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ADAR in Ovarian Cancer: Integrating Mendelian Randomization, Transcriptomic Profiling, and Immunohistochemical Validation to Identify ARHGAP25 and SAMHD1 as Potential Targets

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Abstract

Background and Objective: Ovarian cancer (OC) shows complex pathogenesis and poor prognosis. While Adenosine Deaminase Acting on RNA (ADAR) gene plays roles in multiple tumors, its functions in OC remain unclear. This study investigated ADAR's mechanistic roles in OC through Mendelian randomization (MR) and multi-omics integration to identify key genes and therapeutic targets.

Methods: Transcriptomic data from TCGA (training set) and GSE26712 (validation set) were analyzed. MR assessed ADAR-OC causality. ADAR-associated genes were screened via differential expression, COX regression, and weighted gene co-expression network analysis (WGCNA), with key genes identified through Lasso-Cox and multivariate COX regression. Validation included expression profiling, functional enrichment, protein-protein interaction (PPI) network, immune infiltration, and drug sensitivity analyses. Finally, ADAR protein expression was validated at the tissue level by IHC staining.

Results: ADAR was significantly downregulated in OC ($P < 0.05$), with high expression predicting favorable prognosis. MR confirmed ADAR as a protective factor ($OR < 1$, $P < 0.05$). Among 123 candidate genes, ARHGAP25 and SAMHD1 were identified as key

genes that enriched immune response, antigen presentation and extracellular matrix (ECM) interaction pathways. ADAR expression showed positive correlations with naïve B cells and M2 macrophages, and negative correlations with $\gamma\delta$ T cells and activated mast cells, indicating selective regulation of the tumor microenvironment (TME). ADAR expression predicted sensitivity to 6 anticancer drugs including tamoxifen, etoposide, sorafenib, carboplatin, topotecan, and olaparib ($P < 0.05$). IHC further confirmed that ADAR protein was markedly downregulated in OC tissues, with predominantly cytoplasmic staining, corroborating the transcriptomic findings at the protein level.

Conclusion: ADAR is downregulated in OC and functions as a potential therapeutic targets. Through ARHGAP25, SAMHD1, and immune cell modulation, ADAR regulates cancer initiation, progression, and immune microenvironment

Keywords: ADAR; Ovarian cancer; Mendelian randomization; Bioinformatics; Immune infiltration; Drug sensitivity; Prognostic marker

1 Introduction

Ovarian cancer (OC) is recognized as one of the most lethal gynecologic malignancies affecting women globally, with approximately 314,000 new cases and over 207,000 deaths reported worldwide in 2020 [1,2]. Data from China's National Cancer Centre indicates a year-on-year increase in the incidence of OC [3]. Due to their deep pelvic location and small size, the ovaries produce no characteristic symptoms in the early stages of disease. Patients typically present only with non-specific abdominal pain or bloating, and the majority are diagnosed at an advanced stage [4,5]. The five-year survival rate for late-stage disease is 20–30%, compared to up to 80% for early-stage disease, which represents a significant threat to women's health [6]. Due to insidious early symptoms and the lack of effective screening methods, approximately 70% of OC patients are diagnosed at advanced stages (III/IV), resulting in an overall 5-year survival rate of only 30-50% [7,8]. In contrast, a 5-year survival rate exceeding 90% can be achieved in patients with localized OC diagnosed at early stages [9]. Therefore, the

58 exploration of effective biomarkers for early diagnosis and therapeutic targets is
59 considered to be of great significance for improving the prognosis of OC patients.

60 In recent years, with the rapid development of high-throughput sequencing technologies
61 and multi-omics analyses, RNA editing as an important post-transcriptional modification
62 mechanism has been increasingly recognized for its role in tumor initiation and
63 progression [10]. The Adenosine Deaminase Acting on RNA (ADAR) family is
64 identified as a key class of enzymes mediating adenosine-to-inosine (A-to-I) RNA
65 editing, which influences gene expression, RNA stability, splicing patterns, and protein
66 function through catalyzing the conversion of adenosine to inosine in double-stranded
67 RNA [11]. The ADAR family in mammals is comprised of three members: ADAR1,
68 ADAR2, and ADAR3, among which ADAR1 and ADAR2 have been demonstrated to
69 possess definitive adenosine deaminase activity [12].

70 Accumulating evidence has demonstrated that ADAR expression is dysregulated in
71 multiple malignancies and exerts complex biological functions. ADAR has been shown
72 to be upregulated in various tumors, including hepatocellular carcinoma and non-small
73 cell lung cancer, and is associated with tumor progression and poor prognosis [13,14]. In
74 OC, novel epitopes generated by RNA editing can be presented via HLA molecules,
75 inducing tumor-specific T cell responses, which suggests the potential for RNA editing-
76 based immunotherapy [15]. However, the specific mechanisms underlying ADAR
77 function in OC and its clinical value as a biomarker remain to be further investigated.

78 Traditional observational epidemiological studies are susceptible to confounding factors,
79 reverse causality, and measurement errors, making it difficult to establish definitive
80 causal relationships. Mendelian Randomization (MR) analysis utilizes genetic variants as
81 instrumental variables to infer causal relationships between exposure factors and disease
82 outcomes, effectively overcoming the limitations of conventional studies [16]. In recent
83 years, MR analysis has been widely applied in etiological research on OC, and causal
84 relationships between multiple risk factors and OC risk have been successfully identified
85 [17,18].

Although the important roles of ADAR in various tumors have been revealed by current studies, its causal role and molecular mechanisms in OC remain unclear. We innovatively combined MR analysis with multi-omics data to systematically elucidate the mechanisms underlying ADAR gene function in ovarian cancer (OC) initiation and progression. First, the causal relationship between ADAR gene expression and OC risk is validated through MR analysis. Second, key genes associated with ADAR are screened by combining transcriptomic data, weighted gene co-expression network analysis (WGCNA) and machine learning algorithms. The functional network of ADAR in OC and its clinical application value were revealed through functional enrichment analysis, protein-protein interaction (PPI) network analysis, immune infiltration analysis and drug sensitivity analysis. Additionally, immunohistochemical (IHC) staining was performed on ovarian serous cystadenoma and OC tissues to verify ADAR protein expression. Collectively, our study contributes to a deeper understanding of the potential role of ADAR-related genes in the OC pathogenesis, and provides potential therapeutic targets and possible theories for its early diagnosis and treatment.

2 Methods

2.1 Data Download and Preprocessing

Transcriptomic data from OC patients (n = 308) were downloaded from the TCGA database (<https://www.cancer.gov/ccg/research/genome-sequencing/tcga>), and normal ovarian tissues (CON, n = 88) from GTEx database, merged as the training set. The OC dataset GSE26712 was downloaded from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/profile/>) as validation set, containing 185 OC and 10 CON samples on GPL96 platform. Datasets were preprocessed via R package *inSilicoMerging* for background correction, gene conversion, and normalization.

OC and exposure factor data were downloaded from OpenGWAS database (<https://gwas.mrcieu.ac.uk/>). OC-ID: ieu-a-1120 included 40,941 CON and 25,509 OC samples, with ADAR gene as exposure factor.

2.2 ADAR Gene Expression, Kaplan-Meier Survival Curves, and Clinical Correlation Analysis

ADAR gene expression in OC and CON groups were analyzed using training set data to validate expression patterns. Kaplan-Meier survival curves explored correlations between ADAR expression and OC prognosis, demonstrating ADAR as an independent prognostic biomarker. Clinical data were extracted from TCGA database. Mann-Whitney U test compared age distribution across groups: younger than 40 years (Low), 40-50 years (Middle), and older than 50 years (High). Pearson/Spearman correlation and linear regression assessed ADAR expression-age correlation. Kruskal-Wallis H test compared ADAR expression across clinical stages (II-IV). Statistical significance was set at $P < 0.05$, with box plots and scatter plots for visualization.

2.3 Single-Gene MR Analysis of ADAR

For ADAR eQTL and OC outcome data, the `read_vcf` and `query_gwas` functions from R package "gwasvcf" were used, screening SNPs with $P < 0.05$ as instrumental variables. R^2 and F-statistics were calculated to assess explanatory power and instrument strength, retaining only SNPs with $F > 10$ to avoid weak instrument bias and ensure relevance assumption. The `gwasvcf_to_TwoSampleMR` function from "gwasglue" converted data formats. Outcome data were read via `read_outcome_data` from "TwoSampleMR", with `harmonise_data` function ensuring exposure-outcome allele compatibility. Five algorithms analyzed causal relationships: inverse variance weighted (IVW), MR-Egger regression, weighted median, weighted mode, and simple mode, with IVW as primary reference. Fixed or random effects models were selected based on Cochran's Q test heterogeneity results. Horizontal pleiotropy was assessed via MR-Egger intercept and MR-PRESSO tests. The `mr_singlesnp` function performed independent SNP analysis, with `generate_odds_ratios` function quantifying association strength through odds ratios (OR). Forest plots displayed effect values and 95% CIs, scatter plots assessed exposure-outcome linearity, and funnel plots evaluated publication bias and instrumental variable symmetry. Leave-one-out sensitivity analysis via `mr_leaveoneout` tested individual SNP influence on overall results. Steiger directionality test verified causal direction correctness, excluding reverse causation.

2.4 Differential Expression Analysis and COX Regression Analysis for Screening Differentially Expressed Genes

Based on the training set, the limma package was employed. The Benjamini-Hochberg (BH) method corrected original P-values to calculate false discovery rate (FDR), controlling false-positives from multiple testing. Screening thresholds were $FDR < 0.05$ and $|\log_2(\text{fold change})| > 5$, with volcano plots and heatmaps generated. Gene expression profiles and clinical data from OC patients were obtained from TCGA database. Cox proportional hazards regression was performed for each gene. The intersection of differentially expressed genes and COX-screened genes yielded commonly upregulated and downregulated genes.

2.5 WGCNA and Screening of Significantly Associated Genes

To identify co-expressed gene modules and hub genes associated with OC, WGCNA was performed on the training dataset. Optimal soft threshold β was determined via pickSoftThreshold function analyzing scale-free fit index and mean connectivity. Gene modules were generated through hierarchical clustering and dynamic tree cutting (minimum module genes = 30, deep split = 3, maximum module distance = 0.25). Modules significantly associated with disease ($P < 0.05$) were screened via Pearson correlation. Hub genes were identified using $|\text{gene significance (GS)}| > 0.8$ and $|\text{module membership (MM)}| > 0.7$. The intersection of differentially expressed genes and hub genes yielded candidate genes closely associated with OC.

To screen significantly associated genes, Lasso-Cox regression was performed on candidate gene expression data. Optimal penalty parameter λ was determined via 10-fold cross-validation, with L1 regularization compressing coefficients, eliminating redundant variables, and reducing multicollinearity to obtain the optimal gene subset. Lasso-screened genes were incorporated into multivariate Cox regression to evaluate independence after mutual adjustment, determining significantly associated genes with independent predictive effects on survival ($P < 0.05$). This stepwise strategy reduced high-dimensional data dimensionality while ensuring prognostic relevance and independence, establishing foundation for prognostic risk model construction.

2.6 Expression Validation, Diagnostic Performance Evaluation, and Correlation Analysis of Significantly Associated Genes

To validate significantly associated genes' expression patterns and diagnostic performance, Wilcoxon rank-sum test compared expression levels in training and validation datasets. Receiver operating characteristic (ROC) curve analysis evaluated diagnostic accuracy in both sets. Area under the curve (AUC) was calculated for each gene, with values approaching 1 indicating better performance. Genes with consistent expression across training and external datasets were identified as key genes significantly associated with OC. Expression data for ADAR and key genes were extracted, with Pearson correlation coefficients assessing ADAR-key gene expression correlations. Scatter plots were generated using R "ggplot2" package with R^2 and P-values annotated to demonstrate expression correlation trends.

2.7 Functional Enrichment Analysis of Candidate Genes

Functional enrichment analysis based on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) was performed on candidate genes using the DAVID database (<https://david.ncifcrf.gov>). The enrichment degree of key genes in each KEGG signaling pathway or GO term was calculated, with Fisher's exact test being employed for statistical testing to identify significantly enriched KEGG pathways and GO terms ($P < 0.05$).

2.8 PPI Network Analysis

PPI analysis was performed on the identified candidate genes using the STRING database (<https://cn.string-db.org/>). Subsequently, visualization analysis and topological structure analysis of the PPI network were conducted using Cytoscape software to identify interaction relationships between the ADAR gene and candidate genes associated with OC initiation and progression.

2.9 Immune Infiltration Analysis

CIBERSORTx was used to infer the abundance of 22 immune cell subsets in OC and CON samples via <https://cibersortx.stanford.edu/>. The Wilcoxon rank-sum test compared

immune infiltration between groups. Pearson correlation assessed relationships among immune cells and between ADAR genes and immune cells. The ESTIMATE algorithm calculated tumor microenvironment (TME) scores. Samples were stratified by ADAR expression levels, and stromal, immune, and ESTIMATE scores were visualized by violin plots to evaluate ADAR's impact on TME composition.

2.10 Drug Sensitivity Analysis

Drug sensitivity analysis integrated multi-database data and statistical testing. Drug sensitivity data for OC were obtained from the GDSC database (<https://www.cancerrxgene.org/>), and expression data were retrieved from TCGA. The R package "oncoPredict" was used to predict drug sensitivity for each sample with default parameters, including the "combat" method for batch effect correction. Key drugs were screened through joint GDSC-TCGA analysis, and the Wilcoxon rank-sum test compared IC50 values between different ADAR expression levels to evaluate ADAR's predictive ability for drug response.

2.11 Immunohistochemical validation of ADAR

To validate the expression of the ADAR protein at tissue level, IHC staining was performed on formalin-fixed, paraffin-embedded (FFPE) ovarian tissue sections. Ovarian serocystadenoma samples were used as the control group and OC samples were used as the experimental group.

2.12 Statistical Analysis

Bioinformatics analyses were performed using the R programming language (v 4.2.2). Differences between two groups were compared using the Wilcoxon rank-sum test. A P-value < 0.05 was considered statistically significant.

3 Results

3.1 ADAR Gene Expression, Kaplan-Meier Survival Curves, and Clinical Correlation Analysis

228 According to the results, the ADAR gene was significantly downregulated in the OC
229 group compared to the CON group ($P < 0.05$), with the difference being statistically
230 significant (Figure 1A). To further evaluate the clinical prognostic value of ADAR gene
231 expression levels, Kaplan-Meier survival curve analysis revealed that the overall survival
232 rate of patients in the ADAR high expression group was significantly higher than that of
233 the low expression group (Figure 1B, $P = 0.0046$), with survival differences between the
234 two groups showing significant statistical significance. Furthermore, ADAR expression
235 levels showed no significant association with patient age (Figure 1C) ($P > 0.05$), but were
236 correlated with clinical stage (Figure 1D). The ADAR gene appears to be expressed at
237 low levels in OC, with high expression being associated with a favourable prognosis for
238 patients and correlating with clinical stage. This makes it an important indicator for the
239 prognostic evaluation and stratification of OC patients, providing a vital molecular basis
240 for formulating clinical treatment strategies.

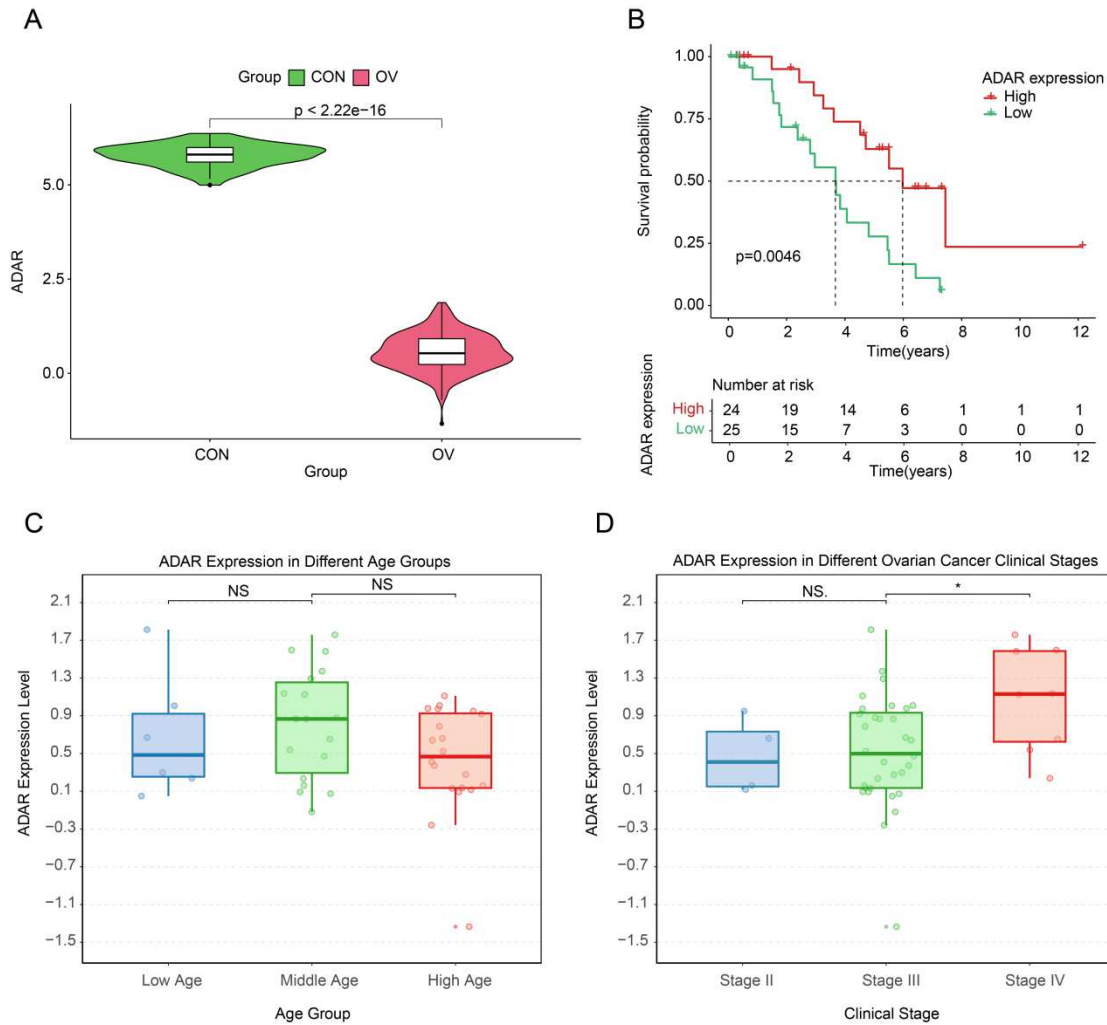


Figure 1 ADAR Gene Differential Analysis and Kaplan-Meier Survival Curve Analysis

(A) ADAR gene differential analysis. (B) Kaplan-Meier survival curve analysis. (C) Box plots of ADAR expression levels in different age groups (Low Age, Middle Age, High Age). (D) Box plots of ADAR expression levels in OC patients at different clinical stages (Stage II, III, IV). (NS: no statistical difference, *: $P < 0.05$, statistically significant difference).

3.2 Single-Genes MR Analysis of ADAR

MR analysis was conducted using five algorithms (Inverse variance weighted test, MR-Egger, weighted median, Simple mode, Weighted mode) with ADAR eQTL as the exposure factor and OC as the outcome. Results primarily referenced the Inverse variance weighted (IVW) method, with reference to subsequent heterogeneity analysis results

from Cochran's Q assessment; random effects IVW was used when heterogeneity was present, and fixed effects IVW was used when heterogeneity was absent.

The F-statistics for all 25 SNP instrumental variables used for the exposure factor were > 10, effectively avoiding weak instrumental variable bias. Figure 2A (the forest plot) displays the individual effect estimates and pooled effects for each SNP instrumental variable. The vast majority of SNP effect estimates lie to the left of the vertical reference line (effect = 0). The pooled effect estimates from the MR Egger and IVW methods are both negative, indicating a consistent association between increased ADAR gene expression and a lower OC risk. The core MR results were as follows: The IVW fitting line (green) exhibited a negative slope (Figure 2B), suggesting that elevated ADAR expression reduces OC risk. This suggests that elevated ADAR expression has a protective causal effect against OC and that the results remain robust across different statistical methods. SNP effect values were symmetrically distributed around the IVW (green) and weighted median (blue) fitting lines (Figure 2B). The estimates of the effects for each SNP were symmetrically distributed, suggesting no significant bias (Figure 2C); the overall effect estimate remained stable after sequentially removing each SNP (Figure 2D).

The reliability verification results are as follows: Heterogeneity test (Table 1), the MR-Egger method ($Q_{pval} = 0.92$) and the IVW method ($Q_{pval} = 0.85$) both returned values greater than 0.05, suggesting no significant heterogeneity. Horizontal pleiotropy test: Egger intercept test (Table 2): $P > 0.05$, indicating no obvious horizontal pleiotropy. MR-PRESSO global test (Table 3): $P < 0.05$, suggesting the presence of potential outlier SNPs, indicating possible horizontal pleiotropy. Leave-one-out analysis showed robust results, with no single SNP dominating the effects. Steiger directionality test (Table 4): $Correct_causal_direction = TRUE$ and $Steiger.p.val < 0.01$, confirming the correct causal direction and ruling out reverse causation

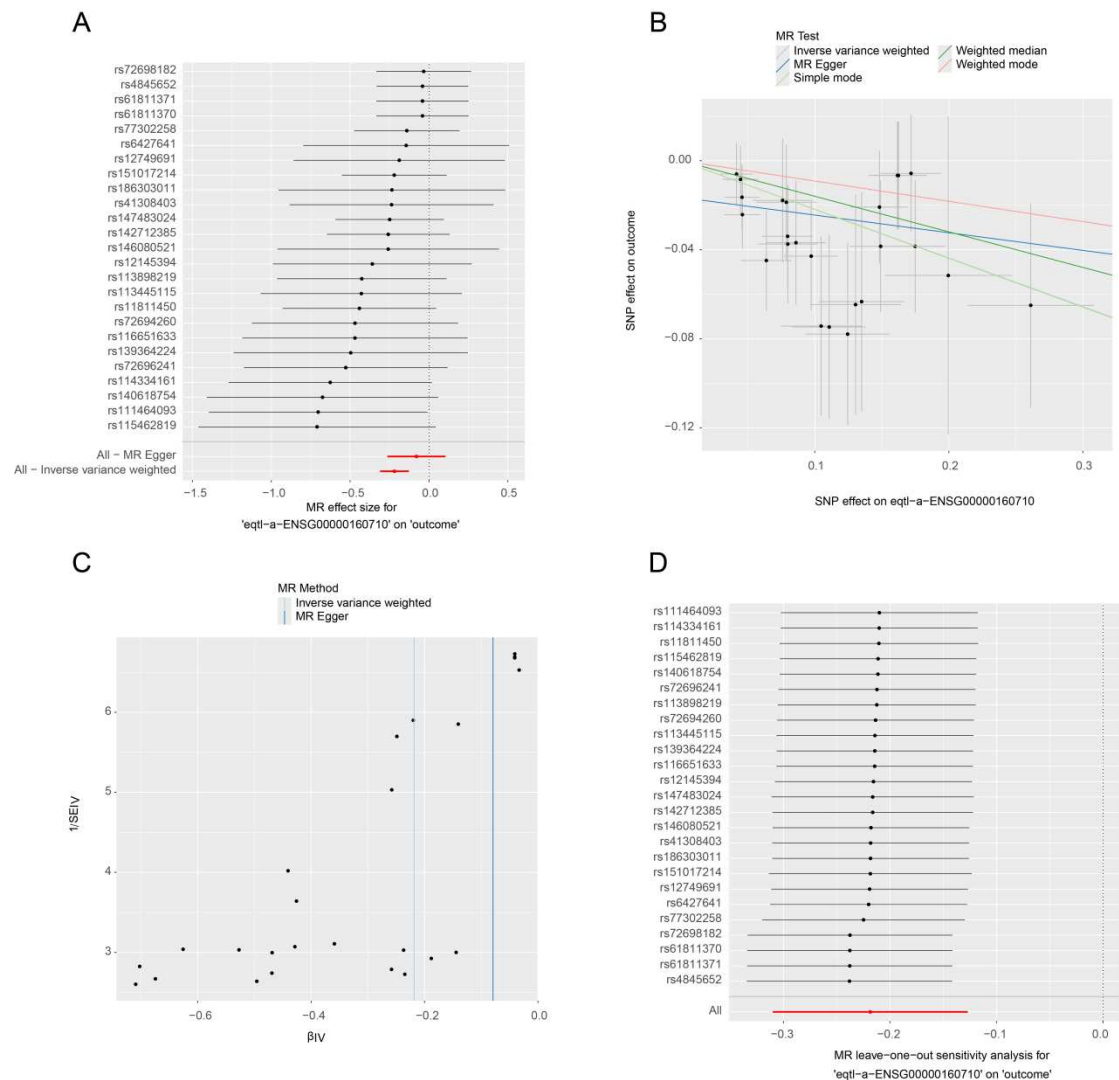


Figure 2 MR Analysis

(A) Forest plot showing MR effect size estimates for each SNP instrument and pooled estimates from MR-Egger and IVW methods. (B) Scatter plot illustrating the concordant negative slopes across five MR methods, which supports a consistent inverse causal association. (C) Funnel plot showing an approximately symmetric SNP distribution, indicating no substantial directional pleiotropy. (D) Leave-one-out analysis confirms that the overall causal estimate remains stable upon sequential removal of each SNP, demonstrating robustness of the results.

Table 1 Heterogeneity Results for Exposure Factors with Causal Relationships

exposure	outcome	method	Q	Q_df	Q_pval
ADAR	OC	MR Egger	14.08	23	0.92

ADAR	OC	Inverse variance weighted	17.00	24	0.85
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Note: The first column represents the exposure factor, the second column represents the outcome, the third column represents different methods for heterogeneity testing, the fourth column represents the Q statistic from Cochran's Q test, the fifth column represents the df value from Cochran's Q test, and the sixth column represents the P-value from Cochran's Q test.

Table 2 Horizontal Pleiotropy Results for Exposure Factors with Causal Relationships

exposure	outcome	egger_intercept	se	p val
ADAR	OC	-0.02	0.01	0.10

Table 3 MR-PRESSO Results for Exposure Factors with Causal Relationships

exposure	outcome	MR_PRESSO_P
ADAR	OC	< 0.05

Table 4 Steiger Directionality Test Results

exposure	outcome	snp_r2.exposure	snp_r2.outcome	correct_causal_direction	steiger_pval
ADAR	OC	0.04	<0.01	TRUE	<0.01

3.3 Differential Expression Analysis and COX Regression Analysis for Screening Differentially Expressed Genes

Compared to the CON group, a total of 8,257 significantly differentially expressed genes were identified in the OC group (817 upregulated, 7,440 downregulated, Figures 3A, 3B); 1,494 significant genes (711 upregulated, 783 downregulated) were screened through COX regression analysis ($P < 0.05$), with 286 alternative genes being obtained after taking the intersection of both (Figures 3C, D).

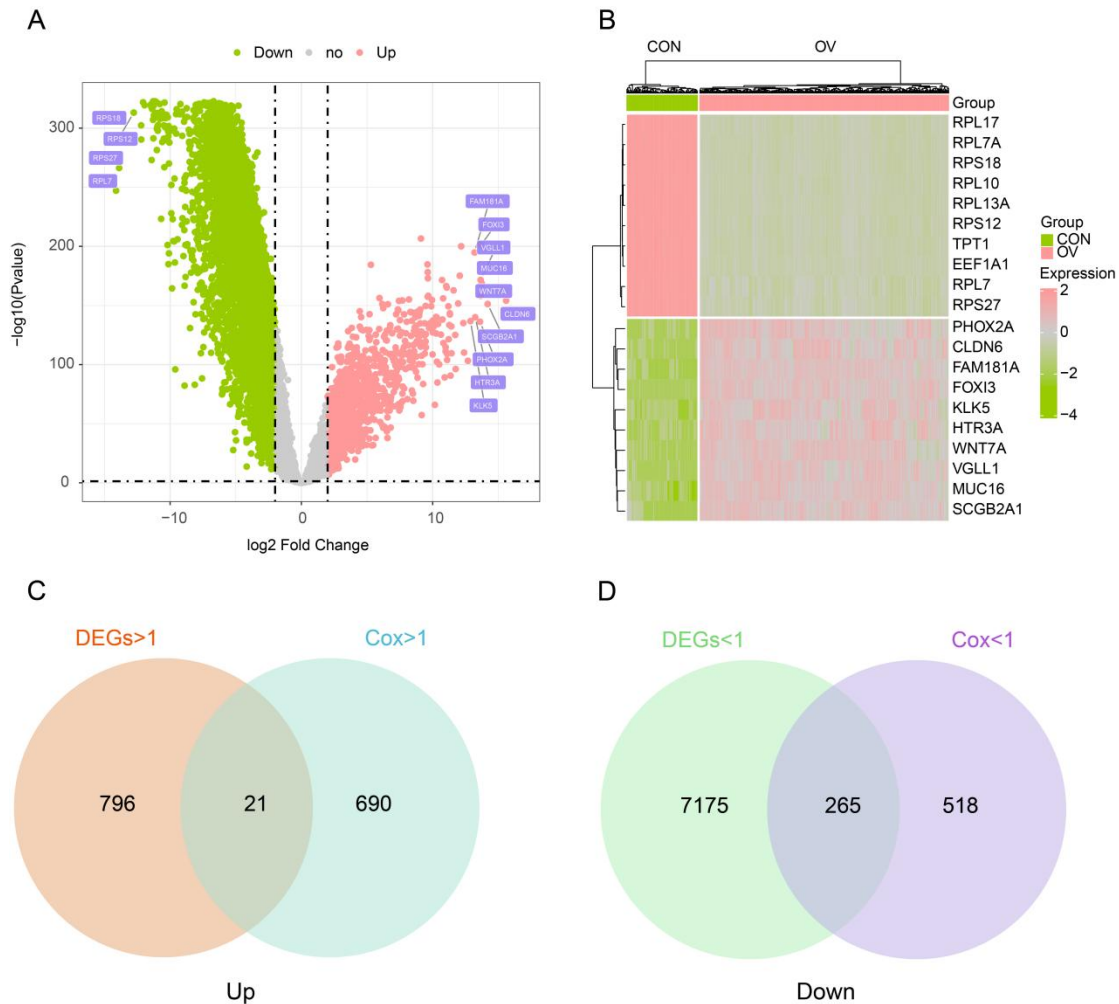


Figure 3 Transcriptomic Data Expression Differences Between OC and CON Groups

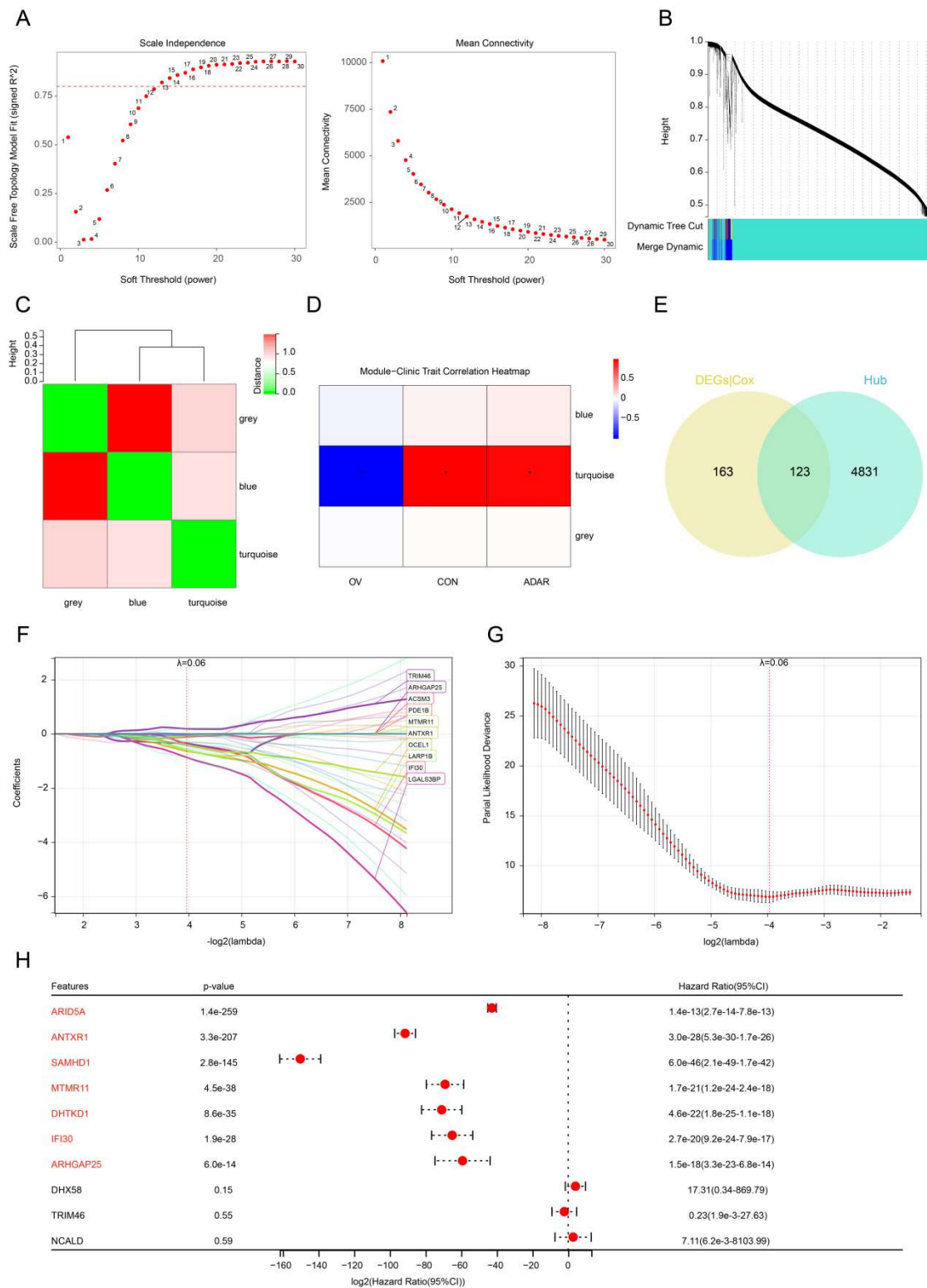
(A) Volcano plot; (B) Heatmap of Top 10 upregulated and downregulated differentially expressed genes. (C) Venn diagram of commonly upregulated genes. (D) Venn diagram of commonly downregulated genes.

3.4 WGCNA and Screening of Significantly Associated Genes

Based on the selected optimal soft threshold ($\beta = 6$, $R^2 = 0.86$), an adjacency matrix was constructed (Figure 4A), which was further transformed into a topological overlap matrix (TOM) to reflect co-expression similarity between genes. Subsequently, based on hierarchical clustering and dynamic tree cutting algorithms, 3 gene modules were generated (Figure 4B), namely blue, turquoise, and grey. Analysis of module eigengene (ME) connectivity showed that the distance between modules was greater than 0.25,

indicating good independence between each module (Figure 4C). Correlation analysis revealed that the turquoise module was significantly correlated with both the ADAR gene and OC phenotype ($P < 0.05$) (Figure 4D), from which 4,954 hub genes related to the ADAR gene were extracted and identified. Through comparison and intersection of alternative genes and hub genes, a total of 123 candidate genes were identified (Figure 4E), which were considered to be ADAR-related co-expressed genes in OC.

Candidate genes were incorporated into the Lasso-Cox regression model, with the optimal penalty parameter λ being determined through 10-fold cross-validation, utilizing the L1 regularization property of Lasso to compress regression coefficients, automatically eliminating redundant variables and reducing multicollinearity effects, thereby screening the optimal gene subset (Figures 4F-G); using these genes as independent variables and patient survival time and outcome status as dependent variables, multivariate COX proportional hazards regression analysis (Figure 4H) results showed that ANTXR1, ARHGAP25, ARID5A, DHTKD1, IFI30, MTMR11, and SAMHD1 genes exhibited significance ($P < 0.05$), and were thus considered to be significantly associated genes.



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Figure 4 WGCNA Analysis and Screening of OC Significantly Associated Genes

(A) WGCNA scale-free fit index; mean connectivity. (B) Gene clustering tree and Dynamic Tree Cutting results. Different colors represent different gene co-expression modules. (C) ME correlation heatmap, showing relationships between different modules. (D) Correlation between MEs and CON group and OC group. Color intensity represents correlation strength. (E) Venn diagram of alternative genes and hub genes. (F-G) Coefficient trajectory (F) and cross-validation curve (G) of LASSO regression. (H) Multivariate COX regression forest plot, showing gene prognostic hazard ratios (HR) and 95% CI. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

3.5 Expression Validation, Diagnostic Performance Evaluation, and Correlation Analysis of Significantly Associated Genes

To evaluate the potential of significantly associated genes as diagnostic markers for OC, the expression levels of ANTXR1, ARHGAP25, ARID5A, DHTKD1, IFI30, MTMR11 and SAMHD1 were examined. In the training set, it was found that these genes were significantly downregulated in the OC group ($P < 0.05$) (Figure 5A). Subsequently, an independent validation set was used to confirm that only the ARHGAP25 and SAMHD1 genes had an expression pattern consistent with the above findings (Figure 5C). The AUC values for both genes were greater than 0.7 (Figures 5B and 5D), indicating that the model was robust and could be generalised. In summary, the expression patterns of ARHGAP25 and SAMHD1 in the OC group showed significant changes, demonstrating stable and effective diagnostic results in both the training and validation sets. This suggests that they could be used as biomarkers for OC diagnosis, which would be important for early diagnosis and clinical applications. To further evaluate the associations between the ADAR gene and the ARHGAP25 and SAMHD1 genes, correlations among these three genes were analyzed in this study. Scatter plots (Figures 5E-G) showed significant linear correlations among the three genes ($P < 0.05$), suggesting close expression correlation between key genes and the ADAR gene.

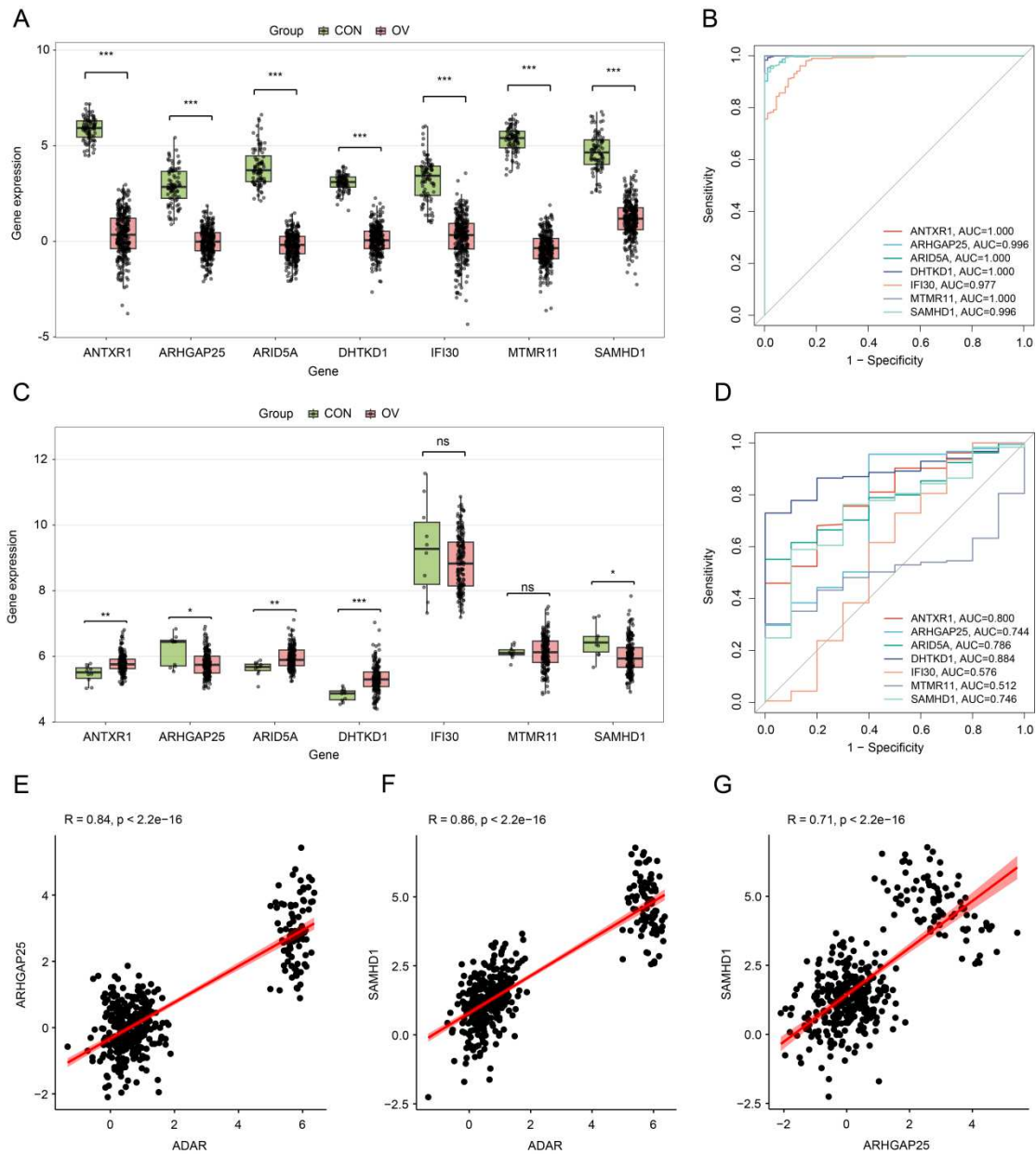


Figure 5 Expression Validation, Diagnostic Performance Evaluation, and Correlation Analysis of Significantly Associated Genes

(A-B) Expression distribution and ROC curve analysis of significantly associated genes in the training set. (C-D) Expression distribution and ROC curve analysis of significantly associated genes in the validation set. (E-G) Correlation scatter plots of ADAR, ARHGAP25, and SAMHD1 genes. Red regression lines show linear relationships, with R^2 values and P-values annotating correlation strength and significance. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

3.6 Functional Enrichment Analysis of Candidate Genes

To gain deeper understanding of the biological functions of these 123 OC-related candidate genes, GO and KEGG enrichment analyses were performed. GO enrichment analysis revealed (Figure 6A) that at the biological process (BP) level, these genes were significantly enriched in immune-related pathways such as defense response to virus, response to virus, regulation of immune effector process, and antigen processing and presentation, suggesting that the immune microenvironment plays an important role in OC initiation and progression; at the cellular component (CC) level, genes were primarily localized to cellular components such as phagocytic vesicle membrane, phagocytic vesicle, endocytic vesicle membrane, MHC protein complex, and external side of plasma membrane, reflecting characteristics of OC cell interactions with immune cells and extracellular matrix remodeling; at the molecular function (MF) level, these genes were involved in functions such as peptide antigen binding, collagen binding, MHC protein binding, proteoglycan binding, and actin binding, further confirming the critical role of antigen presentation and immune recognition in OC.

KEGG pathway enrichment analysis revealed (Figure 6B) that these candidate genes participated in signaling pathways including Autoimmune thyroid disease, AGE-RAGE signaling pathway in diabetic complications, Human cytomegalovirus infection, Graft-versus-host disease, and extracellular matrix (ECM)-receptor interaction. Notably, multiple genes such as SERPINE1, STAT5A, GBP1, TRPV2, ANTXR1, HLA-F, HLA-C, and HLA-E simultaneously participated in regulation of multiple pathways, forming a complex interaction network. These pathways were highly concentrated in immune response, antigen presentation, extracellular matrix interactions, and virus infection-related processes, suggesting that OC initiation and progression are closely associated with immune evasion and TME remodeling.

In summary, functional enrichment analysis demonstrated that these 123 candidate genes constitute a multi-level regulatory network centered on immune regulation, synergistically acting on OC initiation and progression through effects on multiple biological processes including antigen presentation, immune cell infiltration, and extracellular matrix remodeling. Abnormal expression or function of these genes may

lead to tumor immune evasion and microenvironment alterations, providing important theoretical basis and potential targets for OC immunotherapy and targeted intervention.



Figure 6 Functional Enrichment Analysis

(A) GO functional enrichment analysis of candidate genes. (B) Bubble plot of KEGG pathways enriched by candidate genes.

3.7 PPI Network Analysis

To explore the regulatory mechanisms of ADAR in OC initiation and progression, PPI analysis was performed in this study. First, a PPI network comprising 123 candidate genes and the ADAR gene was constructed, which included 119 nodes and 1,074 interaction relationships (Figure 7A), suggesting that these genes may exert synergistic effects through complex PPI networks in OC progression. To further clarify the regulatory role of the ADAR gene, an ADAR gene-centered PPI subnetwork was constructed (Figure 7B). Results showed that the ADAR gene had direct interaction relationships with multiple genes including ARHGAP25, SAMHD1, PABPC1, PARP10, and DHX58, indicating that ADAR may participate in OC progression through regulation of these downstream target genes.

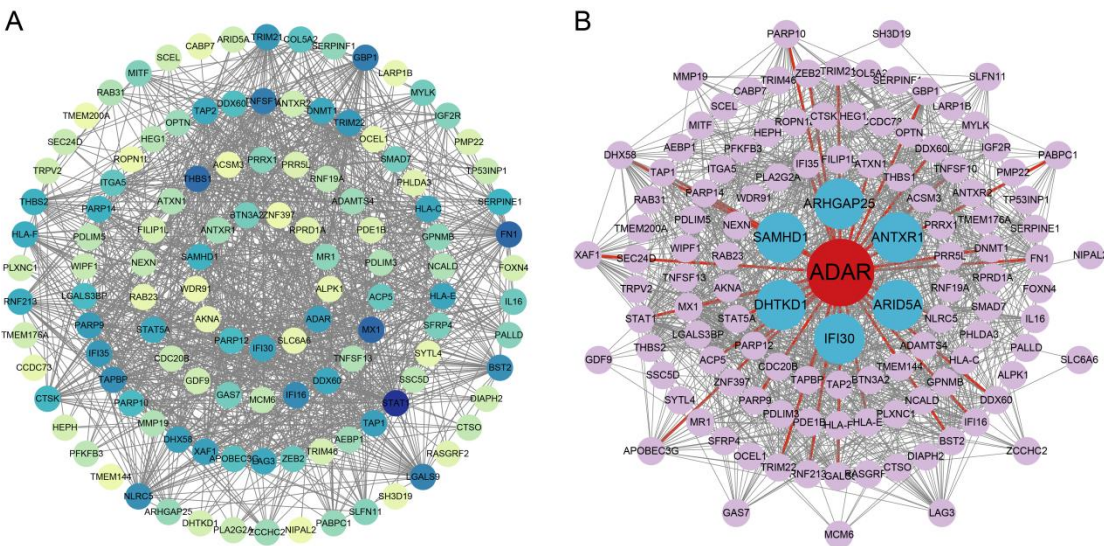


Figure 7 PPI Network Analysis

(A) PPI network of candidate genes. Darker colors indicate higher Degree values of genes in the PPI network. (B) ADAR gene-related PPI network. Red nodes represent the ADAR gene, blue nodes represent significantly characteristic genes, and red lines represent genes directly interacting with the ADAR gene.

3.8 Immune Cell Analysis of ADAR Gene Participation in OC

The CIBERSORT method was employed to analyze immune cell infiltration characteristics of OC and CON groups, with 22 immune cell types being identified. A heatmap presented the infiltration abundance distribution of immune cells in each sample (Figure 8A). Further analysis of immune cell population correlations revealed: Macrophages M1 showed the strongest positive correlation with T cells CD8 ($r = 0.33$, $P < 0.05$), while B cells naive showed the strongest negative correlation with B cells memory ($r = -0.35$, $P < 0.05$) (Figure 8B). Comparison of inter-group infiltration differences showed: Immune cells such as Macrophages M0, Macrophages M1, and T cells regulatory (Tregs) were significantly upregulated in the OC group; Monocytes, Mast cells resting, and B cells naive were significantly downregulated in the OC group (Figure 8C).

Simultaneously, correlation analysis between ADAR gene expression and immune cell infiltration (Figure 8D) showed: ADAR exhibited significant positive correlations with B cells naive, T cells CD4 memory resting, and Macrophages M2, and significant negative correlations with B cells memory, T cells gamma delta, and Mast cells activated ($P < 0.05$). Although ADAR expression was significantly correlated with specific immune cells, no statistical differences were observed in comprehensive TME scores (StromalScore, ImmuneScore, ESTIMATEScore) between high and low ADAR expression groups (Figure 8E).

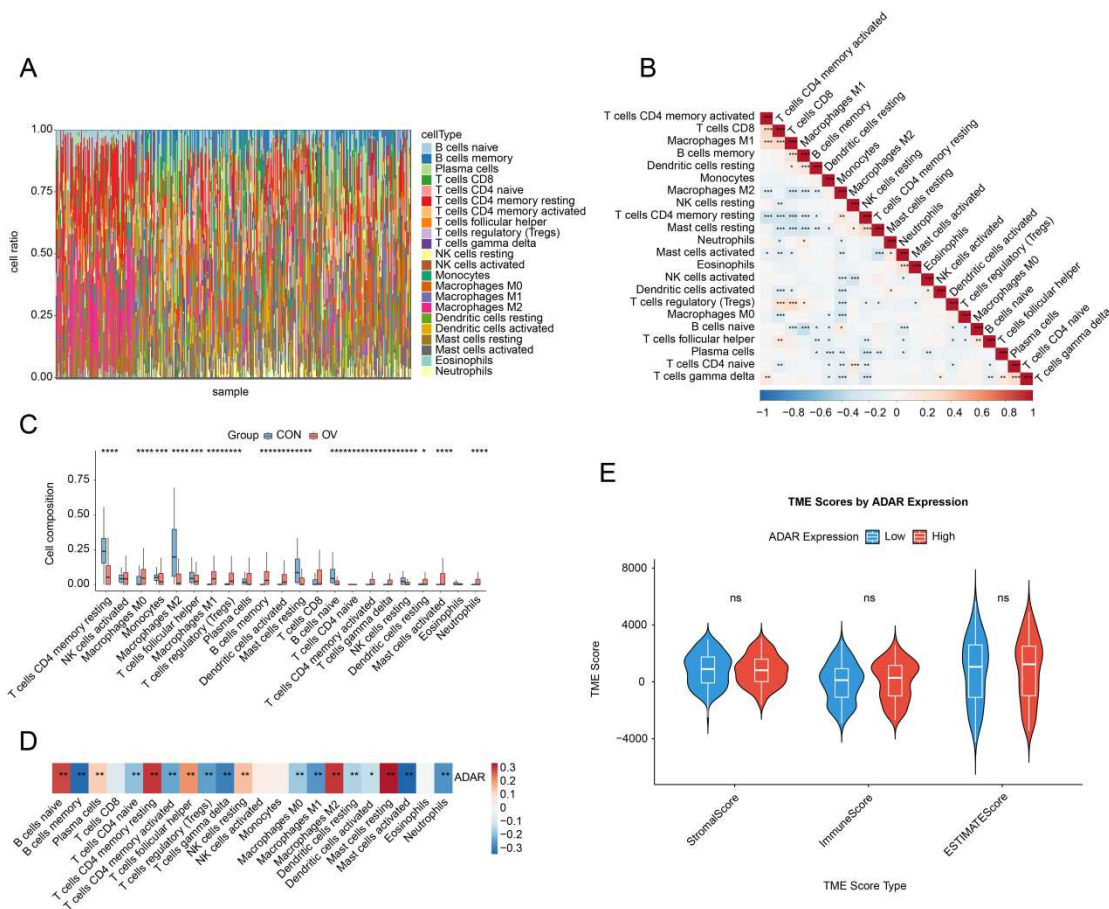


Figure 8 Immune Infiltration Analysis

(A) Infiltration proportions of immune cells among samples. (B) Correlation analysis heatmap between differential immune cells. (C) Differential analysis of immune cell infiltration proportions between OC group and CON group samples. (D) Correlation heatmap between ADAR gene and immune cells. (E) Relationship analysis between ADAR gene expression levels and TIME scores. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns: no statistical difference).

3.9 Drug Sensitivity Analysis

To evaluate the predictive ability of key genes for drug treatment response, expression matrix data were obtained based on the GDSC and TCGA databases in this study, with the R package "oncoPredict" being used to predict drug sensitivity for each cancer patient sample. Default values for all parameters were selected during the analysis process, including using the "combat" method to eliminate batch effects and averaging duplicate gene expression values. Based on anticancer drugs approved by the U.S. Food and Drug

Administration (FDA), 8 clinically commonly used key therapeutic drugs were screened for analysis in this study, including: paclitaxel, tamoxifen, carboplatin, topotecan, etoposide, sorafenib, gemcitabine, and olaparib. Subsequently, the Wilcoxon rank-sum test was employed to compare IC50 value differences for these drugs between ADAR gene high expression group and low expression group patients (Figure 9).

The study results indicate that the expression levels of the ADAR gene can significantly predict differences in patient sensitivity to six drugs, including tamoxifen, carboplatin, topotecan, etoposide, sorafenib, and olaparib ($P < 0.05$). These findings indicate that the ADAR gene can serve as a drug sensitivity prediction biomarker with clinical application value, may provide potential reference for OC clinical treatment, particularly possessing important predictive significance in the precise application of specific targeted therapy and chemotherapy drugs.

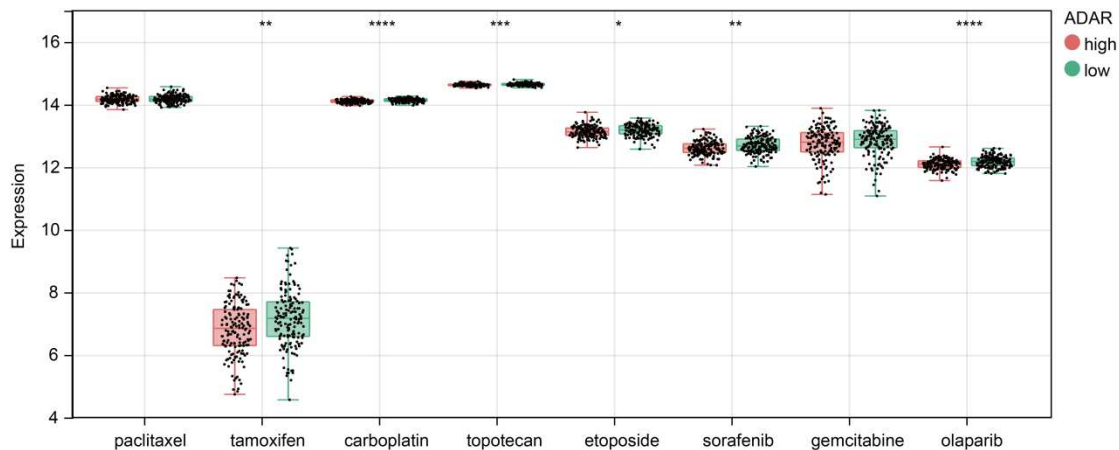


Figure 9 IC50 Differences of 8 Key Drugs Between ADAR Gene High and Low Expression Groups

Red and green dots represent high and low ADAR expression groups, respectively. Higher IC50 values indicate greater drug resistance. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$)

3.10 Immunohistochemical validation of ADAR in OC

To validate the transcriptomic findings at the protein level, IHC staining for ADAR was performed on tissue specimens from ovarian serous cystadenoma (benign control group)

and OC group. Representative images were captured at four magnifications (4×, 10×, 20×, and 40×) to comprehensively evaluate staining intensity and subcellular localization (Figure 10). In the OC group, ADAR immunoreactivity was weak and sparsely distributed, with only scattered faint brown signals observed in the epithelial lining and stroma (Figure 10B). In contrast, the benign control group exhibited markedly stronger and more diffuse ADAR staining, characterized by intense brown DAB signals predominantly localized to the cytoplasm and nuclei of tumor cells throughout the tissue (Figure 10A). These findings indicate that ADAR protein expression is significantly downregulated in OC tissues compared with benign ovarian serous cystadenoma, corroborating the transcriptomic results and supporting the potential role of ADAR as a candidate biomarker for OC.

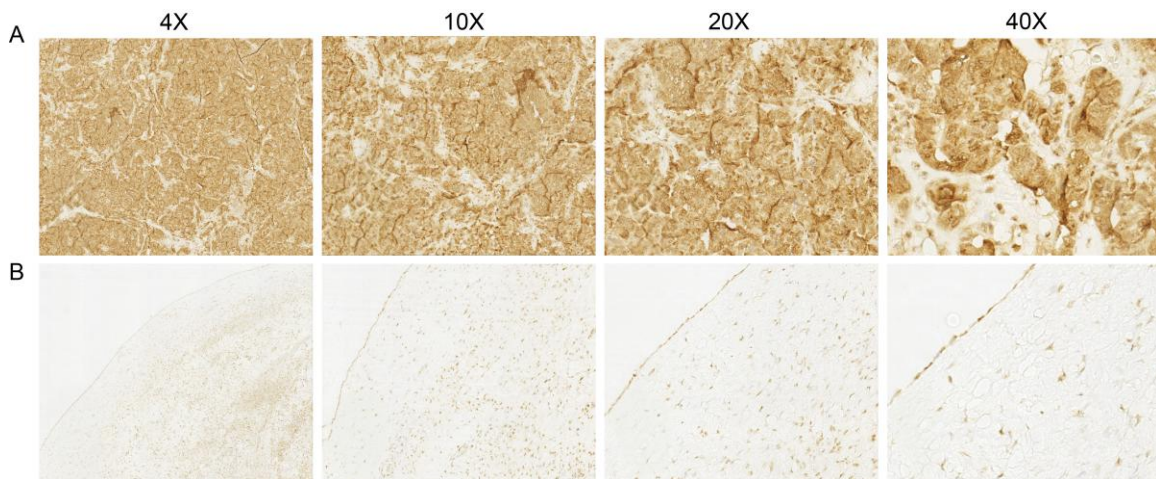


Figure 10 Immunohistochemical validation of ADAR

Representative IHC images showing ADAR expression in ovarian serous cystadenoma (benign control group) and OC group at progressively increasing magnifications (from left to right: 4×, 10×, 20×, and 40×). (A) The benign control group showed strong and diffuse ADAR immunoreactivity, with intense cytoplasmic and nuclear staining widely distributed across tumor cells. (B) The OC group displayed weak and focal ADAR staining, with only a few positive signals distributed along the epithelium and stroma. Brown signal (DAB chromogen) indicates positive ADAR expression.

4 Discussion

OC is one of the most lethal malignancies of the female reproductive system, characterized by aggressive behavior, frequent chemoresistance, recurrence and poor prognosis. Although its incidence ranks behind endometrial and cervical cancers, OC accounts for the highest mortality among gynecologic malignancies [19]. Owing to the anatomical and pathological features of the ovary, most patients present at advanced stages, and current standard care—cytoreductive surgery followed by platinum-based chemotherapy—is limited by high rates of relapse and resistance [8,20]. Tumor metastasis and chemoresistance in OC are governed by complex, multifactorial and polygenic processes [21], reflecting the coordinated dysregulation of cell adhesion, matrix degradation and angiogenesis along the lymphatic, vascular and transcoelomic routes [22,23]. ADAR, a double-stranded RNA-specific adenosine deaminase, edits endogenous dsRNAs and prevents their recognition by innate immune sensors such as MDA5, thereby restraining inappropriate activation of antiviral immunity [10,24,25]. Aberrant ADAR expression has been documented in OC, where dysregulated A-to-I editing may shape tumor-specific antigen repertoires and modulate immune-checkpoint molecules, providing a rationale for refining immunotherapeutic strategies [26]. With the advent of precision medicine, targeting RNA-editing enzymes has emerged as a promising avenue: restoring physiological ADAR activity or restraining its pathological hyperactivation may reverse malignant phenotypes [27,28]. Nevertheless, although ADAR-mediated A-to-I editing is recognized as a key post-transcriptional regulator in cancer, its causal role and downstream effectors in OC remain incompletely defined [10,29]. To address this gap, we integrated MR, transcriptomic analysis, WGCNA and machine learning to interrogate the causal contribution of ADAR in OC, identify ARHGAP25 and SAMHD1 as candidate downstream effectors, and explore its selective regulation of immune subsets and drug responsiveness.

Using MR, we provide genetic evidence supporting a putative causal protective relationship between ADAR expression and OC risk. The observed protective effect appears unlikely to be explained solely by classical tumor-suppressor activity. Instead, ADAR-mediated A-to-I editing may attenuate aberrant activation of the MDA5–MAVS axis by endogenous dsRNAs [24], preserving a balanced type I interferon (IFN-I) tone while limiting the establishment of a chronic immunosuppressive milieu. ADAR-related

transcripts may also undergo selective editing of OC-relevant mRNAs, restraining oncogenic outputs at the post-transcriptional level [30]. These findings provide genetic-epidemiological support for ADAR-targeted strategies and establish a causal-inference framework for downstream transcriptomic exploration.

At the protein level, immunohistochemistry (IHC) was performed on serous cystadenoma and OC tissues to validate ADAR expression. The contrast between control and tumor tissues confirmed assay specificity, while residual benign epithelium within tumor sections served as an internal positive control, supporting the tumor-restricted nature of ADAR downregulation. The predominantly cytoplasmic staining pattern is consistent with the reported subcellular distribution of the constitutively expressed ADAR p110 isoform [31,32]. Together with the mRNA downregulation observed in TCGA and GSE26712 and the protective effect inferred from MR, these IHC findings extend the evidence chain across transcriptomic, causal-genetic and protein-level dimensions, lending histopathological support to the relevance of ADAR as a candidate diagnostic marker and therapeutic target.

For systematic identification of downstream effectors, WGCNA enables genome-wide detection of co-expressed gene clusters and links module eigengenes (MEs) to clinical phenotypes [33]. The turquoise module showed significant correlation with both ADAR expression and OC phenotype, suggesting that its constituent genes may share an ADAR-dependent regulatory context relevant to OC pathogenesis. Multivariate Cox proportional-hazards regression further identified candidates with independent prognostic value and acceptable discriminative performance. By integrating statistical significance, survival relevance, co-expression context and external reproducibility, this strategy provides a reasonable basis for dissecting the ADAR regulatory network.

Among the prioritized candidates, ARHGAP25 and SAMHD1 stood out, both being closely linked to tumor suppression and immune regulation [34–37]. ARHGAP25 encodes a Rho GTPase-activating protein that accelerates GTP hydrolysis on RAC1, thereby restraining cytoskeletal remodeling, lamellipodial protrusion and directional migration [38]. ARHGAP25 has also been shown to exert tumor-suppressive effects through the WNT/ β -catenin and Akt/mTOR pathways [39–42]. Its downregulation may therefore facilitate cell motility and accelerate transcoelomic dissemination of OC.

Beyond its tumor-intrinsic actions, ARHGAP25 modulates phagocytosis and ROS generation in myeloid cells via RAC-dependent mechanisms [39,43]. Reduced ARHGAP25 within the OC microenvironment may thus simultaneously enhance invasiveness and impair innate immune effector function, contributing to disease progression and immune evasion.

SAMHD1, a dNTP triphosphohydrolase, regulates intracellular dNTP pools [44] and promotes homologous recombination (HR)-mediated repair of DNA double-strand breaks (DSBs) by recruiting CtIP (RBBP8) to DSB sites and facilitating end resection, thereby preserving genomic integrity [35]. SAMHD1 deficiency may therefore induce replication stress and genomic instability while simultaneously sensitizing tumor cells to HR-targeted therapies. SAMHD1 also degrades cytosolic DNA fragments arising from replication stress, dampening cGAS–STING-driven IFN-I production [45]. Its loss may sustain low-grade chronic innate signaling that fosters an immunosuppressive context in OC. The strong positive correlations among ADAR, ARHGAP25 and SAMHD1 suggest coordinated action across post-transcriptional regulation and genome-integrity maintenance during OC progression.

Functional enrichment of ADAR-related candidates further supports this mechanistic framework. At the GO level, the enrichment of viral defence responses and antigen processing/presentation pathways, consistent with the function of ADAR-modified MDA5 ligands, highlights the central role of innate immunity and MHC class I molecule presentation within the ADAR network [24,46]. Concurrent enrichment of "negative regulation of cell adhesion" implicates ARHGAP25 in coordinating Rac1-integrin-ECM and TGF- β signaling to modulate adhesion and invasion [47–49]. At the KEGG level, enrichment of the ECM-receptor interaction pathway aligns with the cytoskeletal-remodeling phenotype predicted by ARHGAP25 deficiency and with the established role of ECM dynamics in peritoneal seeding and ascites formation in OC [50], linking molecular dysregulation to its hallmark macroscopic metastatic behavior. As a Rac1 GAP, reduced ARHGAP25 may sustain Rac1 activation and promote aberrant adhesion of tumor cells to peritoneal ECM components (collagen and fibronectin), representing a plausible event in peritoneal dissemination [41,51]. Concurrent SAMHD1 downregulation may compromise antigen presentation, constituting a multilayered

immune-evasion mechanism upstream of conventional checkpoint pathways. Through its dNTPase activity, SAMHD1 clears cytosolic aberrant nucleic acids and restrains inappropriate innate signaling [35,52]. In OC, persistent NLRP3 inflammasome activation drives IL-1 β release, EMT and Wnt/ β -catenin signaling, contributing to platinum resistance and adverse outcomes [53,54], suggesting that SAMHD1 may influence therapeutic responsiveness by shaping NOD-like receptor signaling.

The PPI network places ADAR at a hub position connected to ARHGAP25, SAMHD1, PABPC1 and DHX58, extending its regulatory scope from RNA editing to mRNA stability and innate immune signaling. Interaction with cytoplasmic poly(A)-binding protein 1 (PABPC1)—a central node coupling 3'-end processing, translation initiation and mRNA decay [55]—suggests cooperative control of edited transcripts. The interaction with DHX58 (LGP2), a dsRNA helicase that modulates RIG-I and MDA5 signaling [52], is mechanistically informative: DHX58 can bidirectionally tune antiviral RNA recognition, potentially balancing editing-dependent immune tolerance with dsRNA-triggered activation [24,56]. Imbalance of the ADAR–DHX58 axis in OC may convert acute innate sensing into chronic, low-efficacy IFN-I signaling, fostering chronic inflammation rather than effective antitumor immunity. Collectively, these interactions position ADAR as a regulatory hub integrating RNA metabolism, innate immune recognition and translational control within the OC microenvironment.

Immune-infiltration analysis indicated that the influence of ADAR-related genes on the OC immune microenvironment is subset-selective rather than globally uniform. For instance, M2-polarized tumor-associated macrophages (TAMs) typically exert pro-tumor effects through paracrine IL-10 and TGF- β [57]; however, although high ADAR expression coincided with increased M2 infiltration, A-to-I editing of macrophage transcripts (including those encoding cytokines and their receptors) may reshape polarization, allowing partial preservation of antitumor activity rather than fully immunosuppressive M2 commitment. Positive correlations with naïve B cells and resting CD4 memory T cells suggest a link to adaptive immune memory, in line with the favorable prognosis associated with elevated ADAR-related gene expression. Conversely, negative correlations with $\gamma\delta$ T cells and activated mast cells may reflect restraint of innate inflammatory responses that, if sustained, recruit pro-inflammatory myeloid cells

619 and dampen adaptive antitumor immunity [58,59]. ADAR-related genes may therefore
620 safeguard adaptive antitumor immunity by limiting excessive or persistent innate
621 inflammation. Comparable ESTIMATE scores between groups indicate that this
622 immunoregulatory function is mechanistically selective and warrants single-cell-
623 resolution dissection.

624 Drug-sensitivity predictions were mechanistically coherent and reinforce translational
625 relevance. Higher ADAR-related gene expression correlated with sensitivity to
626 carboplatin and topotecan, agents whose cytotoxicity depends on intact DNA damage
627 response (DDR) [60,61], an association plausibly linked to the HR-related function of
628 SAMHD1 [35]. Concurrent downregulation of ADAR-related genes and SAMHD1 may
629 indicate HR pathway deficiency, paradoxically enhancing susceptibility to DNA-
630 damaging agents. Olaparib, a PARP1/2 inhibitor, exploits HR deficiency (HRD)-driven
631 synthetic lethality and induces G2/M arrest [62]. Tamoxifen, a selective estrogen receptor
632 modulator (SERM), induces apoptosis in OC cells via ER- α blockade and ER/oxidative
633 stress [63]; high ADAR expression may sustain survival through editing of pro-apoptotic
634 transcripts, suggesting that ADAR loss could sensitize cells to tamoxifen-induced
635 apoptosis. Etoposide induces DSBs through topoisomerase II inhibition and depends on
636 ATM/ATR-p53 signaling [64]; ADAR may regulate DDR-related dsRNA homeostasis
637 and DSB repair, with its loss potentially sensitizing cells via repair impairment.
638 Sorafenib, a multi-kinase inhibitor, partly acts through ferroptosis induction [65], and
639 ADAR-mediated editing of GPX4 and other ferroptosis-related transcripts may sustain
640 ferroptosis resistance in OC. Overall, ADAR-related genes appear suited as candidate
641 biomarkers in OC, particularly for stratifying patients toward platinum-based regimens
642 and PARP inhibitors.

643 Several limitations should be acknowledged. First, transcriptomic analyses relied on
644 public datasets with inherent heterogeneity in sample collection, processing platforms
645 and clinical features; despite normalization, residual batch effects cannot be fully
646 excluded. Second, MR analysis used population-level whole-blood eQTL data, which
647 may not faithfully reflect the regulatory context of ovarian tissue; ovary-specific eQTLs
648 would yield more accurate causal estimates. Third, mech

anistic links proposed between ADAR, ARHGAP25, SAMHD1 and the immune microenvironment are inferential, derived from pathway enrichment and correlation analyses, and require functional validation in OC cell lines, patient-derived organoids and in vivo models. Fourth, drug-sensitivity predictions are based on cell line pharmacogenomic databases and may not fully recapitulate in vivo pharmacology. In addition, the present IHC validation is limited by sample size and the absence of normal ovarian tissue as a negative control.

Future studies should prioritize gain- and loss-of-function experiments in OC cell lines [66] to systematically characterize ARHGAP25 and SAMHD1 as ADAR downstream effectors, focusing on migration, invasion, DDR and immune co-culture readouts. Transcriptome-wide A-to-I editing profiling (e.g., ICE-seq) of OC specimens may help identify edited transcripts mediating ADAR's tumor-suppressive effects [67] and uncover neoantigen-generating editing events of immunotherapeutic interest. Spatial transcriptomics could resolve the anatomical relationship between ADAR expression heterogeneity and immune-cell distribution in the architecturally complex OC TME, including ascites and peritoneal metastases [68]. Building on the present IHC findings, prospective validation in larger, well-characterized cohorts using multiplex IHC or immunofluorescence is warranted to evaluate ADAR, ARHGAP25 and SAMHD1 as protein-level diagnostic and prognostic biomarkers. Finally, the pharmacological regulation of ADAR via IFN-I priming or the epigenetic derepression of the ADAR promoter represents a testable therapeutic hypothesis that is worthy of systematic preclinical evaluation.

5 Conclusion

Focusing on OC as a representative pathological model, this study integrated bioinformatic approaches to characterize ADAR expression and its associations with clinicopathological parameters, and identified ARHGAP25 and SAMHD1 as ADAR-related candidates. The findings provide a preliminary framework linking ADAR, ARHGAP25 and SAMHD1 to OC immune evasion, invasion–metastasis and therapeutic resistance, and offer a basis for deepening molecular understanding of OC, stratifying high-risk patients, predicting therapeutic responses and informing the development of

novel interventions. Translation to the clinic, however, will require rigorous pharmacological and safety validation.

Author Contributions

XL and YA conceived, designed and supervised the study. XL and XQ performed the key bioinformatics analyses, interpreted the main findings, and drafted the manuscript. XQ collected and processed the data, and contributed to drafting the manuscript. XL validated the findings. XL and XQ provided critical feedback and helped shape the research. XL, XQ and YA revised the manuscript. All authors discussed the results and approved the final manuscript.

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Availability of data

All datasets are publicly available. Ovarian cancer (OC)-related transcriptomic data were obtained from The Cancer Genome Atlas (TCGA; <https://www.cancer.gov/ccg/research/genome-sequencing/tcga>), the Genotype-Tissue Expression (GTEx) project, and the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). OC outcome data and exposure factor summary statistics were downloaded from the OpenGWAS database (<https://gwas.mrcieu.ac.uk/>). Gene expression profiles and clinical data for ovarian cancer patients were retrieved from TCGA. Functional enrichment analysis of candidate genes, including Kyoto Encyclopedia of Genes and Genomes (KEGG; <https://www.genome.jp/kegg/>) pathway analysis and Gene Ontology (GO; <https://geneontology.org/>) annotation, was conducted using the DAVID database (<https://david.ncifcrf.gov>). Protein-protein interaction (PPI)

analysis of identified candidate genes was performed using the STRING database (<https://cn.string-db.org/>). The type and relative proportion of immune cells in each OC tissue and CON tissue sample were assessed using CIBERSORTx (<https://cibersortx.stanford.edu/>). Drug sensitivity data relevant to OC were downloaded from the Genomics of Drug Sensitivity in Cancer (GDSC) database (<https://www.cancerrxgene.org/>) for pharmacological sensitivity analysis.

Conflicts of Interest

The authors declare no conflicts of interest related to this study.

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