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The Potential Value of RPS27A in Prognosis and Immunotherapy: From Pan-Cancer Analysis to Hepatocellular Carcinoma Validation

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Purpose: Elucidation of the potential value of ribosomal protein S27a (RPS27A) for prognosis and immunotherapy in pan-cancer analysis, and exploration of the oncogenic function of RPS27A on hepatocellular carcinoma (HCC) and macrophage polarization.

Methods: A systematic analysis of the function and mechanism of RPS27A was conducted with R software and multiple public platforms, including UALCAN, HPA, TISIDB, TIMER, cBioPortal, cancerSEA, TIDE, and TIMSO databases. The RPS27A expression in human and mouse liver was detected by immunohistochemistry. The biological behavior of HCC cells was detected in vitro after RPS27A overexpression. The influence of RPS27A on macrophage polarization was detected by the coculturing assay.

Results: RPS27A dysregulation was found in multiple cancer types, and RPS27A level was associated with clinicopathologic features and prognosis in human cancers. RPS27A affected cancer statuses and multiple signaling pathways, such as DNA repair, invasion, IL10 synthesis, and MAPK activation. RPS27A took part in regulations of genomic alterations and heterogeneity and was associated with tumor mutation burden, microsatellite instability, neoantigen and so on. RPS27A expression was connected to the immune subtypes, tumor purity and immune cell infiltration and participated in regulation of the immunotherapy response. RPS27A was upregulated in HCC tissues compared to normal liver tissues. RPS27A overexpression in HCC cells promoted the proliferation, migration, and invasion of cancer cells, and accelerated M2 polarization of macrophage.

Conclusion: RPS27A had the potential to be a biomarker for diagnosis, prognosis and immunotherapy response in pan-cancer, and targeting RPS27A may provide new ideas for cancer immunotherapy.

Keywords: RPS27A, prognosis, immunotherapy, hepatocellular carcinoma, macrophage polarization

Introduction

The ubiquitin-proteasome system is the main protein degradation pathway in eukaryotic cells, which is composed of ubiquitin (Ub), ubiquitin activating-enzyme (E1), ubiquitin-binding enzyme (E2), ubiquitin ligase (E3), 26S proteasome, and deubiquitin enzyme.[1] Ub could covalently bind to the amino acid residues of the target protein under the cascade catalysis of E1/E2/E3 so that Ub labels the target protein and thus is recognized and degraded by the 26S proteasome.[2] Ubiquitylation regulates multiple cellular processes, such as cell proliferation, DNA damage and repair, and stress response.[3,4] There is increasing evidence confirming that ubiquitination is involved in tumor progression through various biological processes.

Ribosomal protein S27a (RPS27A) is a multifunctional ribosomal protein involved in ribosome assembly and protein synthesis.[5] In addition, Ub and RPS27A are co-expressed as fusion proteins, but they function independently after Ub is isolated from RPS27A by deubiquitinating enzymes.[6] Therefore, RPS27A also participates in protein post-translational modification independently through regulating ubiquitylation. The essential role of RPS27A in the genesis

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and development of tumors has been confirmed in multiple cancer types. RPS27A is overexpressed in colon adenocarcinoma (COAD) cell lines and tissues, and RPS27A overexpression in COAD cells induces proliferation promotion and apoptosis inhibition.[7,8] RPS27A is upregulated in the bone marrow of chronic myeloid leukemia (CML) patients, and RPS27A knockdown induces cell cycle arrest and apoptosis.[9,10] RPS27A enhances the proliferation and invasion of renal cancer cells by inhibiting Epstein–Barr virus (EBV)-encoded latent membrane protein 1 degradation.[11] However, the prognostic and therapeutic role of RPS27A in pan-cancer is rarely explored systematically.

This study comprehensively analyzed the expression and prognostic value of RPS27A in human cancers and probed into the biological functions and mechanism of RPS27A in pan-cancer. The relationships between RPS27A level and tumor-infiltrating immune cells and immunotherapy response were also investigated. Finally, we validated the RPS27A function in hepatocellular carcinoma (HCC) cells and explored the influence of RPS27A on macrophage polarization. This study might provide a novel basis for cancer diagnosis, prognosis, and therapy.

Material and Methods

RPS27A Expression in Different Cancer Tissues

The standardized pan-cancer dataset (TCGA Pan-Cancer) was downloaded from the UCSC database (https://xenabrowser.net/ (accessed on 2 July 2024)). RPS27A gene data in each sample was extracted and transformed in log2(x+0.001). The cancer species less than 3 samples were eliminated, and the expression data of 26 cancers was finally demonstrated. RPS27A protein levels in pan-cancer were explored through the UALCAN data analysis portal (https://ualcan.path.uab.edu/ (accessed on 4 July 2024)). The RPS27A protein expression in different cancer and normal tissues through immunohistochemistry was obtained from the Human Protein Atlas database (https://www.proteinatlas.org/ (accessed on 4 July 2024)).

Clinical Value Analysis

The RNAseq and clinical data of different tumors were downloaded from the TCGA database (https://portal.gdc.cancer.gov (accessed on 2 July 2024)). The R packages "stats" and "car" were used to detect the relationship between RPS27A and the pathologic stage through the Kruskal–Wallis test. The "survival" package was utilized to construct the proportional risk hypothesis and performed fitted survival regression, and the surv_cutpoint function in the "survminer" package was performed to screen the optimal cut-off value. The results were visualized through the "ggplot" package. We established a Cox proportional hazards regression model using the Coxph function of the "survival" package to analyze the relationship between RPS27A gene expression and RPS27A and disease-free interval (DFI), disease-specific survival (DSS), and progression-free interval (PFI) in each tumor, and the Log rank test was performed to obtain prognostic significance.

Functional Analysis of RPS27A

The average correlation between the RPS27A gene and functional states in different cancers was detected via the cancerSEA database (http://biocc.hrbmu.edu.cn/CancerSEA/ (accessed on 9 July 2024)), including angiogenesis, apoptosis, cell cycle, differentiation, DNA damage, DNA repair, epithelial-mesenchymal transition (EMT), hypoxia, inflammation, invasion, metastasis, proliferation, quiescence, and stemness.

Gene Set Enrichment Analysis (GSEA) of REP27A

The RNAseq data of tumor samples were downloaded from the TCGA database and then were divided into high/low expression groups according to the median of RPS27A level. The "DESeq2" package was used to analyze the differential expressed genes (DEGs) between high- and low-RPS27A groups. The GSEA was performed by the "clusterProfiler" package according to the DEGs.

Genomic Heterogeneity Analysis

The cBioPortal database (https://www.cbioportal.org/ (accessed on 9 July 2024)) was utilized to detect the RPS27A mutations in the "TCGA PanCancer Atlas Studies". Based on the standardized pan-cancer dataset, the "maftools" package was performed to calculate the tumor mutation burden (TMB) and mutant-allele tumor heterogeneity (MATH) of each sample, and the microsatellite instability (MSI), ploidy, neoantigen (NEO), homologous recombination deficiency (HRD), and loss of heterozygosity (LOH) scores of each cancer were obtained from the previous study.[12] The connections of RPS27A to these scores were calculated by the Pearson test.

Immunogenomic Analyses

Based on the TCGA Pan-Cancer, TCGA-LAML, and TCGA-SKCM-Metastasis, the R software package "ESTIMATE" was used to calculate stromal, immune, and ESTIMATE scores for each patient in each tumor, and the Pearson analysis was conducted to verify the association between RPS27A expression and these three scores. The relationship between RPS27A and immune subtypes of different cancers was explored in the TISIDB portal (http://cis.hku.hk/TISIDB/ (accessed on 11 July 2024)). The "Timer" method in the R package "IOBR" was used to calculate the infiltration scores of B cell, $CD4^+$ T cell, $CD8^+$ T cell, Neutrophil, Macrophage, and DC. The correlation between RPS27A and infiltration score was analyzed using the Pearson method. The gene expressions of RPS27A, CD68, and CFS1R in breast cancer tissue sections by spatial transcriptomics were explored through the SpatialDB database (https://www.spatialomics.org/SpatialDB/ (accessed on 15 July 2024)).

Immunotherapy Response Analysis

The comparison of RPS27A and several well-known biomarkers for immunotherapy response was conducted in the TIDE database (http://tide.dfci.harvard.edu/ (accessed on 19 July 2024)), as well as the connection between RPS27A and T cell dysfunction, immune checkpoint blockade (ICB) and immune cells. The RPS27A expression in tumor models with different ICB responses was detected via the TIMSO database (http://tismo.cistrome.org/ (accessed on 19 July 2024)).

Experimental Methods

The experimental methods are shown in Supplementary Methods.

Results

Differential RPS27A Expressions in Human Cancerous and Normal Tissues

According to the RNAseq of the TCGA database, the RPS27A gene was upregulated in 15 cancer types compared to normal tissues while downregulated in 7 tumors (Figure 1A). Subsequently, we compared the RPS27A protein levels between tumors and normal tissues through the UALCAN database. The results demonstrated that RPS27A protein was overexpressed in ovarian cancer (OV), clear cell renal cell carcinoma (RCC), lung cancer, and liver cancer, but downregulated in breast cancer, uterine corpus endometrial carcinoma (UCEC), pancreatic adenocarcinoma (PAAD), head and neck squamous cell carcinoma (HNSC), and glioblastoma (Figure 1B). RPS27A protein was also explored using the Human Protein Atlas database. As shown in Figure 1C, RPS27A was localized to the nucleoli, endoplasmic reticulum, and cytosol, and a relatively strong positive staining was present in some samples of lung adenocarcinoma (LUAD), stomach adenocarcinoma (STAD), COAD, liver hepatocellular carcinoma (LIHC) and OV compared to corresponding normal tissues, which was similar to the results above. The receiver operating characteristic (ROC) curve confirmed that RPS27A had a potential value in cancer diagnosis, with the area under the curve greater than 0.7 in 12 cancers (Figure S1). These results suggested that RPS27A was dysregulated in most cancer types and might be a novel marker for cancer diagnosis.

Clinical Significance and Prognostic Value of RPS27A in Pan-Cancer

Different molecular subtypes showed different RPS27A expressions in adrenocortical carcinoma (ACC), BRCA, HNSC, kidney renal papillary cell carcinoma (KIRP), brain lower grade glioma (LGG), lung squamous cell carcinoma (LUSC),

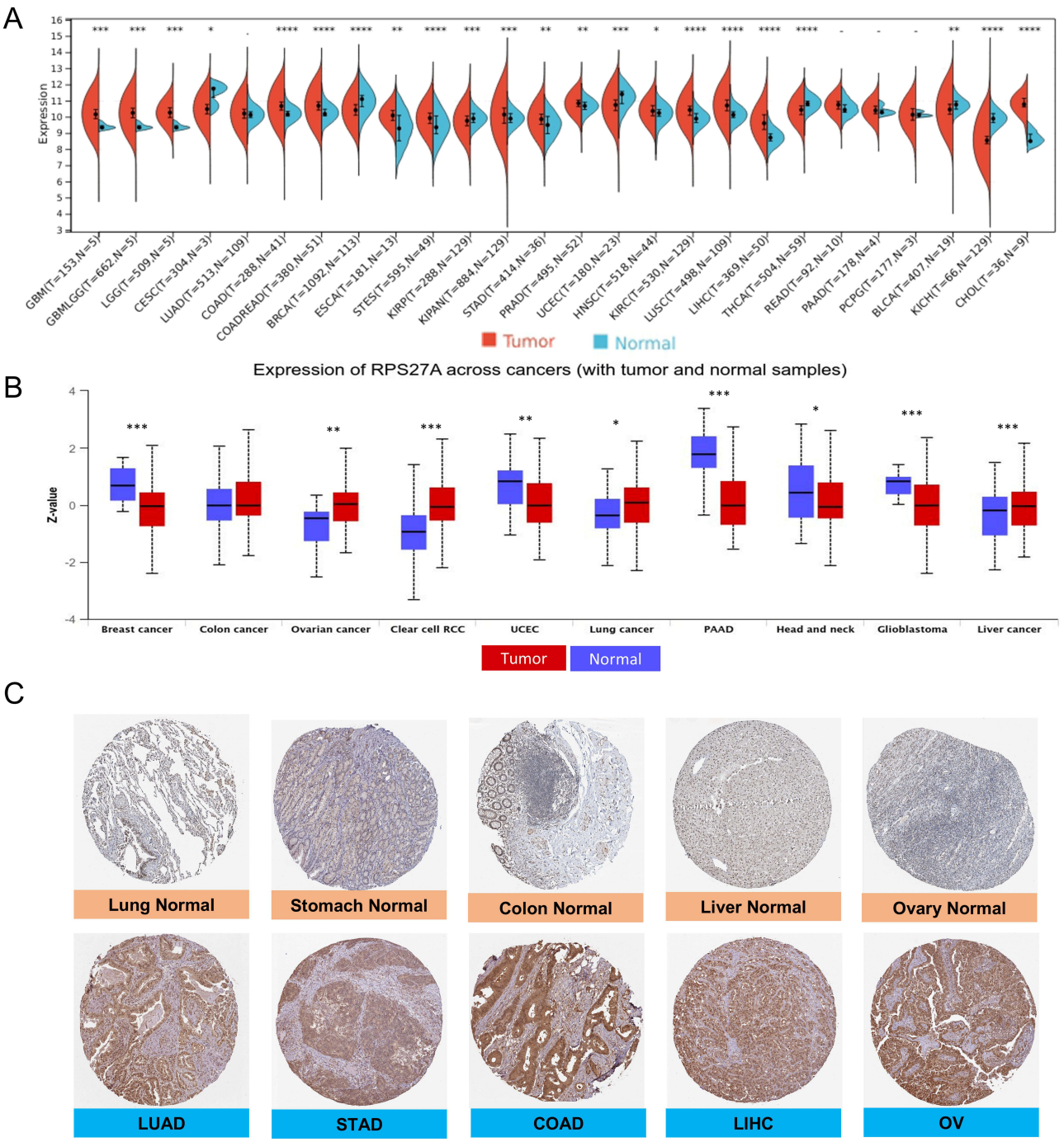

Figure 1 RPS27A expression in pan-cancer. (**A**) RPS27A mRNA expression in human cancer tissues and normal tissues by the TCGA database. (**B**) RPS27A protein expression in human cancer cell lines by the UALCAN database. (**C**) Characteristic images of HOXB7 protein expression in normal and tumor tissues. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

OV, pheochromocytoma, and paraganglioma (PCPG), SKCM, and UCEC (Figure S2). RPS27A also had something to do with the clinical features of cancer patients. For example, high RPS27A level was connected to the advanced pathological stages in LIHC, PCPG, HNSC, testicular germ cell tumor (TGCT), oral squamous cell carcinoma (OSCC), LUAD, and KIRP (Figure 2A).

Cox regression analysis was performed to evaluate the prognostic value of RPS27A in human cancers. As shown in Figure 2B, the patients with high RPS27A levels had worse overall survival (OS) than those with low RPS27A

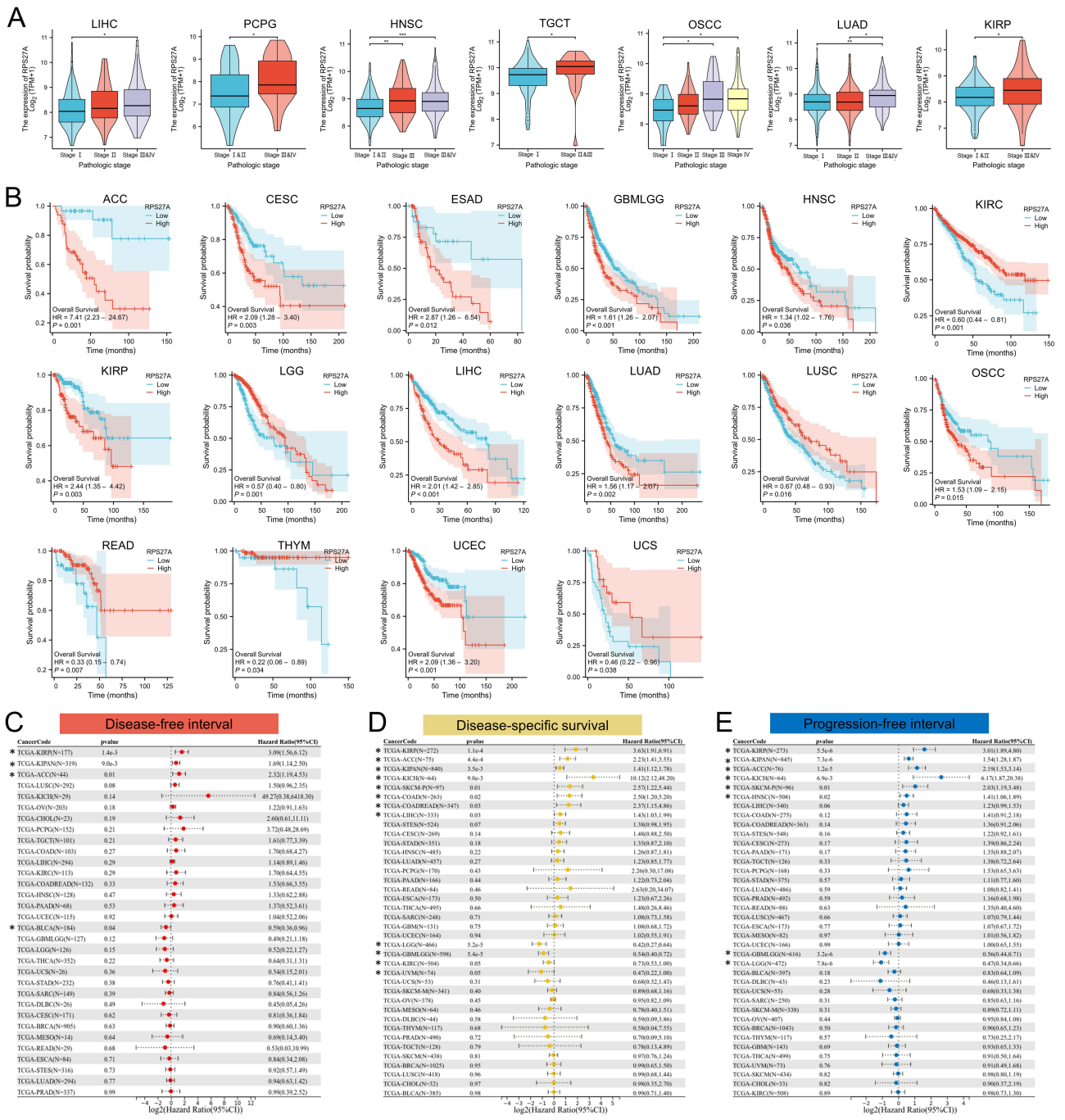

Figure 2 RPS27A value in clinical features and prognosis. (**A**) The association between RPS27A and pathologic stage. (**B**) The overall survival curves of human cancers with different RPS27A expressions. (**C**–**E**) Forest plots were used for pan-cancer analyses of RPS27A and DFI (**C**), DSS (**D**), and PFI (**E**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

expression in ACC, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), esophagus adenocarcinoma (ESAD), Glioma (GBMLGG), HNSC, KIRP, LIHC, LUAD, OSCC, and uterine corpus endometrial carcinoma (UCEC). However, high RPS27A groups in kidney renal clear cell carcinoma (KIRC), LGG, LUSC, rectum adenocarcinoma (READ), thymoma (THYM) and uterine carcinosarcoma (UCS) had better OS than low RPS27A groups (Figure 2B). We also explore the relationship between DFI, DSS, and PFI to eliminate the bias induced by non-tumor factors. In DFI, RPS27A was a protective role for bladder urothelial carcinoma (BLCA), and a risk factor for KIRP, pan-kidney cohort (KIPAN) and ACC (Figure 2C). In DSS, RPS27A was a risk factor for patients with KIRP, ACC, KIPAN,

kidney chromophobe (KICH), primary skin cutaneous melanoma (SKCM-P), COAD, and COADREAD, but the good news for patients with LGG, GBMNLGG, KIRC, and UVM (Figure 2D). Regarding the PFI, RPS27A seemed protective for GBMLEE and LGG, and harmful for KIRP, KIPAN, ACC, KICH, SKCM-P, and HNSC (Figure 2E). These results suggested that RPS27A might regulate the tumor progression and patients' prognosis in multiple cancer types.

Associations Between RPS27A Level and Functional States of Tumors

Now that RPS27A expression had affected the prognosis of multiple cancers, we wondered if RPS27A regulated the tumor cell function to impact cancer progression. The cancerSEA database was utilized to explore the relationship between RPS27A and functional states of cancer cells at the single-cell level. RPS27A was positively associated with DNA damage and DNA repair and negatively connected to differentiation and stemness (Figure 3A). In addition, RPS27A played a different role in different cancers for EMT, invasion, metastasis and quiescence (Figure 3A). Specifically, RPS27A had a positive relationship with DNA repair in BRCA, high-grade glioma (HGG), prostate cancer (PC), colorectal cancer (CRC), RCC, and acute myelocytic leukemia (AML), with invasion in BRCA, LUAD, and AML, with DNA damage in MEL, with metastasis, quiescence, hypoxia, and EMT in LUAD, and with inflammation in RCC (Figure 3B–3M). Meanwhile, RPS27A was negatively related to hypoxia in HGG, HNSC, and CRC, to metastasis in HNSC and MEL, to EMT in PC, to inflammation in CRC, to cell cycle and invasion in retinoblastoma (RB), to hypoxia, stemness, and angiogenesis in RCC, to metastasis, hypoxia, invasion, cell cycle, and apoptosis in Uveal melanoma (UM), to metastasis, angiogenesis, inflammation, and EMT in non-small cell lung cancer (NSCLC), and to quiescence, apoptosis, hypoxia, differentiation, and inflammation in AML (Figure 3B–3M).

Potential Signaling Pathways Associated with RPS27A

To clarify the mechanism for the influence of RPS27A on tumor progression, we conducted the GSEA of 16 cancers in which RPS27A showed significant prognostic value for OS. As shown in Figure 4A–4P, common enrichment pathways were eukaryotic translation elongation, signaling by the B cell receptor, focal adhesion, FCGR3A mediated IL10 synthesis, cell cycle, immunoregulatory interaction between a lymphoid and a non-lymphoid cell, FCERI mediated MAPK activation and so on. These results indicated that RPS27A might be associated with cell proliferation, invasion, MAPK pathway and immune regulation in multiple cancers.

Connection Between RPS27A and Genomic Heterogeneity

Genomic heterogeneity is an important way to analyze cancer, and this study conducted a pan-cancer analysis of RPS27A alterations through the cBioPortal database. RPS27A amplification was common in lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), LUSC, BLCA, OV, and LUAD, whereas mutation was mainly detected in UCEC, SKCM, STAD and PCPG (Figure 5A). A total of 20 mutation sites were found in 156 amino acids, including 16 missenses, 3 inframes, and 1fusion, and K83del was the most common mutation site (Figure 5B). Specifically, we identified mutated genes within the mutation spectrum of RPS27A high/low expression cohorts in LIHC. The top 5 genes were TP53, PCLO, CSMD3, AXIN1 and EYS (Figure 5C).

Considering the influence of genomic heterogeneity on tumor prognosis and therapeutic outcome, the relationships between RPS27A and TMB, MSI, ploidy, MATH, NEO, HRD homologous recombination deficiency (HRD) and LOH were analyzed. We observed a significant positive correlation between RPS27A and TMB in KIPAN, HNSC, ACC, and KICH, and a negative correlation in KIRP and cholangiocarcinoma (CHOL) (Figure 5D). There was a significant positive correlation between RPS27A and MSI in 6 tumors (BRCA, HNSC, OV, TGCT, BLCA, DLBC) and a significant negative correlation in 5 tumors (LGG, COAD, COADREAD, LAML, KIPAN) (Figure 5E). RPS27A had a positive association with ploidy in GBMLGG, LGG, CESC, COAD, COADREAD, STES, KIPAN, LUSC and LIHC and a negative correlation in BRCA, KIRP, THCA and OV (Figure 5F). RPS27A was positively related to MATH in GBM, GBMLGG, LGG, ESCA, STES, KIRP, LUSC, LIHC, TGCT, DLBC, and negatively to KIPAN, KIRC, and BLCA (Figure 5G). RPS27A showed positive connections to NEO in GBM but negative connections in CHOL (Figure 5H). RPS27A was positively correlated to HRD in 13 cancer types, whereas negatively only in SARC (Figure 5I). There was

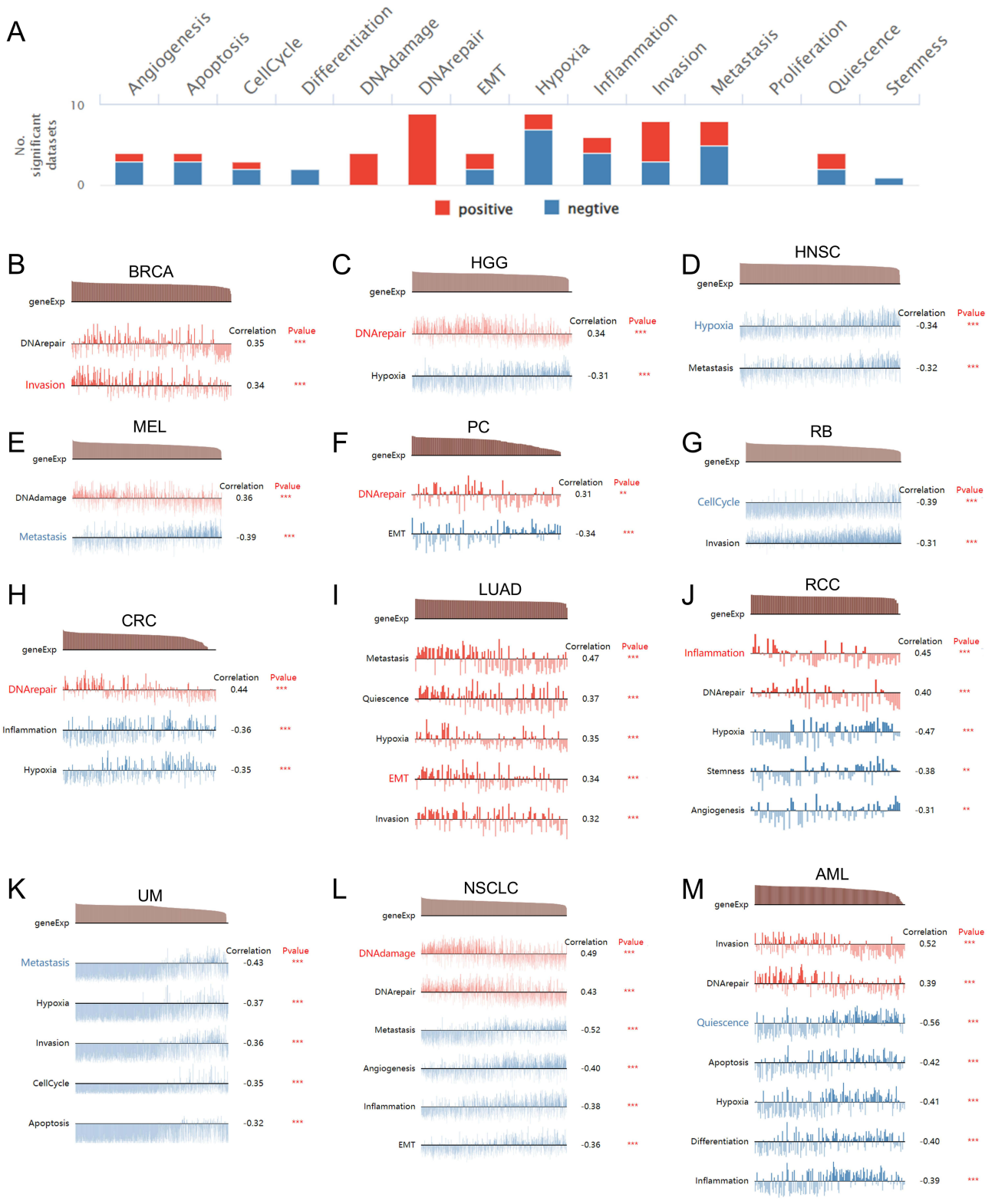

Figure 3 The associations between RPS27A and functional statuses. (**A**) The connections of RPS27A to functional statuses in human cancers. (**B**–**M**) The relationships between RPS27A expression and functional statuses in BRCA (**B**), HGG (**C**), HNSC (**D**), MEL (**E**), PC (**F**), RB (**G**), VRV (**H**), LUAD (**I**), RCC (**J**), UM (**K**), NSCLC (**L**), AML (**M**). ** $P < 0.01$, *** $P < 0.001$.

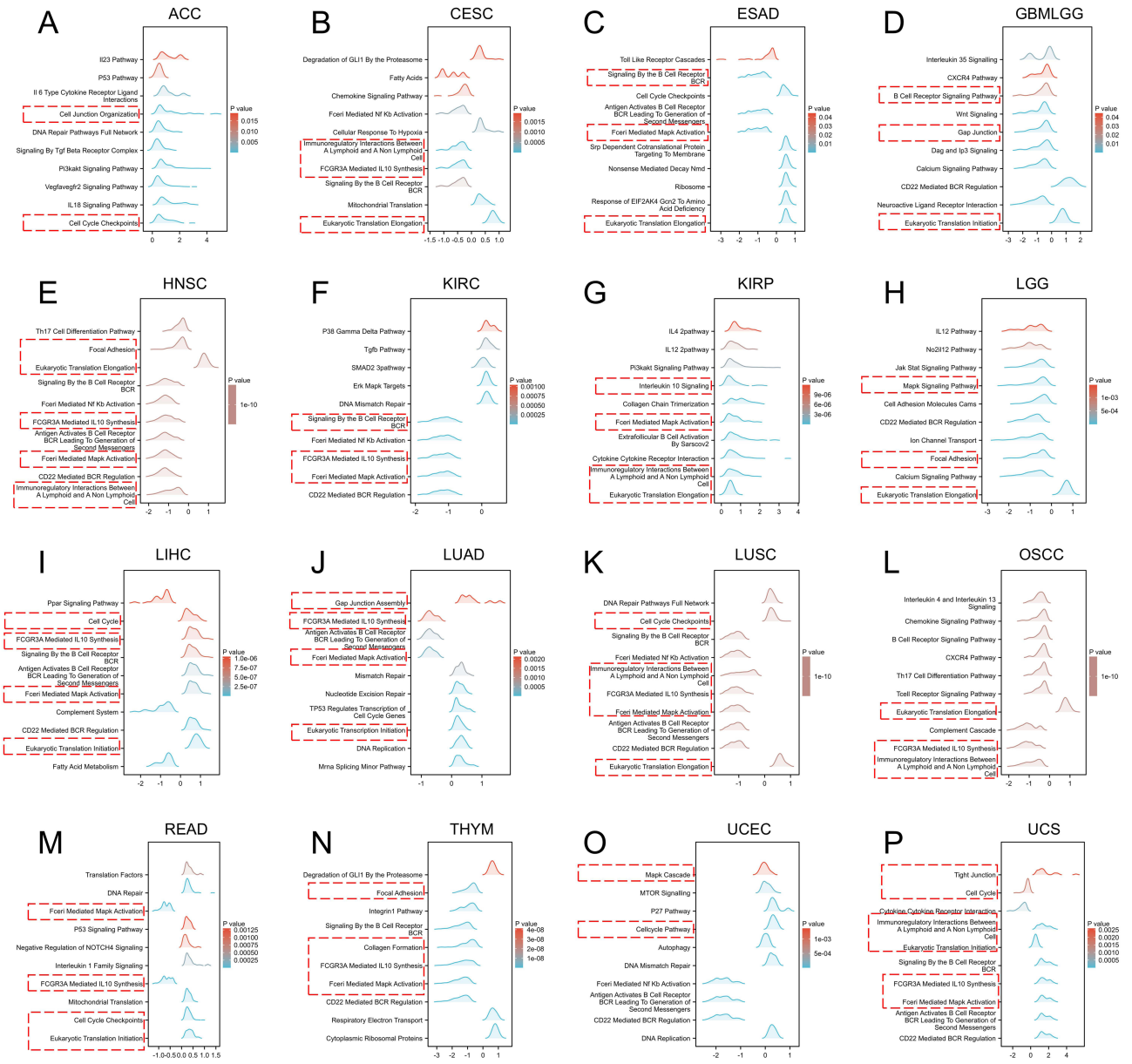

Figure 4 The enrichment pathways of RPS27A-related genes in ACC (**A**), CESC (**B**), ESAD (**C**), GBMLGG (**D**), HNSC (**E**), KIRC (**F**), KIRP (**G**), LGG (**H**), LIHC (**I**), LUAD (**J**), LUSC (**K**), OSCC (**L**), READ (**M**), THYM (**N**), UCEC (**O**), UCS (**P**).

also a positive connection between PRS27A and LOH in BRCA, KIRP, HNSC, LUSC, LIHC, and BLCA, and a negative correlation in KIPAN, KIRC, THYM, THCA, UVM, and ACC (Figure 5J).

Immunogenomic Value of RPS27A in Human Cancers

We observed significant positive associations between RPS27A and tumor purity in 15 tumors, and negative associations only in BRCA, KIPAN, and KICH (Figure 6A). The ESTIMATE method confirmed that RPS27A had a negative association with ESTIMATE score in 20 cancer types and a positive correlation only in BRCA, KIRP, KIPAN and KIRC (Figure 6B), which was mainly in accord with the tumor purity. The RPS27A expression in different immune subtypes of human cancers was assessed by the TISIDB database, demonstrating a close link between RPS27A and immune subtype in 13 cancer types (Figure 6C and Figure S3). These results indicated that the RPS27A level might be related to immune cells in the tumor microenvironment (TME).

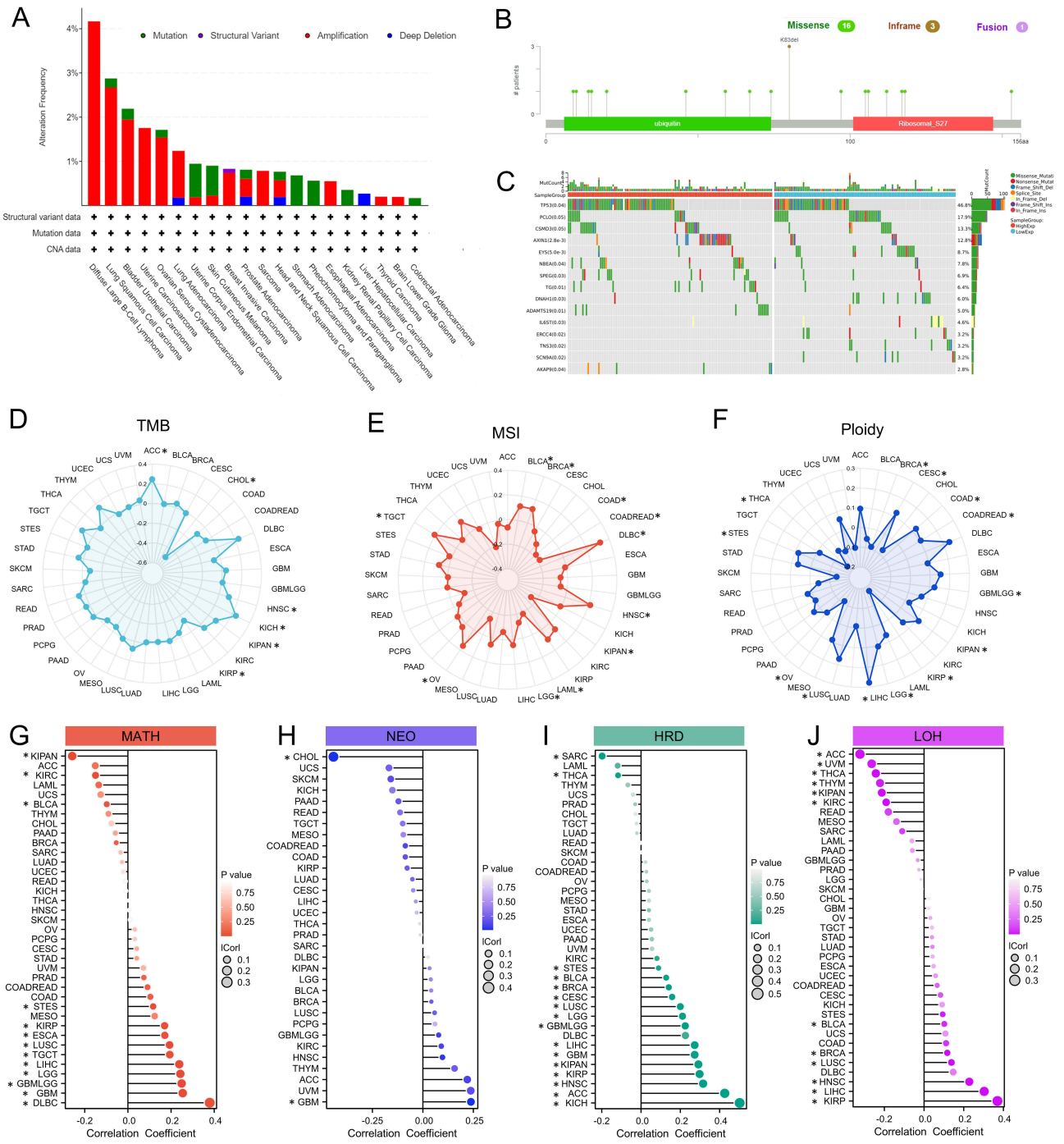

Figure 5 The association between RPS27A expression and genomic heterogeneity. (**A**) Bar chart of RPS27A mutations in human cancers. (**B**) Mutation diagram of RPS27A across protein domains. (**C**) The top 15 genes with the highest frequency of mutations in the high RPS27A group and low RPS27A group in LIHC. (**D–J**) The relationship between RPS27A and TMB (**D**), MSI (**E**), ploidy (**F**), MATH (**G**), NEO (**H**), HRD (**I**), LOH (**J**). * $P < 0.05$.

Subsequently, the association between RPS27A and immune cell infiltration was evaluated by the TIMER method. RPS27A level was positively associated with B cells in 10 tumors, $CD4^+$ T cells in 7 tumors, $CD8^+$ T cells in 10 tumors, neutrophils in 9 tumors, macrophage in 6 tumors, and dendritic cells (DC) in 8 tumors, whereas negatively connected to B cells in 1 tumor, $CD4^+$ T cells in 8 tumors, $CD8^+$ T cells in 6 tumors, neutrophil in 10 tumors, macrophage in 8 tumors, and DCs in 16 tumors (Figure 6D). Specifically, we focused on the relationship between RPS27A and immune cells in LIHC and STAD. The results demonstrated that RPS27A was positively associated with most gene markers of general

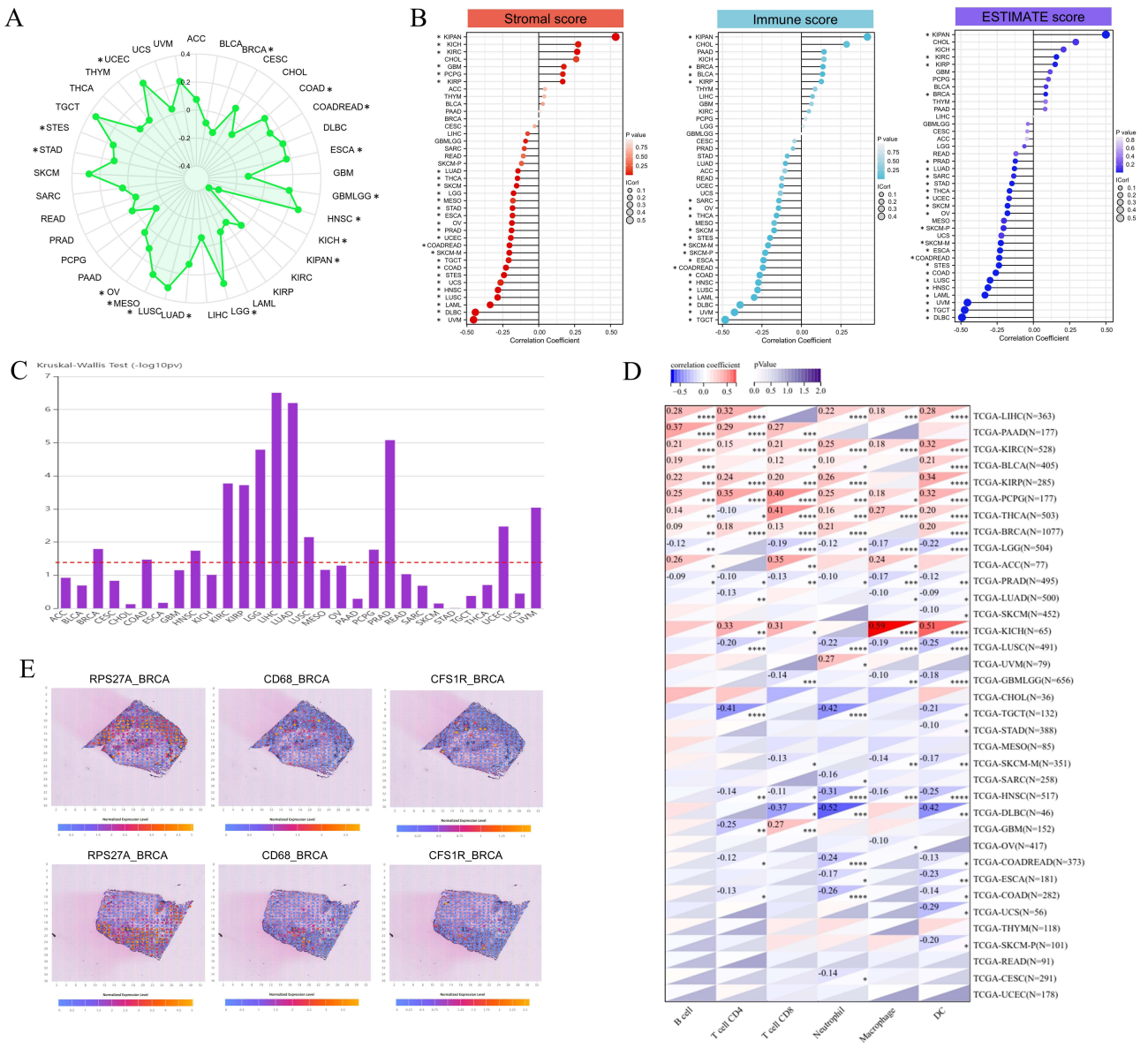

Figure 6 The role of RPS27A in immunoregulation. (**A**) The association between RPS27A and tumor purity. (**B**) The correlations between RPS27A and stromal score, immune score and ESTIMATE SCORE. (**C**) The connection between RPS27A and immune subtypes in the TSIDB database. (**D**) The relationship between PRS27A and immune cells calculated by the Timer method. (**E**) The overlapping patterns of RPS27A, CD68, and CFS1R expression in spatial transcriptomic sections. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

T cell, $CD8^+$ T cell, B cell, monocyte, tumor-associated macrophage (TAM), neutrophil, DC, Th1, and T cell exhaustion in LIHC, while showed negative connection to most gene markers of TAM, M2 macrophage, and Treg in STAD (Table 1). Spatial transcriptomic data demonstrated that the expression region of RPS27A overlaps with the expression region of macrophage marker CD68 and myeloid-derived suppressor cell (MDSC) marker CFS1R In BRCA (Figure 6E).

Relationship Between RPS27A Expression and Immunotherapy Evaluation

Considering the close relationship between RPS27A expression and immune cells in TME, RPS27A may predict the outcome of immunological therapy. The predictive efficiency of RPS27A was compared to other well-known biomarkers of immunotherapy response in the TIDE database, and RPS27A showed an area under the ROC curve more than 0.5 in most studies, in comparison with TIDE, MSI, TMB, CD274, CD8, IFNG, and Merck18 (Figure 7A). Low RPS27A expression was connected to T cell dysfunction phenotype in neuroblastoma (E-MTAB-179); High RPS27A level was

Table 1 Relationships Between RPS27A and Markers of Immune Cell in LIHC and LUSC

Description	Gene Markers	LIHC		STAD	
		R	P	R	P
CD8+ T Cell	CD8A	0.235	<0.001*	−0.086	0.095
	CD8B	0.300	<0.001*	−0.045	0.380
T Cell (general)	CD3D	0.353	<0.001*	0.022	0.673
	CD3E	0.293	<0.001*	−0.096	0.062
	CD2	0.296	<0.001*	−0.102	0.047*
B Cell	CD19	0.222	<0.001*	−0.087	0.090
	CD79A	0.252	<0.001*	−0.068	0.184
Monocyte	CD86	0.328	<0.001*	−0.184	<0.001*
	CSF1R	0.256	<0.001*	−0.343	<0.001*
TAM	CCL2	0.106	0.048*	−0.092	0.073
	CD68	0.219	<0.00*1	−0.353	<0.001*
	IL10	0.207	<0.001*	−0.232	<0.001*
M1	NOS2	−0.133	0.013*	−0.109	0.034*
	IRF5	0.134	0.013*	−0.308	<0.001*
	PTGS2	0.125	0.020*	−0.013	0.796
M2	CD163	0.059	0.271	−0.302	<0.001*
	VSIG4	0.057	0.292	−0.246	<0.001*
	MS4A4A	0.126	0.019*	−0.184	<0.001*
Neutrophils	CEACAM8	0.072	0.182	0.091	0.077
	ITGAM	0.128	0.017*	−0.259	<0.001*
	CCR7	0.127	0.018*	−0.145	0.005*
NK Cell	KIR2DL1	−0.037	0.499	0.064	0.213
	KIR2DL3	0.035	0.514	0.103	0.044*
	KIR2DL4	0.099	0.066	−0.016	0.761
	KIR3DL1	0.003	0.949	−0.009	0.869
	KIR3DL2	0.093	0.083	0.037	0.473
	KIR2DL3	0.035	0.514	0.103	0.044*
	KIR2DS4	−0.023	0.066	−0.005	0.926
Dendritic Cell	HLA-DPB1	0.256	<0.001*	−0.124	0.016*
	HLA-DQB1	0.224	<0.001*	−0.164	0.001*
	HLA-DRA	0.160	0.003*	−0.155	0.002*
	HLA-DPA1	0.183	<0.001*	−0.191	<0.001*
	CD1C	0.258	<0.001*	−0.095	0.064
	NRP1	0.188	<0.001*	−0.209	<0.001*
	ITGAX	0.207	<0.001	−0.245	<0.001*
Th1	TBX21	0.132	0.014*	−0.126	0.014*
	STAT4	0.200	<0.001*	−0.042	0.412
	STAT1	0.237	<0.001*	−0.151	0.003*
	IFNG	0.221	<0.001*	−0.052	0.309
	TNF	0.243	<0.001*	−0.181	<0.001*
	IL12A	0.304	<0.001*	−0.028	0.585
	IL12B	0.093	0.084	−0.109	0.034
Th2	GATA3	0.241	<0.001*	0.004	0.936
	STAT6	0.007	0.903	−0.264	<0.001*
	STAT5A	0.268	<0.001*	−0.235	<0.001*
	IL13	−0.091	0.091	−0.075	0.144
Tfh	BCL6	0.021	0.702	−0.144	0.005*
	IL21	0.046	0.395	−0.129	0.012*

(*Continued*)

Table 1 (Continued).

Description	Gene Markers	LIHC		STAD	
		R	P	R	P
Th17	STAT3	−0.098	0.071	−0.374	<0.001*
	IL17A	0.102	0.057	−0.071	0.170
Treg	FOXP3	−0.147	0.006*	−0.285	<0.001*
	CCR8	0.127	0.019*	−0.201	<0.001*
	STAT5B	0.028	0.601	−0.268	<0.001*
	TGFB1	0.404	<0.001*	−0.212	<0.001*
T cell exhaustion	PDCD1	0.346	<0.001*	−0.171	<0.001*
	CTLA4	0.335	<0.001*	−0.092	0.075
	LAG3	0.206	<0.001*	−0.119	0.020*
	HAVCR2	0.289	<0.001*	−0.228	<0.001*
	GZMB	0.174	0.001*	−0.071	0.169

Notes: *$P<0.05$.

related to worse ICB outcome in melanoma and bladder cancer (ICB_Gide2019_PD1 and ICB_Mariathasan2018_PDL1); RPS27A knockdown had a positive influence on the lymphocyte-mediated tumor killing in melanoma cell and T cell (Freeman 2019 OT1, Patel 2017 2 and Shifrut 2018 Average); Among the cell types promoting T cell exclusion, MDSC had high RPS27A level (Figure 7B). The TISMO database demonstrated that patient groups with different ICB responses had different RPS27A expression levels in 7 cohorts utilizing in-vivo tumor models (Figure 7C). In addition, RPS27A expression was decreased in LLC (lung carcinoma), KPC, and Panco2 (Pancreatic ductal adenocarcinoma) after IFNg treatment, but was inconsistent in B16 (melanoma) (Figure 7D). RPS27A level was also downregulated in CT26 (colorectal carcinoma) after TNFa treatment (Figure 7D).

Influence of RPS27A Overexpression on Tumor Cell Ability and Macrophage Polarization

To assess the results above, the RPS27A expression was detected in human HCC tissues and mouse model. The result confirmed that RPS27A level in HCC tissues was upregulated compared to matched normal tissues (Figure 8A). The mouse model also demonstrated that RPS27A was overexpressed in liver cancer tissues (Figure 8B). Subsequently, we validated the influence of RPS27A on HCC cell function and macrophage polarization. RPS27A level was upregulated in HCC cell lines Huh7 and HLF (Figure 8C). CCK-8 assay and clone formation assay confirmed that RPS27A overexpression promoted the proliferation of HCC cells (Figure 8D). Wound healing assay and Transwell migration assay demonstrated that RPS27A upregulation enhanced the migration of HCC cells (Figure 8E–8G). Transwell invasion assay verified the intensive invasion of HCC cells after RPS27A overexpression (Figure 8H). To clarify if RPS27A level in HCC cells affected the macrophage polarization, macrophage cell-line THP1 was co-cultured with HCC cell lines (Huh7 and HLF) with different RPS27A expressions (Figure 8I). The results confirmed that the HCC cells with high REPS27A level could increase the CD206 expression (M2 marker) and inhibit the CD86 expression (M1 marker) in THP1 (Figure 8J).

Discussion

Due to the demand for rapid proliferation and malignant transformation, tumor cells must have an intensive ability for protein synthesis.[13] As a ribosomal protein, RPS27A is part of ribosomes which act as machines for protein synthesis, and therefore has the potential to regulate tumor progression. In addition, RPS27A has a close relationship with Ub, empowering it to affect ubiquitylation and protein degradation, and then to control multiple processes and pathways. We first detected the mRNA and protein levels in human cancers through TCGA, UALCAN, and HPA databases. RPS27A was dysregulated in most cancer types compared to normal tissues. In addition, high RPS27A expression was associated

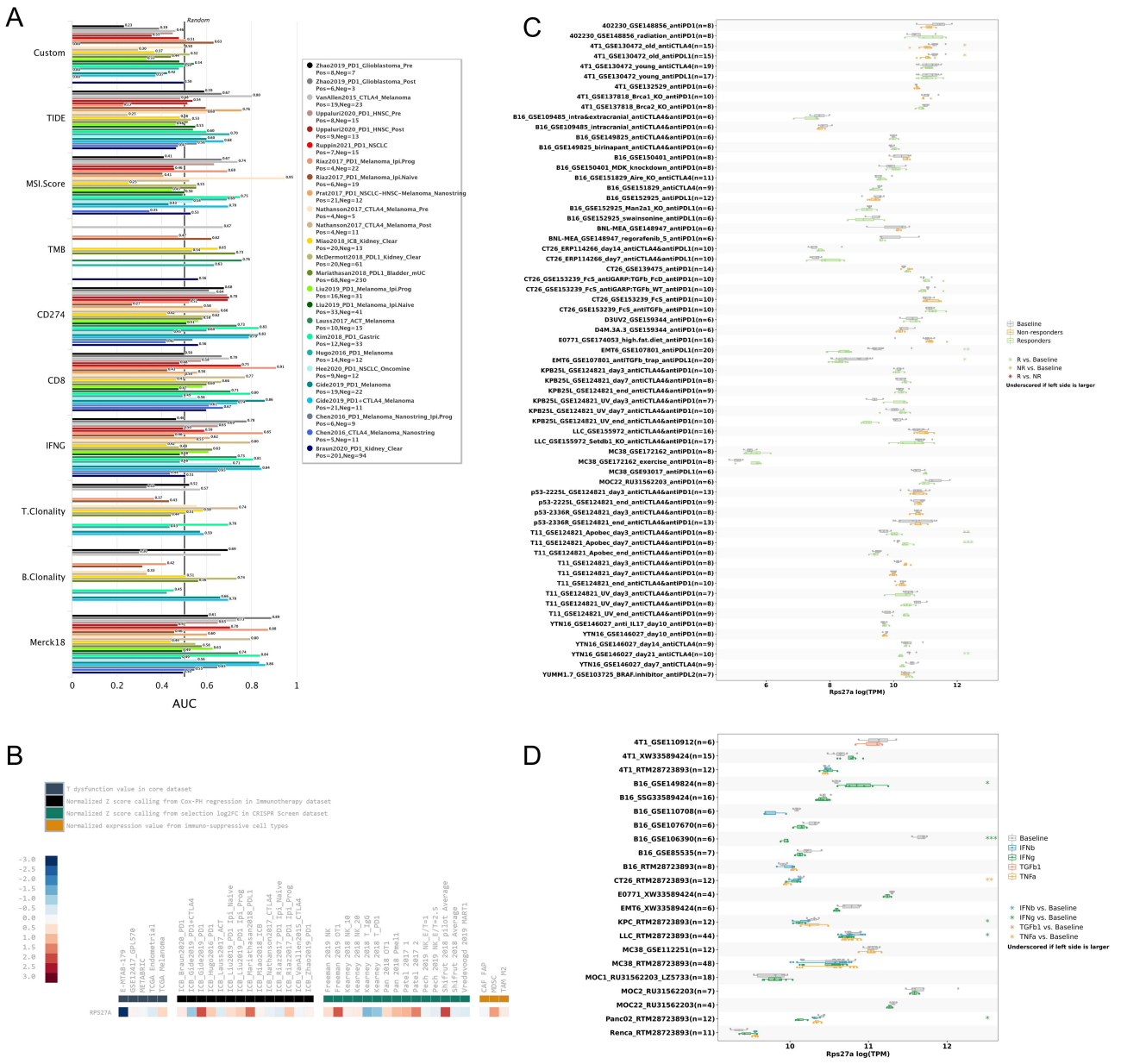

Figure 7 The value of RPS27A in immunotherapy response evaluation. (**A**) The comparison of RPS27A and other published biomarkers for immunotherapy response via the TIDE database. (**B**) The relationship between RPS27A and T cell dysfunction, ICB treatment and immune cells in the TIDE database. (**C**) RPS27A expression in tumor model with ICB treatment based on the TIMSO database. (**D**) RPS27A expression in tumor cell lines with ICB treatment based on the TIMSO database. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

with advanced pathological stage in 7 cancers. Moreover, RPS27A was connected to patients' survival, and was a risk factor in most cases. These results suggested that RPS27A might affect patients' prognosis by regulating tumor progression in some cancer types.

Subsequently, we detected the relevance of RPS27A across 14 functional states in 19 cancers and found that RPS27A was positively associated with DNA damage and repair. And beyond that, RPS27A served a dual function in other cancer states. For example, RPS27A showed a positive connection to invasion in BRCA and AML, but a negative association with invasion in RB and UM. These results indicated that RPS27A might work as an oncogene or suppressor in different cancer types. MAPK is a serine/threonine protein kinase widely found in eukaryotic cells, which can transmit various extracellular stimuli into the cell to mediate numerous cellular activities.[14,15] GSEA result demonstrated RPS27A-related genes enriched in the MAPK signaling pathway in multiple cancer types, both positively and negatively, suggesting that

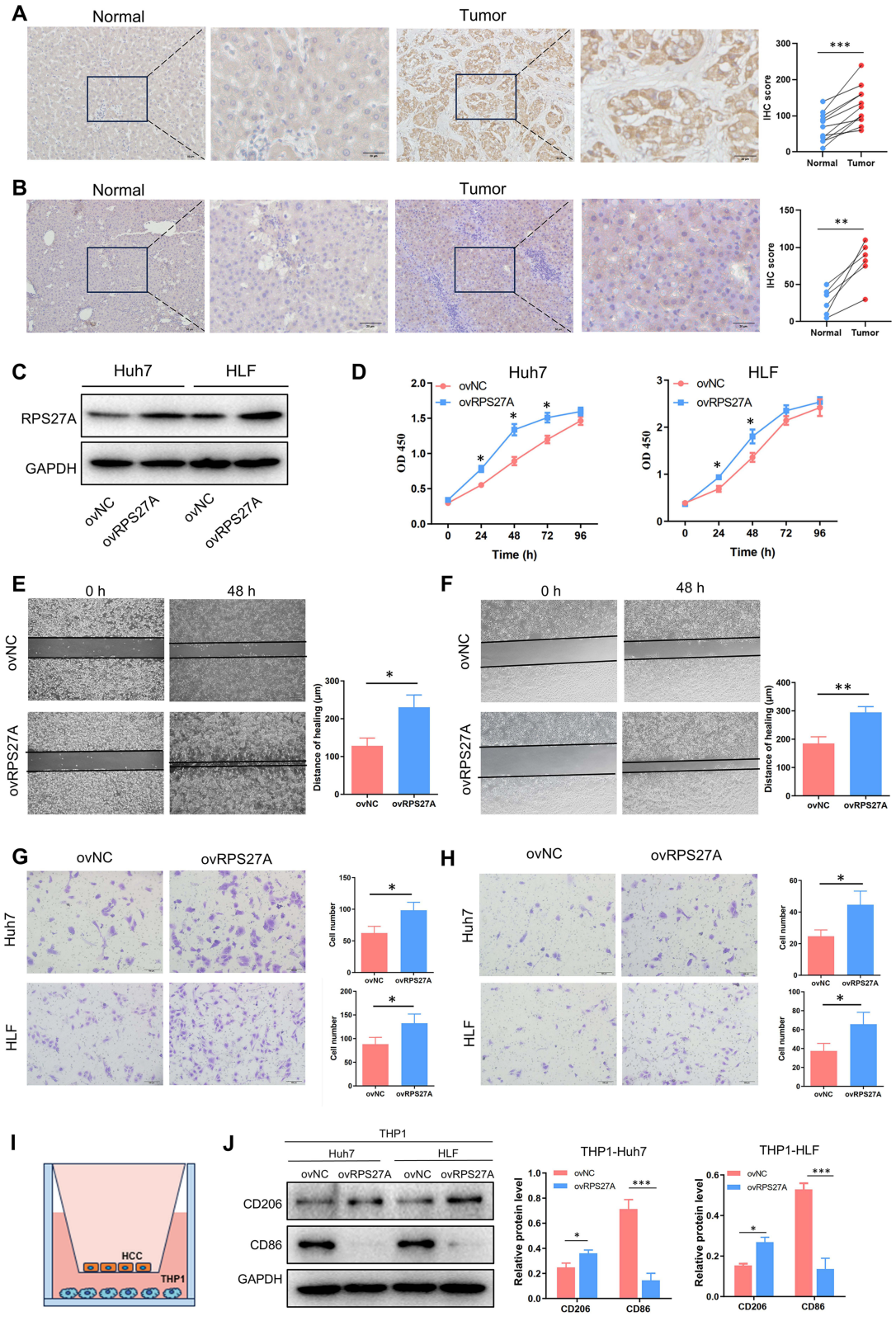

Figure 8 The influence of RPS27A overexpression on HCC activities and macrophage polarization. (**A**) The RPS27A expression in 11 pairs of human HCC tissues and normal liver tissues. (**B**) The RPS27A expression in 6 pairs of mouse HCC tissues and normal liver tissues. (**C**) RPS27A expression detected by Western Blot after overexpression. (**D**) The cell proliferation detected by CCK-8 assay. (**E**) and (**F**): The cell migration of Huh7 (**E**) and HLF (**F**) detected by the wound healing assay. (**G**) The cell migration detected by the Transwell migration assay. (**H**) The cell invasion detected by the Transwell invasion assay. (**I**) The schematic diagram of cell co-culture. (**J**) The protein expressions of macrophage polarization markers detected by the Western Blot. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

RPS27A might regulate the vital genes in the MAPK pathway, and then promote or inhibit tumor progression. Enrichment pathways about B cells and IL10 were also found in most cancers through GSEA. B cells inhibit tumor growth by producing tumor-specific antibodies and presenting tumor antigens. Still, certain B cell subsets and specific antibodies can also inhibit anti-tumor immunity and promote tumor growth.[16–18] IL10 promotes tumor immune escape by reducing the anti-tumor immune response in the tumor microenvironment, such as inhibiting the functions of macrophages and T cells.[19] The common connection of RPS27A to B cells and IL 10 in human cancers indicated that RPS27A might affect tumor development through immunoregulation in TME.

With the progress of sequencing and high-throughput technology, genetic heterogeneity provides more novel insights for cancer research. MATH is an algorithm to assess the genetic heterogeneity of tumor samples based on the mutation frequency of all alleles.[20] Ploidy is a sign of cancer and is associated with chromosomal instability involved in cancer progression.[21] LOH refers to the phenomenon in which a heterozygous allele becomes homozygous and can cause the loss of entire genes and nearby chromosomal regions.[22] Our results demonstrated that RPS27A level had a close relationship with MATH, ploidy, and LOH in multiple cancer types, indicating that RPS27A might affect the genomic alteration and represent the tumor heterogeneity to some extent. TMB refers to the mutations per Mb unit in tumor tissues, and tumors with high TMB express a large number of abnormal proteins (NEO) that can be identified by immune cells and activate immune cells.[23] MSI refers to changes in microsatellite sequence length caused by insertion or deletion mutations during DNA replication.[24] Patients with high TMB always show high MSI, and both TMB and MSI are prognostic markers for ICB therapy.[25] HRD hinders homologous recombination repair and then induces genomic alteration, and HRD status is highly correlated with sensitivity to platinum-based chemotherapeutic drugs and PARP inhibitors.[26] This study confirmed that RPS27A expression was connected to TMB, MSI, NEO, and HRD in multiple cancers, suggesting that RPS27A could work as a novel prognostic biomarker for immunotherapy and chemotherapy.

The possible link between RPS27A and immunoregulation has been frequently mentioned in the results above, so we further investigate the regulatory effects of RPS27A on immune cells. In addition to tumor cells, there are immune, mesenchymal, and other non-tumor cells in tumor tissues. Tumor purity was significantly correlated with clinical features, genomic expression, and biological characteristics of tumors.[27] The tumor purity analysis and ESTIMATE method confirmed that high RPS27A level tended to represent high tumor purity in most cases, which meant few immune cells in TME and weaken insensitivity to immunotherapy. The immune score also verified the negative relationship between RPS27A level and immune cell infiltration. Moreover, immune subtypes of multiple cancers had significantly different levels of RPS27A, further confirming the important role of RPS27A in immune cell regulation.

Then, we investigated the connection of RPS27A to B cell, $CD4^+$ T cell, $CD8^+$ T cell, neutrophil, macrophage, and DC. The results demonstrated that RPS27A was positively associated with most of these immune cells only in LIHC, PAAD, KIRC, BLCA, KIRP, PCPG, THCA, BRCA, and KICH. RPS27A showed negative or no connection to these 6 immune cells in other cancer types. Spatial transcriptomic data of BRCA tissues demonstrated that the region with high RPS27A overlapped the tissues with high CD68 and CFS1R. Therefore, RPS27A might participate in the regulation of macrophage and MDSC. Although macrophages have the potential to kill tumor cells, tumor-associated macrophages in the infiltrating region of tumor tissue promote tumor growth by accelerating angiogenesis and inhibiting effector T cells.[28] MDSC could inhibit the antitumor immune response of macrophages through high CSF1R expression.[29] The association between RPS27A and macrophages might be a new research direction for further study.

Considering the close relationship between RPS27A and immune cells, the value of RPS27A in immunological therapy was explored in the TIDE database. First, RPS27A was assessed for forecast accuracy on ICB response compared to the published biomarkers. RPS27A showed an AUC greater than 0.5 in most ICB cohorts, confirming it to be a satisfactory predictive marker for immunotherapy. The T cell dysfunction in TME was one main obstacle for immunotherapy, where tumor cell killing mainly depends on activated cytotoxic T cell.[30] In neuroblastoma, RPS27A was negatively connected to T cell dysfunction score, meaning that neuroblastoma patients with low RPS27A expression tended to have poor immunotherapy response due to T cell dysfunction. In addition, high RPS27A expression in melanoma and bladder cancer was confirmed to be related to worse ICB outcomes. The TISMO database demonstrated the RPS27A expression in responders and non-responders to ICB therapy. In mammary cancer, RPS27A expression in non-responders decreased compared to baseline (T11), but responders in different models showed different variation

trends (EMT6 and T11). In STAD, responders had a decreased RPS27A compared to baseline. Furthermore, IFNg and TNFa therapy could inhibit the RPS27A expression in multiple cancers. These results suggested that RPS27A has the potential to be a predictive biomarker for immunotherapy response.

Liver cancer is the sixth most common malignancy worldwide, and about 80% of these are HCC.[31] Fatima et al found that the RPS27A level in the mouse HCC model was upregulated in comparison with normal mice.[32] However, Gunasekaran et al reported that RPS27A expression was downregulated in tumor tissues of HCC patients.[33] This study detected the RPS27A expression in liver tissues of both human and mouse and confirmed that RPS27A was overexpressed in HCC tissues. Subsequently, we upregulated the RPS27A expression in HCC cells Huh7 and HLF and detected changes in HCC biological behavior. The results demonstrated that RPS27A overexpression promoted the proliferation, migration, and invasion of HCC cells, similar to the results of published research about other cancers.[10,11,34] Macrophages are the most common immune cells in the HCC microenvironment, accounting for more than 50%.[35] With the stimulation of different microenvironmental signals, macrophages could differentiate into specific cell populations with different phenotypes and functions, M1 and M2.[36] M1 macrophages mainly play an anti-tumor role in promoting inflammation and improving host immune function.[37] M2 macrophages not only lack the function of phagocytizing tumor cells but also promote tumor proliferation, invasion and metastasis, immune escape and angiogenesis.[38] In this study, HCC cells with different PRS27A levels were co-cultured with macrophages, and RPS27A overexpression groups promoted the M2 polarization of macrophages. These results showed that RPS27A in HCC cells promoted the malignant biological behavior of HCC cells, while facilitated HCC progression by inducing M2 polarization.

Although we conducted a comprehensive analysis of RPS27A in pan-carcinoma, there were certain limitations in this study. First, differences in the data from different databases might lead to system bias. Second, the influence of RPS27A on tumor progression in different cancer types needs to be validated in vivo and in vitro. Third, we found that RPS27A is closely related to the immune regulation preliminarily, but the direct evidence to verify the influence of RPS27A on immunotherapy was lacked. Further studies are necessary to confirm the value of RPS27A in prognosis and immunotherapy.

Conclusion

Above all, RPS27A showed significant diagnostic and prognostic value in multiple cancer types and was associated with different cancer statuses and signaling pathways. RPS27A also might affect genomic heterogeneity, immune cell infiltration, and immunotherapy outcomes. Further research about RPS27A is necessary for prognosis and cancer management.

Data Sharing Statement

All data generated or analyzed are included in the current manuscript, and they are available from the corresponding author upon reasonable request.

Ethics Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Affiliated Hospital of Nantong University (2023-L038).

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Author Contributions

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

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Disclosure

The authors report no conflicts of interest in this work.

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