Potential causal roles of immune cells and plasma metabolites in esophageal cancer risk: A Mendelian randomization study with nutritional intervention insights

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ABSTRACT

Background and Objectives: Metabolites, as key mediators of nutrition–immune interactions, have attracted increasing interest in cancer research. However, the causal relationships between immune cells, plasma metabolites, and esophageal cancer, and their potential for guiding nutritional interventions remain unclear. **Methods and Study Design:** We conducted a two-sample Mendelian randomization analysis using the inverse-variance weighted method to evaluate the causal effects of immune cells and plasma metabolites on esophageal cancer. We explored potential intermediary pathways by investigating associations between immune cell traits and plasma metabolites relevant to esophageal cancer risk. To test the robustness of our findings, we also carried out sensitivity analyses. **Results:** We identified 19 immune cell phenotypes associated with esophageal cancer risk (8 protective, 11 risk factors). In addition, 22 plasma metabolites (including 5 ratios) were protective, while 26 metabolites (including 8 ratios) increased risk, highlighting potential targets for nutritional interventions. Our analysis identified four plasma metabolites that were associated with specific immune cell traits relevant to esophageal cancer risk. Sensitivity analyses confirmed the robustness of the findings, with no significant heterogeneity or pleiotropy observed. **Conclusions:** This study provides genetic evidence for potential causal associations among immune cells, plasma metabolites, and esophageal cancer, and identifies observed associations between immune cell traits and plasma metabolites. These findings provide a foundation for precision nutrition and support dietary modification as a promising strategy for prevention and adjunctive therapy.

Key Words: esophageal cancer, Mendelian randomization, immune cells, plasma metabolites, nutrition

INTRODUCTION

Esophageal cancer poses a significant global health challenge and ranks among the most aggressive gastrointestinal cancers.[1] It ranks as the seventh leading cause of cancer-related deaths worldwide, with an estimated 445,100 deaths in 2022, accounting for 4.6% of all cancer fatalities.[2] Early-stage symptoms are often subtle or nonspecific, leading to frequent late-stage diagnosis. Despite advancements in treatment strategies, the prognosis remains poor, with a five-year survival rate of merely 22%.[3] Identifying causal risk factors is therefore critical, as it can shed light on disease mechanisms and inform the development of personalized treatment strategies. In recent years, there has been growing interest in how

nutrition and metabolites influence immune regulation and cancer development. Dietary patterns can influence cancer risk by modulating circulating metabolite levels, whereas metabolites, functioning as key regulators of the immune microenvironment, may act as intermediaries linking nutrition and cancer.[4]

The complex role of immune cells in the tumor microenvironment (TME) during esophageal cancer progression is increasingly recognized. While immune cells can suppress tumor growth through immune surveillance, metabolic reprogramming may contribute to immune evasion. For example, myeloid-derived suppressor cells produce metabolites such as itaconate and methylglyoxal that can affect tumor growth.[5, 6] The research team led by Sidonia Fagarasan discovered that B cells secrete the neurotransmitter γ-aminobutyric acid, which modulates the immune landscape of the TME.[7] Dietary components can influence metabolite levels directly or indirectly. Short-chain fatty acids (e.g., butyrate), microbial fermentation products of dietary fiber, can enhance the anti-tumor activity of CD8+ T cells.[8, 9] Palmitic acid, which is abundant in red meat and processed foods, may promote tumor progression by inducing a pro-inflammatory phenotype in monocytes.[10] Notably, the role of metabolites as mediators between immune cells and esophageal cancer remains largely unexplored. Therefore, further research exploring relationships among immune cells, plasma metabolites, and esophageal cancer risk could provide insights into disease mechanisms and inform nutrition-based preventive strategies.

Although randomized controlled trials are the gold standard for establishing causality, they are often difficult to conduct due to the need for large sample sizes, long follow-up periods, high costs, and ethical considerations.[11] Moreover, their use in nutritional research is further limited by issues such as high heterogeneity and low adherence. In contrast, Mendelian randomization (MR) is an analytical approach that leverages genetic variations as instrumental variables (IVs) to infer causal links between exposures and clinically relevant outcomes. By minimizing confounding and reverse causation, MR has been extensively applied in medical research, offering valuable insights for nutritional studies.[12] Mediation analysis can further assess how exposures affect outcomes through intermediate factors.[13] Integrating insights from immunology and metabolomics into nutritional interventions holds great promise for early prevention and precision treatment of esophageal cancer. Therefore, we leveraged publicly available genome-wide association study (GWAS) datasets (https://gwas.mrcieu.ac.uk/) to perform MR analysis, for the first time, systematically investigating potential associations among immune cells, plasma metabolites, and esophageal cancer. Our study explored potential associations between immune cells, plasma metabolites,

and esophageal cancer, identified key metabolites that may be relevant for dietary interventions, and provided new insights to inform nutrition-based preventive and therapeutic strategies.

MATERIALS AND METHODS

Study design

The study flowchart is shown in Figure 1 (created using Figdraw). We obtained pooled, publicly available GWAS data on immune cells, plasma metabolites, and esophageal cancer. We conducted two-sample MR analyses to explore potential causal relationships. First, we performed MR to assess the causal association between immune cells and esophageal cancer risk, identifying immune cells significantly associated with esophageal cancer. Secondly, MR analysis was performed to assess the causal impact of plasma metabolites on esophageal cancer risk, highlighting metabolites strongly linked to the disease. To explore potential intermediary mechanisms, we performed a sequential analysis. Initially, MR was used to identify metabolites causally influenced by the immune cells of interest. Following this, we assessed the associations of these specific metabolites with esophageal cancer risk. Metabolites showing consistent associations in both steps were proposed as potential candidates for further investigation. This study followed established guidelines for performing MR analyses.[14] A detailed MR checklist is provided in Supplementary Table 1.

Data sources

Our data were obtained from publicly available GWAS databases.[15] We obtained 998 esophageal cancer cases and approximately 4.75×10^5 controls from the GWAS database(GCST90018841), all of whom were of European ancestry and involved approximately 2.42×10^7 single nucleotide polymorphisms (SNPs). Immunocyte signature GWAS (GCST90001391-GCST90002121) covered 731 immunophenotypes derived from approximately 3.76×10^3 individuals of European ancestry in Sardinia.[16] The blood metabolite GWAS (GCST90199621-GCST90201020) was detected by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) in approximately 8.10×10^3 individuals of European ancestry.[17] Detailed information on the GWAS datasets is provided in Supplementary Table 2. To link metabolites to dietary sources, we annotated metabolites using the Human Metabolome Database (HMDB, https://hmdb.ca/). This study was exempt from ethics committee approval as it utilized publicly available genetic data from GWAS databases.

Selection of instrumental variables

In MR, it is crucial that the selected genetic variants accurately represent immune cell and plasma metabolite phenotypes. Therefore, our study utilized SNPs as instrumental variables for MR analysis. A significance threshold of $p < 1 \times 10^5$ was applied for SNPs associated with immune cell and metabolite phenotypes, as well as esophageal cancer, in line with previous MR studies.[18] Independent SNPs were pruned using a linkage disequilibrium threshold of $r^2 < 0.001$, based on the 1000 Genomes Project Phase 3 EUR reference panel.[19] We then calculated F-statistics and excluded SNPs with values below 10 to minimize potential biases from weak instrumental variables.[20] In addition, we calculated the minimum allele frequency (MAF) for each SNP. Although the MAF of some SNPs was low, the reliability of IVs was ensured by F statistic screening.[21] Specific values for MAF and F statistics for selected SNPs are shown in Supplementary Tables 3 and 4.

Statistical analysis

Two-sample Mendelian randomization

To investigate the causal relationships between immune cells, plasma metabolites, and esophageal cancer, this study mainly used the TwoSampleMR, VariantAnnotation, and gwasglue packages within R Software version 4.3.2 (https://www.R-project.org) for two-sample MR analysis.[22] MR analysis typically involves five primary methods: inverse variance weighted (IVW) , MR Egger, weighted median, simple mode and weighted mode.[23-26] Each method has distinct characteristics, but the IVW method is considered the most precise and robust and was therefore used as the primary analytical approach, while the other methods served as complementary analyses.[27] All MR estimates were expressed per 1 standard deviation (SD) increase in immune cell proportions or metabolite levels, and the corresponding odds ratio (OR) reflects the change in esophageal cancer risk per 1-SD increase. Given the large number of MR tests, multiple testing was corrected using the false discovery rate (FDR, Benjamini–Hochberg method), with FDR < 0.05 considered statistically significant. Immune cells or plasma metabolites that also did not show significant heterogeneity or pleiotropy (het Q > 0.05, pleio $p > 0.05$) were included in further analyses. Additionally, heterogeneity and pleiotropy in instrumental variables were assessed using Cochran's Q test and the MR-Egger intercept.[24, 27, 28] Leave-one-out sensitivity analysis was performed to examine the influence of individual instrumental variables. We particularly focused on metabolites associated with significant esophageal cancer risk and their potential clinical and nutritional relevance.

Reverse Mendelian randomization analysis

To explore whether esophageal cancer exerted a causal effect on the identified immune cells ($p < 0.05$), reverse MR analysis was conducted using the same method. If the reverse MR analysis was not significant ($p > 0.05$), no evidence of reverse causality was assumed, and the immune cells were included in the subsequent mediation analysis.

Exploratory analysis of potential intermediary metabolites

We conducted exploratory analyses to investigate potential intermediary associations between immune cells and plasma metabolites in relation to esophageal cancer risk. First, MR analysis was used to estimate associations between immune cells and plasma metabolites (β1). Next, associations between these plasma metabolites and esophageal cancer risk (β2) were assessed, with FDR < 0.05 applied as the significance threshold at each step. As an exploratory calculation, the product of these two estimates (β1 × β2) was used to indicate the direction and potential presence of candidate pathways, without implying a precise quantification of mediation effects. All β coefficients were standardized per 1-SD increase in immune cell proportions or metabolite levels.

RESULTS

Total effect of immune cells on esophageal cancer

A two-sample MR analysis was conducted to assess the causal effect of immune cells on esophageal cancer, with the IVW method as the primary approach. At FDR < 0.05, we identified 19 immunophenotypes significantly associated with esophageal cancer risk, including 8 protective and 11 risk factors. As shown in Figure 2 and Supplementary Table 5, these included 7 immunophenotypes from the TBNK panel, 5 from the B cell panel, 3 from the monocyte panel, 2 from the myeloid cell panel, 1 from the conventional dendritic cells (cDC) panel, and 1 from the Treg panel. Specifically, based on the OR and 95% confidence interval (CI), the following immune cells were inversely associated with esophageal cancer risk: IgD+ CD38- B cell %B cell (OR = 0.908, 95% CI: 0.833-0.989, $p = 0.0267$, FDR = 0.0481), CD62L- HLA DR++ monocyte absolute count (OR = 0.879, 95% CI: 0.774-0.999, $p = 0.0481$, FDR = 0.0481), CD4+ T cell absolute count (OR = 0.889, 95% CI: 0.808-0.979, $p = 0.0162$, FDR = 0.0427), CD28+ CD45RA+CD8dim T cell absolute count (OR = 0.975, 95% CI: 0.955-0.995, $p = 0.0159$, FDR = 0.0427), CD19 on transitional B cell (OR = 0.935, 95% CI: 0.875-0.998, $p = 0.0442$, FDR = 0.0481), CD3 on HLA DR+ T cell (OR = 0.944, 95% CI: 0.9-0.99, $p = 0.0171$, FDR = 0.0427), FSC-A on HLA DR+ CD4+ T cell (OR = 0.886, 95% CI: 0.795-0.988, $p = 0.0293$, FDR = 0.0481), and CD80 on granulocyte (OR = 0.92, 95% CI:

0.849-0.996, p = 0.0395, FDR = 0.0481). Conversely, the following immune cells were positively associated with esophageal cancer risk: IgD+ CD38dim B cell absolute count (OR = 1.06, 95% CI: 1-1.11, p = 0.0382, FDR = 0.0481), CD20- B cell %B cell (OR = 1.12, 95% CI: 1-1.24, p = 0.0462, FDR = 0.0481), CD20- B cell %lymphocyte (OR = 1.08, 95% CI: 1.03-1.13, p = 0.0015, FDR = 0.0293), myeloid dendritic Cell %Dendritic Cell (OR = 1.13, 95% CI: 1.02-1.24, p = 0.0145, FDR = 0.0427), naive CD4-CD8-T cell absolute count (OR = 1.13, 95% CI: 1.02-1.24, p = 0.0162, FDR = 0.0427), CD4+CD8dim T cell %lymphocyte (OR = 1.11, 95% CI: 1.01-1.22, p = 0.0306, FDR = 0.0481), CD33 on basophil (OR = 1.04, 95% CI: 1-1.08, p = 0.0256, FDR = 0.0481), CD16 on CD14- CD16+ monocyte (OR = 1.08, 95% CI: 1.02-1.14, p = 0.0132, FDR = 0.0427), SSC-A on CD14+ monocyte (OR = 1.11, 95% CI: 1.01-1.22, p = 0.0379, FDR = 0.0481), SSC-A on CD8+ T cell (OR = 1.14, 95% CI: 1.03-1.26, p = 0.0084, FDR = 0.0427), and HLA DR on CD33dim HLA DR+ CD11b+ (OR = 1.06, 95% CI: 1-1.11, p = 0.0475, FDR = 0.0481). Cochran's Q statistic and the MR-Egger intercept test showed no evidence of heterogeneity or pleiotropy (Supplementary Tables 6 and 7). Leave-one-out analysis demonstrated minimal changes after sequentially removing each SNP (Fig. S1), indicating that the results are robust. Using the IVW reverse MR approach, no evidence of reverse causality was observed (p > 0.05, Supplementary Figure 2 and Supplementary Table 8).

Effect of plasma metabolites on esophageal cancer

Tissue, blood, urine, and feces are commonly used biological matrices in metabolomics research. Among these, blood is particularly valuable because it is easily accessible and metabolically diverse, making it well-suited for identifying circulating biomarkers relevant to cancer screening. We applied MR to investigate the causal relationships between 1,091 plasma metabolites (including 309 metabolite ratios) and esophageal cancer risk. Our analysis identified 30 known metabolites (16 associated with increased risk and 14 with reduced risk), 5 unknown metabolites (2 risk-associated and 3 protective), and 13 metabolite ratios (8 risk-associated and 5 protective) that showed significant associations with esophageal cancer risk (Figure 3 and Supplementary Table 9). Specifically, among lipids, 12 species were significantly associated with esophageal cancer: maltotriose, 5-dodecenoate (12:1n7), carnitine C14, hyocholate, linolenate [alpha or gamma; (18:3n3 or 6)], 1-palmitoyl-2-stearoyl-GPC (16:0/18:0), 1-stearoyl-2-arachidonoyl-GPE (18:0/20:4), and myristate (14:0) were positively associated, whereas 1-stearoyl-GPI (18:0), 3-methyladipate, 5alpha-pregnan-diol disulfate, and sphingomyelin (d17:2/16:0, d18:2/15:0) were negatively associated. For amino

acid metabolites, elevated levels of 8-methoxykynurenate, N-lactoyl phenylalanine, dimethylglycine, alanine, and glycine correlated with increased risk, while higher concentrations of 3-hydroxy-2-ethylpropionate, N-acetyl-isoputreanine, thyroxine, and tyrosine exhibited protective effects. Among xenobiotics: 2,3-dihydroxyisovalerate and (S)-a-amino-omega-caprolactam were positively associated with esophageal cancer risk, whereas tartronate (hydroxymalonate), 3-hydroxy-2-methylpyridine sulfate, 4-acetylcatechol sulfate (1), and gluconate were negatively associated with esophageal cancer risk. Among cofactors and vitamins: trigonelline and 1-methylnicotinamide were negatively associated with esophageal cancer risk, while one peptide (gamma-glutamylglycine) was positively associated with esophageal cancer risk. Among metabolite ratios: spermidine / 5-methylthioadenosine (MTA), arachidonate (20:4n6) / oleate/vaccenate (18:1), glycine / serine, adenosine 5'-diphosphate (ADP) / sulfate, glycine / phosphate, isoleucine / phosphate, mannose / glycerol, and arachidonate (20:4n6) / linoleate (18:2n6) were positively associated with esophageal cancer risk, whereas ADP / mannose, phosphate / fructose, phosphate / linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1], phosphate / linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2], and glucose / mannose were negatively associated with esophageal cancer risk. Sensitivity analyses indicated no pleiotropy or heterogeneity (Supplementary Tables 10 and 11), and leave-one-out tests confirmed robustness of the results (Supplementary Figure 3).

Potential intermediary role of plasma metabolites in the association between immune cells and esophageal cancer

To explore potential intermediary associations between immune cells and plasma metabolites in relation to esophageal cancer risk, we conducted exploratory analyses. In preliminary analyses, we identified 19 immune cell traits, 35 plasma metabolites, and 13 metabolite ratios significantly associated with esophageal cancer. We next evaluated the causal effects of these 19 immune cell traits on the identified metabolites. Using the IVW MR method, we detected 8 significant immune cell–metabolite associations (FDR < 0.05, Figure 4 and Supplementary Table 12), yielding β1 estimates. Subsequently, we applied the same approach to assess the associations of these metabolites on esophageal cancer, obtaining β2 estimates (FDR < 0.05, Figure 5 and Supplementary Table 13). Sensitivity analyses confirmed the robustness of these results, showing no evidence of heterogeneity or horizontal pleiotropy (Supplementary Tables 14–17, Supplementary Figures 4 and 5).

We further conducted exploratory analyses to investigate potential intermediary associations between immune cells and plasma metabolites in relation to esophageal cancer risk. As an exploratory evaluation, candidate pathways involving specific plasma metabolites were highlighted, with details of the quantitative calculations provided in the Table S18. Notably, four metabolite-related pathways were prioritized based on significant associations ($p < 0.05$; Figure 6). For example, CD3 on HLA DR+ T cells may exert a protective effect by lowering 2,3-dihydroxyisovalerate levels, whereas SSC-A on CD8+ T cells may increase risk through elevated 3-hydroxy-2-ethylpropionate. Likewise, HLA DR on CD33dim HLA DR+ CD11b+ cells may enhance risk by increasing 8-methoxykynurenate levels. These findings suggest that immune cells may contribute to esophageal carcinogenesis through specific plasma metabolites and offer novel insights into the underlying biological pathways.

DISCUSSION

It is well recognized that the biological behavior of tumors is shaped not only by their intrinsic properties but also by the immune cells infiltrating the tumor microenvironment. In esophageal cancer, the immune cell composition of the tumor microenvironment differs markedly from that of adjacent normal tissue, suggesting a pivotal role for immune cells in tumor initiation and progression.[29] To investigate their causal relationships with esophageal cancer, we performed a two-sample MR analysis and identified 19 immune cell phenotypes significantly associated with disease risk. Metabolites, as downstream effectors, are a key mechanism through which immune cells exert their biological influence.[30] To further determine whether these 19 immune cell phenotypes influence esophageal cancer via metabolites, we identified 22 plasma metabolites (including 5 metabolite ratios) that were protective against esophageal cancer, and 26 plasma metabolites (including 8 metabolite ratios) that were associated with an increased risk of esophageal cancer. We then assessed the causal effects of these 19 immune cell phenotypes on the 48 identified metabolites. Exploratory analyses further highlighted four metabolite-related pathways as candidate intermediaries. These findings suggest that plasma metabolites could act as intermediaries linking immune cells to esophageal cancer risk, offering preliminary insights into a potential immune–metabolite–cancer axis.

In this study, we employed MR analysis to investigate the causal relationship between immune cells and esophageal cancer. Notably, previous studies have identified IgD+CD38-B cells as protective and IgD+CD38dim B cells as linked to higher esophageal cancer risk, which is consistent with our findings.[31] Zhang et al. also reported significantly elevated levels

of myeloid dendritic cell infiltration in the tumor microenvironment of pancreatic cancer.[32] Similarly, we found that the proportion of myeloid dendritic cells may increase esophageal cancer risk. CD4+T cells are well-known for their antitumor effects, and in our study, higher absolute counts of CD4+ T cells were associated with lower esophageal cancer risk. CD4+CD8dim T cells represent a small fraction of total CD3+ T cells in peripheral blood, and their function remains largely unclear.[33] Interestingly, we observed that a higher percentage of CD4+CD8dim T cell% lymphocyte was associated with increased esophageal cancer risk, whereas Wang et al. reported that CD8dim% T cells were linked to reduced breast cancer risk.[34] In our study, CD3 on HLA-DR+ T cells appeared to reduce esophageal cancer risk, although this cell population is significantly elevated in ovarian cancer.[35] Basophils are associated with worse overall survival in colorectal cancer, yet Constantinescu et al. found that higher basophil counts were protective against colorectal cancer using MR analysis.[36, 37] Low preoperative circulating basophil counts have been linked to poor prognosis in postoperative esophageal cancer patients. In contrast, we found that higher CD33 expression on basophils was positively associated with esophageal cancer risk.[38] The role of immune cells in the TME may vary depending on the local inflammatory context and can differ markedly across solid tumor types.

The development of esophageal cancer is closely associated with changes in multiple metabolite levels. In this study, we systematically examined plasma metabolites associated with esophageal cancer risk and integrated their endogenous/exogenous origins and potential dietary sources (HMDB) to propose possible nutritional intervention strategies (Supplementary Table 19). Among lipid metabolites, certain exogenous or partially exogenous fatty acids, such as 5-dodecenoate (12:1n7), carnitine C14, 1-palmitoyl-2-stearoyl-GPC (16:0/18:0), and myristate (14:0) were positively associated with esophageal cancer risk, suggesting that high intake of dairy products, animal fats, and foods rich in long-chain saturated fatty acids may elevate risk. In contrast, endogenous lipids (e.g., 1-stearoyl-GPI (18:0), 3-methyladipate, 5alpha-pregnan-diol disulfate) and some lipids derived from animal foods (e.g., sphingomyelin (d17:2/16:0, d18:2/15:0)) appeared to have protective effects, potentially by supporting lipid metabolic homeostasis and dietary phospholipid balance. For amino acids and their metabolites, those associated with high protein intake (e.g., dimethylglycine, alanine, glycine) increased risk, whereas metabolites linked to endocrine balance and essential amino acid metabolism (e.g., thyroxine, tyrosine, N-acetyl-isoputreanine) showed protective effects, indicating that the type and amount of dietary protein may influence the metabolic profile and disease risk. Exogenous cofactors and

polyphenol-related metabolites (e.g., trigonelline, 3-hydroxy-2-methylpyridine sulfate, 4-acetylcatechol sulfate) were protective effects, suggesting potential benefits of consuming coffee, vitamin B6, and polyphenol-rich foods. Moreover, metabolite ratios reflect the balance of energy, amino acids, and fatty acids and may be modulated by adjusting protein sources, fatty acid composition, and intake of sugars or phosphate. Overall, integrating dietary sources with the endogenous/exogenous characteristics of metabolites provides a scientific basis for esophageal cancer prevention: reducing high-fat animal foods, moderating protein intake, and increasing consumption of plant-based foods rich in polyphenols, vitamin B6, and dietary fiber may help lower risk by modulating key metabolite levels.

Tumor cells enhance nutrient uptake, consume oxygen, increase the acidity of the TME, and upregulate the production of pro-tumor metabolites, thereby creating an immunosuppressive TME that promotes tumor progression and immune evasion.[39] Emerging evidence indicates that immune cells can also influence tumor progression by modulating metabolites.[40] The Hasim team reported differential metabolite distributions in esophageal cancer, which may aid early diagnosis.[41] However, the precise mechanisms by which immune cells affect esophageal cancer via metabolites remain unclear. In this study, we for the first time explored potential pathways through which immune cell exposures may be associated with esophageal cancer risk via plasma metabolites. Exploratory analyses suggested that CD3 on HLA DR+ T cells may be linked to lower levels of 2,3-dihydroxy-5-methylvalerate, whereas SSC-A on $CD8^+$ T cells and HLA DR on CD33dim HLA DR+ CD11b+ cells may be associated with higher levels of 3-hydroxy-2-ethylpropionate and 8-methoxykynurenate, respectively. These findings highlight that distinct immune cell subsets could influence the TME through specific metabolite patterns, potentially affecting esophageal cancer development. Importantly, our results provide novel insights into immune–metabolic interactions and lay a theoretical foundation for future investigations into the dynamic interplay between immune cells and metabolites within the TME, guiding subsequent functional experiments and translational research.

This study is the first to systematically explore the potential causal links between immune cells, plasma metabolites, and esophageal cancer using MR analysis. We highlight the potential intermediary role of plasma metabolites in the immune–metabolism–cancer axis, providing preliminary insights into candidate pathways rather than precise quantification of mediation effects. However, several limitations should be noted. This work also lays a foundation for metabolism-based dietary interventions, emphasizing the importance of individualized nutrition strategies for high-risk populations. First, the GWAS dataset for

esophageal cancer included only 998 cases, which limits statistical power to detect modest effects (OR = 1.05-1.10) and may have missed weaker associations. Second, the publicly available dataset lacked detailed demographic information, restricting subgroup analyses, and was predominantly derived from individuals of European ancestry, limiting the generalizability to other ethnic groups. Third, the observed associations between immune cells and esophageal cancer may involve additional mediators that were not captured in our exploratory analyses, and the quantitative estimates should be interpreted cautiously. Future directions include leveraging larger GWAS datasets and integrating multi-omics data, combined with experimental validation, to further elucidate the role of immune–metabolic pathways in esophageal cancer. Multivariable MR (MVMR) approaches could model immune cells and metabolites jointly, controlling for confounding between exposures, while more robust MR methods such as MR Robust Adjusted Profile Score (MR-RAPS) or Genome-wide Summary-data-based Mendelian Randomization (GSMR) may strengthen causal inference. Additionally, attention to ethnic differences in metabolite levels is warranted to develop nutritional strategies with broader applicability.

Conclusion

In summary, this study employed MR analysis to investigate the causal relationships between immune cells and plasma metabolites in esophageal cancer. Our exploratory analysis suggests that plasma metabolites may play a mediating role in this process. These findings not only deepen our understanding of esophageal cancer pathogenesis but also provide new perspectives for nutritional prevention and management, potentially guiding personalized dietary strategies for high-risk populations. Looking ahead, combining clinical trials with nutritional research could enable dietary interventions targeting specific metabolites to serve as innovative approaches for both prevention and adjunct therapy in esophageal cancer.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

All authors declare that there are no conflicts of interest.

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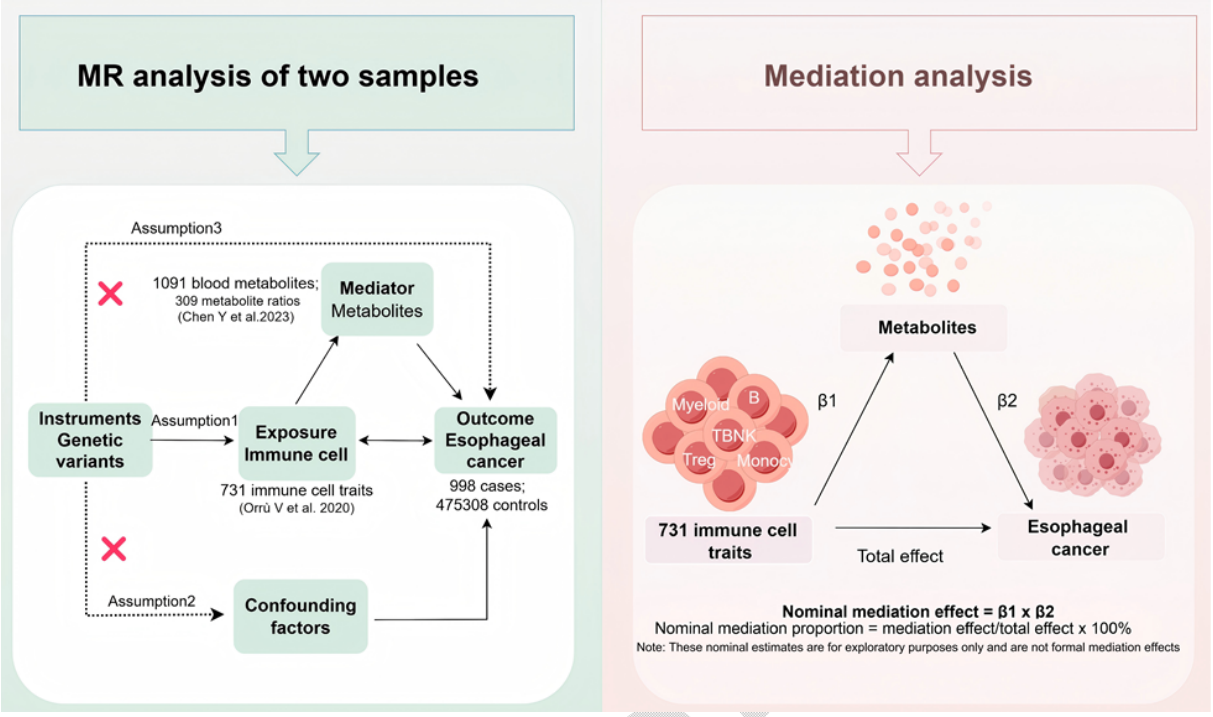

Figure 1. Design of a MR study of plasma metabolites mediating immune cells to esophageal cancer. Assumptions: (1) instrumental variables are associated with the exposure; (2) not affected by confounders; (3) influence the outcome only through the exposure. Note: Estimates are exploratory and indicate potential direction and relative strength of intermediary pathways, not formal mediation effects.

panel	exposure	outcome	method	nsnp	β	p	OR(95% CI)	p.adjusted_FDR
B cell	IgD+ CD38dim B cell Absolute Count	Esophageal cancer	IVW	25	0.0536	**0.0382**	1.06 (1 to 1.11)	**0.0481**
	IgD+ CD38− B cell %B cell	Esophageal cancer	IVW	24	−0.0969	**0.0267**	0.908 (0.833 to 0.989)	**0.0481**
	CD20− B cell %B cell	Esophageal cancer	IVW	19	0.11	**0.0462**	1.12 (1 to 1.24)	**0.0481**
	CD20− B cell %lymphocyte	Esophageal cancer	IVW	19	0.0747	**0.0015**	1.08 (1.03 to 1.13)	**0.0293**
	CD19 on transitional B cell	Esophageal cancer	IVW	26	−0.0677	**0.0442**	0.935 (0.875 to 0.998)	**0.0481**
TBNK	Naive CD4−CD8− T cell Absolute Count	Esophageal cancer	IVW	20	0.12	**0.0162**	1.13 (1.02 to 1.24)	**0.0427**
	CD4+ T cell Absolute Count	Esophageal cancer	IVW	24	−0.117	**0.0162**	0.889 (0.808 to 0.979)	**0.0427**
	CD4+ CD8dim T cell %lymphocyte	Esophageal cancer	IVW	22	0.104	**0.0306**	1.11 (1.01 to 1.22)	**0.0481**
	CD3 on HLA DR+ T cell	Esophageal cancer	IVW	28	−0.0579	**0.0171**	0.944 (0.9 to 0.99)	**0.0427**
	FSC−A on HLA DR+ CD4+ T cell	Esophageal cancer	IVW	17	−0.121	**0.0293**	0.886 (0.795 to 0.988)	**0.0481**
	CD80 on granulocyte	Esophageal cancer	IVW	33	−0.0837	**0.0395**	0.92 (0.849 to 0.996)	**0.0481**
	SSC−A on CD8+ T cell	Esophageal cancer	IVW	19	0.133	**0.0084**	1.14 (1.03 to 1.26)	**0.0427**
Treg	CD28+ CD45RA+ CD8dim T cell Absolute Count	Esophageal cancer	IVW	49	−0.0254	**0.0159**	0.975 (0.955 to 0.995)	**0.0427**
Myeloid	CD33 on basophil	Esophageal cancer	IVW	23	0.0401	**0.0256**	1.04 (1 to 1.08)	**0.0481**
	HLA DR on CD33dim HLA DR+ CD11b+	Esophageal cancer	IVW	22	0.0539	**0.0475**	1.06 (1 to 1.11)	**0.0481**
Monocyte	CD62L− HLA DR++ monocyte Absolute Count	Esophageal cancer	IVW	19	−0.129	**0.0481**	0.879 (0.774 to 0.999)	**0.0481**
	CD16 on CD14− CD16+ monocyte	Esophageal cancer	IVW	22	0.0729	**0.0132**	1.08 (1.02 to 1.14)	**0.0427**
	SSC−A on CD14+ monocyte	Esophageal cancer	IVW	23	0.101	**0.0379**	1.11 (1.01 to 1.22)	**0.0481**
cDC	Myeloid Dendritic Cell %Dendritic Cell	Esophageal cancer	IVW	28	0.118	**0.0145**	1.13 (1.02 to 1.24)	**0.0427**

Figure 2. Forest plots showing causal effects of immune cells on esophageal cancer. IVW: inverse variance weighted; OR: odds ratio; CI: confidence interval; FDR: false discovery rate

superpathway	exposure	outcome	method	nsnp	β	p	OR(95% CI)	p.adjusted_FDR
Lipid	Maltotriose levels	Esophageal cancer	IVW	24	0.215	**0.0053**	1.24 (1.07 to 1.44)	**0.0346**
	1−stearoyl−GPI (18:0) levels	Esophageal cancer	IVW	18	−0.231	**0.0456**	0.794 (0.633 to 0.995)	**0.0475**
	5−dodecenoate (12:1n7) levels	Esophageal cancer	IVW	15	0.349	**0.0090**	1.42 (1.09 to 1.84)	**0.0346**
	Carnitine C14 levels	Esophageal cancer	IVW	22	0.238	**0.0292**	1.27 (1.02 to 1.57)	**0.0421**
	Hyocholate levels	Esophageal cancer	IVW	21	0.132	**0.0228**	1.14 (1.02 to 1.28)	**0.0387**
	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	Esophageal cancer	IVW	24	0.267	**0.0331**	1.31 (1.02 to 1.67)	**0.0428**
	3−methyladipate levels	Esophageal cancer	IVW	18	−0.292	**0.0039**	0.747 (0.612 to 0.911)	**0.0346**
	5alpha−pregnan−diol disulfate levels	Esophageal cancer	IVW	23	−0.123	**0.0380**	0.884 (0.787 to 0.993)	**0.0437**
	1−palmitoyl−2−stearoyl−gpc (16:0/18:0) levels	Esophageal cancer	IVW	32	0.157	**0.0109**	1.17 (1.04 to 1.32)	**0.0346**
	1−stearoyl−2−arachidonoyl−GPE (18:0/20:4) levels	Esophageal cancer	IVW	32	0.111	**0.0345**	1.12 (1.01 to 1.24)	**0.0428**
	Sphingomyelin (d17:2/16:0, d18:2/15:0) levels	Esophageal cancer	IVW	29	−0.504	**0.0308**	0.604 (0.382 to 0.955)	**0.0423**
	Myristate (14:0) levels	Esophageal cancer	IVW	23	0.274	**0.0111**	1.32 (1.06 to 1.63)	**0.0346**
Amino acid	3−hydroxy−2−ethylpropionate levels	Esophageal cancer	IVW	29	−0.254	**0.0016**	0.776 (0.663 to 0.908)	**0.0346**
	8−methoxykynurenate levels	Esophageal cancer	IVW	26	0.203	**0.0043**	1.22 (1.07 to 1.41)	**0.0346**
	N−lactoyl phenylalanine levels	Esophageal cancer	IVW	16	0.299	**0.0108**	1.35 (1.07 to 1.7)	**0.0346**
	N−acetyl−isoputreanine levels	Esophageal cancer	IVW	38	−0.153	**0.0197**	0.858 (0.754 to 0.976)	**0.0379**
	Thyroxine levels	Esophageal cancer	IVW	22	−0.262	**0.0109**	0.77 (0.629 to 0.942)	**0.0346**
	Dimethylglycine levels	Esophageal cancer	IVW	33	0.136	**0.0496**	1.15 (1 to 1.31)	**0.0496**
	Tyrosine levels	Esophageal cancer	IVW	29	−0.299	**<0.001**	0.742 (0.625 to 0.88)	**0.0292**
	Alanine levels	Esophageal cancer	IVW	22	0.225	**0.0128**	1.25 (1.05 to 1.5)	**0.0346**
	Glycine levels	Esophageal cancer	IVW	22	0.118	**0.0285**	1.13 (1.01 to 1.25)	**0.0421**
Xenobiotics	Tartronate (hydroxymalonate) levels	Esophageal cancer	IVW	20	−0.277	**0.0234**	0.758 (0.597 to 0.963)	**0.0387**
	2,3−dihydroxyisovalerate levels	Esophageal cancer	IVW	27	0.149	**0.0208**	1.16 (1.02 to 1.32)	**0.0379**
	3−hydroxy−2−methylpyridine sulfate levels	Esophageal cancer	IVW	21	−0.263	**0.0385**	0.769 (0.599 to 0.986)	**0.0437**
	4−acetylcatechol sulfate (1) levels	Esophageal cancer	IVW	18	−0.241	**0.0334**	0.786 (0.629 to 0.981)	**0.0428**
	(S)−a−amino−omega−caprolactam levels	Esophageal cancer	IVW	13	0.297	**0.0347**	1.35 (1.02 to 1.77)	**0.0428**
	Gluconate levels	Esophageal cancer	IVW	24	−0.216	**0.0103**	0.805 (0.683 to 0.95)	**0.0346**
Cofactors and vitamins	Trigonelline levels	Esophageal cancer	IVW	25	−0.134	**0.0428**	0.874 (0.768 to 0.996)	**0.0464**
	1−methylnicotinamide levels	Esophageal cancer	IVW	19	−0.249	**0.0201**	0.779 (0.631 to 0.962)	**0.0379**
Peptide	Gamma−glutamylglycine levels	Esophageal cancer	IVW	25	0.126	**0.0277**	1.13 (1.01 to 1.27)	**0.0421**
Unknown	X−12906 levels	Esophageal cancer	IVW	18	0.287	**0.0175**	1.33 (1.05 to 1.69)	**0.0379**
	X−13728 levels	Esophageal cancer	IVW	19	−0.288	**0.0165**	0.75 (0.593 to 0.949)	**0.0379**
	X−23639 levels	Esophageal cancer	IVW	23	0.235	**0.0391**	1.27 (1.01 to 1.58)	**0.0437**
	X−23780 levels	Esophageal cancer	IVW	27	−0.207	**0.0106**	0.813 (0.693 to 0.953)	**0.0346**
	X−19141 levels	Esophageal cancer	IVW	25	−0.106	**0.0123**	0.899 (0.828 to 0.977)	**0.0346**
Metabolite ratio	Spermidine to 5−methylthioadenosine (MTA) ratio	Esophageal cancer	IVW	19	0.228	**0.0081**	1.26 (1.06 to 1.49)	**0.0346**
	Adenosine 5'−diphosphate (ADP) to mannose ratio	Esophageal cancer	IVW	18	−0.235	**0.0213**	0.791 (0.648 to 0.966)	**0.0379**
	Arachidonate (20:4n6) to oleate to vaccenate (18:1) ratio	Esophageal cancer	IVW	20	0.13	**0.0257**	1.14 (1.02 to 1.28)	**0.0412**
	Glycine to serine ratio	Esophageal cancer	IVW	25	0.116	**0.0470**	1.12 (1 to 1.26)	**0.0480**
	Phosphate to fructose ratio	Esophageal cancer	IVW	17	−0.169	**0.0386**	0.845 (0.72 to 0.991)	**0.0437**
	Adenosine 5'−diphosphate (ADP) to sulfate ratio	Esophageal cancer	IVW	25	0.176	**0.0097**	1.19 (1.04 to 1.36)	**0.0346**
	Glycine to phosphate ratio	Esophageal cancer	IVW	28	0.12	**0.0298**	1.13 (1.01 to 1.26)	**0.0421**
	Isoleucine to phosphate ratio	Esophageal cancer	IVW	28	0.218	**0.0435**	1.24 (1.01 to 1.54)	**0.0464**
	Phosphate to linoleoyl−arachidonoyl−glycerol (18:2 to 20:4) [2] ratio	Esophageal cancer	IVW	26	−0.139	**0.0210**	0.87 (0.773 to 0.979)	**0.0379**
	Phosphate to linoleoyl−arachidonoyl−glycerol (18:2 to 20:4) [1] ratio	Esophageal cancer	IVW	23	−0.167	**0.0162**	0.847 (0.739 to 0.97)	**0.0379**
	Mannose to glycerol ratio	Esophageal cancer	IVW	22	0.27	**0.0130**	1.31 (1.06 to 1.62)	**0.0346**
	Glucose−to−mannose ratio	Esophageal cancer	IVW	25	−0.204	**0.0042**	0.815 (0.709 to 0.937)	**0.0346**
	Arachidonate (20:4n6) to linoleate (18:2n6) ratio	Esophageal cancer	IVW	25	0.145	**0.0198**	1.16 (1.02 to 1.31)	**0.0379**

Figure 3. MR analysis depicting the relationship between metabolites and esophageal cancer. IVW: inverse variance weighted; OR: odds ratio; CI: confidence interval; FDR: false discovery rate.

exposure	outcome	method	nsnp	β1	p	OR(95% CI)	p.adjusted_FDR
CD20− B cell %B cell	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	IVW	19	−0.0552	**0.0095**	0.946 (0.908 to 0.987)	**0.0473**
CD28+ CD45RA+ CD8dim T cell Absolute Count	Dimethylglycine levels	IVW	47	0.0107	**0.0045**	1.01 (1 to 1.02)	**0.0112**
CD3 on HLA DR+ T cell	2,3−dihydroxyisovalerate levels	IVW	27	−0.0407	**0.0056**	0.96 (0.933 to 0.988)	**0.0278**
FSC−A on HLA DR+ CD4+ T cell	Adenosine 5'−diphosphate (ADP) to sulfate ratio	IVW	17	0.0813	**0.0045**	1.08 (1.03 to 1.15)	**0.0227**
CD16 on CD14− CD16+ monocyte	Phosphate to linoleoyl−arachidonoyl−glycerol (18:2 to 20:4) [2] ratio	IVW	20	0.0377	**0.0034**	1.04 (1.01 to 1.06)	**0.0170**
SSC−A on CD14+ monocyte	X−12906 levels	IVW	24	0.0517	**0.0089**	1.05 (1.01 to 1.09)	**0.0148**
SSC−A on CD8+ T cell	3−hydroxy−2−ethylpropionate levels	IVW	17	−0.0593	**0.0024**	0.942 (0.907 to 0.979)	**0.0122**
HLA DR on CD33dim HLA DR+ CD11b+	8−methoxykynurenate levels	IVW	21	0.0517	**0.0010**	1.05 (1.02 to 1.09)	**0.0051**

Figure 4. Forest plot illustrating the causal effects of immune cells on plasma metabolites. IVW: inverse variance weighted; OR: odds ratio; CI: confidence interval; FDR: false discovery rate.

superpathway	exposure	outcome	method	nsnp	β2	p	OR(95% CI)	p.adjusted_FDR
Lipid	Linolenate [alpha or gamma; (18:3n3 or 6)] levels	Esophageal cancer	IVW	24	0.267	**0.0331**	1.31 (1.02 to 1.67)	**0.0379**
Amino acid	Dimethylglycine levels	Esophageal cancer	IVW	33	0.136	**0.0496**	1.15 (1 to 1.31)	**0.0496**
	3−hydroxy−2−ethylpropionate levels	Esophageal cancer	IVW	29	−0.254	**0.0016**	0.776 (0.663 to 0.908)	**0.0125**
	8−methoxykynurenate levels	Esophageal cancer	IVW	26	0.203	**0.0043**	1.22 (1.07 to 1.41)	**0.0174**
Xenobiotics	2,3−dihydroxyisovalerate levels	Esophageal cancer	IVW	27	0.149	**0.0208**	1.16 (1.02 to 1.32)	**0.0280**
Metabolite ratio	Adenosine 5'−diphosphate (ADP) to sulfate ratio	Esophageal cancer	IVW	25	0.176	**0.0097**	1.19 (1.04 to 1.36)	**0.0259**
	Phosphate to linoleoyl−arachidonoyl−glycerol (18:2 to 20:4) [2] ratio	Esophageal cancer	IVW	26	−0.139	**0.0210**	0.87 (0.773 to 0.979)	**0.0280**
Unknown	X−12906 levels	Esophageal cancer	IVW	18	0.287	**0.0175**	1.33 (1.05 to 1.69)	**0.0280**

Figure 5. MR analysis of plasma metabolites highly associated with immune cells and esophageal cancer. IVW: inverse variance weighted; OR: odds ratio; CI: confidence interval; FDR: false discovery rate

exposure	outcome	method	nsnp	β	p	OR(95% CI)	p.adjusted_FDR
CD3 on HLA DR+ T cell	2,3−dihydroxyisovalerate levels	IVW	27	−0.0407	**0.0056**	0.96 (0.933 to 0.988)	**0.0278**
2,3−dihydroxyisovalerate levels	Esophageal cancer	IVW	27	0.148	**0.0208**	1.16 (1.02 to 1.32)	**0.0280**
CD3 on HLA DR+ T cell	Esophageal cancer	IVW	28	−0.0579	**0.0171**	0.944 (0.9 to 0.99)	**0.0427**
SSC−A on CD14+ monocyte	X−12906 levels	IVW	24	0.0517	**0.0089**	1.05 (1.01 to 1.09)	**0.0148**
X−12906 levels	Esophageal cancer	IVW	18	0.287	**0.0175**	1.33 (1.05 to 1.69)	**0.0280**
SSC−A on CD14+ monocyte	Esophageal cancer	IVW	23	0.101	**0.0379**	1.11 (1.01 to 1.22)	**0.0481**
SSC−A on CD8+ T cell	3−hydroxy−2−ethylpropionate levels	IVW	17	−0.0593	**0.0024**	0.942 (0.907 to 0.979)	**0.0122**
3−hydroxy−2−ethylpropionate levels	Esophageal cancer	IVW	29	−0.254	**0.0016**	0.776 (0.663 to 0.908)	**0.0125**
SSC−A on CD8+ T cell	Esophageal cancer	IVW	19	0.133	**0.0084**	1.14 (1.03 to 1.26)	**0.0427**
HLA DR on CD33dim HLA DR+ CD11b+	8−methoxykynurenate levels	IVW	21	0.0517	**0.0010**	1.05 (1.02 to 1.09)	**0.0051**
8−methoxykynurenate levels	Esophageal cancer	IVW	26	0.203	**0.0043**	1.22 (1.07 to 1.41)	**0.0174**
HLA DR on CD33dim HLA DR+ CD11b+	Esophageal cancer	IVW	22	0.0539	**0.0475**	1.06 (1 to 1.11)	**0.0481**

Figure 6. Exploratory analysis of plasma metabolites potentially linking immune cells to esophageal cancer. IVW: inverse variance weighted; OR: odds ratio; CI: confidence interval; FDR: false discovery rate