International Journal of Women's Health **Dovepress**

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Proteome-Wide Mendelian Randomization Analysis to Identify Potential Plasma Biomarkers and Therapeutic Targets for Epithelial Ovarian Cancer Subtypes

Qianhan Lin[1], Jiajia Li[1], Yating Sun[1], Zulimire Abudousalamu[1], Mengyang Xue[1], Liangqing Yao[2], Mo Chen[1]

[1]Department of Gynecologic Oncology, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, 200011, People's Republic of China; [2]Department of Gynecologic Oncology, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, 510005, People's Republic of China

Correspondence: Liangqing Yao, Department of Gynecologic Oncology, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, 510005, People's Republic of China, Email yaoliangqing@163.com; Mo Chen, Department of Gynecologic Oncology, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, 200011, People's Republic of China, Email chenmo_nicy@163.com

Background: Epithelial ovarian cancer (EOC) remains an unmet medical challenge due to its insidious onset, atypical symptoms, and increasing resistance to conventional chemotherapeutic agents. It is imperative to explore novel biomarkers and generate innovative target drugs.

Methods: To identify potential proteins with causal association to EOC subtypes, we conducted a Mendelian Randomization (MR) analysis using 15,419 protein quantitative trait loci (pQTLs) associated with 2015 proteins. Bayesian colocalization analysis, Summary-data-based MR, and Heterogeneity in Dependent Instruments tests were employed for validation. Enrichment and druggability analyses were performed to assess the biological significance and therapeutic potential of identified proteins.

Results: Our analysis identified 455 unique proteins associated with at least one EOC subtype, with 14 protein-cancer associations confirmed by further validation. Ten proteins were prioritized as potential therapeutic targets, including α1B-glycoprotein (A1BG) and ephrin-A1 (EFNA1), which interact with the known drug targets human epidermal growth factor receptor 2 (HER2) and vascular endothelial growth factor receptor (VEGFR).

Conclusion: This study elucidated the plasma proteins causally associated with EOC subtypes, potentially offering easily detectable biomarkers and promising therapeutic targets. A1BG and EFNA1 were identified as druggable targets and confirmed to correspond with current pharmacological targets. Targeting these proteins in drug development potentially offers an avenue for innovative treatment strategies.

Keywords: drug target prediction, novel circulation biomarkers, epithelial ovarian cancer, Mendelian randomization, plasma proteins

Introduction

Ovarian cancer (OC) represents a significant public health concern, accounting for the leading cause of mortality among gynecological malignancies.[1] According to the most recent statistics, there were 324,398 new cases and 206,839 deaths reported globally in 2022.[2] Among these, China accounted for 61,100 new cases and 32,600 deaths.[3] In the United States, it is estimated that there would be 19,680 new cases and 12,740 related deaths in 2024.[4] OC encompasses a diverse group of neoplasms, with epithelial OC (EOC) representing a prevalent variant. EOC is further subdivided into five primary subtypes: high-grade serous (HGSOC), endometrioid (ENOC), clear cell (CCOC), low-grade serous (LGSOC), and mucinous (MOC). Each subtype is distinguished by a specific trait of genetic, epigenetic, and proteomic attributes, which collectively modulate its pathogenicity, prognostic indicators, and susceptibility to therapeutic interventions.[5,6] The insidious onset, coupled with nonspecific symptoms, results in the majority of patients presenting with advanced or metastatic disease when diagnosed. In clinical practice, key biomarkers include CA-125, HE4, and BRCA1/2 mutations.[7] CA-125, a marker that is widely utilized for the monitoring of

Received: 10 September 2024
Accepted: 5 December 2024
Published: 21 December 2024

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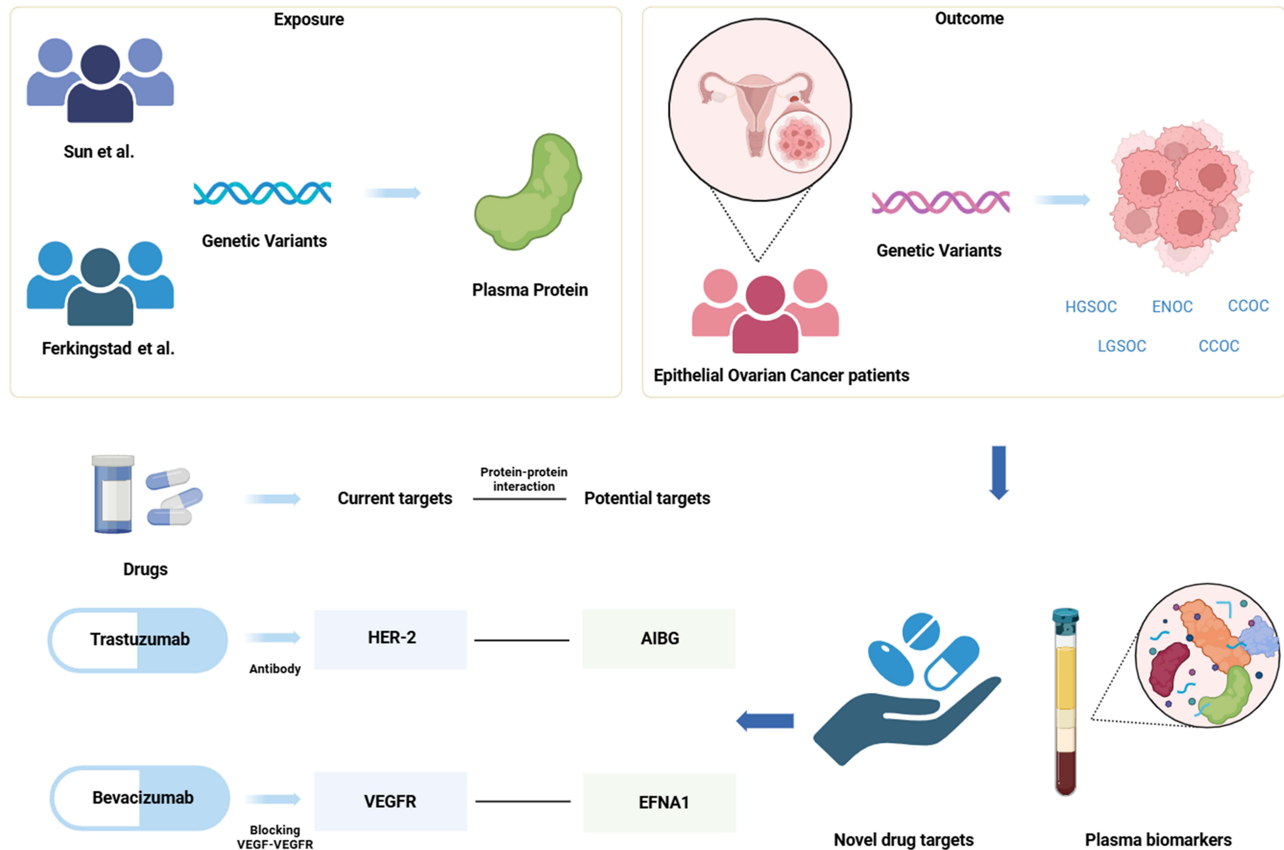
Graphical Abstract



treatment response and recurrence, has been demonstrated to exhibit limitations in terms of sensitivity and specificity.[8] Approximately 50%-60% of patients with early-stage disease exhibit elevated CA-125 levels.[9] Moreover, elevated CA-125 levels have been documented in non-malignant conditions, such as endometriosis. HE4 exhibits greater specificity, enabling more accurate distinction between benign and malignant pelvic masses more accurately. However, its sensitivity for early-stage detection is limited. BRCA1/2 genes mutation testing is vital for genetic risk assessment, as it provides guidance for the implementation of preventive strategies for individuals with an elevated risk of developing the disease. It should be noted, however, that this is not a diagnostic tool for OC itself. Despite their utility, these biomarkers have limitations in terms of sensitivity and specificity for early detection. Furthermore, debulking surgery, which represents the primary treatment approach, is typically followed by adjuvant chemotherapy, which traditionally employs platinum-based agents. Despite an initially favorable responses to chemotherapy, the majority of patients with advanced disease would experience recurrence within 5 years and subsequently develop resistance to chemotherapy drugs with an increasing number of chemotherapies. The evolution of chemoresistance represents a formidable obstacle in the management of EOC. Identifying readily detectable biomarkers and potent drug targets represents an imperative direction for future research.

Plasma proteins, as a class of molecules with multiple biological functions, play a pivotal role in disease diagnosis, therapy, and drug development. In the field of oncology, plasma proteins have emerged as a compelling research direction due to their involvement in critical processes such as tumor growth, metastasis, angiogenesis, and immune evasion. The analysis of the plasma proteome provides insights into protein expression patterns associated with specific tumor types, thereby offering novel avenues for early diagnosis and prognostic assessment.[10] The convenience of blood testing enables rapid assessment of therapy

outcomes through plasma protein biomarkers. Furthermore, owing to their pivotal roles, plasma proteins present a distinctive possibility for targeted therapeutic intervention in cancer treatment.

Mendelian randomization (MR) is an epidemiological approach that employs genetic variations as instrumental variables to ascertain the causality between exposure and disease outcome.[11] One of the principal advantages of this approach is its capacity to reduce the effects of confounders, reverse causality, and measurement errors, which are common in traditional observational studies. As the scale of high-throughput proteomics broadens, genome-wide association studies (GWAS) of plasma protein have the potential to uncover a multitude of protein quantitative trait loci (pQTLs) associated with protein levels.[12] Proteome-wide MR represents an emerging field of proteomics integrated with MR methods, which facilitates the elucidation of causal relationships between protein and disease outcomes. The utilization of genetic variations as instrumental variables to investigate the relationship between protein levels and disease susceptibility enables proteome-wide MR to uncover potential causal links, thereby pinpointing novel drug targets with a higher probability of therapeutic success. The application of proteome-wide MR offers new avenues for research in the field of drug development. By identifying genetic variants associated with specific protein levels and linking them to disease outcomes, researchers can gain a more comprehensive understanding of the role of proteins in the pathogenesis of diseases and devise targeted treatment strategies. Proteome-wide MR represents a distinctive and invaluable tool for investigating drug targets and disease mechanisms, thereby facilitating the development of more efficacious treatments and enriching the landscape of drug development.

Nevertheless, there is a paucity of studies has employed proteome-wide MR in EOC. Only a few studies have attempted to explore plasma proteins associated with EOC pathogenesis by MR.[13,14] These studies had relatively narrow sample exposures and failed to adequately account for the molecular variability between different EOC subtypes, instead generalizing them as a single disease entity. In contrast, our study incorporates two large-scale proteomics programs and meticulously delineates the distinct subtypes, thereby ensuring the stability and reliability of the results.

Materials and Methods

Study Design

To identify plasma proteins potentially associated with the development of different subtypes of EOC, we leveraged circulating pQTL data from two recent large-scale proteomic studies as exposure variables, and GWAS data for five EOC subtypes as outcome variables. MR and sensitivity analyses were conducted and 569 protein-cancer associations were identified. To validate the causal relationships of the proteins, Bayesian colocalization analysis, summary data-based MR (SMR) analysis, and heterogeneity in dependent instruments (HEIDI) test were employed. Colocalization analysis was performed on 569 protein-cancer associations to assess shared genetic variation between these proteins and cancer traits, and to investigate their causal relationships. From this analysis, 9 protein-cancer associations with causal relationships were identified out of the 569 associations. Moreover, SMR analysis and HEIDI analysis, unique MR methods estimating the impact of genetic variation on phenotypes using summary data, provided valuable insights even in the absence of direct colocalization evidence. By including these 569 protein-cancer associations in SMR and HEIDI analyses, 8 additional protein-cancer associations were identified. By combining the results from both methods, a total of 14 protein-cancer associations (involving 13 unique proteins) were identified as the most relevant research focus concerning EOC. Enrichment analyses were undertaken to identify the functional roles and pathways associated with causal proteins. Additionally, a drug target evaluation was conducted to ascertain whether the proteins identified as causally related are existing or prospective drug targets, whereas protein-protein interaction (PPI) analysis was employed to explore potential biological connections with established drug targets. A further exploration utilizing bioinformatics analysis served to confirm the diagnosis and prognosis value of identified protein in HGSOC tissues. An independent dataset was employed to explore the expression levels of causal proteins in HGSOC ascites. The methodology of our research is depicted in Figure 1.

Study Exposures

In the application of MR to probe the causal links between plasma protein and cancer, this study employed pQTLs as genetic instruments. The data for pQTLs were extracted from two recently large-scale proteomics GWAS.[12,15] Information on these two studies was specifically outlined in Additional file 1: Table S1.

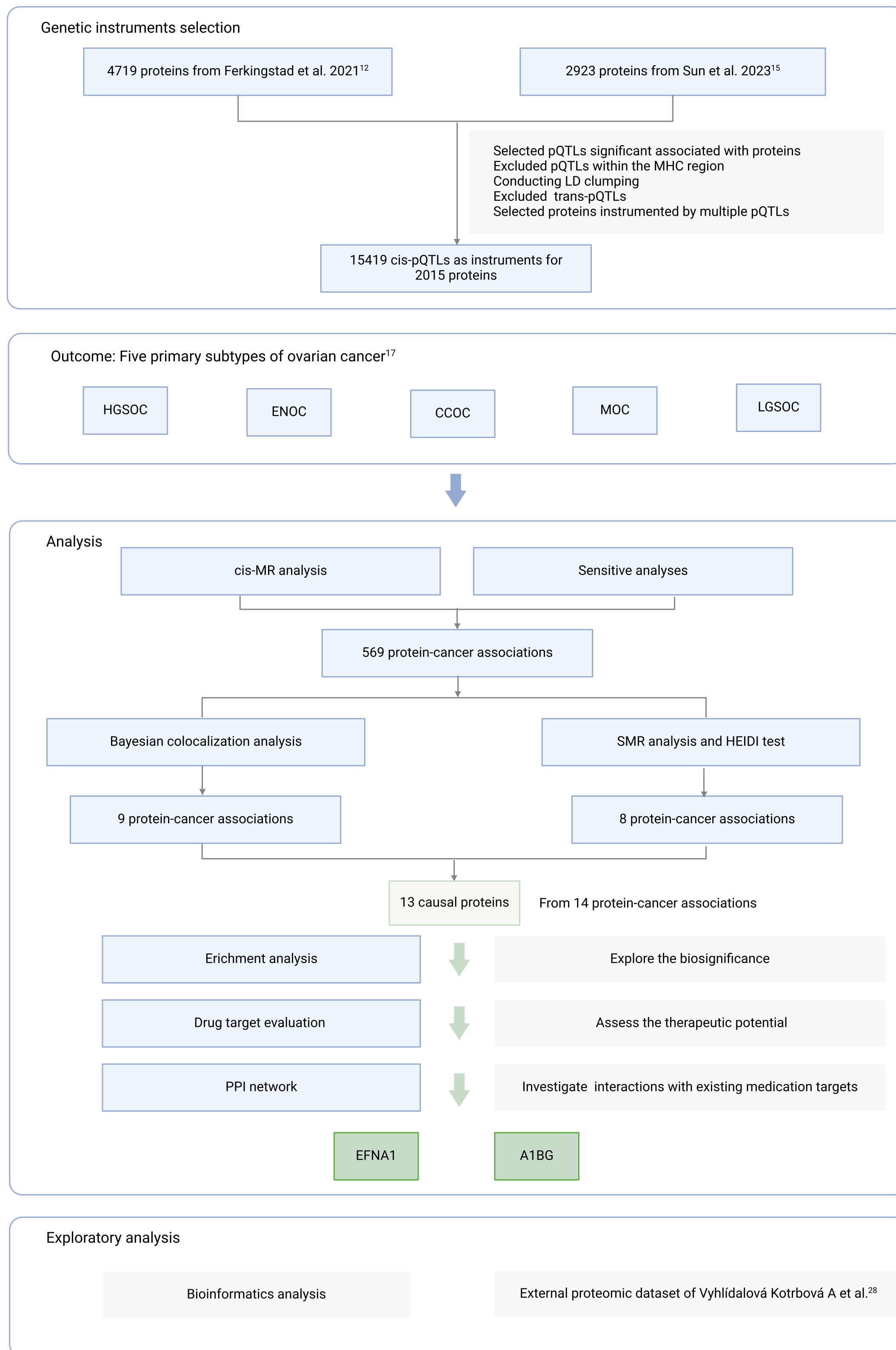


Figure 1 Workflow of the study design.

Abbreviations: pQTL, protein quantitative trait loci; MHC, major histocompatibility complex; LD, linkage disequilibrium; HGSOC, high-grade serous ovarian carcinoma; ENOC, endometrioid ovarian carcinoma; CCOC, clear cell ovarian carcinoma; MOC, mucinous ovarian carcinoma; LGSOC, low-grade serous ovarian carcinoma; MR, Mendelian randomization; SMR, Summary-data-based Mendelian randomization; HEIDI, Heterogeneity in dependent instruments; PPI, protein-protein interaction; EFNA1, Ephrin-A1; AIBG, Alpha-1B-glycoprotein.

The plasma pQTLs were selected according to the following criteria:[16:] First, pQTLs were included based on surpassing a statistical significance threshold of $P < 5\times10^{-8}$ to focus exclusively on associations supported by robust statistical support. Second, single nucleotide polymorphisms (SNPs) within the major histocompatibility complex region on chromosome 6 (26 to 34 Mb) were excluded to avert the confounding effects of linkage disequilibrium (LD). Third, the independence of pQTLs was ascertained through LD clumping with $r^2>0.01$ and a distance criterion of < 10000 kb. Fourth, cis-pQTLs, identified as the leading SNP within a 2-megabase upstream or downstream of a protein-coding gene's transcription start site, were distinguished from trans-pQTLs found outside this zone. The selection of cis-pQTLs as genetic instruments was predicated on their presumed direct influence on gene expression, thus reducing the likelihood of horizontal pleiotropy.[16] Last, proteins were instrumented by multiple SNPs, enhancing the robustness of the genetic instruments and the reliability of the causality.

Study Outcomes

GWAS data for EOC patients were accessed via the Ovarian Cancer Association Consortium[17] (https://gwas.mrcieu.ac.uk/). This study focused on 5 specific EOC subtypes, which included HGSOC (13,037 cases and 40,941 controls), ENOC (2018 cases and 40,941 controls), CCOC (1366 cases and 40,941 controls), LGSOC (1012 cases and 40,941 controls) and MOC (2566 cases and 40,941 controls). These subtypes were extracted as outcomes in this study.

Proteome-Wide MR Analysis

To ascertain the causal relationship between circulation proteins and EOC subtypes, we employed MR analyses using the "TwoSampleMR" software package (https://github.com/MRCIEU/TwoSampleMR). The Inverse Variance Weighted (IVW) method was used to generate effect estimates.[18] Subsequently, the P-values were subjected to stringent adjustment for multiple comparisons using the false discovery rate (FDR) method with the Benjamini-Hochberg approach. A significant cut-off was declared at P adjust < 0.05, ensuring that findings deemed significant were robust against the heightened risk of type I errors associated with multiple testing.

Sensitivity Analyses

To verify the reliability and stability of these protein-cancer associations and identify potential biases resulting from heterogeneity and horizontal pleiotropy, sensitivity analyses were conducted. Firstly, the heterogeneity test was carried out, with P > 0.05 serving as the threshold. Additionally, the pleiotropy test was performed to exclude the possibility of horizontal pleiotropy. A P-value greater than 0.05 was indicative of the absence of significant horizontal pleiotropy, The sensitivity analyses satisfied the prerequisite of MR, reinforcing the credibility of our findings.

Bayesian Colocalization Analysis

To ascertain the probability that the same genetic variant is responsible for both the protein level and the cancer phenotype, Bayesian colocalization analysis was employed utilizing the "coloc" package (https://github.com/chr1swallace/coloc).[19] Bayesian colocalization analyses consisted of five hypotheses: H0: No association with either trait. H1: Association with plasma protein but not cancer risk. H2: Association with cancer risk but not plasma protein. H3: Association with both traits, but with different causal variants. H4: Association with both traits, with shared causal variants (colocalization).[20] The analysis outputs posterior probabilities for each hypothesis, the posterior probability of hypothesis 4 (PPH4) $\geq$0.75 was considered strong evidence of colocalization, while $0.5 \leq \text{PPH4} < 0.75$ was considered as moderate strong evidence.[21] Proteins identified as having strong or moderate evidence of colocalization suggest a shared causal variant with the cancer phenotype, rather than being merely associated due to LD.

SMR Analysis and HEIDI Tests

To further validate our findings, we applied SMR analysis as a rigorous method for investigating the causal associations between protein levels and cancer risk.[22] A significance threshold for the SMR analysis was established at an FDR-adjusted P-value of less than 0.05. A result below this threshold would suggest a significant association, indicating that changes in protein levels may have a causal role in the development of cancer. In order to complement the SMR analysis, HEIDI tests

were conducted in order to discern whether the associations detected could be attributed to shared genetic variation or LD. By setting a P value greater than 0.05 as the criterion for the HEIDI test, it was aimed to exclude the possibility that LD is driving the observed associations, thereby reinforcing the evidence for a potential causal relationship.

Enrichment Analysis

To elucidate the intricate relationships among the MR-identified proteins, we conducted a thorough Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis utilizing the R package "ClusterProfiler" (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html). We established a threshold for significance where enrichment results boasting a P adjust value of less than 0.05 were considered statistically significant.

Mapping of the MR-Prioritized Proteins with Drug Targets

To assess the therapeutic potential of the causal proteins, we cross-referenced the causal proteins with the druggable genes designed by Finan et al,[23] There are 4479 genes identified by Finan et al as targets of existing or potential drug targets, which indicates they are druggable.

In addition, we turned to the Therapeutic Target Database[24](http://db.idrblab.net/ttd/),and the DrugBank database[25] (https://go.drugbank.com/) to validate the druggability. The Therapeutic Target Database provides information about known and explored therapeutic protein targets and their corresponding drugs. Similarly, the DrugBank database is a comprehensive resource that contains detailed drug data, including chemical, pharmacological, and pharmaceutical information, along with their targets.

PPI Network Investigates the Interactions with Existing Targets

To investigate the druggable MR-prioritized proteins' interactions with existing medication targets, we constructed PPI networks. This was achieved using the Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/) and Cytoscape, setting the minimum required interaction score to 0.4.[26]

Bioinformatics Analysis and RT-qPCR analysis

The main data of ovarian cancer RNA sequencing profiles and corresponding clinical information were obtained from the TCGA database and GTEx database.[27] A total of 427 hGSOC tissues and 88 normal tissues were included using R (v 4.3.0) for analysis and visualization. The survival analysis was carried out with the Kaplan-Meier plotter. A P-value < 0.05 was considered statistically significant. RNA of tissues was extracted using TRIzol (Invitrogen) and was reversely transcribed to complementary DNA (cDNA) via the PrimeScript RT reagent Kit (Vazyme). qPCR was carried out using FastStart Universal SYBR Green Master MIX (Vazyme) on QuantStudio 3 Real-Time PCR system. All results have been repeated three times.

External Validation

The external proteomic dataset of Vyhlídalová Kotrbová et al was incorporated into our analysis to ascertain the expression levels of the causal proteins identified in our study.[28]

Results

Proteome-Wide MR Analysis Estimates the Causal Effect on EOC Subtypes

To estimate the causal effect of plasma proteins on five EOC subtypes, A total of 15419 SNPs associated with 2015 proteins (1558 unique proteins) were used as exposures, and the incidence of five EOC subtypes as outcomes (Additional file 1: Table S2). 1264 protein-cancer associations were identified at an FDR-corrected threshold (Additional file 1: Table S3 and Figure 2). Overall, 569 protein-cancer associations passed the sensitivity analyses (Additional file 1: Table S4), including 455 unique proteins associated with at least one EOC subtype (Figure 3). These 455 proteins were designated as MR-identified proteins. For those 81 proteins demonstrated associations with multiple EOC subtypes (Additional file 1: Table S5), notably, over one-third of the proteins (35 out of 81) exhibited contrasting effects on tumor development across different subtypes (Additional file 2: Figure S1). This finding indicates the substantial molecular heterogeneity inherent among EOC subtypes and implies that they cannot be

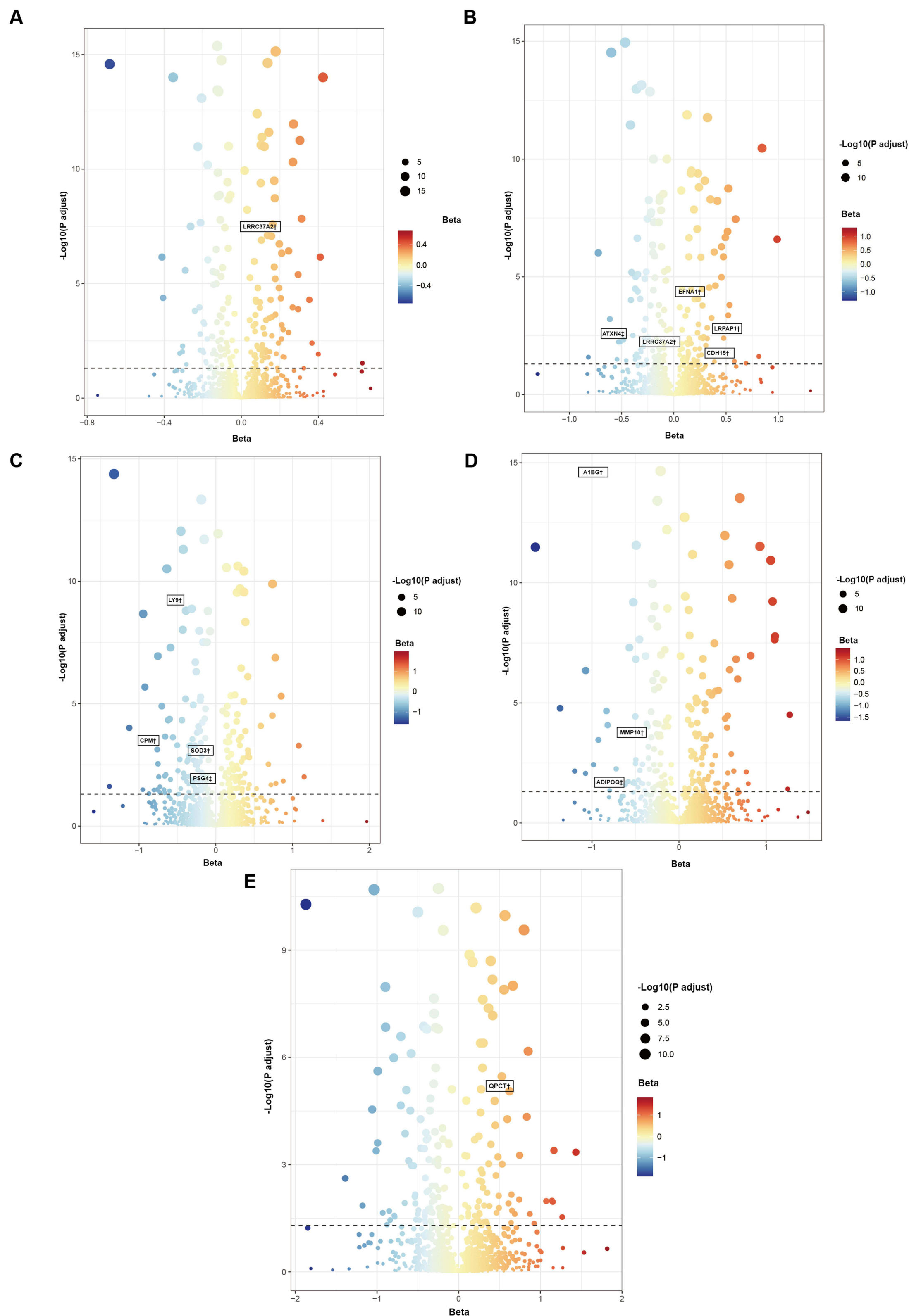


Figure 2 Volcano plot showing results from CIS-Mendelian Randomization. A total 1264 causal proteins were identified as associated with tumors, causal proteins related to HGSOC risk (**A**), ENOC risk (**B**), CCOC risk (**C**), MOC risk (**D**), and LGSOC risk (**E**) were presented. Source of the instruments: † represents Sun et al,[15] ‡ represents Ferkingstad et al,[12] The color of the dots represents the beta value; dot size represents the−log10 adjusted P value.

Abbreviations: CIS-MR, Cis-Mendelian randomization; HGSOC, high-grade serous ovarian carcinoma; ENOC, endometrioid ovarian carcinoma; CCOC, clear cell ovarian carcinoma; MOC, mucinous ovarian carcinoma; LGSOC, low-grade serous ovarian carcinoma.

simply analyzed and discussed as a whole. The data highlights the necessity for targeted investigations into each subtype to fully understand underlying mechanisms linking plasma proteins to cancer pathogenesis.

Colocalization Analysis Identified Nine Protein-Cancer Associations

To verify that the plasma protein and cancer risk shared common genetic variants, we employed the Bayesian colocalization analysis for MR-identified causal proteins. Among 569 protein-cancer associations, nine were supported by strong or moderate strong colocalization evidence (Additional file 1: Table S6).

SMR and HEIDI Tests Verified Eight Protein-Cancer Associations

To further substantiate the MR-identified protein-cancer associations, we implemented SMR and HEIDI tests, which provided valuable insights even in the absence of direct colocalization evidence. Thus, 569 protein-cancer associations identified by MR analysis were included in SMR and HEIDI tests. As shown in Additional file 1: Table S7, eight protein-cancer associations met the significance threshold in the SMR test (P adjust < 0.05), and passed the HEIDI test (P > 0.05). The concatenation of the protein-cancer associations exhibiting colocalization evidence and those passing SMR and HEIDI tests yielded 14 protein-cancer associations, each with compelling causal evidence (Additional file 1: Table S8 and Figure 4), as well as 13 unique causal proteins collectively designated as MR-prioritized proteins (Table 1).

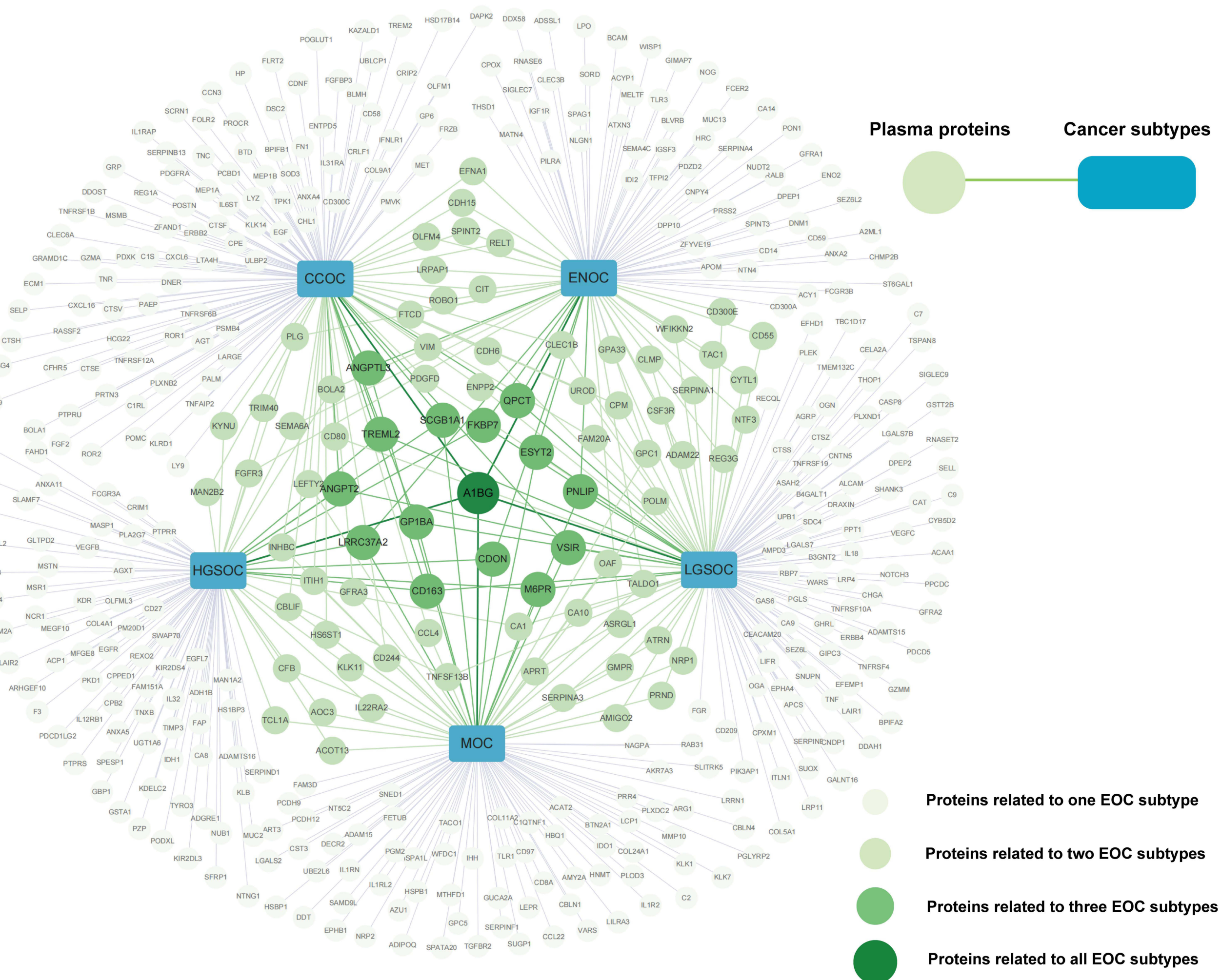


Figure 3 569 protein-cancer associations were identified by Mendelian Randomization and passed the sensitivity analyses, including 81 proteins that demonstrated associations with multiple EOC subtypes.

Abbreviations: HGSOC, high-grade serous ovarian carcinoma; ENOC, endometrioid ovarian carcinoma; CCOC, clear cell ovarian carcinoma; MOC, mucinous ovarian carcinoma; LGSOC, low-grade serous ovarian carcinoma.

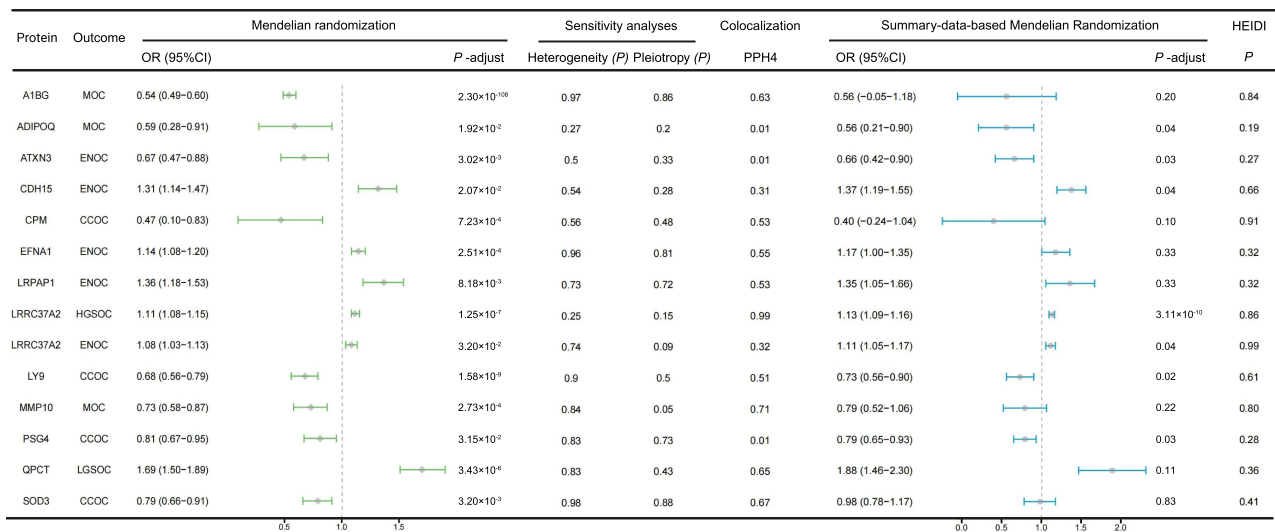


Figure 4 Protein–cancer associations prioritized by MR, Sensitive analysis, Colocalization, SMR, and HEIDI test using cis-pQTLs. 14 Proteins, including 13 unique proteins which prioritized by MR and passed Colocalization or SMR and HEIDI test were identified as core causal proteins.

Abbreviations: MR, Mendelian randomization; SMR, Summary-data-based Mendelian randomization; HEIDI, Heterogeneity in dependent instruments; pQTL, protein quantitative trait loci; HGSOC, high-grade serous ovarian carcinoma; ENOC, endometrioid ovarian carcinoma; CCOC, clear cell ovarian carcinoma; MOC, mucinous ovarian carcinoma; LGSOC, low-grade serous ovarian carcinoma.

Enrichment Analysis of the MR-Identified Proteins

We further performed enrichment analyses to explore the biosignificance of MR-identified proteins. Several pathways biologically relevant to cancer were enriched in GO pathway analysis, such as regulation of positive chemotaxis,

Table 1 Protein–Cancer Associations Prioritized by MR, Sensitive Analysis, Colocalization, SMR, and HEIDI Test Using Cis-pQTLs

UniProt	Protein Full Name	Outcome	Association with Cancer Risk
A6NM11	Leucine-rich repeat-containing protein 37A2 (LRRC37A2)	HGSOC, ENOC	Positive
P20827	Ephrin-A1 (EFNA1)	ENOC	Positive
P30533	Alpha-2-macroglobulin receptor-associated protein (LRPAP1)	ENOC	Positive
P14384	Carboxypeptidase M (CPM)	CCOC	Negative
Q9HBG7	T-lymphocyte surface antigen Ly-9 (LY9)	CCOC	Negative
P08294	Extracellular superoxide dismutase [Cu-Zn] (SOD3)	CCOC	Negative
P04217	Alpha-1B-glycoprotein (A1BG)	MOC	Negative
P09238	Stromelysin-2 (MMP10)	MOC	Negative
Q16769	Glutaminyl-peptide cyclotransferase (QPCT)	LGSOC	Positive
P55291	Cadherin-15 (CDH15)	ENOC	Positive
P54252	Ataxin-3 (ATXN3)	ENOC	Negative
Q00888	Pregnancy-specific beta-1-glycoprotein 4 (PSG4)	CCOC	Negative
Q15848	Adiponectin (ADIPOQ)	MOC	Negative

Abbreviations: MR, Mendelian randomization; SMR, Summary-data-based Mendelian randomization; HEIDI, Heterogeneity in dependent instruments; pQTL, protein quantitative trait loci; HGSOC, high-grade serous ovarian carcinoma; ENOC, endometrioid ovarian carcinoma; CCOC, clear cell ovarian carcinoma; MOC, mucinous ovarian carcinoma; LGSOC, low-grade serous ovarian carcinoma.

regulation of cell adhesion, cell proliferation, cell growth, and proliferation of immune cells. Further to this, some signaling pathways including epidermal growth factor receptor tyrosine kinase inhibitor resistance, phosphoinositide 3-kinase-protein kinase B signaling pathway, and extracellular matrix-receptor interaction were enriched in KEGG pathway analysis. (Additional file 1: Table S9-13, Additional file 2: Figure S2).

Evaluating the Druggability of MR-Prioritized Proteins

Given the pivotal role that proteins play in therapeutic targeting, our objective was to ascertain whether the prioritized proteins can be utilized for therapeutic purposes. To this end, we conducted a comparative analysis between the prioritized proteins and the druggable genes of Finan et al. As shown in Table 2 and Additional file 1: Table S14, nine proteins were annotated as potential druggable, including 1 Tier 1, 3 Tier 2, 4 Tier 3A, and 1 Tier 3B. In addition, according to the Therapeutic Target database and DrugBank database, 8 of the 22 proteins were annotated as targets of existing or potential drugs. Overall, ten proteins were identified as drug targets or to be druggable.

Investigating PPI of the MR-Prioritized Proteins with Existing Medication Targets

The PPI networks elucidated interactions between two druggable prioritized proteins and established drug targets (Figure 5). Specifically, α1B-glycoprotein (A1BG) and ephrin-A1 (EFNA1) are both involved in known interactions with human epidermal growth factor receptor 2 (HER2), which was the direct target of the monoclonal antibody Trastuzumab. In addition, EFNA1's interaction with vascular endothelial growth factor receptor (VEGFR) has been documented. Bevacizumab, which is widely used in EOC treatment, functions by inhibiting the VEGF/VEGFR interaction through its competitive binding to VEGF.

Table 2 List of the Prioritized Proteins That Were Drug Targets or to Be Druggable

UniProt	Protein Full Name	Druggability Tier[a]	Target Type[b]	Existing Target Drugs[c]
P20827	Ephrin-A1 (EFNA1)	Tier 3A	-	-
P30533	Alpha-2-macroglobulin receptor-associated protein (LRPAP1)	Tier 3A	-	-
P14384	Carboxypeptidase M (CPM)	Tier 2	Literature-reported Target	Yes
Q9HBG7	T-lymphocyte surface antigen Ly-9 (LY9)	Tier 3A	Literature-reported Target	-
P08294	Extracellular superoxide dismutase [Cu-Zn] (SOD3)	Tier 1	-	Yes
P04217	Alpha-1B-glycoprotein (A1BG)	Tier 3B	-	Yes
P09238	Stromelysin-2 (MMP10)	Tier 2	Patented-recorded Target	Yes
Q16769	Glutaminyl-peptide cyclotransferase (QPCT)	Tier 2	Clinical trial Target	Yes
P54252	Ataxin-3 (ATXN3)	-	Clinical trial Target	Yes
Q15848	Adiponectin (ADIPOQ)	Tier 3A	Literature-reported Target	-

Notes: [a]Based on the druggable genes designed by Finan et al [b]Based on the Therapeutic target database. [c]Based on the Therapeutic target database and the DrugBank database.

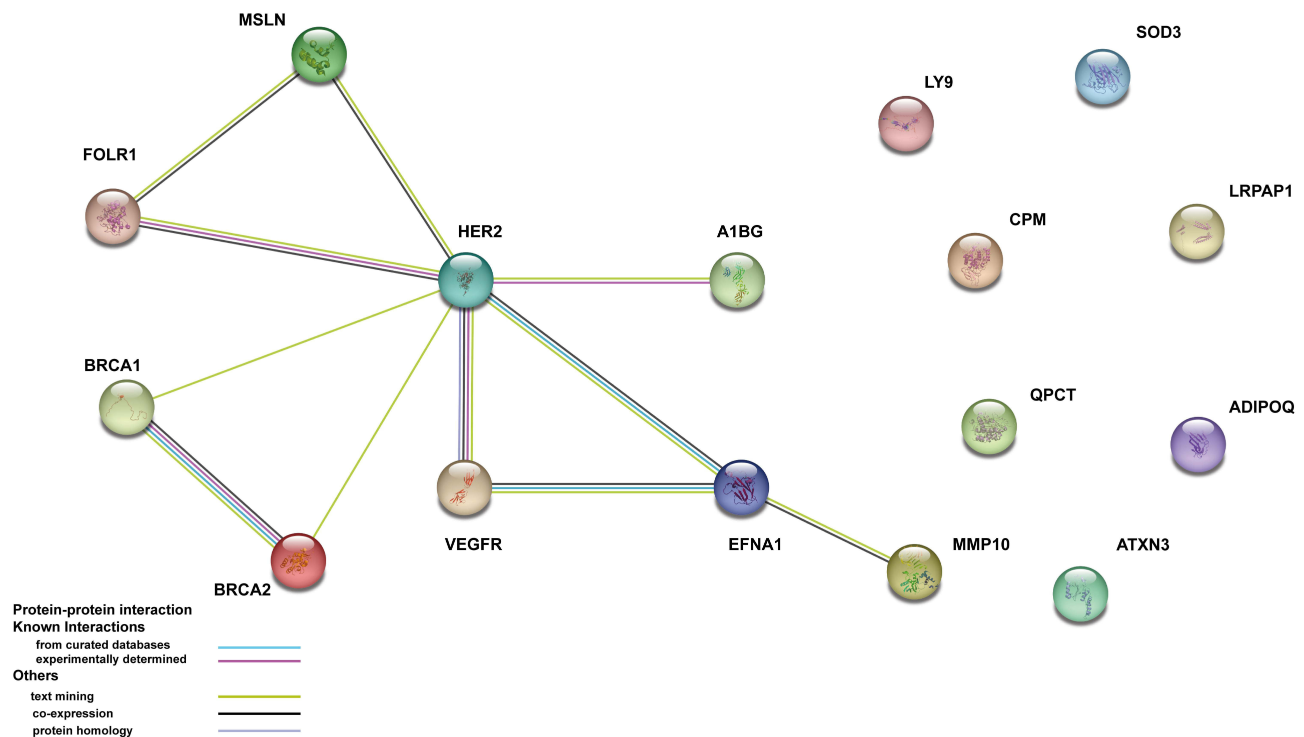


Figure 5 PPI network between existing drug targets with druggable causal proteins revealed interactions between AIBG and HER-2, and also between EFNA1 and both HER-2 and VEGFR.

Abbreviations: PPI, protein-protein interaction; A1BG, α1B-glycoprotein; HER2, human epidermal growth factor receptor 2; EFNA1, ephrin-A1; VEGFR, vascular endothelial growth factor receptor.

EFNA1 and A1BG Were Aberrantly Upregulated in HGSOC Tissues and Negative Related to Clinical Outcomes

Bevacizumab (VEGF inhibitor) has been widely used in EOC, particularly HGSOC, with promising results. Although HER2 amplifications are not common in EOC, antibody-drug conjugates (ADCs) targeting HER2 have demonstrated promising therapeutic efficacy in HER2-positive OC patients, underscoring the significance of personalized treatment strategies. To further assessed the expression of EFNA1 and A1BG in HGSOC tissues, we conducted a comparative analysis using the TCGA and GTEx databases. EFNA1 was observed to be markedly elevated in HGSOC tissues ($P < 0.001$) (Figure S3A). In addition, as shown in Figure S3B, its expression is associated with a higher clinical stage ($p<0.05$). In contrast to MR analysis of plasma A1BG acted as a negative factor for EOC, AIBG mRNA level in HGSOC tissues is elevated compared to normal tissues ($P < 0.05$), while not related to clinical stage (Figure S3C-S3D). Receiver operating characteristic (ROC) analysis was conducted by calculating the area under the curve (AUC). ROC analysis indicated that EFNA1 (AUC = 0.927, confidence interval (CI) = 0.894–0.960) could serve as a crucial parameter for distinguishing between normal and tumor tissues in HGSOC compared to A1BG (AUC = 0.596, CI = 0.542–0.651) (Figure S3E-S3F). The prognostic value of EFNA1 and A1BG was evaluated by Kaplan-Meier survival analysis, which revealed a clear correlation between high EFNA1 expression and significantly inferior overall survival (OS) (P=0.0025, hazard ratio (HR) = 1.42, CI=1.13–1.79). A comparable trend was noted for progression-free survival (PFS) (P=0.017, HR = 1.31, CI=1.05–1.63) (Figure S3G). Regarding A1BG, a high mRNA level was significantly associated with poor PFS (P<0.001, HR = 1.52, CI=1.26–1.84), whereas no significant association was found with OS (P=0.09, HR = 1.19, CI=0.97–1.46) (Figure S3H). The mRNA expression of EFNA1 was also upregulated in HGSOC patients' tissues (Figure S3I-S3J).

External Validation in HGSOC Ascites

To further verify the causal proteins identified, we cross-reference our data with the ascites proteins from Vyhlídalová Kotrbová A et al and successfully confirmed the causal links of AIBG and ADIPOQ with EOCs, as detailed in Table S15. It is important to note that within the external validation frame, eight among the 13 causal proteins were not measured. The majority of the measurable proteins exhibited consistent trends with our findings even the primary findings did not reach statistical significance.

Discussion

EOC presents significant challenges and highlights the urgent need for novel biomarkers and therapeutic targets. However, associations between plasma proteins and EOC risk remain unclear. Our research employed a multi-tiered approach combining MR, sensitivity analyses, colocalization, SMR, HEIDI tests, enrichment analyses, PPI network, and assessment of druggability to comprehensively investigate the causality of 2015 plasma proteins from two recently published large proteomics GWAS on five EOC subtypes. In summary, based on the MR and sensitivity analyses, 569 protein-cancer associations were identified and 14 compelling protein-cancer associations were further validated by colocalization, SMR, and HEIDI tests, thus ensuring robustness. Subsequently, to elucidate the underlying functional relevance among these proteins, we conducted enrichment analyses. The assessment of druggability yielded a total of ten druggable protein targets, which are expected to provide an avenue for innovative treatment strategies. In addition, Of the MR-prioritized proteins, A1BG and EFNA1 were identified to correspond with current pharmacological targets and EFNA1 was further confirmed to be an excellent indicator for diagnosis and prognosis prediction in HGSOC tissue by bioinformatics analysis.

EFNA1 serves as a ligand for the ephrin type-A receptor (EphA) receptor, and its aberrant expression is associated with OC malignancy and prognosis.[29] In breast cancer, EFNA1 knockdown has been shown to reduce endothelial cell migration and tumor angiogenesis in vivo.[23] Previous studies have shown that the soluble form of EFNA1 can competitively bind to EphA2 on cell membranes and stimulate endocytosis, thereby reducing membrane-bound EphA2 and promoting tumor lung metastasis.[30] A neutralizing antibody that inhibits the interaction between EphA1/A2 and hepatocyte growth factor A1 has successfully suppressed lung metastasis.[31] Our findings are consistent with the above studies that soluble EFNA1 may serve as a biomarker for cancer and a promising candidate for molecularly targeted therapy. Furthermore, the PPI network highlighted the interactions with HER2 and VEGFR. Specific inhibitors that disrupt the Eph/ephrin interaction are under extensive investigation. Given these findings, further research is imperative to elucidate the precise role and underlying mechanisms of EFNA1 in OC. It is crucial to highlight that EFNA1 is a highly potent plasma marker and causal protein for ENOC. However, there is a paucity of studies investigating the expression levels of EFNA1 in ENOC tissues, which represents a crucial avenue for further research within the ENOC population. It is important to note that, with the exception of HGSOC, other subtypes of OC are relatively uncommon in clinical practice. Consequently, there is a paucity of clinical and fundamental research data on therapeutic targets for these particular subtypes, including bevacizumab. Although our study has preliminarily revealed a causal relationship between EFNA1 and ENOC, as well as the interaction between EFNA1 and VEGFR, further research is required to gain a deeper understanding of these findings in the ENOC patient population and biological models. Additionally, our findings indicate that EFNA1 expression is elevated in HGSOC tissues, suggesting its potential as a therapeutic target. However, it should be emphasized that this does not qualify as a plasma marker for HGSOC. This requires further investigation to elucidate the underlying reasons for this phenomenon.

Besides, A1BG, as a binding partner of cysteine-rich secretory protein 3, is recognized for its presence in the exocrine secretions and secretory granules of neutrophils. The specific function of A1BG within the context of tumors remains elusive; however, it is purported to contribute to innate immunity, subsequently facilitating the activation of the adaptive immune system via a process termed antigen presentation.[32] In our study, A1BG emerged as a protective factor across five subtypes, demonstrating a particularly strong negative correlation in MOC risk. Additionally, the PPI network revealed interactions between A1BG and HER2, the latter of which has been identified as a promising target for both Trastuzumab treatment and antibody-drug conjugates. It is important to emphasize that HER2 amplification is not a common occurrence in MOC. It is therefore essential to conduct a more comprehensive investigation into the therapeutic efficacy and potential mechanisms of A1BG as a drug target in the CCOC population. This should entail a personalized analysis of HER2 amplification and an acknowledgement that its protective impact may not be exclusively

dependent on HER2 amplification. In addition, in HGSOC tissues, there is an increasing trend in mRNA levels of A1BG compared to normal ovaries. The inconsistent expression patterns of A1BG in plasma protein levels and tissue mRNA levels require further validation and investigation through subsequent studies to elucidate the precise role and underlying mechanisms of A1BG in OC.

Our investigation further pinpointed certain prioritized proteins that, despite lacking prior associations with OC in previous studies, might contribute to the tumor's progression, particularly those identified as druggable targets. Low-density lipoprotein receptor-related protein (LRP) associated protein 1 (LRPAP1) acts as a chaperone for LRP1.[33] Previous studies have demonstrated the pro-tumor effects of LRPAP1 in several cancers.[34,35] LRPAP1 has also been identified as a B cell receptor autoantigen that triggers proliferation in mantle cell lymphoma.[36] However, the impact of LRPAP1 on ovarian tumors has not yet been investigated. Recently, Li et al demonstrated that secreted LRPAP1 is able to bind and induce degradation of type I interferon (IFN) receptor 1 (IFNAR1) to promote viral evasion of cellular innate immunity.[37] IFNAR1 is a subunit of the receptor for type I IFN, which binds to type I IFN and activates the JAK-STAT signaling pathway, essential for anti-tumor immunity.[38] Li et al also demonstrated that α2M could inhibit LRPAP1 and stabilize IFNAR1.[37] Our results strengthen the above studies and highlight secreted LRPAP1 as a causal protein of ENOC. Targeting LRPAP1 might activate innate anti-tumor immunity, potentially enabling cancer patients to benefit from type I IFN-based anti-tumor immunotherapy. Besides, although glutaminyl-peptide cyclotransferase (QPCT) has not yet been studied in OC, previous research has shown that it plays a specific role in promoting tumor cell proliferation, migration, and angiogenesis, while also inhibiting apoptosis in thyroid and renal cell carcinomas. Studies have shown that QPCT may be associated with tumor cell resistance. QPCT is up-regulated by NF-κB, which promotes resistance to Sunitinib in renal cell carcinoma patients.[39] It has also been found to reduce adriamycin sensitivity in breast cancer.[40] Additionally, it has been found that QPCT's isoform QPCTL could mediate pE formation at the N-terminus of signal-regulated protein alpha (SIRPα) binding site cluster differentiation cluster 47 (CD47) protein and contribute to the CD47-SIRPα myeloid immune checkpoint, which by suppressing immune surveillance in cancer cells to evade phagocytosis by macrophages.[41,42] Our findings are consistent with the above studies that QPCT is a positive causal protein for LGSOC. This highlights the avenue for future studies, which might focus more on the specific mechanism of QPCT in LGSOC, emphasizing the promotion of cancer progression, drug resistance, and immunosuppression. T-lymphocyte surface antigen Ly-9 (LY9) is ubiquitously expressed in various immune cell types[43,44] and plays intricate roles in the regulation of complex immune networks.[45] Previous studies have elucidated a dual role for LY9 in the context of cancer. However, its function in OC remains elusive. Our preliminary results suggest that LY9 may act as a tumor suppressor in CCOC. In addition, upregulation of LY9 has been associated with a BRCAness phenotype and may mediate the response to poly ADP-ribose polymerase inhibitors.[46] This highlights the importance of LY9 as a target of interest for future research efforts. In addition, extracellular superoxide dismutase (SOD3) encodes a member of the SOD protein family. SOD is an antioxidant enzyme that catalyzes the conversion of superoxide radicals into hydrogen peroxide and oxygen, thereby protecting tissues from oxidative stress and preventing the occurrence and development of tumors.[47] Our findings are consistent with the above studies that consider SOD3 as an anticancer factor. ATXN3 is a ubiquitin hydrolase, which is an enzyme that cleaves ubiquitin molecules from protein substrates.[48] Despite limited investigation into the role of ATXN3 in EOC, there is growing evidence that ubiquitination and deubiquitination may play a complex and pivotal bidirectional role in tumor biology. It has been demonstrated that ATXN3 exerts anti-tumor effects in human colon cancer by enhancing cell apoptosis induced by galectin-9.[49] Our findings are in accordance with the aforementioned studies, which indicate that ATXN3 functions as a negative causal protein for ENOC. Furthermore, previous studies have indicated that ATXN3 may function as a positive regulator of PD-L1 transcription,[50] thereby suggesting its potential to enhance the efficacy of immune checkpoint PD-1 therapy. ADIPOQ is primarily secreted by adipocytes and has been demonstrated to have a negative correlation with body fat mass and visceral obesity. Previous studies have demonstrated that the intratumoral administration of adiponectin results in the disruption of tumor vasculature and caspase-3-mediated intratumoral cell apoptosis in mouse fibrosarcoma model.[51] Furthermore, adiponectin receptor agonist ADIPOQ has been shown to induce apoptotic cell death and suppress proliferation in OC cells. Our findings are consistent with the aforementioned studies and additionally suggest that circulating ADIPOQ may serve as a protective factor.[52] MMP10, a member of the matrix metalloproteinases (MMPs) family, has been demonstrated to be overexpressed in tumor tissues and to play a pivotal role in the processes of invasion and metastasis, as evidenced by previous studies on OC histology.[53] However, there is a paucity of research on the plasma protein levels of MMP10 in MOC. Further research is required to provide a more comprehensive explanation. Similarly, previous researches have demonstrated

a correlation between the expression of CPM and the progression of diverse cancers.[54,55] While, in our study, circulating CPM was identified as exhibiting a negative correlation with CCOC. More research is needed to offer a more thorough explanation.

Given that EOC, located deep within the pelvis, often begins insidiously and lacks early typical symptoms, it poses a challenge for early detection. Furthermore, the efficacy of chemotherapy is hindered by resistance to anticancer drugs. In addition, the molecular diversity among these subtypes necessitates a tailored approach to drug development and treatment. Consequently, there is an urgent need to identify easily detectable biomarkers and novel therapeutic targets for individuals with different EOC subtypes. Plasma proteins' involvement in tumor growth and metastasis renders them attractive targets for precision cancer therapies. The altered expression of these proteins within the tumor microenvironment provides unique opportunities for therapeutic intervention and drug delivery. Additionally, blood's accessibility versus other tissues makes circulating proteins appealing candidates for easily detectable biomarkers and drug targets, facilitating both the development and application of targeted treatments. As a relatively well-established epidemiological method for testing causality, MR can effectively reduce the influence of confounding factors and achieve superior credibility. In recent years, with the continuous development of large-scale proteomics and GWAS, MR has become a novel tool for exploring circulating protein biomarkers as well as potential therapeutic targets. Proteome-wide Mendelian randomization analysis has become the focus of current clinical research trends, but relevant studies in the field of EOC are still limited. To the best of our knowledge, only sporadic studies have investigated the causal protein for EOC using proteome-wide Mendelian randomization analysis. In one study, 732 plasma proteins derived from a single proteomic GWAS were used as exposures, and total EOC was used as the outcome.[13] This study only looked at total EOC, but did not explicitly account for the different molecular profiles of the subtypes and did not yield positive results. In another study, researchers did not account for potential biases associated with using trans-pQTLs as genetic instruments, incorporating all pQTLs into the analysis without differentiating them based on their localization into cis- and trans-pQTLs. Similarly, molecular differences among EOC subtypes were not considered, with EOC being treated as a homogeneous entity and defined as the outcome.[14] In our research, we incorporated two extensive datasets of 2015 plasma proteins. To minimize the potential for horizontal pleiotropy, we exclusively utilized cis-pQTLs as our genetic instruments. Our analysis was structured around the distinct subtypes of EOC, emphasizing both the similarities and heterogeneity among the various subtypes. This approach aimed to enhance both the applicability and reliability of our findings.

Despite the current widespread use of CA-125 and HE4 as OC biomarkers, and the potential for the mutational status of the BRCA1/2 gene to guide the use of PARP inhibitors, there are still several limitations. CA-125 is primarily utilized for monitoring treatment response and recurrence. Additionally, HE4 and BRCA1/2 mutations have demonstrated limitations in early detection, diagnostic specificity, and risk assessment. In contrast, the newly identified proteins have the potential to enable early disease detection due to their causal associations with EOC subtypes and offer valuable insights into disease progression and treatment response. This would provide both diagnostic and prognostic advantages. Furthermore, recently discovered proteins such as A1BG and EFNA1 have been found to interact with known drug targets, including HER2 and VEGFR. This presents significant potential for the development of innovative therapeutic strategies. This not only enhances their utility as biomarkers but also reveals new opportunities for drug development and personalized therapies, which could signify a substantial advancement compared to current biomarkers.

Nevertheless, the study was conducted exclusively within European populations, necessitating further validation to determine if these findings are applicable to other ethnic groups. This study emphasizes the significant potential of several causal proteins as readily detectable biomarkers. It is imperative that future research conduct in-depth validation of the identified proteins in a broader, independent cohort of OC patients, particularly targeting different subtypes. This encompasses an evaluation of their viability as plasma biomarkers in the early stages of the disease, as well as an assessment of their sensitivity and specificity, which is particularly crucial to EOC, given the lack of effective early screening methods. Moreover, further research is required to investigate the potential of plasma proteins for monitoring disease progression and predicting treatment response in patients with EOC. These endeavors have the potential to markedly enhance patient prognosis by facilitating earlier interventions and more personalized treatment plans. Moreover, given the interaction of A1BG and EFNA1 with known drug targets such as HER2 and VEGFR, future researches should concentrate on elucidating their precise mechanisms of action and biological pathways in the development of OC, including the analysis of both plasma and tissue samples. This will provide a robust scientific basis for the development of innovative therapeutic drugs.

Conclusions

To sum up, through two-sample MR analyses, our research has pinpointed a multitude of circulating proteins with a causal link to EOC subtypes. Additionally, our study offers insights into the credibility of the MR results and highlights potential therapeutic targets for EOC by employing a structured analytical approach. Notably, EFNA1 was identified as a druggable target and confirmed to correspond with current pharmacological targets, potentially offering an avenue for innovative treatment strategies. Future research is imperative to delve into the pathogenic roles and the intrinsic biological processes associated with these causal proteins in EOC.

Abbreviations

A1BG, α1B-glycoprotein; CCOC, Clear cell ovarian carcinoma; CD47, cluster differentiation cluster 47; EFNA1, Ephrin-A1; ENOC, Endometrioid ovarian carcinoma; EOC, Epithelial ovarian carcinoma; EphA, Ephrin type-A receptor; FDR, False discovery rate; GO, Gene Ontology; GWAS, Genome-wide association studies; HEIDI, Heterogeneity in Dependent Instruments; HER2, Human epidermal growth factor receptor 2; HGSOC, High-grade serous ovarian carcinoma; IFN, Type I interferon; IFNAR1, Type I interferon receptor 1; IVW, Inverse Variance Weighted; KEGG, Kyoto Encyclopaedia of Genes and Genomes; LD, Linkage disequilibrium; LGSOC, Low-grade serous ovarian carcinoma; LRPAP1, Low-density lipoprotein receptor-related protein associated protein 1; LY9, T-lymphocyte surface antigen Ly-9; MOC, Mucinous ovarian carcinoma; MR, Mendelian Randomization; OC, Ovarian cancer; PPH4, Posterior probability of hypothesis 4; PPI, Protein-protein interaction; pQTL, Protein quantitative trait loci; QPCT, Glutaminyl-peptide cyclotransferase; SIRPα, Signal-regulated protein alpha; SMR, Summary-data-based Mendelian Randomization; SNP, Single nucleotide polymorphism; SOD, Superoxide dismutase; SOD3, Extracellular superoxide dismutase; VEGFR, Vascular endothelial growth factor receptor.

Data Sharing Statement

The GWAS summary data of proteins are available at http://ukb-ppp.gwas.eu and https://www.decode.com/summarydata/. GWAS summary data of HGSOC are available at https://gwas.mrcieu.ac.uk/datasets/ieu-a-1121/. Further information is available from the corresponding author upon request.

Ethical Approval and Consent to Participate

This research was approved by the Obstetrics and Gynecology Hospital, Fudan University (Ethics number: kyy2021-114).

Acknowledgments

We would like to thank all the participants from two proteomic studies and the UK Biobank, the institutions and their staff for providing data.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the National Natural Science Foundation of China (82002750 to Mo Chen) and Shanghai Pujiang Programme (23PJD009 to Mo Chen).

Disclosure

The authors declare no conflicts of interest.

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