Overexpression Of RNPEPL1 Predicts Poor Overall Survival In Ovarian Carcinoma

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Research Article

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Abstract

Background. RNPEPL1 (arginyl aminopeptidase-like 1) belongs to the M1 family of zinc metallopeptidases. We aimed to explore the role of RNPEPL1 in the occurrence and progression of ovarian carcinoma (OV).

Methods: We analyzed the expression level and prognostic value of RNPEPL1 in OV across Oncomine, GEO, TCGA and Human Protein Atlas (HPA) database and identified its prognostic value by the Cox regression analysis and Kaplan–Meier method. We utilized GESA software to perform GO and KEGG analyses. We also utilized TIMER to evaluate the relevance between RNPEPL1 and tumor-infiltrating immune cells. Finally, relevance between RNPEPL1 and immune-related genes was examined by Pearson correlation analysis.

Results: RNPEPL1 was overexpressed in OV tissues compared with normal ovary tissue. Moreover, high RNPEPL1 expression was related with poor OV survival in TCGA. Cox analyses suggested that overexpression of RNPEPL1 was an independent risk factor for overall survival(OS) of OV. Results of KEGG analysis suggested that genes were mainly enriched in T cell receptor signaling pathway, B cell receptor signaling pathway and natural killer cell mediated cytotoxicity when the expression level of RNPEPL1 is low. Besides, RNPEPL1 expression is associated with infiltrating levels of CD4+ T cells, B cells, macrophage, neutrophils and dendritic cells. And, the level of dendritic cells is correlated with OS of OV. RNPEPL1 expression was significantly correlated with the immune signaling pathway of antigen processing and presentation.

Conclusions: RNPEPL1 is a novel independently prognostic biomarker of OV and was related to immune infiltration. Especially, the tight correlation between RNPEPL1 and antigen processing and presentation may be the potential hub of anti-tumor immunity and one of the essential factors influencing survival.

Introduction

Ovarian carcinoma (OV) ranks fifth among the causes of death in female and ranks first among the mortality rates of all gynecologic malignant tumors[1]. There were 295,400 women diagnosed with OV and 184,800 women died of ovarian cancer around the world in 2018, [2]. In the United States, there are 21,410 new cases and 13,770 deaths of ovarian cancer predicted in 2021[3]. Surgery and chemotherapy are the main treatments for OV. Despite development in these treatments, the survival rate of OV has only improved slightly. The 5-year survival rate is only 38.9% in China and 48% in the United States for OV[4]. Epithelial OV accounts for 90% among all pathological types and serous carcinoma is the most common subtype[5]. OV is usually diagnosed in advanced stage when the mortality rate is high due to the anatomical features of the ovary[6]. Exploration for valid adjuvant therapeutics is based on a better comprehending of its progression biology. Reliable biomarkers, as a potential improvement for OV patients including screening, diagnosis, response to therapy, prognosis and survival, are urgently required.

RNPEPL1(arginyl aminopeptidase-like 1) belongs to the M1 family of zinc metallopeptidases[7]. Most M1 family of zinc metallopeptidases are aminopeptidases and exopeptidases. They hydrolyze amino acid residues sequentially from the N-terminus region of protein substrate or peptide. Inhibitors of these enzymes are promising in many treatment circumstances, such as inflammation[8], hypertension[9], and even some cancers[10, 11]. The intron 5 is an alternative splicing event of RNPEPL1 and was speculated to generate a mRNA isoform including an immature stop codon, and it would finally generate a curtal protein product deficient in catalytic activity[7]. There were only ovary and thymus that did not generate mRNA containing intron 5[7]. Hence, ovary and thymus would not neither produce the truncated protein of RNPEPL1 nor lose catalytic activity. Whether RNPEPL1 plays a role in the occurrence and progression of OV is still unknown either. With the progression of sequencing technique, bioinformatics can be utilized to understand and discover new biomarkers of cancer[12–14]. Therefore, we used GEO, TCGA and other bioinformatic database to explore the relation betweent RNPEPL1 and OV and the possible function of RNPEPL1 in the occurrence and progression of OV.

Materials And Methods

Data resource and Description

The sequencing data of RNPEPL1 were downloaded from three gene datasets (GSE18521, GSE40595, and GSE38666) containing OV tumor and normal ovary samples from the Gene Expression Omnibus (GEO). RNA-sequencing gene expression and clinical data of 375 OV patients from The Cancer Genome Atlas (TCGA) official website were retrieved on October 15, 2020.

Identification of RNPEPL1 expression

The expression values of RNPEPL1 were normalized using the quantile method of Robust Multichip Analysis (RMA). Expression values were rendered as log2-transformed values using the R affy package. Student's t-test was used to analyze the expression difference of RNPEPL1 by the limma package. In the Oncomine database, studies comparing RNPEPL1 between OV tumor and normal samples of ovary were set with a threshold of fold change ≥ 2, $P \leq 1E\text{-}4$ and top 10% gene rank. Protein expression of RNPEPL1 of OV tumor, normal ovary samples and normal lymph node detected by immunohistochemical method was acquired from the Human Protein Atlas (HPA) website.

Survival analysis

The clinicopathological characteristics of 375 OV patients relevant to the overall survival (OS) of OV patients were identified by the Cox regression analysis and Kaplan–Meier method. The cut-off point of RNPEPL1 expression value was the median value. Based on the median value, patients were divided into RNPEPL1-high and RNPEPL1-low level groups. Log-rank test was used to determine the difference between high and low level groups of RNPEPL1 by R package "survival". The association between RNPEPL1 expression and OS in different clinicopathological groups was also analyzed.

The correlations between clinicopathological characteristics and RNPEPL1 expression

Associations between RNPEPL1 expression and clinicopathological characteristics were analyzed by a non-parametric test (the Wilcoxon test was performed when the data were classified into two groups; the Kruskal–Wallis test was conducted when the data were classified into three or four groups).

GO and KEGG analysis by GSEA

We used GESA software (version 4.1.0) to conduct KEGG and GO analyses. According to the expression level of RNPEPL1, OV samples from TCGA were divided into 2 different groups. Permutation analysis (1,000 permutations) was used to identify the thresholds for significance. Metrics for ranking the key mRNAs were computed ground on Pearson's correlation coefficient. The minimum and maximum sizes of gene sets were set as 10 and 500. The results with a nominal $p < 0.05$ and an FDR value < 0.25 were identified as statistically significant.

The relevance between RNPEPL1 and infiltrating immune cells

Tumor Immune Estimation Resource (TIMER) was used to evaluate the relevance between RNPEPL1 and immune cells[15,16]. "Gene" module of TIMER was performed to analyze the association between enrichment of six main types of immune cells infiltrates and RNPEPL1 expression, including CD8+ T cells, CD4+ T cells, B cells, neutrophils, dendritic cells and macrophages in OV using tumor purity-corrected partial Spearman's correlation. The prognostic value of each immune cells was determined using Kaplan-Meier analysis.

Immune-related genes enrichment analysis

All immune-related genes (IRGs) from the ImmPort database were downloaded. Pearson correlation analysis was used to select the RNPEPL1-related IRGs. The IRGs that are significantly related to the expression of RNPEPL1 in TCGA dataset were remained and used as the candidates for the enrichment analyses. The most significantly enriched IRGs gene ontology terms and the corresponding genes were exhibited using a cross-link analysis.

Statistical analysis

Paired t test was used to analyze the expression difference by R software. In R software survival package (version 3.1–11), "coxph" method was used to conduct Multivariate Cox regression. RNPEPL1 expression in different clinicopathological groups were analyzed by a non-parametric test using GraphPad Prism software. Pearson correlation analysis and cross-link analysis were performed using R software. The scatter plots, box plots and survival plots were graphed using GraphPad Prism software (version 5.0). A p value less than 0.05 was considered as significantly different.

Results

RNPEPL1 expression comparison

The expression of RNPEPL1 in OV tumor samples was significantly higher than normal ovary samples in GSE18521, GSE40595, and GSE38666 (all $P < 0.0001$, Figure 2). In the Oncomine database, the meta-analysis of RNPEPL1 expression in 8 analyses showed that RNPEPL1 was overexpressed in OV tissues ($P = 4.49E-04$, Figure 3I). RNPEPL1 was significantly overexpressed in OV ($P = 3.75E-10$, Figure 3E), ovarian serous adenocarcinoma ($P = 0.002$, Figure 3A; $P = 0.041$, Figure 3H), ovarian mucinous adenocarcinoma ($P = 8.06E-05$, Figure 3D; $P = 9.77E-05$, Figure 3F), ovarian clear cell adenocarcinoma ($P = 8.18E-04$, Figure 3C; $P = 0.003$, Figure 3G) and ovarian endometrioid adenocarcinoma ($P = 0.004$, Figure 3B). Immunohistochemistry staining of RNPEPL1 was positive in OV samples (Figure 4) with a positive rate of 90.90%(10/11). Expression of RNPEPL1 was not detected in normal ovary tissues and normal lymph node (Figure 4).

Survival analysis

There were 375 OV patients included in the survival analysis. The main characteristics of them were summarized in Table 1. The median age of the diagnosed patients was 58 years. The results suggested that RNPEPL1 was significantly associated with the OS of OV (HR = 1.572, 95% CI =1.185-2.086; $P = 0.002$) in univariate Cox regression analysis. At the same time, several clinicopathological factors, including age (HR = 1.019, 95% CI = 1.006-1.032; $P = 0.005$), FIGO stage (HR = 1.425, 95% CI = 1.054-1.926; $P = 0.021$) and residual disease (HR = 1.229, 95% CI = 1.068-1.414; $P = 0.004$) were significantly correlated with OS of OV. In the multivariate Cox regression analysis, age (HR = 1.018, 95% CI = 1.004-1.033; $P = 0.011$), FIGO stage (HR = 1.412, 95% CI = 1.022-1.953; $P = 0.037$), residual disease (HR = 1.173, 95% CI = 1.013-1.359; $P = 0.033$) and RNPEPL1 (HR = 1.622, 95% CI = 1.207-2.181; $P = 0.001$) were significantly related to OS (Table 2). Overexpression of RNPEPL1 was an independent risk factor for OS of OV.

Table 1. The characteristics of patients in the TCGA-OV cohort

Characteristic	Total (375)	Percentage (%)
Age		
≤ 58	185	49.33%
> 58	190	50.67%
FIGO stage		
Stage I/II	21	5.60%
Stage III/IV	349	93.07%
unknown	5	1.33%
Grade		
G1	1	0.27%
G2	42	11.20%
G3	320	85.33%
unknown	12	3.20%
Residual disease		
No Macroscopic disease	67	17.87%
1-10 mm	171	45.60%
11-20 mm	26	6.93%
>20 mm	70	18.67%
unknown	41	10.93%
Venous invasion		
No	39	10.40%
Yes	62	16.53%
unknown	274	73.07%
Lymphatic invasion		
No	48	12.80%
Yes	100	26.67%
unknown	227	60.53%

TCGA: The Cancer Genome Atlas; OV: ovarian carcinoma.

Table 2. Univariate analysis and multivariate analysis of the correlation of RNPEPL1 expression and important clinical characteristics with survival among ovarian carcinoma patients

Parameter	Univariate analysis			Multivariate analysis		
Overall survival	HR	95%CI	P-value	HR	95%CI	P-value
Age	1.019	1.006-1.032	**0.005**	1.018	1.004-1.033	**0.011**
FIGO stage	1.425	1.054-1.926	**0.021**	1.412	1.022-1.953	**0.037**
Grade	1.391	0.913-2.120	0.125			
Residual disease	1.229	1.068-1.414	**0.004**	1.173	1.013-1.359	**0.033**
Venous invasion	0.803	0.424-1.518	0.499			
Lymphatic invasion	1.337	0.788-2.271	0.282			
RNPEPL1	1.572	1.185-2.086	**0.002**	1.622	1.207-2.181	**0.001**

HR: hazard ratio; CI: confidence interval. Bold values indicate p-value < 0.05.

Overexpression of RNPEPL1 in OV tumor tissues was significantly correlated with poor OS (P = 0.0016, Figure 5A) in OV patients. Besides, a subgroup analysis suggested that the overexpression of RNPEPL1 in OV tumor tissue was correlated with reduced 5 year (P = 0.0179, Figure 5B) and 10 years survival (P = 0.0068, Figure 5C).

In different clinicopathological groups, overexpression of RNPEPL1 was correlated with poor survival in patients with age ≤ 58 years (P = 0.0450, Figure 6A), while no significant difference was found in patients with age ▯ 58 years (P = 0.191, Figure 6B). Moreover, overexpression of RNPEPL1 significantly correlated with poorer OS in patients with late stage (P = 0.0009, Figure 6D) , high grade (P = 0.0032, Figure 6E) and ≥1mm residue disease (P = 0.0038, Figure 6H), but not determined as risk factors for OV patients with early stage (P = 0.3819, Figure 6C), low grade (P = 0.4486, Figure 6F) and no macroscopic disease(P = 0.1839, Figure 6G).

The correlations between RNPEPL1 expression and clinicopathological characteristics

The analysis of clinical data and gene expression data downloaded from TCGA-OV showed that the expression of RNPEPL1 in younger patients (≤ 58 years old) was significantly higher than older patients (> 58 years old) (P < 0.0001, Figure 7A). RNPEPL1 was highly expressed in early stage (P < 0.0001, Figure 7B) and low grade (P < 0.0001, Figure9C) patients. RNPEPL1 increased with venous invasion (P < 0.0001, Figure 7D) and lymphatic invasion(P < 0.0001, Figure 7E). RNPEPL1 was significantly distributed differently in different groups of residue disease and highly expressed in patients with 1mm-10mm and 10mm-20mm residue disease classification (P < 0.0001, Figure 7F).

GO and KEGG analyses by GSEA

The results of KEGG analysis showed that genes were mainly enriched RNA degradation, RNA polymerase, spliceosome, oxidative phosphorylation and ribosome when the expression level of RNPEPL1 is high, genes were mainly enriched in T cell receptor signaling pathway, B cell receptor signaling pathway, apoptosis, natural killer cell mediated cytotoxicity and cell adhesion molecules cams when the expression level of RNPEPL1 is low (Figure 8). The results of GO analysis suggested that genes were mainly enriched in spliceosomal snrnp assembly, U2 type spliceosomal complex, nuclear transcribed mRNA catabolic process, viral gene expression, and polysome when the expression level of RNPEPL1 is high, genes were mainly enriched in specific granule, positive regulation of NF kappab transcription factor activity, macrophage activation, actin cytoskeleton reorganization, negative regulation of toll like receptor signaling pathway when the expression level of RNPEPL1 is low (Figure 9).

Correlation between immune cells and RNPEPL1 expression

The analysis of TIMER database showed that RNPEPL1 expression levels correlated with B cells (r = 0.099, P = 3.08e-02), CD8+ T cells (r = 0.035, P =4.39e-01), CD4+ T cells (r = −0.002, P =9.37e-01), macrophage (r = -0.083, P =6.90e-02), neutrophils (r = -0.044, P =3.32e-01) and dendritic cells (r = -0.047, P =3.09e-01) (Figure 10A). The prognostic value of the six types of immune cells for OV was shown in Figure 10B, low level of dendritic cells (P = 0.039) can predict poor survival of OV (Figure 10B).

Immune-related genes enrichment analysis

There are 2483 immune-related genes (IRGs) extracted from the ImmPort website. A total of 249 IRGs are significantly related with the expression of RNPEPL1 in TCGA dataset (Table 3). The most significantly correlated IRGs gene ontology terms and the corresponding genes were exhibited using a cross-link analysis. The expression of RNPEPL1 was significantly correlated with the immune signaling pathway of antigen processing and presentation, antimicrobials, TCR signaling pathway, BCR signaling pathway and natural killer cell cytotoxicity (Figure 11).

Table 3. RNPEPL1-related immune genes

Description	Gene ID
Antigen_processing_and_presentation	CD4/CIITA/CD74/RELB/HLA-E/PSMC6/CD1C/HLA-DPA1/HLA-DPB1/HLA-DQA1/SEM1/PSMC1/ECPAS/CTSB/HLA
Antimicrobials	GRK2/AHNAK/TLR7/IRF5/CYBB/FURIN/PML/CD14/MAVS/IL1B/HCK/CCR5/PRDX2/TGFB1/UNC93B1/PTK2B/OL
BCR signaling pathway	INPP5D/CHP1/VAV2/PIK3CD/VAV1/PTPN6/FCGR2B/CARD11/RAC2/LYN/NFATC2/NFKBIE/NFATC1/PIK3R3/IKBK
Chemokine_receptors	PLXNB2/PTAFR/CX3CR1/PLXNA1/PLXND1/PLAUR/PLXNA3/C5AR1/PLXNB1/FPR1/CXCR3/ACKR1/TYMP/SEMA
Chemokines	TYMP/SEMA4D/SEMA4B/SEMA3B/C3/SBDS
Cytokine_receptors	CSF1R/IL10RA/IL17RA/CSF2RA/NPR1/C3AR1/SDC3/TNFRSF14/CSF2RB/PPARD/PPARA/TNFRSF1B/RARG/OGF
Cytokines	CSF1/TNFSF12/CMTM4/TNFSF13/TXLNA/LTBP3/NRTN/BMP1/EBI3/OSM/LRSAM1/PDGFB/VEGFB/NGF/ESM1
NaturalKiller_cell_cytotoxicity	ITGB2/ITGAL/LCP2/SH3BP2/FCGR3A/SHC1/PRF1/TYROBP/GRB2/FYN/PRKCA
TCR signaling pathway	PTPRC/CBLC/CD3E/CDK4/PRKCQ/TRAC

BCR: B cell receptor; TCR: T cell receptor.

Discussion

RNPEPL1 was significantly overexpressed in different histologic types of OV. RNPEPL1 may play a role in the progression of OV. Moreover, Immunohistochemistry staining results showed that RNPEPL1 was positive in OV samples but was not detected in normal ovary tissues and normal lymph node (Fig. 4). This is in accordance with the expression difference of RNPEPL1 at mRNA level. Furthermore, RNPEPL1 may be used as a new pathologic diagnostic index to distinguish normal ovary and OV. The negative expression of RNPEPL1 in normal lymph node may help doctors judge the lymph node metastasis of OV which is important in the definition of tumor stage.

RNPEPL1 was an independent prognostic biomarker in OV. Overexpression of RNPEPL1 can predict poor OS for OV. This also implies that RNPEPL1 may take part in the progression of OV. RNPEPL1 could be used as a prognostic biomarker in OV patients with age ≤ 58 years, late stage, high grade and ≥ 1mm residue disease. Usually, there is no obvious symptom of OV in the early stages. The occurrence of OV is hidden[17]. Most serous OV patients are diagnosed at stage III (51%) or IV (29%)[5], the prognostic value of RNPEPL1 in late stage patients is more meaningful due to the high proportion and low survival rate of late stage. Patients with less residue disease experience longer survival time[18, 19]. How to predict survival time for patients with residue disease is of significant importance due to the low survival rate of them. The high expression of RNPEPL1 in patients with venous invasion and lymphatic invasion reflects that RNPEPL1 may be correlated with the invasion of OV.

The results of GO and KEGG analysis implies that RNPEPL1 may take part in the process of gene transcription and tumor immunity in OV. Genes were mainly enriched in T cell receptor signaling pathway, B cell receptor signaling pathway and natural killer cell mediated cytotoxicity when the expression level of RNPEPL1 is low (Fig. 6B). It implies that overexpression of RNPEPL1 may inhibit both specific immunity and nonspecific immunity. And, that may explain the poor prognosis of OV patients with high expression level of RNPEPL1. RNPEPL1 belongs to the M1 family of zinc metallopeptidases. The family has functions of breakdown of peptides and recycle of amino acids from usual cellular proteolysis[20], adjustment of signaling cascades through hydrolyzing peptide hormones[21] and management of peptides exhibited on class I MHC molecules[22, 23]. RNPEPL1 may have a similar function of the processing of peptides exhibited on class I MHC molecules and plays a role in tumor immune escape of OV.

The CD4 + T cells, dendritic cells, neutrophils and macrophage all play a positive role in anti-tumor immunity process. RNPEPL1 was associated with all the six main infiltrating immune cells which indicate that RNPEPL1 is strongly immune-related, suggesting that RNPEPL1 may inhibit the process of anti-tumor immunity. Moreover, we found that when the amount of dendritic cells is high, patients will have longer survival time. Whereas, overexpression of RNPEPL1 was correlated with low amount of dendritic cells. RNPEPL1 may affect the progression of OV through inhibiting the process of anti-tumor immunity. Expression of RNPEPL1 was significantly correlated with the immune signaling pathway of antigen processing and presentation, antimicrobials, TCR signaling pathway, BCR signaling pathway and natural killer cell cytotoxicity (Fig. 11). The results further proved the immune-related function of RNPEPL1. The result further indicates that RNPEPL1 may affect the progression of OV through inhibiting the process of anti-tumor immunity.

Under normal circumstances, tumor cells could be recognized by the immune system based on tumor-associated antigens and eliminated[24]. One of the features of cancer is that tumor cells get the ability of avoiding attacks from the immune system[25]. Tumor immune escape is indispensable for the development of tumor. There are also some successful cases of cancer immunotherapy by activating immune system[26]. Tumor with high tumor mutation burden is more sensitive to immunotherapy[27, 28] The accumulation of mutation induced the expression of tumor-specific antigen. Antigen processing and presentation is one of the most important steps of anti-tumor immunity. Our results suggested that the expression of RNPEPL1 is significantly correlated with antigen processing and presentation. RNPEPL1 may affect the progression of OV through affecting antigen processing and presentation. The specific antigen of tumor will bind with MHC1 molecules on the surface of tumor cell and be recognized by CD8 + lymphocyte to trigger anti-tumor immune response[29]. Although there is no report about the impact of RNPEPL1 on MHC1 molecules, Members of this family have been implicated in the processing of peptides exhibited on class I MHC molecules[22, 23]. RNPEPL1 may have similar function of processing of peptides exhibited on class I MHC molecules, play a role in tumor immune escape of OV, promote the progression and affect survival of OV patients.

There are also some limitations in our research. The data of this study was retrospective data. The clinical value of RNPEPL1 needs to be verified by more prospective data. Experimental data is lacking in this study to explain the correlation between RNPEPL1 and the possible mechanism in OV. More research is required to illuminate the potential value of RNPEPL1 in the diagnosis and treatment of OV.

Conclusions

RNPEPL1 is a novel gene correlated with the prognosis of OV. It has rarely been reported before and even never been reported in cancer. We first explore the expression and possible function of RNPEPL1 in OV. RNPEPL1 is a new diagnostic and prognostic biomarker of OV. Further experiment on the mechanism of promoting OV progression and whether it could inhibit anti-tumor immunity through regulating immunity is needed. If the antigen processing and presentation of OV could be promoted through inhibiting RNPEPL1, RNPEPL1 may also be a potential immunotherapy target for OV.

Declarations

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Availability of data and materials

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Authors' contributions

JMC contributed to the research design and manuscript writing. JCC performed statistical analysis and drafted the manuscript. YZP provided experimental guidance. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

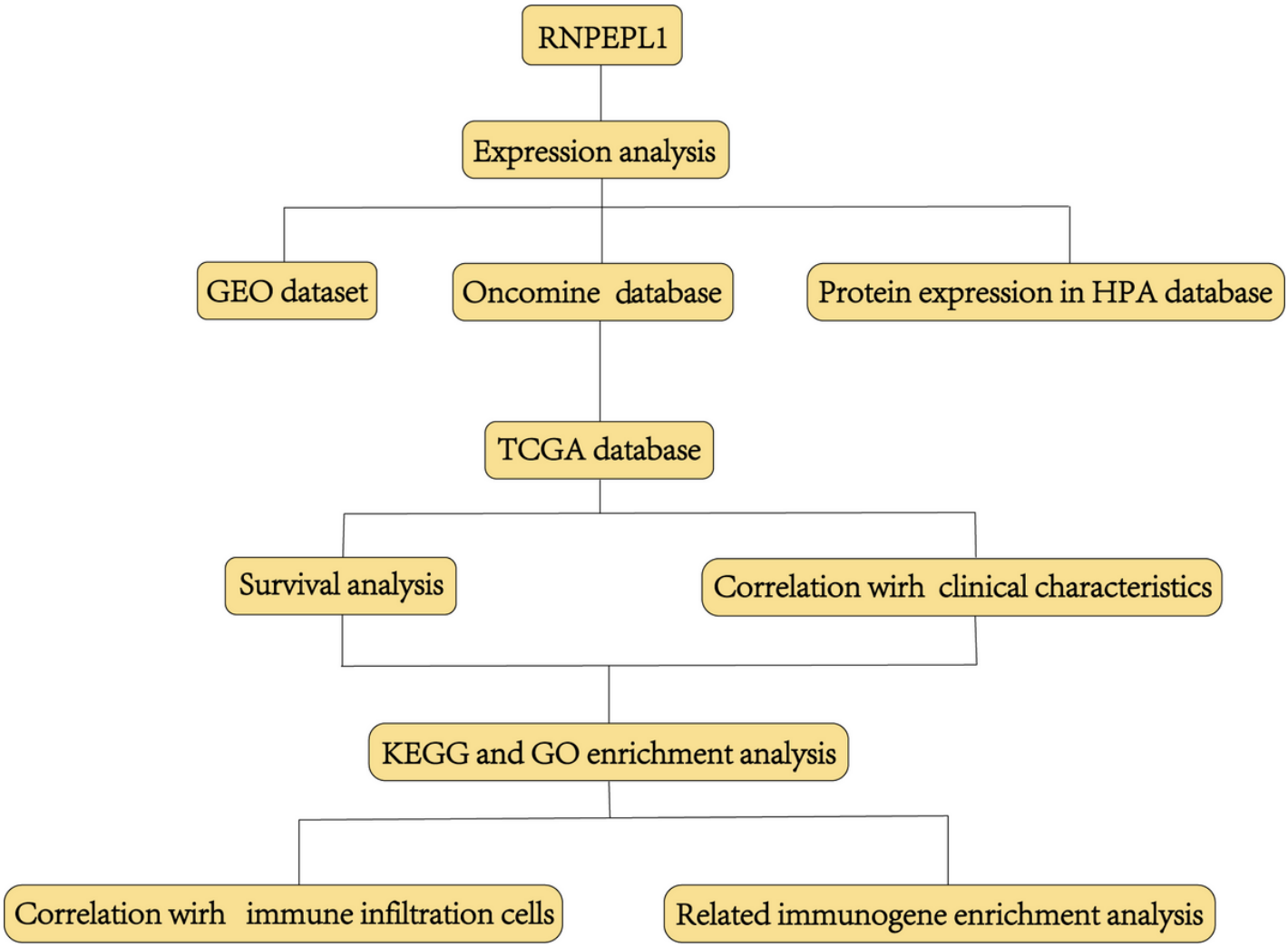


Figure 1

Flow chart of study design

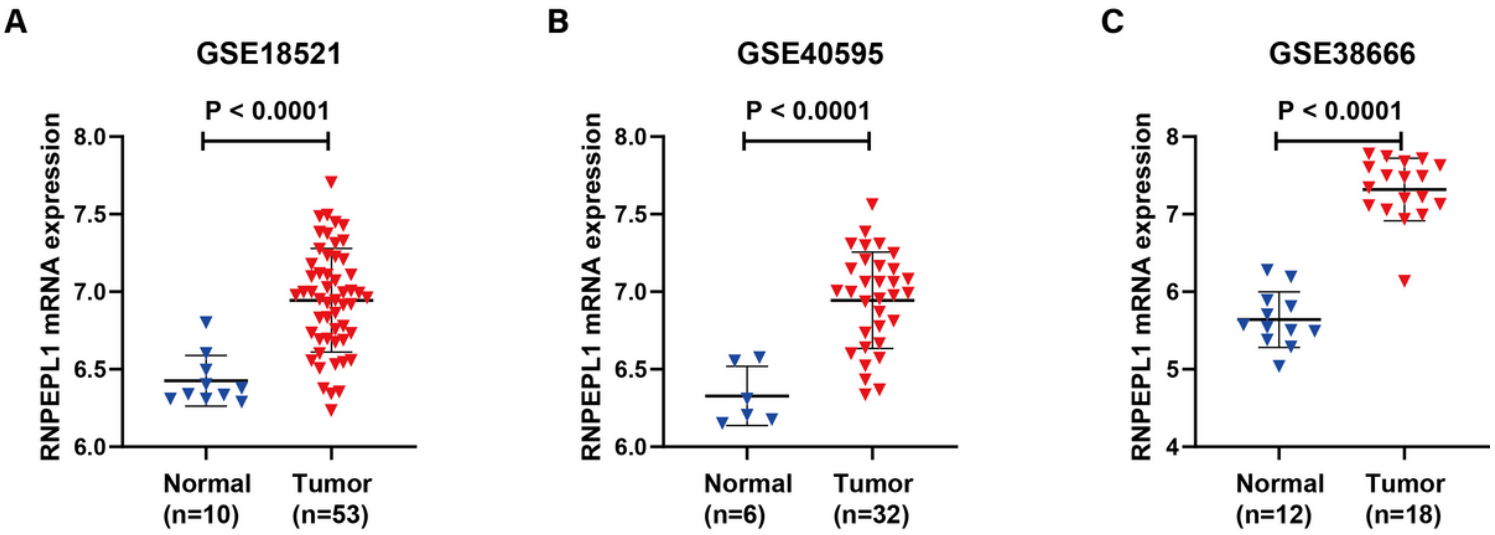
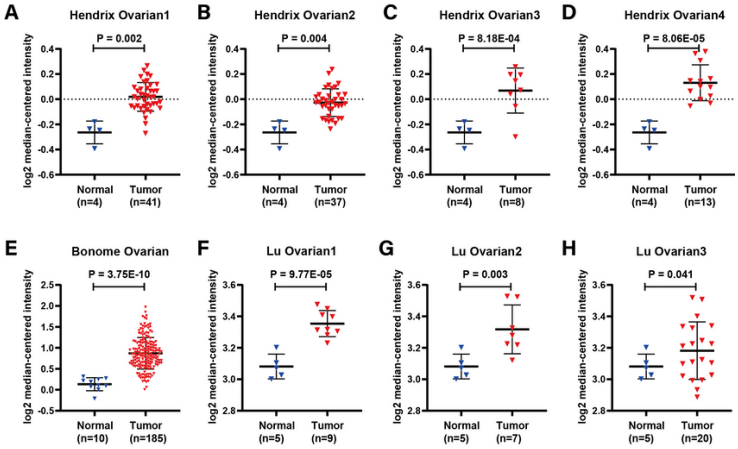


Figure 2

mRNA expression levels of RNPEPL1 between tumor and normal samples in OV patients on the GEO database series including GSE18521 (A), GSE40595 (B),GSE38666 (C). OV, Ovarian carcinoma.



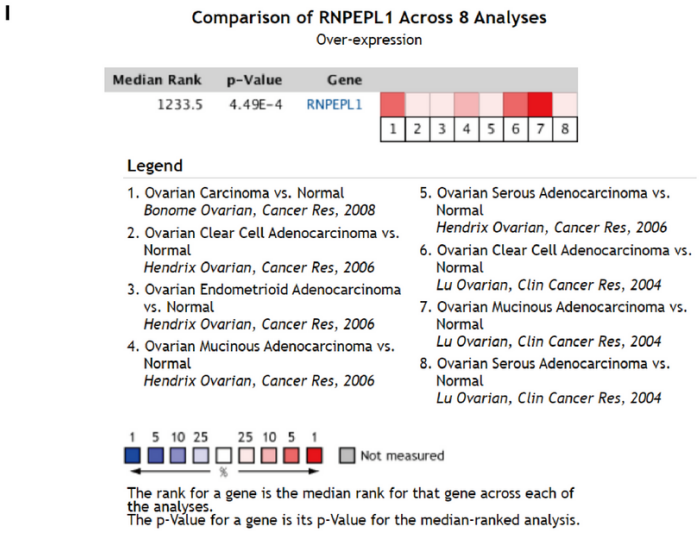


Figure 3

Comparison of RNPEPL1 mRNA expression levels across 8 analyses in the Oncomine database. A, Hendrix ovarian 1: ovarian serous adenocarcinoma, B, Hendrix ovarian 2: ovarian endometrioid adenocarcinoma, C, Hendrix ovarian 3: ovarian clear cell adenocarcinoma, D, Hendrix ovarian 4: ovarian mucinous adenocarcinoma, E, Bonome ovarian: ovarian carcinoma, F, Lu ovarian 1: ovarian mucinous adenocarcinoma, G, Lu ovarian 2: ovarian clear cell adenocarcinoma, H, Lu ovarian 3: ovarian serous adenocarcinoma, I, Meta-analysis of PRKACB expression in 8 analyses.

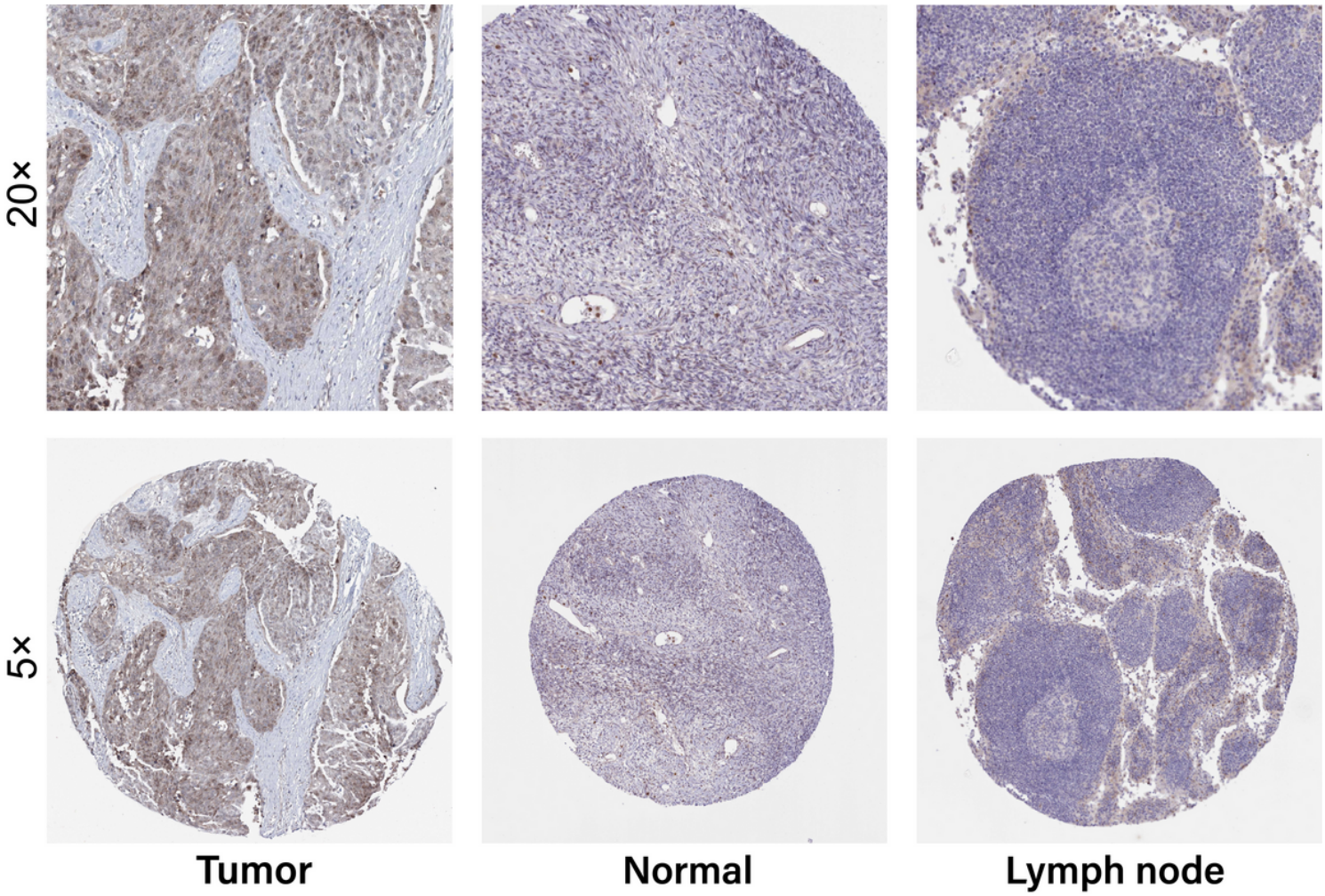


Figure 4

Gene expression of RNPEPL1 was assessed using immunohistochemistry in normal ovary, OV tissues and lymph node. Protein levels of Protein levels of RNPEPL1 in tumor tissue (staining: Medium; intensity: Moderate; quantity: > 75%; Location: Nuclear). RNPEPL1 in normal tissue (staining: Not detected; intensity: Negative; quantity: None; Location: None). RNPEPL1 in lymph node (staining: Not detected; intensity: Negative; quantity: None; Location: None). OV, Ovarian carcinoma.

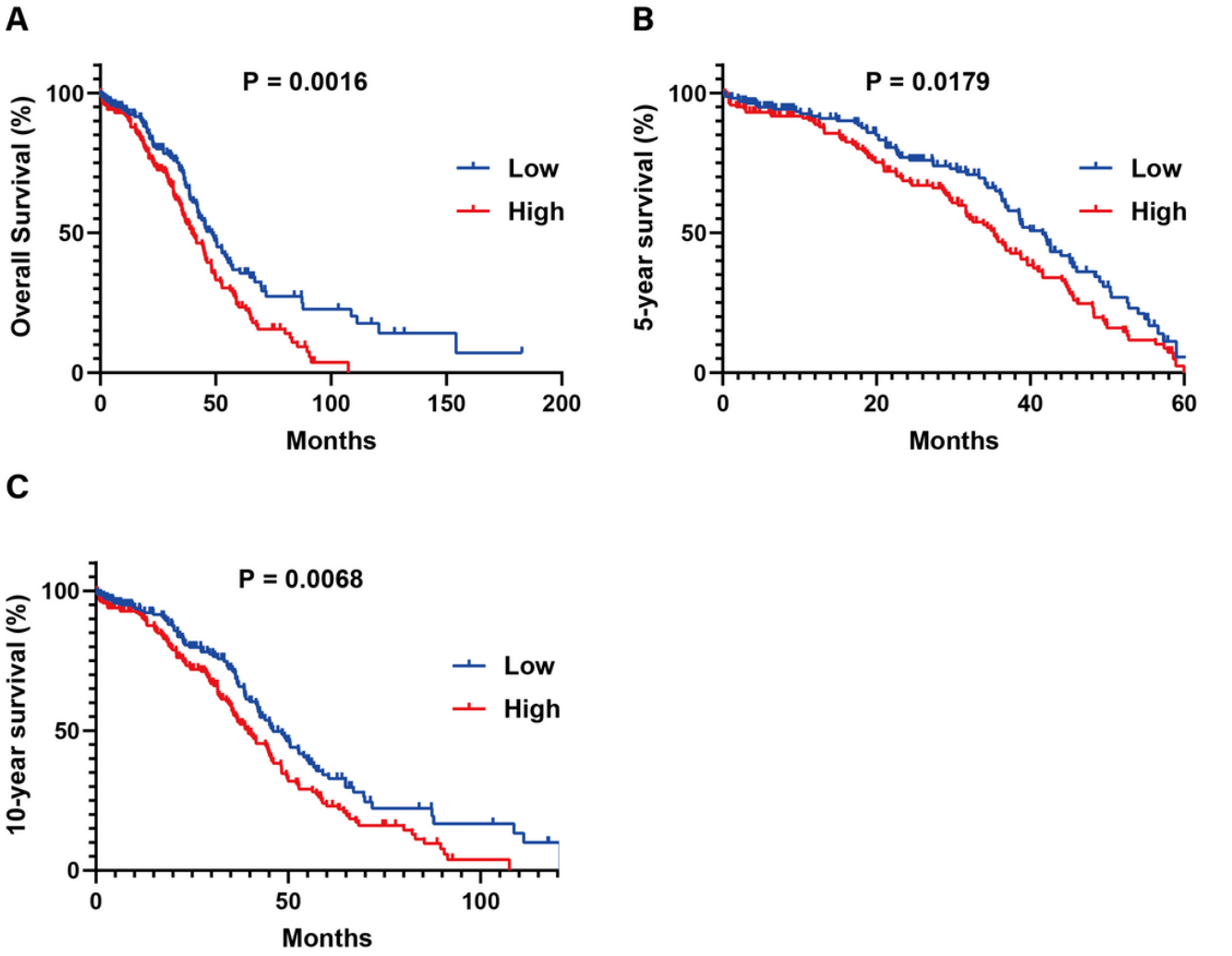


Figure 5

Overall survival of OV patients grouped by RNPEPL1 median cutoff in TCGA database; overall (A), 5-year (B) and 10-year(C) survival comparison between high and low RNPEPL1 groups. OV, Ovarian carcinoma.

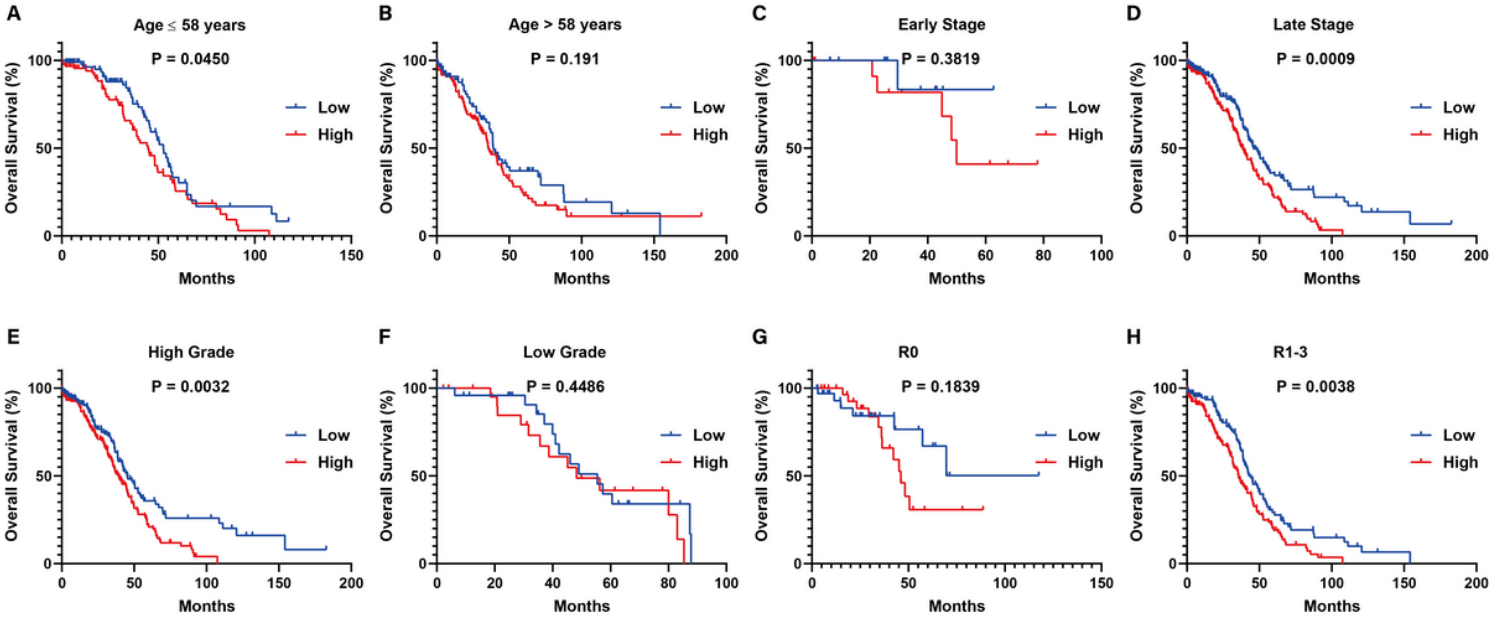


Figure 6

Subgroup analyses of overall survival comparison in different populations, age (A, B), residue disease (C, D), FIGO stage (E, F), tumor grade (G, H), venous invasion (I, J) and lymphatic invasion (K, L) with RNPEPL1 median cutoffs in OV patients. OV, Ovarian carcinoma.

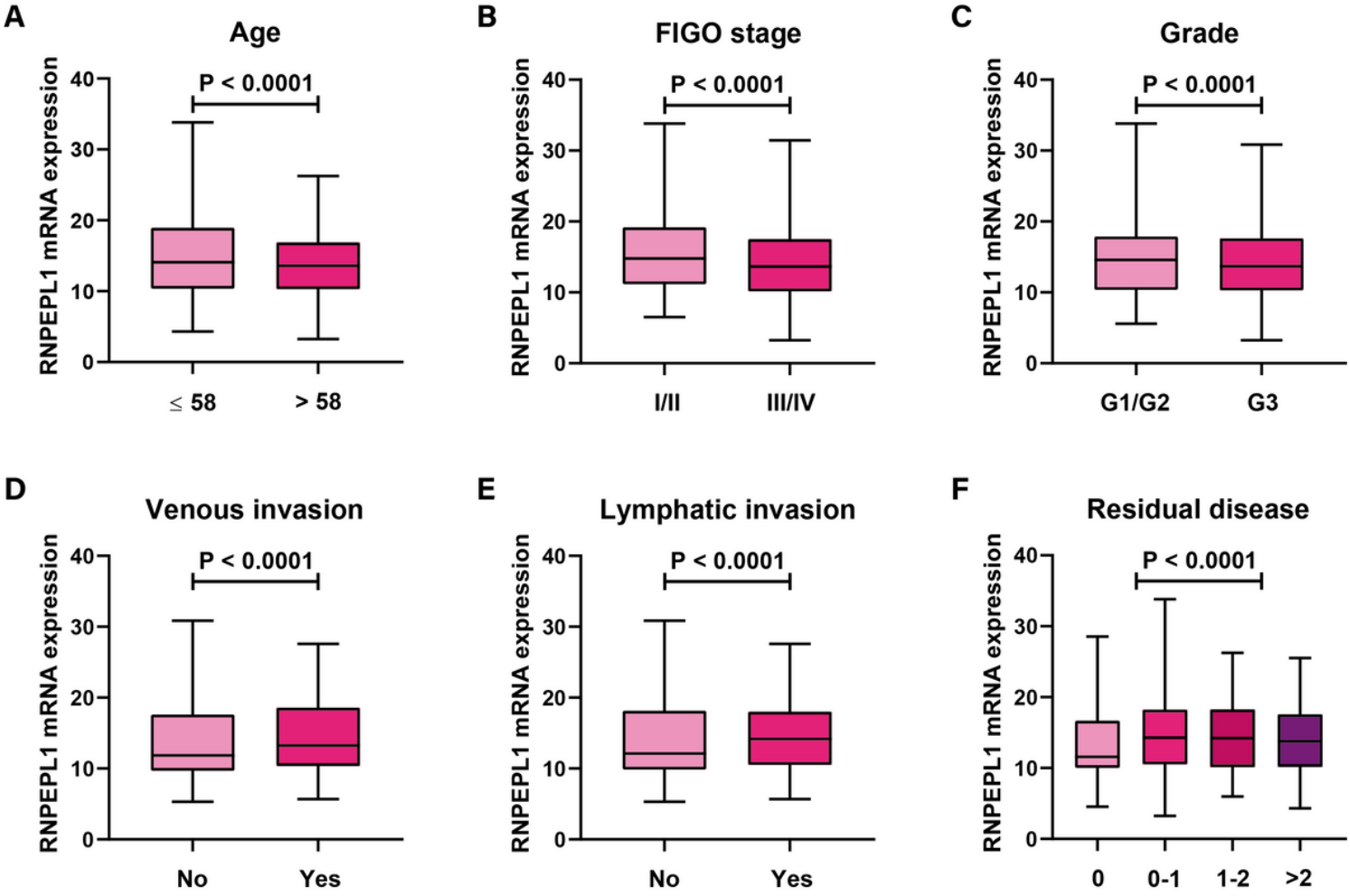


Figure 7

RNPEPL1 expression in sub-groups of OV clinical characteristics. (A, B, C, D, E) RNPEPL1 expression distribution analyses stratified based on age, FIGO stage, tumor grade venous invasion and lymphatic invasion (Wilcoxon test). (F) RRM2 expression distribution analyses stratified based on residue disease (Kruskal-Wallis test). P-value < 0.05 was used to assess differences. OV, Ovarian carcinoma.

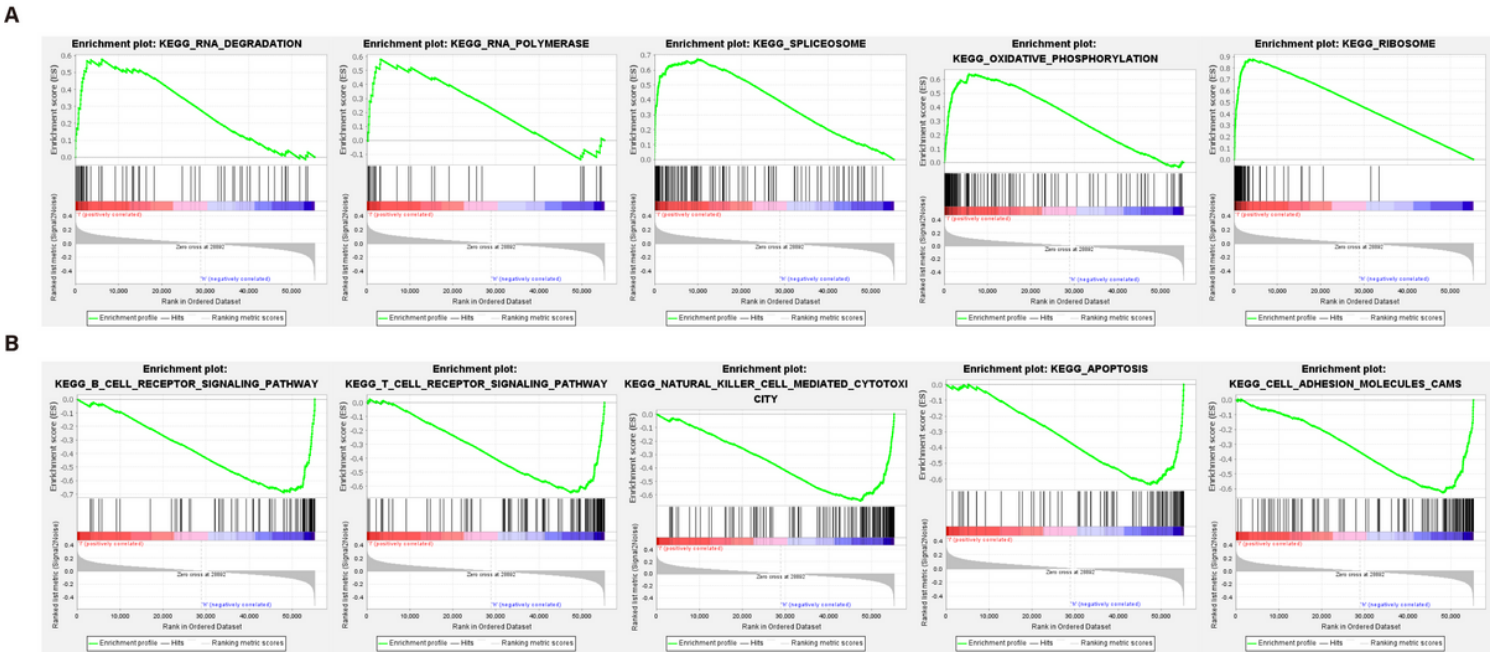


Figure 8

KEGG biological function enrichment analyses of RNPEPL1 related genes from GSEA. (A)The five most functional gene sets enriched in OV samples with RNPEPL1 highly expressed, (B)The five most functional gene sets enriched in OV samples with low expression of RNPEPL1. OV, Ovarian carcinoma; GSEA, Gene Set Enrichment Analysis

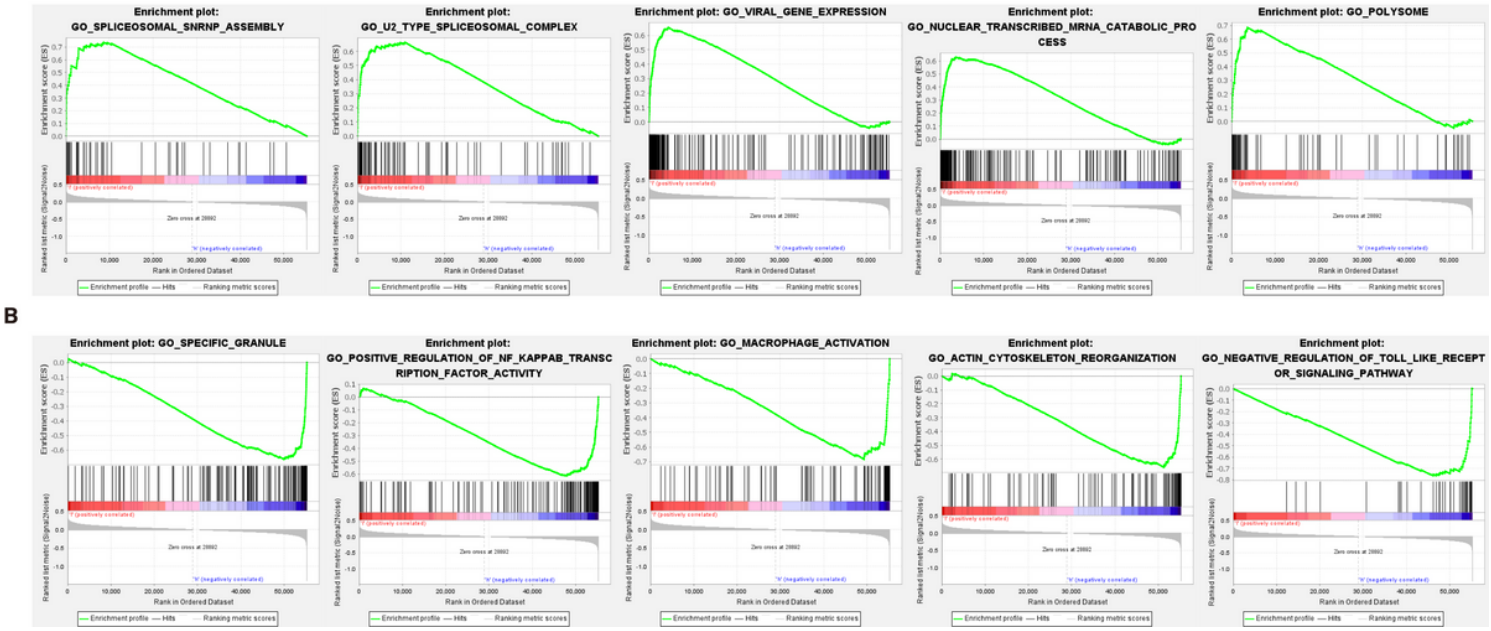
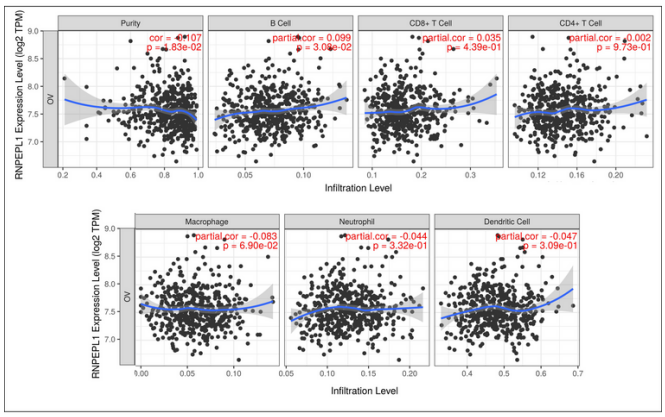


Figure 9

GO biological function enrichment analyses of RNPEPL1 related genes from GSEA. (A)The five most functional gene sets enriched in OV samples with RNPEPL1 highly expressed, (B)The five most functional gene sets enriched in OV samples with low expression of RNPEPL1.OV, Ovarian carcinoma; GSEA, Gene Set Enrichment Analysis

A



B

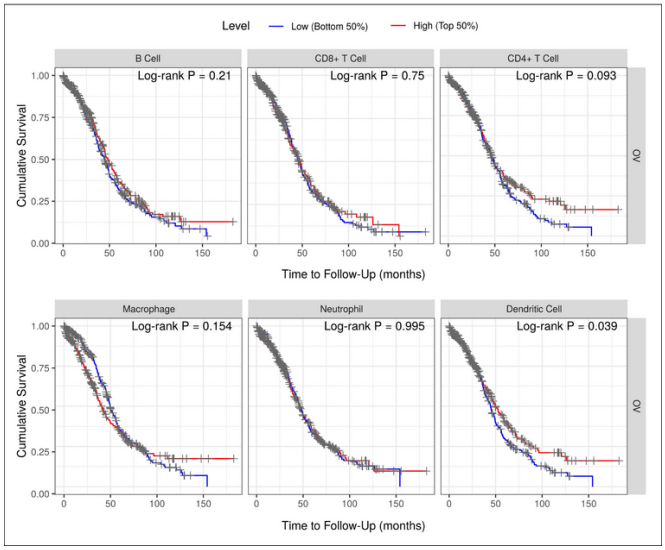


Figure 10

Correlation analysis between RNPEPL1 expression and six types of infiltrating immune cells in OV. (A) Correlation of RNPEPL1 expression with six types of immune infiltration cells obtained from TIMER (purity-corrected Spearman test). (B) Overall survival curve of each of the six types of immune cells produced by Kaplan-Meier estimator from TIMER. Survival differences are compared between patients with high and low (grouped according to median) infiltrating of each kind of immune cells; OV, Ovarian carcinoma; TIMER: The Tumor Immune Estimation Resource

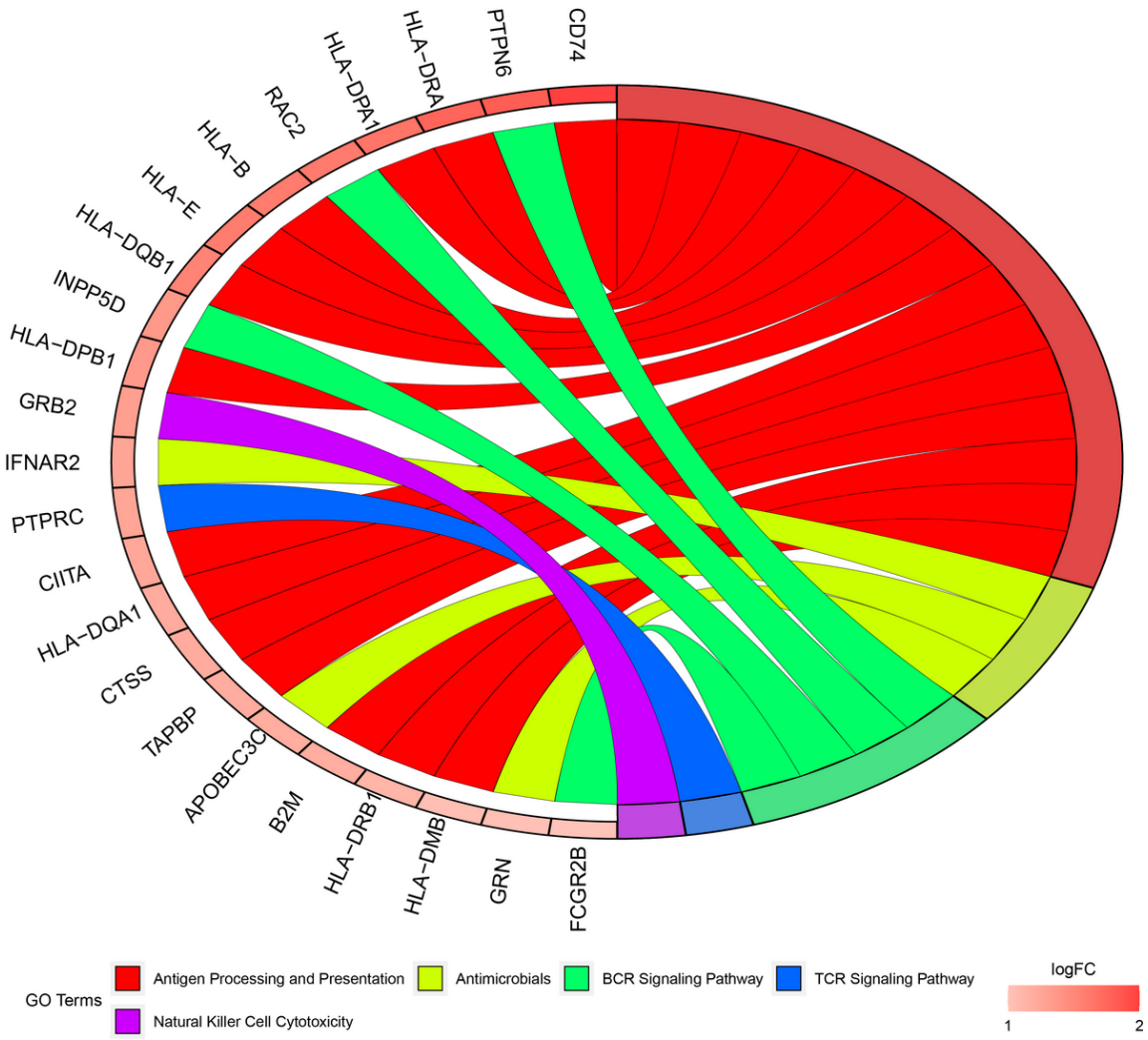


Figure 11

RNPEPL1-related immunogene enrichment analysis. The left side is the gene (the shade of the colour represents the gene's fold change), and the right side is the different gene terms. Connected bands indicate that a gene is in its corresponding gene ontology terms.