Original Article

Unveiling the atlas of associations between 1,400 plasma metabolites and 24 tumors: Mendelian randomization analyses

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Background: Association between plasma metabolites and pan-cancer remains controversial. Herein, we performed a two-sample Mendelian randomization (MR) analysis to verify whether there is a causal relationship between the two and to point the way for cancer metabolism research.

Methods: In our research, we downloaded 1,400 plasma metabolites from a large genome-wide association study (GWAS). We also obtained GWAS summary statistics for 24 types of cancers from the publicly available GWAS database, totaling 5,003,410 European individuals. We mainly used the fixed/random-effects inverse variance-weighted (IVW) method for two-sample MR analysis.

Results: In a combined sample of 291,202 cancer cases and 4,712,208 controls, a total of 55 plasma metabolites were identified as causally associated with nine types of cancer as a result of our MR analysis [P<0.05, false discovery rate (FDR) <0.2], including methionine sulfone, gamma-glutamylcitrulline, alliin, tetradecanedioate, hexadecanedioate, glutarate, ceramide, linolenoylcarnitine, hydroxypalmitoyl sphingomyelin, 1-palmitoyl-2-linoleoyl-glycerylphosphorylcholine (1-palmitoyl-2-linoleoyl-GPC), 3-acetylphenol sulfate, retinol (vitamin a) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) ratio, etc. Reverse MR analysis revealed a causal relationship between lung cancer and the only plasma metabolite, 1-palmitoyl-2-linoleoyl-GPC (P<0.05, FDR <0.2).

Conclusions: Our study provides a comprehensive atlas of cancer-related plasma metabolites, offering possible targets for cancer detection, as well as a reference for future research on tumorigenesis mechanisms and therapeutic targets.

Keywords: Mendelian randomization (MR); plasma metabolites; metabolism; pan-cancer; cancer detection

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Introduction

Cancer has been a threat to human health since its discovery (1). In 2023, it was expected to have 1,958,310 new cancer cases and 609,820 cancer deaths in the United States (2), and malignant tumors are the major cause of death among people aged 45 to 64 years (3). In the current times, although malignant tumors have gone through several iterations of treatment modalities, they remain one of the major risk factors for patient mortality (1-3). As humans continue to fight against tumors, our understanding of the complex pathogenesis of tumors has improved and plasma tumor detection has become possible (4,5). Plasma tumor testing is used to find tumor-associated plasma substances in a patient's blood to provide a basis for tumor diagnosis (4). However, plasma cancer detection also faces a number of problems, including how to differentiate between the genetic mutations and metabolic changes that result from normal physical aging and those that result from tumors (6-9). This requires specific markers that are causally related to the onset of malignant tumors. Specific markers of malignant tumors can be divided into tissue and serological sources, depending on how they are detected (10). It is worth highlighting that liquid phase markers (serologic markers/plasma markers) have the advantage of being more sensitive, safer, and less damaging to patients compared to histologic markers (11).

Some of the existing cancer plasma markers are malignant cell metabolites, such as neuron-specific enolase (NSE) (12), which marks small cell lung cancer (SCLC), polyamine (13) and alpha-fetoprotein (AFP) (14,15), which are associated with hepatocellular carcinoma (HCC), suggesting that we can look for new plasma markers from tumor metabolism. Classically, aberrant phospholipid metabolism is one of the features of tumor metabolism (16,17), and even an important hallmark of tumors (18). Phospholipid metabolism regulates the composition of cell membranes, modulates tumor cell energy accumulation, influences cell signaling, governs tumor immunity, and plays an important role in the growth, metastasis, and resistance of tumor cells to drugs (19). Next, disorders of glucose metabolism occur in various types of malignant tumors (20). Inadequate blood supply within the tumor leads to a hypoxic environment that induces hypoxia-inducible factors 1 and 2 (expression of HIF-1 and HIF-2), which in turn leads to the expression of glucose transporter proteins, GLUT1 and GLUT3, which promotes glucose uptake and allows glucose to enter into the glycolytic pathway. This enhanced glycolysis promotes tumor cell migration, induces angiogenesis, and mediates chemoresistance (21,22). With the reprogramming of glycolysis, in the absence of glucose, amino acids can be involved in metabolism, such as elevated levels of glutamine metabolism (23,24). Precursors of glutamine and its metabolites can regulate signaling pathways, proliferation, and metastasis of tumor cells by modulating the tricarboxylic acid cycle (TCA cycle) in tumor cells (25,26). In addition, the role of altered lipid metabolism in tumor growth has attracted attention (27). Unlike other metabolites that are directly involved in energy metabolism, lipid metabolism mainly provides biomolecules for metabolites (28,29). In conclusion, with the development of metabolomics, more and more new metabolites have been defined, and it is practical and effective to explore the causality of tumorigenesis through a metabolic perspective (30-32).

Observational studies are one of the commonly used methods when exploring causal relationships in research subjects (33), but often vary from the results of randomized controlled trials (RCTs) when confronted with real-world issues due to confounding factors and reverse causation (33). However, RCTs require long-term follow-up with a large clinical cohort and can be expensive (34-37). Mendelian randomization (MR) analysis is an emerging analytical method that further assesses the reliability of causality by adding genetic variation as instrumental variables (IVs) between exposure and outcome to overcome the limitations of RCTs and observational studies (38). Although some MR analyses suggest a causal relationship between metabolites and cancer, to our knowledge there have been

Highlight box

Key findings

- This study revealed the causal relationship between some plasma metabolites and several cancers.

What is known and what is new?

- Abnormal changes of different tumor markers in plasma can predict the occurrence, development and treatment prognosis of the corresponding tumor.
- Our results show that different plasma metabolite changes predict different cancers, and we provide a comprehensive tumor-associated plasma metabolite mapping.

What is the implication, and what should change now?

- The tumor-associated plasma metabolite profiles in this study provide targets for plasma cancer detection and suggest basic oncology research approaches.

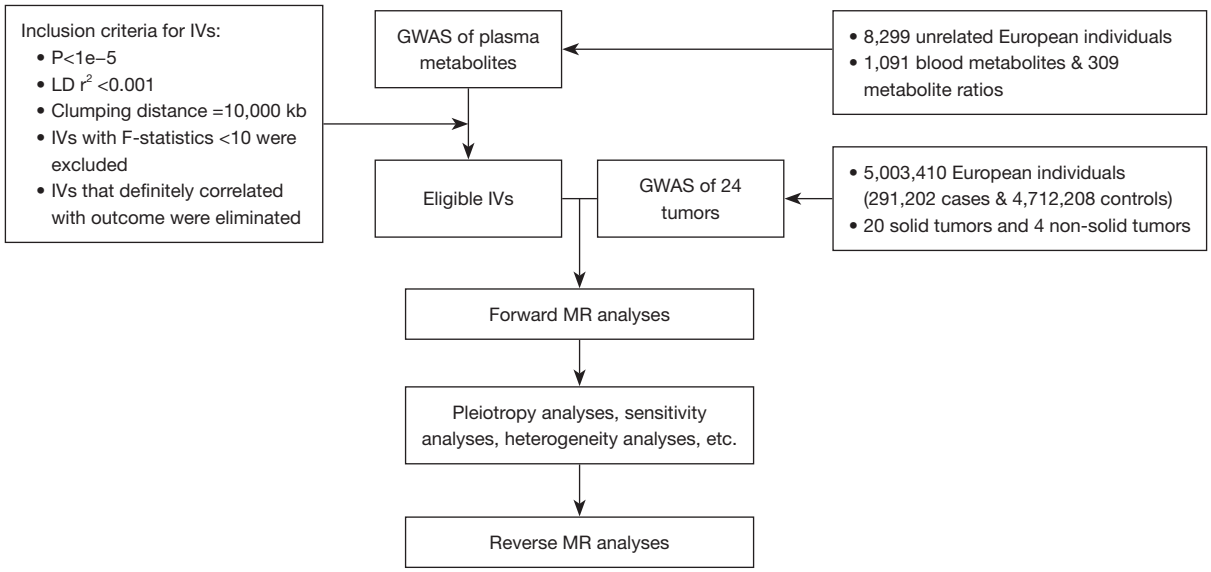

Figure 1 The study design. Flowchart of the study. GWAS, genome-wide association study; IVs, instrumental variables; MR, Mendelian randomization; LD, linkage disequilibrium.

no comprehensive MR analyses exploring the causality between plasma metabolites and pan-cancers (39). In this study, we used the most current and comprehensive human plasma metabolomics data to explore causal relationships with 24 common human tumors, with the aim of obtaining plasma metabolites that could be used for cancer detection and providing a reference for research on tumorigenesis mechanisms and therapeutic targets. We present this article in accordance with the STROBE-MR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-359/rc).

Methods

Study design

In this study, a two-sample MR analysis was used to identify causal relationships between 1,400 plasma metabolites (including 1,091 blood metabolites and 309 metabolite ratios) and 24 types of cancers (including 20 solid tumors: bladder cancer, brain cancer, breast cancer, cervical cancer, colorectal cancer, corpus uteri cancer, endometrial cancer, esophageal adenocarcinoma, gastric cancer, liver cancer, lung cancer, malignant melanoma, malignant neoplasm of head and neck, oral cavity cancer, oropharynx cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, thyroid cancer; and 4 non-solid tumors: chronic lymphocytic leukemia, diffuse large B-cell lymphoma, Hodgkin lymphoma, multiple myeloma). From substantial genome-wide association studies (GWASs), 5,003,410 European individuals, including 291,202 cancer cases and 4,712,208 controls across 24 types of cancer were included. Considering the fact that the study used open-access summary data, no further participant consent or ethical approval was required. In order to minimize the potential bias in results, IVs must satisfy three core assumptions: (I) IVs exhibit a significant association with the exposure; (II) genetic IVs are independent of potential confounders; and (III) IVs influence outcome only through exposure. The directionality of the causal linkage was further determined with a bidirectional MR design, which ruled out a potential reverse causal linkage. A comprehensive study design was presented in *Figure 1*. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Plasma metabolites data

GWAS summary statistics for plasma metabolites were obtained from the GWAS Catalog (https://www.ebi.ac.uk/gwas/, accession numbers from GCST90199621 to GCST90201020; table available at https://cdn.amegroups.cn/static/public/tcr-24-359-1.xlsx) (40). A cohort of 8,299 unrelated European individuals participating in the metabolomics study underwent genome-wide genotyping

Table 1 Information of cancer-specific GWAS datasets

Cancer type	Resource	Population	Sample size (n=5,003,410)	
			Cases (n=291,202)	Controls (n=4,712,208)
Bladder cancer	FINNGEN R9	European	2,053	287,137
Brain cancer	FINNGEN R9	European	764	287,137
Breast cancer	IEU OpenGWAS project: ieu-a-1126	European	122,977	105,974
Cervical cancer	IEU OpenGWAS project: ukb-b-8777	European	1,889	461,044
Chronic lymphocytic leukemia	FINNGEN R9	European	624	287,133
Colorectal cancer	IEU OpenGWAS project: ebi-a-GCST90018808	European	6,581	463,421
Corpus uteri cancer	FINNGEN R9	European	1,967	167,189
Diffuse large B-cell lymphoma	FINNGEN R9	European	1,010	287,137
Endometrial cancer	IEU OpenGWAS project: ebi-a-GCST006464	European	12,906	108,979
Esophageal adenocarcinoma	IEU OpenGWAS project: ebi-a-GCST003739	European	4,112	17,159
Gastric cancer	FINNGEN R9	European	1,307	287,137
Hodgkin lymphoma	FINNGEN R9	European	780	299,952
Liver cancer	IEU OpenGWAS project: ebi-a-GCST90092003	European	775	1,332
Lung cancer	IEU OpenGWAS project: ieu-a-966	European	11,348	15,861
Malignant melanoma	FINNGEN R9	European	3,960	286,874
Malignant neoplasm of head and neck	FINNGEN R9	European	2,131	287,137
Multiple myeloma	IEU OpenGWAS project: ieu-b-4957	European	601	372,016
Oral cavity cancer	IEU OpenGWAS project: ieu-b-94	European	1,223	2,928
Oropharynx cancer	IEU OpenGWAS project: ebi-a-GCST012242	European	1,119	2,329
Ovarian cancer	IEU OpenGWAS project: ieu-a-1120	European	25,509	40,941
Pancreatic cancer	FINNGEN R9	European	1,416	287,137
Prostate cancer	IEU OpenGWAS project: ebi-a-GCST006085	European	79,148	61,106
Renal cell carcinoma (female)	GWAS Catalog: GCST008225	European	1,992	3,095
Renal cell carcinoma (male)	GWAS Catalog: GCST008226	European	3,227	4,916
Thyroid cancer	FINNGEN R9	European	1,783	287,137

GWAS, genome-wide association study.

and had their circulating plasma metabolites measured in the Canadian Longitudinal Study of Aging (CLSA). Batch-normalized metabolites levels were used in the study, and metabolites with missing measurements were detected in less than 50% of samples (n=1,091). According to the Human Metabolome Database (HMDB), 309 metabolite pairs share enzymes or transporters. By dividing one metabolite's batch-normalized measurement value by the other metabolite's measurement in the same individual, the metabolite ratio was calculated for each pair of metabolites.

Resource of 24 types of cancers

Data from publicly available databases were used to generate summary statistics on GWAS for cancer outcomes. The cancer outcomes included a total of 5,003,410 European individuals (291,202 cancer cases and 4,712,208 controls, *Table 1*). FinnGen studies, R9 release (https://

www.finngen.fi), a research project involving European descent participants, supplied summary statistics for bladder cancer (2,053 cases, 287,137 controls), brain cancer (764 cases, 287,137 controls), chronic lymphocytic leukemia (624 cases, 287,133 controls), corpus uteri cancer (1,967 cases, 167,189 controls), diffuse large B-cell lymphoma (1,010 cases, 287,137 controls), gastric cancer (1,307 cases, 287,137 controls), Hodgkin lymphoma (780 cases, 299,952 controls), malignant melanoma (3,960 cases, 286,874 controls), malignant neoplasm of head and neck (2,131 cases, 287,137 controls), pancreatic cancer (1,416 cases, 287,137 controls), and thyroid cancer (1,783 cases, 287,137 controls). IEU Open GWAS project (https://gwas.mrcieu.ac.uk/) provided breast cancer (122,977 cases, 105,974 controls), cervical cancer (1,889 cases, 461,044 controls), colorectal cancer (6,581 cases, 463,421 controls), endometrial cancer (12,906 cases, 108,979 controls), esophageal adenocarcinoma (4,112 cases, 17,159 controls), liver cancer (775 cases, 1,332 controls), lung cancer (11,348 cases, 15,861 controls), multiple myeloma (601 cases, 372,016 controls), oral cavity cancer (1,223 cases, 2,928 controls), oropharynx cancer (1,119 cases, 2,329 controls), ovarian cancer (25,509 cases, 40941 controls), and prostate cancer (79,148 cases, 61,106 controls). The renal cell carcinoma dataset (male: 3,227 cases, 4,916 controls; female: 1,992 cases, 3,095 controls) was downloaded from GWAS Catalog (https://www.ebi.ac.uk/gwas/). *Table 1* provided detailed information.

Selection of IVs

For forward MR analysis, the significance level of IVs associated with plasma metabolites was set to 1×10^{-5}. For obtaining IVs with independent loci, we set the linkage disequilibrium (LD) threshold of $r^2<0.001$ and clumping distance =10,000 kb in 1,000 Genomes European data using the "TwoSampleMR" package. We calculated F-statistics to verify IV strength and mitigate weak instrumental bias, F-statistics greater than 10 were considered to be sufficiently strong for the correlation between IVs and exposure. F-statistics were calculated through the following formula: $F = R^2*(n - k - 1)/k*(1 - R^2)$, where "n" signifies the sample size, "k" denotes the number of IVs, and "R^2" indicates the portion of the exposure variance elucidated by the IVs (41). We adopted the following formula to calculate R^2 for more than 10 single nucleotide polymorphisms (SNPs): $R^2=2*(beta^2)*EAF*(1 - EAF)/[2*(beta^2)*EAF*(1 - EAF) + 2*(SE^2)*N*EAF*(1 - EAF))$; whereas for less than 10 SNPs we used: $R^2=2*(beta^2)*EAF*(1 - EAF)$, where EAF identifies the effect allele frequency, beta denotes the effect size of SNP on exposure, N is the sample size, and SE is the standard error of the beta (42). Last but not least, we used "PhenoScanner" (http://www.phenoscanner.medschl.cam.ac.uk/) to eliminate SNPs that definitely correlated with cancer risk factors.

In the reverse MR analysis, we used plasma metabolites with positive results in forward MR as the outcome and corresponding cancers as the exposure to explore the causality of each. We screened for IVs related to cancers at threshold ($P<5\times10^{-8}$ for breast cancer, colorectal cancer, endometrial cancer, lung cancer, and ovarian cancer; $P<1\times10^{-6}$ for bladder cancer and pancreatic cancer; $P<1\times10^{-5}$ for brain cancer, cervical cancer, and oropharynx cancer). The remaining steps of the IVs screening in reverse MR analysis were similar to that described above for forward MR analysis.

Statistical analysis

All statistical analyses were conducted with R software (version 4.3.1) using the package "TwoSampleMR" (version 0.5.7). To evaluate the causal relationship between 1,400 plasma metabolite and 24 types of cancers, the fixed/random-effects inverse variance-weighted (IVW) method, weighted median method, simple mode, weighted mode, and MR-Egger regression were mainly performed. The IVW method, which provides the most precise effect estimates, was performed as the primary analysis in this study and for almost all MR-analysis (43-45). Multiple correction of the IVW P value was performed by the false discovery rate (FDR) method. The Cochran's Q test was conducted to scrutinize the heterogeneity of the selected SNPs. In cases where significant heterogeneity emerged ($P<0.05$), a random-effects IVW method was chosen; if not, a fixed-effects IVW method was applied (46). Moreover, we implemented the MR-Egger intercept test to detect horizontal pleiotropy (statistically significant if $P<0.05$) (47). Leave-one-out analysis was performed to assess whether there was a pleiotropic effect on the results after the removal of individual SNP (48). In addition, the MR pleiotropy residual sum and outlier (MR-PRESSO) test which conducted a global test of heterogeneity was used to identify potential outliers in each SNP and obtain a corrected association result after removing the potential outliers (49). In order to increase the reliability and stability of the results, we used five criteria for screening positive results: (I) P value of IVW was less than 0.05; (II) P value of FDR-corrected IVW was less than 0.2; (III) the trend of

OR values of the five MR analytical methods was consistent; (IV) MR-Egger intercept test P value was greater than 0.05; (V) MR-PRESSO global test P value was greater than 0.05. Lastly, reverse MR analysis was conducted to investigate any reverse causal relationship between 10 types of cancers and plasma metabolites.

Results

Causality of plasma metabolites on tumorigenesis

Following a series of rigorous quality control procedures as described above, 34,843 SNP associated with 1,400 plasma metabolites were selected as IVs for MR analysis (table available at https://cdn.amegroups.cn/static/public/tcr-24-359-2.xlsx). Based on a set of rigorous screening criteria, we identified suggestive evidence that 2 plasma metabolites were associated with bladder cancer risk, 1 plasma metabolite with Brain cancer risk, 6 plasma metabolites with Breast cancer risk, 12 plasma metabolites with cervical cancer risk, 7 plasma metabolites with colorectal cancer risk, 10 plasma metabolites with endometrial cancer risk, 3 plasma metabolites with lung cancer risk, 1 plasma metabolite with oropharynx cancer risk, 10 plasma metabolites with ovarian cancer risk, and 3 plasma metabolites with pancreatic cancer risk (*Figure 2*). Summary information for IVs used in these significant results was listed in the online table (available at https://cdn.amegroups.cn/static/public/tcr-24-359-3.xlsx). An analysis of five MR analysis methods and sensitivity analysis further confirmed the robustness of the causal associations (table available at https://cdn.amegroups.cn/static/public/tcr-24-359-4.xlsx). Specifically, both the MR-Egger intercept test and the MR-PRESSO global test ruled out the possibility of horizontal pleiotropy (*Table 2*). Forest plots, funnel plots, leave-one-out plots, and scatter plots also indicated the stability of the results (Figures S1-S4).

Genitourinary systems

In the urinary system, a decrease in 5-acetylamino-6-formylamino-3-methyluracil levels [odds ratio (OR) =0.837, 95% confidence interval (CI): 0.765–0.916, P=1.07E–04, FDR =0.075) and an increase in paraxanthine to 5-acetylamino-6-formylamino-3-methyluracil ratio (OR =1.215, 95% CI: 1.113–1.327, P=1.36E–05, FDR =0.019) were positively associated with bladder carcinogenesis (*Figure 2*). In the reproductive system, 32 metabolites were causally associated with cervical, endometrial, and ovarian cancers. As presented in *Figure 2*, we found that N-alpha-acetylornithine levels (OR =1.002, 95% CI: 1.001–1.003, P=1.02E–04, FDR =0.020), N-acetylleucine levels (OR =1.001, 95% CI: 1.000–1.001, P=1.51E–04, FDR =0.023), N-acetyltyrosine levels (OR =1.001, 95% CI: 1.000–1.001, P=5.25E–05, FDR =0.018), N-acetyl-L-glutamine levels (OR =1.001, 95% CI: 1.000–1.001, P=8.13E–05, FDR =0.019), N-acetylarginine levels (OR =1.001, 95% CI: 1.000–1.001, P=2.55E–05, FDR =0.018), N-acetylcitrulline levels (OR =1.001, 95% CI: 1.000–1.001, P=3.94E–05, FDR =0.018), and N-acetyl-1-methylhistidine levels (OR =1.001, 95% CI: 1.000–1.001, P=1.36E–04, FDR =0.023) increased the risk of cervical cancer, while alliin levels (OR =0.999, 95% CI: 0.998–1.000, P=1.64E–03, FDR =0.191), methionine sulfone levels (OR =0.999, 95% CI: 0.998–0.999, P=1.45E–05, FDR =0.018), gamma-glutamylcitrulline levels (OR =0.998, 95% CI: 0.997–0.999, P=5.04E–04, FDR =0.070), X-24518 levels (OR =0.999, 95% CI: 0.998–1.000, P=1.33E–03, FDR =0.168), and N-delta-acetylornithine levels (OR =0.999, 95% CI: 0.999–1.000, P=7.65E–05, FDR =0.019) were negatively associated with the risk of cervical cancer. Meanwhile, we identified a total of ten plasma metabolites that were positively associated with the risk of endometrial cancer, including tetradecanedioate (C14-DC) levels, hexadecanedioate (C16-DC) levels, 5alpha-androstan-3alpha,17beta-diol monosulfate (1) levels, 1-oleoyl-glycero-3-phosphorylglycerol (GPG) (18:1) levels, glycodeoxycholate 3-sulfate levels, 1-linoleoyl-GPG (18:2) levels, octadecenedioate (C18:1-DC) levels, glutarate (C5-DC) levels, X-22509 levels, and adenosine 5'-diphosphate (ADP) to pantothenate ratio. As for ovarian cancer, 10 plasma metabolites were statistically associated with the risk of ovarian cancer, including 4 protective risk factors [2R,3R-dihydroxybutyrate levels, linolenoylcarnitine (C18:3) levels, N-lactoyl phenylalanine levels, and spermidine to N-acetylputrescine ratio] and 6 risk factors [5-acetylamino-6-amino-3-methyluracil levels, ceramide (d18:1/14:0, d16:1/16:0) levels, hydroxypalmitoyl sphingomyelin [d18:1/16:0(OH)] levels, X-12221 levels, X-12410 levels, and 5-acetylamino-6-formylamino-3-methyluracil levels]. In summary, our results revealed specific plasma metabolites associated with the risk of genitourinary tumors and provided new perspectives for human understanding of the pathogenesis of genitourinary tumors.

Cancer type (Outcome)	Plasma metabolites/metabolite ratios (Exposure)	N.SNP	P (IVW)	FDR (IVW)	OR (95% CI)
Bladder cancer	5-acetylamino-6-formylamino-3-methyluracil levels	21	1.07E−04	0.075	0.837 (0.765–0.916)
	Paraxanthine to 5-acetylamino-6-formylamino-3-methyluracil ratio	25	1.36E−05	0.019	1.215 (1.113–1.327)
Brain cancer	Adenosine 5'-monophosphate (AMP) to flavin adenine dinucleotide (FAD) ratio	16	9.69E−06	0.014	1.894 (1.427–2.514)
Breast cancer	Docosadienoate (22:2n6) levels	19	6.61E−04	0.154	1.083 (1.035–1.134)
	Myristoleate (14:1n5) levels	21	1.74E−04	0.081	1.103 (1.048–1.160)
	Cysteinylglycine disulfide levels	19	5.45E−05	0.076	1.059 (1.030–1.088)
	3-bromo-5-chloro-2,6-dihydroxybenzoic acid levels	26	1.06E−03	0.185	0.949 (0.919–0.979)
	Histidine to pyruvate ratio	23	9.14E−04	0.183	1.062 (1.025–1.100)
	Caffeine to paraxanthine ratio	26	6.47E−04	0.154	0.938 (0.904–0.973)
Cervical cancer	N-alpha-acetylornithine levels	5	1.02E−04	0.020	1.002 (1.001–1.003)
	Alliin levels	6	1.64E−03	0.191	0.999 (0.998–1.000)
	Methionine sulfone levels	15	1.45E−05	0.018	0.999 (0.998–0.999)
	Gamma-glutamylcitrulline levels	7	5.04E−04	0.070	0.998 (0.997–0.999)
	N-acetylleucine levels	7	1.51E−04	0.023	1.001 (1.000–1.001)
	X-24518 levels	6	1.33E−03	0.168	0.999 (0.998–1.000)
	N-acetyltyrosine levels	5	5.25E−05	0.018	1.001 (1.000–1.001)
	N-acetyl-L-glutamine levels	10	8.13E−05	0.019	1.001 (1.000–1.001)
	N-acetylarginine levels	11	2.55E−05	0.018	1.001 (1.000–1.001)
	N-acetylcitrulline levels	7	3.94E−05	0.018	1.001 (1.000–1.001)
	N-acetyl-1-methylhistidine levels	12	1.36E−04	0.023	1.001 (1.000–1.001)
	N-delta-acetylornithine levels	7	7.65E−05	0.019	0.999 (0.999–1.000)
Colorectal cancer	1,2-dipalmitoyl-GPC (16:0/16:0) levels	27	7.37E−06	0.005	1.133 (1.073–1.197)
	1-palmitoyl-2-stearoyl-GPC (16:0/18:0) levels	32	1.05E−05	0.005	1.119 (1.065–1.177)
	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) levels	32	6.24E−04	0.109	1.076 (1.032–1.121)
	1-oleoyl-2-linoleoyl-GPE (18:1/18:2) levels	33	9.02E−04	0.140	0.929 (0.889–0.970)
	1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6) levels	31	4.04E−06	0.005	1.083 (1.047–1.120)
	Retinol (Vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	23	1.19E−04	0.033	0.903 (0.857–0.951)
	Arachidonate (20:4n6) to linoleate (18:2n6) ratio	24	1.58E−04	0.033	1.112 (1.053–1.175)
Endometrial cancer	Tetradecanedioate (C14-DC) levels	18	8.78E−04	0.146	1.106 (1.042–1.174)
	Hexadecanedioate (C16-DC) levels	22	4.94E−08	0.160	1.120 (1.045–1.201)
	5alpha-androstan-3alpha,17beta-diol monosulfate (1) levels	21	1.37E−03	<0.001	1.148 (1.092–1.206)
	1-oleoyl-GPG (18:1) levels	26	1.24E−03	0.158	1.104 (1.039–1.172)
	Glycodeoxycholate 3-sulfate levels	29	2.09E−03	0.196	1.086 (1.030–1.144)
	1-linoleoyl-GPG (18:2) levels	20	1.61E−04	0.057	1.134 (1.062–1.211)
	Octadecenedioate (C18:1-DC) levels	23	2.10E−03	0.196	1.104 (1.036–1.175)
	Glutarate (C5-DC) levels	23	3.01E−04	0.082	1.175 (1.077–1.283)
	X-22509 levels	20	3.50E−04	0.082	1.153 (1.066–1.246)
	Adenosine 5'-diphosphate (ADP) to pantothenate ratio	15	1.62E−04	0.057	1.184 (1.084–1.293)
Lung cancer	5alpha-androstan-3beta,17alpha-diol disulfate levels	23	4.56E−05	0.022	1.193 (1.096–1.298)
	1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels	27	2.66E−07	<0.001	0.822 (0.763–0.886)
	1-stearoyl-2-arachidonoyl-GPC (18:0/20:4) levels	32	1.34E−04	0.047	1.094 (1.045–1.145)
Oropharynx cancer	3-acetylphenol sulfate levels	15	4.04E−05	0.057	0.448 (0.305–0.657)
Ovarian cancer	5-acetylamino-6-amino-3-methyluracil levels	34	2.72E−05	0.038	1.116 (1.060–1.174)
	2R,3R-dihydroxybutyrate levels	30	2.87E−04	0.100	0.905 (0.857–0.955)
	Ceramide (d18:1/14:0, d16:1/16:0) levels	26	2.65E−04	0.100	1.122 (1.055–1.194)
	Linolenoylcarnitine (C18:3) levels	21	8.39E−04	0.186	0.876 (0.810–0.947)
	N-lactoyl phenylalanine levels	16	1.17E−03	0.186	0.849 (0.769–0.937)
	Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH)) levels	31	1.40E−03	0.196	1.096 (1.036–1.160)
	X-12221 levels	23	1.20E−03	0.186	1.130 (1.050–1.217)
	X-12410 levels	25	9.89E−04	0.186	1.114 (1.045–1.187)
	5-acetylamino-6-formylamino-3-methyluracil levels	22	1.07E−04	0.111	1.071 (1.031–1.113)
	Spermidine to N-acetylputrescine ratio	20	1.85E−04	0.100	0.893 (0.841–0.947)
Pancreatic cancer	X-18888 levels	27	3.15E−04	0.183	1.334 (1.140–1.560)
	X-24494 levels	17	4.43E−04	0.183	0.722 (0.602–0.866)
	Retinol (Vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	24	1.19E−04	0.125	1.365 (1.168–1.596)

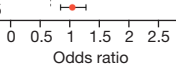

Figure 2 Significant MR results of causal effects between plasma metabolites and cancer risk. N.SNP, the number of SNPs used as IVs; SNP, single-nucleotide polymorphism; IVs, instrumental variables; IVW, inverse variance-weighted; FDR, false discovery rate; OR, odds ratio; CI, confidence interval; MR, mendelian randomization; GPC, glycerylphosphorylcholine; GPG, glycerophosphoethanolamine; GPE, glycero-3-phosphorylglycerol.

Table 2 The horizontal pleiotropy test results of causal effects between plasma metabolites and cancer risk

Cancer type (outcome)	Plasma metabolites/metabolite ratios (exposure)	MR-Egger intercept test P value	MR-PRESSO global test P value
Bladder cancer	5-acetylamino-6-formylamino-3-methyluracil levels	0.791	0.492
	Paraxanthine to 5-acetylamino-6-formylamino-3-methyluracil ratio	0.566	0.327
Brain cancer	Adenosine 5'-monophosphate (AMP) to flavin adenine dinucleotide (FAD) ratio	0.901	0.855
Breast cancer	Docosadienoate (22:2n6) levels	0.740	0.302
	Myristoleate (14:1n5) levels	0.226	0.137
	Cysteinylglycine disulfide levels	0.167	0.526
	3-bromo-5-chloro-2,6-dihydroxybenzoic acid levels	0.501	0.332
	Histidine to pyruvate ratio	0.261	0.872
	Caffeine to paraxanthine ratio	0.930	0.068
Cervical cancer	N-alpha-acetylornithine levels	0.220	0.499
	Alliin levels	0.195	0.406
	Methionine sulfone levels	0.770	0.943
	Gamma-glutamylcitrulline levels	0.963	0.429
	N-acetylleucine levels	0.567	0.837
	X-24518 levels	0.537	0.891
	N-acetyltyrosine levels	0.912	0.729
	N-acetyl-L-glutamine levels	0.554	0.647
	N-acetylarginine levels	0.936	0.968
	N-acetylcitrulline levels	0.845	0.811
	N-acetyl-1-methylhistidine levels	0.433	0.595
	N-delta-acetylornithine levels	0.744	0.846
Colorectal cancer	1,2-dipalmitoyl-GPC (16:0/16:0) levels	0.814	0.783
	1-palmitoyl-2-stearoyl-GPC (16:0/18:0) levels	0.927	0.570
	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) levels	0.585	0.105
	1-oleoyl-2-linoleoyl-GPE (18:1/18:2) levels	0.054	0.074
	1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6) levels	0.176	0.470
	Retinol (vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	0.674	0.585
	Arachidonate (20:4n6) to linoleate (18:2n6) ratio	0.206	0.183
Endometrial cancer	Tetradecanedioate (C14-DC) levels	0.146	0.554
	Hexadecanedioate (C16-DC) levels	0.608	0.119
	5alpha-androstan-3alpha,17beta-diol monosulfate (1) levels	0.638	0.429
	1-oleoyl-GPG (18:1) levels	0.588	0.334
	Glycodeoxycholate 3-sulfate levels	0.183	0.313
	1-linoleoyl-GPG (18:2) levels	0.109	0.598

Table 2 (*continued*)

Table 2 (*continued*)

Cancer type (outcome)	Plasma metabolites/metabolite ratios (exposure)	MR-Egger intercept test P value	MR-PRESSO global test P value
	Octadecenedioate (C18:1-DC) levels	0.053	0.236
	Glutarate (C5-DC) levels	0.211	0.854
	X-22509 levels	0.886	0.966
	Adenosine 5'-diphosphate (ADP) to pantothenate ratio	0.833	0.794
Lung cancer	5alpha-androstan-3beta,17alpha-diol disulfate levels	0.361	0.662
	1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels	0.584	0.602
	1-stearoyl-2-arachidonoyl-GPC (18:0/20:4) levels	0.569	0.579
Oropharynx cancer	3-acetylphenol sulfate levels	0.189	0.561
Ovarian cancer	5-acetylamino-6-amino-3-methyluracil levels	0.555	0.431
	2R,3R-dihydroxybutyrate levels	0.997	0.913
	Ceramide (d18:1/14:0, d16:1/16:0) levels	0.144	0.740
	Linolenoylcarnitine (C18:3) levels	0.680	0.462
	N-lactoyl phenylalanine levels	0.596	0.889
	Hydroxypalmitoyl sphingomyelin [d18:1/16:0(OH)] levels	0.736	0.761
	X-12221 levels	0.286	0.787
	X-12410 levels	0.385	0.375
	5-acetylamino-6-formylamino-3-methyluracil levels	0.224	0.940
	Spermidine to N-acetylputrescine ratio	0.129	0.734
Pancreatic cancer	X-18888 levels	0.602	0.980
	X-24494 levels	0.269	0.401
	Retinol (vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	0.303	0.924

MR, Mendelian randomization; PRESSO, pleiotropy residual sum and outlier; GPC, glycerylphosphorylcholine; GPG, glycerophosphoethanolamine; GPE, glycero-3-phosphorylglycerol.

Digestive systems

In the digestive system, 1,2-dipalmitoyl-glycerylphosphorylcholine (1,2-dipalmitoyl-GPC) (16:0/16:0) levels (OR =1.133, 95% CI: 1.073–1.197, P=7.37E–06, FDR =0.005), 1-palmitoyl-2-stearoyl-GPC (16:0/18:0) levels (OR =1.119, 95% CI: 1.065–1.177, P=1.05E–05, FDR =0.005), 1-stearoyl-2-arachidonoyl-glycerophosphoethanolamine (GPE) (18:0/20:4) levels (OR =1.076, 95% CI: 1.032–1.121, P=6.24E–04, FDR =0.109), 1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6) levels (OR =1.083, 95% CI: 1.047–1.120, P=4.04E–06, FDR =0.005), and arachidonate (20:4n6) to linoleate (18:2n6) ratio (OR =1.112, 95% CI: 1.053–1.175, P=1.58E–04, FDR =0.033) were positively correlated with the risk of colorectal cancer, while 1-oleoyl-2-linoleoyl-GPE (18:1/18:2) levels (OR =0.929, 95% CI: 0.889–0.970, P=9.02E–04, FDR =0.140) and retinol (vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) ratio (OR =0.903, 95% CI: 0.857–0.951, P=1.19E–04, FDR =0.033) were negatively associated with colorectal cancer (*Figure 2*, *Table 2*). In addition, high X-18888 levels (OR =1.334, 95% CI: 1.140–1.560, P=3.15E–04, FDR =0.183) and high retinol (vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) ratio (OR =1.365, 95% CI: 1.168–1.596, P=1.19E–04, FDR =0.125) could promote the onset of pancreatic cancer, while X-24494 levels (OR =0.722, 95% CI: 0.602–0.866, P=4.43E–04, FDR =0.183) could be a protective factor against pancreatic

cancer (*Figure 2*, *Table 2*). Notably, the retinol (vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) ratio plays diametrically opposed roles in colorectal and pancreatic cancers, which may suggest differences in metabolic levels pathway between the two cancers. Our results illustrate that in digestive system tumors, different tissue origins have different metabolic backgrounds, which may suggest new solutions for screening cancers of the digestive tract.

Other cancers

We also found that high 5alpha-androstan-3beta,17alpha-diol disulfate levels (OR =1.193, 95% CI: 1.096–1.298, P=4.56E−05, FDR =0.022) and high 1-stearoyl-2-arachidonoyl-GPC (18:0/20:4) levels (OR =1.094, 95% CI: 1.045–1.145, P=1.34E−04, FDR =0.047) were causally associated with lung cancer development, whereas 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels (OR =0.822, 95% CI: 0.763–0.886, P=2.66E−07, FDR <0.001) was a protective factor for lung cancer. As for breast cancer, 6 plasma metabolites were statistically associated with the risk of breast cancer, including 2 protective factors (3-bromo-5-chloro-2,6-dihydroxybenzoic acid levels and Caffeine to paraxanthine ratio) and 4 risk factors [docosadienoate (22:2n6) levels, myristoleate (14:1n5) levels, cysteinylglycine disulfide levels, and histidine to pyruvate ratio]. Meanwhile, 3-acetylphenol sulfate levels (OR =0.448, 95% CI: 0.305–0.657, P=4.04E−05, FDR =0.057) were negatively associated with the risk of oropharynx cancer (*Figure 2*, *Table 2*). In brain cancer, adenosine 5'-monophosphate (AMP) to flavin adenine dinucleotide (FAD) ratio (OR =1.894, 95% CI: 1.427–2.514, P=9.69E−06, FDR =0.014) is a risk factor for tumorigenesis (*Figure 2*, *Table 2*). This suggests that high energy conversion rate metabolism is selected for brain cancer. Therapeutic agents targeting the correction of excessive metabolism may yield favorable results in brain cancer.

In conclusion, our results indicated that tumors from different tissue sources have different metabolic profiles and different metabolites. At the same time, different plasm metabolites also respond to different lifestyle habits of the population, and poor lifestyle habits are responsible for metabolic derangement and causing tumorigenesis. Our study provides a basis for the health management of the population. Finally, our results demonstrate a causal relationship between different plasm metabolites and different tumors, which makes non-invasive cancer screening possible.

Causality of tumorigenesis on plasma metabolites

To explore the causal effects of cancers on plasma metabolites, reverse MR analyses were conducted and 1,390 SNP associated with 10 tumors were selected as IVs for reverse MR analysis (table available at https://cdn.amegroups.cn/static/public/tcr-24-359-5.xlsx). Remarkably, we screened the only plasma metabolite 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels (OR =0.902, 95% CI: 0.836–0.973, P=0.008, FDR =0.024) with causality by IVW method in lung cancer patients with FDR value less than 0.2 (*Figure 3A*). Meanwhile, both the MR-Egger intercept test and the MR-PRESSO global test ruled out the possibility of horizontal pleiotropy (table available at https://cdn.amegroups.cn/static/public/tcr-24-359-6.xlsx). In addition, the OR values calculated by the five methods were in the same direction (*Figure 3A*,*3B*). Methodological scatter plots, forest plots of effect sizes, leave-one-out plots, and funnel plots all indicated reliable results (*Figure 3B-3E*). Interestingly, the plasm metabolite 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels had a negative causal association with lung cancer in forward MR above, which is consistent with the results of reverse MR analyses. This suggests that lecithin metabolism may play a pivotal role in both lung cancer development and progression. In summary, our results may point to a novel way of understanding the specific mechanisms of lung cancer development.

Discussion

Based on a large amount of publicly available genome-wide data, we explored causal associations between 1,400 plasma metabolites and 24 cancers. To the best of our knowledge, this is the first MR analysis to explore the causal relationship between plasma metabolites and pan-cancer. It was found that 53 metabolites had substantial causal associations with ten malignancies of different tissue origins in this study (FDR <0.2, IVW method). These plasma metabolites are not only star molecules in glucose metabolism, fatty acid metabolism, and amino acid metabolism but are also involved in phospholipid metabolism, caffeine metabolism, and amino acid acetylation. In conclusion, our results demonstrate the diversity of metabolic profiles of malignant tumors and provide a molecular basis for cancer detection and drug targeting.

In cervical cancer, 11 plasma metabolites were associated with amino acid metabolism (X-24518 role is currently unknown) and eight plasma metabolites carried N-terminal

A

Cancer type (Exposure)	Plasma metabolites/metabolite ratios (Outcome)	N.SNP	Method	P value	FDR	OR (95% CI)
Lung cancer	1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels	5	MR Egger	0.464	0.696	0.877 (0.646–1.192)
Lung cancer	1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels	5	Weighted median	0.040	0.120	0.910 (0.832–0.996)
Lung cancer	1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels	5	Inverse variance weighted	0.008	0.024	0.902 (0.836–0.973)
Lung cancer	1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels	5	Simple mode	0.349	0.976	0.931 (0.815–1.063)
Lung cancer	1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels	5	Weighted mode	0.145	0.436	0.910 (0.822–1.008)

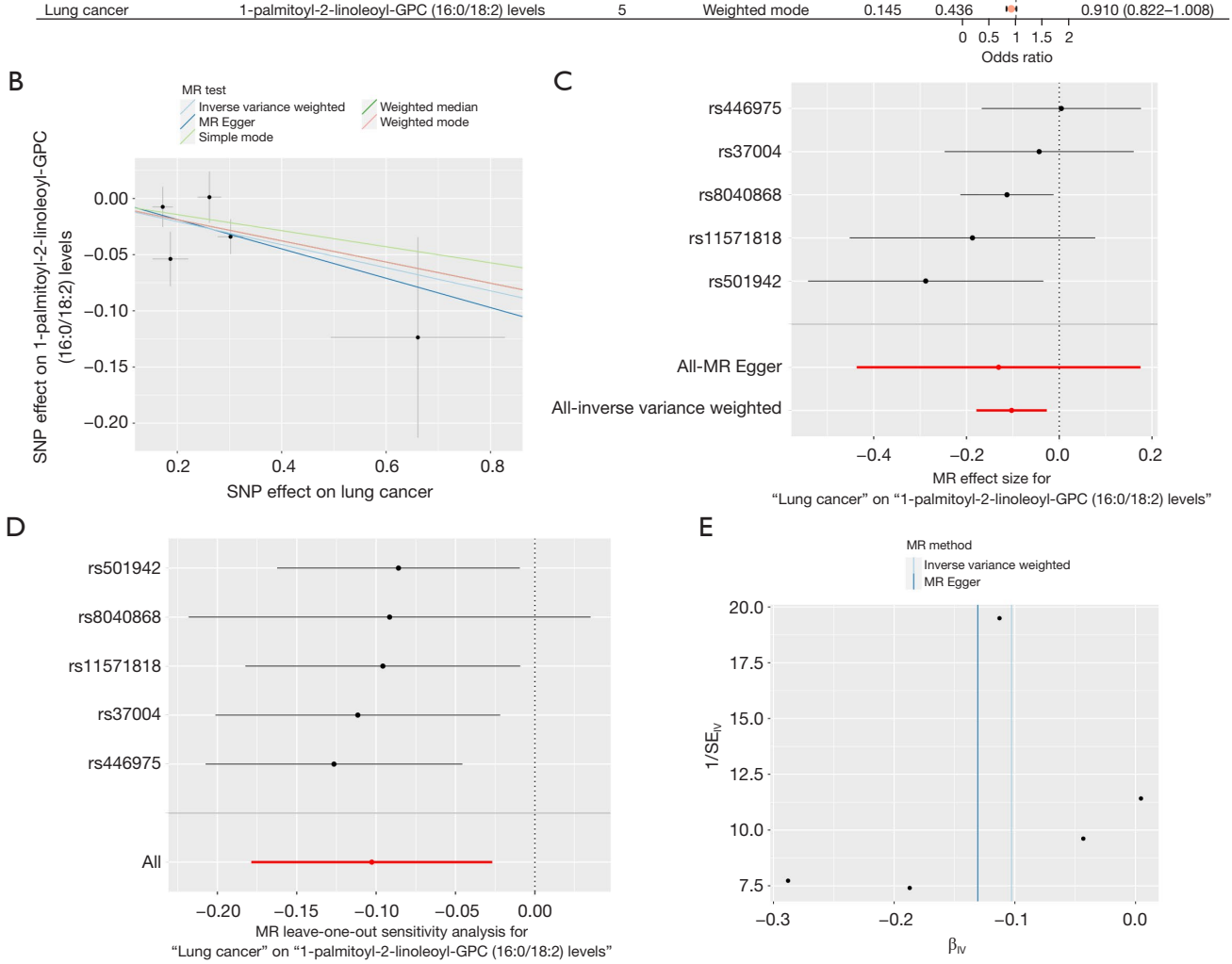

Figure 3 Significant results of reverse MR. (A) MR results of causal effects between lung cancer risk and 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels. (B) Scatter plots of causality, the slope of each line corresponding to the estimated MR effect in different models. (C) Forest plot with the estimated MR effect of each IV in IVW and MR-Egger models. (D) Leave one out of sensitivity tests. Calculate the MR results of the remaining IVs after removing the IVs one by one. (E) Funnel plot from genetically predicted 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels on lung cancer. N.NSP, the number of SNPs used as IVs; SNP, single-nucleotide polymorphism; IVs, instrumental variables; FDR, false discovery rate; OR, odds ratio; CI, confidence interval; MR, Mendelian randomization; SE, standard error; GPC, glycerylphosphorylcholine.

amino acid acetylation, suggesting a specific relationship between cervical cancer and protein acetylation modifications. Protein acetylation is a widespread and reversible post-translational protein modification that regulates biological behaviors such as cellular metabolism, division, and signaling by acetylating thousands of histones and non-histone proteins in cells through lysine deacetylases (KDACs) and lysine acetyltransferases (KATs) (50). In our results, eight plasma metabolites with N-terminal acetylation were causally associated with

cervical cancer, but in inverse MR analysis, cervical cancer was not causally associated with all 11 plasma metabolites, suggesting that amino acid acetylation may be associated with cervical carcinogenesis only. Human papilloma virus (HPV), the main cause of cervical cancer, can induce H3K9 acetylation and affect chromatin modification through its early genes, E6 and E7, which in turn directly or indirectly affects apoptosis, proliferation, and growth, leading to tumorigenesis (51-54), which is consistent with our results. Three other causally related plasma metabolites, gamma-glutamylcitrulline, methionine sulfone, and alliin, have also been shown to have inhibitory effects on other malignant tumors (55-59), which provides a sidebar to the potential of these three metabolites to inhibit cervical cancer, consistent with our results.

In endometrial cancer, the scenario changes. 1-linoleoyl-GPG (18:2) is a regulator of fatty acid metabolism, which can promote tumorigenesis by regulating key enzymes of lipid metabolism (60,61). Zhao *et al.* demonstrated that abnormal fatty acid metabolism can directly promote endometrial cancer formation and invasive migration of endometrial cancer cells through mTOR signaling by observing the intestinal flora and spatial visualization of fatty acids (62). Mozihim *et al.* and Wang *et al.* also illustrated the critical role of uncontrolled excess fatty acid metabolism in the development of endometrial cancers from the perspectives of sexual hormone metabolism and fatty acid metabolism regulatory enzymes, respectively (63-67). In our study, both 1-linoleoyl-GPG (18:2) and tetradecanedioate were risk factors for endometrial cancer, which may be due to the fact that the transporter protein tetradecanedioate can promote tumorigenesis by participating in fatty acid metabolism (63-66). Glycodeoxycholate 3-sulfate is a bile acid metabolite, and its elevation in plasma is also predictive of endometrial cancer, in line with established studies (67,68). In addition, it has been shown that endometrium under the environment of uncontrolled glutarate metabolism develops abnormalities of decidualization, and this abnormality not only leads to miscarriage but also the development of endometrial cancer (69). This is also illustrated by Our results that there is a causal relationship between high plasma glutarate levels and the development of endometrial cancer. Moreover, our results suggest that the metabolite hexadecanedioate (C16-DC), which also leads to abnormal endometrial decidualization, is also causally associated with the development of endometrial cancer (FDR <0.2, IVW method), which is the first report of this kind to the best of our knowledge.

In this study, ovarian cancer was causally linked to a variety of plasma metabolites, including amino acid metabolism, lipid metabolism, and caffeine metabolism (the role of X-12221 and X-12410 is unknown at this time). In our results, both 5-acetylamino-6-formylamino-3-methyluracil and 5-acetylamino-6-formylamino-3-methyluracil are products of caffeine metabolism (70) and our results that the more of these metabolites, the higher the probability of developing ovarian cancer. In previous prospective studies, caffeine intake predicted a better prognosis in ovarian cancer (71), and the same results were obtained in *in vitro* experiments (72). However, whether caffeine intake has a causal relationship with the occurrence of ovarian cancer is still controversial in current studies (73,74). We speculate that caffeine and its metabolites may play different roles in ovarian carcinogenesis and progression, which needs to be explored in further studies. Meanwhile, the relationship between caffeine and metabolites also needs more explicit evidence. Sphingomyelin is composed of ceramide with either choline phosphate or ethanolamine phosphate attached to the hydroxyl group (75). Our results show that both hydroxypalmitoyl sphingomyelin [d18:1/16:0(OH)] levels and ceramide (d18:1/14:0, d16:1/16:0) levels can promote ovarian carcinogenesis. Existing studies illustrate that sphingomyelin can contribute to poor prognosis by activating AKT/mTOR/4EBP1 (75), mediate cisplatin resistance in ovarian cancer by altering ceramide 1-phosphate (C1P) activity (76), and enhance the viability of tumor cells in harsh metabolic environments (77), they proved the reliability of our results on the other hand.

Our results indicate that breast cancer is similarly associated with multiple metabolic pathways, including caffeine metabolism, histidine-pyruvate-mediated glucose metabolism, and lipid metabolism. In breast cancer, we used the caffeine-to-paraxanthine ratio to evaluate the direction of the caffeine metabolic pathway. Paraxanthine is an intermediate in the caffeine metabolic pathway, which serves as a substrate for the synthesis of caffeine or continues to be metabolized into 5-acetylamino-6-amino-3-methyluracil and 5-acetylamino-6-formylamino-3-methyluracil (70). Our results showed that the caffeine to paraxanthine ratio was inversely related to the occurrence of breast cancer. Jiang *et al.* and Zheng *et al.* conducted a meta-analysis of 37 published studies and found that caffeine intake was negatively associated with the risk of breast cancer in postmenopausal women (78,79). Bellerba *et al.* used metformin to lower breast cancer recurrence

rates and found that paraxanthine was lower in a population with low breast cancer recurrence rates (80). These studies partially explain our results from both caffeine and paraxanthine perspectives. Histidine is one of the essential amino acids, which can be partially synthesized in adults through the gluconeogenic pathway (pyruvate as a raw material), can protect tumor cells to a certain extent from harsh environments, and has some potential as a plasma marker (81). In this study, we used histidine to pyruvate ratio to show the possible direction of histidine metabolism and found a positive correlation with breast cancer development, which is consistent with the results of existing studies (81), which may serve as a new prognostic marker for tumor patients.

In bladder cancer, our results could not circumvent the caffeine metabolic pathway. As mentioned above, 5-acetylamino-6-formylamino-3-methyluracil is one of the end products of the caffeine metabolic pathway, while paraxanthine is an intermediate product of caffeine metabolism (70). Cui *et al.* found that low levels of 5-acetylamino-6-formylamino-3-methyluracil levels in urine were associated with the development of bladder cancer by first giving caffeine to subjects and then testing their urine (82). Landi *et al.* explained the effect of caffeine on bladder carcinogenesis by detecting changes in the levels of 5-acetylamino-6-formylamino-3-methyluracil levels in the blood of subjects after caffeine consumption by affecting the activity of cytochrome P4501A2 (CYP1A2), indicating that acetylamino-6-formylamino-3-methyluracil is a protective factor against bladder cancer (83). In our results, there was a causal relationship between low levels of 5-acetylamino-6-formylamino-3-methyluracil levels and bladder carcinogenesis, and a causal relationship between high paraxanthine to 5-acetylamino-6-formylamino-3-methyluracil ratio and bladder cancer, and these results are consistent with the above studies (84). However, we note that all causal relationships between caffeine metabolic pathways and tumors avoid the role of caffeine itself. Further studies are needed to clarify the specific role of caffeine and its metabolites in the body and *in vitro*.

Retinol (vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) ratio plays different roles in colorectal and pancreatic cancer. Our results indicate that the retinol (vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) ratio is inversely associated with colorectal carcinogenesis but promotes pancreatic cancer. A meta-analysis has demonstrated that retinol is a protective factor against colorectal carcinogenesis (85), as well as *in vitro* experiments demonstrating that retinol can reduce chronic inflammation of the intestinal mucosa and decrease the incidence of colorectal cancer by inducing foxp3+ regulatory T cells to regulate T cells into the Th1/Th2/Th17/Tregs pathway (86), and in a mouse model, a retinol deficiency leads to dysregulation of T-cell responses, leading to colitis and colorectal cancer (87), in partial agreement with our findings. On the other side, retinol promotes pancreatic development and maintains the normal secretory function of the pancreas (88), and loss of retinol leads to pancreatic astrocyte apoptosis and pancreatic fibrosis (89). The question arises then as to what role the plasma metabolite linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) plays in pancreatic cancer. It has been shown that linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) may be a product formed by esterification of retinol, an intracellular storage form of retinol (90). Linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) may have roles in regulating the ability of the pancreas to metabolize lipids (91) and regulating neutrophil chemotaxis in some tumors (92). However, to the best of our knowledge, there is no evidence to indicate a specific role for linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) in pancreatic and colorectal cancers. Our results suggest that the direct use of retinol (vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) ratio to predict tumorigenesis may be more reliable than the use of one of the plasma metabolites alone.

Disorders of lipid metabolism are the main protagonists of lung cancer. Available evidence indicates that in lung cancer, 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) plays a critical role in regulating tumor cell metabolism (93). Unsaturated short-chain fatty acids are reduced in tumor cell membranes, whereas the increase in saturated long-chain fatty acids is to protect cells from oxidative stress that arises during the development of various cancers (93,94), consistent with our results that 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2), which is a saturated long-chain fatty acid, is a protective factor in lung cancer. Fatty acid metabolism has a precise predictive effect for specific malignant tumors, and our inverse MR results also obtained a causal relationship between lung cancer and 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2). Our results suggest that lung carcinogenesis may lead to decreased 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) levels, implying that 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) may be involved in both lung carcinogenesis and progression, and is a novel and valuable target throughout the whole process of lung cancer prediction, diagnosis, and treatment. Loss of

1-palmitoyl-2-linoleoyl-GPC to an L-α-GPC (also known as choline glycerophosphate) and attachment of a 3-acetyl-rac-glycerol to form 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG), an inhibitory factor in lung cancer (95-97). PLAG inhibits tumor progression in lung cancer animal models by promoting the degradation of adenosine 2B receptors (A2BRs) (96), and PLAG also modulates PAR2/EGFR transactivation by accelerating protease-activated receptor 2 (PAR2) degradation to attenuate metastatic activity in lung cancer (97). In our results, 1-palmitoyl-2-linoleoyl-GPC, which shares the same motif as PLAG, has similar inhibitory effects on lung cancer as PLAG in both forward MR and reverse MR. Unfortunately, there is no definite evidence for the relationship between 1-palmitoyl-2-linoleoyl-GPC and PLAG in metabolism and its direct effect on lung cancer. In conclusion, our results on 1-palmitoyl-2-linoleoyl-GPC are very rare and give a new perspective on plasma cancer detection in lung cancer. Furthermore, our results also suggest that 1-stearoyl-2-arachidonoyl-GPC (18:0/20:4) levels are a high-risk factor for lung carcinogenesis. In existing studies, 1-stearoyl-2-arachidonoyl-GPC (18:0/20:4) can regulate cell growth via exosomes, which in turn promotes lung carcinogenesis (98), is consistent with findings in a mouse model (99), validating the reliability of our results. Our results also revealed a positive correlation between 5alpha-androstan-3beta,17alpha-diol disulfate levels and lung carcinogenesis, which is the first report to our knowledge.

In oropharynx cancer, our results showed that 3-acetylphenol sulfate levels were the only protective factor, which was similarly demonstrated in a recent RCT study (100). 3-acetylphenol sulfate levels are a promising marker for oropharynx cancer with potential as markers for plasma cancer detection in cancer. The major difference between tumor cells and normal tissue cells resides in their higher energy demand and higher metabolic efficiency, and it is no exception in brain cancer (101-103). AMP and FAD are both important energy metabolism intermediates, which can directly participate in electron transfer, form electron chains, and directly generate energy (104-107). The difference is that the same amount of AMP provides more energy and is more efficient than FAD (108,109). Maher *et al.* used [U-13 C]-labeled glucose to trace the metabolic processes of brain cancer and obtained the result that brain cancer has a high metabolic rate (101); Martínez-Reyes *et al.* revisited the high metabolic profile of brain cancer again from the perspective of the mitochondrial electron transport chain (ETC) (102). Based on our findings, AMP to FAD ratio is a risk factor for brain cancer, reflecting the high energy demand of brain cancer, and is a strong corroboration of the results of existing studies (101,102). Notably, in addition to energy metabolism, FAD is also associated with the activity of several biological enzymes, such as spermine oxidase (SMO), which can lead to tumor progression by regulating the biological behavior of tumor cells (110). On the other hand, AMP can directly participate in the activation of adenosine monophosphate-activated protein kinase (AMPK), and then regulate the PI3K signaling pathway, mTOR signaling pathway, Wnt signaling pathway, etc., which can directly affect the survival of tumor cells and play a two-way regulatory role of "inhibiting cancer when cancer is absent and promoting cancer cell growth when cancer is present" (111). This demonstrates the superiority of using ratio rather than AMP or FAD alone to predict brain cancer. Rather than taking into account individual metabolites, we only consider the overall effect. It could be a source of new markers.

There have been several MR articles published investigating the relationship between plasma metabolites and tumors. Despite this, they use fewer types of plasma metabolites than we do, and our data sources are more current and comprehensive. Additionally, they tend to focus on a specific tumor or group of tumors, which can lead to one-sided results due to individual differences. Finally, we used the ratio between plasma metabolites to illustrate their relationship with tumorigenesis, which can avoid the disadvantage that some plasma metabolites play multiple roles in the organism and cannot be judged alone. The overall effect of plasma metabolites on malignant tumors can be more accurately described by our approach, which considers the overall effect. In conclusion, our work provides the most comprehensive atlas to date of the causal relationships between 1,400 plasma metabolites and 24 cancers with the expectation of providing novel ideas for specific tumor plasma markers, plasma cancer detection, and therapeutic targets.

We used published large genome-wide cohorts for our two-sample MR analyses, which is statistically efficient and statistically reliable. Genetic IVs and causal inference were used in this study to derive conclusions. There were no confounding factors such as horizontal polytomies or other confounding factors that affected the results. There are, however, still limitations to our study. First, horizontal polyvalence cannot be completely ruled out even when multiple methods of quality control were conducted. Second, the lack of individual information on participants

prevented us from further stratifying the population. Third, due to the study's European database, the conclusions cannot be generalized to other races, which limits our results' generalizability. Finally, we adapted more flexible thresholds for assessing the results, which may result in more false positives, but this simultaneously enabled us to assess plasma metabolites' association with tumors in a more comprehensive manner.

Conclusions

In summary, our MR study found that there is a causal relationship between plasma metabolites and cancer and that they may play different roles at different times of tumor growth. In addition, our study provides a comprehensive map of plasma metabolites associated with pan-cancer and suggests an important role of biometabolism in tumorigenesis and development, showing the direction of basic cancer research and providing possible targets for plasma cancer detection.

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Footnote

Reporting Checklist: The authors have completed the STROBE-MR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-359/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-359/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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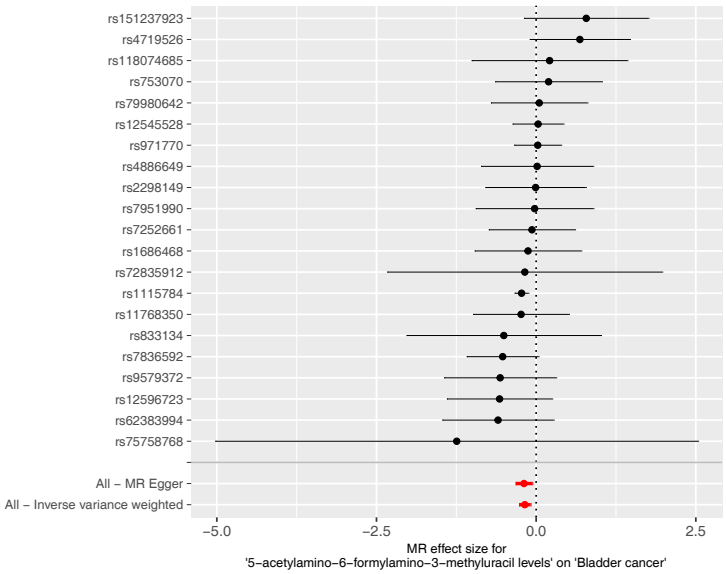

Figure S1 Forest plots of the significant results in forward MR analyses. MR, Mendelian randomization.

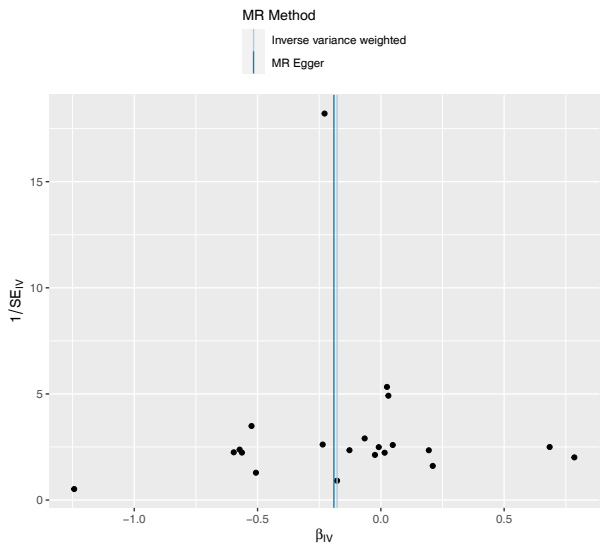

Figure S2 Funnel plots of the significant results in forward MR analyses. MR, Mendelian randomization; SE, standard error.

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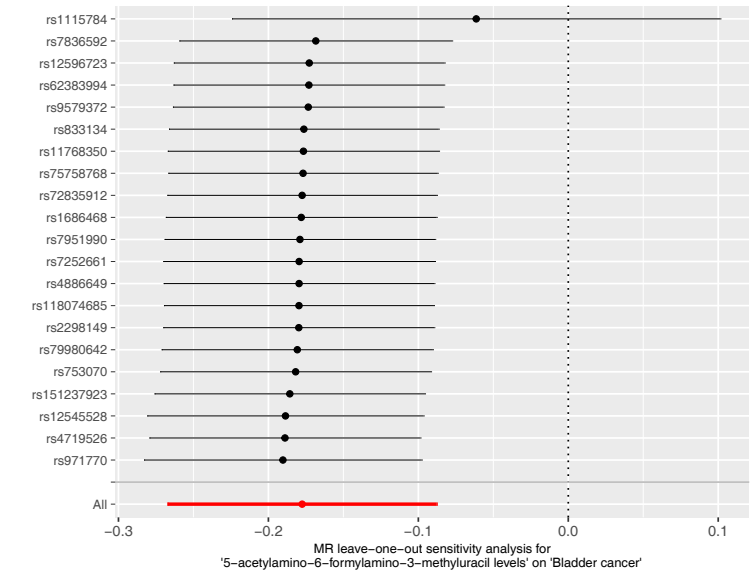

Figure S3 Leave-one-out plots of the significant results in forward MR analyses. MR, Mendelian randomization.

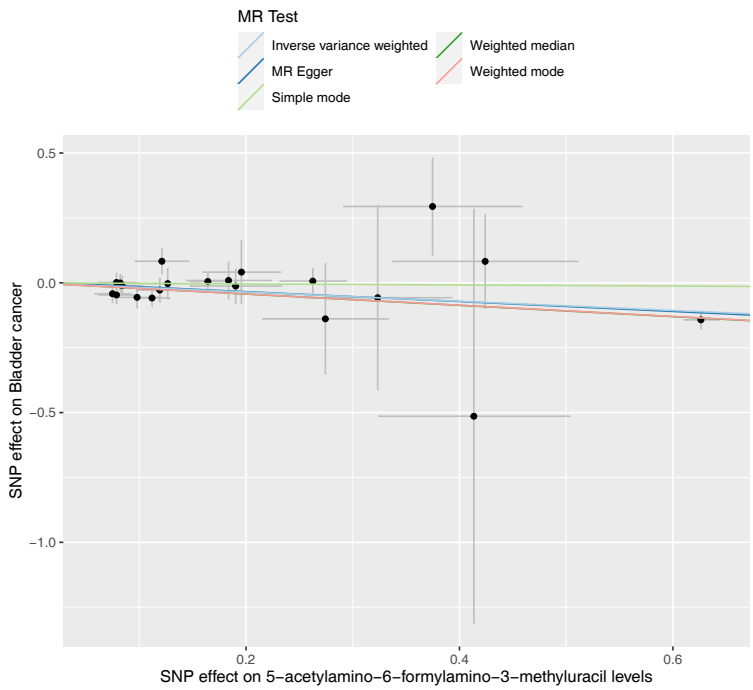

Figure S4 Scatter plots of the significant results in forward MR analyses. MR, Mendelian randomization.

https://dx.doi.org/10.21037/tcr-24-359