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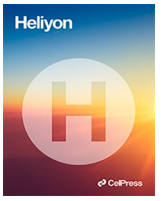

Research article

Causal relationships between immune cells, inflammatory factors, serum metabolites, and hepatic cancer: A two-sample Mendelian randomization study

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ABSTRACT

Background: Observational studies and clinical trials suggest associations between immune cells, inflammatory factors, serum metabolites, and hepatic cancer. However, the causal relationships between these factors and hepatic cancer remain to be established.
Objective: To explore the causal relationships between immune cells, inflammatory factors, serum metabolites, and hepatic cancer.
Methods: This study employed comprehensive two-sample Mendelian randomization (MR) utilizing publicly available genetic data (GWAS) to analyze causal relationships between 731 immune cell traits, 91 inflammatory factors, 1400 serum metabolites, and hepatic cancer. The primary analysis used inverse variance-weighted (IVW) MR, with additional sensitivity tests to assess the validity of causal relationships.
Results: After correction for heterogeneity and horizontal pleiotropy, in exploring the causal relationships between immune cell groups and hepatic cancer, we found that Terminally Differentiated $CD4^{-}CD8^{-}$ T cell %T cell was negatively associated with hepatic cancer, serving as a protective factor, while Effector Memory $CD4^{-}CD8^{-}$ T cell %$CD4^{-}CD8^{-}$ T cell was positively associated with hepatic cancer, acting as a risk factor. In investigating the causal relationships between inflammatory factors and hepatic cancer, C–C motif chemokine 19 levels were positively associated with hepatic cancer, representing a risk factor, while Interleukin-10 levels were negatively associated with hepatic cancer, acting as a protective factor. Regarding the causal relationships between serum metabolites and hepatic cancer, (N(1) + N(8))-acetylspermidine levels were negatively associated with hepatic cancer, serving as a protective factor, while 1-(1-enyl-palmitoyl)-GPC (p-16:0) levels were positively associated with hepatic cancer, acting as a risk factor.
Conclusion: Our MR analysis indicates causal relationships between immune cells, inflammatory factors, serum metabolites, and hepatic cancer. However, further validation is needed to assess

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the potential of these immune cells, inflammatory factors, and serum metabolites as preventive or therapeutic targets for hepatic cancer.

1. Background

Hepatic cancer is one of the most common cancers globally and a major cause of cancer-related deaths. Immune cells and inflammatory factors are closely associated with the development of hepatic cancer, playing crucial regulatory roles throughout its progression. Serum metabolites, being products of metabolic processes within the body, also exhibit certain relationships with the occurrence and development of hepatic cancer.

The role of immune cells in hepatic cancer has garnered widespread attention. T lymphocytes, B lymphocytes, natural killer (NK) cells, myeloid cells, and other immune cells may play pivotal roles in tumor initiation, development, and response to treatment. Immune cells can prevent tumor formation and development by recognizing and eliminating cancer cells [1]. However, when the complex balance of the immune system is disrupted, inhibitory immune cells in the microenvironment assist cancer cells in immune evasion, promoting tumor development. The degree of local inflammatory response in hepatic cancer is closely correlated with tumor progression. Inflammatory factors, as important regulatory molecules, exert complex effects in the development of hepatic cancer. Inflammatory states can activate tumor-associated signaling pathways, promoting tumor proliferation, invasion, and metastasis. Inflammatory factors can also facilitate the development of hepatic cancer by activating immune cells, stimulating angiogenesis, and altering the cell cycle. Furthermore, immune cells can regulate inflammatory factors to promote tumor growth [2]. Serum metabolites are small organic molecules widely present in the body, and their levels are influenced by genetics, environment, and physiological conditions. Studies have indicated that levels of serum metabolites, such as amino acids, lipid metabolism disturbances, and cholesterol, are closely related to the occurrence and development of hepatic cancer [3].

However, the causal relationships between immune cells, inflammatory factors, and serum metabolites in the development of hepatic cancer are currently unclear. Genome-Wide Association Studies (GWAS) are a method used to identify genetic variations associated with specific diseases or traits across multiple individuals. GWAS can identify genetic variations related to both exposures and outcomes, providing insights into their potential causal relationships. Additionally, GWAS can help uncover shared genetic pathways or mechanisms associated with exposures and outcomes, further enhancing the understanding of disease etiology and aiding in the discovery of new therapeutic targets. This study aims to explore the causal relationships between immune cells, inflammatory factors, serum metabolites and hepatic cancer through a two-sample Mendelian randomization (MR) analysis utilizing GWAS. The goal is to further uncover the mechanisms of hepatic cancer development, providing new theoretical foundations for the prevention and treatment of hepatic cancer.

2. Materials and methods

2.1. Study design

We conducted a two-sample MR analysis to assess the causal relationships between 731 immune cells, 91 inflammatory factors, 1400 serum metabolites, and hepatic cancer. MR employs genetic variants as proxies for risk factors; therefore, instrumental variables (IVs) in causal inference must satisfy three key assumptions: (1) genetic variants are strongly correlated with the exposure; (2) genetic variants are unrelated to potential confounders between exposure and outcome (i.e., no horizontal pleiotropy); (3) genetic variants influence the outcome only through the exposure and not through other pathways.

2.2. GWAS data source

We selected single nucleotide polymorphisms (SNPs) significantly associated with immune cells, inflammatory factors, and serum metabolites as IVs. Comprehensive summary statistics for GWAS of immune cells, inflammatory factors, and serum metabolites were obtained from the catalog database (https://www.ebi.ac.uk/gwas/publications/37563310) GWAS catalog [4–6]. We included 731 immune cell phenotypes (GCST0001391 to GCST0002121), 91 inflammatory factors (GCST90274758 to GCST90274848), and 1400 serum metabolites (GCST90199621 to GCST90201020). The immune phenotypes comprised 389 median fluorescence intensities (MFI), 192 relative cell (RC), 118 absolute cell (AC), and 32 morphological parameters (MP), with the original GWAS conducted using data from 3757 European individuals without overlapping cohorts. Inflammatory factors were obtained from the latest integrated GWAS summary data, including 14,824 Europeans from 11 cohorts, covering 91 systemic inflammation-regulating factors measured using the Olink Target Inflammation Panel and subjected to genome-wide pQTL localization. Serum metabolites included 1091 serum metabolites and 309 metabolite ratios, sourced from 8299 individuals in the Canadian Longitudinal Study on Aging (CLSA) cohort.

2.3. Hepatic cancer GWAS data source

We downloaded the GWAS data for hepatic cancer (GWAS ID: ebi-a-GCST90018858) reported in the IEU Open GWAS database (https://gwas.mrcieu.ac.uk/). The study population consisted of Europeans, and ethical approval was unnecessary as the data were from public databases.

2.4. Instrumental variables (IVs) selection

We screened SNPs strongly correlated with exposure factors as IVs to test the causal relationship between exposure factors and outcomes. For the three exposure factors—immune cells, inflammatory factors, and serum metabolites—GWAS datasets were used to extract SNPs as IVs, and SNPs as exposure factors were required to reach a genome-wide statistical significance threshold (5×10^{-8}). If the number of IVs obtained was less than 5, we lowered the threshold to $P < 5 \times 10^{-6}$, following the method of previous MR studies [7], to discover potential causal associations between exposure factors and hepatic cancer. Additionally, to alleviate bias caused by linkage disequilibrium (LD), we limited SNPs with clump_kb > 10000 and clump_r [2] < 0.001, selecting only the SNP with the strongest impact on the outcome as the genetic instrument. We calculated the R^2 for each SNP in the IVs using the formula $R^2 = 2 \times (1\text{-MAF}) \times \text{MAF} \times \beta$ [2], where R^2 represents the degree to which the instrument explains the exposure, MAF is the minimum allele frequency, and β is the allele effect value. Subsequently, we calculated the F value, $F=R^2 \times (n\text{-}k\text{-}1)/[k \times (1\text{-} R^2)]$, where n is the total sample size and k is the number of instrumental variables. Finally, we removed weak instrumental variables bias by including only SNPs with F > 10 in the MR analysis.

2.5. Statistical analysis

In order to assess the causal relationships between immune cells, inflammatory factors, serum metabolites, and hepatic cancer, we utilized selected SNPs as IVs and conducted analyses using the "Mendelian Randomization" package [8]. The primary methods employed for estimation of the causal effects of exposures on outcomes included the conventional fixed-effects inverse variance weighting (IVW) [9], the weighted median (WM) method [10], and the MR-Egger method. The results were visualized through forest plots. Cochran's Q test was used for heterogeneity testing, and $P < 0.05$ indicated heterogeneity. If $P > 0.05$, suggesting heterogeneity in the results, the random-effects IVW method was used instead of the fixed-effects IVW method to correct for heterogeneity [9,11]. The IVW method was reported to be slightly stronger under certain conditions than other methods [10]. However, since it assumes that all instrumental variables are effective, we used the Weighted Median method [10], and Leave-One-Out sensitivity test [12] for sensitivity analysis, sequentially removing the influence of each SNP, to assess the robustness of causal effect estimates. The Weighted Median method is more robust to outliers. The MR-Egger regression method was used for intercept testing to assess horizontal pleiotropy [13,14]. When $P < 0.05$, there is evidence of horizontal pleiotropy, and the remaining SNP list after removing horizontal pleiotropy SNPs was used for subsequent MR analysis. In addition, scatterplots and funnel plots were used for visualization. Scatterplots show results unaffected by outliers, while funnel plots demonstrate the robustness of the correlation and absence of heterogeneity. Only MR analyses with SNP counts ≥3 were included as valid results. All statistical analyses were performed using R 4.2.3 (http://www.Rproject.org), R studio software, and R packages, with an alpha level of 0.05 for significance testing.

3. Results

3.1. Causal effects of immune cell exposure on the onset of hepatic cancer

To explore the causal impact of immune cells on hepatic cancer, MR analysis was employed, with IVW, Weighted median, and MR-Egger as the primary analytical methods. GWAS datasets were utilized to extract SNPs related to immune cells as IVs. We applied a criterion of $P < 5 \times 10^{-8}$ for screening SNPs as exposure factors and identified a total of 355 SNPs as IVs to assess the causal relationship between immune cells and hepatic cancer (Supplementary Table 1).

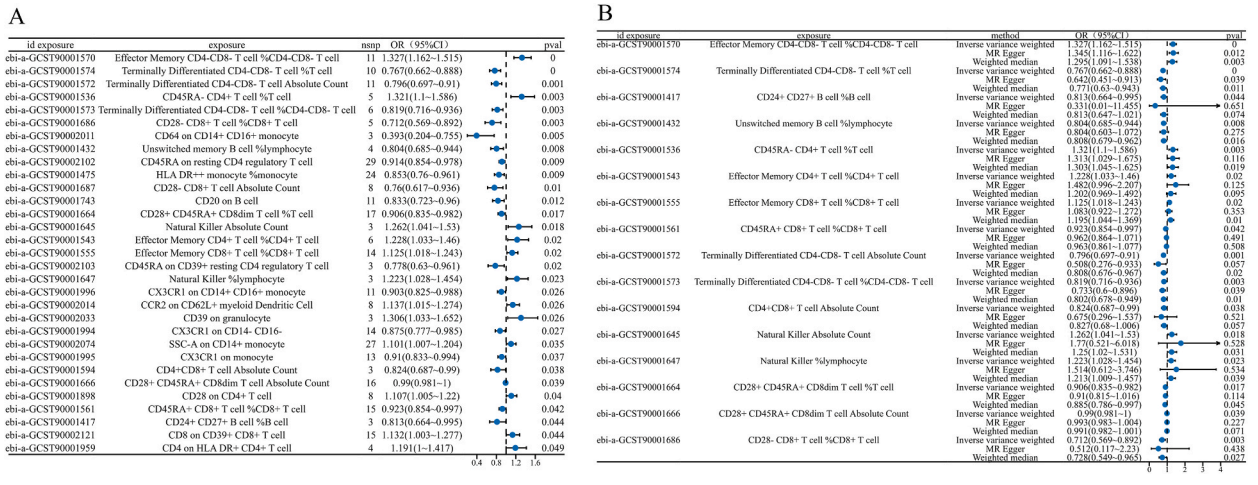

Fig. 1. The forest plot illustrating the causal relationship between immune cell characteristics and Hepatic cancer (A: Forest plot using the IVW method in MR analysis; B: Forest plot using the IVW, Weighted median, and MR-Egger methods in MR analysis).

Following heterogeneity testing, horizontal pleiotropy examination, and sensitivity analysis (specific results in Section 3.4), IVW method identified 31 immune cell types causally related to hepatic cancer (Fig 1A). Specifically, Terminally Differentiated $CD4^{-}CD8^{-}$ T cell %T cell (ebi-a-GCST90001574) (IVW: OR = 0.767, 95 % CI = 0.662–0.888, $P = 0.000$; Weighted Median: OR = 0.771, 95 % CI = 0.63–0.943, $P = 0.011$; MR Egger: OR = 0.642, 95 % CI = 0.451–0.913, $P = 0.039$) exhibited a causal relationship with hepatic cancer, acting as a protective factor. Terminally Differentiated $CD4^{-}CD8^{-}$ T cell %$CD4^{-}CD8^{-}$ T cell (ebi-a-GCST90001573), $CD28^{-}$ $CD8^{+}$ T cell %$CD8^{+}$ T cell (ebi-a-GCST90001686), CD64 on $CD14^{+}$ $CD16^{+}$ monocyte (ebi-a-GCST90002011), among others, also demonstrated protective effects against hepatic cancer. Additionally, Effector Memory $CD4^{-}CD8^{-}$ T cell %$CD4^{-}CD8^{-}$ T cell (ebi-a-GCST90001570) (IVW: OR = 1.327, 95 % CI = 1.162–1.515, $P = 3.03 \times 10\hat{}-5$; Weighted Median: OR = 1.295, 95 % CI = 1.091–1.538, $P = 3.164 \times 10\hat{}-3$; MR Egger: OR = 1.35, 95 % CI = 1.116–1.622, $P = 0.012$) exhibited a causal relationship with hepatic cancer, acting as a risk factor. $CD45RA^{-}$ $CD4^{+}$ T cell %T cell (ebi-a-GCST90001536), Effector Memory $CD4^{+}$ T cell Absolute Count (ebi-a-GCST90001542), among others, were also identified as risk factors for hepatic cancer. The MR results from IVW, Weighted Median, and MR-Egger methods were visualized with a forest plot containing 16 immune cell types (Fig 1B).

3.2. Causal effects of inflammatory factors exposure on the onset of hepatic cancer

To explore the causal impact of inflammatory factors on hepatic cancer, MR analysis was employed, with the primary analytical methods being the IVW, Weighted Median, and MR-Egger method. GWAS data sets were utilized to extract SNPs related to inflammatory factors as IVs. The selected SNPs for exposure factors were required to have a significance level below the genome-wide threshold (5×10^{-8}). However, due to the limited number of SNPs initially selected for inflammatory factors, the threshold was relaxed to $P < 5 \times 10^{-6}$. A total of 159 SNPs were then screened as IVs for evaluating the causal relationship between inflammatory factors and hepatic cancer (Supplementary Table 2), aiming to discover a more comprehensive causal association between inflammatory factors and hepatic cancer.

Following heterogeneity testing, sensitivity, and horizontal pleiotropy (specific results in Section 3.4), the IVW method identified 6 inflammatory factors with a causal relationship with hepatic cancer (Fig 2A). Notably, C–C motif chemokine 19 levels (GCST90274765) (IVW: OR = 1.258, 95%CI = 1.090–1.452, $P = 1.677 \times 10\hat{}(-3)$; Weighted median: OR = 1.260, 95%CI = 1.068–1.486, $P = 6.15 \times 10\hat{}(-3)$; MR Egger: OR = 1.327, 95%CI = 1.073–1.642, $P = 0.018$) and Fms-related tyrosine kinase 3 ligand levels (GCST90274791) (IVW: OR = 1.290, 95%CI = 1.045–1.593, $P = 0.018$; Weighted median: OR = 1.405, 95%CI = 1.052–1.876, $P = 0.021$; MR Egger: OR = 1.806, 95%CI = 1.093–2.981, $P = 0.029$) were identified as risk factors for hepatic cancer, showing statistical significance across all three analysis methods. Similarly, Interleukin-13 levels (GCST90274799) and Interleukin-4 levels (GCST90274813) were also identified as risk factors for hepatic cancer. In contrast, Interleukin-10 levels (GCST90274795) (IVW: OR = 0.638, 95%CI = 0.453–0.899, $P = 0.010$; Weighted median: OR = 0.809, 95%CI = 0.521–1.255, $P = 0.344$; MR Egger: OR = 0.888, 95%CI = 0.376–2.097, $P = 0.791$) demonstrated a causal relationship with hepatic cancer in the IVW method, indicating a protective effect. Monocyte chemoattractant protein-3 levels (GCST90274823) (IVW: OR = 0.742, 95%CI = 0.556–0.989, $P = 0.042$; Weighted median: OR = 0.666, 95%CI = 0.465–0.954, $P = 0.027$; MR Egger: OR = 0.664, 95%CI = 0.279–1.580, $P = 0.368$) showed a protective effect against hepatic cancer according to both the IVW and Weighted Median methods. The MR results from the IVW, Weighted Median, and MR-Egger methods were visualized in a forest plot (Fig 2B).

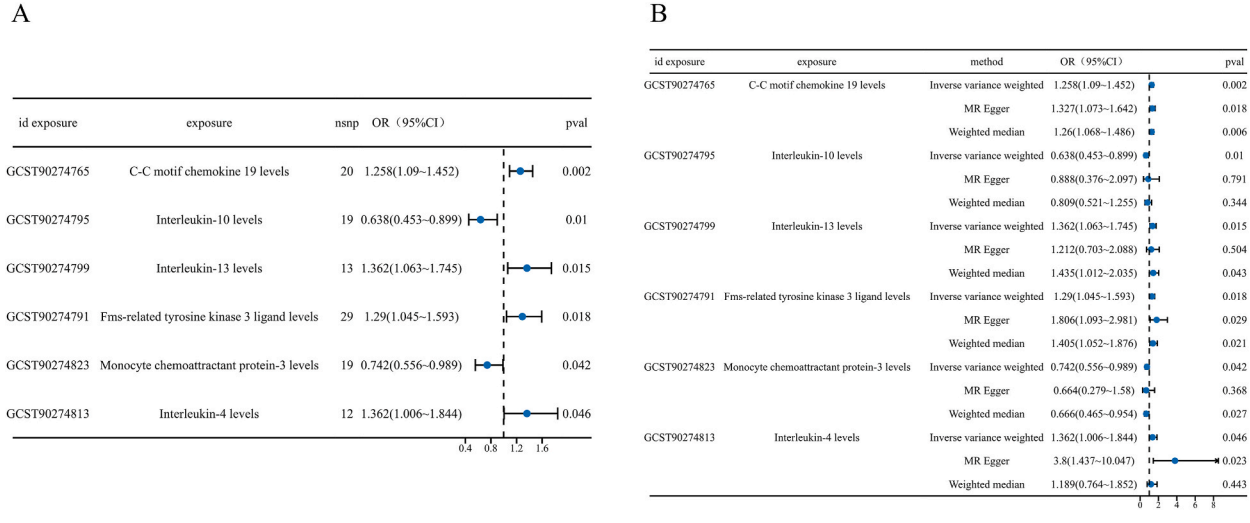

Fig. 2. Forest Plot Illustrating the Causal Relationship Between Inflammatory Factors and Hepatic cancer (A: Forest plot using the IVW method in MR analysis; B: Forest plot using the IVW, Weighted median, and MR-Egger methods in MR analysis).

3.3. Causal effects of serum metabolite exposure on the onset of hepatic cancer

To investigate the causal impact of serum metabolite exposure on the development of hepatic cancer, MR analysis was employed, utilizing the IVW, Weighted Median, and MR-Egger method as the primary analytical approaches. GWAS datasets were utilized to extract SNPs related to serum metabolites as IVs. SNPs were selected as exposure factors based on a significance level below 5×10^{-8}. A total of 73 SNPs were screened as IVs to assess the causal relationship between serum metabolites and hepatic cancer (Supplementary Table 3), aiming to discover the causal association between serum metabolites and hepatic cancer.

Following heterogeneity testing, sensitivity analysis, and assessment of horizontal pleiotropy (specific results in Section 3.4), the IVW method identified 19 serum metabolites with a causal relationship with hepatic cancer (Fig 3A). Specifically, (N(1) + N(8))-acetylspermidine levels (GCST90200153) were identified as a protective factor for hepatic cancer (IVW: OR = 0.787, 95%CI = 0.652–0.950, P = 0.013; Weighted median: OR = 0.758, 95%CI = 0.611–0.940, P = 0.012; MR Egger: OR = 0.762, 95%CI = 0.383–1.517, P = 0.483). Similarly, 1-palmitoyl-2-linoleoyl-gpc (16:0/18:2) levels (GCST90200330), 6-bromotryptophan levels (GCST90200201), Campesterol levels (GCST90199725), and others were identified as risk factors for hepatic cancer. Furthermore, 1-(1-enyl-palmitoyl)-GPC (p-16:0) levels (GCST90199899) were found to be causally associated with l hepatic cancer as a risk factor (IVW: OR = 1.327, 95%CI = 1.000–1.760, P = 0.049; Weighted median: OR = 1.356, 95%CI = 1.001–1.838, P = 0.049; MR Egger: OR = 1.461, 95%CI = 0.483–4.421, P = 0.623). Additionally, 2-stearoyl-GPE (18:0) levels (GCST90199928), 3beta-hydroxy-5-cholestenoate levels (GCST90200014), and Benzoate to oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] ratio (GCST90200987) were also identified as risk factors for hepatic cancer. Visualization of the MR results from the IVW, Weighted Median, and MR-Egger methods was achieved through a forest plot (Fig 3B).

4. Reliability assessment of results

4.1. Heterogeneity testing

Cochran's Q tests conducted for MR-Egger and IVW methods revealed heterogeneity in ebi-a-GCST90001687 and ebi-a-GCST90001475 ($P < 0.05$) within the immune cell group. As a result, the random-effects IVW method was employed to correct for heterogeneity instead of the fixed-effects IVW method. There was no heterogeneity in inflammatory factors, serum metabolites and other immune cells ($P > 0.05$). Scatter plots demonstrated that the results were not influenced by outliers. The funnel plot confirmed the robustness of the correlations, indicating no heterogeneity (Supplementary Tables 4–6, Supplementary Figs. 1–15).

4.2. Sensitivity testing

Sensitivity analyses, including Weighted Median and Leave-One-Out tests, were performed during the SNP selection process for instrumental variables. The results indicated the reliability of the MR findings (Supplementary Figs. 16–23).

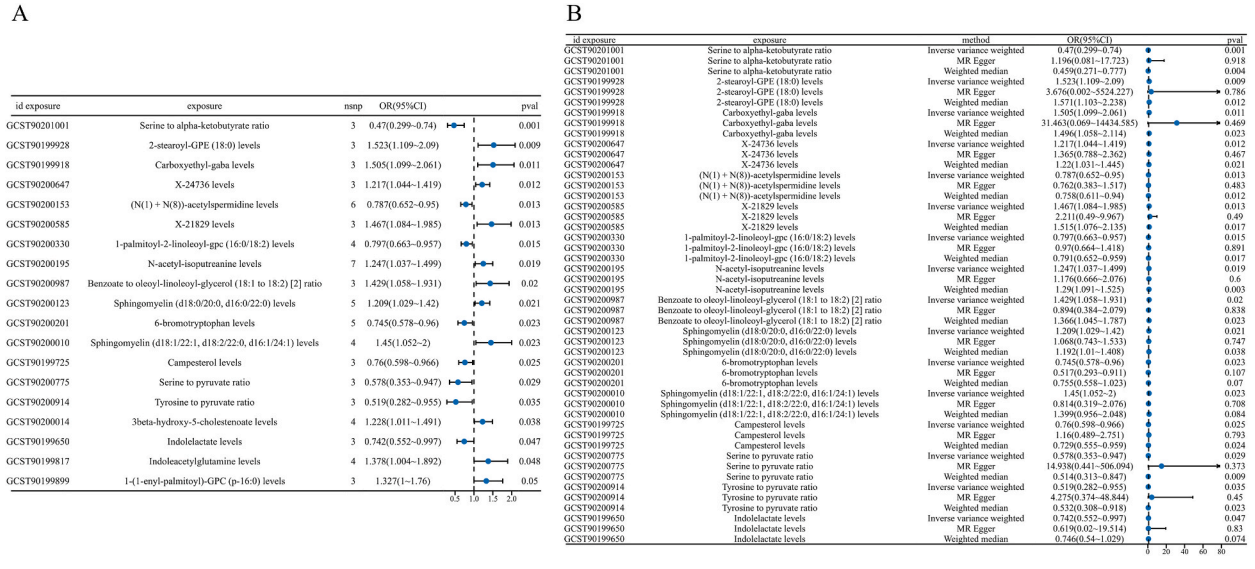

Fig. 3. Forest Plot Illustrating the Causal Relationship Between Serum Metabolites and Hepatic cancer (A: Forest plot using the IVW method in MR analysis; B: Forest plot using the IVW, Weighted median, and MR-Egger methods in MR analysis).

4.3. Horizontal pleiotropy testing

MR-Egger regression was employed for intercept tests to evaluate horizontal pleiotropy. The results showed that CD20 on IgD + CD38dim B cell in the immune cell group and Stem cell factor levels (GCST90274833) in the inflammation factors exhibited $P < 0.047$, indicating horizontal pleiotropy and were subsequently excluded. For the remaining immune cells, inflammatory factors, and serum metabolites in MR analysis showed $P > 0.05$, suggesting minimal influence of genetic polymorphism on the MR results (Supplementary Tables 7–9).

5. Discussion

Based on a substantial amount of publicly available genetic data, we investigated the causal relationships among 731 immune cell traits, 91 inflammatory factors, and 1400 serum metabolites with hepatic cancer. We identified causal relationships between certain immune cells, inflammatory factors, and serum metabolites with hepatic cancer. Additionally, we discovered that 31 immune cells, 6 inflammatory factors, and 19 serum metabolites may serve as potential risk factors for hepatic cancer. These findings could potentially have an impact on the implementation of public health interventions aimed at reducing the risk of hepatic cancer.

In the study of the causal relationship between immune cells and hepatic cancer, we identified 31 immune cells, including Terminally Differentiated $CD4^{-}CD8^{-}$ T cell %T cell, Effector Memory $CD4^{-}CD8^{-}$ T cell %$CD4^{-}CD8^{-}$ T cell, and $CD28^{-}$ $CD8^{+}$ T cell % $CD8^{+}$ T cell, that exhibited causal relationships with hepatic cancer. An increasing number of studies have revealed that immune cells such as T lymphocytes, B lymphocytes, natural killer (NK) cells, and myeloid cells may play crucial roles in the initiation, progression, and response to treatment of tumors. Their manifestations include either inhibiting or activating the progression of tumors. Immunocytes prevent tumor formation and growth by recognizing and eliminating cancer cells, with CD4 T cells, CD8 T cells, NK cells, and other innate immune cells demonstrated to inhibit tumor growth. Tconv cells can exert cytotoxic functions to kill tumor cells or secrete tumor necrosis factor (TNF) to inhibit tumor growth [15]. However, when the complex immune balance is disrupted, inhibitory immune cells in the microenvironment assist cancer cells in immune evasion, promoting tumor initiation and progression. When immune cells are continually exposed to tumors, the factors they produce contribute to various aspects of carcinogenesis, including directly promoting tumor growth and angiogenesis, recruiting inflammatory cells and cytokines, as well as facilitating tumor metastasis, etc. T effector (T^{+}EFF) cells have long been considered a crucial mediator of tumor protection [16]. Additionally, immune cells can regulate inflammatory factors to promote tumor growth. In non-Hodgkin B-cell lymphoma, B lymphocytes produce IL-10, inhibiting the phagocytic/effector activity of macrophages to increase tumor growth [17]. Many studies have suggested that the distribution and functional status of immune cells in hepatic cancer can significantly influence the clinical outcomes of tumors. For instance, tumor-infiltrating lymphocytes in hepatic cancer can help predict tumor prognosis [18]. Regulatory T-cell have been shown to increase in the peripheral blood of hepatocellular carcinoma (HCC) patients and infiltrate into the tumor, serving as an independent prognostic factor associated with shorter survival and poorer prognosis for HCC. Tumor-associated macrophages (TAMs), predominantly M2-type macrophages in the HCC microenvironment, are believed to promote tumor initiation, progression, and metastasis. Meanwhile, TAMs can produce IL-6, exacerbating the immunosuppressive environment, and the upregulation of IL-6 expression can further increase the content of IL-10 in myeloid-derived suppressor cells (MDSCs). This positive feedback loop between TAMs and MDSCs promotes the immunosuppressive effects in HCC [19]. NK cells have a clear relationship with the development of hepatic cancer and can inhibit its progression. Studies speculate that high activity of NK cells in patients with hepatic cancer is advantageous for reducing the risk of postoperative recurrence and obtaining a better prognosis. In recent years, immunotherapy has made significant progress in the treatment of advanced HCC. The combined application of various treatment techniques and cellular immunotherapy has achieved favorable treatment outcomes.

In the study on the causal relationship between inflammatory factors and hepatic cancer, we identified a causal relationship between hepatic cancer and six inflammatory factors, including C–C motif chemokine 19 levels, Fms-related tyrosine kinase 3 ligand levels, and Monocyte chemoattractant protein-3 levels, etc. Inflammatory factors, produced by activated inflammatory cells, constitute a class of biologically active small-molecule proteins or peptides. They are typically secreted into the bloodstream and play a role in regulating the body's immune system response and maintaining physiological balance. The most common inflammatory factors include interleukins (IL), tumor necrosis factor (TNF), and interferons (IFN), among others. Recent studies have indicated that inflammatory factors play a crucial role in the occurrence, development, invasion, and metastasis of tumors, considered essential biological characteristics of malignant tumors. Approximately 15 %–20 % of malignancies are attributed to infections and uncontrollable inflammation. For instance, inflammatory bowel disease is associated with colorectal cancer, HBV infection is linked to liver cancer, H. pylori infection is significantly correlated with gastric cancer, Epstein-Barr virus infection causes nasopharyngeal cancer, and HPV virus infection leads to cervical cancer. The inflammatory environment is closely related to the tumor microenvironment. In the tumor microenvironment, numerous inflammatory factors, such as IL-1, IL-6, IL-12, IL-17, TNF-α, and TGF-β, are present. These factors not only recruit inflammatory cells to the tumor site, amplifying the inflammatory effects, but also stimulate tumor cell growth and metastasis, promoting the formation of tumor blood vessels and lymphatic vessels. Specifically, IL-6 can induce the expression of microRNA-21 in a STAT3 signaling pathway-dependent manner, thereby promoting cell proliferation and inhibiting apoptosis. Inflammatory factors such as TNF-alpha can activate transcription factors in precancerous cells, including NF-kB signaling, Signal Transducer and Activator of Transcription 3 (STAT3), and Activator Protein-1 (AP-1), further regulating the growth and migration of tumor cells, playing a crucial role in the initiation and progression of tumors. In addition, inflammatory factors can induce carcinogenesis or mutations in tumor suppressor genes. Prolonged inflammatory states expose cells to an oxidative stress environment, increasing the likelihood of DNA mutations and consequently elevating the risk of tumor development. TNF-α, IFN-β, and other factors

can stimulate the expression of microRNA-155, thereby inducing tumor formation. Inflammatory factors are also potential tumor markers. Researchers have developed a tool to assess the inflammatory burden in cancer patients called the Inflammatory Burden Index (IBI). The IBI, a combination of C-reactive protein, neutrophils, and lymphocytes, has been experimentally validated as a potential biomarker for predicting the prognosis of cancer patients [20]. The crucial role of inflammatory factors in cancer makes them potential targets for tumor therapy. Currently, there are promising drugs targeting inflammatory factors or their signaling pathways in cancer treatment. For instance, IL-1 inhibitors and IL-6 ligand-blocking antibodies have shown promising efficacy in clinical trials [21]. IL-1 inhibitors are being explored for the treatment of multiple myeloma and melanoma, while IL-6 ligand-blocking antibodies are in clinical trials for multiple myeloma, prostate cancer, and renal cell carcinoma. Inflammatory factors also play a significant role in the development of hepatic cancer. A meta-analysis study suggests that the TNF-α G-308A polymorphism increases the risk of hepatic cancer in Asians [22]. TNF-α and IL-6 contribute to obesity-mediated hepatic cancer development by promoting the development of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) [23]. TNF-α can induce AIF-1+ CSF1R + MSCs to create an inflammatory microenvironment and promote hepatic cancer development [24]. On the other hand, some inflammatory factors have tumor-suppressive functions. For example, IFN-α and IFN-β can inhibit tumor angiogenesis, preventing tumor growth and spread [25]. They can also enhance immune cell cytotoxicity against tumors, thereby inhibiting tumor progression. Studies, such as the work by Yanmeizhi Wu et al. [26], have summarized the research progress on immune checkpoint inhibitors regulating inflammatory factors to control anti-cancer responses. This highlights the functional role of inflammatory factors in immune checkpoint pathways like programmed cell death-1 (PD-1), participating in the regulation of programmed cell death ligand-1 (PD-L1) expression to activate immune responses against tumor progression.

In the research on the causal relationship between serum metabolites and hepatic cancer, we found that 19 serum metabolites, including (N(1) + N(8))-acetylspermidine levels, 1-palmitoyl-2-linoleoyl-gpc (16:0/18:2) levels, and 1-(1-enyl-palmitoyl)-GPC (p-16:0) levels, are causally related to hepatic cancer. Serum metabolites are small molecules produced in normal physiological and pathological processes, including proteins, carbohydrates, lipids, etc. Their concentrations reflect various biochemical processes occurring in the organism. When the body experiences disease, this normal metabolic balance is disrupted. The formation of tumors is often associated with an imbalance in various substance metabolisms in the body. The rapid growth of tumors and the consumption of large amounts of energy lead to a decrease in specific metabolites in the serum. Additionally, metabolic products released during tumor cell death may differ from those released during normal cell death, and these differences may be reflected in the serum. Previous studies have suggested that serum metabolites may be both a potential cause of tumor occurrence and markers for early detection, providing possibilities for screening, prevention, and early diagnosis of tumors. One category of serum metabolites has been widely used in cancer diagnosis and treatment. For example, the elevation of certain tumor markers may indicate the presence of tumors in the body. Although these tumor markers have high specificity, their sensitivity is relatively low, limiting their application in early cancer screening and diagnosis. Jesus M. Banales et al. [27] found that changes in the concentrations of specific metabolites in the serum can help distinguish intrahepatic cholangiocarcinoma from HCC or primary sclerosing cholangitis, aiding in the early diagnosis of these diseases. In breast cancer, changes in ER/PR and Her-2/neu are used to predict disease progression. In recent years, increasing evidence suggests a close association between serum metabolites and hepatic cancer. Some metabolites have been found to be related to the occurrence and metastasis of hepatic cancer. For instance, abnormal levels of acetylated tyrosine, glutamine, and lipid metabolism products in the serum of hepatic cancer patients have been observed, and these changes may be closely related to the growth and metastasis of tumors [3]. Furthermore, serum metabolites can also be used for monitoring hepatic cancer treatment and assessing prognosis, which is of great significance for formulating individualized treatment plans and improving treatment effectiveness. Ana P. Gomes [28] and the research team found that the serum metabolite methylmalonic acid (MMA) can induce the expression of SOX4, causing transcriptional reprogramming and making hepatic cancer cells more aggressive, indicating that MMA is a promising late-stage hepatic cancer treatment target. Jinkai Liu [29], using a comprehensive metabolomics approach, discovered that metabolites in the portal vein serum of HCC patients, such as DL-3-phenyllactic acid, L-tryptophan, glycocholic acid, and 1-methylnicotinamide, are associated with impaired liver function and poorer survival. Linoleic acid and phenol in the portal vein serum of HCC patients significantly inhibit the proliferation of hepatic cancer cells. It is speculated that these substances act as protective metabolites against hepatic cancer.

This study conducted a two-sample MR analysis based on published results from a large-scale GWAS cohort. Several notable advantages were evident in this research. Firstly, the study relied on genetic instrumental variables and employed various MR analysis techniques, minimizing the impact of confounding factors and reverse causation as much as possible. Secondly, compared to prospective cohort studies or randomized controlled trials, MR analysis is more accessible in acquiring public data, thereby reducing the time and cost required for the research. Thirdly, having a large sample size of European individuals contributes to higher statistical efficiency. Nevertheless, our study has certain limitations. Firstly, despite conducting multiple sensitivity analyses, it is challenging to fully assess horizontal pleiotropy. Secondly, since the study is based on a European database and lacks a validation cohort, the conclusions may not be generalized to other ethnic groups, and further research is needed to confirm its applicability to other populations. Thirdly, in order to incorporate more inflammatory factors into the MR analysis, we adopted a more lenient threshold for assessing results, which may introduce some false positives but allows for a more comprehensive evaluation of the strong associations between inflammatory factors and hepatic cancer. Finally, GWAS cannot directly provide information on gene expression, protein function, or metabolic pathway changes. However, integrating GWAS data with genetics and proteomics can offer a more comprehensive understanding of the biological pathways involved in disease development and progression.

6. Conclusion

In summary, we conducted a two-sample MR analysis to assess the causal relationships between 731 immune cell, 91 inflammatory factors, 1400 serum metabolites, and hepatic cancer. Our study results indicate robust associations between various immune cells, inflammatory factors, and serum metabolites with hepatic cancer. This offers new insights into the complex relationships between immune cells, inflammatory factors, serum metabolites, and hepatic, providing potential strategies for disease prevention and treatment. The findings contribute to a fresh perspective and methodological approach in exploring the etiology of hepatic cancer, but further experimental and clinical observations are needed to validate these associations.

Provide a data availability statement

The datasets generated and analyzed during the current study are available in the catalog database (https://www.ebi.ac.uk/gwas/publications/37563310).

Ethics approval

Review and approval by an ethics committee was not needed for this study because the data were from public databases.

CRediT authorship contribution statement

Hongyao Chen: Writing – original draft, Software, Data curation, Conceptualization. **Renyi Yang:** Writing – original draft, Software, Data curation. **Jingting Zhang:** Writing – original draft, Data curation. **Jincheng Tang:** Writing – original draft, Data curation. **Xiaopeng Yu:** Methodology, Data curation. **Wanshuang Zhou:** Visualization, Data curation. **Kexiong Li:** Writing – review & editing. **Wei Peng:** Writing – review & editing. **Puhua Zeng:** Writing – review & editing.

Declaration of competing interest

The authors unequivocally state that they possess no known competing financial interests or personal relationships that could have conceivably influenced the integrity or outcomes of the work presented in this paper.

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Appendix A. Supplementary data

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References

[1] T. Ahrends, J. Borst, The opposing roles of CD4+ T cells in anti-tumour immunity, Immunology 154 (2018) 582–592.
[2] S. Inoue, W.W. Leitner, B. Golding, D. Scott, Inhibitory effects of B cells on antitumor immunity, Cancer Res. 66 (2006) 7741–7747.
[3] D. Du, et al., Metabolic dysregulation and emerging therapeutical targets for hepatocellular carcinoma, Acta Pharm Sin B 12 (2022) 558–580.
[4] V. Orrù, et al., Complex genetic signatures in immune cells underlie autoimmunity and inform therapy, Nat. Genet. 52 (2020) 1036–1045.
[5] J.H. Zhao, et al., Genetics of circulating inflammatory proteins identifies drivers of immune-mediated disease risk and therapeutic targets, Nat. Immunol. 24 (2023) 1540–1551.
[6] Y. Chen, et al., Genomic atlas of the plasma metabolome prioritizes metabolites implicated in human diseases, Nat. Genet. 55 (2023) 44–53.
[7] R. Xu, T. Zheng, C. Ouyang, X. Ding, C. Ge, Causal associations between site-specific cancer and diabetes risk: a two-sample Mendelian randomization study, Front. Endocrinol. 14 (2023) 1110523.
[8] O.O. Yavorska, S. Burgess, MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data, Int. J. Epidemiol. 46 (2017) 1734–1739.

[9] S. Burgess, A. Butterworth, S.G. Thompson, Mendelian randomization analysis with multiple genetic variants using summarized data, Genet. Epidemiol. 37 (2013) 658–665.
[10] J. Bowden, G. Davey Smith, P.C. Haycock, S. Burgess, Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator, Genet. Epidemiol. 40 (2016) 304–314.
[11] F.D. Greco M, C. Minelli, N.A. Sheehan, J.R. Thompson, Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome, Stat. Med. 34 (2015) 2926–2940.
[12] S. Burgess, J. Bowden, T. Fall, E. Ingelsson, S.G. Thompson, Sensitivity analyses for robust causal inference from mendelian randomization analyses with multiple genetic variants, Epidemiology 28 (2017) 30–42.
[13] J. Bowden, G. Davey Smith, S. Burgess, Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression, Int. J. Epidemiol. 44 (2015) 512–525.
[14] S. Burgess, S.G. Thompson, Interpreting findings from Mendelian randomization using the MR-Egger method, Eur. J. Epidemiol. 32 (2017) 377–389.
[15] H. Braumüller, et al., T-helper-1-cell cytokines drive cancer into senescence, Nature 494 (2013) 361–365.
[16] B.M. Seeley, et al., Potent effector function of tumor-sensitized L-selectin(low) T cells against subcutaneous tumors requires LFA-1 co-stimulation, Otolaryngol. Head Neck Surg. 124 (2001) 436–441.
[17] M. Horikawa, V. Minard-Colin, T. Matsushita, T.F. Tedder, Regulatory B cell production of IL-10 inhibits lymphoma depletion during CD20 immunotherapy in mice, J. Clin. Invest. 121 (2011) 4268–4280.
[18] W. Yao, et al., The prognostic value of tumor-infiltrating lymphocytes in hepatocellular carcinoma: a systematic review and meta-analysis, Sci. Rep. 7 (2017) 7525.
[19] Q. Zhang, et al., Landscape and Dynamics of Single Immune Cells in Hepatocellular Carcinoma, Cell 179 (2019) 829–845.e20.
[20] R. Song, et al., Prognostic value of inflammation-immunity-nutrition score and inflammatory burden Index for hepatocellular carcinoma patients after hepatectomy, JIR 15 (2022) 6463–6479.
[21] K.A. Donovan, L.L. Moon-Tasson, J.A. Lust, Interplay between IL-1, IL-6 and IL-17 in IL-1 receptor antagonist (IL-1Ra) treated multiple myeloma patients, Blood 120 (2012), 1874–1874.
[22] H.K. Verma, N. Merchant, L.V.K.S. Bhaskar, Tumor necrosis factor-alpha gene promoter (TNF-α G-308a) polymorphisms increase the risk of hepatocellular carcinoma in Asians: a meta-analysis, Crit. Rev. Oncog. 25 (2020) 11–20.
[23] N. Damavandi, S. Zeinali, Association of xenobiotic-metabolizing enzymes (GSTM1 and GSTT 1), and pro-inflammatory cytokines (TNF-α and IL-6) genetic polymorphisms with non-alcoholic fatty liver disease, Mol. Biol. Rep. 48 (2021) 1225–1231.
[24] C. Zong, et al., AIF1 + CSF1R + MSCs, induced by TNF-α, act to generate an inflammatory microenvironment and promote hepatocarcinogenesis, Hepatology 78 (2023) 434–451.
[25] X. Chen, K. Wang, Mesenchymal Stem cells carrying IFN-α and IFN-β overexpression genes inhibit non-small cell lung cancer via the JAK/STAT pathway, J. Evol. Biochem. Physiol. 59 (2023) 1136–1149.
[26] Y. Wu, S. Yu, H. Qiao, Understanding the functional inflammatory factors involved in therapeutic response to immune checkpoint inhibitors for pan-cancer, Front. Pharmacol. 13 (2022) 990445.
[27] J.M. Banales, et al., Serum metabolites as diagnostic biomarkers for cholangiocarcinoma, hepatocellular carcinoma, and primary sclerosing cholangitis, Hepatology 70 (2019) 547–562.
[28] A.P. Gomes, et al., Age-induced accumulation of methylmalonic acid promotes tumour progression, Nature 585 (2020) 283–287.
[29] J. Liu, et al., Integrative metabolomic characterisation identifies altered portal vein serum metabolome contributing to human hepatocellular carcinoma, Gut 71 (2022) 1203–1213.