Previous reports have substantiated crucial and pivotal role of inflammation in the occurrence and development of HCC, underscoring robust associations between proteins involved in inflammation and pathways of hepatic inflammatory. According to our investigation, the results showed that IL-17 A demonstrated a negative association with the likelihood of HCC, while TNF-β demonstrated a positive association with the likelihood of HCC. IL-17 A, which is commonly called IL-17, belongs to the family of interleukin-17, which is served as the main inflammatory mediator and is currently the most clearly investigated and explained member [22–24]. IL-17 A is predominantly produced by Th17 cells, while cell types such as NK cells, γ δ T cells, CD8+ T cells, mast cells, neutrophils, and macrophages also exhibit IL-17 A expression [25]. IL-17 A is the main executor of Th17 cell, which can promote the occurrence of inflammation and acts as an significant role in autoimmune diseases, tumors, as well as transplant rejection [26]. Additionally, it has been demonstrated that the abnormal expression of IL-17 A plays a role in the onset and advancement of various malignant tumors. IL-17 A may act as a tumor suppressor gene or as a proto-oncogene through different signaling pathways in multiple forms of cancer [27–29]. The superfamily of tumor necrosis factor (TNF) consists of 19 members, most of which bind to the corresponding receptor in a trimeric form to activate downstream signaling pathway transduction, thus participating in a multitude of inflammatory and immune responses. Among them, the most thorough and extensive study is tumor necrosis factor α (TNF-α), which is recognized that has a close relationship with the occurrence of ankylosing spondylitis, rheumatoid arthritis, inflammatory enteritis, multiple sclerosis, psoriasis, acute hepatitis and other diseases [30, 31]. TNF-β is another important member in the superfamily of TNF, and the most important biological activity of TNF-β shared with TNF-α is the activation of non-classical and classical NF-κB cascade signaling pathway transduction [32]. TNF-β has a broad spectrum of biological activities, ranging from regulating the procedure of secondary lymphoid organs ontogeny to regulating the immune response for specific microorganisms [33]. TNF-β mainly expresses in CD4+ type I T helper cells (T helper cell 1, TH 1), NK cells, B cells, CD8+ cells, and macrophages. It has a special role in occurrence, development and function of immune system, especially in the development of lymphoid organs, maintenance of lymph, microenvironment, host defense and inflammation [34]. Thus, our findings affirm the inverse correlation between IL-17 A levels and HCC susceptibility, alongside the direct correlation observed between TNF-β levels and HCC risk.

The interrelationship between the tumors and immune system is one of the most studied topics in the field of cancer. Previously, cancer was considered useful only for the tumor itself; however, we now know that it requires support from cytokines and different cells, namely, the tumor microenvironment (TME). Among the components constituting the TME, immune cells tasked with supporting the tumor exert a crucial role in the TME [35, 36]. Although immune cells have the ability to recognize and exterminate nascent cancer cells during immunosurveillance for cancer under normal conditions, they have the possibility to be influenced by various factors in the presence of tumors during cancer immunoediting. The observation that immune cells can function as guardians of tumors (anti-tumor immunity) or supporters of tumors

Table 4 Mediation effect of immune cells mediating inflammatory proteins on HCC

Exposure	Mediator	Outcome	Total effect	Mediator effect	Direct effect	SE	Z	Mediation effect proportion	Confidence interval
TNF-beta levels	CD25 on B cell	Liver cancer	0.23	0.01	0.22	0.02	0.48	3.19%	(–0.02,0.04)
TNF-beta levels	CD25 on IgD+ CD38dim B cell	Liver cancer	0.23	0.01	0.22	0.02	0.45	2.98%	(–0.02,0.04)
TNF-beta levels	CD25 on IgD+ B cell	Liver cancer	0.23	0.01	0.22	0.01	0.57	3.29%	(–0.02,0.03)
Interleukin-17A levels	CD4 on CD39+ secreting CD4 regulatory T cell	Liver cancer	–1.40	0.07	–1.48	0.06	1.24	–5.14%	(–0.04,0.19)

(pro-tumor immunity) enables them to become a "double-edged sword" in the TME. Immune cells include different cell populations: white blood cells (granulocytes, monocytes, T lymphocytes and B lymphocytes), mast cells, platelets, red blood cells, dendritic cells (DC) and innate lymphocytes (ILC). Garnelo et al. [37] discovered that upon depletion of B lymphocytes marked by CD20, there was an observed up-regulation in PD-1 expression on CD8+ T lymphocytes, indicating a potential regulatory role of CD20+ B lymphocytes in CD8+ T lymphocyte activation. It has been demonstrated that regulatory B lymphocytes (Breg) exert negative modulation of immune responses through the secretion of IL-10 and TGF-β. This mechanism serves to attenuate Th2 and Th1 development while promoting differentiation of regulatory T lymphocytes (Treg), which results in impaired immune function of CD8+ T lymphocytes and reduced numbers of CD4+ T lymphocytes. T lymphocyte-mediated immune response is central to tumor monitoring and clearance, and enhancing the specific immune response of T lymphocytes to hepatoma cells is able to reduce the risk of HCC. Studies have demonstrated that within HCC tumor tissue, CD8+ T lymphocytes exhibit differentiation into cytotoxic T lymphocytes, characterized by their ability to produce cytokines and cytotoxic enzymes such as perforin and granzyme B. These effector functions enable the eradication of cancer cells and the subsequent development into memory T lymphocytes [38]. Apart from CD8+ T lymphocytes, CD4+ T lymphocytes have also been shown to exert a pivotal role in control of tumor progression. Findings from several studies suggest that in animal model of nonalcoholic steatohepatitis (NASH), initiation of apoptosis in CD4+ T lymphocytes could potentially promote the progression of HCC [39]. CD4+ T lymphocytes can exert their immune function by activating CD8+ T lymphocytes and secreting cytokines. Meanwhile, naive CD4+ T lymphocytes are able to express transcription factors such as GATA-3, T-bet and Bcl6 upon induction of specific cytokines, and then differentiate into various cell subtypes, including Th9, Th1, Th17, Th2, Th22 and Tfh [40]. Moreover, cytotoxic CD4+ T lymphocytes were also detected in anti-tumor responses, suggesting that these cells may exhibit a complementary function to CD8+ T lymphocytes in vivo to exert anti-tumor effects [41]. As a class of T lymphocyte subsets that regulate autoimmune responses, Treg plays a pivotal role in HCC tumor immunity. As an immunosuppressive and anti-inflammatory cell, Treg has the ability to inhibit the impact of various cytokines (IL-6 and IL-17) on T lymphocytes and the anti-tumor function of effector T lymphocytes by high expression of related immunosuppressive molecules (such as TIM-3 and LAG-3) [42, 43].

Besides, the findings from Mikael et al. underscored the unique role of CD25+ B lymphocytes in antigen presentation among B cells, revealing stringent control over the regulation of CD25 expression [44]. Compared to CD25+ CD4+ T cells, CD25+ B lymphocytes acted as an enhancer of the immune response. Moreover, CD25+ B lymphocytes were characterized by a surface adorned with immunoglobulin, priming them for antigen engagement and subsequent activation of T cells. The result in our work revealed that CD4 on $CD39^{+}$ secreting CD4 regulatory T cell, CD25 on IgD^{+} CD38 dim B cell, CD25 on IgD^{+} B cell, and CD25 on B cell had a negative association with the risk of HCC.

Since our results indicated that causal relationships of 2 inflammatory proteins as well as 4 immune cells with the risk of HCC, we hypothesized that inflammatory proteins affect the risk of HCC by modulating immune cells, and our result of the mediation effect of immune cells mediating inflammatory proteins on HCC supported the hypothesis.

Together, our research elucidated how inflammatory proteins and immune cells are causally linked to the susceptibility to developing HCC.; and the involvement of immune cell-mediated inflammatory proteins in HCC risk underscores novel insights into the intricate molecular mechanisms underlying HCC progression, potentially reshaping approaches to early diagnosis.

Certain limitations persist in this study: (1) The GWAS dataset utilized in this study predominantly comprises data from individuals of European ancestry, potentially introducing bias in estimations and impacting the generalizability of the study findings; (2) The data collected in this study are cross-sectional, and considering the time-dependence of disease incidence, the lack of longitudinal data on exposure measures may cause partial bias to the analysis results; (3) The assumptions of independence and exclusion restriction cannot be fully evaluated in this study, which may lead to bias. The relatively small number of HCC cases (n = 128) may also impact the results.

Our study may also be influenced by the "winner's curse". In GWAS (Genome-Wide Association Studies) or other genetic association studies, associations may be partially or wholly attributable to random error due to limited sample sizes and multiple comparisons. When these genetic variants are used as instrumental variables in MR analysis, a true association strength that is lower than initially reported can lead to erroneous conclusions regarding the causal relationship between exposure and outcome. Future research should replicate the strength of initial associations in independent samples to confirm their robustness.

Interestingly, our MR study results differ from the conclusions of experimental and clinical studies. This discrepancy may stem from the limitations inherent in MR, including uncertainties in genetic variant selection and effect size, constraints related to sample size and statistical power, the complexity of gene-environment interactions, disease heterogeneity, and the broad definitions of phenotypes, as well as methodological differences. Together, these limitations

provide a reasonable explanation for the observed differences. Future research will aim to integrate diverse evidence, increase sample sizes, optimize genetic variant selection, delve deeper into interactions, and enhance methodological innovations to more accurately elucidate the causal relationships in complex diseases.

Future research directions include the development of inflammatory protein modulators, exploration of immune cell therapies, multi-omics integrated analyses to identify novel biomarkers, and the design of clinical trials to validate the efficacy and safety of these treatments. This work aims to deepen our understanding of disease mechanisms within the complex interactions of genetic and environmental factors.

Author contributions Y.I.W. and Z.J.T.: contributed to manuscript writing, conducted literature searches, and participated in data cleaning, processing, and data visualization. Y.C. and S.K.W.: contributed to data processing and assisted in interpreting the results of data analysis. Y.I.W. and Z.J.T.: participated in manuscript revisions and proofreading. Y.C., S.K.W. and D.H.Y.: provided major contributions, including project design, determination of research direction, supervised data analysis, and oversaw the entire research process.

Data availability The data that support the findings of this study are available from the GWAS Catalog Database (https://www.ebi.ac.uk/gwas/studies/).

Declarations

Competing interests Not applicable.

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