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ORIGINAL RESEARCH

LARS1 is a Prognostic Biomarker and Exhibits a Correlation with Immune Infiltrates in Hepatocellular Carcinoma

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Purpose: To study the relationship between LARS1 expression and immune infiltration and prognosis in hepatocellular carcinoma (HCC).

Patients and Methods: The clinical characteristics together with LARS1 expression levels were obtained from the TCGA database. Immunohistochemistry confirmed LARS1 expression levels in paraneoplastic and tumor tissues. To investigate LARS1-related downstream molecules, a network of protein-protein interactions (PPIs) and the Gene Ontology (GO)/Kyoto Encyclopedia of Genes and Genomes (KEGG) were built. Furthermore, gene set enrichment analysis (GSEA) was used to analyze the pathways associated with LARS1 expression, whereas Single-sample GSEA (ssGSEA) was applied to perform an association study between immune infiltration and LARS1 gene expression. The TISCH Database and the TISIDB database were used to compare the difference of LARS1 expression in hepatocellular carcinoma and immunomodulators.

Results: In comparison to that in normal tissues, the LARS1 expression level was elevated in tumor tissues. LARS1 expression exhibited substantial correlation with AFP, Histologic grade, pathologic stage, Residual tumor, and Vascular invasion in HCC. Higher LARS1 expression in HCC was linked to lower progression-free survival (PFS), disease-specific survival (DSS), and overall survival (OS). According to the GO/KEGG study, the important biological process (neutral lipid metabolic process), cellular component (triglyceride-rich plasma lipoprotein), molecular functions (lipase inhibitor activity), and KEGG pathway (cholesterol metabolism) could be a probable function mechanism in promoting HCC. Various pathways as per GSEA revealed that they were enriched in samples with elevated LARS1 expression. The expression level of LARS1 in malignant tumor cells after immunotherapy was significantly higher than that before immunotherapy. LARS1 was also remarkably linked to the infiltration level and the immunomodulators.

Conclusion: LARS1 can be used as a biomarker of HCC, which is associated to immune infiltration of HCC.

Keywords: hepatocellular carcinoma, leucyl-tRNA synthetase 1, bioinformatics analysis, biomarker, the cancer genome atlas

Introduction

Hepatocellular carcinoma (HCC) represents 75% of all malignancies affecting the liver,[1] making it the eighth most prevalent and third major cause of cancer death.[2] The overall prognosis of hepatocellular carcinoma has improved with the advent of the targeting and immunology era, but its 5-year overall survival rate remains low, particularly for patients with advanced hepatocellular carcinoma.[3,4] There is an urgent need for new targets in current clinical studies to better predict the overall prognosis of patients.

We are aware that the tumor microenvironment (TME) is a sophisticated ecosystem that is crucial to the growth and spread of cancer.[5] Immune cells like macrophages and immune cells infiltrate and proliferate, respectively in the TME. Additionally, angiogenesis is induced in TME, and TME is reportedly strongly linked to the development, survival, and metastasis of tumor tissues.[6] The tumor microenvironment of liver cancer is often immunosuppressive, resulting in

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immune escape and resistance to immunotherapy. Immune checkpoint inhibitors (ICIS) can transform the tumor immune microenvironment from cancer-promoting to anti-cancer by targeting inhibitory immune receptor signals.[7] Tumor immunotherapy has greatly changed the clinical treatment of HCC, immunotherapy approaches are increasingly focused on cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and apoptotic process protein 1(PD-1) monoclonal antibody, because these antibodies lock into the immune checkpoint inhibition pathway.[8] An immunomodulator is a substance that helps the body respond to disease by regulating and controlling the immune system. Immunomodulators includes immunoinhibitors and immunostimulators, More and more studies had shown that different immune phenotypes in solid tumors are closely related to gene types.[9,10]

A Protein-coding gene, Leucyl-tRNA synthetase 1 (LARS1), also named LARS, is a member of the aminoacyl-tRNA synthetase class I family. L-leucine ligation to tRNA is catalyzed by the encoded enzyme. It is present in the cytoplasm as part of a multisynthetase complex, and its C-terminal domain interacts with the arginine tRNA synthetase. LARS1 also controls protein synthesis, metabolism, autophagy, and cell growth by regulating the mechanistic target of rapamycin complex 1 (mTORC1) pathway.[11] LARS1 has been illustrated to play essential roles and might be potential therapy targets in various tumors, like Colon cancer,[12] Lung cancer,[13,14] Osteosarcoma[15] and breast cancer.[16] However, especially for hepatocellular carcinoma, the tumor prognosis of LARS1 is rarely reported. To further clarify whether changes in the tumor microenvironment in patients with HCC are associated with specific markers associated with patient prognosis, we conducted this study, hoping to bring new therapeutic ideas and methods for HCC diagnosis and management.

Materials and Methods

Download of TCGA, GTEx Database

The TCGA yielded 424 transcriptome gene expression profiles and provided clinical information on HCC. For further analysis, the 374 RNA-seq data that contained clinical information were transformed into transcripts per million reads (TPM) format. Missing value is defined as clinical information that is unavailable or unclear. The tumor samples were classified into two distinct categories, that is, low- and high-expression groups as per the LARS1 expression. The differentially expressed genes were identified using both Htseq Counts and the DESeq2-package. The thresholds for a statistical difference were determined at log2-fold change (log2 Fc) >0.585 as well as adjusted P value < 0.05. The GTEx database was used to retrieve the normal liver samples.

CPTAC Database Analysis

The degree of protein expression of LARS1 in HCC has been evaluated utilizing the clinical proteomic tumor analysis consortium (CPTAC) in the UALCAN database. At the same time, the differential methylation levels of LARS1 in HCC can also be found in the website.

Immunohistochemical (IHC) Assay

From August to September 2023, two patients with HCC who were identified at the first hospital affiliated with Bengbu Medical College provided tumor tissues that had been formalin-fixed and paraffin-embedded. We performed immunohistochemical verification of the expression levels of LARS1 in these two samples, and this experiment required antibody. The antibodies we used are as follows: a polyclonal rabbit antibody (Proteintech, cat. no. 21146-1-AP; IHC: 1:100–1:400).

GO/KEGG Analysis and the Development of the PPI Network

Bioconductor package "clusterProfiler" was utilized in the GO/KEGG analysis. On the other hand, to screen for single-gene correlations associated with LARS1 from the TCGA-HCC database, the R package (v3.6.3) was adopted. The *z*-test and the Pearson correlation value (≥0.5 or ≤-0.3, and P-value<0.001) examined the correlations between the amount of LARS1 expression and its co-expressed genes. The STRING database and Cytoscape (v3.9.1) facilitated the development of the PPI network.

The GSEA

In the realm of computational analysis, GSEA stands out as a technique that thoroughly considers the full gene expression matrix to derive meaningful insights. GSEA was used in this investigation to construct an orderly list of all genes based on their link to LARS1 expression and facilitate the analysis of 424 sample expression patterns. A total of 1000 gene set permutations were executed. The C2.cp.v7.2.symbols.gmt served as a reference gene set. Following correlation, the GSEA cutoff value of P<0.05 and FDR of <0.25 denote the statistical significance. The pathways enriched in every phenotype were sorted by the normalized enrichment score (NES) as well as an adjusted P-value. The GSEA enrichment was evaluated and visualized with the aid of the ClusterProfiler version 3.14 package.

Examination of Immune Infiltration

The single-sample GSEA (ssGSEA) approach was involved in examining the infiltration status of 24 immune cell types in the tumor.[17] The investigation of immune cell infiltration between LARS1 high- and low-expression groups, as well as the Pearman connection between LARS1 and the 24 categories of immune cells, was described above.

Tumor Immune Single-Cell LARS1 Expression and TISIDB

The Tumor Immune Single-cell Hub (TISCH), a database for single-cell RNA sequencing (scRNA-seq), was used to download single-cell gene expression data from all available cell types. Three cell type annotations were utilized to display the particular expression of LARS1 (LARS) from the Single-cell profile of LIHC_GSE125449, including immunological, malignancy, stromal, and treated with PD-L1 or CTLA-4, and the LARS1 expression visualization was accomplished using R package Seurat. The TISIDB database is a platform that merges disparate sources of data to investigate interactions between tumors and the immune system.[18] This database could help researchers uncover insights regarding how cancers and immune cells interact, identify new immunotherapy targets, and forecast responses to immunotherapies. It would be a useful tool in research encompassing immunology and its treatment. The TISIDB database was used in this study to look at the relationship of LARS1 with 13 immunostimulators and 15 immunoinhibitors in HCC.

Statistical Analysis

The R (version 3.6.3) was used in the execution of all statistical analyses. On the other hand, concerning unpaired and paired samples, the Wilcoxon rank-sum test and the Wilcoxon signed-rank test were utilized, respectively. The receiver operating characteristic (ROC) curve investigated whether LARS1 expression could serve as a diagnostic marker. The Kruskal–Wallis, as well as Wilcoxon signed-rank tests, examined the association between clinicopathological features and LARS1 expression. The chi-square, as well as the Fisher exact tests, were employed to ascertain the link between LARS1 expression and clinicopathological characteristics. We further utilized the Kaplan-Meier technique together with the Cox regression to analyze the relevance of LARS1 expression in prognosis. The multivariate Cox regression incorporated variables that exhibited statistical significance ($P < 0.05$) in the univariate analysis.

Result

Exploring the Link Between LARS1 Expression and Clinical Characteristics

We examined the expression data contained in the TCGA database to confirm if LARS1 expression had an impact on patients with hepatocellular cancer. The Immunohistochemistry findings affirmed that the levels of the LARS1 protein antibody were elevated in HCC (Figure 1E) than in the equivalent adjacent normal liver tissue (Figure 1F), confirming the variation in LARS1 expression levels (P <0.001) (Figure 1A and B). ROC curves ascertained LARS1's diagnostic utility, and its area under the curve (AUC) was 0.962, implying that it might be useful as a diagnostic biomarker (Figure 1G).

The Chi-square test or Fisher exact test yielded consistent outcomes. And The link between LARS1 expression and clinical features was also examined utilizing the Kruskal–Wallis test and the Wilcoxon signed-rank test (Table 1). A greater grade of Pathology stage ($P < 0.05$), AFP ($P < 0.05$), Histological grade ($P < 0.001$), Residual tumor ($P < 0.05$), and vascular invasion ($P < 0.05$) were all positively link to an increase in LRAS1 expression (Figure 2A–E). Moreover, the logistic regression showed that LARS1 expression was also closely related to clinical characteristics, including Pathologic T stage (OR = 1.525, 95%confidence

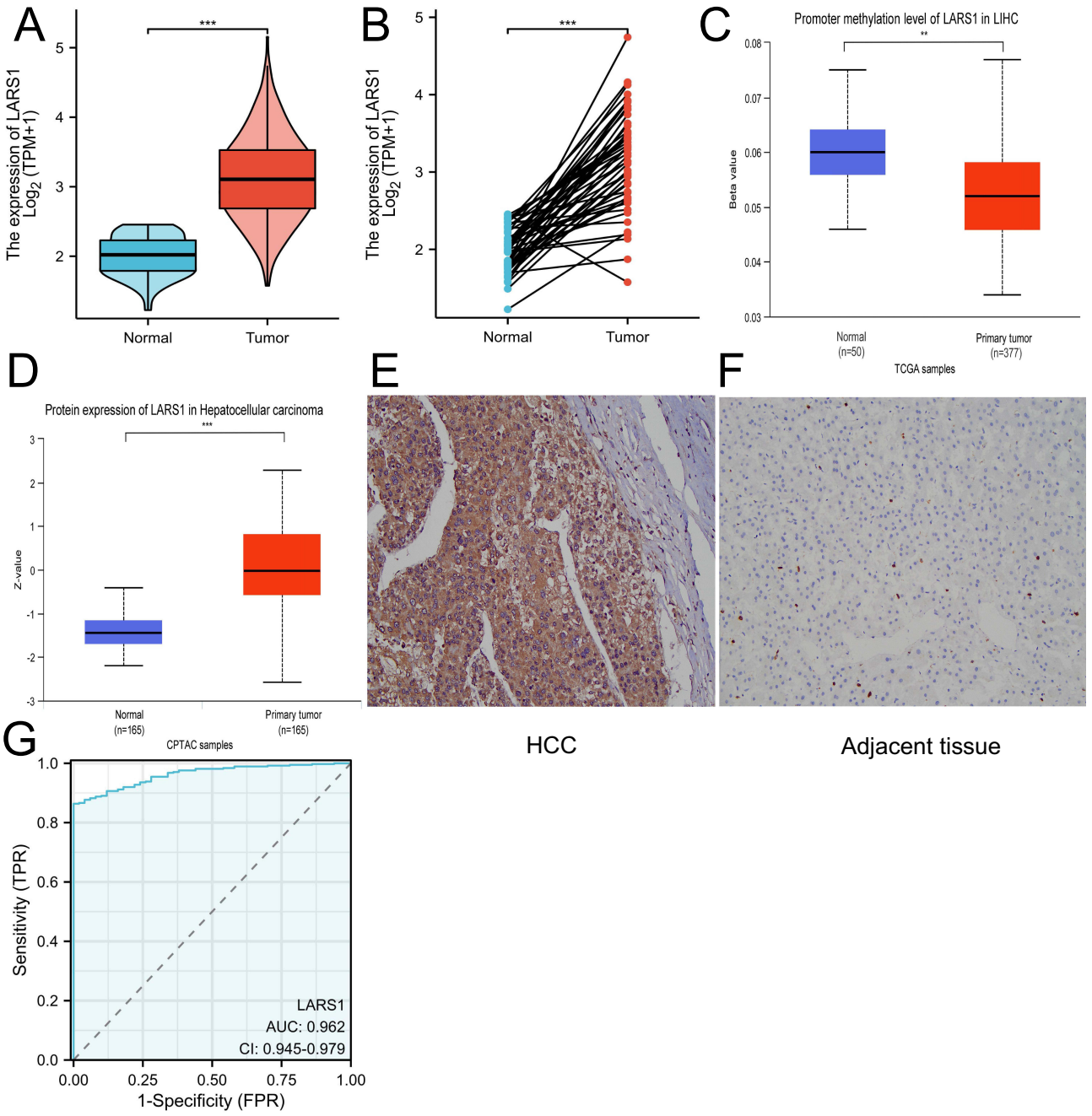


Figure 1 Relationship between LARS1 expression and Hepatocellular carcinoma (HCC). (**A**) Differential expression of LARS1 between tumor tissues and normal tissues. (***represents P<0.001). (**B**) Differential expression of LARS1 between tumor tissues and matched para-cancerous tissues. (***represents P<0.001). (**C**) The Promoter methylation level of LARS1 in HCC tissues and adjacent normal liver tissues. (**represents P<0.01). (**D**) The protein level of LARS1 in HCC tissues and adjacent normal liver tissues. (***represents P<0.001). (**E** and **F**) The results of IHC between the adjacent tissue and HCC. (**G**) Diagnostic value of LARS1 expression in HCC.

interval (CI): 1.013–2.296, P =0.043), Tumor status (OR = 1.566, 95%confidence interval (CI): 1.026–2.390, P =0.038), Histologic grade (OR = 2.186, 95%confidence interval (CI): 1.419–3.370, P < 0.001), and AFP (OR = 1.778, 95%confidence interval (CI): 1.013–3.118, P = 0.045) (Table 2).

Methylation and Protein Expression Levels of LARS1 in HCC

Following analysis of the levels of methylation of LARS in LIHC and normal tissues from the TCGA database, it was affirmed that the level of promoter methylation of LARS1 for tumor tissues was lower when compared to that for normal

Table 1 Link Between LARS1 Expression and Clinical Characteristics in HCC

Characteristics	Low Expression of LARS1	High Expression of LARS1	P value
n	187	187	
Pathologic T stage, n (%)			0.116
T1	101 (27.2%)	82 (22.1%)	
T2	44 (11.9%)	51 (13.7%)	
T3&T4	40 (10.8%)	53 (14.3%)	
Pathologic M stage, n (%)			0.594
M0	131 (48.2%)	137 (50.4%)	
M1	3 (1.1%)	1 (0.4%)	
Pathologic stage, n (%)			0.092
Stage I	96 (27.4%)	77 (22%)	
Stage II	43 (12.3%)	44 (12.6%)	
Stage III	35 (10%)	50 (14.3%)	
Stage IV	4 (1.1%)	1 (0.3%)	
Tumor status, n (%)			0.037
Tumor free	111 (31.3%)	91 (25.6%)	
With tumor	67 (18.9%)	86 (24.2%)	
Gender, n (%)			0.224
Female	55 (14.7%)	66 (17.6%)	
Male	132 (35.3%)	121 (32.4%)	
Age, n (%)			0.499
<= 60	85 (22.8%)	92 (24.7%)	
> 60	101 (27.1%)	95 (25.5%)	
BMI, n (%)			0.976
<= 25	91 (27%)	86 (25.5%)	
> 25	82 (24.3%)	78 (23.1%)	
Residual tumor, n (%)			0.045
R0	170 (49.3%)	157 (45.5%)	
R1&R2	5 (1.4%)	13 (3.8%)	
Histologic grade, n (%)			0.002
G1	36 (9.8%)	19 (5.1%)	
G2	98 (26.6%)	80 (21.7%)	
G3	47 (12.7%)	77 (20.9%)	
G4	5 (1.4%)	7 (1.9%)	
AFP (ng/mL), n (%)			0.043
<= 400	120 (42.9%)	95 (33.9%)	
> 400	27 (9.6%)	38 (13.6%)	
Albumin (g/dl), n (%)			0.346
< 3.5	40 (13.3%)	29 (9.7%)	
≥ 3.5	119 (39.7%)	112 (37.3%)	
Prothrombin time, n (%)			0.889
<= 4	107 (36%)	101 (34%)	
> 4	45 (15.2%)	44 (14.8%)	
Child-Pugh grade, n (%)			0.160
A	115 (47.7%)	104 (43.2%)	
B&C	15 (6.2%)	7 (2.9%)	
Vascular invasion, n (%)			0.149
No	116 (36.5%)	92 (28.9%)	
Yes	52 (16.4%)	58 (18.2%)	
OS event, n (%)			0.009
Alive	134 (35.8%)	110 (29.4%)	
Dead	53 (14.2%)	77 (20.6%)	

(*Continued*)

Table 1 (Continued).

Characteristics	Low Expression of LARS1	High Expression of LARS1	P value
DSS event, n (%)			0.057
No	151 (41.3%)	136 (37.2%)	
Yes	32 (8.7%)	47 (12.8%)	
PFI event, n (%)			0.049
No	105 (28.1%)	86 (23%)	
Yes	82 (21.9%)	101 (27%)	

tissues (P < 0.01) (Figure 1C). We also discovered from the CPTAC database that LARS1 protein expression levels are remarkably elevated in HCC tissues in comparison to normal tissues (P < 0.001) (Figure 1D).

The Impact of LARS1 on the Prognostic of HCC

After delving into the expression of LARS1 in HCC, we validated the prognostic value of LARS1 in HCC by survival analyses utilizing K-M curves. As presented in the pictures, LARS1 expression was negatively linked to OS, DSS, and PFI (OS: HR = 1.755, 95% confidence interval (CI):1.229–2.451, P = 0.002. DSS: HR = 1.786,95% confidence interval (CI):1.146–2.781, P = 0.010. PFI: HR = 1.501,95% confidence interval (CI):1.122–2.008, P = 0.006). We additionally develop an OS nomogram to integrate LARS1 and other prognostic factors, such as tumor status, pathologic T stage. The calibration curve examined performance of the nomogram of LARS1, and 0.653 was calculated to be the C-index of OS

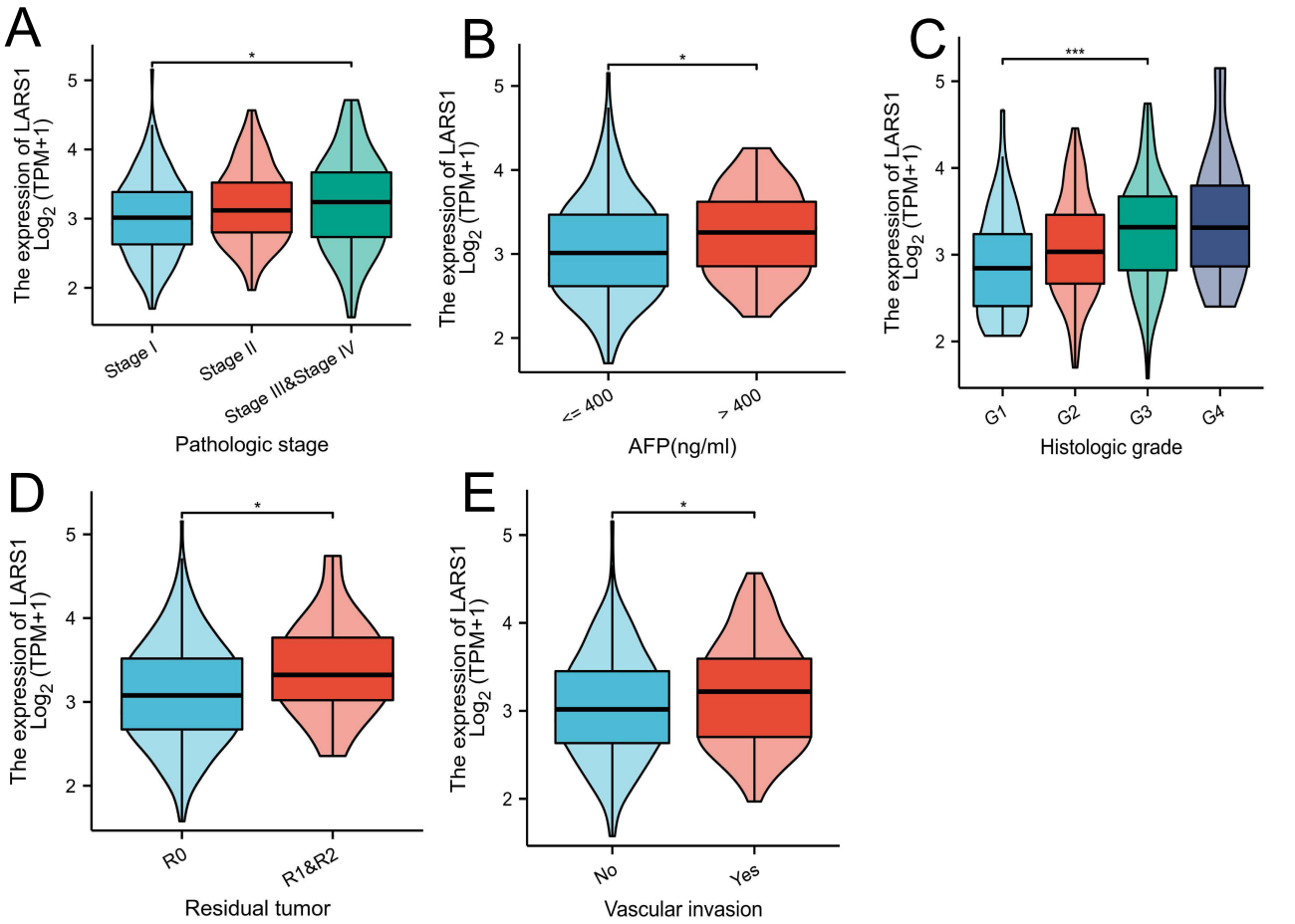


Figure 2 Correlation between LARS1 expression and clinical characteristics. (**A**) Pathologic stage. (*represents P<0.05). (**B**) AFP. (*represents P<0.05). (**C**) Histologic grade. (***represents P<0.001). (**D**) Residual tumor. (*represents P<0.05) (**E**) Vascular invasion. (*represents P<0.05).

Table 2 Logistic Regression Analysis of LARS1 Expression Association with Clinical Pathological Characteristics

Characteristics	Total (N)	OR (95% CI)	P value
Pathologic T stage (T2&T3&T4 vs T1)	371	1.525 (1.013–2.296)	0.043
Pathologic M stage (M1 vs M0)	272	0.319 (0.033–3.103)	0.325
Tumor status (With tumor vs Tumor free)	355	1.566 (1.026–2.390)	0.038
Gender (Male vs Female)	374	0.764 (0.495–1.180)	0.225
Age (> 60 vs <= 60)	373	0.869 (0.579–1.305)	0.499
Residual tumor (R1&R2 vs R0)	345	2.815 (0.981–8.077)	0.054
Histologic grade (G3&G4 vs G1&G2)	369	2.186 (1.419–3.370)	< 0.001
AFP (ng/mL) (> 400 vs <= 400)	280	1.778 (1.013–3.118)	0.045
Child-Pugh grade (B&C vs A)	241	0.516 (0.202–1.315)	0.166
BMI (> 25 vs <= 25)	337	1.007 (0.656–1.544)	0.976
Albumin (g/dl) (≥ 3.5 vs < 3.5)	300	1.298 (0.754–2.235)	0.346
Prothrombin time (> 4 vs <= 4)	297	1.036 (0.630–1.702)	0.889
Vascular invasion (Yes vs No)	318	1.406 (0.885–2.236)	0.149

(Figure 3A–E). In univariate analysis, Pathologic T stage, Tumor status, and LARS1 expression level was affirmed to influence the prognosis of patients with HCC ($P < 0.05$). A subsequent multivariate Cox regression analysis unveiled that pathologic T stage, Tumor status, and LARS1 expression emerged as independent prognostic risk factors of OS (HR =

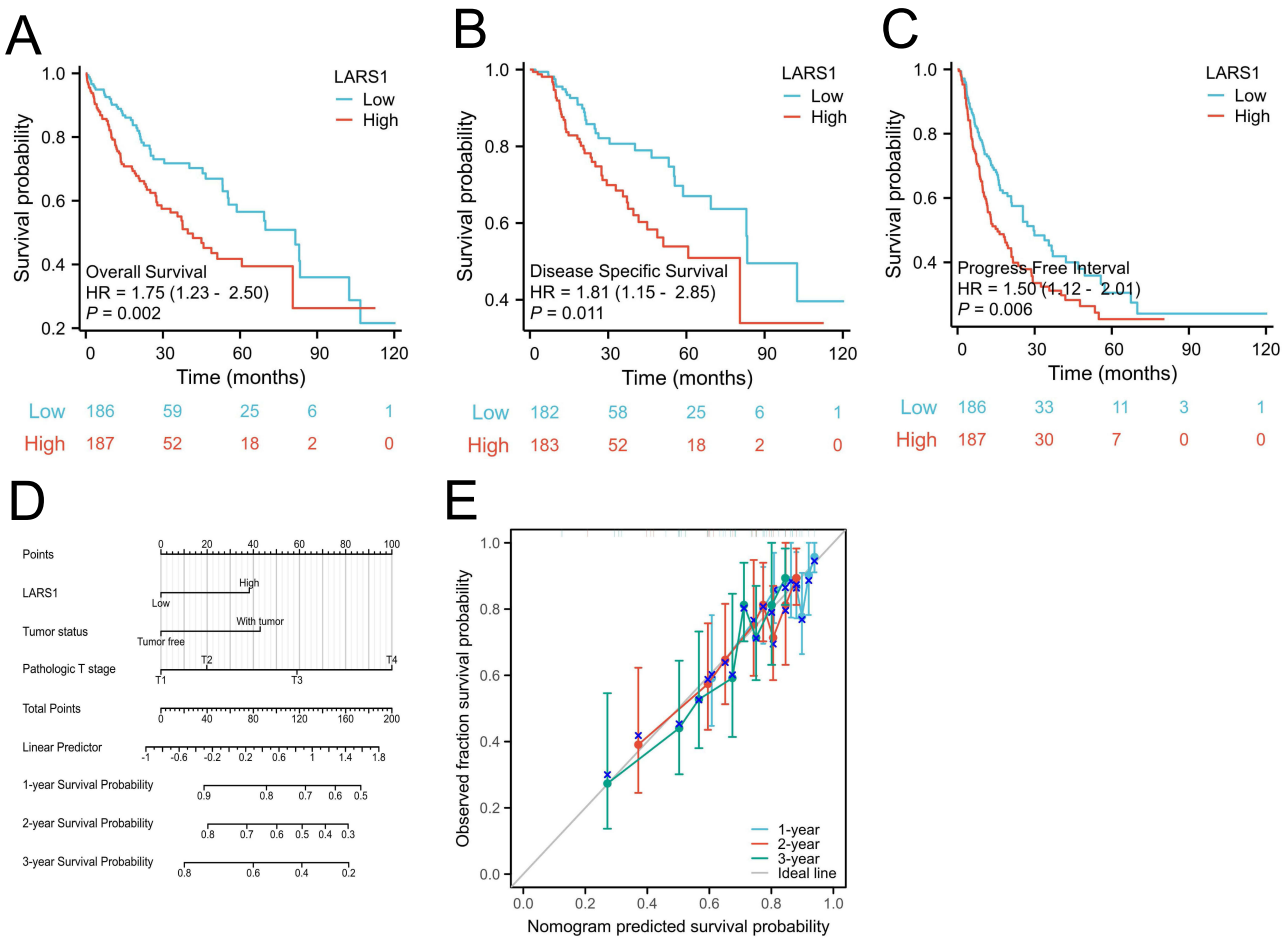


Figure 3 The expression level and survival analysis of LARS1 in HCC. (**A**) Overall Survival (OS). (**B**) Disease Specific Survival (DSS). (**C**) Progress Free interval (PFI). (**D**) Nomogram for predicting the 1-,3-, and 5-year overall survival rates. (**E**) The calibration curve of the nomogram.

1.728, 95% CI: 1.184–2.523) among patients with HCC (Table 3). The above findings implied that the LARS1's expression levels were linked to HCC patients' prognosis.

GO/KEGG and PPI Network Development for Genes Linked to LARS1 in HCC

In total, 4234 genes were affirmed to be linked to LARS1 expression in TCGA-HCC patients using single-gene correlation analysis (89 of the 4234 genes were negatively linked to LARS1 and 4145 of the 4234 genes were positively correlated with LARS1). A heatmap depicting the top 20 genes that are negatively or positively linked to LARS1 (Figure 4A). GO/KEGG

Table 3 Univariate and Multivariate Cox Proportional Hazards Analysis for LARS1 Expression

Characteristics	Total (N)	Univariate Analysis		Multivariate Analysis	
		Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)	P value
LARS1	373	1.755 (1.232–2.499)	0.002	1.728 (1.184–2.523)	0.005
Low	186				
High	187				
Pathologic T stage	370	2.949 (1.982–4.386)	< 0.001	2.457 (1.611–3.747)	< 0.001
T1	183				
T2	94				
T3&T4	93				
Tumor status	354	2.317 (1.590–3.376)	< 0.001	1.865 (1.266–2.748)	0.002
Tumor free	202				
With tumor	152				
Pathologic M stage	272		0.050		
M0	268				
M1	4	4.077 (1.281–12.973)	0.017		
Gender	373	0.793 (0.557–1.130)	0.204		
Female	121				
Male	252				
Age	373	1.205 (0.850–1.708)	0.293		
<= 60	177				
> 60	196				
BMI	336	0.798 (0.550–1.158)	0.234		
<= 25	177				
> 25	159				
Residual tumor	344	1.604 (0.812–3.169)	0.203		
R0	326				
R1&R2	18				
Histologic grade	368	1.162 (0.686–1.969)	0.792		
G1	55				
G2	178				
G3	123				
G4	12				
AFP (ng/mL)	279	1.075 (0.658–1.759)	0.773		
<= 400	215				
> 400	64				
Albumin (g/dl)	299	0.897 (0.549–1.464)	0.665		
< 3.5	69				
≥ 3.5	230				
Vascular invasion	317	1.344 (0.887–2.035)	0.169		
No	208				
Yes	109				
Child-Pugh grade	240	1.643 (0.811–3.330)	0.194		
A	218				
B&C	22				

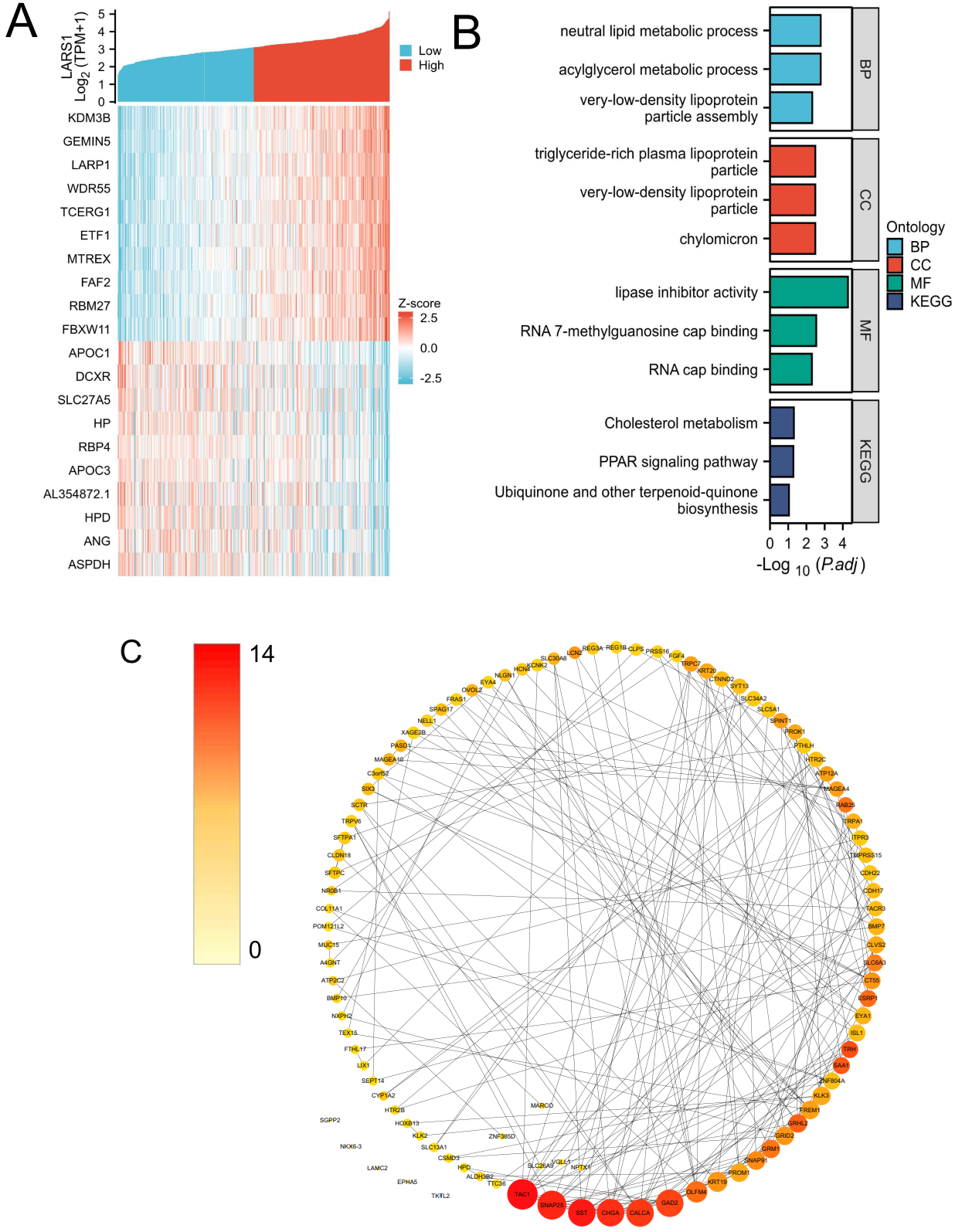


Figure 4 Network construction for LARS1 correlated genes in HCC. (**A**) Top 10 genes of positively or negatively correlated with LARS1 were shown in Heatmap. (**B**) GO analysis and KEGG pathway reveal the underlying mechanism of LARS1 in the promotion of HCC. (*P*.adj represents adjusted P values). (**C**) The PPI network of LARS1 interaction partners generated by STRING and Cytoscape, The color represents the degree score.

analysis was used to investigate major biological functions and pathways. According to GO analysis, the important biological process (neutral lipid metabolic process), cellular component (triglyceride-rich plasma lipoprotein), and molecular functions (lipase inhibitor activity) may all play a role in LARS1-related biology. It was affirmed by the KEGG pathway analysis that the PPAR signaling pathway, Cholesterol metabolism, Ubiquinone, and other terpenoid quinone biosynthesis are substantially enriched by the LARS1 co-expressed genes (Figure 4B). In addition, using STRING and Cytoscape, the top 100 genes that were either negatively or positively link to LARS1 were examined to build the PPI network (Figure 4C).

GSEA Shows the LARS1-Related Pathways in HCC

The most significant enrichment signaling pathway with the high LARS1 gene expression as per the NE, was selected (Table 4). The findings of the GSEA analysis inferred that the increased expression of the LARS1 phenotype was primarily mediated by the Retinoblastoma Gene in Cancer, Kinesins, Mitotic Prometaphase, Resolution of Sister Chromatid, Gastric Cancer Network 1, Endoderm Differentiation, MET Activates PTK2 Signaling, RAC1 GTPASE Cycle, and Ciliopathies (Figure 5A–I).

LARS1 Expression is Linked to HCC-Related Immune Infiltration Level

The distinct indicators of cancer survival are the levels of immune infiltration. We explored whether HCC-related immune infiltration level is correlated with the expression of LARS1. We discovered that LARS1 correlates positively with T helper cells, Tcm, Th2 cells, Tem, NK CD56bright cells, TFH, Macrophages, and NK cells while negatively relating to aDC, Eosinophils, iDC, Tgd, T cells, CD8 T cells, TReg, Mast cells, B cells, Neutrophils, Th17 cells, Cytotoxic cells, pDC, Th1 cells, and DC (Figure 6A). The following cells were ascertained to exhibit substantial variations in the expression levels of LARS1 among infiltrating immune cells via further analysis: pDC, Neutrophils, B cells, CD8 T cells, DC, CD56dim cells, T cells, T helper cells, Tem, cytotoxic cells, Tcm, Th17 cells, Th2 cells, and Tgd. (Figure 6B–O).

Effect of immunotherapy on the expression of LARS1 in various cells of HCC and its expression is linked to the immunomodulators in HCC

By analyzing the expression levels of LARS1 in hepatocellular carcinoma samples from the TISCH database, we found that after immunotherapy (PD-L1 or CTLA-4), the expression level of LARS1 in malignant tumor cells was remarkably different from that in untreated malignant tumor cells ($P < 0.05$) (Figure 7). Immunomodulators refer to substances involved in altering the function of the immune system. As per our research, LARS1 was remarkably linked to immunoinhibitors ($P < 0.05$) like CD274 (PD-L1) (rho = −0.127), LAG3 (rho = −0.155), PDCD1LG2 (rho = −0.197), TGFBR1 (rho = −0.114), TIGIT (rho = −0.115), KDR (rho = −0.131), CSF1R (rho = −0.127), IDO1 (rho = −0.115), BTLA (rho = −0.168), CD160 (rho = −0.106), and CD244 (rho = −0.132). However, no substantial link between the expression level of LARS1 and that of CTLA4 and PDCD1 (PD-1) was affirmed (Figure 8A–M). Moreover, LARS1's expression was affirmed to exhibit a close link to immunostimulators ($P < 0.05$) like TNFRSF8 (rho = −0.196), TNFSF13

Table 4 GSEA Results

Description	setSize	Enrichment Score	NES	P-value	Q-value	Rank
WP RETINOBLASTOMA GENE IN CANCER	87	0.622868215	2.71441	0.001677852	0.00913894	8376
REACTOME KINESINS	61	0.66095067	2.69785	0.001718213	0.00913894	7180
REACTOME MITOTIC PROMETAPHASE	203	0.541391286	2.68268	0.001547988	0.00913894	9127
REACTOME RESOLUTION OF SISTER CHROMATID COHESION	126	0.57312832	2.67445	0.001569859	0.00913894	8442
WP GASTRIC CANCER NETWORK 1	27	0.761169116	2.64598	0.001811594	0.00913894	6346
WP ENDODERM DIFFERENTIATION	142	0.55780763	2.64395	0.001560062	0.00913894	8934
REACTOME MET ACTIVATES PTK2 SIGNALING	30	0.744008528	2.63147	0.001801802	0.00913894	5357
REACTOME RAC1 GTPASE CYCLE	184	0.531804685	2.61636	0.001519757	0.00913894	10,144
WP CILIOPATHIES	177	0.534442382	2.60400	0.001545595	0.00913894	9087

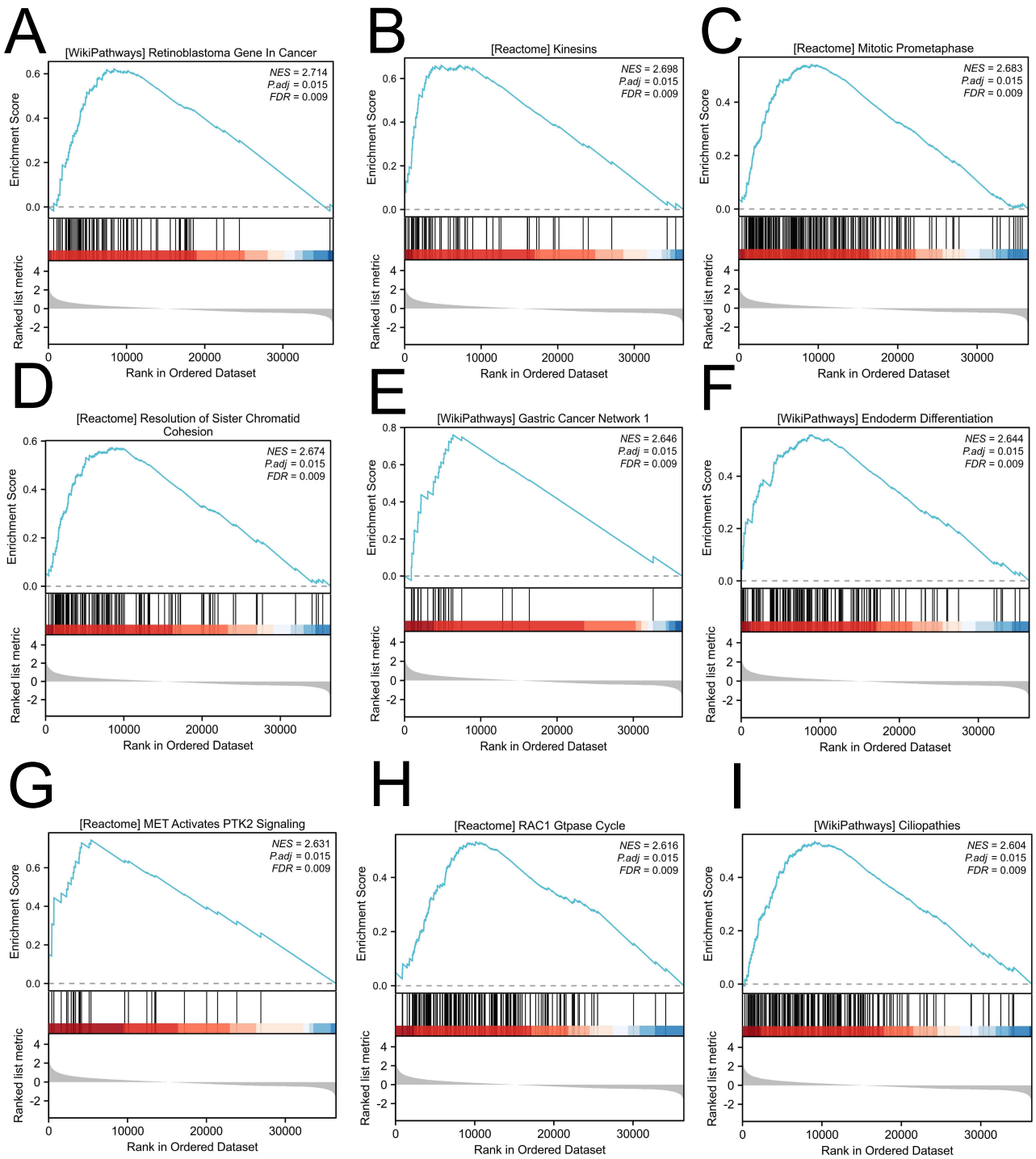


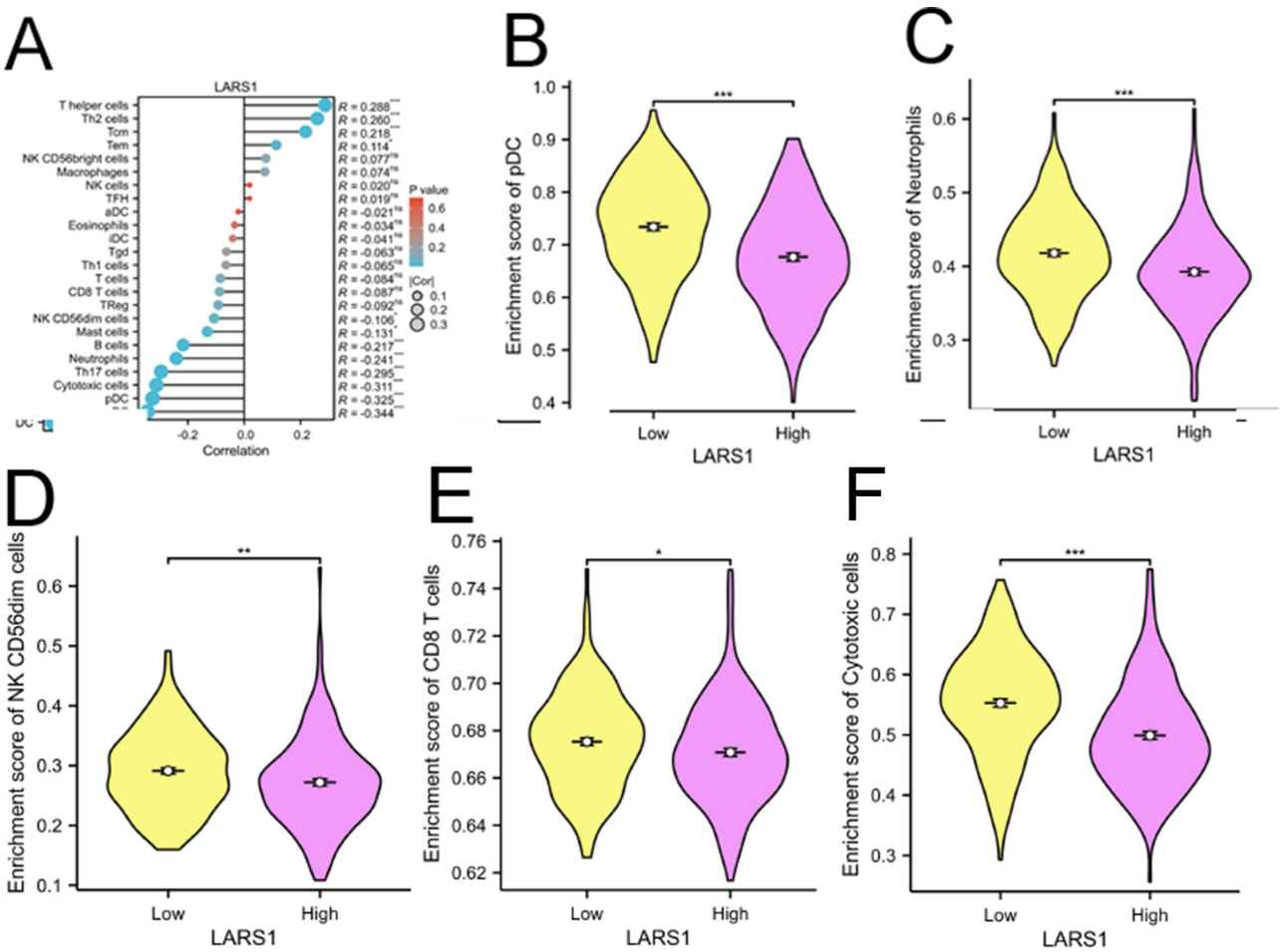
Figure 5 GSEA enrichment analysis results. (**A**) Retinoblastoma Gene in cancer. (**B**) Kinesins. (**C**) Mitotic Prometaphase. (**D**) Resolution of sister Chromatid Cohesion. (**E**) Gastric Cancer Network 1. (**F**) Endoderm Differentiation. (**G**) MET Activates PTK2 Signaling. (**H**) RAC1 Gtpase Cycle. (**I**) Ciliopathies.
Abbreviations: FDR, false discovery rate; NES, normalized Enrichment Score; (P)adj, represents adjusted P values.

(rho = −0.243), LTA (rho = −0.108), PVR (rho = 0.136), TMEM173 (rho = −0.182), CXCR4 (rho = −0.123), IL6 (rho = −0.232), IL2RA (rho = −0.113), KLRK1 (rho = −0.224), CD28 (rho = −0.182), CD48 (rho = −0.167), CD86 (rho = −0.123), CXCL12 (rho = −0.172), C10orf54 (rho = −0.166), and CD27 (rho = −0.178) (Figure 9A–O). LARS1, through these findings, is intimately engaged in the modulation of the immune interaction, and as a result, it has the potential to modulate tumor immune escape.

Discussion

A common treatment approach known as immunotherapy has been utilized in the treatment of several cancers such as HCC. Immunological cell infiltration in the immunological microenvironment has been linked to HCC immunosurveillance and immunotherapy.[19,20] Amino acids not only participate in the composition of proteins, but also participate in protein metabolism.[21] The mTORC1 pathway is related to the intracellular availability of amino acids and is also closely related to the occurrence and development of cancer.[22] LARS1 has been discovered as an intracellular leucine sensor involved in the activation of the mechanistic target of the rapamycin kinase (mTOR) signaling pathway.[23,24] LARS1 senses intracellular leucine concentration and mediates amino acid-induced mTORC1 activation.[25] The activation of mTORC1 is closely related to cancer, so the inhibition of LARS1 may be helpful in cancer treatment. We speculated whether LARS1 expression levels in HCC are related to tumor growth and development by the activation of mTOR signaling pathway.

Following analysis of bioinformatics, we discovered that LARS1 expression was enhanced in tumor samples from HCC patients than in normal liver tissue, affirming that LARS1 is involved in carcinogenesis. Simultaneously, the study discovered that an increase in LARS1 expression was inversely connected to the survival rate of individuals with hepatocellular carcinoma. In comparison to patients with low expression levels of LARS1, patients with higher LARS1 expression levels exhibited remarkably lower OS, DSS, and PFI. These results may help us evaluate the overall prognosis of HCC patients with high levels of LARS1 expression in the future.



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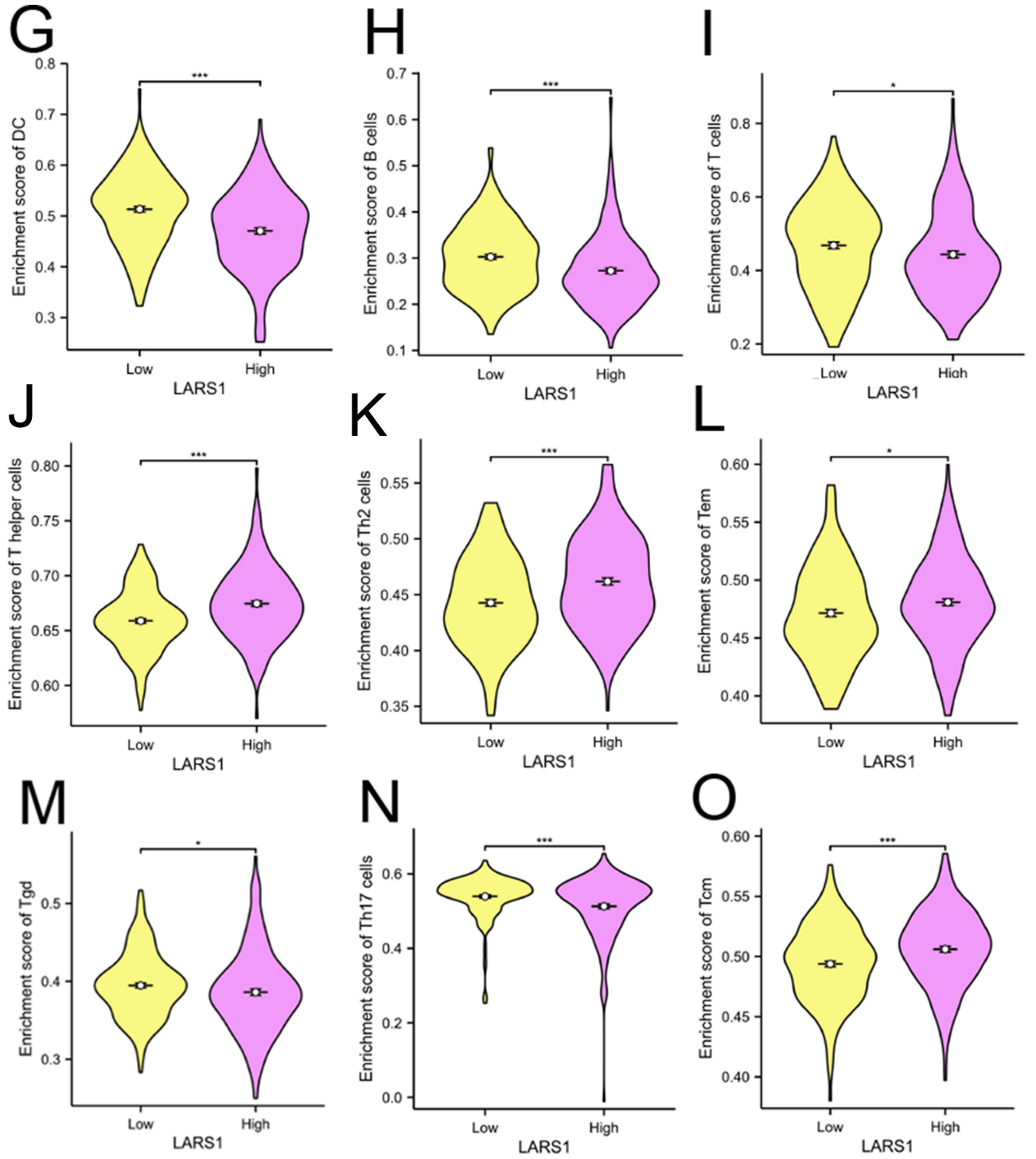


Figure 6 Relationship between LARS1 expression and immune infiltration. (**A**) Relationship between LARS1 expression and immune cells. (**B–O**) Different immune cell subpopulations in the high and low expression groups of LARS1. (ns represents No Significance, |Cor| represents the size of the dot and the absolute value of the correlation coefficient, *represents P<0.05, ***represents P<0.001).

In this investigation, we also discovered that pathological stage, histological grade, and AFP levels were greater in patients with high-expression LARS1 hepatocellular carcinoma. This shows that the clinical characteristics of patients may be directly related to the expression of LARS1. The data from the TCGA database was utilized for GSEA to facilitate further investigations on the impact of LARS1 on HCC. The findings showed that genes with higher expression

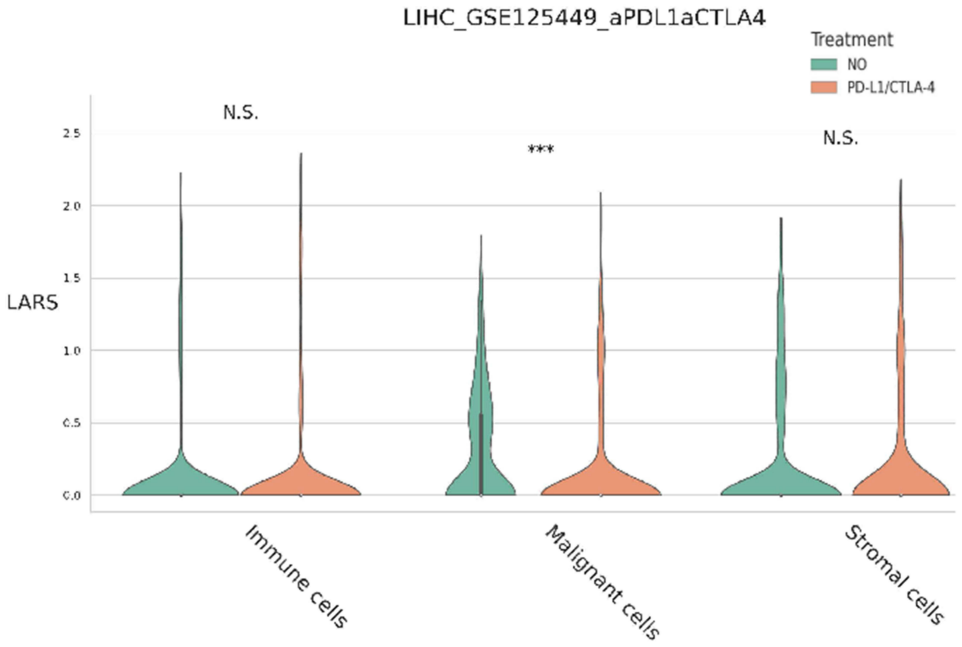
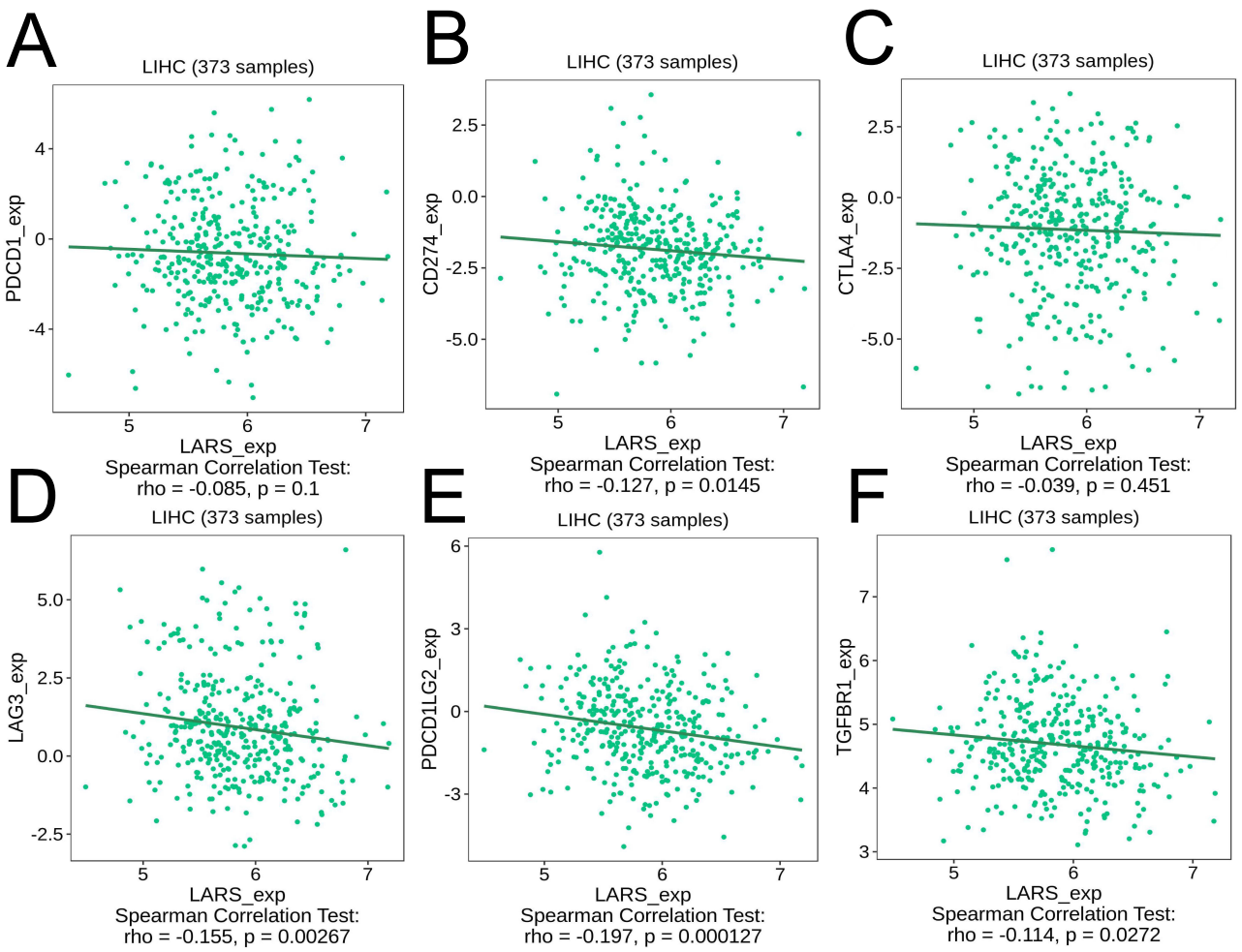


Figure 7 Compare the differences in LARS1 expression levels in Immune cells, Malignant cells, and stromal cells among HCC patients in the CTLA-4 or PD-L1 treatment group and the untreated group. (LIHC_GSE125449_aPDL1aCTLA4 reprents Single-cell transcriptome information in the GSE125449 dataset related to LIHC patients receiving PDL-1 or CTLA4 treatment, (N) S represents No Significance, ***represents P<0.001).



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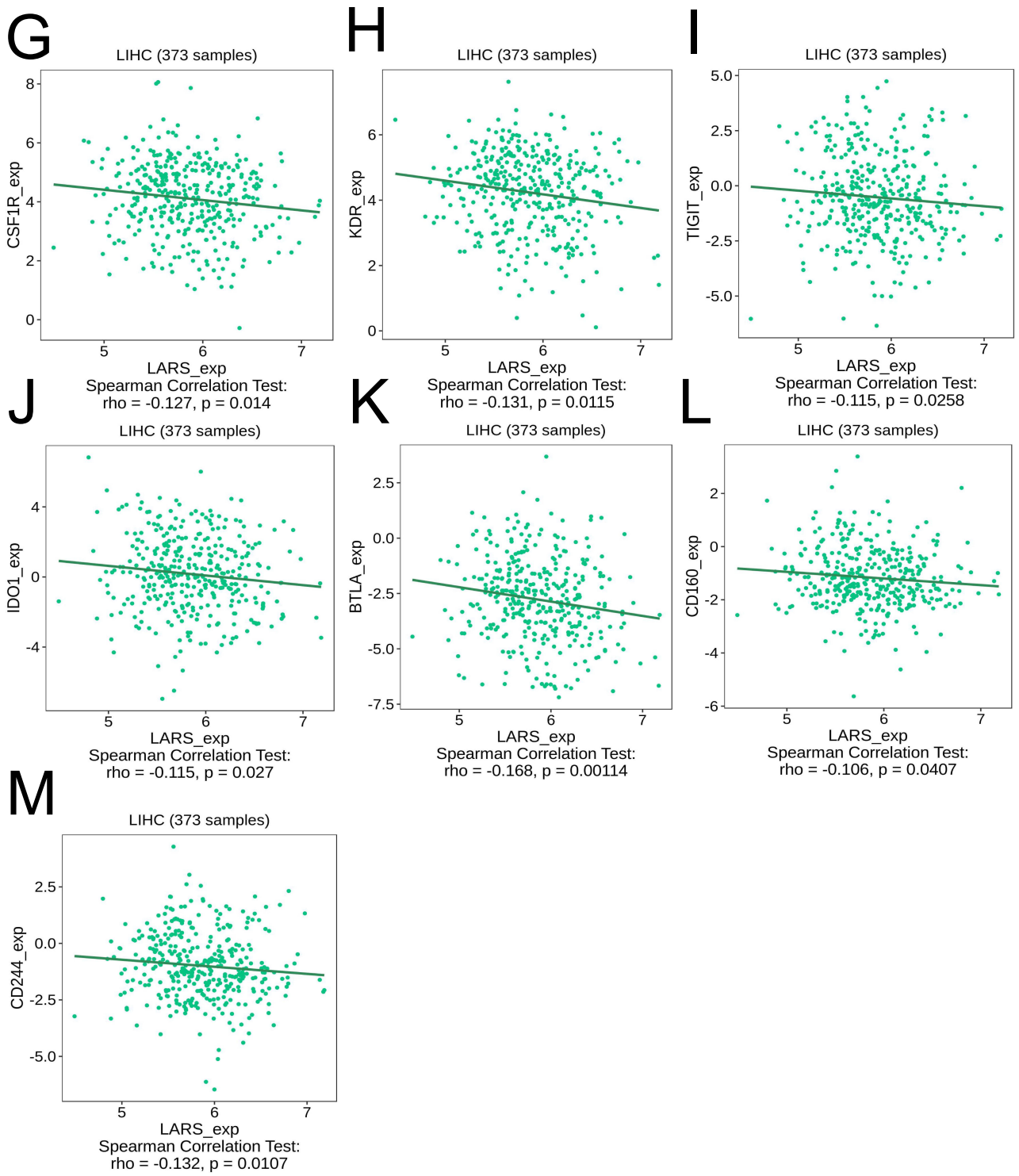
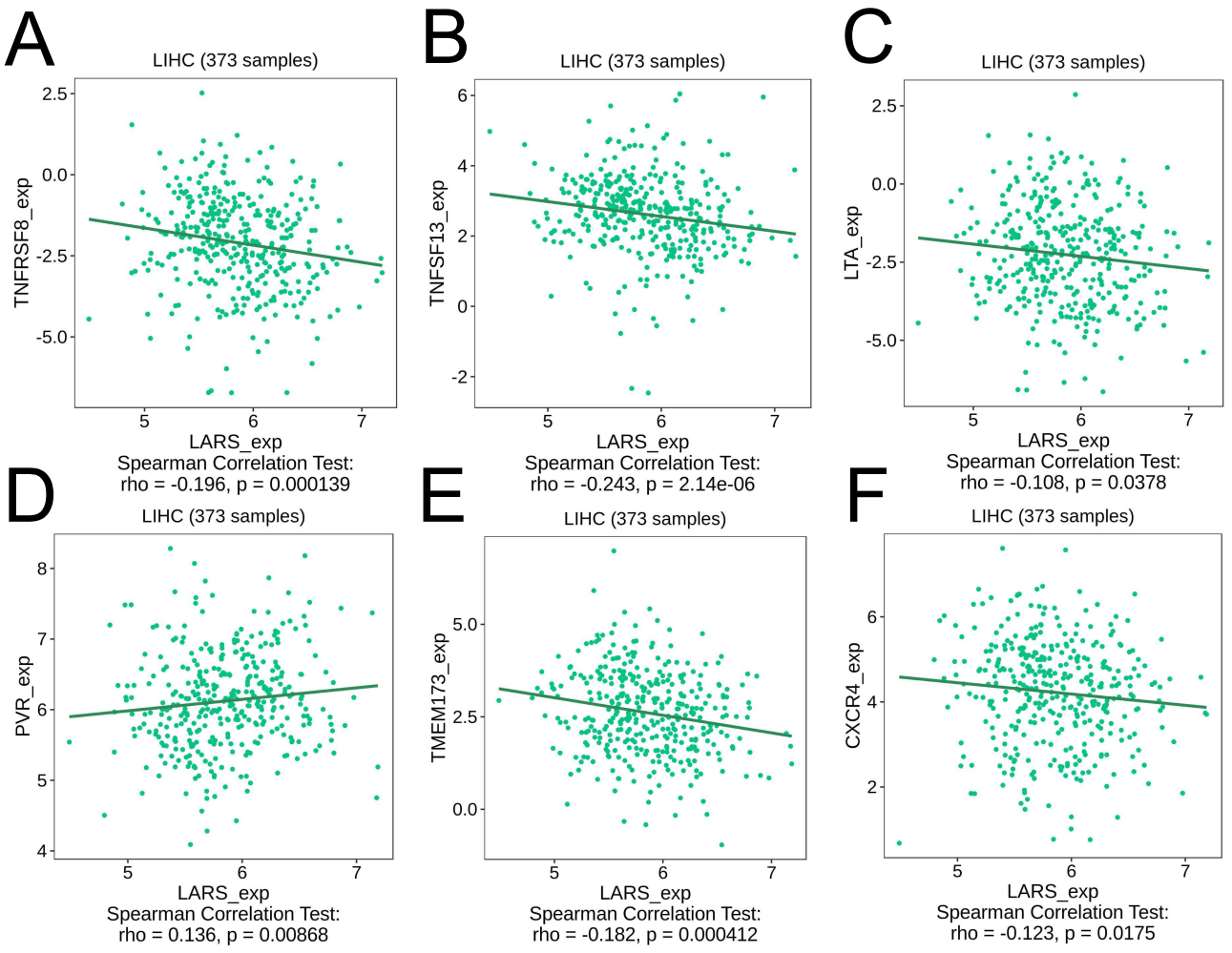


Figure 8 The expression of LARS1 (LARS) is associated with immunoinhibitors in HCC. (**A**) CTLA4. (**B**) CD274. (**C**) PDCD1. (**D**) LAG3. (**E**) PDCD1LG2. (**E**) TGFBR1. (**G**) TIGIT. (**H**) KDR. (**I**) CSF1R. (**J**) IDO1. (**K**) BTLA. (**L**) CD160. (**M**) CD244. (rho represents rank correlation coefficient, LARS_exp represents the expression level of LARS, CTLA4_exp represents the expression level of CTLA4, CD274_exp represents the expression level of CD274, PDCD1_exp represents the expression level of PDCD1, LAG3_exp represents the expression level of LAG3, PDCD1LG2_exp represents the expression level of PDCD1LG2, TGFBR1_exp represents the expression level of TGFBR1, TIGIT_exp represents the expression level of TIGIT, KDR_exp represents the expression level of KDR, CSF1R_exp represents the expression level of CSF1R, IDO1_exp represents the expression level of IDO1, BTLA_exp represents the expression level of BTLA, CD160 _exp represents the expression level of CD160, CD244_exp represents the expression level of CD244).

were greatly enriched in pathways linked to carcinogenesis, including the Retinoblastoma Gene in Cancer and the Gastric Cancer Network 1. In conclusion, there is a substantial link between the expression level of LARS1 and the morbidity and prognosis of HCC.

The TME in HCC is composed of multiple immune cell subpopulations, which are closely related to tumor development. Tumor-infiltrating lymphocytes are composed of T cells, B cells, and NK cells, and tumor-infiltrating lymphocytes participate in the anti-tumor immunity. In order to get over the limitations of computation methods, we scrutinized the link between LARS1 expression and immune cell levels by screening the transcriptome data. This might aid in illustrating how the immune system infiltrates malignancies. This study found that a substantial negative correlation between LARS1 expression and aDC, Eosinophils, iDC, Tgd, Th1 cells, T cells, CD8 T cells, pDC, TReg, NK CD56dim cells, Mast cells, B cells, Neutrophils, Th17 cells, Cytotoxic cells, and DC was discovered. Especially in cancer immunology, these immune cells have a significant impact. There is no doubt that the majority of immune cells have the potential to improve therapeutic results. The immune cells may promote or inhibit tumor progression by changing the tumor immune microenvironment.[26] However, the results of some studies on pathways related to the tumor immune microenvironment were still uncertain or unsatisfactory. Compared with immunotherapy alone, targeted therapy combined with immunotherapy has become the first-line treatment for advanced HCC. The mTOR pathway related drug combined with immunotherapy involved in this study may provide a new treatment idea for clinical use.

We discovered that LARS1's expression level in malignant tumor cells was considerably greater in HCC patients after immunotherapy (PD-L1 or CTLA-4) than before immunotherapy by scanning the TISCH database. Immunomodulators are



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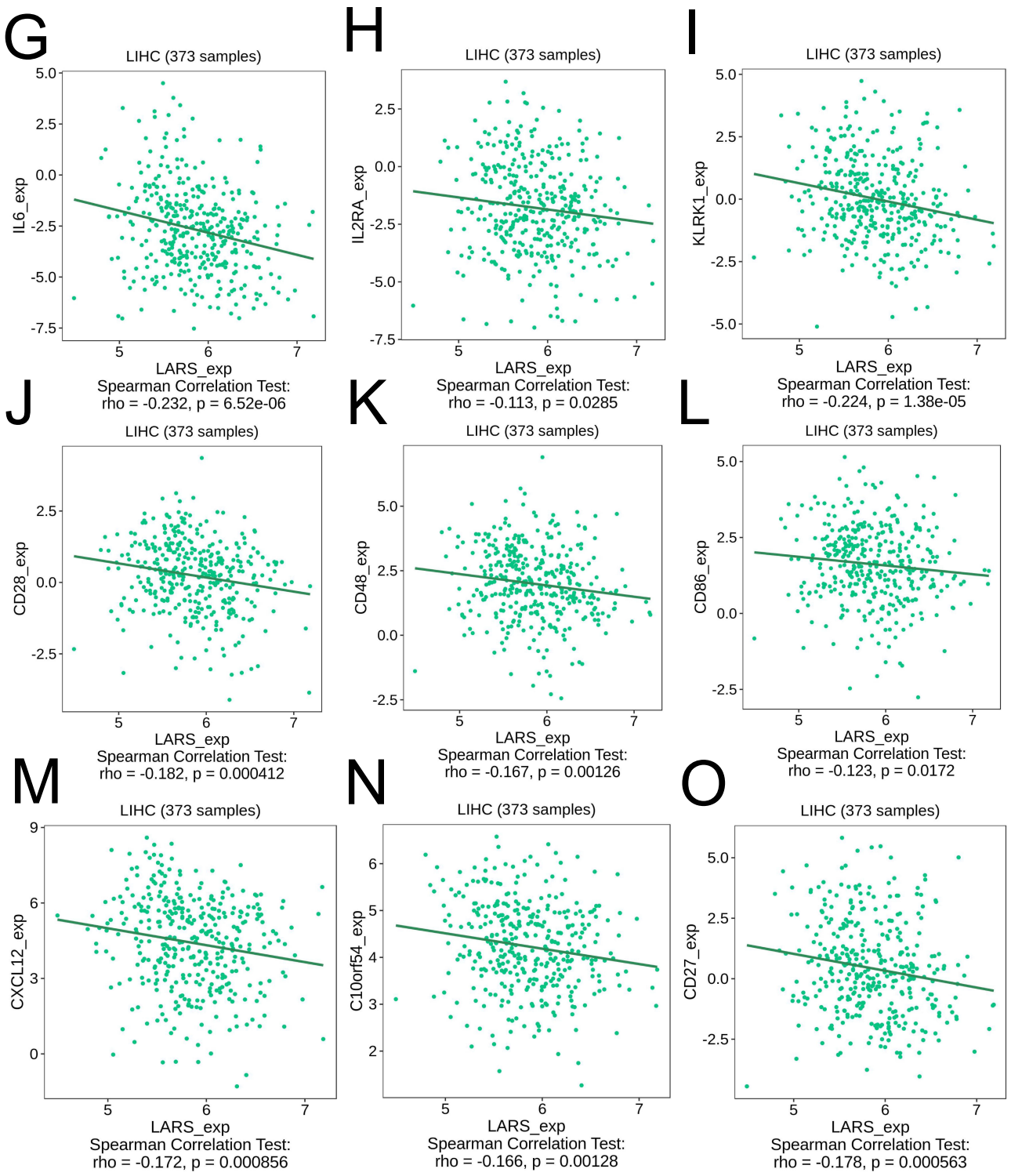

Figure 9 The expression of LARS1 (LARS) is associated with immunostimulators in HCC. (**A**) TNFRSF8. (**B**) TNFSF13. (**C**) LTA. (**D**) PVR. (**E**) TMEM173. (**F**) CXCR4. (**G**) IL6. (**H**) IL2RA. (**I**) KLRK1. (**J**) CD28. (**K**) CD48. (**L**) CD86. (**M**) CXCL12. (**N**) C10orf54. (**O**) CD27. (rho represents rank correlation coefficient, LARS_exp represents the expression level of LARS, TNFRSF8_exp represents the expression level of TNFRSF8, TNFSF13_exp represents the expression level of TNFSF13, LTA_exp represents the expression level of LTA, PVR_exp represents the expression level of PVR, TMEM173_exp represents the expression level of TMEM173, CXCR4_exp represents the expression level of CXCR4, IL6 _exp represents the expression level of IL6, IL2RA_exp represents the expression level of IL2RA, KLRK1 _exp represents the expression level of KLRK1, CD28_exp represents the expression level of CD28, CD48_exp represents the expression level of CD48, CD86_exp represents the expression level of CD86, CXCL12_exp represents the expression level of CXCL12, C10orf54_exp represents the expression level of C10orf54, CD27_exp represents the expression level of CD27).

chemicals that impact the immune system's activity. We also discovered no significant link between LARS1 expression levels and CTLA-4 and PDCD1 (PD-1), implying that PD-L1 therapy may be a factor influencing LARS1 levels in HCC malignant tumors. Furthermore, our study discovered a significant link between PVR and the level of LARS1. We also discovered that PVR's expression level was strongly linked to the prognosis of various malignancies, with high expression of PVR in HCC patients indicating a poor prognosis.[27–30] These findings indicate that LARS1 may engage in the pathogenesis, development, and prognosis of HCC.

Despite the fact that our study discovered that LARS1 and HCC are closely associated in terms of clinicopathologic features, prognosis, and tumor microenvironment, there are significant gaps. First and foremost, we employed only a single database to execute our analysis, and the findings require cross-validation by different databases. Second, because the data were not validated by in vitro as well as in vivo investigations, we were unable to confirm its precise molecular mechanism. As a result, we may miss some key biological facts in our research.

Conclusion

Higher expression of LARS1 is linked to poorer outcomes in HCC. Further research on the correlation between LARS1 expression and immune infiltration inferred that LARS1 can act as a novel target for HCC-related immunotherapy.

Data Sharing Statement

The TCGA database (http://portal.gdc.cancer.gov/), The GTEx database (https://gtexportal.org/), the UALCAN database (https://ualcan.path.uab.edu/), STRING database (http://cn.string-db.org/), The Tumor Immune Single-cell Hub (TISCH) database (http://tisch.comp-genomics.org/home/), and the TISIDB database (http://cis.hku.hk/TISIDB/index.php) are the sources of datasets utilized and evaluated in the study.

Statement of Ethics

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Bengbu Medical University (approve No.2022322). All patients provided the informed written consent. And The study complied with the Declaration of Helsinki.

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Disclosure

The authors claimed that they have no competing interests in this work.

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