


Decaprenyl Diphosphate Synthase Subunit I (PDSSI): A Potential Prognostic Biomarker and Immunotherapy-Target for Hepatocellular Carcinoma

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Purpose: PDSSI (decaprenyl diphosphate synthase subunit 1) plays an important role in the progression of several types of tumor. However, the biological functions of PDSSI remain unclear in patients with hepatocellular carcinoma (HCC). In this study, we attempted to determine the role of PDSSI in predicting the survival and efficacy of immunotherapy for HCC patients.

Methods: We analyzed the expression of PDSSI in pan-cancer by Tumor Immune Estimation Resource (TIMER) and UALCAN database. Next, we investigated the correlations between the expression and potential prognostic value of PDSSI in pan-cancer by Gene Expression Profiling Interactive Analysis (GEPIA) and Kaplan-Meier plotter and further confirmed our finding in our study of 139 patients with HCC. Furthermore, we correlated expression patterns with the presence of immune cell infiltrates and immune regulatory molecules in HCC tissue by TIMER. Finally, its potential immune-related mechanism was explored by Gene Set Enrichment Analysis (GSEA).

Results: Multiple datasets demonstrate that PDSSI is up-regulated in HCC tissues compared with adjacent tissues, which was validated at mRNA (databases) and protein levels (our cohort). Patients with higher PDSSI expression had shorter overall survival and relapse-free survival. In addition, PDSSI expression was positively related to early recurrence and served as an independent poor prognostic factor for HCC. Patients with higher PDSSI expression had lower CD8+ T cells in HCC tissue, and PDSSI deteriorates T cell exhaustion by promoting T cell surface inhibitory receptors' secretion and immunosuppressive cell proliferation. Furthermore, PDSSI was positively correlated with the WNT, TGF β , VEGF, and other signaling pathways in HCC.

Conclusion: PDSSI is a potential prognostic biomarker and immunotherapy target for hepatocellular carcinoma.

Keywords: decaprenyl diphosphate synthase subunit 1, hepatocellular carcinoma, prognosis, tumor microenvironment

Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies and ranks third in cancer-related mortality globally.¹ HCC accounts for 90% of cases of liver cancer.² Although significant progress in clinical diagnosis and comprehensive treatment has partially increased patients' longevity, the prognosis of patients with HCC is still inferior because patients with advanced liver cancer account for about 50%.² A recent study has shown that programmed cell death protein-1-targeted immunotherapy with nivolumab or pembrolizumab is associated with good survival in patients with advanced hepatocellular carcinoma.³ In addition, TGF β signal-targeted immunotherapy showed promising efficacy in inhibiting cancer progression.⁴ So HCC may be a promising candidate for immunotherapy. But the effective target for hepatocellular carcinoma is still unclear. Searching for a reliable immunotherapeutic target for HCC is warranted.

Decaprenyl diphosphate synthase subunit 1 (PDSS1) was considered a gene related to the synthesis of coenzyme Q.⁵ Several studies showed that PDSS1 speed up the progression of some tumors, such as cutaneous melanoma, bladder cancer, breast cancer, and HCC.^{6–11} Bioinformatics studies suggest that PDSS1 may be associated with hypoxia in the tumor microenvironment of HCC.^{9–11} However, it is not yet clear whether PDSS1 acts as a prognostic marker in HCC and if its expression is related to the tumor immune infiltration.

Using bioinformatics tools and laboratory studies, we attempted to clarify the potential function of PDSS1 in HCC by evaluating the correlation between PDSS1 expression and prognosis, as well as the tumor microenvironment in HCC patients. Multiple datasets suggested that up-regulation of PDSS1 predicts poor prognosis and maybe a potential immunotherapeutic target for patients with HCC.

Materials and Methods

Tumor Immune Estimation Resource (TIMER) Database Analysis

TIMER is a comprehensive resource for systematical analysis of immune infiltrates across different types of cancer (<http://timer.cistrome.org/>) and allows users comprehensively to explore tumor immunological, clinical, and genomic features.¹² The present study investigated the levels of PDSS1 mRNA expression in different cancer tissues and adjacent tissues in TIMER. Furthermore, we explored the correlation between PDSS1 expression and immune cells infiltration and tumor immune microenvironment by TIMER.

UALCAN Databases Analysis

UALCAN is a comprehensive and interactive web portal to analyze publicly available cancer databases in-depth, including The Cancer Genome Atlas (TCGA) gene expression dataset (<http://ualcan.path.uab.edu/index.html>). Specifically, it allows users to investigate the relative expression of a query gene across tumor and normal tissue samples, as well as correlate gene expression with clinicopathologic features or the prognosis of patients.¹³ In the present study, we performed the levels of PDSS1 mRNA expression in pan-cancer and corresponding normal tissues.

Gene Expression Profiling Interactive Analysis (GEPIA), Kaplan–Meier (KM) Plotter Analysis

GEPIA is a web server (<http://gepia.cancer-pku.cn/>) using a standard processing pipeline for analyzing the RNA sequencing expression from the TCGA and Genotype-Tissue Expression projects.¹⁴ The KM plotter (<http://kmplot.com/analysis/>) can assess the effect of 54k genes (mRNA, miRNA, protein) on survival in 21 types of cancer.^{15,16} In the present study, the survival analysis based on PDSS1 mRNA expression in 33 different types of cancer and 21 different types of cancer was explored by GEPIA and the KM plotter, respectively. In addition, the KM-plotter was used to investigate the survival analysis of patients with HCC based on immune cells infiltration, such as B cells, macrophages, CD4+ T cells, and CD8+ T cells. (http://kmplot.com/analysis/index.php? p=service and cancer=pancancer_rnaseq).

Gene Set Enrichment Analysis (GSEA) Analysis

Gene Set Enrichment Analysis (GSEA) is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states.¹⁷ In the present study, we explored the correlation between PDSS1 mRNA expression (the RNA seq from TCGA: <https://portal.gdc.cancer.gov/>) and immune inflammation signaling pathways by GSEA 4.1.0.

Immunohistochemistry Analysis of Liver Sections

Tissue samples were fixed in 10% formalin, embedded in paraffin, cut into 4 μm-thick sections, and stained with immunohistochemistry according to standard procedures. Tumor sections of hepatocellular carcinoma were stained with the appropriate PDSS1 antibody (Thermo Fisher, #: PA558217), and both the intensity and percentage (signal: 0 for negative; 1 for weak staining; 2 for intermediate staining; and 3 for strong staining; percentage: 0 for 0%; 1 for 1–25%; 2 for 26–50%; 3 for 51–75%; and 4 for >75%) of immunostaining were taken into consideration when analyzing the data.

The ultimate quantitation of each sample was obtained by multiplying these two values (intensity score and percentage score). The case was defined as a low expression if the score was 4 or less, and other cases were defined as high expression.

Patients and Specimens

139 samples from patients with HCC were collected in the Hospital. Ten patients were lost to follow-up. The study was performed to comply with the Declaration of Helsinki principles.

Statistical Analysis

Independent samples *t*-test was employed to compare the difference between two groups, and the one-way ANOVA was used for three groups or more. KM survival analysis and Log rank tests account for the overall survival. The results were defined as statistical significance if $P < 0.05$. SPSS software (Version 27.0; IBM, Armonk, NY, USA) was used in all our analyses.

Results

PDSSI is Up-Regulated in HCC Tissues

TIMER and UALCAN databases were used to analyze the mRNA expression of PDSSI in different cancer. The results of TIMER in the pan-cancer analysis showed that PDSSI expression was up-regulated in 13 types of tumors, including hepatocellular carcinoma; however, it was down-regulated in only two types of tumors (Figure 1A). The results of UALCAN suggested that PDSSI mRNA expression was up-regulated in bladder urothelial carcinoma, invasive breast carcinoma, cervical squamous cell carcinoma, endocervical adenocarcinoma, cholangiocarcinoma, esophageal carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, prostate adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma (STAD), but down-regulated in uterine corpus endometrial carcinoma and thyroid carcinoma (Figure 1B).

In order to confirm the expression status of the PDSSI protein in HCC tissues, immunohistochemistry assays were further conducted to examine the expression pattern of PDSSI in 139 paired human HCC specimens and non-neoplastic liver tissues. Consistent with the results of TIMER and UALCAN, we also found that PDSSI was significantly up-regulated in HCC tissue ($P < 0.01$). Furthermore, it is primarily expressed in the cytoplasm and nucleus (Figure 1C).

High PDSSI Expression Predicts a Poor Prognosis in HCC Patients

First, we explored the prognostic value of PDSSI mRNA expression in 33 types of cancers by GEPIA. We found that HCC patients with high PDSSI expression had poorer overall survival (OS) (HR=1.8, $P < 0.001$) and relapse-free survival (RFS) (HR=1.6, $P < 0.01$) (Figure 2A). In addition, high expression of PDSSI was associated with poorer OS in adrenocortical carcinoma, sarcoma (SARC), and mesothelioma, but had the opposite effect in the brain lower grade glioma, STAD, and THYM (Supplementary Figure 1).

Next, we confirmed the prognostic value of PDSSI mRNA expression in 21 types of cancers by KM plotter. In line with the results obtained from GEPIA, high expression of PDSSI was correlated with shorter OS (HR=2.12, $P < 0.0001$) and RFS (HR=1.57, $P < 0.01$) in HCC patients (Figure 2B). Moreover, an increased level of PDSSI expression was correlated with shorter OS in invasive breast carcinoma, kidney renal clear cell carcinoma, lung adenocarcinoma, SARC, and uterine corpus endometrial carcinoma. But a high level of PDSSI expression had longer OS in esophageal squamous cell carcinoma, STAD, and thymoma (Supplementary Figure 2).

Furthermore, the association between the levels of PDSSI protein expression and OS or RFS in HCC patients was also investigated in our cohort. The results were certified that a high protein level of PDSSI expression was significantly associated with worse OS (HR=1.969, $P < 0.01$) and poor RFS (HR=1.858, $P < 0.05$) in patients with HCC (Figure 2C).

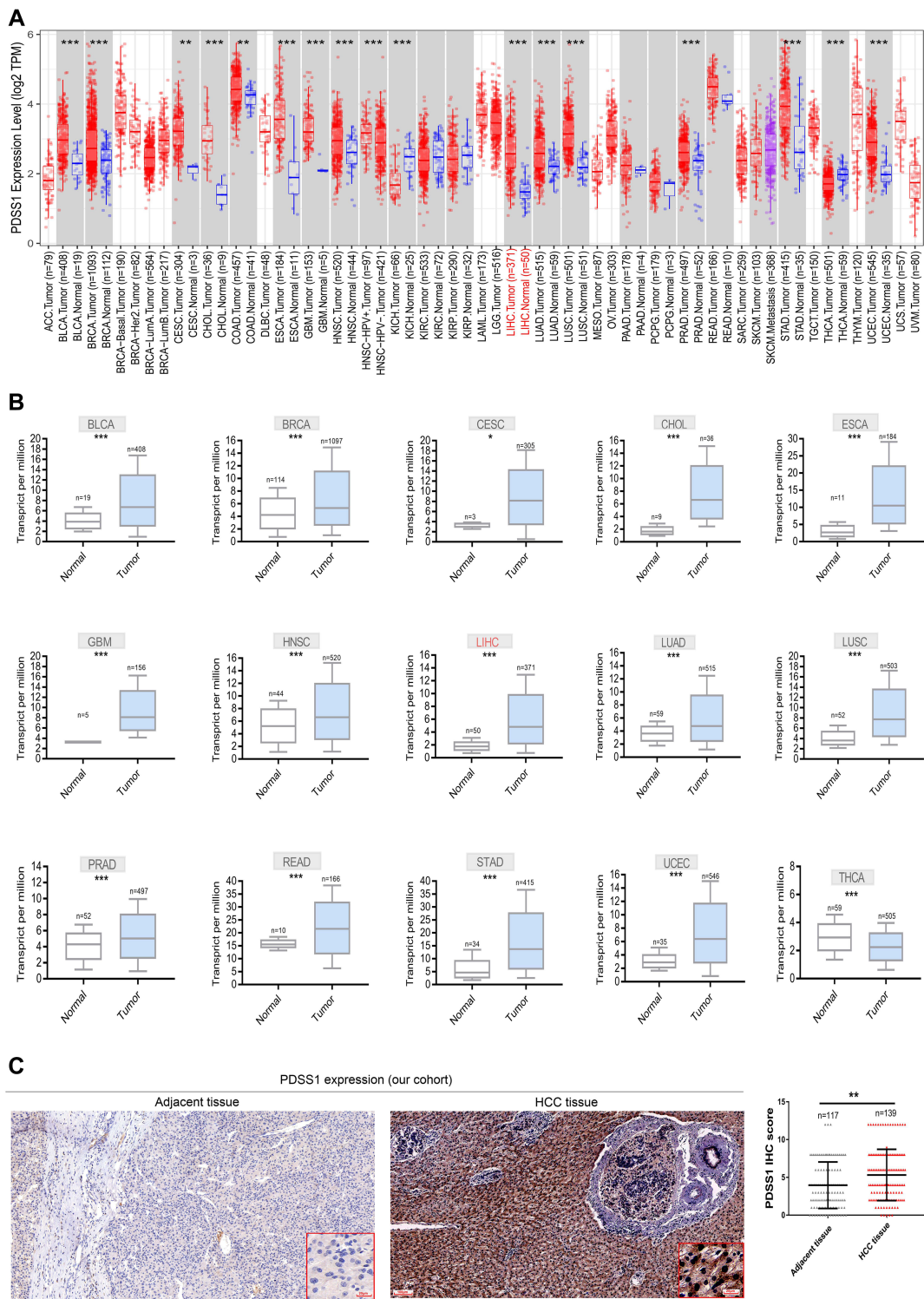


Figure 1 The levels of PDSS1 expression in human tumors. (The mRNA expression of PDSS1 in different types of human tumors was analyzed by Tumor Immune Estimation Resource (TIMER) (A) and UALCAN (B). There are representative immunohistochemistry staining images with a PDSS1 antibody in HCC tumor tissues and adjacent tissues in our cohort (C). High expression tumors: Bladder urothelial carcinoma (BLCA); Breast invasive carcinoma (BRCA); Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC); Liver hepatocellular carcinoma (LIHC); Colon adenocarcinoma (COAD); Esophageal carcinoma (ESCA); Glioblastoma multiforme (GBM); Brain Lower Grade Glioma (LGG); Head and neck squamous cell carcinoma (HNSC); Head and neck squamous cell carcinoma-HPV neg (HNSC- HPV neg); Liver hepatocellular carcinoma (LIHC); Lung adenocarcinoma (LUAD); Lung squamous cell carcinoma (LUSC); Prostate adenocarcinoma (PRAD); Stomach adenocarcinoma (STAD); Uterine Corpus Endometrial Carcinoma (UCEC). Low expression tumors: Kidney chromophobe (KICH); Thyroid carcinoma (THCA). No difference tumors: Breast invasive carcinoma-Basal (BRCA-Basal); Breast invasive carcinoma-Her2 (BRCA- Her2); Breast invasive carcinoma-Luminal (BRCA- Luminal); Kidney renal clear cell carcinoma (KIRC); Kidney renal papillary cell carcinoma (KIRP); Pancreatic adenocarcinoma (PAAD); Pheochromocytoma and Paraganglioma (PCPG); Rectum adenocarcinoma (READ). *Indicates P-value <0.05; **Indicates P-value < 0.01; ***Indicates P-value < 0.001.)

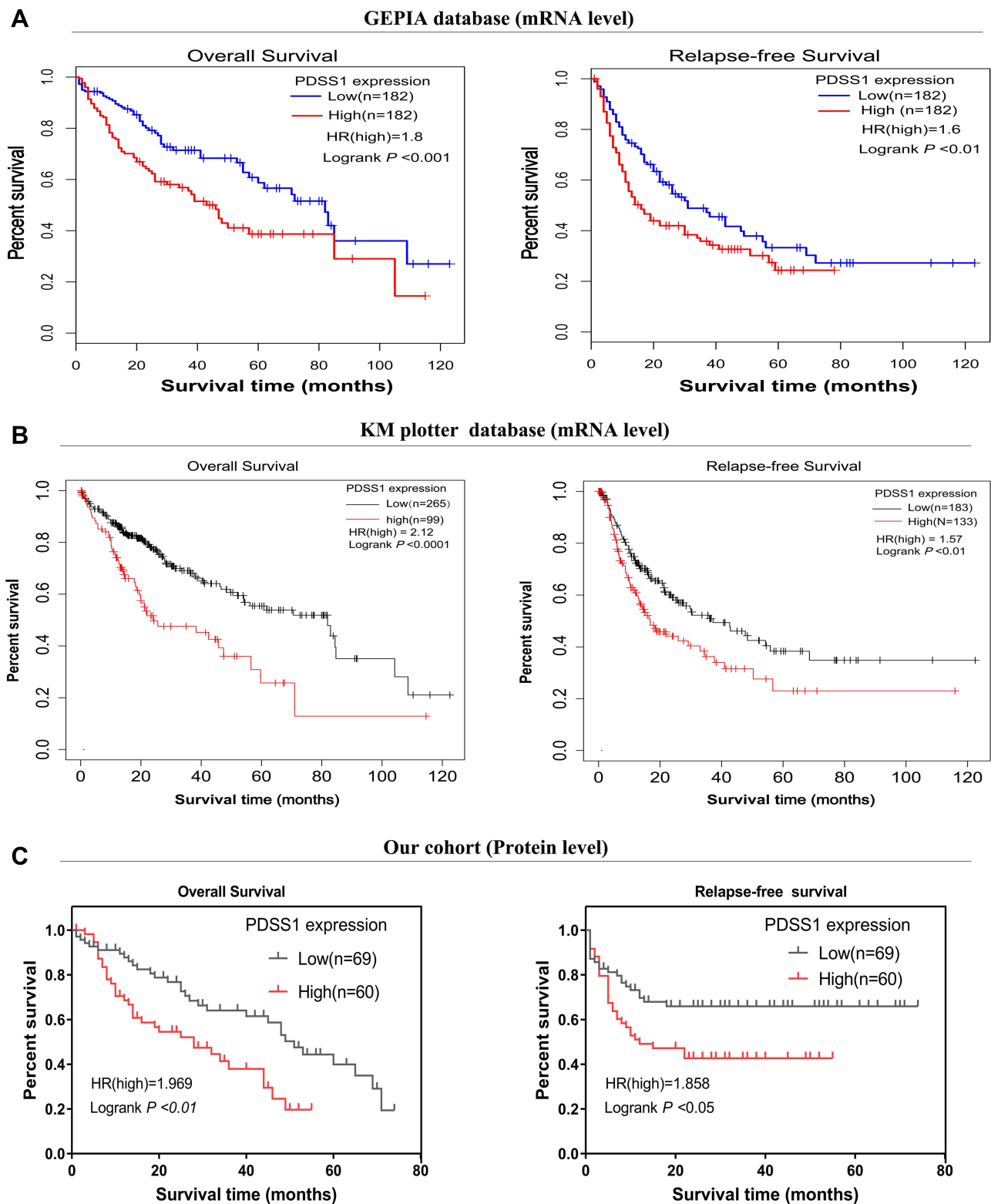


Figure 2 Up-regulated PDSS1 predicts a poor prognosis in HCC patients. The correlation between PDSS1 mRNA expression and survival of patients with HCC was analyzed by Gene Expression Profiling Interactive Analysis (GEPIA) and the Kaplan–Meier (KM) plotter (**A** and **B**). The correlation between PDSS1 protein expression and survival of patients with HCC was analyzed by our cohort. (**C**).

Abbreviations: OS, Overall survival; RFS, relapse-free survival.

PDSS1 is an Independent Risk Factor for HCC Patients

To verify the functions of PDSS1 genes, we analyzed the PDSS1 expression in our cohort with 15 widely recognized clinicopathological parameters, including age, gender, HBsAg, alpha-fetoprotein, alanine aminotransferase, aspartate aminotransferase, cirrhosis, Child-Pugh grade, tumor size, tumor number, tumor thrombus, tumor capsule, tumor grade, tumor stage, and tumor early recurrence. We found PDSS1 was associated with aspartate aminotransferase and early recurrence of HCC ($P < 0.05$) (Table 1). These data suggested that PDSS1 is associated with aggressive behavior in HCC patients.

Next, we explored the prognostic potential of PDSS1 expression for OS from our present cohort by univariate and multivariate Cox regression analyses. The univariate analysis suggested that poor Child-Pugh grade (HR = 2.767, $P < 0.01$), bigger tumor size (HR = 2.064, $P < 0.001$), tumor thrombus (HR = 5.03, $P < 0.001$), tumor numbers (HR = 2.527, $P < 0.001$), vascular invasion (HR = 3.333, $P < 0.001$), advanced tumor stage (HR = 3.999, $P < 0.001$) and high PDSS1 expression (HR = 2.06, $P < 0.01$) significantly contribute to the worse OS (Table 2). The multivariate analysis demonstrated that high expression of PDSS1 was an independent risk factor for shorter OS in HCC patients (HR = 1.759, $P < 0.05$) (Table 2). The above studies suggested that an increased level of PDSS1 expression caused an adverse prognosis and may speed up tumor progression of patients with HCC.

Up-Regulation of PDSS1 Reduces the Level of CD8+T Cells Infiltration in Tumor

The infiltration of immune cells in a tumor is closely related to tumor progression and prognosis^{18,19}. Particularly, differentiated CD8+T cells in tumor tissue exert powerful antitumor effects; patients with an elevated level of CD8+T cell infiltration in tumors have a good prognosis.²⁰ We explored the relationship between PDSS1 expression and immune cells infiltration of B cell, macrophages, myeloid dendritic cell, CD4+ T cell, NK cell, and CD8+ T cell in HCC tissue by TIMER. The studies showed that an increased level of PDSS1 was positively correlated with the tumor-infiltration of B cells, macrophages, myeloid dendritic cells, and CD4+ T cells. In contrast, it reduced the level of CD8+T cell infiltration in tumors ($P < 0.05$) (Figure 3A). Nevertheless, differences were not found in NK cell or tumor purity ($P > 0.05$) (Figure 3A). Our present study also demonstrated that PDSS1 was expressed in the tumor microenvironment (Figure 1C).

To explore the relationship between immune cells infiltration and worse prognosis of HCC patients with elevated PDSS1 expression, we performed the KM plotter to analyze the survival of PDSS1 expression based on the immune cell subgroup. The studies suggested that up-regulation of PDSS1 was a significant indicator of poor prognosis in HCC patients regardless of whether enriched or decreased of B cell, macrophages, CD4+ T cell ($P < 0.01$) (Supplementary Figure 3A–C) or CD8+ T cell ($P < 0.01$) (Figure 3B).

Up-Regulation of PDSS1 Induces CD8+ T Cell Exhaustion by Promoting the Immunosuppressive Microenvironment

The decrease or exhaustion of CD8+ T cells is correlated with the tumor immunosuppressive microenvironment.^{21,22} Moreover, the tumor immunosuppressive microenvironment is mainly regulated by cell surface inhibitory receptors, immunoregulatory cell populations, and soluble factors.^{21,22} We used TIMER to explore the correlation between PDSS1 expression and immunosuppressive microenvironment from regulatory factors. The studies showed that high PDSS1 expression promoted the secretion of programmed cell death protein 1 (PD-1; rho = 0.154, $P < 0.01$), cytotoxic T lymphocyte-associated antigen-4 (CTLA4; rho = 0.182, $P < 0.001$), hepatitis A virus cellular receptor 2 (HAVCR2; r = 0.212, $P < 0.0001$), T cell immunoreceptor with Ig and ITIM domains (TIGIT; rho = 0.154, $P < 0.01$) (Figure 4A). The expression of these cell surface inhibitory receptors would deteriorate the function of CD8+ T cells.²¹ The primary immunosuppressive cells in the HCC microenvironment are myeloid-derived suppressor cells (MDSC), regulatory T cells (Treg), and tumor-associated macrophages (TAM).²⁰ The studies from TIMER indicated that PDSS1 expression was positively correlated with immune biomarker sets^{23,24} of MDSC (STAT3, CD80, ITGAM, CD33), Treg (CTLA4, FOXP3, CCR4, CCR8), and TAM (PD-1, CD68, CCR2, IL-10) (Figure 4B). Furthermore, soluble molecules, including immunosuppressive cytokines such as transforming growth factor- β (TGF β), IL-2, IL-7, and IL-10, are the third class of signals that regulate T cell exhaustion.²⁵

Table I PDSSI is Correlated with Early Tumor Recurrence in HCC Patients

Characteristics	n	PDSSI		χ^2	P
		Low	High		
Gender					
Female	23	14 (60.9%)	9 (39.1%)	0.645	0.422
Male	116	60 (51.7%)	56 (48.3%)		
Age (years)					
≤50	48	28 (58.3%)	20 (41.7%)	0.765	0.382
>50	91	46 (50.5%)	45 (49.5%)		
HBsAg					
Negative	17	11 (64.7%)	6(35.3%)	1.203	1.312
Positive	122	63 (51.6%)	59 (48.4%)		
AFP (μg/mL)					
≤20	54	29 (53.7%)	25 (46.3%)	0.108	0.743
≥20	69	35 (50.7%)	34 (49.3%)		
ALT (U/L)					
≤50	97	56 (57.7%)	41 (42.3%)	1.680	0.195
>50	42	18 (42.9%)	24 (57.1%)		
AST (U/L)					
≤40	82	50 (61.0%)	32 (39%)	4.810	0.028
>40	57	24 (42.1%)	33 (57.9%)		
Cirrhosis					
No	54	30 (55.6%)	24 (44.4%)	0.191	0.662
Yes	85	44 (51.8%)	41 (48.2%)		
Child-Pugh grade					
A	121	67 (55.4%)	54 (44.6%)	1.710	0.191
B	18	7 (38.9%)	11 (61.1%)		
Tumor size (cm)					
≤5	66	38 (57.6%)	28 (42.4%)	0.950	0.330
>5	73	36 (49.3%)	37 (50.7%)		
Tumor number					
Single	102	58 (56.9%)	44 (43.1%)	2.023	0.155
Multiple	37	16 (43.2%)	21 (56.8%)		
Tumor thrombus					
No	130	71 (54.6%)	59 (45.4%)	1.531	0.216
Yes	9	3 (33.3%)	6 (66.7%)		
Tumor capsule					
No	22	13 (59.1%)	9 (40.9%)	0.355	0.551
Yes	115	60(52.2%)	55 (47.8%)		
Tumor grade					
1–2	43	23 (46.4%)	20 (53.6%)	0.066	0.797
3–4	90	46 (17.9%)	44 (82.1%)		
Tumor stage (AJCC)					
I–II	109	61 (56.0%)	48 (44.0%)	1.507	0.220
III–IV	30	13 (43.3%)	17 (56.7%)		
Early recurrence					
No	78	48 (61.5%)	30 (38.5%)	5.541	0.019
Yes	54	22 (40.7%)	32 (59.3%)		

Notes: The correlation between PDSSI expression and clinicopathological parameters (age, gender, HBsAg, alpha-fetoprotein, alanine aminotransferase, aspartate aminotransferase, cirrhosis, Child-Pugh grade, tumor size, tumor number, tumor thrombus, tumor capsule, tumor grade, tumor stage, and early recurrence) were analyzed by data set of HCC from our cohort. Early recurrence indicates tumors were detected within 2 years after surgical operation. The bolded numbers indicate significance at the *P*-value < 0.05 level.

Table 2 PDSSI is an Independent Risk Factor for Patients with Hepatocellular Carcinoma

Characteristics	Univariate Analysis		Multivariate Analysis	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Age (year)				
≥50 vs.<50	1.390(0.849–2.277)	0.203		
Gender				
Male vs Female	2.051(1.078–3.902)	0.083		
Cirrhosis				
Cirrhosis vs Non- Cirrhosis	0.645(0.381–1.098)	0.076		
Grade classification				
Grade 1–2 vs Grade 3–4	0.983(0.596–1.621)	0.944		
Tumor capsule				
No vs Incomplete/ Complete	0.550(0.201–1.504)	0.115		
Child-Pugh				
B vs A	2.767(1.146–6.685)	<0.001	1.980(0.971–4.040)	0.06
Tumor size				
≥5cm vs.<5cm	2.064(1.269–3.357)	<0.01	1.380(0.758–2.514)	0.292
Tumor thrombus				
Yes vs No	5.03(0.737–34.31)	<0.001	1.424(0.488–4.155)	0.518
Tumor number				
Multiple vs Single	2.527(1.278–4.996)	<0.001	0.776(0.236–2.550)	0.676
Vascular invasion				
Vascular invasion vs None	3.333(1.629–6.818)	<0.001	2.241(0.963–5.213)	0.061
Stage classification				
Stage I–II vs Stage III–IV	3.999(1.778–8.990)	<0.001	2.073(0.497–8.655)	0.317
PDSSI expression				
High vs Low.	2.06(1.242–3.417)	<0.01	1.759(1.021–3.033)	0.042

Notes: The parameters from our cohort, including age, gender, HBsAg, alpha-fetoprotein, alanine aminotransferase, aspartate aminotransferase, cirrhosis, Child-Pugh grade, tumor size, tumor number, tumor thrombus, tumor capsule, tumor grade, tumor stage, early recurrence, and PDSSI expression in HCC were used for univariate Cox regression analyses and significant factors ($P < 0.05$) were used to further multivariate Cox regression analysis. The bolded numbers indicate significance at the P -value < 0.05 level.

These soluble molecules would accelerate the disintegration of CD8+ T cell cells.^{21,22,25} The data from TIMER indicated that PDSSI expression was positively correlated with secretion of TGF β , IL-2, IL-7, and IL-10 (Figure 4C).

Up-Regulation of PDSSI is Correlated with Multiple Immune-Related Signaling Pathways

To explore the potential immune-related signaling pathways of PDSSI, we used GSEA to analyze the RNA seq from TCGA. The studies suggested PDSSI expression was positively correlated with the WNT signaling pathway, P53 signaling pathway, TGF β signaling pathway, toll-like receptor signaling pathway, VEGF signaling pathway, nod-like receptor signaling pathway, FC epsilon RI signaling pathway, FC gamma R mediated phagocytosis, and MAPK signaling pathway ($P < 0.05$) (Figure 5). These pathways are associated with immune tolerance, immune inflammation, and inflammatory factors of tumors.^{26–29}

Discussion

Up-regulation of PDSSI speed up the progression of some tumors.^{7,8} Overexpression of circPDSSI caused increased proliferation, migration, and invasion rates of urothelial bladder cancer cells.⁷ High expression of PDSSI promoted the metastasis of triple-negative breast cancer and suggested patients had a shorter OS.⁸ Moreover, an increased level of PDSSI was positively correlated with the hypoxic microenvironment in tumors of HCC; the hypoxia of the tumor microenvironment inhibits tumor immune infiltration.^{9,10} In the present study, we found that PDSSI expression levels were associated with prognosis in different tumors. Elevated expression of PDSSI was correlated with tumor early

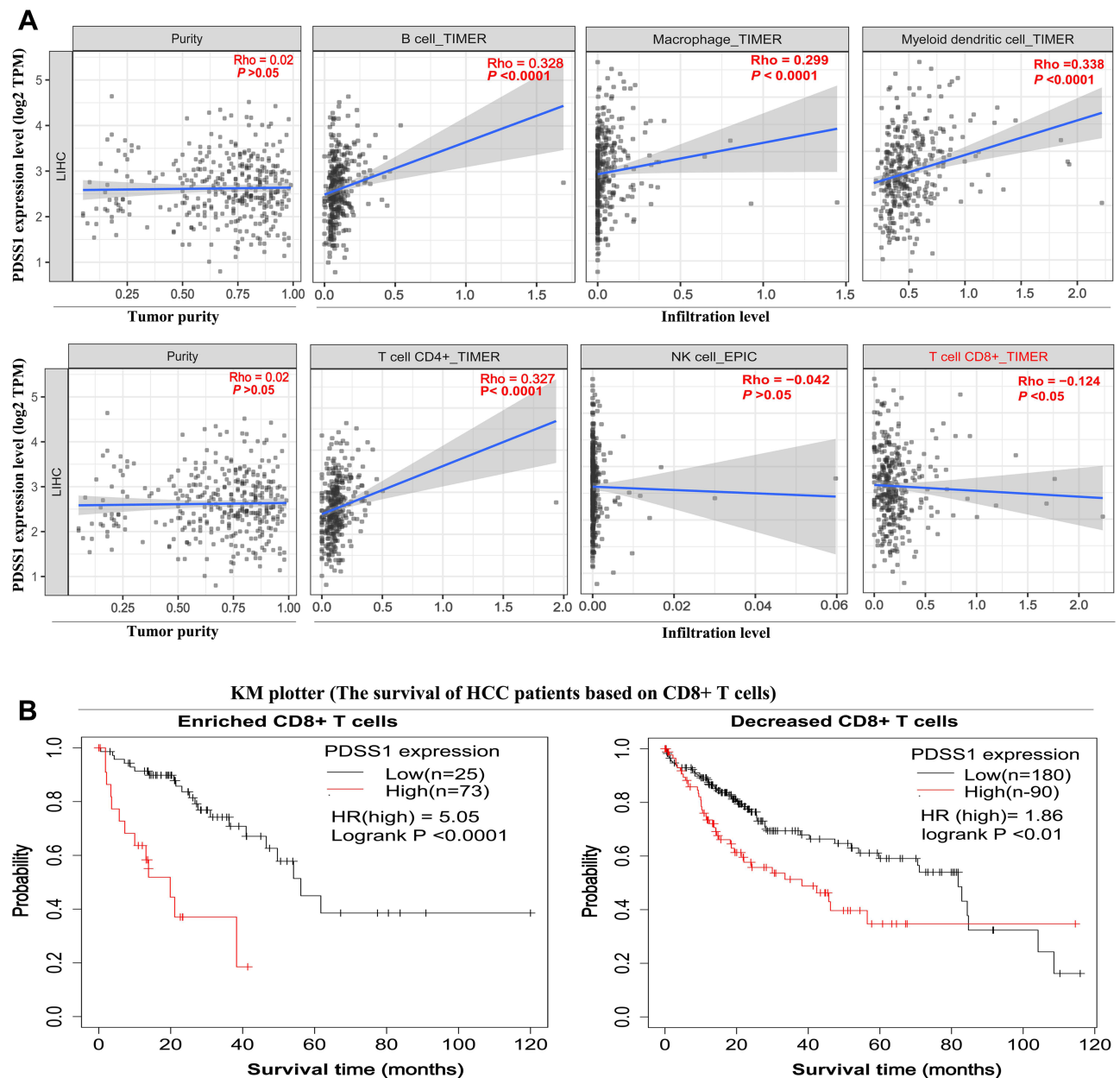


Figure 3 Up-regulated PDSS1 is associated with tumor immune infiltration in HCC patients. The correlation between PDSS1 expression and immune cells (B cells, macrophages, myeloid dendritic cells, CD4+ T cells, natural killer (NK) cells, and CD8+ T cells) were analyzed by Tumor Immune Estimation Resource (TIMER) (A). Kaplan-Meier survival analysis of the high and low group of PDSS1 expression in patients with HCC based on the number of tumor-infiltrating CD8+ T cells (B).

recurrence and identified as an independent prognostic factor for patients with HCC. Up-regulation of PDSS1 decreased the number of CD8+ T cells in tumor tissue and could induce T cell exhaustion by promoting the secretion of immunosuppressive markers in patients with HCC. Up-regulation of PDSS1 enhanced some immune-related signaling pathways, such as WNT, TGF β , and VEGF. Our studies identified that PDSS1 served as a prognostic biomarker and is a potential target of immunotherapy for HCC patients.

The aberrant expression and prognostic value of PDSS1 in pan-cancers remain unknown. In our present study, the results from TIMER and UALCAN databases consistently demonstrated the up-regulated PDSS1 in bladder urothelial carcinoma, breast invasive carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, cholangiocarcinoma, esophageal carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, prostate adenocarcinoma and stomach, and STAD.

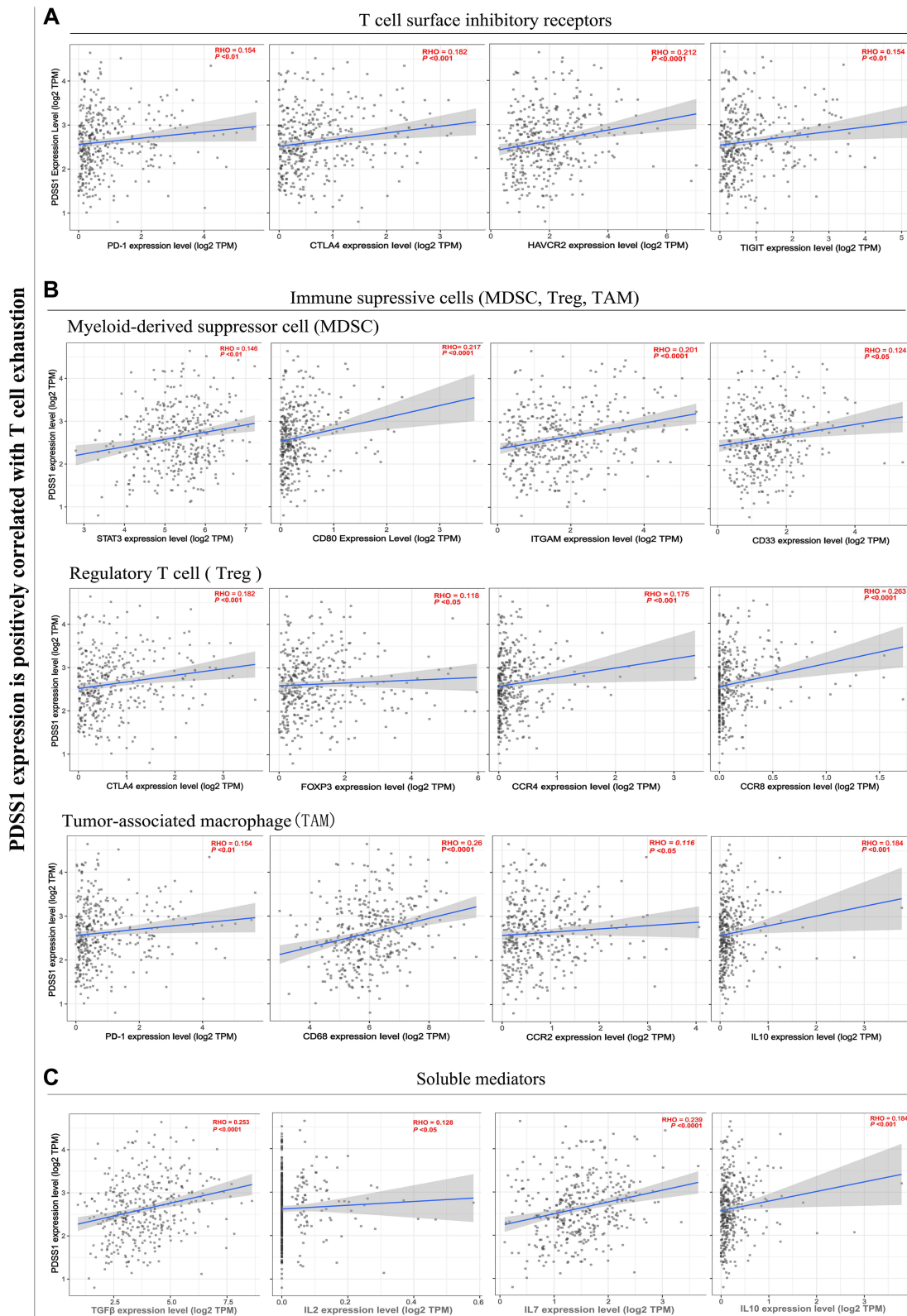


Figure 4 Up-regulated PDSS1 is positively correlated with tumor immunosuppressive microenvironment involved in T cell exhaustion. Correlation of PDSS1 expression with T cell surface inhibitory receptor (PD-1, CTLA4, HAVCR2, and TIGIT) (A). Correlation of PDSS1 expression with immunosuppressive cells (myeloid-derived suppressor cells, regulatory T cells, tumor-associated macrophages) (B). Correlation of PDSS1 expression with soluble mediators (TGFβ, IL-2, IL-7, IL-10) (C).

Immune inflammation-related signaling pathways

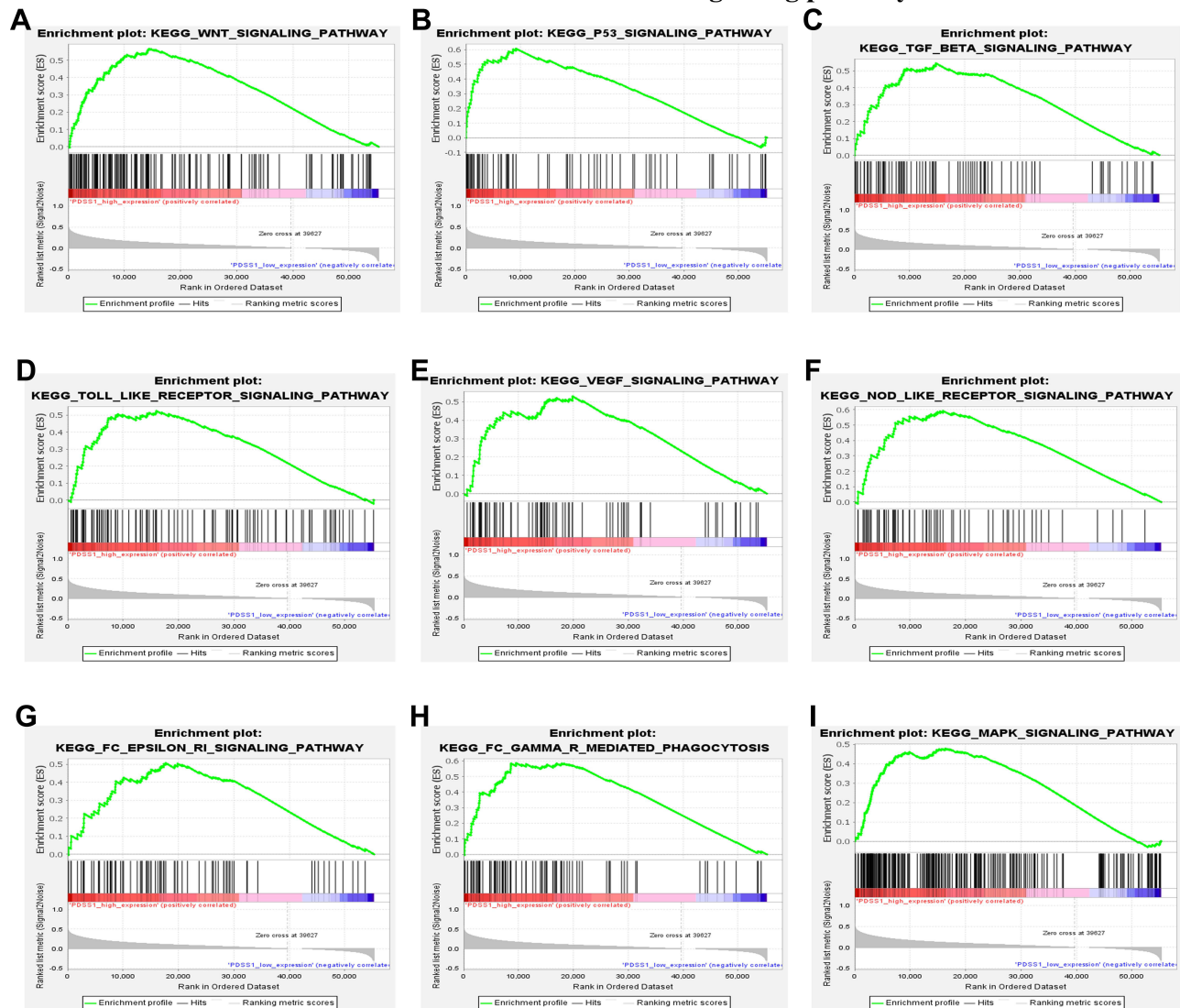


Figure 5 Up-regulated PDSS1 is significantly correlated with nine immune inflammation-related signaling pathways in HCC. The RNA seq from TCGA was analyzed by Gene Set Enrichment Analysis (GSEA). Wnt signaling pathway (A); P53 signaling pathway (B); TGF β signaling pathway (C); toll-like receptor signaling pathway (D); VEGF signaling pathway (E); nod-like receptor signaling pathway (F); FC epsilon RI signaling pathway (G); FC gamma R mediated phagocytosis (H); MAPK signaling pathway (I).

Moreover, GEPIA and the KM plotter consistently identified the expression of PDSS1 as a prognostic biomarker for SARC, STAD, thymoma, and HCC. We further explored the relationship between clinicopathological parameters and PDSS1 expression to provide more insight into the biological role of PDSS1 in the progression of HCC. The studies indicated that higher expression of PDSS1 is associated with a more advanced tumor stage, and identified PDSS1 expression as an independent risk factor for poor OS in patients with HCC. The above studies indicated PDSS1 played a vital role in the progression of HCC and could be used as a predictor for outcomes of patients with HCC.

Tumor-infiltrating immune cells, especially CD8⁺ T cells, and tumor microenvironment are closely related to the prognosis of patients with HCC.^{30–32} Therefore, we investigated the influence of PDSS1 expression on tumor immune infiltration in HCC. The decreased number of tumor-infiltrating CD8⁺ T cells deteriorated host immune defense against tumor progression and poor prognosis of HCC.^{33,34} Our studies revealed that high expression of PDSS1 reduced the number of CD8⁺T cells in HCC tissue. HCC patients with increased expression of PDSS1 had poor OS, despite increased or decreased levels of CD8⁺ T cells in tumor tissue. An increased level of PDSS1 induced T cell exhaustion mainly through three aspects: the secretion of T cell surface inhibitory receptors and soluble cytokines, and the proliferation and

migration of immunosuppressive cells in the tumor microenvironment of HCC. However, a decrease or exhaustion of T cells may cause uncontrolled tumor growth and worsen the prognosis among patients with HCC.^{35,36}

The progression of hepatocellular carcinoma often occurs in the activation background of cancer-promoting immune signals.³⁷ The activation of WNT signaling induces tumor immune tolerance, limiting the antitumor response and promoting the maintenance of Cancer Stem Cells.^{28,29} Excessive TGF β stimulation will induce tumor progression.²⁷ Our studies indicated that high expression of PDSS1 enhances the activity of multiple immune signaling pathways, including WNT, TGF β , VEGF, and other immune signaling pathways in HCC. These studies showed that increased expression of PDSS1 may promote the proliferation, invasion, and metastasis of hepatocellular carcinoma cells through the above pathways.

In summary, our study demonstrated that up-regulation of PDSS1 might help predict the poor prognosis for patients with HCC. PDSS1 can speed up the progression of HCC by reducing T cells' immune infiltration and regulating immune signals. PDSS1-targeted immunotherapy may be a promising strategy for patients with HCC.

Abbreviations

HCC, hepatocellular carcinoma; PDSS1, decaprenyl diphosphate synthase subunit 1; TIMER, Tumor Immune Estimation Resource; GEPIA, Gene Expression Profiling Interactive Analysis; GSEA, Gene set enrichment analysis; STAD, stomach adenocarcinoma; OS, overall survival; RFS, relapse-free survival; SARC, sarcoma.

Ethics Statement and Consent to Participate

The study was performed to comply with the Declaration of Helsinki principles and was approved by the First Affiliated Hospital of Jinan University Office for Protection of Human Subjects. Written informed consent was obtained from patients or their guardian.

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Disclosure

The authors declare that they do not have any competing interests in this work.

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