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Analysis

Integrating Mendelian randomization and multi-transcriptomic analyses to unveil the genetic association risk of regulatory T cell-mediated free cholesterol and gastric cancer

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Abstract

Background Evidence from observational studies suggests an association between free cholesterol and gastric cancer. Immune cells play a crucial role in the tumor microenvironment of gastric cancer, and free cholesterol can influence immune cells in various ways, thereby impacting gastric cancer. The mechanisms by which free cholesterol regulates and activates the immune response to exert antitumor effects, as well as the causal relationship between free cholesterol and gastric cancer, remain unclear.

Methods We employed a two-sample Mendelian randomization (MR) approach to investigate the causal relationship between 233 metabolites and gastric cancer. Additionally, we validated our findings using data from GWAS databases of similar traits. Using publicly available genetic data, we analyzed the causal relationship between 731 types of immune cells and gastric cancer. Furthermore, we explored the mediating role of regulatory T cells in the causal relationship between free cholesterol and gastric cancer through multivariable Mendelian randomization. Finally, we validated our results using data from the TCGA database and single-cell sequencing data.

Findings We found a causal relationship between free cholesterol levels and gastric cancer (odds ratio [*OR*] = 0.89, confidence interval [CI] = 0.81–0.98, P < 0.05). We also observed a causal relationship between free cholesterol levels and regulatory T cells (odds ratio [*OR*] = 0.86, confidence interval [CI] = 0.75–0.98, P < 0.05), and between regulatory T cells and gastric cancer (odds ratio [*OR*] = 1.04, confidence interval [CI] = 1.01–1.07, P < 0.05). Additionally, our multivariable Mendelian randomization analysis indicated that regulatory T cells mediate the causal relationship between free cholesterol levels and gastric cancer. Furthermore, through single-cell sequencing analysis and data analysis from the TCGA database, we found that the expression of the free cholesterol uptake protein LDLR is negatively correlated with Treg infiltration, which further influences the occurrence and development of gastric cancer.

Interpretation Our analysis indicates a causal relationship between free cholesterol levels and gastric cancer, with regulatory T cells acting as mediators. Modulating free cholesterol levels to influence regulatory T cells may offer new insights and prospects for the prevention and treatment of gastric cancer.

Keywords ScRNA-seq · Mendelian randomization · Gastric cancer · Regulatory T cells · Free cholesterol

Fanyu Peng and Haitao Liu have contributed equally to this work.

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1 Introduction

Gastric cancer is the fifth most common cancer worldwide and the third leading cause of cancer-related deaths. Risk factors for the condition include Helicobacter pylori infection, age, high salt intake, and a diet low in fruits and vegetables [1]. Less than a century ago, gastric cancer was the most common cancer in the United States and worldwide [2]. Although its global incidence has decreased over the past century, gastric cancer remains a major killer globally [3]. Gastric cancer remains one of the leading causes of cancer-related mortality worldwide, with complex etiological factors including genetic, environmental, and metabolic components [4, 5]. Metabolic dysregulation, particularly in lipid metabolism, has been implicated in the pathogenesis of various cancers, including gastric cancer [6]. Emerging evidence suggests that cholesterol metabolism, specifically, plays a significant role in cancer development and progression [7]. Cholesterol, a fundamental component of cell membranes, is involved in various cellular processes such as signaling, growth, and differentiation [8]. Dysregulated cholesterol levels have been linked to carcinogenesis through mechanisms that involve alterations in cell membrane composition, signaling pathways, and immune cell modulation.

Recent studies have highlighted the potential causal relationship between specific cholesterol traits and gastric cancer [9]. Mendelian randomization (MR) studies, which utilize genetic variants as instrumental variables to infer causality, provide a robust framework for examining these associations while minimizing confounding and reverse causation [10]. Utilizing this approach, our study aims to elucidate the causal relationships between cholesterol traits, immune cell phenotypes, and gastric cancer.

Immune cells, particularly regulatory T cells (Tregs), play a crucial role in maintaining immune homeostasis and preventing autoimmunity [11]. However, in the context of cancer, Tregs can contribute to immune evasion by suppressing anti-tumor immune responses. The interplay between lipid metabolism and immune regulation is an emerging area of interest, with studies suggesting that cholesterol levels can influence the function and infiltration of immune cells, including Tregs, in the tumor microenvironment [12].

This study utilizes MR to assess the causal relationships between 233 metabolites and gastric cancer, with a particular focus on cholesterol traits. We employed the inverse-variance weighted (IVW) method to identify significant associations and validated our findings using various datasets. Horizontal pleiotropy testing and Leave-One-Out (LOO) analysis was conducted to ensure the robustness of our results. Additionally, we investigated the causal relationship between immune cell phenotypes, particularly Tregs, and gastric cancer. Furthermore, we explored the potential mediating role of Tregs in the relationship between free cholesterol and gastric cancer. Finally, we performed transcriptome sequencing and deconvolution analysis to examine the correlation between cholesterol uptake-related protein expression and Treg infiltration in gastric cancer tissue.

This comprehensive approach aims to elucidate the complex interactions between cholesterol metabolism, immune regulation, and gastric cancer, providing novel insights into the pathogenesis of this malignancy and identifying potential targets for therapeutic intervention.

2 Methods

2.1 Data sources

The summary statistics for each immunophenotype can be publicly accessed from the GWAS Catalog (Accession numbers from GCST90001391 to GCST90002121).

The GWAS data for 233 metabolites were sourced from previously published literature [13]. To validate the results, we used GWAS data for cholesterol traits with similar characteristics, registered in the GWAS Catalog under Accession numbers GCST90025953, GCST90092988, and met-d-L_LDL_FC. The GWAS data for gastric cancer traits were registered under Accession number GCST90018849 in the GWAS Catalog.

2.2 Two-sample Mendelian randomization analysis

We employed two-sample MR to estimate the causal effects of metabolites on gastric cancer and regulatory T cells. The two-sample MR approach involves the following steps.

Selection of instrumental variables (IVs): Single nucleotide polymorphisms (SNPs) associated with free cholesterol levels at genome-wide significance ($P < 1 \times 10^{-5}$) were selected as IVs. These SNPs were pruned for linkage disequilibrium (LD) using a threshold of $r^2 < 0.01$ to ensure independence.

Effect estimates: The genetic associations (beta coefficients and standard errors) of these IVs with gastric cancer and regulatory T cells were obtained from their respective GWAS datasets.

MR analysis: The causal effects were estimated using the inverse-variance weighted (IVW) method, which provides a combined estimate of the causal effect from multiple SNPs, accounting for their precision. Sensitivity analyses using MR-Egger regression and weighted median methods were also performed to assess the robustness of the results.

2.3 Multivariable Mendelian randomization analysis

To explore the potential mediating role of regulatory T cells in the relationship between free cholesterol levels and gastric cancer, we conducted multivariable MR (MVMR) analysis [14]. MVMR allows for the estimation of direct and indirect effects by including multiple exposures in the model.

Selection of IVs for MVMR: SNPs associated with both free cholesterol levels and regulatory T cells were selected. These SNPs were again pruned for LD to ensure independence.

MVMR analysis: The direct effect of free cholesterol levels on gastric cancer, adjusting for regulatory T cells, was estimated. Similarly, the indirect effect mediated through regulatory T cells was quantified. The analysis was performed using the IVW method extended for multiple exposures.

All statistical analyses were conducted using R (version 4.3.3) with the packages TwoSampleMR (version 0.5.1).

2.4 scRNA-seq data

The scRNA-seq data of human lung cancer patients were downloaded from the GEO database with the Accession number: GSE131907 and GSE173896. We utilized the Seurat R package to identify distinct cell types and examine variations in immune cell infiltration. Cells that met specific criteria, such as having fewer than 200 genes, over 5000 genes, or more than 20% mitochondrial expression, were excluded from the analysis.

2.5 TCGA data

We obtained gene expression data from patients with gastric cancer (STAD) from The Cancer Genome Atlas (TCGA) database.

To estimate the abundance of regulatory T cells within the tumor microenvironment, we employed the CIBERSORT algorithm:

CIBERSORT analysis: Gene expression profiles were input into the CIBERSORT algorithm to infer the composition of immune cell subtypes, including regulatory T cells (Tregs).

Statistical analysis: We conducted statistical analyses to assess the association between Treg abundance and clinical parameters, such as tumor stage and patient survival outcomes.

2.5.1 Input data preparation

Matrix construction: Gene expression matrix derived from TCGA STAD dataset.

Reference signature matrix: Provided by the CIBERSORT algorithm, representing gene expression profiles of immune cell subtypes.

2.5.2 Algorithm execution

Execution: CIBERSORT was executed using default settings and LM22 signature matrix for deconvolution of Treg and other immune cell proportions.

Output Interpretation: Generated results were interpreted to quantify Treg infiltration levels and compare across different patient groups.

2.5.3 Statistical validation

Correlation analysis: Correlation between Treg abundance and clinical variables (e.g., tumor stage, survival outcomes) was assessed using appropriate statistical tests.

Visualization: Results were visualized using graphs and plots to illustrate associations and differences in Treg infiltration based on clinical parameters.

This methodology allowed for the comprehensive assessment of regulatory T cell involvement in gastric cancer using computational deconvolution techniques applied to large-scale genomic datasets.

3 Results

3.1 Discussion on the causal relationship between metabolites and gastric cancer

The flow chart about the findings of the study was displayed in Fig. 1**.** We assessed whether 233 metabolites, derived from recently published literature, exhibit a causal relationship with gastric cancer. Primarily employing the IVW method, our results indicated that three cholesterol traits are causally associated with gastric cancer (Fig. 2 and Table S1). To further confirm our findings, we validated them using data from other studies with similar traits. Despite the change in datasets, the causal relationship between the three cholesterol traits and gastric cancer persisted (Fig. 3 and Table S2). We validated the reliability of our results through horizontal pleiotropy testing and Leave-One-Out (LOO) analysis. Horizontal pleiotropy testing using the MR-Egger method yielded p-values for the MR-Egger regression intercept greater than 0.05, indicating no evidence of horizontal pleiotropy for the three cholesterol traits with respect to gastric cancer **(**Table S3). LOO analysis demonstrated consistent trends across all included SNPs in our analysis (Figure S1). Subsequently, we employed different methods to assess whether the results for the three cholesterol traits and gastric cancer exhibit consistent directions (Fig. 4). The results revealed consistent directional associations between free cholesterol levels and gastric cancer across five methods. However, Total cholesterol levels and the association of Total cholesterol levels with gastric cancer exhibited inconsistent results when using the Simple mode method, suggesting potential instability in these results. Furthermore, we conducted reverse Mendelian randomization analysis with gastric cancer as the exposure and Free cholesterol levels as the outcome, which indicated no causal relationship between the two (Table S4). In conclusion, our findings suggest a causal relationship between free cholesterol levels and gastric cancer.

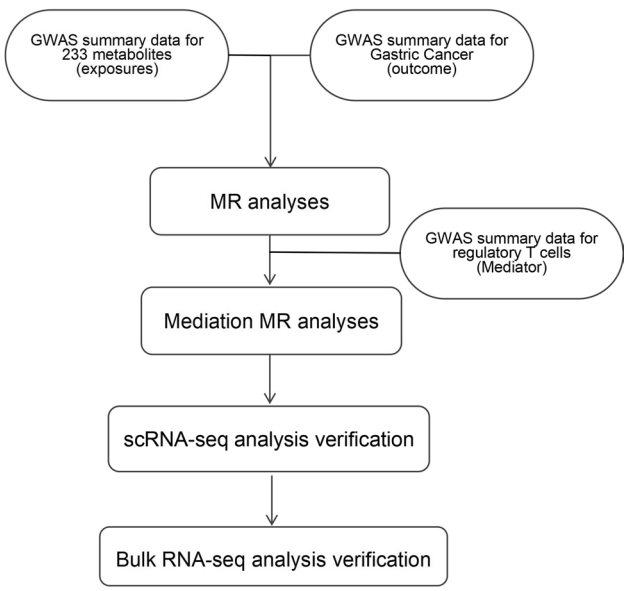


Fig. 1 Study design flowchart

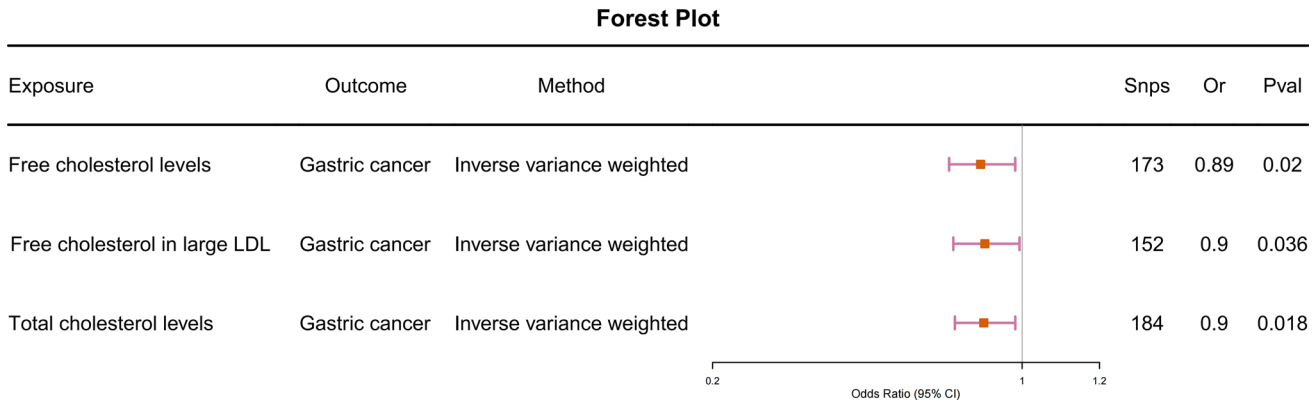


Fig. 2 Using IVW methods to analyze the causal association between cholesterol and gastric cancer

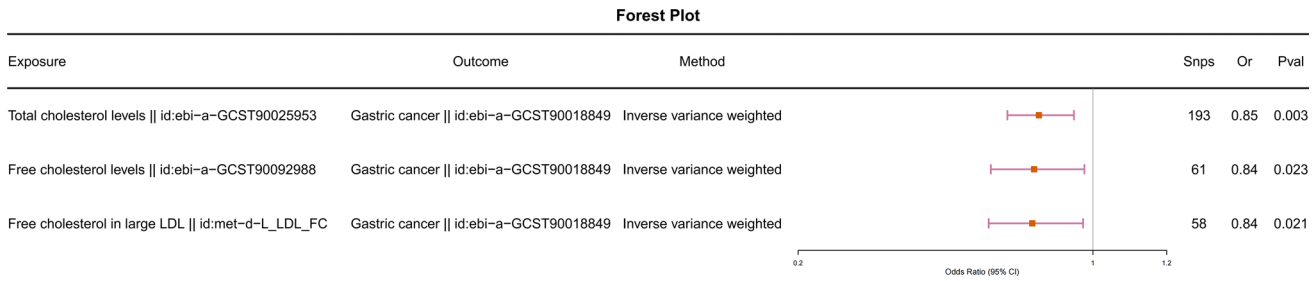


Fig. 3 Using IVW methods to analyze the causal association between cholesterol and gastric cancer using different datasets

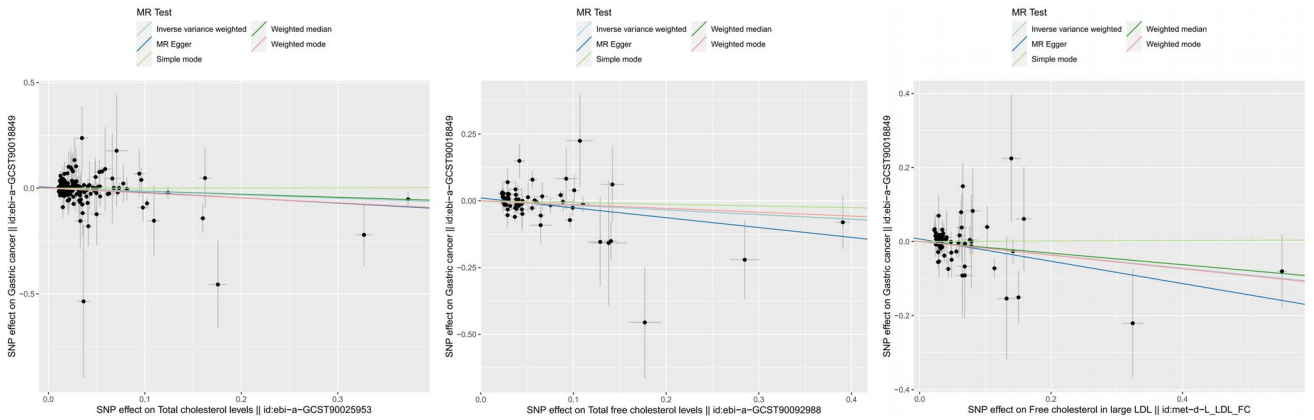


Fig. 4 Scatter plot illustrating the robustness of the causal relationship between cholesterol and gastric cancer analyzed using multiple methods

3.2 Discussion on the causal relationship between immune phenotypes and gastric cancer

To investigate the causal relationship between immune cells and gastric cancer, we conducted a Mendelian randomization study using 731 immune cell phenotypes as exposures and gastric cancer as the outcome. The results revealed that 61 immune cell phenotypes may be causally associated with gastric cancer (p-value < 0.05) (Table S5). Among them, the Secreting CD4 regulatory T cell %CD4 regulatory T cell (Treg) phenotype was found to be causally associated with gastric cancer (odds ratio [*OR*] = 1.03, 95% confidence interval [CI] = 1.01–1.07, P-value < 0.05) **(**Fig. 5**)**. Horizontal pleiotropy testing and Leave-One-Out (LOO) analysis validated the robustness of our results (Figure S2 and Table S6). Scatter plots further supported our results across multiple methods (Figure S3). Reverse Mendelian randomization still supports our results (Table S7). These findings collectively indicate a causal relationship between Treg and gastric cancer.

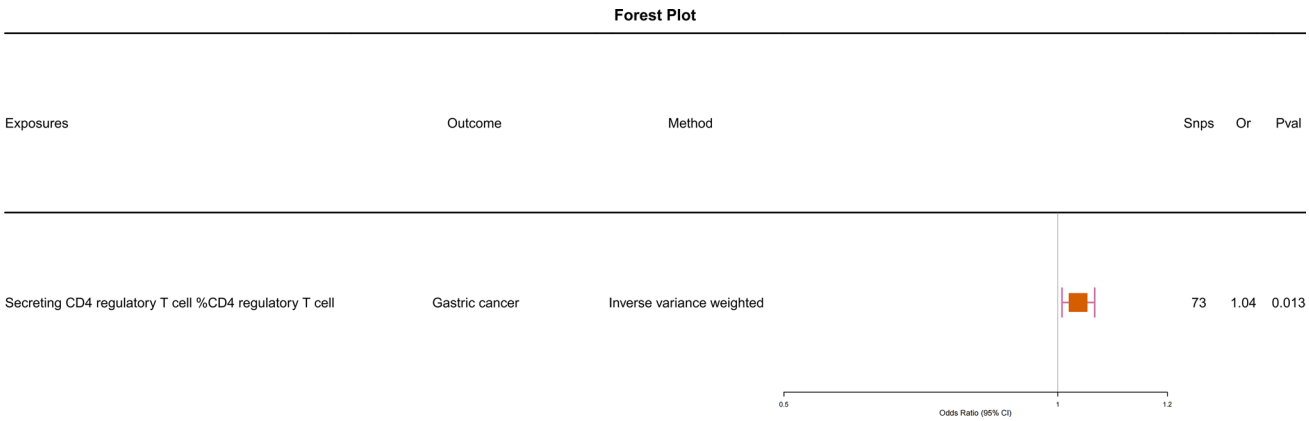


Fig. 5 Using IVW methods to analyze the causal association between regulatory T cells (Tregs) and gastric cancer

3.3 Discussion on the causal relationship between free cholesterol and regulatory T cells

To investigate the causal relationship between free cholesterol and regulatory T cells, we conducted a Mendelian randomization study, with Free cholesterol levels as the exposure and regulatory T cell phenotypes as the outcome. The results revealed a causal relationship between Free cholesterol levels and regulatory T cells (CD4 + T cell, odds ratio [*OR*] = 0.86, 95% confidence interval [CI] = 0.76–0.98, P-value < 0.05) (Fig. 6 and Table S8). Horizontal pleiotropy testing and Leave-One-Out (LOO) analysis validated the robustness of our results (Figure S4 and Table S9). Scatter plots further supported our results across multiple methods (Figure S5). Reverse Mendelian randomization still supports our results (Table S10). These findings collectively indicate a causal relationship between Free cholesterol levels and regulatory T cells.

3.4 Mediating role of regulatory T cells in the causal relationship between free cholesterol and gastric cancer

The aforementioned results indicate causal relationships between free cholesterol and gastric cancer, between free cholesterol and regulatory T cells, and between regulatory T cells and gastric cancer, suggesting that regulatory T cells may mediate the causal relationship between free cholesterol and gastric cancer. To explore the potential mediating effect, we conducted a multivariable Mendelian randomization analysis with regulatory T cells and free cholesterol as exposures and gastric cancer as the outcome. The results revealed that regulatory T cells and free cholesterol still exhibit causal relationships with gastric cancer (Fig. 7 and Table S11). These findings suggest that regulatory T cells mediate the causal relationship between free cholesterol and gastric cancer.

3.5 Transcriptome sequencing reveals a negative correlation between LDLR expression and treg infiltration

To further investigate the mediating role of Tregs in the causal relationship between free cholesterol and gastric cancer, we conducted a deconvolution analysis using gastric cancer data from the TCGA database. We observed the correlation between genes encoding proteins involved in cellular free cholesterol uptake (LDLR, VLDLR, NPC1, NPC2, LPR1) and

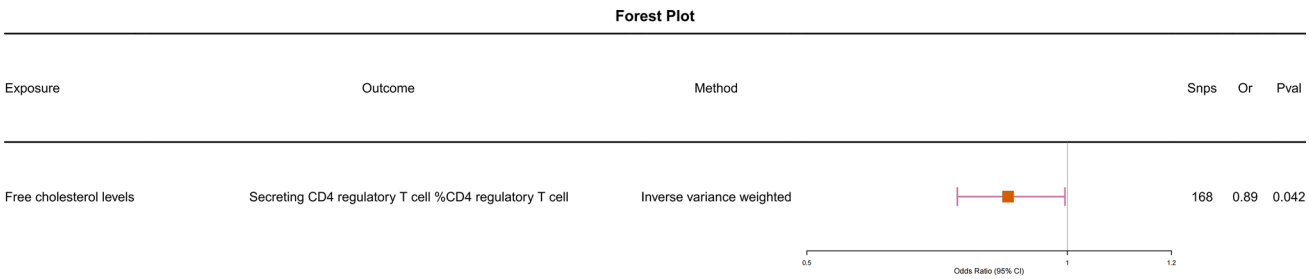


Fig. 6 Using IVW methods to analyze the causal association between free cholesterol levels and regulatory T cells (Tregs)

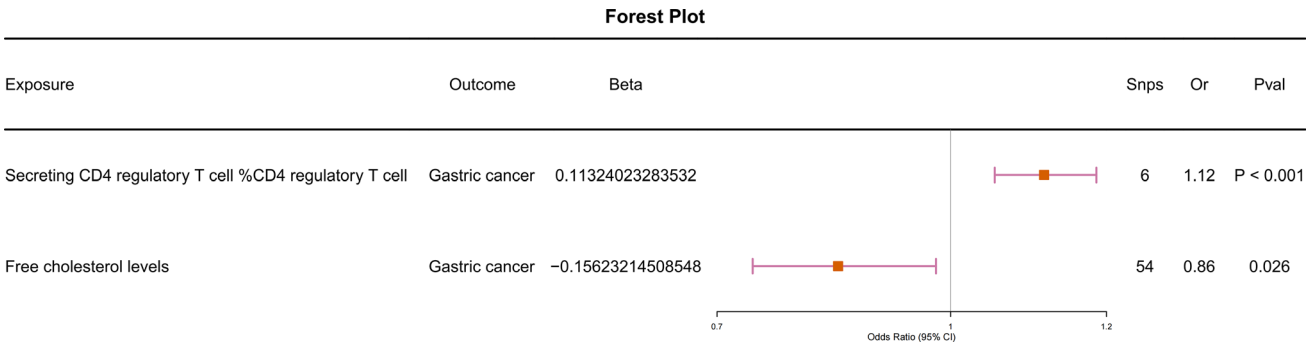


Fig. 7 Multivariable Mendelian randomization analysis of the causal relationship among free cholesterol levels, regulatory T cells (Tregs), and gastric cancer

various cell types. The results showed that the expression levels of LDLR, VLDLR, and LRP1 were negatively correlated with Treg cells (Fig. 8A). High expression levels of cholesterol uptake-related proteins LDLR, VLDLR, and LRP1 may indicate increased free cholesterol uptake and decreased Treg infiltration.

To further confirm the relationship between LDLR, VLDLR, LPR1, and Treg infiltration, we analyzed single-cell data of gastric cancer. To obtain high-quality T cells, we subsetted the single-cell data (CD3D > 0 | CD3E > 0 | CD3G > 0). Further dimensionality reduction and clustering (Fig. 8B), along with cell marker identification (Fig. 8C), revealed several T cell subpopulations, including proliferating T cells with high MKI67 expression (MKI67_T), naïve T cells with high TCF7 expression (Naive_T), effector T cells with high GZMK expression (Teff), regulatory T cells with high FOXP3 expression (Treg), gamma delta T cells with high TRDC expression, Th17 cells with high IL17 A expression, and T cells with high HSPA1 A expression (HSPA1 A_T).

We examined Treg infiltration in normal gastric tissue and tumor tissue and found higher levels of Treg infiltration in tumor tissue (Fig. 8D). We then assessed the expression levels of LDLR, VLDLR, and LRP1 in Tregs and found that LDLR had the highest expression level, while VLDLR and LRP1 were expressed at lower levels (Fig. 8E). Finally, we examined LDLR expression in Tregs in normal gastric tissue and tumor tissue and found that LDLR expression was lower in tumor tissue (Fig. 8F). These findings suggest that LDLR expression is negatively correlated with Treg levels and that high LDLR expression in Tregs is associated with lower infiltration.

4 Discussion

The findings of our study elucidate the complex interplay between metabolites, immune phenotypes, and gastric cancer, emphasizing the causal relationships among free cholesterol levels, regulatory T cells (Tregs), and gastric cancer. Our results suggest that Tregs play a mediating role in the causal relationship between free cholesterol and gastric cancer, supported by comprehensive analyses including Mendelian randomization (MR) and transcriptome sequencing.

Our findings demonstrate that free cholesterol levels act as a protective factor against gastric cancer, consistent with a previous cohort study based on the Korean population. Higher levels of high-density lipoprotein cholesterol (HDL-C) are associated with a lower risk of gastric cancer in the general population, including both males and females. Additionally, among the elderly, subjects with elevated low-density lipoprotein cholesterol (LDL-C) levels have a reduced risk of gastric cancer [15]. Previous studies also indicate that increased HDL-C is associated with lower mortality risk in both men and women [16]. Furthermore, postoperatively, HDL-C may serve as an independent prognostic factor for gastric cancer [17].

To elaborate on the relationship between cholesterol and gastric cancer, it is important to highlight the potential mechanisms by which cholesterol influences tumorigenesis and progression. Cholesterol is a crucial component of cell membranes and is involved in cell signaling, membrane microdomain formation, and the regulation of cell proliferation [18]. Some studies suggest that HDL-C exerts antioxidative and anti-inflammatory effects, which may reduce cancer risk by mitigating oxidative stress and inflammatory responses [19]. Conversely, LDL-C might affect cancer risk through different mechanisms; its protective role in the elderly could be related to its involvement in cellular metabolism and immune regulation [20]. Additionally, dysregulation of cholesterol metabolism may contribute to cancer development by affecting the tumor microenvironment, promoting tumor cell invasion, and metastasis [21].

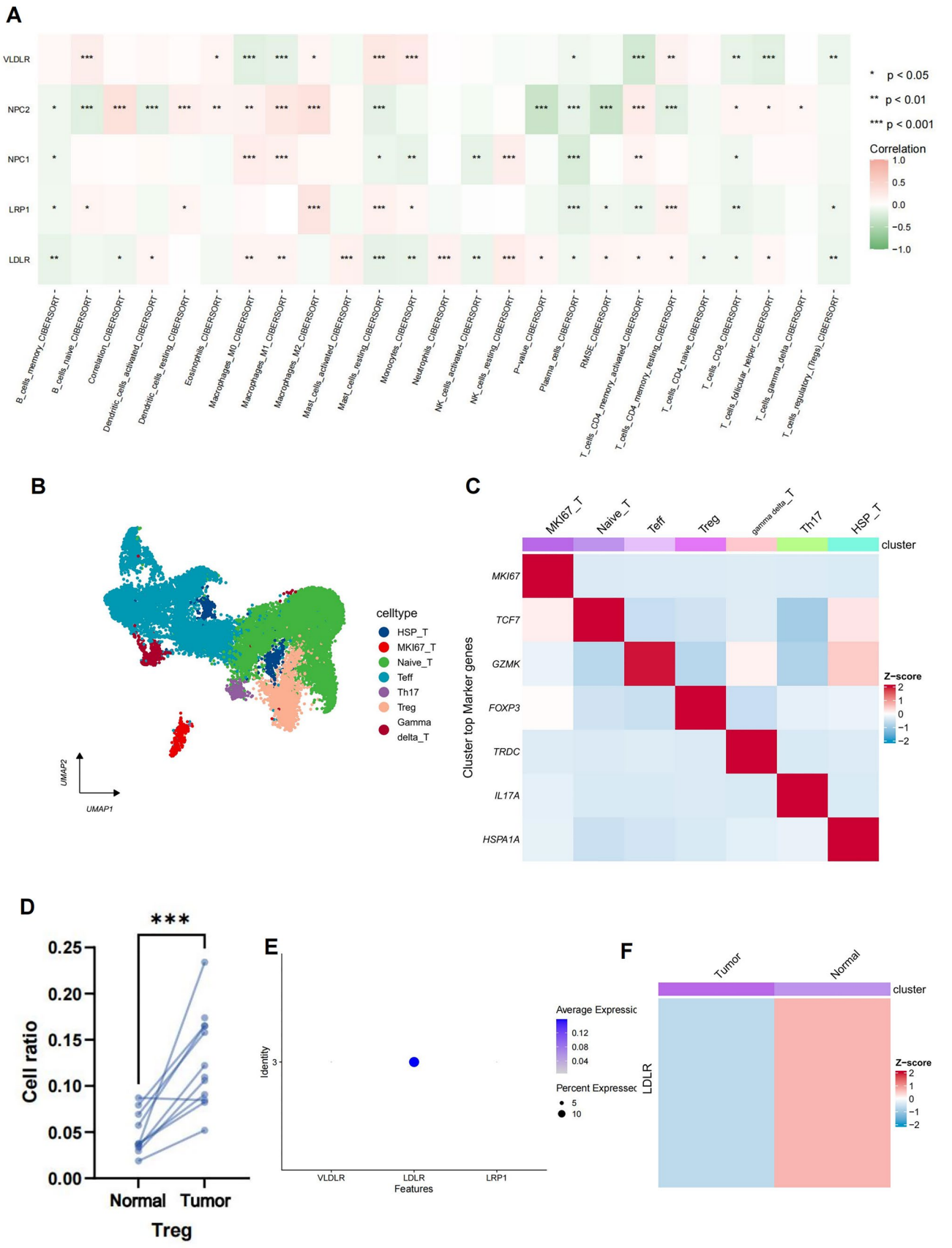
Fig. 8 **A** Deconvolution analysis using the CIBERSORT method applied to the TCGA database, showing the correlation between cell types and genes encoding cholesterol uptake-related proteins. **B** UMAP plot displaying the distribution of cell types in gastric cancer and normal gastric tissues. **C** Heatmap depicting the expression levels of marker genes across different cell types. **D** Proportion of Treg infiltration in gastric cancer and normal gastric tissues. **E** Expression levels of three cholesterol uptake-related protein-coding genes in Tregs. **F** Expression of LDLR in Tregs within gastric cancer and normal gastric tissues

Our results indicate that regulatory T cells (Tregs) are a risk factor for gastric cancer, playing a crucial role in its onset and progression. Tregs, a subset of T lymphocytes, are significantly elevated in gastric cancer patients compared to controls, suggesting their potential role in tumor progression [22]. Studies have shown a positive correlation between Treg cells and CD8 +T cells in gastric cancer, with a marked expression of Tregs in tumor tissues. Patients with high FOXP3 expression have a lower overall survival rate compared to those with low FOXP3 expression, further supporting the importance of Tregs in gastric cancer progression [23]. Additionally, Tregs interact with monocytes in gastric cancer patients, potentially promoting tumor growth and spread by suppressing anti-tumor immune responses [24]. To further elaborate on the relationship between regulatory T cells and gastric cancer, it is important to highlight the role of Tregs in the tumor microenvironment and their potential mechanisms. Tregs suppress host anti-tumor immune responses by expressing inhibitory molecules such as CTLA-4 and PD-1, and by secreting inhibitory cytokines like IL-10 and TGF-β [25]. This suppression hampers the function of effector T cells and natural killer (NK) cells, allowing tumor cells to evade immune surveillance and enhancing their growth and metastasis [26]. Moreover, Tregs can alter the cytokine profile within the tumor microenvironment, promoting tumor cell survival and proliferation. Thus, regulatory T cells play a vital role in immune evasion and tumor progression in gastric cancer, making them a potential therapeutic target [27].

Through multivariable Mendelian randomization analysis, we demonstrated that regulatory T cells (Tregs) mediate the causal relationship between free cholesterol levels and gastric cancer. Additionally, multi-transcriptomic analysis revealed that low-density lipoprotein receptor (LDLR) plays a critical role in this process. Specifically, LDLR expression is negatively correlated with Treg infiltration and shows low expression in Tregs within the tumor microenvironment. These findings are consistent with previous studies. In LDLR-deficient mouse models, the number of immune cells in the lymph nodes decreased, while the proportion of Treg cells significantly increased [28]. As the proportion of Treg cells increased, the percentage of effector and effector memory T cells declined, further supporting the key role of LDLR in regulating the tumor immune microenvironment. As a crucial cholesterol transporter, LDLR not only plays a key role in cholesterol metabolism but may also influence tumor progression by regulating the distribution and function of immune cells. Low LDLR expression might lead to cholesterol metabolism disorders, thereby promoting the accumulation and activation of Treg cells [29]. Treg cells inhibit the activity of effector T cells, weakening the body's anti-tumor immune response, allowing tumor cells to evade immune surveillance, and enhancing their growth and metastatic potential [30]. Therefore, understanding the interaction mechanisms between LDLR and Treg cells in the tumor microenvironment can help uncover new mechanisms of gastric cancer development and provide a theoretical basis for new therapeutic strategies.

This study has several notable limitations. First, while Mendelian randomization analysis and multi-omics data uncovered associations between cholesterol metabolism and immune regulation, the findings were derived from a European population cohort, raising questions about their generalizability to other ethnic groups or geographic regions. Second, although LDLR expression was negatively correlated with Treg infiltration, the precise molecular mechanisms by which LDLR modulates Treg differentiation and function through cholesterol metabolic pathways remain incompletely understood. Third, the reliance on observational data and murine models limits causal inference in human systems, as dynamic in vivo interactions and long-term effects require validation through prospective cohort studies and clinical intervention trials. Additionally, the study did not account for potential interactions between LDLR/Treg pathways and other immune cell subsets (e.g., Th17 cells, macrophages) or metabolic intermediates (e.g., bile acids) within the tumor microenvironment, which may influence the complexity of disease mechanisms. Lastly, environmental factors (e.g., diet, Helicobacter pylori infection) and gene-environment interactions were not fully evaluated, potentially confounding causal relationships. Future research should integrate multi-omics technologies, single-cell sequencing, and large-scale cross-ethnic cohorts to unravel the intricate cholesterol-immune axis in gastric carcinogenesis.

Our study provides compelling evidence for a multifaceted causal relationship between free cholesterol levels, Treg infiltration, and gastric cancer risk. The inverse association between cholesterol uptake proteins (LDLR) and Treg infiltration highlights the complex interplay between metabolic and immune pathways in cancer development. These findings underscore the potential for targeting metabolic pathways to modulate immune responses as a therapeutic strategy in gastric cancer. Further research is needed to elucidate the precise mechanisms underlying these relationships and to explore potential interventions that can disrupt the metabolic-immune axis in cancer.


A
VLDLR
NPC2
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LRP1
LDLR
* p < 0.05
** p < 0.01
*** p < 0.001
Correlation
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−0.5
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B_cells_memory_CIBERSORT
B_cells_naive_CIBERSORT
Correlation_CIBERSORT
Dendritic_cells_activated_CIBERSORT
Dendritic_cells_resting_CIBERSORT
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Macrophages_M0_CIBERSORT
Macrophages_M1_CIBERSORT
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Author contributions Fanyu Peng and Haitao Liu contributed to the conception, design, analysis, draft writing and interpretation of data for the work. Yesong Guo and Jing Wen contributed to the conception, analysis, and interpretation of data for the work. Yanhong Luo and Yizhi Ge contributed to research design, data analysis, results interpretation, paper writing, and critical revision of this manuscript.

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Data availability The information of GWAS catalog databases can be found at the following website: https://gwas.mrcieu.ac.uk/. Further inquiries and the statistical can be directed to the corresponding authors.

Declarations

Ethics approval and consent to participate This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication All authors approved the final version and agreed to be accountable for all aspects of the work.

Competing interests The authors declare no competing interests.

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