

SCNN1A Overexpression Correlates with Poor Prognosis and Immune Infiltrates in Ovarian Cancer

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Introduction: Ovarian cancer (OV) is a common malignancy affecting women globally; recognizing useful biomarkers has been one of the key priorities. Since *SCNN1A* was reported to be relevant to tumor progression in a variety of cancers, but rarely in ovarian cancer, we explored the roles of *SCNN1A* in OV.

Methods: RNA sequencing data from TCGA and GEO were utilized to analyze the expression of *SCNN1A* and related differentially expressed genes (DEGs) in ovarian cancer. We performed GO, GSEA and immune cell infiltration analysis on *SCNN1A*-associated DEGs. Correlation of *SCNN1A* methylation levels and its mRNA expression was analyzed by cBioPortal and UCSC Xena databases. To assess the prognostic impact of *SCNN1A*, Kaplan–Meier plot analysis and Cox regression analysis were performed; ROC curves and nomogram were also plotted.

Results: Compared to normal tissues, *SCNN1A* was highly expressed in ovarian cancer. The methylation level of *SCNN1A* negatively correlated with the *SCNN1A* expression. Moreover, high expression of *SCNN1A* was correlated with poor prognosis in OV patients and associated with immune infiltrates.

Conclusion: High *SCNN1A* expression could be a promising biomarker for poor outcomes in OV and correlated with tumor immune cells infiltration. The findings might help illuminate the function of *SCNN1A* in tumorigenesis and lay a foundation for further research.

Keywords: ovarian cancer, *SCNN1A*, biological function, prognostic biomarker, immune infiltrates

Introduction

Ovarian cancer (OV) is one of the main causes of death from gynecologic malignancies faced by women. It represents around 295,414 new cases and 184,799 deaths worldwide each year.¹ The most common type of ovarian cancer is epithelial ovarian cancer (EOC), which accounts for approximately 80–90% of OV cases. The mortality of OV is largely attributed to the difficulties in early detection and treatment resistance. Although the best first-line treatment includes debulking surgery and platinum-paclitaxel chemotherapy, maintenance treatment with poly ADP-ribose polymerase (PARP) inhibitors, bevacizumab, and drugs targeting homologous recombination deficiency, the majority of patients with advanced ovarian cancer will ultimately relapse.^{2,3} Despite advances in OV treatment, long-term prognosis has remained poor, with a 5-year survival rate approximately 47%.⁴ Therefore, new prognostic predictors and better therapeutic targets are urgently needed for ovarian cancer patients.

The epithelial sodium channel (ENaC) is an ion channel famous for its function in Na⁺ reabsorption in epithelia, which comprises the following three subunits: *SCNN1A*, *SCNN1B*, and *SCNN1G*.⁵ Of these proteins, *SCNN1A* expression levels are the highest. In addition, a study confirmed that DNA methylation patterns correlate with *SCNN1A* expression.⁶ Briefly, their relationship is either high *SCNN1A* expression level/low DNA methylation or low-*SCNN1A* expression level/high DNA methylation. Recently, new functions about ENaC have been reported, including the underlying roles in tumor. Previous researches have proved that dysregulated *SCNN1A* expression is significantly correlated with the progression of multiple cancer types, including neuroblastoma,⁷ pulmonary neuroendocrine tumors,⁸ pancreatic

cancer,⁹ and osteosarcoma.¹⁰ A recent paper from Ware et al indicated that *SCNN1A* expression is correlated with inferior prognosis in breast cancer and can regulate the proliferation of breast cancer cells.¹¹ Moreover, some findings suggested that *SCNN1A* overexpression can promote cell proliferation, migration and predict unfavorable prognosis in ovarian cancer by modulating epithelial-to-mesenchymal transition (EMT).¹² Nonetheless, its functions in cancer has not been widely studied, with few related researches on *SCNN1A* in ovarian cancer. To this end, it is worth in-depth research the potential function and the prognostic value of *SCNN1A* in OV.

In our study, TCGA, GEO, GEPIA, HPA, and Kaplan–Meier plotter websites were utilized to analyze *SCNN1A* expression and its association with prognosis. Additionally, we investigated the molecular mechanisms of *SCNN1A* dysregulation, including DNA methylation, copy number variation, and somatic cell mutations in cBioPortal and UCSC Xena database. Moreover, we established a nomogram to forecast the patients' prognosis. In order to understand the biological function of *SCNN1A* in the pathogenesis of OV, differential expression gene (DEG) analysis, gene ontology (GO) enrichment analysis and GSEA analysis were carried out. Additionally, TIMER and GEPIA websites were utilized to probe into the relationship between *SCNN1A* and infiltrating immune cells and their correlated gene marker sets. Our study indicates that *SCNN1A* may serve as a biomarker of adverse prognosis and immune cells' infiltration in OV.

Materials and Methods

TCGA Datasets

TCGA (<https://genome-cancer.ucsc.edu/>) is currently the world's largest database of cancer genetic information, providing clinical and pathological information on 33 types of cancer. TCGA opens the era of molecular biology of tumors and precision medicine, and the database is free and open to researchers for cancer-related research.

The GEO Database

The GEO database contains high-throughput gene expression data uploaded by research institutions all over the world and is one of the most comprehensive public gene expression data resources (<https://www.ncbi.nlm.nih.gov/geo/>). In our study, we downloaded the gene expression profile microarray datasets GSE26712, GSE12470 and GSE27651 containing ovarian cancer tissues and normal tissues.

Gene Expression Profiling Interactive Analysis (GEPIA)

The GEPIA website, developed by Peking University, integrates TCGA and GTEx datasets to analyze gene expression in various tumor and normal samples (<http://gepia.cancer-pku.cn/index.html>).¹³ In this study, expression analysis of *SCNN1A* was assessed by TCGA-OV datasets. The relationship between *SCNN1A* expression and various immune cell markers was also studied in GEPIA.

The Human Protein Atlas (HPA) Database

The HPA (<http://www.proteinatlas.org/>) offers information about human gene expression profiles at the protein level in normal and tumor tissues.¹⁴ In this study, we explored HPA website to validate the *SCNN1A* protein expression between normal ovarian tissue and ovarian cancer tissue through immunohistochemical staining by antibody HPA012743.

DEGs Between *SCNN1A*-High and -Low Expression OV Cohorts

DEGs between *SCNN1A*-high and *SCNN1A*-low OV patients from TCGA dataset were recognized using unpaired Student's *t*-test by DESeq2 (3.8) package.¹⁵ The genes with statistical significance should meet the following conditions: $P < 0.05$ and $|\log_2 \text{Fold change}| > 1$. All the DEGs were displayed in the volcano plot.

SCNNIA-Interacting Genes and Proteins Analysis

We utilized GeneMANIA (<http://www.genemania.org>) to build the *SCNNIA* gene interaction network. The STRING website (<https://string-db.org/>) is an online searchable database of protein interactions. It is easy to operate and powerful in visualization. The protein–protein interaction (PPI) network of *SCNNIA* was constructed via STRING.¹⁶

cBioPortal

The cBioPortal for Cancer Genomics includes a sizable cancer genomics dataset with visualization, download, and analysis functions.¹⁷ We selected 3 ovarian cancer datasets with 1680 patients for further study. The genomic alteration types and alteration frequency of *SCNNIA* in ovarian cancer were measured by “OncoPrint” mode and “Cancer Types Summary” mode. Besides, the relationship between *SCNNIA* methylation level and *SCNNIA* gene expression was performed in “Plots” module. And the OS of *SCNNIA* was analyzed by the “Comparison/Survival” mode.

UCSC Xena

UCSC Xena, a cancer genomics data analysis platform, offers the visualization and analysis of multiple omics data from cancer samples (<http://xena.ucsc.edu/>). The website integrates some large public datasets, such as TCGA, ICGC, TARGET, GTEx, CCL, etc.¹⁸ This database allows the analysis of gene expression, protein expression, gene methylation, copy number variation, and somatic mutations.

Gene Ontology (GO) Enrichment Analysis

Metascape is a vehicle for gene annotation and pathway analysis (<http://metascape.org>).¹⁹ In the present study, Metascape was utilized as a tool to identify the enrichment process and pathway of *SCNNIA*-related DEGs. To obtain statistically significant differences, the threshold conditions included: $P < 0.01$, a minimum count of 3, and enrichment factor >1.5 .

Gene Set Enrichment Analysis (GSEA)

GSEA was carried out by R package ClusteProfiler (3.6.3) to clarify the functional and pathway differences between the high- and low- *SCNNIA* expression cohorts.²⁰ The MsigDB database of GSEA website was utilized to obtain the data set of “c2.cp.v7.2.symbols.gmt”. The number of analysis permutations was set to 1000, and the significant enrichment gene sets should meet the following conditions: adjusted P -values <0.05 and FDR q -values <0.25 .

Kaplan-Meier Plotter Database Analysis

Kaplan–Meier Plotter website (<http://kmplot.com>), an online database containing gene expression data and survival information of 1657 clinical ovarian cancer cases, was utilized to evaluate the prognostic value of *SCNNIA* in ovarian cancer. OV patients were divided into two groups (high- and low-*SCNNIA* group) according to median expression and analyzed for overall survival (OS) and progression-free survival (PFS) with hazard ratio (HR) and Log rank p -value.

TIMER Database Analysis

TIMER software is a program for the analysis of immune cell infiltration in a wide range of cancers (<https://cistrome.shinyapps.io/timer/>).²¹ We initially used this website to measure differences in *SCNNIA* expression levels in OV and then evaluated the relationship between the *SCNNIA* expression status and the grade of infiltration by particular immune cell subsets. Finally, we evaluated how *SCNNIA* expression associated with the expression of particular immune infiltrating cell subset markers.

Immune Infiltration Analysis by Single-Sample GSEA (ssGSEA)

Immune infiltration analysis of OV specimens was achieved using ssGSEA by the GSVA package²² in R for 24 types of immune cells. Additionally, the relationship between *SCNNIA* expression and immune cell infiltration was assessed

using Spearman test, and the infiltration level of immune cells was compared for high- and low-*SCNN1A* expression cohorts through Wilcoxon rank sum test.

Statistical Analysis

All statistical analysis and plots were applied by R Version 3.6.3 and R package ggplot2 was used to visualize expression differences. Kaplan–Meier plots and GEPIA outcomes were presented with HR and P or Cox P-value by a Log rank test. Besides, the correlation of gene expression was estimated using Spearman test. *P*-value <0.05 was regarded statistically significant. The ROC curve was created to assess the diagnostic performance of *SCNN1A* expression by pROC package.

Results

SCNN1A Was Upregulated in OV

Firstly, we evaluated the expression levels of *SCNN1A* in pan-cancer data from TCGA and GTEx. Analysis showed that *SCNN1A* was highly expressed in 13 tumors, including BRCA, CESC, CHOL, COAD, ESCA, KICH, LAML, LUAD, OV, PAAD, READ, THYM, and UCEC. In contrast, its expression was low in ACC, HNSC, KIRC, KIRP, LIHC, LUSC, PRAD, SKCM, and THCA (Figure 1A). Using GEPIA website, we identified that *SCNN1A* mRNA expression was significantly elevated in ovarian cancer (*p*-value <0.01, |Log2FC| > 1) (Figure 1B) and there was a strong association between *SCNN1A* mRNA levels and inferior OS (*p*-value <0.002, Figure 1C).

Secondly, ROC curve was utilized to assess the diagnostic capability of *SCNN1A* between OV and normal ovary tissue. And the area under the curve (AUC) of *SCNN1A* was 0.990 (95% CI: 0.981–0.999), indicating that *SCNN1A* may be a potentially moderate identification molecule for OV (Figure 1D). In addition, we validated the *SCNN1A* expression level in OV using three GEO datasets (GSE26712, GSE12470 and GSE27651) and found that *SCNN1A* mRNA level was all remarkably higher in OV tissue compared to normal ovarian tissue (Figure 1E–G). Immunohistochemical staining from HPA also showed that *SCNN1A* protein was increased in ovarian cancer tissues, as presented in Figure 2A and B. In brief, the above results suggested that both mRNA and protein level of *SCNN1A* were up-regulated in ovarian cancer.

Functional Enrichment Analysis of High- and Low-*SCNN1A* Expression Samples

Identification of DEGs in OV

In order to identify the underlying mechanisms of *SCNN1A* promoting tumor, we analyzed DEGs in the high- and low-*SCNN1A* expression cohorts. A total of 658 DEGs were identified, involving 91 up-regulated genes and 567 down-regulated genes. And the expression of DEGs was presented in a volcano plot (Figure 2C).

Identifying *SCNN1A*-Interacting Genes and Proteins

We explored STRING website to generate the protein–protein interaction (PPI) network of *SCNN1A* (Figure 2D). There were 42 edges and 11 nodes, involving *NEDD4L*, *SCNNIG*, *SCNNIB*, *NEDD4*, and *SGK1* (Figure 2D). We built a gene–gene interaction network between *SCNN1A* and the neighboring genes via GeneMania. And the outcome revealed that 20 genes were closely related to *SCNN1A*, including *SCNNIB*, *SCNNIG*, *NEDD4L*, *WNK3*, and *WNK2* (Figure 2E).

Association Between *SCNN1A* Mutation, Hypomethylation, and Prognosis in OV

cBioPortal was employed to analyse the expression and mutation of *SCNN1A* in OV. We analyzed genetic alteration in *SCNN1A* and its relationship with the survival of OV patients. The mutation rate of *SCNN1A* gene was high in OV patients (Figure 3A). Of the 1680 OV patients, 131 had genetic alterations, with a mutation rate of 8%. We also found that majority of the alterations of *SCNN1A* in ovarian cancer patients are gene amplifications. In addition, the result revealed that genetic alteration in *SCNN1A* was correlated with lower OS of OV patients (Figure 3B). The findings suggested that the genetic mutation of *SCNN1A* gene can influence OV patients' prognosis. Additionally, we explored the association between *SCNN1A* gene expression and gene methylation, and the outcome indicated that *SCNN1A* expression was strongly negatively associated with gene methylation ($R = -0.39$, $P < 0.001$) in OV (Figure 3C).

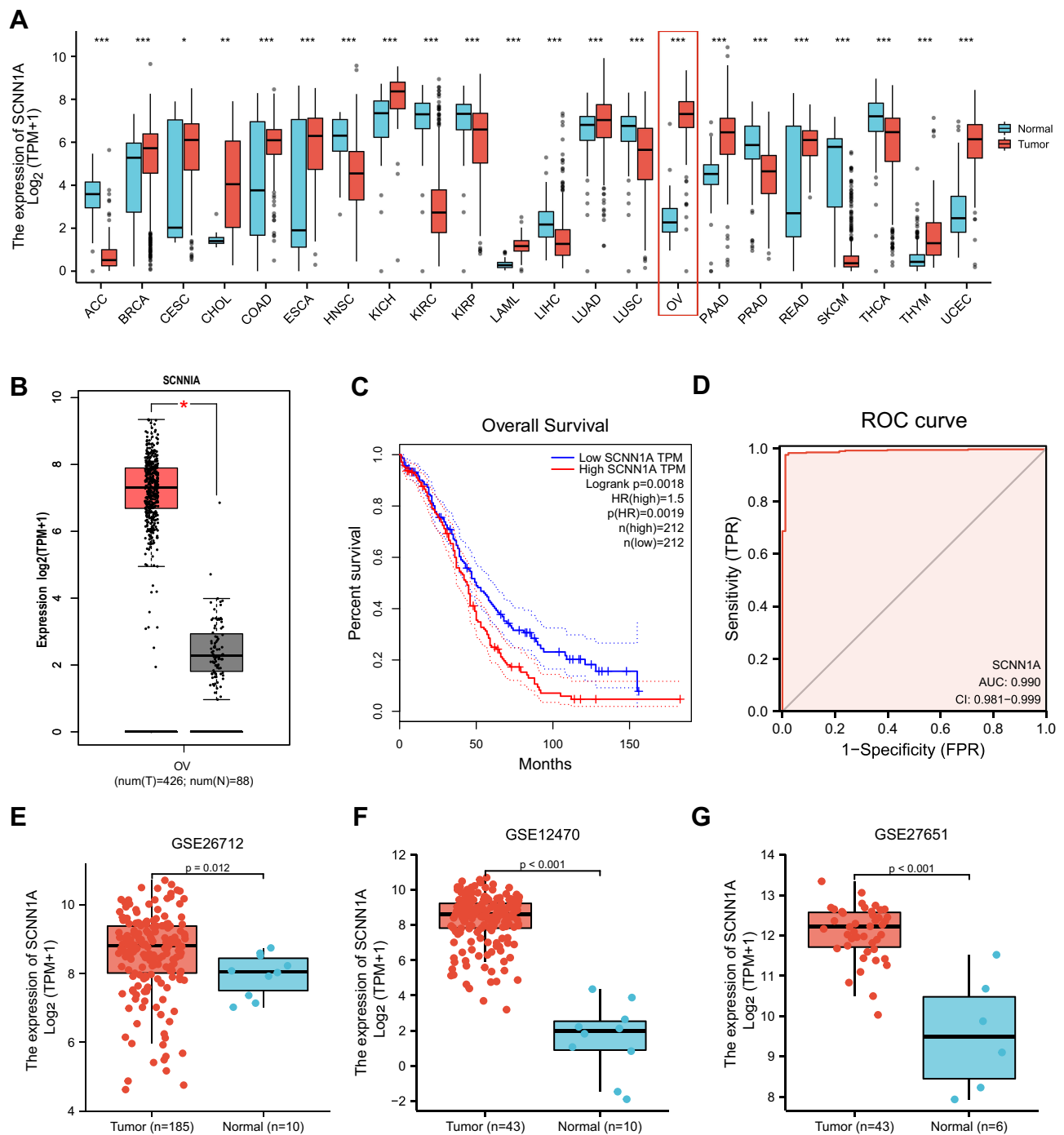


Figure 1 The expression of *SCNN1A* in ovarian cancer. **(A)** The mRNA expression of *SCNN1A* in pan-cancer from TCGA datasets. **(B)** Increased expression of *SCNN1A* in ovarian cancer. **(C)** Kaplan–Meier analysis of overall survival in GEPIA. **(D)** ROC curve analysis assessing the performance of *SCNN1A* for OV diagnosis. **(E–G)** *SCNN1A* expression in tumor and normal tissues in ovarian cancer from GSE26712, GSE12470 and GSE27651 (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Abbreviations: ACC, adrenocortical carcinoma; BRCA, breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma.

Mutation, Copy Number Variation, and Methylation of *SCNN1A*

SCNN1A expression was remarkably increased in OV. We explored the reasons of increased *SCNN1A* levels. As is known to all, DNA methylation, gene mutation, and copy number variation are closely related to genetic and epigenetic regulation and are

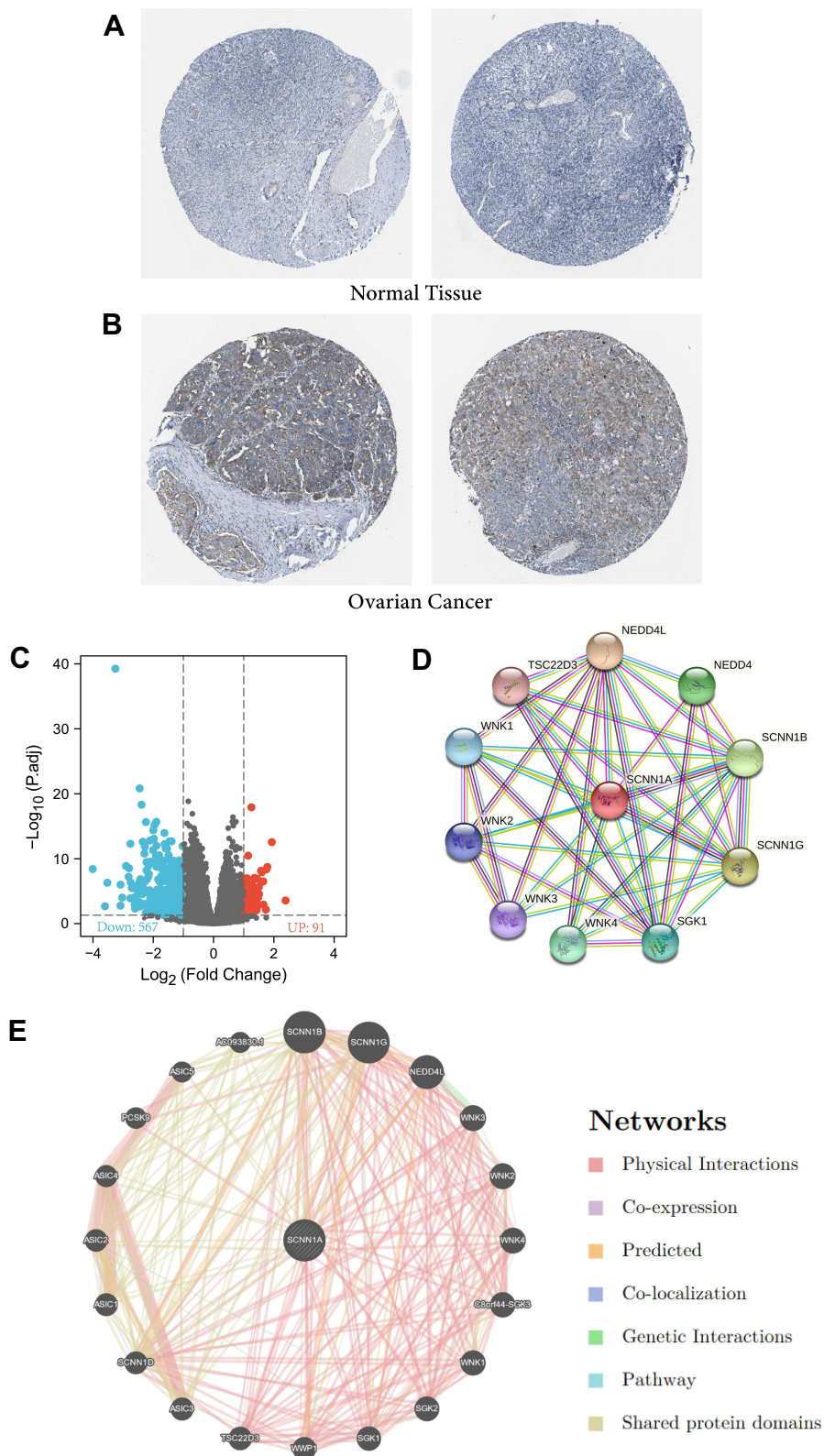


Figure 2 The protein expression of *SCNN1A* in ovarian cancer, *SCNN1A*-related differentially expressed genes (DEGs) and *SCNN1A*-correlated proteins and genes. **(A and B)** The protein levels of *SCNN1A* in normal ovarian tissue and OV based on Human Protein Atlas. **(C)** Volcano plot of DEGs between the high- and low-*SCNN1A* expression groups. **(D)** The protein–protein interaction network of *SCNN1A* via STRING. **(E)** The gene–gene interaction network of *SCNN1A* via GeneMania.

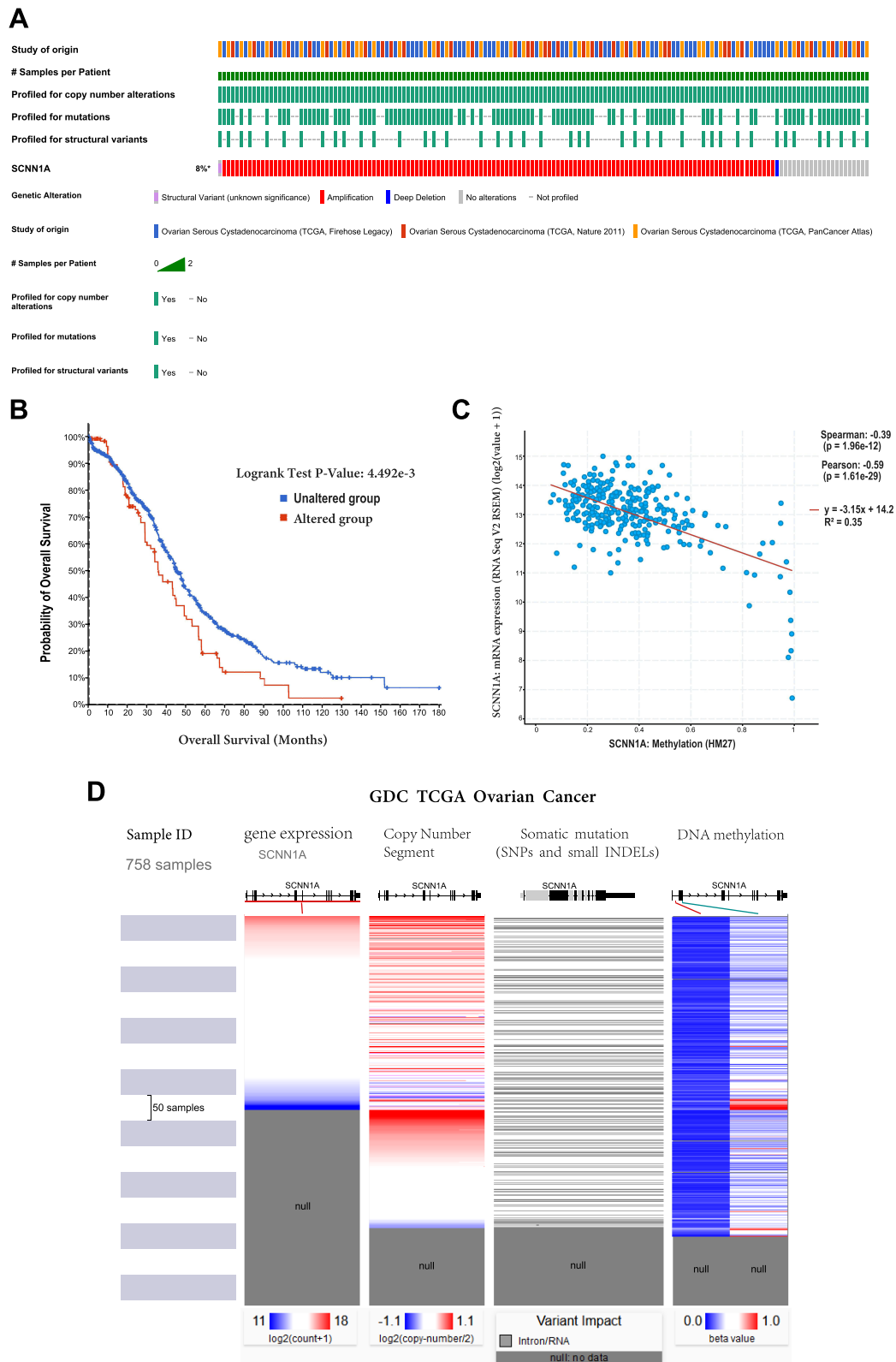


Figure 3 The gene alteration and methylation of *SCNN1A* in OV. **(A and B)** Genetic alteration in *SCNN1A* and its correlation with OS of OV patients. **(C)** The relationship between *SCNN1A* methylation and its expression level. **(D)** Mutation, CNV, and methylation analysis of *SCNN1A* in OV via UCSC Xena.

highly relevant to tumorigenic process. We validated the DNA methylation, gene mutation, CNV status of *SCNN1A* in OV through UCSC Xena. The results revealed that the level of *SCNN1A* mRNA was related to CNV, somatic mutation and DNA methylation in OV (Figure 3D). Consequently, we considered that CNV, somatic mutation and DNA methylation might facilitate the elevated level of *SCNN1A* in OV.

Functional Enrichment of *SCNN1A* Related DEGs in OV

To assess the function of *SCNN1A*-related DEGs in OV patients, we used “Metascape” software. As shown in Figures 4A–C, we found that several pathways associated with *SCNN1A* were enriched, involving metabolic process, immune system process, and cellular process (GO: 0008152, GO: 0002376, GO: 0009987).

GSEA Identifies *SCNN1A*-Related Signaling Pathways

In order to recognize *SCNN1A*-associated signaling pathways in OV, GSEA analysis was carried out between *SCNN1A*-high and -low expression cohorts to identify significant differences (adjusted $P < 0.05$, FDR q value < 0.25) in enrichment of MSigDB Collection (C2.all.v7.0.symbols.gmt). And the most significant pathways were M phase, cell cycle checkpoints, cellular senescence, epigenetic regulation of gene expression, signaling by nuclear receptors, and transcriptional regulation by *RUNX1* (Figure 5A–F).

Association of *SCNN1A* Expression and Clinicopathological Characteristics in Ovarian Cancer Patients

Clinical characteristics and gene expression data of 379 patients with ovarian cancer were gathered from TCGA database. The main clinical characteristics included age, race, FIGO stage, primary therapy outcome, histologic stage, anatomic neoplasm subdivision, venous invasion, lymphatic invasion, tumor residual, and tumor status. According to the mean value of *SCNN1A* level, OV patients were separated into high- and low-*SCNN1A* expression cohorts (Table 1).

Effect of High *SCNN1A* Expression on Prognosis of OV Patients with Different Clinicopathological Status

To better comprehend the correlation and mechanisms of *SCNN1A* expression in OV, we performed univariate Cox analysis to study the relationship between *SCNN1A* expression and clinical characteristics in OV (Figure 6). The result revealed that higher *SCNN1A* expression was related to a shorter OS (HR: 1.585; 95% CI: 1.221–2.057; $P < 0.001$). Other clinicopathologic factors associated with low overall survival included age (> 60), primary therapy outcome (PD&SD&PR), tumor residual (RD) and tumor status (with tumor). And the clinicopathologic variables that associated with inferior disease-specific survival (DSS) consisted race, primary therapy outcome (PD&SD&PR), and tumor residual (with tumor).

Prognostic Performance of *SCNN1A* in the Ovarian Cancer Clinicopathological Subgroups

Subsequently, we tried to ascertain the predictive value of *SCNN1A* for clinical outcomes in a few clinicopathological subgroups. Cox regression analysis was performed in specific subgroups. As presented in the forest diagram (Figure 7A), *SCNN1A* was a strong risk factor for OS in patients of age ≤ 60 years (HR = 1.75, $P = 0.003$), FIGO stage III–IV (HR = 1.57, $P = 0.001$), histologic stage G3–G4 (HR = 1.59, $P = 0.001$), anatomic neoplasm subdivision (bilateral) (HR = 1.45, $P = 0.02$), and tumor status (with tumor) (HR = 1.36, $P = 0.032$). Moreover, similar observations occurred for disease-specific survival (Figure 7B). Kaplan–Meier survival curves of high- and low- *SCNN1A* expression in different subgroups of ovarian cancer patients can be seen in Supplementary Figure 1.

Construction of a Prognostic Model Based on *SCNN1A* and Clinical Variables

Moreover, we validated the prognostic value of *SCNN1A* expression in ovarian cancer by Kaplan–Meier plotter website. High *SCNN1A* level was related to poorer prognosis in OV (OS: HR=1.15, $P = 0.037$; PFS: HR=1.34, $P = 1.1e-05$) (Figure 8A and B). Then, a prognostic nomogram was built based on the statistically significant prognostic factors in Cox regression analysis, and we plotted the calibration curves to check on the validity of the nomogram. FIGO stage, primary

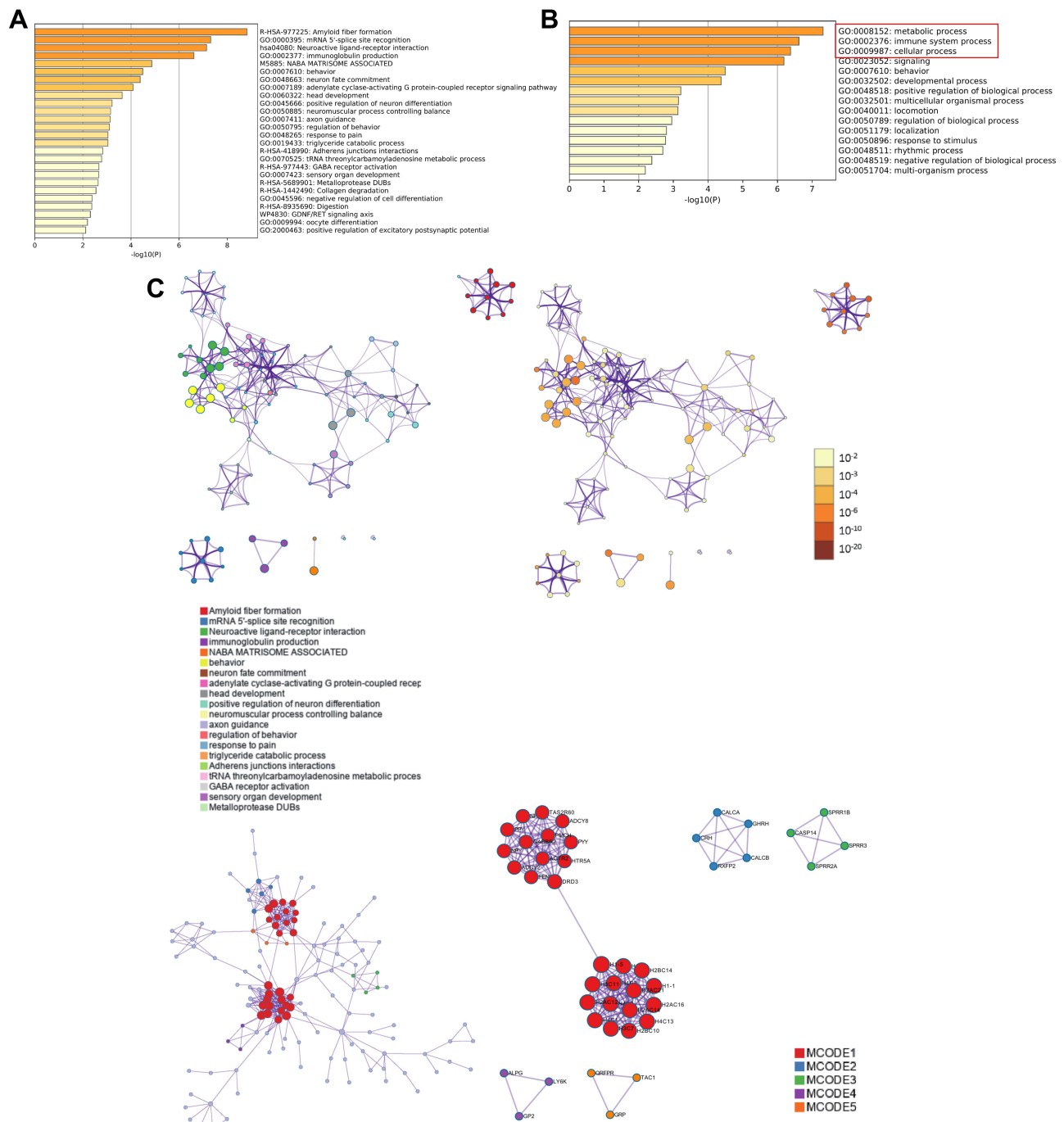


Figure 4 Functional enrichment analysis of differentially expressed genes (DEGs) in ovarian cancer patients with distinct *SCNN1A* levels via Metascape database. (A and B) The gene ontology (GO)-enriched terms colored according to P-values. (C) Network of GO-enriched terms colored according to clusters and P-values.

therapy outcome, tumor status, as well as *SCNN1A*, were involved in the nomogram to forecast overall survival (Figure 8C). A higher point on the nomogram meant a worse prognostic factor. We explored the prediction efficiency of this nomogram, and the outcome showed that the C-index of this model was 0.712 (CI: 0.693–0.732), indicating the prediction efficiency of this nomogram was moderately precise. Calibration curves of 3 and 5 years showed a satisfactory performance for our constructed nomogram (Figure 8D and E). In conclusion, the nomogram can be a better model for forecasting survival of OV patients compared to single prognostic factors.

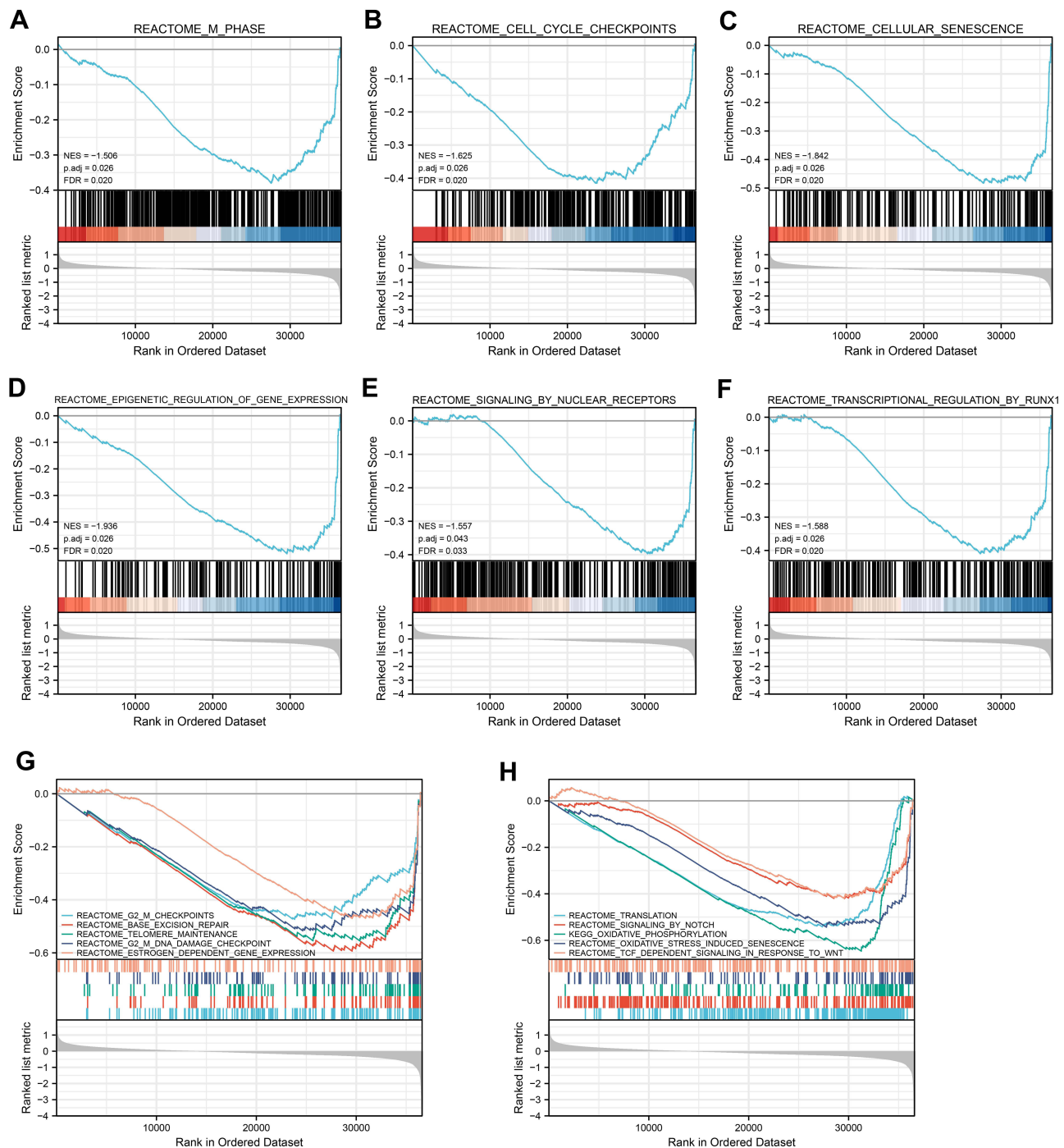


Figure 5 Enrichment graphs from GSEA. Some signaling pathways were differentially enriched in OV patients upon high- and low-*SCNN1A* expression. **(A)** M Phase. **(B)** Cell cycle checkpoints. **(C)** Cellular senescence. **(D)** Epigenetic regulation of gene expression. **(E)** Signaling by nuclear receptors. **(F)** Transcriptional regulation by *RUNX1*. **(G)** G2 M checkpoints, base excision repair, telomere maintenance, estrogen dependent gene expression. **(H)** Translation, signaling by notch, oxidative stress induced senescence, TCFdependent signaling in response to Wnt.

Abbreviations: ES, enrichment score; NES, normalized enrichment score; ADJ p-Val, adjusted P-value; FDR, false discovery rate.

Prognostic Analysis of *SCNN1A* Expression Based on Immune Cells in OV Patients

Because the expression of *SCNN1A* is significantly associated with immune system process and inferior prognosis in OV, we examined whether *SCNN1A* level influenced the survival of OV due to immune infiltration. We conducted Kaplan-Meier plotter analysis of *SCNN1A* expression in OV by basophils, B cells, CD4+ memory T cells, CD8+ T cells, eosinophils, macrophages, mesenchymal stem cells, NK T cells, Treg T cells, Th1 cells, Th2 cells. As presented in

Table I Clinical Characteristics of the Ovarian Cancer Patients (TCGA)

Characteristic	SCNN1A Expression		P-value
	Low	High	
No.	189	190	
Age			0.714
≤60 years	106 (28%)	102 (26.9%)	
>60 years	83 (21.9%)	88 (23.2%)	
Race			0.070
Asian	4 (1.1%)	8 (2.2%)	
Black or African American	8 (2.2%)	17 (4.7%)	
White	172 (47.1%)	156 (42.7%)	
FIGO stage			0.777
Stage I	1 (0.3%)	0 (0%)	
Stage II	13 (3.5%)	10 (2.7%)	
Stage III	148 (39.4%)	147 (39.1%)	
Stage IV	27 (7.2%)	30 (8%)	
Primary therapy outcome			0.320
PD	15 (4.9%)	12 (3.9%)	
SD	12 (3.9%)	10 (3.2%)	
PR	18 (5.8%)	25 (8.1%)	
CR	124 (40.3%)	92 (29.9%)	
Histologic grade			0.967
G1	1 (0.3%)	0 (0%)	
G2	23 (6.2%)	22 (6%)	
G3	164 (44.4%)	158 (42.8%)	
G4	0 (0%)	1 (0.3%)	
Anatomic neoplasm subdivision			0.085
Unilateral	59 (16.5%)	43 (12%)	
Bilateral	120 (33.6%)	135 (37.8%)	
Venous invasion			0.747
No	22 (21%)	19 (18.1%)	
Yes	31 (29.5%)	33 (31.4%)	
Lymphatic invasion			0.128
No	29 (19.5%)	19 (12.8%)	
Yes	46 (30.9%)	55 (36.9%)	
Tumor residual			0.566
NRD	36 (10.7%)	31 (9.3%)	
RD	131 (39.1%)	137 (40.9%)	
Tumor status			0.157
Tumor free	43 (12.8%)	29 (8.6%)	
With tumor	131 (38.9%)	134 (39.8%)	

Figure 9A and B, ovarian cancer patients with high level of *SCNN1A* had a low survival in most of the subgroups. The above results indicate that *SCNN1A* might influence the survival of OV patients in part through immune infiltration.

The Correlation Between *SCNN1A* Expression and Immune Infiltration

Since that both GO and prognostic analysis revealed that *SCNN1A* might be involved in tumor immune process, we performed ssGSEA to explore the association between the *SCNN1A* mRNA expression and the infiltration level of 24 immune cells. The relationship between immune cells infiltration and the *SCNN1A* mRNA expression is presented in Figure 10. The outcome suggested that *SCNN1A* mRNA expression was closely linked to the infiltration of Tcm cells ($R = 0.240$, $P < 0.001$), Th17 cells

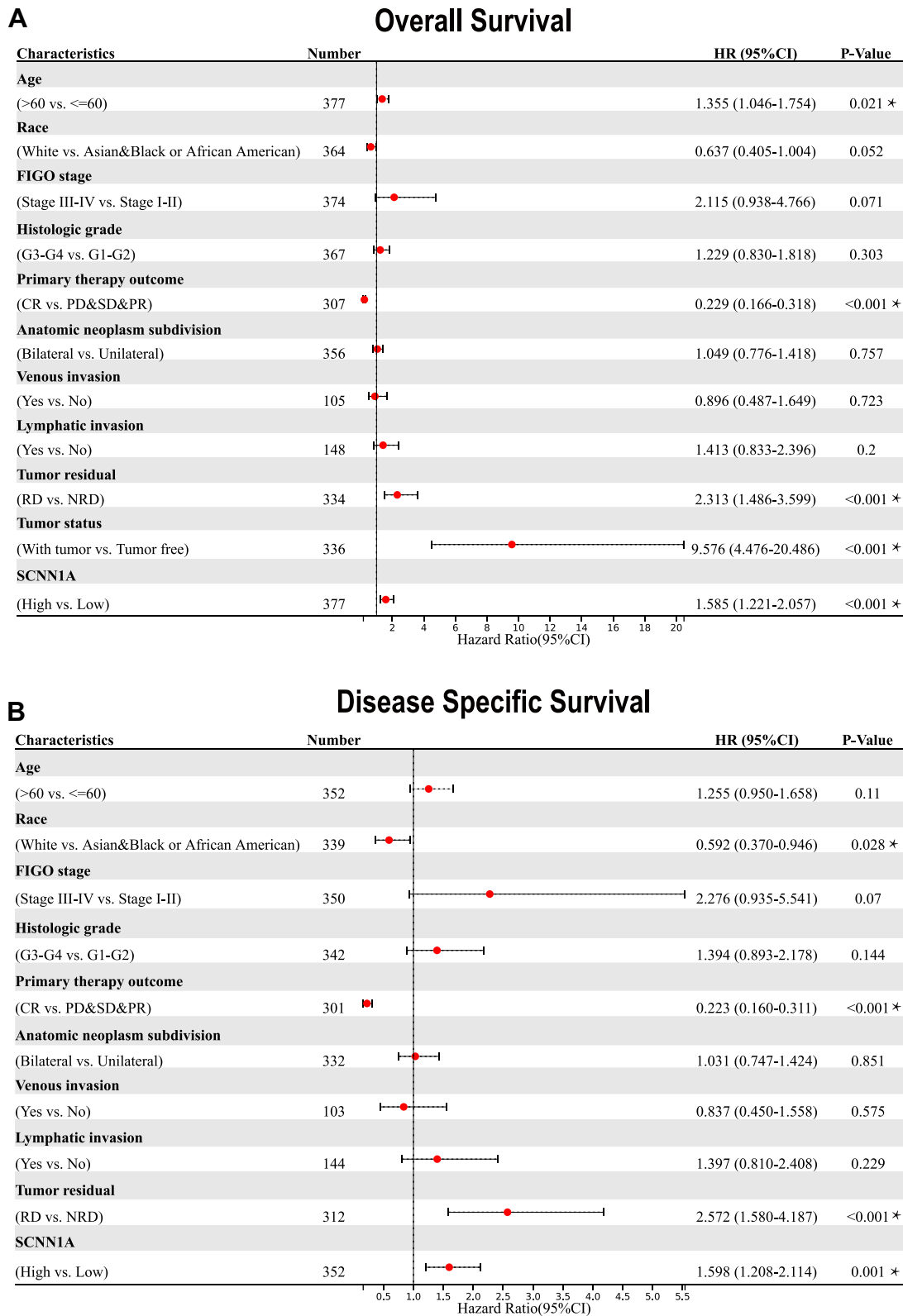
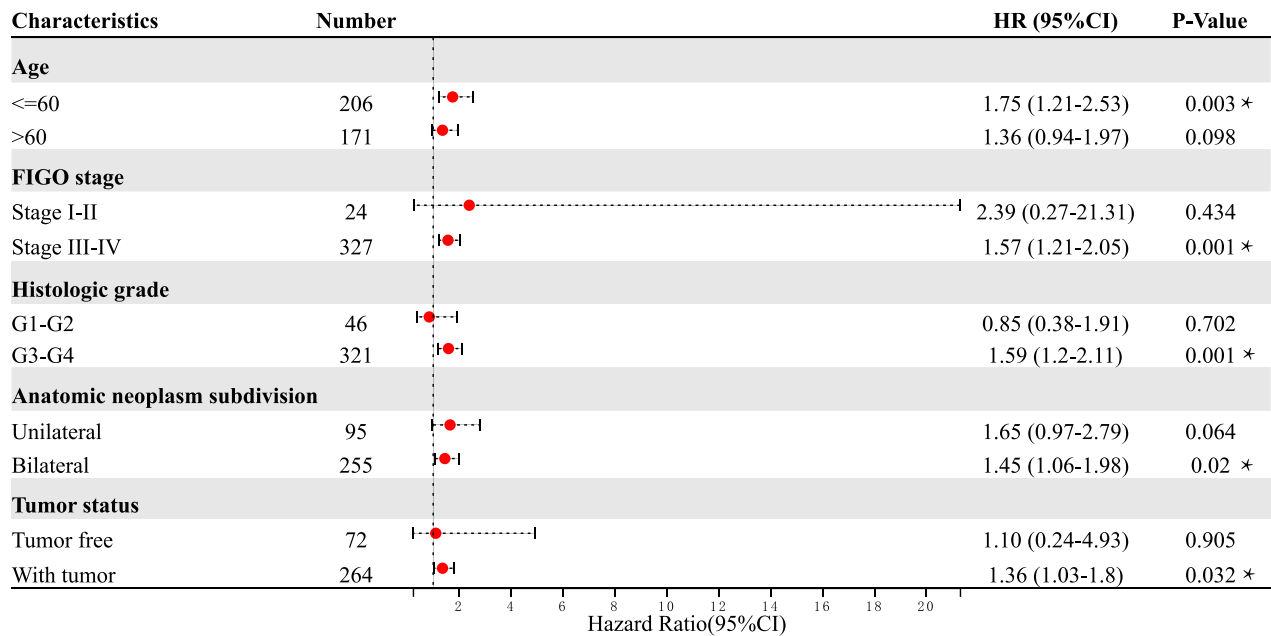


Figure 6 Forest plots of the univariate Cox regression analysis for OV patients in TCGA. (A) Overall survival. (B) Disease-specific survival. *P < 0.05.

A

Overall Survival



B

Disease Specific Survival

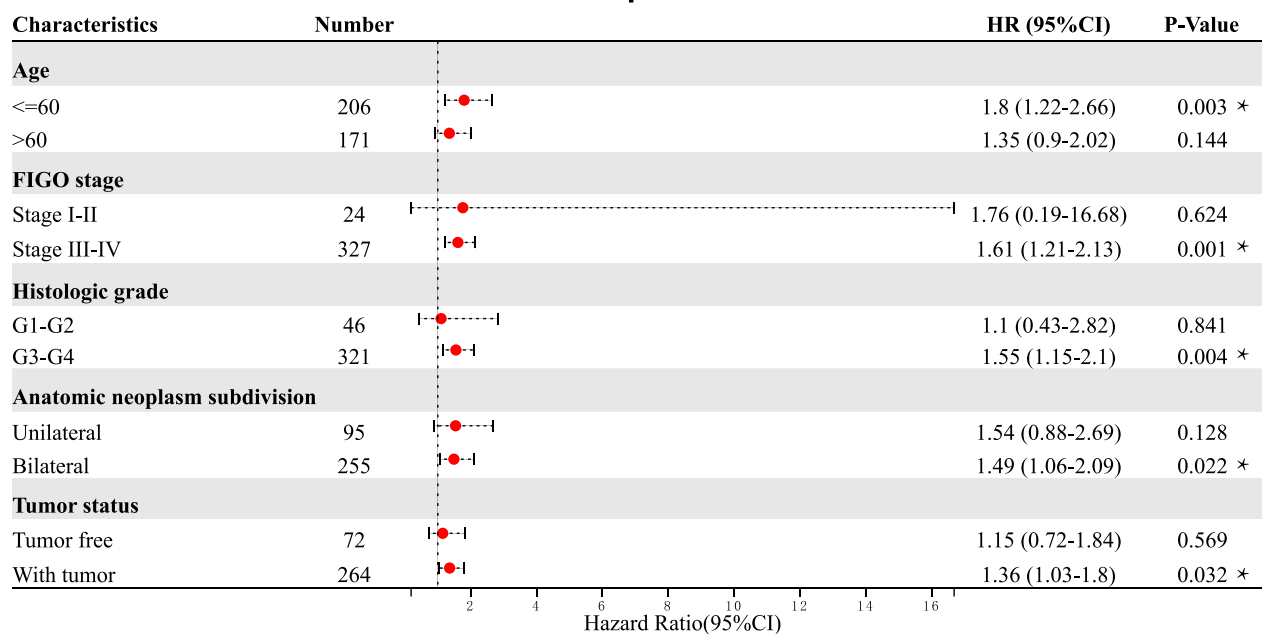


Figure 7 Correlation of *SCNN1A* mRNA expression level and prognosis in OV patients with different clinicopathological factors. **(A)** Overall survival. **(B)** Disease-specific survival. * $P < 0.05$.

($R = 0.160$, $P = 0.002$), Tem cells ($R = 0.150$, $P = 0.004$), NK cells ($R = 0.110$, $P = 0.035$) and Eosinophils ($R = 0.120$, $P = 0.025$). ssGSEA also displayed a negative correlation between *SCNN1A* expression and Th2 cells' infiltration ($R = -0.170$, $P = 0.001$).

Relationships Between *SCNN1A* Expression and Immune Markers

The association between *SCNN1A* expression and tumor-infiltrating immune cell gene marker levels in OV tissues was evaluated through TIMER. The outcome revealed that the *SCNN1A* level in OV tissues related to immune markers of

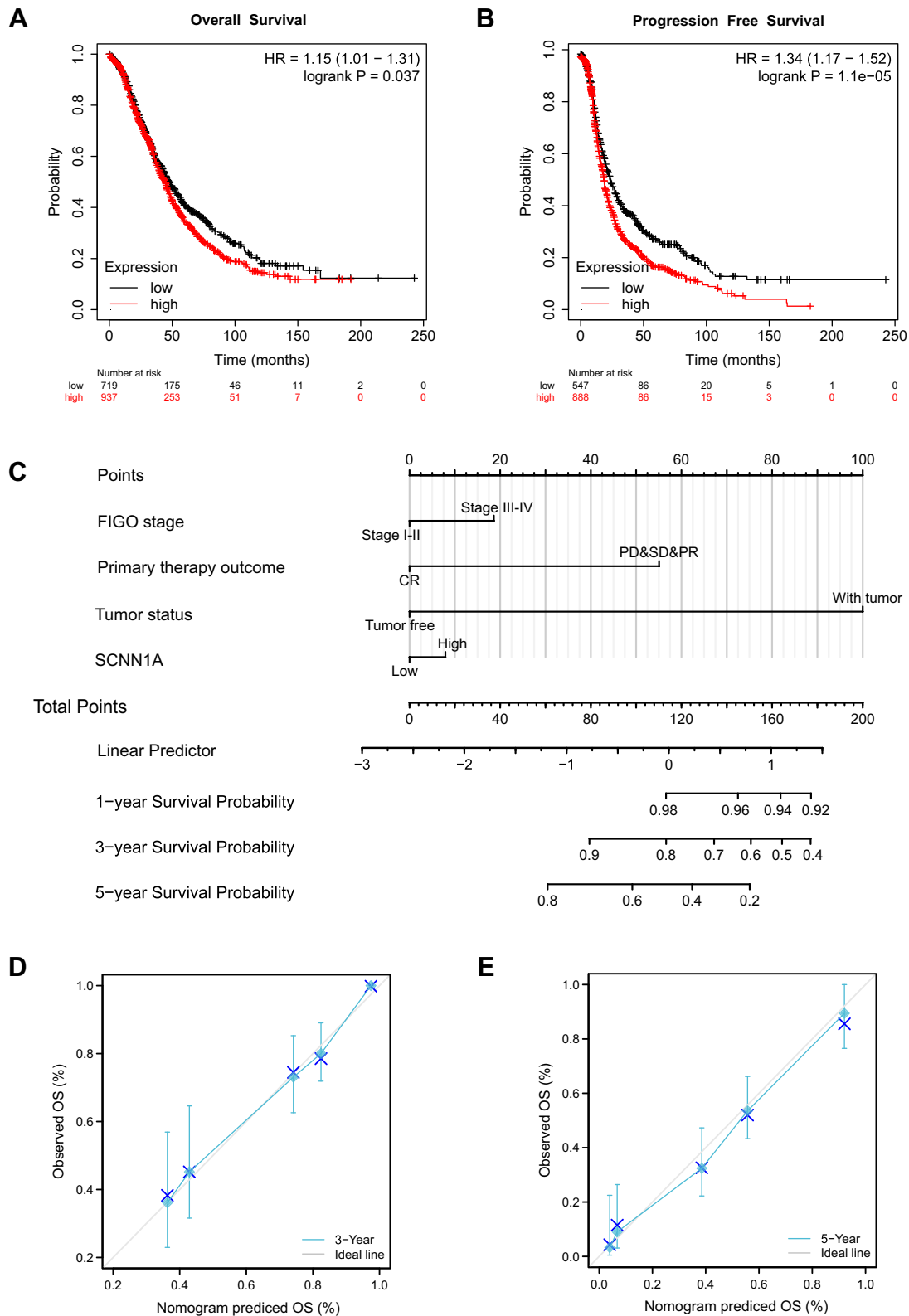


Figure 8 Survival curve assessing the prognostic value of *SCNN1A* and an *SCNN1A* expression-based nomogram. (A and B) Survival curves of OS and PFS. (C) Nomogram of 1-, 3-, and 5-year overall survival for OV patients. (D and E) Calibration plots of the nomogram for predicting the OS likelihood.

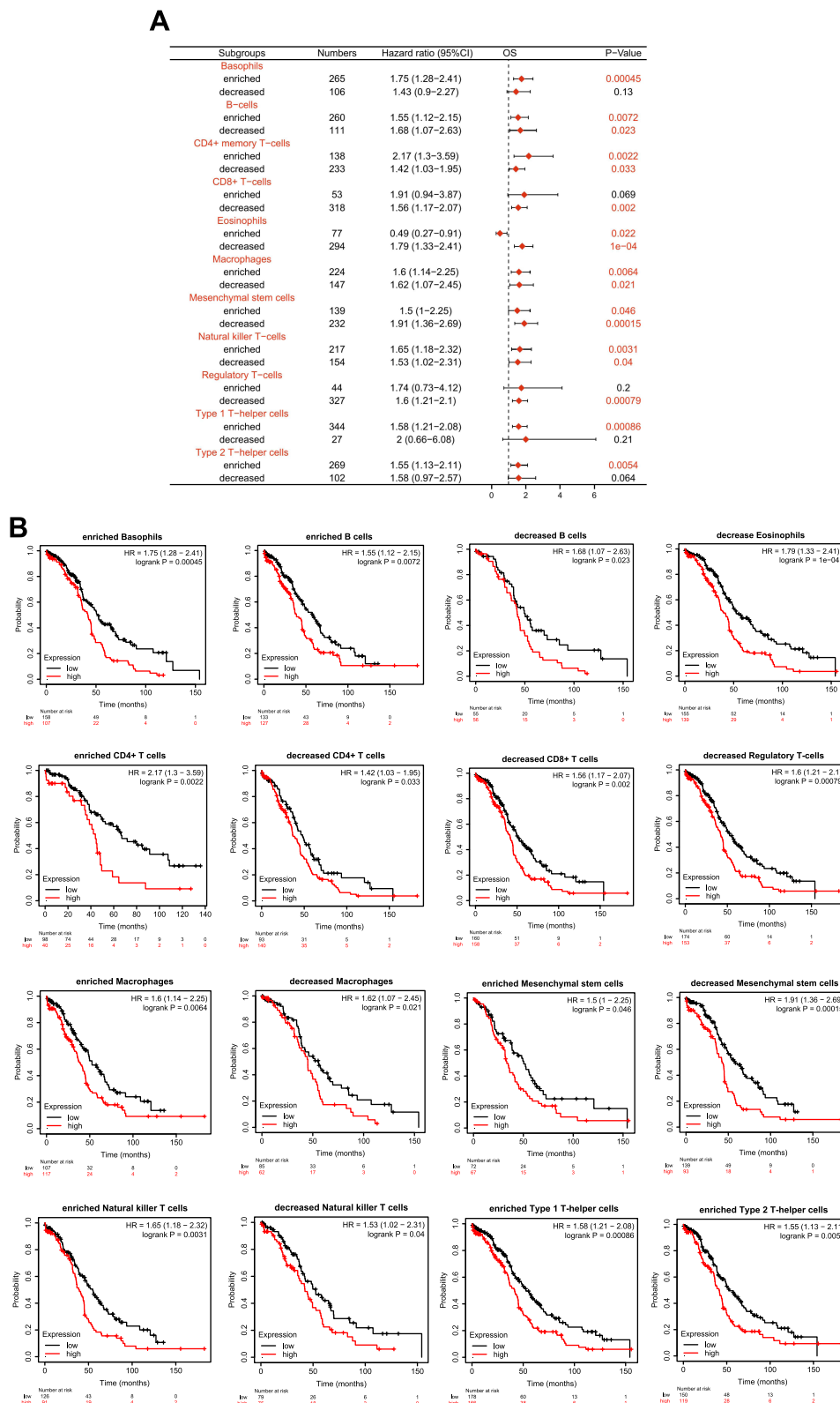


Figure 9 Kaplan-Meier survival curves according to high and low level of *SCNN1A* in different immune cell subgroups in OV patients. **(A)** The forest plot reveals the prognostic value of *SCNN1A* level based on various immune cell subgroups. **(B)** Association between *SCNN1A* expression and OS in various immune cell subgroups.

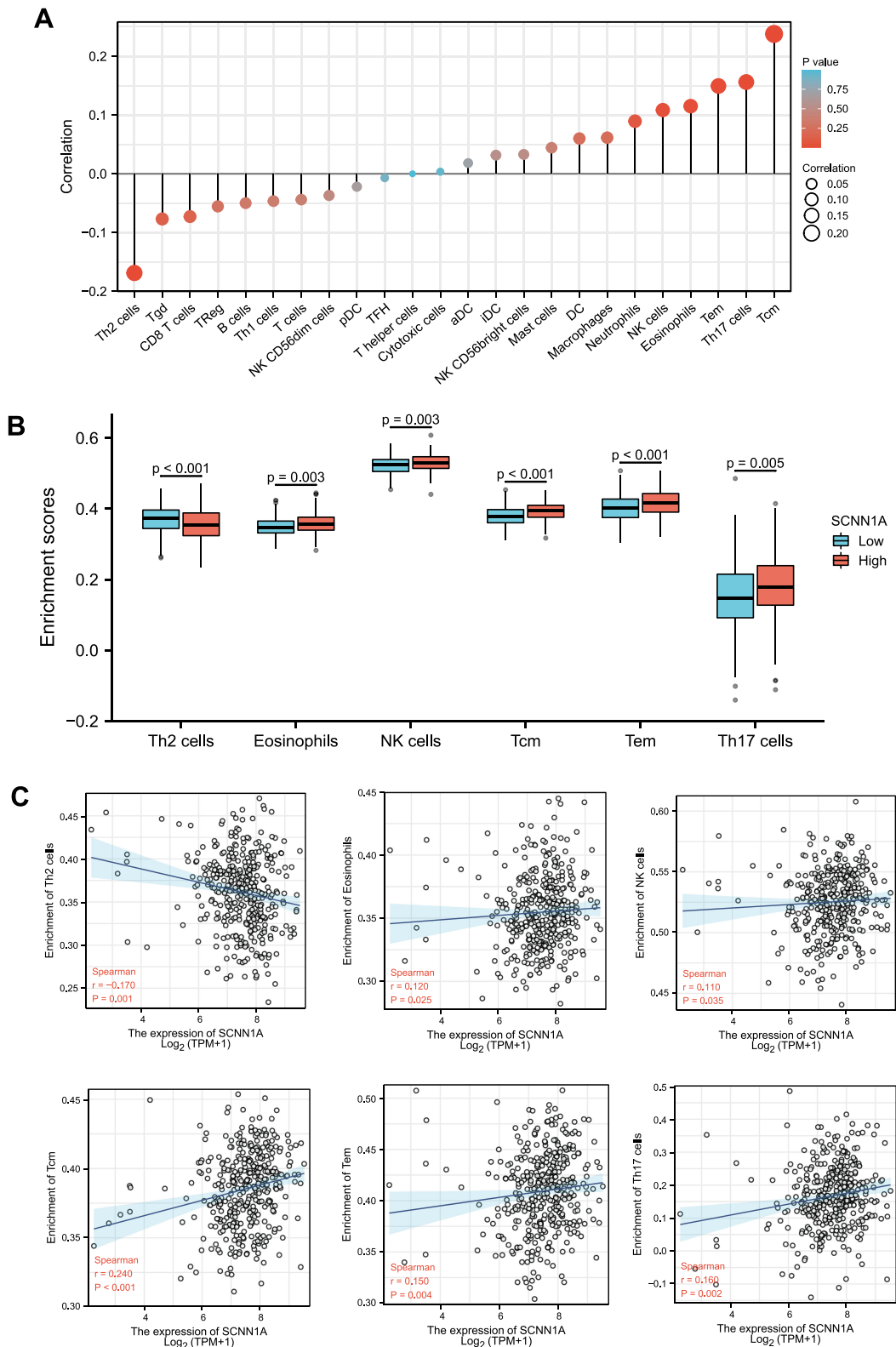


Figure 10 Association of immune cell infiltration and *SCNN1A* expression in OV patients. **(A)** Relationships among infiltration levels of 24 immune cell types and *SCNN1A* expression. **(B and C)** Box plots and correlation diagrams displaying the difference of Th2 cells, eosinophils, NK cells, Tcm, Tem, Th17 cells infiltration level between *SCNN1A*-high and -low cohorts.

Abbreviations: Th, T helper cells; Th2 cells, type 2 Th cells; Tgd, T gamma delta; TReg, regulatory T cells; Th1 cells, type 1 Th cells; pDC, plasmacytoid DCs; TFH, T follicular helper; aDC, activated DC; iDC, immature DC; DC, dendritic cell; NK cells, natural killer cells; Tem, T effector memory; Th17 cells, type 17 Th cells; Tcm, T central memory.

monocytes, M1 Macrophage, M2 Macrophage, T cell (general), TAM, Tfh, Th2, Th17, and neutrophil cells (Table 2 and Supplementary Figure 2). Furthermore, we evaluated the association between *SCNN1A* expression and these markers in OV by GEPIA, which revealed similar relationship between *SCNN1A* and markers of monocytes, neutrophils, TAMs, Th1, Th2, Treg, and T cell exhaustion to those in TIMER (Table 3 and Supplementary Figure 3). Thus, these results support our conjecture that *SCNN1A* expression in OV is related to immune cell infiltration in various ways, which helps to interpret the differences in patients' survival.

Discussion

Due to the lack of early typical clinical symptoms and effective diagnostic methods, most OV patients are diagnosed at late stages.²³ Additionally, a high rate of disease recurrence contributes to inferior outcomes in the majority of OV patients. Although surgery and treatment drugs have improved, the latest treatments still do not improve survival in ovarian cancer patients.²⁴ Hence, it is of great significance to further study the molecular mechanisms of OV pathogenesis and develop new therapeutic methods for improving prognosis. It is imperative to search stable and reliable biomarkers for advancing the prognosis of ovarian cancer. In our bioinformatics analysis, we focused on epithelial ovarian cancer, which accounts for the majority of ovarian cancer cases. *SCNN1A* codes one of the subunits of the epithelial sodium channel (ENaC), a sodium-permeable ion channel expressed in epithelial tissues. Previous researches have revealed that *SCNN1A* has multiple functional roles in a variety of malignancies. In ovarian cancer, whether *SCNN1A* is involved in tumor progression and tumor immunity remains to be studied. Although some studies have found

Table 2 Correlation Analysis Between *SCNN1A* and Gene Markers of Immune Cells in TIMER

Description	Gene Markers	None		Purity	
		Cor	P	Cor	P
Monocyte	CD86	0.046	0.428	0.098	0.121
	CD115	0.118	*	0.192	**
M1 Macrophage	iNOS	0.005	0.931	0.014	0.823
	IRF5	0.206	***	0.208	***
M2 Macrophage	COX2	0.028	0.623	0.019	0.769
	CD163	0.113	*	0.192	**
T cell (general)	VSIG4	0.049	0.398	0.102	0.107
	MS4A4A	0.013	0.82	0.076	0.235
	CD3D	-0.113	*	-0.128	*
TAM	CD3E	-0.04	0.493	-0.047	0.4572
	CD2	-0.059	0.305	-0.065	0.311
	CCL2	-0.138	*	-0.137	*
Tfh	CD68	0.039	0.502	0.095	0.137
	IL10	-0.031	0.586	-0.003	0.963
	BCL6	0.233	***	0.21	***
Th2	IL21	0.053	0.356	0.069	0.277
	GATA3	0.014	0.806	0.035	0.587
	STAT6	0.29	***	0.315	***
Th17	STAT5A	0.237	***	0.261	***
	IL13	-0.058	0.313	-0.019	0.765
	STAT3	0.301	***	0.363	***
Neutrophils	IL17A	-0.009	0.875	-0.019	0.766
	CD66b	0.09	0.119	0.07	0.272
	CD11b	0.115	*	0.176	**
	CCR7	-0.028	0.626	-0.05	0.437

Note: *P<0.05, **P<0.01, ***P<0.001.

Table 3 Correlation Analysis Between SCNN1A and Marker Genes of Immune Cells in GEPIA

Description	Gene Markers	Tumor		Normal	
		R	P	R	P
Monocyte	CD86	0.15	**	-0.014	0.89
	Neutrophils	CD11b	0.28	***	0.0013
TAM	CCR7	0.059	0.23	-0.017	0.88
	CD68	0.16	***	0.19	0.076
Th1	IL-10	0.13	**	-0.012	0.91
	IFN- γ (IFNG)	-0.0032	0.95	-0.083	0.44
Th2	STAT1	0.18	***	-0.035	0.75
	T-bet (TBX21)	0.1	*	0.064	0.55
Treg	TNF- α (TNF)	0.068	0.16	0.048	0.66
	STAT6	0.36	***	-0.21	0.054
T cell exhaustion	CCR8	0.0029	0.95	0.3	**
	STAT5B	0.25	***	-0.34	**
T cell exhaustion	TGF- β (TGFB1)	0.14	**	0.13	0.23
	CTLA4	0.026	0.6	0.17	0.12
	PD-1 (PDCD1)	0.024	0.62	0.075	0.49
	TIM-3 (HAVCR2)	0.16	***	0.0097	0.93

Note: *P<0.05, **P<0.01, ***P<0.001.

that *SCNN1A* is overexpression and is a potential biomarker of OV,^{12,25} the prognostic value and the potential function of *SCNN1A* in OV were unclear and not fully understood.

In the present study, we carried out prognosis and clinical correlation analysis to investigate the underlying diagnostic and prognostic value of *SCNN1A*; the biological functions were determined by molecular interaction network analysis and enrichment analysis. The biological roles of *SCNN1A* were assessed by Metascape database and GSEA. We performed Kaplan–Meier analysis, Cox regression analysis and nomograms to assess the predictive value of *SCNN1A* for clinical outcomes in OV patients. Finally, single-sample GSEA was conducted to study the relationship between *SCNN1A* and immune cell infiltration.

Herein, we discovered that both mRNA and protein level of *SCNN1A* were overexpressed in ovarian cancer (Figures 1 and 2A, B). Significantly, high *SCNN1A* expression was correlated with poorer OS, progression-free survival (PFS), and disease-specific survival (DSS) of ovarian cancer patients. Moreover, the ROC curve for *SCNN1A* discrimination of OV diagnosis had an AUC of 0.990, firmly implying that *SCNN1A* was a promising biomarker for ovarian cancer (Figure 1D). In addition, high *SCNN1A* expression was correlated with inferior survival of OV in FIGO stage III–IV, histologic stage G3–G4, anatomic neoplasm subdivision (bilateral), and tumor status (with tumor) subgroups, with higher HR for poor OS and DSS when *SCNN1A* was highly expressed in OV (Figure 7). We discovered that *SCNN1A* expression remained a strong prognostic predictor in these subgroups, indicating that *SCNN1A* was independent of these important clinicopathological factors. In this article, a nomogram plot was constructed to visually display the relationship between four clinicopathologic variables (FIGO stage, primary treatment outcome, tumor status, and *SCNN1A*) and 1-, 3-, and 5-year overall survival. After the evaluation of C-index, ROC and calibration curves, the *SCNN1A*-based nomogram showed pleasing performance (Figure 8).

As is known to all, DNA methylation is a frequent epigenetic mechanism that occurs in a variety of cancers.²⁶ Improvement in technology to study DNA methylation has begun to uncover how epigenetics contributes to the etiology, chemotherapy response and prognosis of OV patients.²⁷ Although there are a lot of mechanisms that can lead to the upregulation of gene level, hypomethylation is one of the major regulatory mechanisms of gene expression. By exploring cBioPortal and UCSC Xena databases, we found that *SCNN1A* DNA amplification and hypomethylation may conduce to *SCNN1A* upregulation, and *SCNN1A* DNA amplification and genetic alteration are related to inferior outcome (Figure 3). Thus, differentially methylated genes may serve as promising biomarkers or therapeutic targets in ovarian cancer.

Considering the limited functional data on *SCNN1A*, we executed functional enrichment analysis based on GO and GESA. The results revealed that *SCNN1A* may participate in metabolic process, immune system process, and cellular process (Figure 4). These findings were consistent with the ssGSEA results shown in this study and reinforced the link between *SCNN1A* and immune infiltrates (Figure 10). Moreover, GSEA displayed that *SCNN1A* was significantly associated with M phase, cell cycle checkpoints, cellular senescence, epigenetic regulation of gene expression, signaling by nuclear receptors, and transcriptional regulation by *RUNX1* (Figure 5). A disrupted cell cycle is a hallmark of many tumors. Disruption of the cell cycle can lead to abnormal cell proliferation, which is closely associated with tumors, including ovarian cancer. Cellular senescence has been reported to be associated with several aspects of ovarian cancer.²⁸ Platinum chemotherapy induces cellular senescence, which promotes chemoresistance by inducing the production of cancer stem-like cells (CSCs).²⁹ *RUNX1* was upregulated in ovarian cancer and high expression of *RUNX1* is related to lower survival in OV patients. A study confirmed that knockdown of *RUNX1* in ovarian cancer cells can remarkably inhibited cell proliferation, cell migration and invasion.³⁰ In addition, Xiao et al constructed *RUNX1*-knockout clones using CRISPR/Cas9 technology and found that depletion of *RUNX1* gene promoted cisplatin-induced apoptosis in ovarian cancer cells.³¹ These findings will help to better understand the mechanism of *SCNN1A* and *RUNX1* in OV.

Tumor microenvironment (TME) comprises tumor cells, stromal cells, extracellular matrix, and infiltrating immune cells.³² Some studies have covered different important roles of tumor-infiltrating immune cells (TIICs) in tumor development. TIICs develop a complex network of intercellular interaction that helps facilitate and sustain the immunosuppressive microenvironment and foster immune escape, thereby contributing to tumor progression.^{33,34} We revealed an underlying association between *SCNN1A* expression and immune cell infiltration. Our results demonstrated that *SCNN1A* expression in OV was positively associated with multiple types of immune cell infiltration, for example, Tcm, Tem, Th17, NK, and eosinophil cells (Figure 10). However, *SCNN1A* expression was negatively related to Th2 cells. Recent studies have reported that eosinophils infiltrate multiple tumors and are equipped to regulate tumor progression either directly by interacting with tumor cells or indirectly by shaping the TME. In ovarian cancer, higher eosinophil count was predictive of both recurrence and mortality.³⁵ NK cells are cytotoxic lymphocytes and characterized by strong antitumor activities, involving production of IFN- γ and direct cytotoxicity against cancer cells. Latest studies have shown that abnormal molecules caused by the ovarian cancer TME could interfere with the antitumor response of NK cells. For instance, some researchers have found and characterized a group of mature NK cells that overexpressed programmed death 1 (PD-1) in the ascites of OV patients.^{36,37} PD-1+ NK cells show inferior proliferative reaction to exogenous cytokines and display diminished antitumor activity involving decreased cytokine production and reduced cytotoxic/degranulation activity.³⁶ Tumor-infiltrating Tcm cells have been described in a variety of cancers and they often display dysfunctional phenotypes correlated with tumor progression. This could be interpreted that excessive neoantigen exposure induced functional changes in Tcm cells, shifting the anti-tumor response toward non-responsiveness. Besides, a remarkable association between *SCNN1A* and various immune cell marker set was noticed in ovarian cancer (Tables 2 and 3). In addition, *SCNN1A* could influence the survival of ovarian cancer patients partly via immune cell infiltration (Figure 9). The above observations highlight the apparent role of *SCNN1A* in tumorigenesis and suggest that *SCNN1A* might play a vital role in the regulation of immune cell infiltration in ovarian cancer. Nonetheless, the exact functions of *SCNN1A* in the TME still need to be fully explored.

Although this study enhanced our comprehension of the correlation between *SCNN1A* and ovarian cancer, there were several limits. Firstly, the expression and prognostic implication of *SCNN1A* were determined using online public databases, and further studies with clinical samples are required to confirm these results. Secondly, to further explore the detailed mechanism of the effect of *SCNN1A* on immune infiltration in OV, in vivo/vitro experiments need to be designed.

In conclusion, *SCNN1A* mRNA and protein expression were overexpressed in OV, while methylation of *SCNN1A* was decreased in OV. This research provides new insights into the potential mechanisms of OV tumorigenesis and reveals *SCNN1A* as a promising diagnostic and prognostic biomarker for OV. Our results provide an underlying mechanism that *SCNN1A* expression may mediate tumor immunity by modulating the infiltration of immune cells in OV. Taken together,

this study suggests that *SCNN1A* may influence the prognosis of OV patients by interacting with infiltrating immune cells. The biological impact of *SCNN1A* in OV remains to be further verified.

Ethics Statement

The study has been approved by the Ethics Committee of Guangxi Medical University Hospital and is considered to be in compliance with the requirements of medical ethics. Approval number: LW2021101. This trial was conducted in accordance with the Declaration of Helsinki.

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Author Contributions

All authors made significant contributions to conception and design, data acquisition, or data analysis and interpretation; participated in the drafting of the article or made critical revisions to important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be responsible for all aspects of the work.

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Disclosure

The authors state that there are no conflicts of interest about this study.

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