Experimental Research

INTERNATIONAL JOURNAL OF SURGERY

OPEN

Unravelling the molecular landscape of endometrial cancer subtypes: insights from multiomics analysis

Yufei Shen, BSc[a,c,d], Yan Tian, PhD[a,c,d], Jiashan Ding, MSc[a,c,d], Zhuo Chen, MD[a,c,d], Rong Zhao, MD[a,c,d], Yingnan Lu, BSc[a,c,d], Lucia Li, BSc[a,c,d], Hui Zhang, BSc[a,c,d], Haiyue Wu, BSc[a,c,d], Xi Li, PhD[b,c,e,*], Yu Zhang, MD, PhD[a,c,d,*]

Background: Endometrial cancer (EC) as one of the most common gynecologic malignancies is increasing in incidence during the past 10 years. Genome-Wide Association Studies (GWAS) extended to metabolic and protein phenotypes inspired us to employ multiomics methods to analyze the causal relationships of plasma metabolites and proteins with EC to advance our understanding of EC biology and pave the way for more targeted approaches to its diagnosis and treatment by comparing the molecular profiles of different EC subtypes.
Methods: Two-sample mendelian randomization (MR) was performed to investigate the effects of plasma metabolites and proteins on risks of different subtypes of EC (endometrioid and nonendometrioid). Pathway analysis, transcriptomic analysis, and network analysis were further employed to illustrate gene-protein-metabolites interactions underlying the pathogenesis of distinct EC histological types.
Results: The authors identified 66 causal relationships between plasma metabolites and endometrioid EC, and 132 causal relationships between plasma proteins and endometrioid EC. Additionally, 40 causal relationships between plasma metabolites and nonendometrioid EC, and 125 causal relationships between plasma proteins and nonendometrioid EC were observed. Substantial differences were observed between endometrioid and nonendometrioid histological types of EC at both the metabolite and protein levels. The authors identified seven overlapping proteins (RGMA, NRXN2, EVA1C, SLC14A1, SLC6A14, SCUBE1, FGF8) in endometrioid subtype and six overlapping proteins (IL32, GRB7, L1CAM, CCL25, GGT2, PSG5) in nonendometrioid subtype and conducted network analysis of above proteins and metabolites to identify coregulated nodes.
Conclusions: Our findings observed substantial differences between endometrioid and nonendometrioid EC at the metabolite and protein levels, providing novel insights into gene-protein-metabolites interactions that could influence future EC treatments.

Keywords: endometrial cancer, mendelian randomization, plasma metabolites, plasma proteins

Background

Endometrial cancer (EC) is one of the most common gynecologic malignancies, with its incidence steadily increasing globally. In 2020, there were 417 000 new cases reported worldwide[1]. With the development of socio-economic factors and changes in people's lifestyles, the incidence of EC has been increasing annually, showing a trend towards affecting younger individuals[2,3].

EC comprises various histological types. The WHO classified EC into several pathological types, including endometrioid carcinoma, serous carcinoma, clear cell carcinoma, mixed carcinoma, undifferentiated carcinoma, carcinosarcoma, and other rare types[4,5]. Traditionally, the endometrioid carcinoma is the most common histological type. Tumors in this category tend to be well-differentiated, with a high positivity for estrogen and progesterone receptors, resulting in a favorable prognosis with a 5-year survival rate of 85.6%[6]. The pathogenesis of this type is mainly associated with prolonged estrogen stimulation in the absence of progesterone antagonism[7]. However, nonendometrioid histological type EC, including serous carcinoma, clear cell carcinoma, and others, usually exhibit high malignancy and poor differentiation. It carries a high risk of recurrence, with a less favorable prognosis and a 5-year survival rate of 58.8%[6]. The etiology of nonendometrioid EC remains unclear, possibly involving

HIGHLIGHTS

- This research showed the gene-protein-metabolites interactions underlying the pathogenesis of distinct EC histological types and laid the foundation for more targeted approaches to its diagnosis and treatment.

[a]Department of Gynecology, Xiangya Hospital, Central South University, [b]Department of Clinical Pharmacology, Xiangya Hospital, Central South University, [c]National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, [d]Gynecological Oncology Research and Engineering Center of Hunan Province and [e]Institute of Clinical Pharmacology, Central South University, Hunan Key Laboratory of Pharmacogenetics, Changsha Hunan, People's Republic of China

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

*Corresponding author. Address: Department of Gynecology, Xiangya Hospital, Central South University, 87 Xiangya Road, Changsha 41008 Hunan, People's Republic of China. Tel.: +86 731 897 537 18. E-mail: xyzhangyu@csu.edu.cn (Y. Zhang), and Department of Clinical Pharmacology, Xiangya Hospital, Central South University, Changsha, Hunan 410008, People's Republic of China. Tel.: +86 731 848 053 80. E-mail: bayern@csu.edu.cn (X. Li).

International Journal of Surgery (2024) 110:5385–5395

Received 14 February 2024; Accepted 10 May 2024

Supplemental Digital Content is available for this article. Direct URL citations are provided in the HTML and PDF versions of this article on the journal's website, www.lww.com/international-journal-of-surgery.

Published online 22 May 2024

http://dx.doi.org/10.1097/JS9.0000000000001685

genetic factors[8]. For patients with this type of EC, in addition to standard surgical treatment, additional adjuvant therapies such as radiation, chemotherapy, or immunotherapy are often required[9]. Currently, there is a relatively limited researches on the pathogenic mechanisms of EC, especially nonendometrioid histological types. Therefore, there is an urgent need for more in-depth studies to address these knowledge gaps.

In recent years, there are growing evidence that metabolic dysregulation and protein regulatory networks have a close association with the development of EC. Previous studies have found that models composed of specific metabolic biomarkers can accurately differentiate between endometrioid subtype and nonendometrioid subtype EC[10,11]. However, due to sample size limitations and confounding factors, the causal effect of blood metabolites and proteins on EC still cannot be confirmed. Simultaneous analysis of metabolomics and proteomics through integrated approaches can uncover biological pathogenic mechanisms at both protein and metabolic levels[12]. Currently, no relevant research exists in the field of EC; thus, it is worthwhile to conduct in-depth exploration of differential proteins and metabolites associated with EC by combining multiomics methods, enabling a more comprehensive understanding of its underlying mechanisms.

Mendelian randomization (MR) can utilize genomic data for causal inference through employing genetic variants as instrumental variables to estimate the causal effects of exposure phenotypes on desired outcome phenotypes[13]. Because of the random assignment of genetic variants occurs during meiosis, variations in exposure phenotypes are expected to be unaffected by confounding factors[14,15]. The significant advancements in large-scale Genome-Wide Association Studies (GWAS) and the development of analytical methods for MR have contributed to the robust estimation of causal effects. Further, the integration of multiomics methods, along with MR, presents a powerful approach for investigating the molecular landscape of disease[16]. By combining transcriptomics, proteomics, and metabolomics, this methodology will offer a comprehensive exploration of EC's molecular characteristics and provide us with valuable insights into the complex regulatory networks driving EC development and progression.

Inspired by the given dataset of metabolites and proteins atlas, we hereby analyzed the causal relationships of plasma metabolites and proteins with EC, seeking to identify metabolic and protein pathways that might contribute to the development of the EC and unraveling the molecular difference between two EC histological subtypes. We aim to advance our understanding of EC biology and pave the way for more targeted approaches to its diagnosis and treatment by comparing the molecular profiles of different EC subtypes.

Material and methods

Study design

We systematically assessed the causal association of human plasma metabolites and plasma proteins with the risk of EC using a two-sample MR design. The performance of a convincing MR study should comply with three fundamental assumptions. Genetic information for plasma metabolites, plasma proteins, and EC was obtained from independent GWAS datasets separately to avoid sample overlap. The overview of this study was presented in Figure 1.

GWAS data sources for EC

The GWAS summary statistics of EC were taken from a large-scale study consisting of the Endometrial Cancer Association Consortium, the epidemiology of endometrial cancer consortium, and the UK Biobank[17] and were publicly available via GWAS catalog (https://www.ebi.ac.uk/gwas/). This study encompassed 121 885 participants of European ancestry, including 12 906 cases of EC and 108 979 controls. These EC cases were further classified into EC of endometrioid histology (8 758 cases) and EC with nonendometrioid histology (serous carcinoma, carcinosarcoma, clear cell carcinoma, or mucinous carcinoma) (1 230 cases) according to the histological subtype of EC.

GWAS data sources for plasma metabolites

The GWAS summary statistics involving 1 091 blood metabolites and 309 metabolite ratios were obtained from the study by Chen *et al*.[18]. This is the currently most comprehensive analysis of human metabolites, and the full summary statistics of which were publicly available via GWAS catalog (https://www.ebi.ac.uk/gwas/). 8 192 European ancestry individuals were included in this GWAS analysis.

GWAS data sources for plasma proteins

The GWAS summary statistics of 3 283 proteins were obtained from the genomic atlas of the human plasma proteome by Sun *et al*.[19]. This study identified 1 927 genetic associations with 1 478 proteins from 3 301 European ancestry individuals and the data were available from GWAS catalog (https://www.ebi.ac.uk/gwas/).

Genetic instruments selection

The selection of instrumental variables (IVs) in this MR analysis was based on the three fundamental assumptions. Firstly, for each metabolite/protein, we extracted SNPs with association thresholds at $P < 0.5 \times 10^{-8}$. Secondly, independent variants were identified using a clumping procedure implemented in R software, in which a linkage-disequilibrium threshold of $R2 < 0.001$ within a 10 000 kilobase (kb) distance based on European ancestry reference data from the 1000 Genomes Project. All palindromic SNPs were dropped. Finally, to quantitatively verify whether the selected SNPs were strong instruments, we calculated the proportion of phenotypic variation explained and the F statistic of instruments for each metabolite/protein. Typically, a threshold of $F > 10$ was suggested for further MR analysis.

MR statistical analyses

The causal associations between metabolites/proteins and EC for this MR analysis were mainly estimated using a standard inverse variance weighted (IVW) or Wald ratio method. *Q*-test for IVW and MR-Egger was used to detect potential violations of the assumption by the heterogeneity of the association between individual IVs. MR-Egger was applied to estimate horizontal pleiotropy according to its intercept. Additional analyses of MR methods with different modeling assumptions and strengths were applied (MR-Egger, weighted median, weighted mode, simple

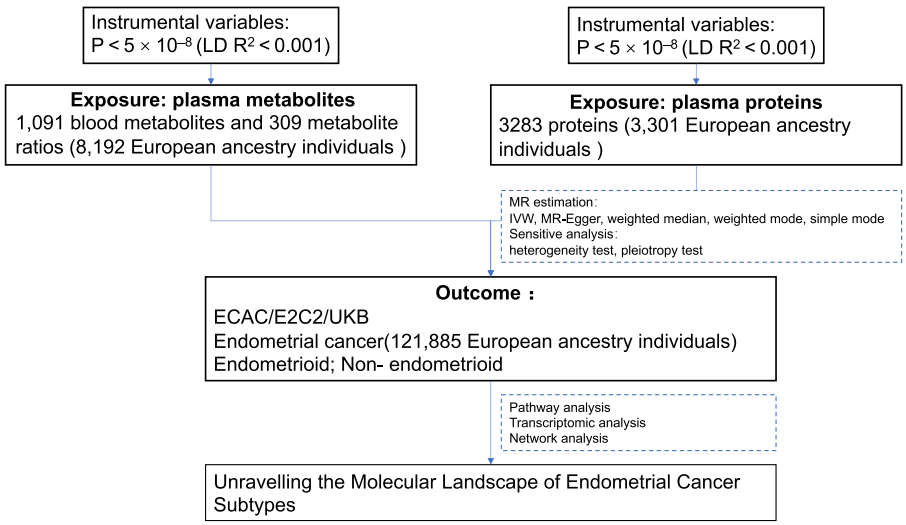

Figure 1. Overview of this study. In this study, we use a two-sample MR method to assess the causal association of human plasma metabolites and plasma proteins with the risk of EC. Genetic information for plasma metabolites, plasma proteins and EC was obtained from GWAS datasets. The MR analysis was estimated using IVW, MR-Egger, weighted median, weighted mode, simple mode, and sensitive analysis was also done. EC, endometrial cancer; MR, mendelian randomization; GWAS, Genome-Wide Association Studies; IVW, inverse variance weighted.

mode) to increase the stability and robustness of the results[14]. For the MR results, the significant level was set at 0.05, and multiple-testing significance was determined at each feature level using Bonferroni correction ($P < 0.05/n$, where n is the number of metabolites/proteins). Statistical analyses were performed in R4.3.1 software, and all analyses were performed using the 'TwoSampleMR' package and the 'mendelian randomization' package.

Pathway analysis

Metabolic pathways were analyzed via the web-based Metaconflict 4.0. (https://www.metaboanalyst.ca/)[20]. Protein pathways were analyzed via the web-based DAVID bioinformatics resources (https://david.ncifcrf.gov/tools.jsp)[21]. The significant level for pathway analysis was set at 0.10. Pathway illustrations were generated using the 'ggplot2' R package.

Integration of transcriptomic analysis in the TCGA database

UCEC expression data and baseline characteristics information were downloaded from the TCGA database (https://portal.gdc.cancer.gov/), including 245 endometrioid histological type, 138 nonendometrioid histological type, and 35 normal tissue samples. Differential analysis was conducted using HTSeq-Counts, and R4.3.1 software was employed for analyzing differentially expressed genes (DEGs). DEGs were selected with criteria of $P < 0.05$ and $|\log2(FC)| > 2$ with the DESeq2 R package. We next overlapped significant proteins identified in previous proteomic study using MR with the DEGs obtained from TCGA. The overlap allowed for the identification of crucial proteins and genes associated with different histological type of EC.

Network analysis

The causal associations between plasma metabolites and proteins were estimated using IVW method. Each node represents a protein (circle) or a metabolite (square), where the size of each node is proportional to the number of its related node (network influence). An edge exists when it connects two nodes with a $P < 0.05$ (solid edge means protein to metabolite correlations and dashed edge means metabolite to protein correlations). The length of the edge is proportional to the inverse of beta value. Network plots of proteins and metabolites were generated with the igraph R package.

Results

Causal effects of metabolites on EC

We estimated the causal association between these metabolites/metabolite ratios and EC and identified a total of 171 suggestive associations ($P < 0.05$), 65 suggestive associations in overall EC, 66 suggestive associations in endometrioid histology EC, and 40 suggestive associations in nonendometrioid histology EC, respectively (Fig. 2A–C; Table S1, Supplemental Digital Content 1, http://links.lww.com/JS9/C641). Using the IVW method, four causal associations with multiple-testing corrected significance could be observed. Genetic predicted a higher abundance of androstenediol (3beta,17beta) monosulfate was significantly associated with an increased risk of overall EC (IVW beta = 0.63, 95% CI: 0.50–0.77, $P = 3.65 \times 10^{-6}$). A higher abundance of androstenediol (3beta,17beta) monosulfate and 5alpha-androstan-3alpha,17-beta-diol disulfate was significantly associated with an increased risk of endometrioid histology EC (IVW beta = 0.73, 95% CI: 0.56–0.89, $P = 6.32 \times 10^{-6}$; IVW beta = 0.38, 95% CI: 0.29–0.47, $P = 3.10 \times 10^{-5}$, respectively). A higher abundance of 4-methylhexanoylglutamine was significantly associated with an increased risk of nonendometrioid histology EC (IVW beta = 1.11, 95% CI: 0.84–1.38, $P = 3.95 \times 10^{-5}$) (Table S1, Supplemental Digital Content 1, http://links.lww.com/JS9/C641). Cochran Q test indicated no heterogeneity was

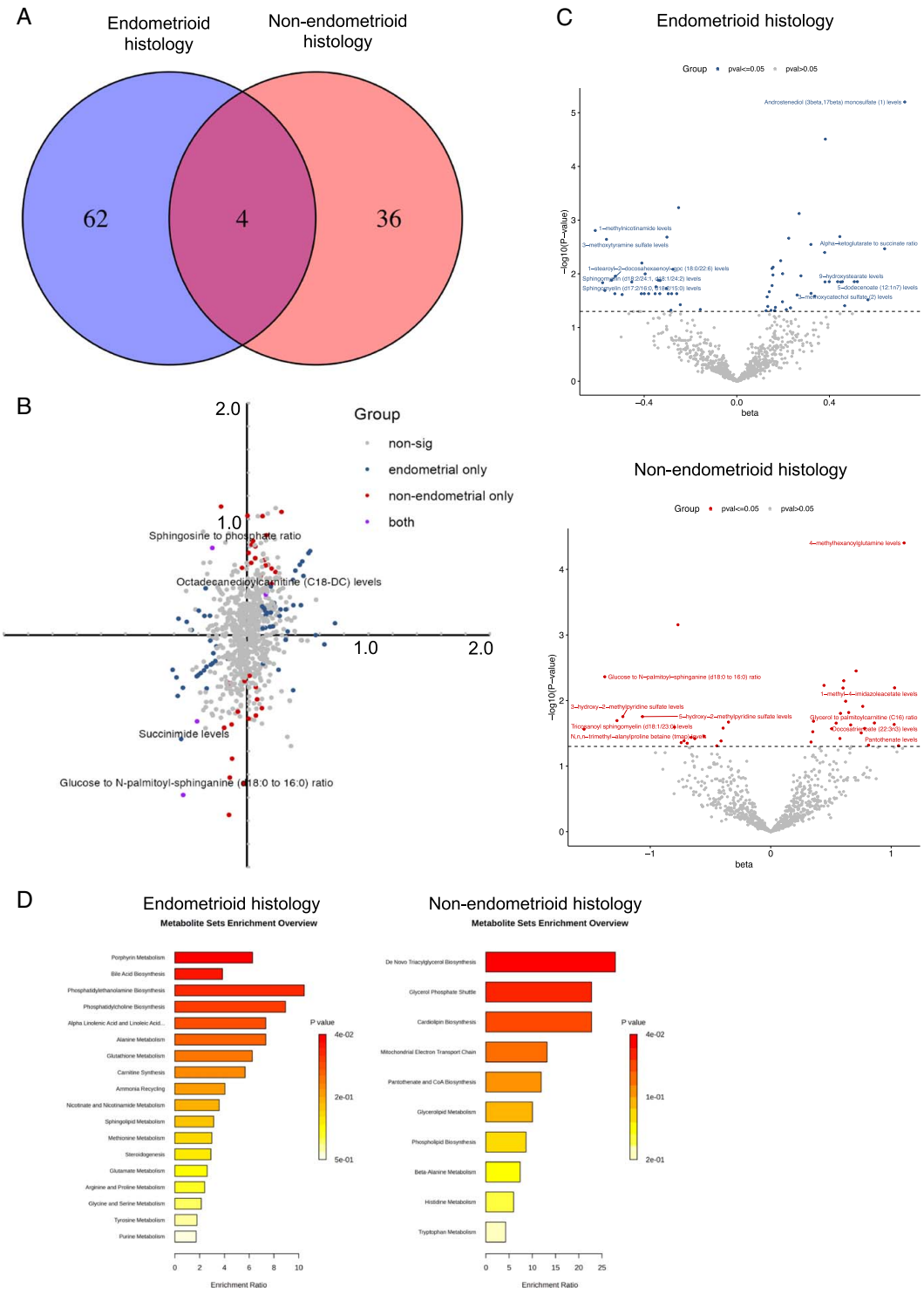

Figure 2. Causal effects of metabolites on EC. A. Venn diagram summarizing the differential and overlapping metabolites. B. Plot of beta values of metabolites to increase the risk of endometrial subtype EC, nonendometrial subtype EC, and both. The significant level was set at 0.05. C. Volcano plot of causal effects of metabolites on EC. Beta values represent the extent of increase or decrease in the risk of two subtypes of EC associated with a particular metabolite. D. Metabolic pathway analysis. EC, endometrial cancer.

detected (Table S2, Supplemental Digital Content 1, http://links.lww.com/JS9/C641). MR-Egger intercept analysis suggested that there was no potential horizontal pleiotropy (Table S3, Supplemental Digital Content 1, http://links.lww.com/JS9/C641). The core set of proteins consisted of four metabolites associated with both endometrioid subtype and nonendometrioid subtype while 62 and 36 were unique to endometrioid subtype or nonendometrioid subtype, respectively (Fig. 2A). The octadecanedioylcarnitine (C18-DC) level was positively associated with both endometrioid subtype and nonendometrial subtype, while succinimide level and glucose to N-palmitoyl-sphinganine (d18:0 to 16:0) ratio were negatively associated with both endometrioid subtype and nonendometrial subtype (Fig. 2B).

Metabolic pathway analysis

The metabolic pathway analysis identified three significant metabolic pathways in endometrioid histology EC and six significant metabolic pathways in nonendometrioid histology EC (Fig. 2D, Table S4, Supplemental Digital Content 1, http://links.lww.com/JS9/C641). There is no overlap of metabolic pathways between two histological types of EC.

Causal effects of proteins on EC

To further elucidate the differences in the pathogenic mechanisms between endometrioid histological types and nonendometrioid histological types of EC, we conducted additional analysis using GWAS data sources for plasma proteins. We estimated the causal association between these plasma proteins and EC and identified a total of 257 suggestive associations ($P < 0.05$), 132 suggestive associations in endometrioid subtype, and 125 suggestive associations in nonendometrioid subtype, respectively (Fig. 3A–C; Table S5, Supplemental Digital Content 1, http://links.lww.com/JS9/C641). Using the IVW method, two causal associations with multiple-testing corrected significance could be observed. Genetic predicted a higher abundance of Interleukin-2 receptor subunit beta levels (IL2RB) and Lymphocyte activation gene 3 protein levels (LAG3) was significantly associated with an decreased risk of endometrioid EC (Wald ratio beta = −0.70, 95% CI: −0.83–−0.58, $P = 2.25 \times 10^{-8}$; Wald ratio beta = −0.71, 95% CI: −0.84–−0.58, $P = 2.25 \times 10^{-8}$, respectively). Cochran Q test indicated no heterogeneity was detected (Table S6, Supplemental Digital Content 1, http://links.lww.com/JS9/C641). MR-Egger intercept analysis suggested that there was no potential horizontal pleiotropy (Table S7, Supplemental Digital Content 1, http://links.lww.com/JS9/C641). The core set of proteins consisted of 14 proteins associated with both endometrioid subtype and nonendometrioid subtype while 118 and 111 were unique to endometrioid subtype or nonendometrioid subtype, respectively (Fig. 3A). The top five positive-associated proteins with both endometrioid subtype and nonendometrial subtype were RAB14, SLC41A2, CFI, APLP2, QSOX2 and the top five negative-associated proteins with both types were TUFT1, TJP1, ICAM2, IL2RB, and LAG3 (Fig. 3B).

Protein pathway analysis

The GO enrichment analysis revealed the difference of biological processes, cellular components, and molecular functions in either endometrioid subtype or nonendometrioid subtype (Fig. 3D). The protein pathway analysis identified 6 significant KEGG pathways in endometrioid histology EC and 22 significant KEGG pathways in nonendometrioid histology EC (Fig. 3D, Table S8, Supplemental Digital Content 1, http://links.lww.com/JS9/C641). The overlap of protein pathways between two histological types of EC were Cytokine-cytokine receptor interaction pathway and MAPK signaling pathway.

Integration of transcriptomic analyses in the TCGA database

We compared DEGs in the TCGA database with proteins associated with EC in preceding MR results (Fig. 4A–C). We identified seven overlapping proteins (RGMA, NRXN2, EVA1C, SLC14A1, SLC6A14, SCUBE1, and FGF8) in endometrioid subtype and six overlapping proteins (IL32, GRB7, L1CAM, CCL25, GGT2, and PSG5) in nonendometrioid subtype (Fig. 4D). We also displayed the common KEGG pathways regulated in endometrial subtype and nonendometrial subtype at both the RNA and protein levels (Fig. 4E). In endometrial subtype and nonendometrial subtype, there were two and three pathways, respectively, regulated at both the RNA and protein levels. For the endometrial subtype, PI3K-Akt signaling pathway and MAPK signaling pathway was regulated at two levels. For the nonendometrial subtype, it was the Cytokine-cytokine receptor interaction, Protein digestion and absorption, and cAMP signaling pathway.

Network analysis

We finally performed network analysis of the overlapping proteins and the metabolites associated with EC in preceding MR results to identify coregulated nodes. Figure 5 showed the network analysis highlighting the most correlated metabolites and proteins in endometrial subtype and nonendometrial subtype. In endometrial subtype, there were many related metabolites with SCUBE1, RGMA, and EVA1C, underlining the importance of these proteins in the pathogenesis of endometrial EC through different metabolism pathways. For nonendometrial subtype, PSG5, L1CAM, GGT2, and IL32 were the important proteins that involved in the pathogenesis of nonendometrial EC through interaction with metabolites, which showed a difference of the coregulated network between two histological subtypes of EC. The identification of notable differences in the coregulated network between endometrioid and nonendometrioid subtype is significant in understanding the molecular mechanisms underlying these two subtypes of EC.

Discussion

In this study, we unraveled the causal relationships of plasma metabolites and proteins with two different histological subtypes of EC. For endometrial subtype, we found 66 causal relationships between plasma metabolites and endometrial EC, and two of which were observed with multiple-testing corrected significance. We also found 132 causal relationships between plasma proteins and endometrial EC and only IL2RB and LAG3 were multiple-testing corrected significant. For nonendometrial subtype, 40 causal relationships between plasma metabolites and nonendometrial EC were observed, and 4-methylhexanoylglutamine was the only multiple-testing corrected significantly associated metabolite. We also observed

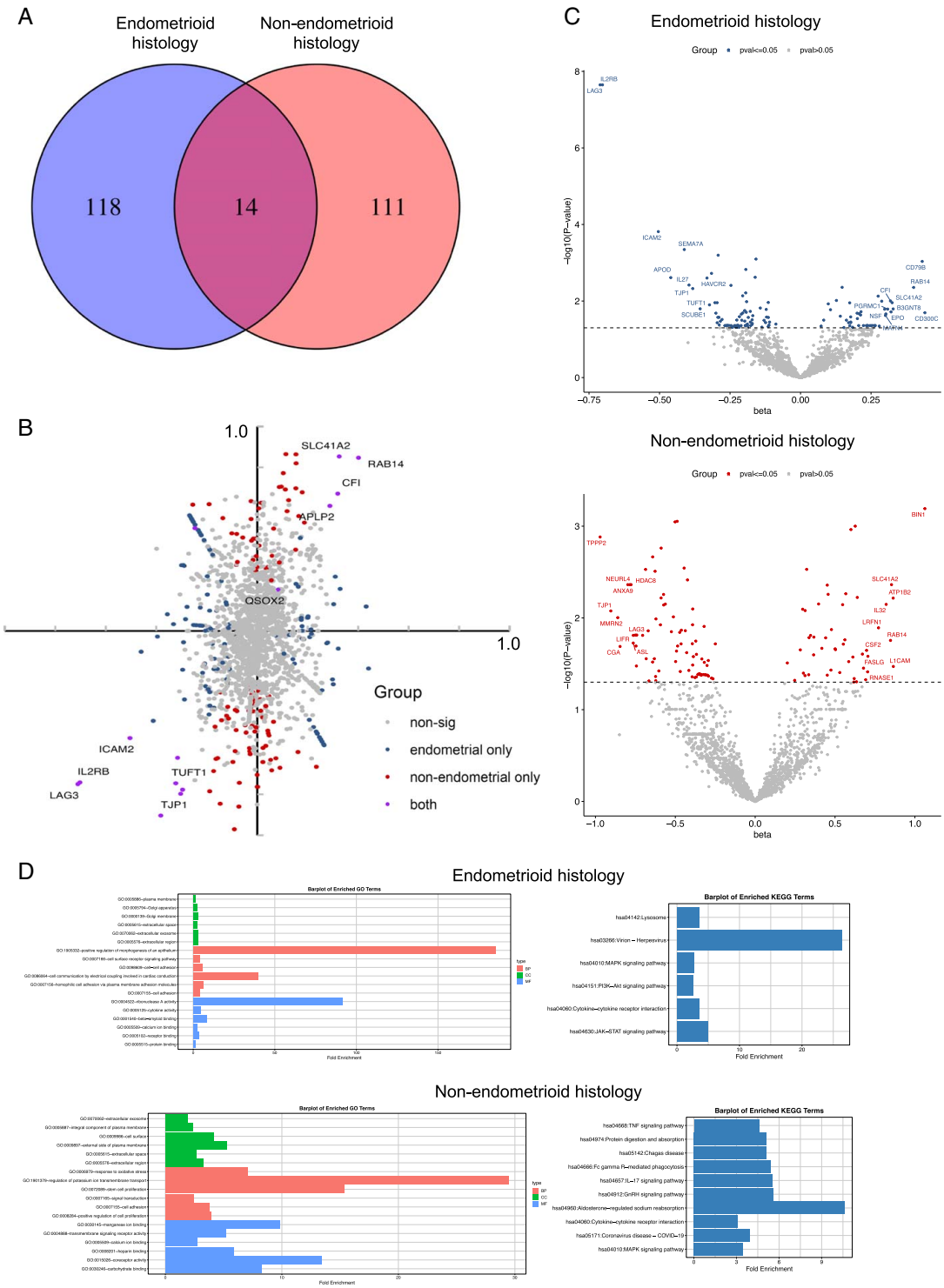

Figure 3. Causal effects of proteins on EC. A. Venn diagram summarizing the differential and overlapping proteins. B. Plot of beta values of proteins to increase the risk of endometrial subtype EC, nonendometrial subtype EC, and both. The significant level was set at 0.05. C. Volcano plot of causal effects of proteins on EC. Beta values represent the extent of increase or decrease in the risk of two subtypes of EC associated with a particular protein. D. Protein pathway analysis. EC, endometrial cancer.

125 causal relationships between plasma proteins and nonendometrial EC and none of them were observed with multiple-testing corrected significance. Despite a limited number of common metabolite pathways and protein pathways, substantial differences were observed between endometrioid and nonendometrioid histological types of EC at both the metabolite and protein levels. To further validate the previous results, we overlapped significant plasma proteins with the

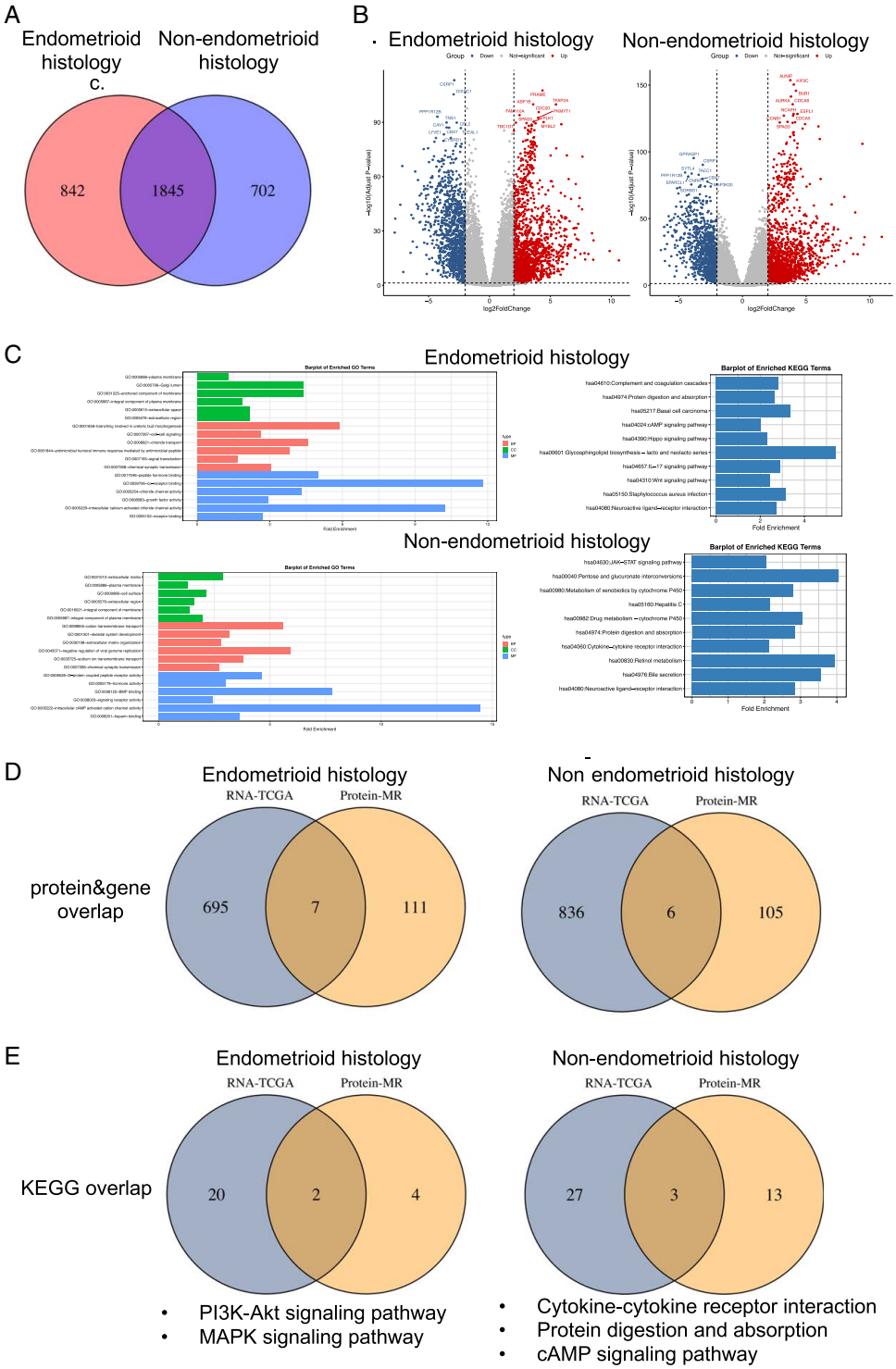

Figure 4. Integration of transcriptomic analyses in the TCGA database. A. Venn diagram summarizing the differential and overlapping differentially expressed genes (DEGs). B. Volcano plot of DEGs of two subtypes of EC. C. GO and KEGG pathway analysis. D. Overlap of DEGs and proteins. e Overlap of KEGG pathways. DEGs, differentially expressed genes; GO, gene ontology; KEGG, kyoto encyclopedia of genes and genomes.

DEGs obtained from TCGA to identify the crucial proteins associated with different histological type of EC. We identified seven overlapping proteins (RGMA, NRXN2, EVA1C, SLC14A1, SLC6A14, SCUBE1, and FGF8) in endometrioid subtype and six overlapping proteins (IL32, GRB7, L1CAM, CCL25, GGT2, and PSG5) in nonendometrioid subtype and we finally added network analysis of above proteins and metabolites to identify coregulated nodes. To our knowledge, this is the first study to analyze the causal relationships of plasma metabolites and proteins with EC.

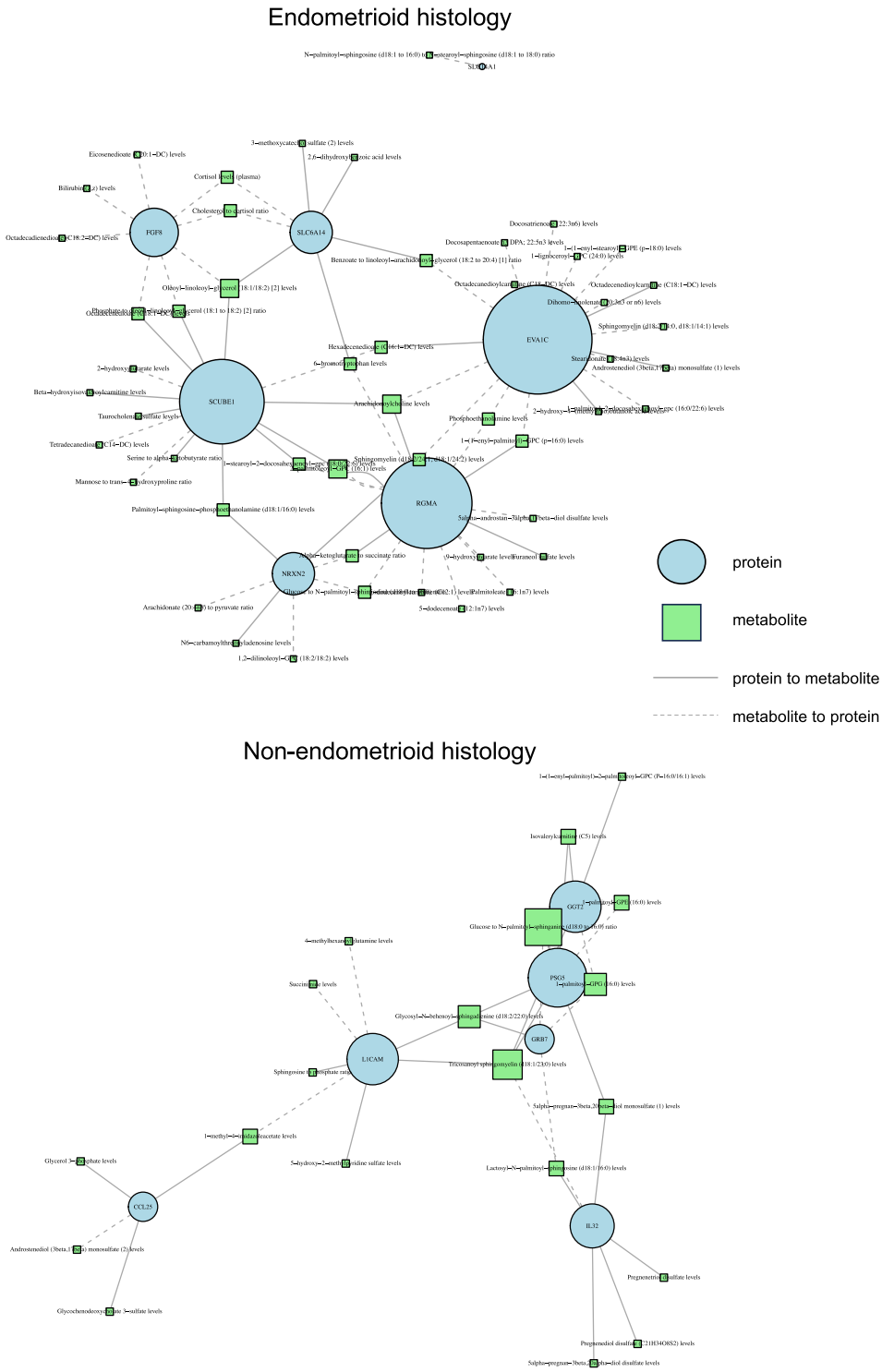

Figure 5. Network analysis. The causal associations between plasma metabolites and proteins. Each node represents a protein (circle) or a metabolite (square), where the size of each node is proportional to the number of its related node. An edge exists when it connects two nodes with a $P < 0.05$ (solid edge means protein to metabolite correlations and dashed edge means metabolite to protein correlations). The length of the edge is proportional to the inverse of beta value.

EC has been recognized as a metabolism-related disease as it can be found in combination with a variety of metabolic disorders, including hypertension, diabetes, and obesity[4]. Currently, a number of EC-related metabolites and metabolic pathways have been continuously discovered and verified[22,23]. Our study confirmed the presence of EC metabolic profiles and identified the difference of key metabolites and metabolic pathways between endometrial subtype and nonendometrial subtype.

The metabolic derivatives of androgens, Androstenediol (3beta,17beta) monosulfate, and 5alpha-androstan-3alpha,17-beta-diol disulfate were found to be significantly associated with an increased risk of endometrioid histology EC, suggesting a potential role of androgen in the development of endometrial EC. Previous studies have found that androgen metabolites, including testosterone and dehydroepiandrosterone sulfate were all risk factors of EC and were potential prognostic markers of EC[24,25]. This may be due to the fact that androgens can be converted into estradiol under the action of aromatase, and long-term estrogen is a risk factor for EC[26]. Nevertheless, the role of androgens in EC remains contradictory and how important are androgens in determining the fate of EC is still unknown. 4-methylhexanoylglutamine, a kind of glutamine metabolites, was found to be a risk factor of nonendometrioid EC in our study. Currently, various studies have found that glutamine metabolism plays an important role in tumor development, exerting its influence through intricate interactions with the tumor microenvironment across various cancer types, such as glioma, ovarian cancer, breast cancer, and prostate cancer[27–29]. Our results showed the potential role of glutamine metabolism in the development of nonendometrioid EC and additional research was necessary to verified this underlying relationship.

Among the overlapping proteins in the endometrial subtype, sodium-dependent and chloride-dependent neutral and basic amino acid transporter B(0 +) (SLC6A14) protein is a membrane-bound amino acid transporter, playing a crucial role in the transportation of specific amino acids, with a particular emphasis on neutral amino acids. Its malfunctioning is correlated with several pathological states and it is upregulated in many solid tumors[30]. As a result, we suggest that SLC6A14 may play a significant role in the pathogenesis of endometrial EC by through the amino acid metabolism. Fibroblast growth factor 8 (FGF8), belonging to the Fibroblast Growth Factor (FGF) family, is another overlapping protein. This family includes a variety of proteins that regulate cell growth, differentiation, and development. Some studies have suggested that FGF8 may play a role in promoting the invasion and migration of certain tumors[31,32]. Signal peptide, CUB, and EGF-like domain-containing protein 1 (CUBE1) is a transmembrane protein with a signal peptide, a CUB domain, an EGF-like domain, and a compression region[33]. Some studies have found that CUBE1 can enhance the malignancy and stemness of tumor cells and can be diagnostic and prognostic markers of some tumors[34,35]. Repulsive guidance molecule A (RGMA) is a membrane-associated protein primarily functions in the central nervous system. Concerning studies have demonstrated that RGMA is linked to the pathogenesis of prostate cancer, colon cancer, and breast cancer[36,37]. Protein eva-1 homolog C (EVA1C) is a membrane protein-coding gene, and only one study has investigated its function in the immuno-oncologic interactions[38]. The above proteins have not been investigated in the area of EC. Further research is needed to explore their roles and potential contributions to endometrial EC. Concerning the overlapping proteins in the nonendometrial subtype, the Gamma-Glutamyltransferase 2 (GGT2) protein functions as an enzyme involved in the metabolism of glutathione[39]. Hence, we postulate that GGT2 could play a substantial role in the pathogenesis of nonendometrial EC by influencing glutathione metabolism. Growth factor receptor-bound protein 7 (GRB7) typically interacts with growth factor receptors such as HER2, EGFR, and plays a regulatory role in processes such as cell growth, differentiation, and migration[40]. The expression of GRB7 is often closely correlated with HER2 expression[41,42]. Additionally, in conjunction with unpublished data from our research group, it was observed that there is a higher prevalence of HER2-positive patients in nonendometrioid subtype compared to endometrioid subtype. This suggests a potential close association between GRB7, HER2, and the occurrence and development of nonendometrioid EC and relative researches are needed. Pregnancy-Specific Beta-1-Glycoprotein 5 (PSG5) protein is expressed during pregnancy. The specific biological functions of the PSG family are not fully understood[43]. C-C motif chemokine 25 (CCL25) and Interleukin-32 (IL32) both play crucial roles in regulating the immune response[44,45], indicating the potential relevance of immune therapy in nonendometrial EC.

There are still some limitations in this study. First, due to the restriction of the GWAS data, we could only divide the EC into the endometrioid subtype and nonendometrioid subtype instead of the molecular subtype of EC, which is currently the standard classification of EC[46]. Nevertheless, our findings remained relevant, aligning with the classical Bokhman classification in rough[46]. Secondly, the GWAS data were from various populations and databases, which may differ in their design, cohort, and quality. Consequently, there might be inherent biases across different GWAS datasets, potentially impacting the reliability of our results. Despite these limitations, our study still contributes to the in-depth understanding of EC etiology and appeals to further investigation to validate and refine our findings. The future research direction was to validate the results through cellular, animal, and clinical samples, stratified by distinct molecular subtypes, thereby enhancing the clinical applicability of this study. And the search for crucial plasma proteins and metabolites in the pathogenesis of EC holds potential for future prevention, diagnosis, and treatment strategies, underscoring the significance of this study.

Conclusion

To conclude, this study revealed the causal relationships of plasma metabolites and proteins with two EC subtypes. Significant associations were identified, with notable differences observed between endometrioid and nonendometrioid EC at both metabolite and protein levels. The findings offer novel insights into gene-protein-metabolites interactions, potentially influencing future EC treatments.

Ethical approval

All the data of the article came from the public database, and did not require ethics approval.

Consent

None.

Sources of funding

The study was supported by National Key Research and Development Program of China (No. 2021YFC2701202), the National Natural Science Foundation of China

(No. U20A20368) and the National Natural Science Foundation of China (No. 82073323) for Y. Z. Hunan Provincial Natural Science Foundation of China (No.2021JJ31074) and the National Natural Science Foundation of China (No. 82373974) for X. L.

Author contribution

Y.Z. and X.L.: conceptualization; Y.Z., X.L., and Y.S.: methodology; X.L., Y.S., Y.T., J.D., and Z.C.: formal analysis; R.Z., Y.L., L.L., H.Z., H.W.: investigation; Y.Z. and X.L.: resources; Y.S.: writing – original draft; Y.Z. and X.L.: writing – review and editing.

Conflicts of interest disclosure

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Research registration unique identifying number (UIN)

1. Name of the registry: not applicable.
2. Unique identifying number or registration ID: not applicable.
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): not applicable.

Guarantor

Yu Zhang and Xi Li.

Data availability statement

The original data used in this article is all available publicly. Further inquiries can be directed to the corresponding author.

Provenance and peer review

My paper was not invited.

Acknowledgements

Assistance with the study: The authors thank all the consortium studies for making the summary association statistics data publicly available.
Presentation: none.

References

[1] Sung H, Ferlay J, Siegel RL, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49.
[2] Siegel RL, Miller KD, Wagle NS, *et al*. Cancer statistics, 2023. CA Cancer J Clin 2023;73:17–48.
[3] Xia C, Dong X, Li H, *et al*. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chin Med J (Engl) 2022;135:584–90.
[4] Crosbie EJ, Kitson SJ, McAlpine JN, *et al*. Endometrial cancer. Lancet 2022;399:1412–28.
[5] Makker V, MacKay H, Ray-Coquard I, *et al*. Endometrial cancer. Nat Rev Dis Primers 2021;7:88.
[6] Concin N, Matias-Guiu X, Vergote I, *et al*. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. Int J Gynecol Cancer 2021;31:12–39.
[7] Suryo Rahmanto Y, Shen W, Shi X, *et al*. Inactivation of Arid1a in the endometrium is associated with endometrioid tumorigenesis through transcriptional reprogramming. Nat Commun 2020;11:2717.
[8] Zheng W, Xiang L, Fadare O, *et al*. A proposed model for endometrial serous carcinogenesis. Am J Surg Pathol 2011;35:e1–14.
[9] Vanbraband J, Van Damme N, Silversmit G, *et al*. Practice patterns, time trends and quality of care of uterine cancer in Belgium: an analysis of the EFFECT database. Gynecol Oncol 2023;180:70–8.
[10] Troisi J, Sarno L, Landolfi A, *et al*. Metabolomic signature of endometrial cancer. J Proteome Res 2018;17:804–12.
[11] Cummings M, Massey KA, Mappa G, *et al*. Integrated eicosanoid lipidomics and gene expression reveal decreased prostaglandin catabolism and increased 5-lipoxygenase expression in aggressive subtypes of endometrial cancer. J Pathol 2019;247:21–34.
[12] Benson MD, Eisman AS, Tahir UA, *et al*. Protein-metabolite association studies identify novel proteomic determinants of metabolite levels in human plasma. Cell Metab 2023;35:1646–60.
[13] Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet 2014;23(R1):R89–98.
[14] Skrivankova VW, Richmond RC, Woolf BAR, *et al*. Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement. JAMA 2021;326:1614–21.
[15] Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. JAMA 2017;318:1925–6.
[16] Weith M, Beyer A. The next step in mendelian randomization. Elife 2023;12:e86416.
[17] O'Mara TA, Glubb DM, Amant F, *et al*. Identification of nine new susceptibility loci for endometrial cancer. Nat Commun 2018;9:3166.
[18] Chen Y, Lu T, Pettersson-Kymmer U, *et al*. Genomic atlas of the plasma metabolome prioritizes metabolites implicated in human diseases. Nat Genet 2023;55:44–53.
[19] Sun BB, Maranville JC, Peters JE, *et al*. Genomic atlas of the human plasma proteome. Nature 2018;558:73–9.
[20] Chong J, Xia J. Using MetaboAnalyst 4.0 for metabolomics data analysis, interpretation, and integration with other omics data. Methods Mol Biol 2020;2104:337–60.
[21] Sherman BT, Hao M, Qiu J, *et al*. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res 2022;50(W1):W216–21.
[22] Huang P, Fan X, Yu H, *et al*. Glucose metabolic reprogramming and its therapeutic potential in obesity-associated endometrial cancer. J Transl Med 2023;21:94.
[23] Byrne FL, Poon IKH, Modesitt SC, *et al*. Metabolic vulnerabilities in endometrial cancer. Cancer Res 2014;74:5832–45.
[24] Zhao B, Wang Z, Liu D, *et al*. Genetically predicted serum testosterone and risk of gynecological disorders: a mendelian randomization study. Front Endocrinol (Lausanne) 2023;14:1161356.
[25] Dahmani C, Caron P, Simonyan D, *et al*. Circulating adrenal 11-oxygenated androgens are associated with clinical outcome in endometrial cancer. Front Endocrinol (Lausanne) 2023;14:1156680.
[26] Gjorgoska M, Rizner TL. Integration of androgen hormones in endometrial cancer biology. Trends Endocrinol Metab 2022;33:639–51.
[27] Yang R, Li X, Wu Y, *et al*. EGFR activates GDH1 transcription to promote glutamine metabolism through MEK/ERK/ELK1 pathway in glioblastoma. Oncogene 2020;39:2975–86.
[28] House RJ, Tovar EA, Essenburg CJ, *et al*. NF1 deficiency drives metabolic reprogramming in ER + breast cancer. Mol Metab 2024;80:101876.
[29] Yang L, Venneti S, Nagrath D. Glutaminolysis: a hallmark of cancer metabolism. Annu Rev Biomed Eng 2017;19:163–94.
[30] Nałęcz KA. Amino Acid Transporter SLC6A14 (ATB0, +) - A Target in Combined Anti-cancer Therapy. Front Cell Dev Biol 2020;8:594464.
[31] Chandra KB, Kumar V, Ranjan S, *et al*. Unveiling the significance of FGF8 overexpression in orchestrating the progression of ovarian cancer. Int J Mol Sci 2023;24:14239.
[32] Song M, Xing X. miR-6742-5p regulates the invasion and migration of lung adenocarcinoma cells via mediating FGF8/ERK12/MMP9/MMP2 signaling pathway. Aging (Albany NY) 2023;15:53–69.

[33] Lin Y-C, Sahoo BK, Gau S-S, *et al*. The biology of SCUBE. J Biomed Sci 2023;30:33.
[34] Zhao J, Li R, Li J, *et al*. CAFs-derived SCUBE1 promotes malignancy and stemness through the Shh/Gli1 pathway in hepatocellular carcinoma. J Transl Med 2022;20:520.
[35] Sahoo BK, Lin Y-C, Tu C-F, *et al*. Signal peptide-CUB-EGF-like repeat-containing protein 1-promoted FLT3 signaling is critical for the initiation and maintenance of MLL-rearranged acute leukemia. Haematologica 2023;108:1284–99.
[36] Li Y, Liu H-T, Chen X, *et al*. Aberrant promoter hypermethylation inhibits RGMA expression and contributes to tumor progression in breast cancer. Oncogene 2022;41:361–71.
[37] Yu M, Cao H, Yang J, *et al*. EIF4A3-regulated hsa_circ_0001445 can inhibit the progression of laryngeal squamous cell carcinoma via hsa-miR-432-5p-dependent up-regulation of RGMA expression. Cell Cycle 2023;22:2038–56.
[38] Hu Z, Qu S. EVA1C is a potential prognostic biomarker and correlated with immune infiltration levels in WHO Grade II/III Glioma. Front Immunol 2021;12:683572.
[39] Mitrić A, Castellano I. Targeting gamma-glutamyl transpeptidase: a pleiotropic enzyme involved in glutathione metabolism and in the control of redox homeostasis. Free Radic Biol Med 2023;208: 672–83.
[40] Zhu G, Cai H, Xiao Q, *et al*. GRB7 plays a promoting role in the progression of gastric cancer. BMC Cancer 2023;23:1262.
[41] Cao D, Xu H, Li L, *et al*. Molecular characteristics of gastric cancer with ERBB2 amplification. Heliyon 2023;9:e18654.
[42] Rojhannezhad M, Soltani BM, Vasei M, *et al*. Functional analysis of a putative HER2-associated expressed enhancer, Her2-Enhancer1, in breast cancer cells. Sci Rep 2023;13:19516.
[43] Plouzek CA, Leslie KK, Stephens JK, *et al*. Differential gene expression in the amnion, chorion, and trophoblast of the human placenta. Placenta 1993;14:277–85.
[44] Yu H, Mei Y, Dong Y, *et al*. CCR9-CCL25 mediated plasmacytoid dendritic cell homing and contributed the immunosuppressive microenvironment in gastric cancer. Transl Oncol 2023;33: 101682.
[45] Hough JT, Zhao L, Lequio M, *et al*. IL-32 and its paradoxical role in neoplasia. Crit Rev Oncol Hematol 2023;186:104011.
[46] Suarez AA, Felix AS, Cohn DE. Bokhman Redux: Endometrial cancer 'types' in the 21st century. Gynecol Oncol 2017;144: 243–9.