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ORIGINAL RESEARCH

High PD-L2 Expression Is Associated with Better Disease-Free Survival in Patients with Intrahepatic Cholangiocarcinoma

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Objective: PD-L2 is expressed in various cancer tissues. Currently, the value of PD-L2 expression in intrahepatic cholangiocarcinoma (ICC) tissues remains unclear. This study investigated the prognostic and clinical significance of PD-L2 expression in ICC.

Methods: This research employs The Cancer Genome Atlas (TCGA) database to examine the expression profiles of PD-L2 in ICC and to evaluate its prognostic significance. Immunohistochemistry detected PD-L2 expression in 66 postoperative ICC tissues and paired adjacent tissues. Correlations between PD-L2 expression and clinicopathological data were analyzed. Kaplan-Meier survival analysis and Cox proportional hazards regression model were used to evaluate the prognostic value of PD-L2.

Results: Bioinformatics analysis shows that high expression of PD-L2 dominates in early ICC patients and is closely associated with tumor recurrence. High PD-L2 expression is associated with longer progression free survival (PFS) (P=0.015). Immunohistochemistry confirmed that the expression of PD-L2 was significantly increased in tumor tissue compared to surrounding tissue. Overexpression of PD-L2 is associated with early TNM staging (P=0.014). Univariate analysis identified preoperative symptoms, number of tumors, tumor size, tumor differentiation, peritumoral integrity, surgical margins, TNM stage, and preoperative CA125 level as associated with overall survival (OS) (all $P < 0.05$). Preoperative symptoms, number of tumors, peritumoral integrity, surgical margins, TNM stage, preoperative CA125, and PD-L2 expression were associated with disease-free survival (DFS) (all $P < 0.05$). Multivariate analysis determined tumor differentiation and TNM stage as independent prognostic factors for OS (both $P < 0.05$). Surgical margins and PD-L2 expression levels were independent prognostic factors for DFS (both $P < 0.05$).

Conclusion: PD-L2 expression is predominantly high in patients with early-stage ICC. PD-L2 expression serves as an independent predictor of DFS in patients with ICC following hepatectomy. ICC patients with elevated PD-L2 expression levels exhibit delayed relapse.

Keywords: intrahepatic cholangiocarcinoma, programmed death ligand-2, immunohistochemistry, bioinformatics, prognosis

Introduction

Intrahepatic cholangiocarcinoma (ICC) is a malignant tumor originating from the epithelial cells of the intrahepatic bile ducts. Its incidence rate is second only to that of hepatocellular carcinoma, accounting for 10–15% of primary malignant liver tumors.[1–3] In recent years, both the incidence and mortality rates of ICC have continuously increased.[4,5] Owing to the lack of distinct clinical symptoms in ICC patients during the early stages, the majority of patients are diagnosed at an advanced stage of cancer and miss the opportunity for surgical treatment, resulting in a low rate of surgical resection.[6] Even after effective radical surgical resection, the overall 5-year survival rate is only 20% to 35%.[7,8] Unfortunately, most patients with ICC eventually relapse and die from the disease even after standard treatment.[8,9] Therefore, the clinical prognosis of ICC patients is

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very poor, and the search for novel biomarkers and new targets for treatment is particularly important for improving the early diagnosis rate of ICC and the prognosis of patients, thus reducing mortality.

Programmed death ligand-2 (PD-L2) is a common immune signaling molecule belonging to the B7 family. It plays a powerful biological role in the tumorigenesis process, such as promoting tumor invasion and triggering tumor chemotherapy resistance.[10] Tumor cells induce the expression of PD-L2, which facilitates the evasion of immune surveillance, thereby preventing the identification and elimination of cancer cells and subsequently promoting tumor proliferation, invasion, and metastasis.[11–13] Several studies have shown that PD-L2 is overexpressed and highly correlated with the clinical prognosis of patients with various solid tumors, including lung adenocarcinoma,[14] esophageal cancer,[15] gastric cancer,[16] hepatocellular carcinoma,[17] pancreatic ductal adenocarcinoma,[18] and bladder cancer.[19] These findings indicate that PD-L2 plays an important role in the tumorigenesis, invasion and metastasis of multiple malignancies. PD-L2 is a promising target for immunotherapy, and understanding its mechanism is expected to improve the treatment and prognosis of related malignant tumors.

However, there are no relevant studies on the effects of PD-L2 on ICC.Therefore, in this study, we examined PD-L2 expression in ICC by bioinformatics analysis and immunohistochemical staining. We analyzed the correlation of PD-L2 expression with clinicopathological characteristics and its prognostic significance in ICC patients after hepatectomy.

Materials and Methods

Material Collection and Data Sources

We downloaded the RNA-seq data (level 3) and corresponding clinical information of 35 ICC patients from TCGA dataset (The Cancer Genome Atlas, https://portal.gdc.com). R software version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) was used to conduct the statistical analyses. We extract data from the raw compression package to obtain detailed clinical information and expression data file, which formed the basis for information integration, resulting in a dataset containing 44 samples. PDCD1LG2 (PD-L2) gene expression was detected in 35 ICC tissue samples and 9 paracancerous control samples. Each sample included the corresponding basic information (sex, age, race) and clinical information (stage, survival time, survival status, etc.) of the ICC patients.

Prognostic and Diagnostic Roles of PD-L2

TCGA dataset was used to examine whether PD-L2 expression was related to survival time and survival status in ICC patients. The Log-rank test was used to compare survival differences between patients in the high PD-L2 expression group and those in the low PD-L2 expression group. On the basis of the Log-rank test and univariate Cox proportional hazard regression, a Kaplan–Meier curve was plotted to calculate the P value, hazard ratio (HR) and 95% confidence interval (CI). We used time ROC (v4.0.3) analysis to compare the prediction accuracy of the PD-L2 gene. The relationship between PD-L2 expression and the prognosis of patients with ICC was investigated using univariate and multivariate analyses. In addition, we calculated the area under the diagnostic curve (AUC) on the basis of PD-L2 expression data. Second, the R software package ggalluvial was used to construct a Sankey map of the pTNM stage, tumor recurrence, PD-L2 expression and survival status of patients. We then used the maftools software package in R software to download and visualize the mutation data, and the Spearman correlation analysis was performed between individual genes and pathway scores. The genes contained in the corresponding pathway were collected and analyzed using the R software GSVA package. Finally, the Spearman method was also used to analyze the correlation between PD-L2 (PDCD1LG2) gene expression and MSI /TMB. All analysis methods and R software packages used were based on R software version 4.0.3 (R Foundation for Statistical Computing, 2020). $P < 0.05$ was considered statistically significant.

Patients and Samples

Our study was reviewed and approved by the Ethics Committee of the Affiliated Hospital of Guilin Medical University (Medical Ethics Approval Number: 2022YJSLL-13). Written informed consent was waived given the retrospective nature of this study. This study collected clinicopathological data from patients diagnosed with ICC after surgical treatment at the Department of Hepatobiliary and Pancreatic Surgery, Affiliated Hospital of Guilin Medical University, from August 2016 to

June 2023. Based on the implementation of strict inclusion and exclusion criteria, 66 patients who met the requirements were selected as study objects.

The inclusion criteria were as follows: patients aged 18–85 years who were diagnosed with mass-type ICC by CT, magnetic resonance imaging (MRI) or clinicopathology; who were not receiving chemotherapy, radiotherapy, interventional therapy or biological targeted immunotherapy before surgery; who had no clinical symptoms of underlying infection; who had no history of other malignancies or radical excision therapy; and who had complete clinicopathological and follow-up information. Patients with Child–Pugh grade C liver function, severe complications and even death in the short term after surgery, palliative surgery or loss to follow-up were excluded. Clinicopathological data, including age, sex, preoperative symptoms, history of cholelithiasis, cirrhosis, HBsAg, intraoperative blood transfusion, number of tumors, tumor diameter, tumor differentiation, envelope integrity, surgical margin, TNM stage, absolute neutrophil-to-lymphocyte ratio (NLR), serum γ-glutamine transferase (r-GT), serum carcinoembryonic antigen (CEA), serum cancer antigen 19–9 (CA19-9), serum cancer antigen 125 (CA125) and the expression level of PD-L2 in tumor tissue, were obtained. TNM stage for ICC patients was determined using the American Joint Committee on Cancer (AJCC) Eighth edition.

All patients with ICC underwent monitoring every three months during the first two years postsurgery, followed by surveillance every six months thereafter. OS was defined as the time interval from the date of surgery to the date of death or the end of follow-up, and DFS was defined as the time interval from the date of surgery to the date of tumor recurrence. The follow-up was concluded as of June 2023. The follow-up rate was 80.5% (66/82 patients).

Immunohistochemistry (IHC)

The IHC procedure was performed as follows: (1) Formalin-fixed, paraffin-embedded (FFPE) ICC tumor tissues and paired peritumoral tissues sections with a width of 4 μm were used. (2) The tissue sections were soaked in citric acid buffer (pH 3.5) for 10 minutes for antigen retrieval. (3) Then, the samples were moved into a 3% H_2O_2 solution and soaked for 5 minutes to block endogenous peroxidase activity. (4) Anti-PD-L2 antibodies (diluted at 1:200, Abcam, USA) were incubated with the tissue slides overnight at 4 °C. (5) The slides were incubated with secondary antibodies at room temperature for 15 minutes. (6). The slides were then visualized using 3,3'-diaminobenzidine chromogen, counter-stained with hematoxylin, dehydrated using graded ethanol and mounted with coverslips.

The IHC staining results were independently observed by two experienced senior pathologists under a microscope to view the stained sections, which were scored via a double-blind method. When the results were inconsistent, a third senior pathologist rescored and interpreted the data. The scores were evaluated according to previous standards.[20] Specifically, the staining intensity was evaluated using a 4-point scale, which included 0 points/no positive staining, 1 point/light yellow, 2 points/brown yellow, and 3 points/tan, in which a score of 2 to 3 was defined as high expression, and a score of 0 to 1 was defined as low expression.

Statistical Analysis

The chi-square test was used to analyze the correlation between PD-L2 expression and clinicopathological features. The Kaplan–Meier method was subsequently used to analyze the correlation between high and low PD-L2 expression and OS or DFS in ICC patients. A multivariate Cox risk regression model was constructed to analyze the effects of PD-L2 expression and clinicopathological parameters on the prognosis of ICC patients. All the statistical analyses were performed using IBM SPSS Statistics 22.0 software, and $P < 0.05$ was considered statistically significant.

Results

High PD-L2 Expression Is Associated with a Good Prognosis in ICC

Bioinformatics analysis revealed that ICC patients with higher expression of PD-L2 had longer OS, but the difference in OS was not statistically significant (data from TCGA, Figure 1A). However, patients with ICC with high PD-L2 expression had longer PFS than those with low PD-L2 expression ($P = 0.015$, Figure 1B). The PD-L2 level has a certain predictive value for PFS in ICC. Specifically, the AUC for the 1-year prognosis of PD-L2 (PDCD1LG2) for

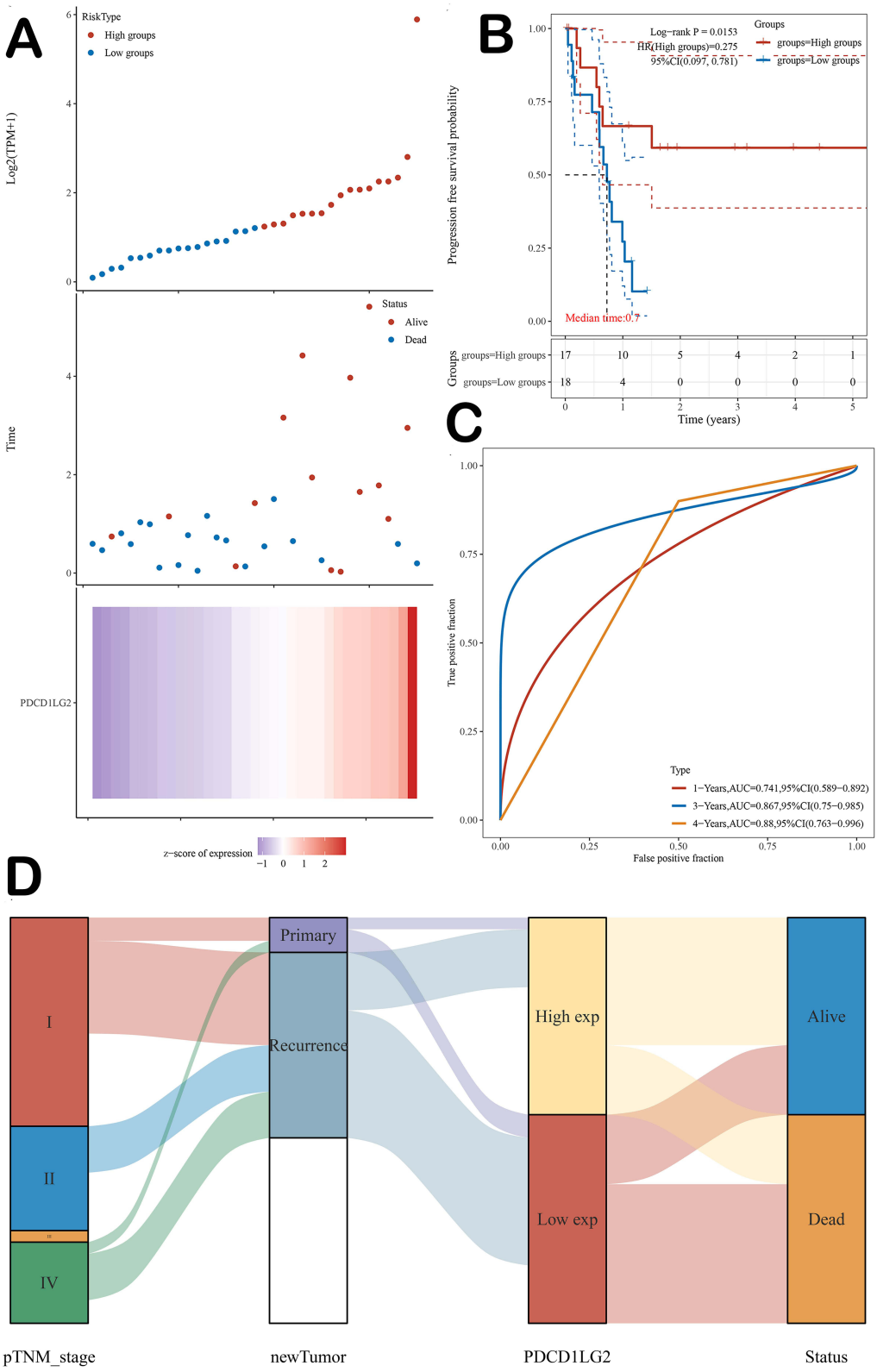

Figure 1 The bioinformatics analysis results showed the expression and prognostic value of PD-L2 in ICC. (**A**) Relationship between PD-L2 expression and survival status or time. The top panel shows the scatter plot of PD-L2 expression from low to high. The middle panel represents the scatter plot distribution of survival time and survival status corresponding to PD-L2 expression in different samples, and the bottom panel shows the heatmap of PD-L2 expression. (**B**) Progression-free survival curves of ICC patients in different PD-L2 expression groups. (**C**) Receiver operating characteristic (ROC) curves and areas under the curve (AUCs) of the prognostic value of PD-L2 expression in ICC patients at 1, 3 and 4 years (data from TCGA database). (**D**) Sankey diagram of pTNM stage, tumor recurrence, PD-L2 (PDCD1LG2) expression, and survival in ICC patients (each column represents a variable; lines between columns indicate correlations) (data from TCGA database).

PFS was 0.741 (95% CI, 0.589–0.892), the AUC for 3-year prognosis was 0.867 (95% CI, 0.750–0.985), and the AUC for 4-year prognosis was 0.88 (95% CI, 0.763–0.996) (Figure 1C).

The Sankey chart shows the pTNM stage, tumor recurrence, PD-L2 expression and survival status of ICC patients (Figure 1D). The curves between each column represent their relationships. The third column indicates that high PD-L2 expression is primarily observed in earlier stages (I–II) of ICC, with relatively lower expression of PD-L2 in advanced stages, and that low expression of PD-L2 is closely associated with ICC recurrence. The fourth column indicates that patients with high PD-L2 expression had a greater proportion of survival than did those with low PD-L2 expression.

PD-L2 (PDCD1LG2) expression was positively correlated with tumor apoptosis scores, tumor inflammation signature (TIS) scores, EMT scores, and ECM-related gene scores (Figure 2A–D, all $P < 0.05$). Additionally, PD-L2 expression in ICC was negatively correlated with MSI ($P = 0.038$, $r = -0.350$, Figure 2E). However, PD-L2 expression was not correlated with TMB ($P = 0.132$, Figure 2F).

PD-L2 Expression Is Associated with the Clinicopathological Characteristics of ICC Patients

A total of 66 patients with pathologically confirmed ICC who underwent surgery at the Affiliated Hospital of Guilin Medical University between 2016 and 2023 were included in this study. The patients included 46 men and 20 women. Owing to the small number of patients (only 4 patients, 6.1%), this study did not analyze the correlation between postoperative chemotherapy and prognosis in ICC patients. The clinicopathological characteristics of the patients are summarized in Table 1.

PD-L2 protein expression was examined using immunohistochemistry (IHC). Positive PD-L2 staining is primarily observed in the cell nucleus and cytoplasm, with negative expression indicated by the absence of staining. PD-L2 expression levels were significantly greater in ICC tumor tissues compared with paired paraneoplastic tissues (Figure 3). On the basis of the PD-L2 expression in ICC as determined by IHC, patients were categorized into high PD-L2 expression groups and low PD-L2 expression groups (Table 1). Among the 66 patients with ICC, 28 (42.4%) had high PD-L2 expression, and 38 (57.6%) had low PD-L2 expression (Table 1 and Figure 4). The chi-square test revealed that PD-L2 expression in ICC was correlated with tumor TNM stage. Specifically, higher PD-L2 expression was noted in the TNM stages I–II group than in the IIIA-IIIB group, and this difference was statistically significant ($\chi^2 = 6.045$, $P = 0.014$). However, patient age, sex, preoperative symptoms, history of cholelithiasis, liver cirrhosis, HBsAg status, number of tumors, tumor diameter, tumor differentiation, integrity of the tumor capsule, preoperative r-GT levels, preoperative CEA levels, preoperative CA125 levels, and preoperative CA19-9 levels were not significantly correlated with PD-L2 expression (all $P > 0.05$).

PD-L2 Expression Is Associated with the Clinical Survival of ICC Patients After Hepatectomy

Univariate analysis revealed that preoperative symptoms ($P = 0.015$), tumor number ($P = 0.015$), tumor size ($P = 0.049$), differentiation ($P = 0.012$), existing capsulation ($P = 0.003$), surgical margin ($P < 0.001$), TNM stage ($P < 0.001$), and preoperative CA125 level ($P = 0.034$) were prognostic factors for OS in patients with ICC (Table 2 and Figure 5). The factors influencing DFS in ICC patients included preoperative symptoms ($P = 0.041$), tumor number ($P = 0.013$), encapsulation ($P = 0.048$), surgical margin ($P < 0.001$), TNM stage ($P < 0.001$), preoperative CA125 level ($P = 0.011$), and PD-L2 status ($P = 0.042$) (Table 2 and Figures 6, 7). In addition, the 1- and 3-year OS rates of the PD-L2 high-expression group were 61.8% and 31.4%, respectively, which were significantly greater than those of the PD-L2 low-expression group (47.7% and 5.6%, respectively). However, the difference was not statistically significant ($P = 0.056$). The same trend was observed for the 1- and 3-year DFS rates of the PD-L2 high- and PD-L2 low-expression groups (53.3% and 17.9% vs 28.8% and 0.0%, respectively, $P = 0.042$) (Table 2 and Figure 7). Multivariate analysis revealed that tumor differentiation (hazard ratio [HR], 1.950; 95% confidence interval [CI], 1.040 to 3.656; $P = 0.037$) and TNM stage (HR, 4.745; 95% CI, 2.299 to 9.796; $P < 0.001$) were independent prognostic factors for OS. The independent

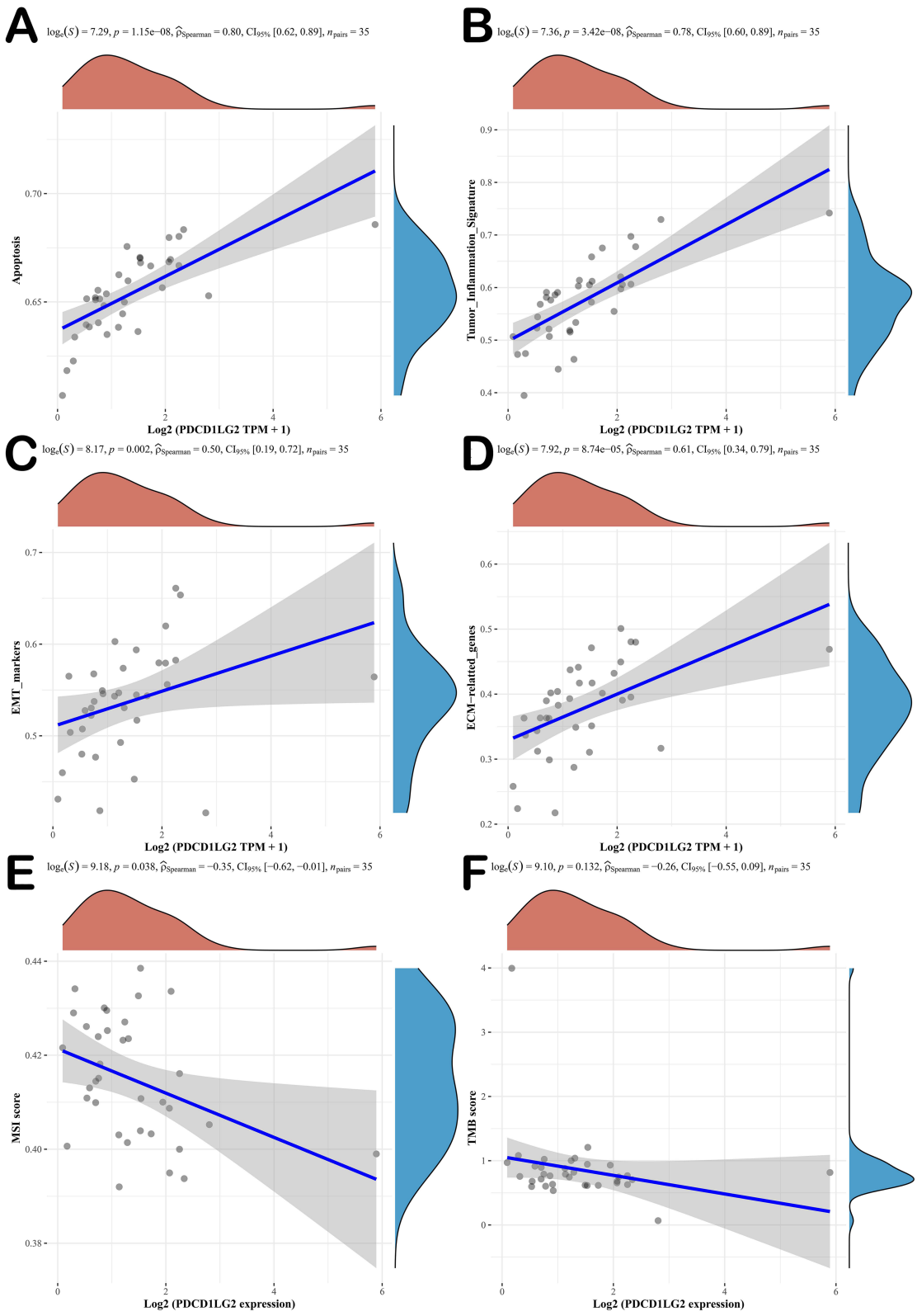

Figure 2 Spearman correlation analysis of PD-L2 (PDCD1LG2) expression in ICC with apoptosis (**A**) the tumor inflammation signature (**B**) EMT markers (**C**) ECM-related genes (**D**) microsatellite instability (MSI, **E**) and the tumor mutational load (TMB, **F**) (data from TCGA database).

Table 1 Correlation of PD-L2 Expression with Clinicopathologic Parameters in ICC (n = 66)

Category	Subcategory	No	PD-L2		χ^2	*r*	*P*
			Low (n = 38)	High (n =28)			
Age(years)	≤59	34	19(50.0%)	15(53.6%)	0.082	−0.035	0.774
	> 59	32	19(50.0%)	13(46.4%)			
Sex	Female	20	14(36.8%)	6(21.4%)	1.813	0.166	0.178
	Male	46	24(63.2%)	22(78.6%)			
Preoperative symptoms	No	21	13(34.2%)	8(28.6%)	0.236	0.060	0.627
	Yes	45	25(65.8%)	20(71.4%)			
History of cholelithiasis	No	60	35(94.6%)	25(89.3%)	0.633	0.099	0.426
	Yes	6	3(5.4%)	3(10.7%)			
Cirrhosis	No	53	32(84.2%)	21(75.0%)	0.865	0.114	0.352
	Yes	13	6(15.8%)	7(25.0%)			
HBsAg	Negative	46	27 (71.1%)	19 (67.9%)	0.078	0.034	0.780
	Positive	20	11 (28.9%)	9 (32.1%)			
Tumor number	Single	41	23(60.5%)	18(64.3%)	0.097	−0.038	0.756
	Multiple	25	15(39.5%)	10(35.7%)			
Tumor size (cm)	≤ 5	46	27 (71.1%)	19 (67.9%)	0.078	0.034	0.780
	> 5	20	11 (28.9%)	9 (32.1%)			
Tumor Differentiation	Well/Moderate	42	22 (57.9%)	20 (71.4%)	1.276	−0.139	0.259
	Poor	24	16 (42.1%)	8 (28.6%)			
Capsulation	No	28	14 (36.8%)	14 (50.0%)	1.143	−0.132	0.285
	Yes	38	24 (63.2%)	14 (50.0%)			
TNM	I–II	38	17 (44.7%)	21 (75.0%)	6.045	−0.303	0.014
	IIIA-IIIB	28	21 (55.3%)	7 (25.0%)			
r-GT (U/L)	≤ 50	18	9 (23.7%)	9 (32.1%)	0.582	−0.094	0.446
	> 50	48	29 (76.3%)	19(67.9%)			
CEA (ng/mL)	≤ 5	42	22 (57.9%)	20 (71.4%)	1.276	−0.139	0.259
	> 5	24	16 (42.1%)	8(28.6%)			
CA125 (IU/mL)	≤ 35	53	29 (76.3%)	24 (85.7%)	0.900	−0.117	0.343
	> 35	13	9 (23.7%)	4 (14.3%)			
CA19-9 (IU/mL)	≤ 35	29	16 (42.1%)	13 (46.4%)	0.122	−0.043	0.727
	> 35	37	22 (57.9%)	15 (53.6%)			

Abbreviations: ICC, intrahepatic cholangiocarcinoma; HBsAg, hepatitis B surface antigen; TNM, tumor node metastasis; r-GT, serum γ-glutamine transferase; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA19-9, carbohydrate antigen 19–9.

prognostic indicators for DFS were surgical margin (HR, 0.280; 95% CI, 0.136 to 0.575; P = 0.001) and PD-L2 expression (HR, 0.506; 95% CI, 0.273 to 0.939; P =0.031) (Table 3).

Discussion

At present, ICC remains a refractory malignancy with a poor clinical prognosis, even after patients have undergone curative hepatectomy. Postoperative local or distant recurrence and metastasis are the primary factors contributing to the low postoperative survival rates of patients with ICC.[21,22] Identifying new effective biomarkers and therapeutic targets to improve the early diagnosis rate of ICC and identifying patients at high risk of recurrence and metastasis after hepatectomy are highly important for improving clinical outcomes. In the present study, we found that PD-L2 expression is an effective indicator for predicting early recurrence in patients with ICC after hepatectomy.

PD-L2 was first described in 2001 as the second ligand for the PD-1 receptor. It is a member of the B7 family and is classified as a type I transmembrane protein featuring an immunoglobulin (Ig)-like V domain and an Ig-like C2 domain in its extracellular region.[23] PD-L1 and PD-L2 share approximately 40% of their amino acid sequences.[10] Although

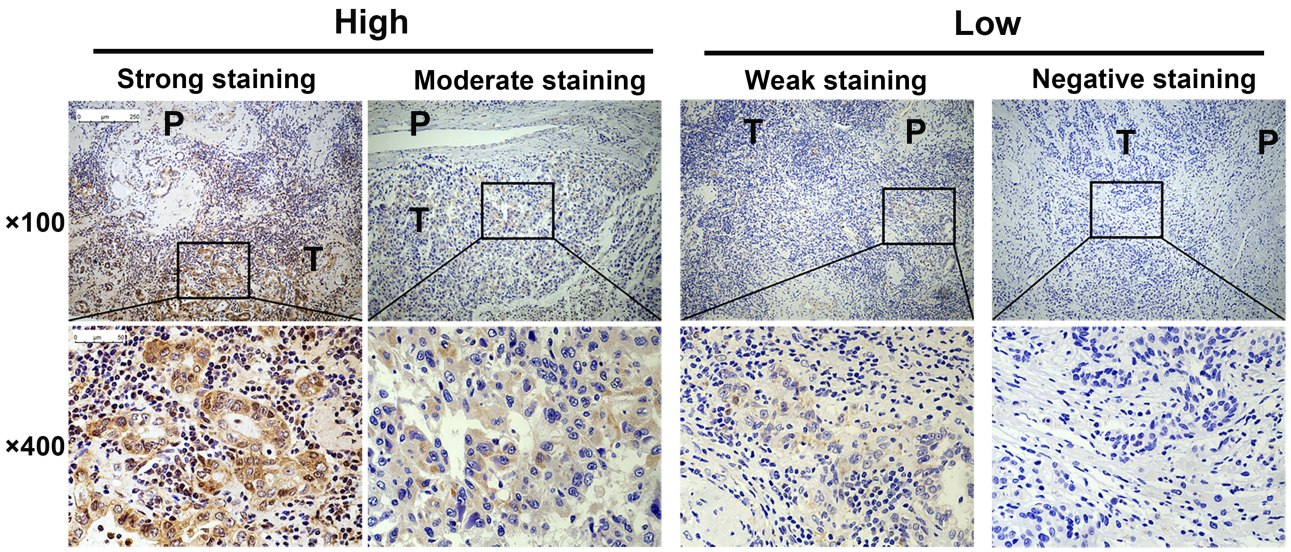

Figure 3 Representative image of immunohistochemical staining of PD-L2 expression (magnification ×100 and ×400).
Abbreviations: P, peritumoral tissue; T, tumor tissue.

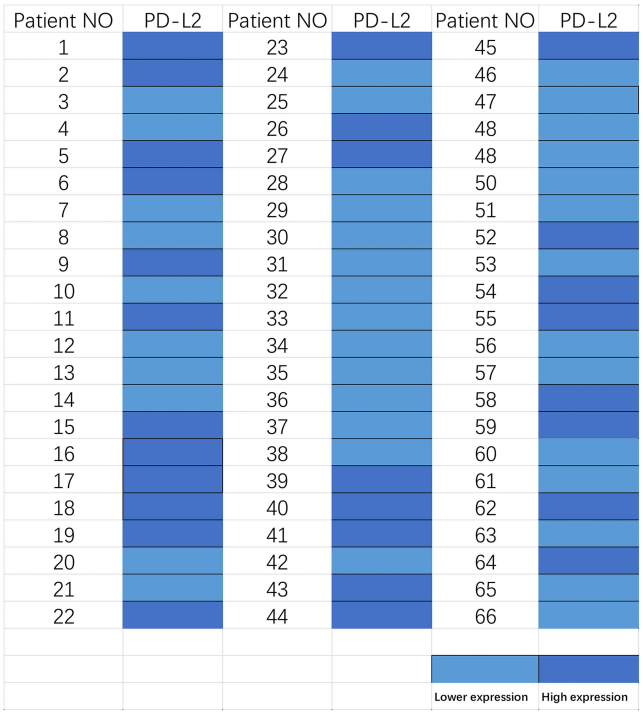

Figure 4 General view of PD-L2 expression in ICC tissues (n=66).

PD-L2 is homologous to PD-L1, these two ligands exhibit distinct expression levels across various tissues and possess different mechanisms of action, potentially harboring some nonoverlapping functions.[24] PD-1 receptors located in T cells and in the cells of tumors and immune infiltration ligands, such as PD-L1 and PD-L2, interact, regulate the T-cell-mediated immune response, and may play a role in escaping immune surveillance in human tumors.[11] Immunotherapy targeting the PD-1 axis includes the use of monoclonal antibody agents to block the interaction between the receptor and PD-L1 and PD-L2 and the use of binders to block the interaction between the ligand and PD-1, has led to breakthroughs in the clinical response of a variety of human cancers.[25,26] Currently, the selection of patients who are eligible for PD-1

Table 2 Univariate Analysis of Prognostic Factors Associated with OS and DFS in ICC (Including 1,3-year Cumulative Survival Analysis) (n = 66)

Category	No	OS		*P*	DFS		*P*
		1-yr	3-yrs		1-yr	3-yrs	
Age (years)							
≤ 59	34	53.2%	20.2%	0.830	34.0%	11.3%	0.829
> 59	32	54.3%	0.0%		48.5%	0.0%	
Sex							
Female	20	68.0%	19.2%	0.252	53.1%	0.0%	0.838
Male	46	47.0%	14.3%		34.3%	16.0%	
History of cholelithiasis							
No	60	50.4%	14.9%	0.367	39.6%	8.6%	0.479
Yes	6	80.0%	53.3%		60.0%	30.0%	
Preoperative symptoms							
No	21	72.2%	34.7%	0.015	58.7%	11.2%	0.041
Yes	45	45.7%	7.1%		32.1%	8.0%	
Cirrhosis							
No	53	57.2%	10.2%	0.988	39.2%	5.9%	0.664
Yes	13	42.0%	31.5%		35.5%	17.9%	
HBsAg							
Negative	46	61.4%	17.9%	0.780	44.2%	7.0%	0.549
Positive	20	35.9%	9.0%		24.9%	12.5%	
Tumor number							
Single	41	67.9%	20.0%	0.015	47.4%	10.7%	0.013
Multiple	25	28.9%	0.0%		29.1%	0.0%	
Tumor size (cm)							
≤5	46	63.5%	14.3%	0.049	46.4%	10.6%	0.173
>5	20	30.6%	12.2%		25.8%	6.5%	
Tumor Differentiation							
Well/Moderate	42	72.1%	19.0%	0.012	47.2%	8.1%	0.050
Poor	24	26.4%	9.9%		28.6%	8.6%	
Capsulation							
No	28	37.3%	0.0%	0.003	27.3%	0.0%	0.048
Yes	38	67.7%	24.7%		51.2%	11.0%	
Surgical margin							
R0	53	66.9%	20.2%	<0.001	51.1%	10.9%	<0.001
R1	13	7.70%	0.0%		0.0%	0.0%	
TNM							
I–II	38	74.6%	27.0%	<0.001	63.3%	13.1%	<0.001
IIIA-IIIB	28	28.7%	0.0%		7.70%	0.0%	
Intraoperative blood transfusion							
No	37	47.6%	10.3%	0.205	30.9%	16.5%	0.075
Yes	29	63.3%	21.8%		53.5%	11.4%	
NLR							
≤2.350	33	57.6%	8.1%	0.837	44.5%	0.0%	0.634
>2.350	33	50.2%	21.5%		35.6%	15.6%	
r-GT (U/L)							
≤50	18	49.8%	24.9%	0.374	51.4%	10.3%	0.575
>50	48	55.3%	12.4%		37.2%	9.3%	
CEA (ng/L)							
≤5	42	55.9%	11.2%	0.751	42.7%	10.4%	0.409
>5	24	50.6%	0.0%		36.7%	0.0%	

(*Continued*)

Table 2 (Continued).

Category	No	OS		P	DFS		P
		1-yr	3-yrs		1-yr	3-yrs	
CA19-9 (IU/mL)							
≤35	29	56.2%	14.0%	0.895	44.5%	6.50%	0.921
>35	37	51.9%	16.4%		37.5%	10.9%	
CA125 (IU/mL)							
≤35	53	62.8%	16.7%	0.034	46.6%	14.5%	0.011
>35	13	20.5%	10.3%		15.4%	0.0%	
PD-L2							
Low	38	47.7%	5.6%	0.056	28.8%	0.0%	0.042
High	28	61.8%	31.4%		53.3%	17.9%	

Abbreviations: OS, overall survival; DFS, disease-free survival; ICC, intrahepatic cholangiocarcinoma; HBsAg, hepatitis B surface antigen; TNM, tumor node metastasis; NLR, Neutrophil-Lymphocyte Ratio; r-GT, serum γ-glutamine transferase; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19–9; CA125, carbohydrate antigen 125; PD-L2, Programmed Death Ligand 2.

axis-targeted therapy is focused on the assessment of PD-L1 expression in tumors by means of immunohistochemical testing. Despite the significant utility of PD-L1 as a predictive biomarker in some tumor types, a subset of PD-L1-positive patients have a poor response to anti-PD-L1 axis therapy, whereas some PD-L1-negative patients exhibit a good response.[27,28] These findings suggest that molecular interactions with PD-1 ligands other than PD-L1, such as PD-L2, may be relevant in predicting the clinical reactivity of these treatments. In addition, PD-L1 blockade does not block the PD-1/PD-L2 interaction, which may allow tumors to evade the antitumor immune response.[29] Although PD-L2 has a 2–6 times greater affinity for PD-1 than does PD-L1,[30] PD-L2 has received much less attention in human tumor research than PD-L1. At present, immunotherapy for ICC is in its early stages. Given the poor prognosis of ICC and the lack of highly effective treatment options, this study explores the possible role of PD-L2 in ICC. The results of this study revealed that PD-L2 is highly expressed and strongly related to better DFS in ICC patients. PD-L2 overexpression in ICC patients may indicate later postoperative recurrence or metastasis. Our data subsequently revealed that ICC patients with high PD-L2

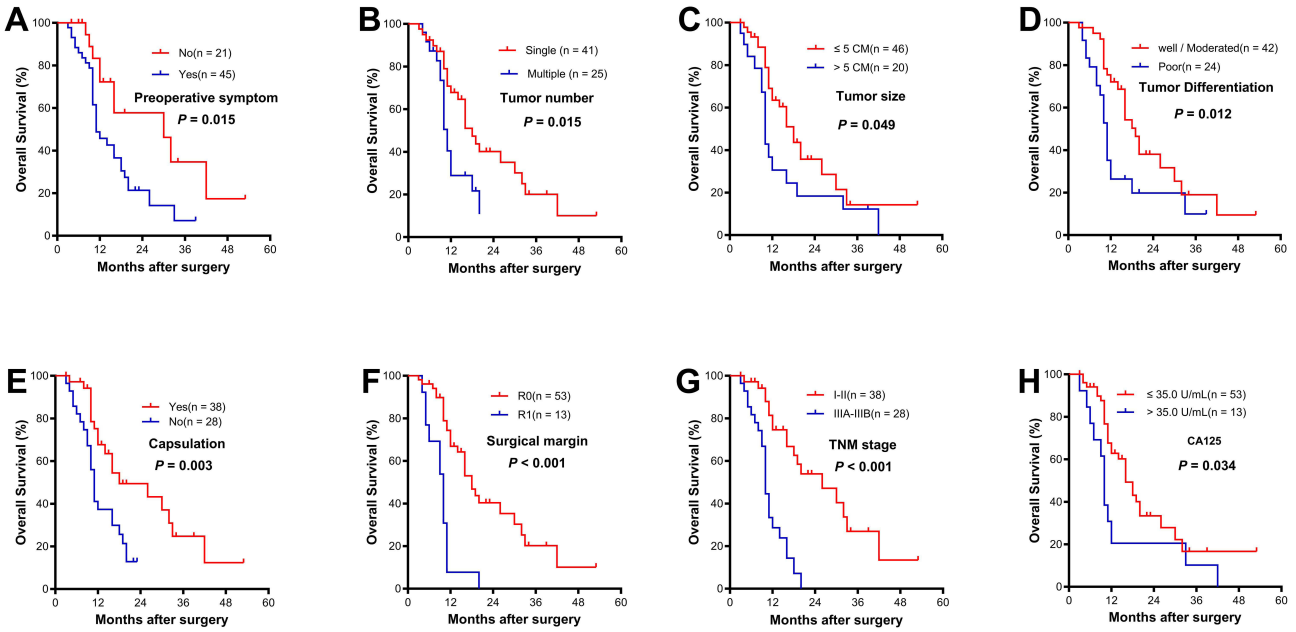

Figure 5 Kaplan–Meier univariate survival analysis of overall survival (OS) in patients with ICC. Preoperative symptoms (**A**) tumor number (**B**) tumor size (**C**) tumor differentiation (**D**) existing capsulation (**E**) surgical margin (**F**) TNM stage (**G**) preoperative CA125 level (**H**) were prognostic factors of overall survival in ICC (all $P < 0.05$).

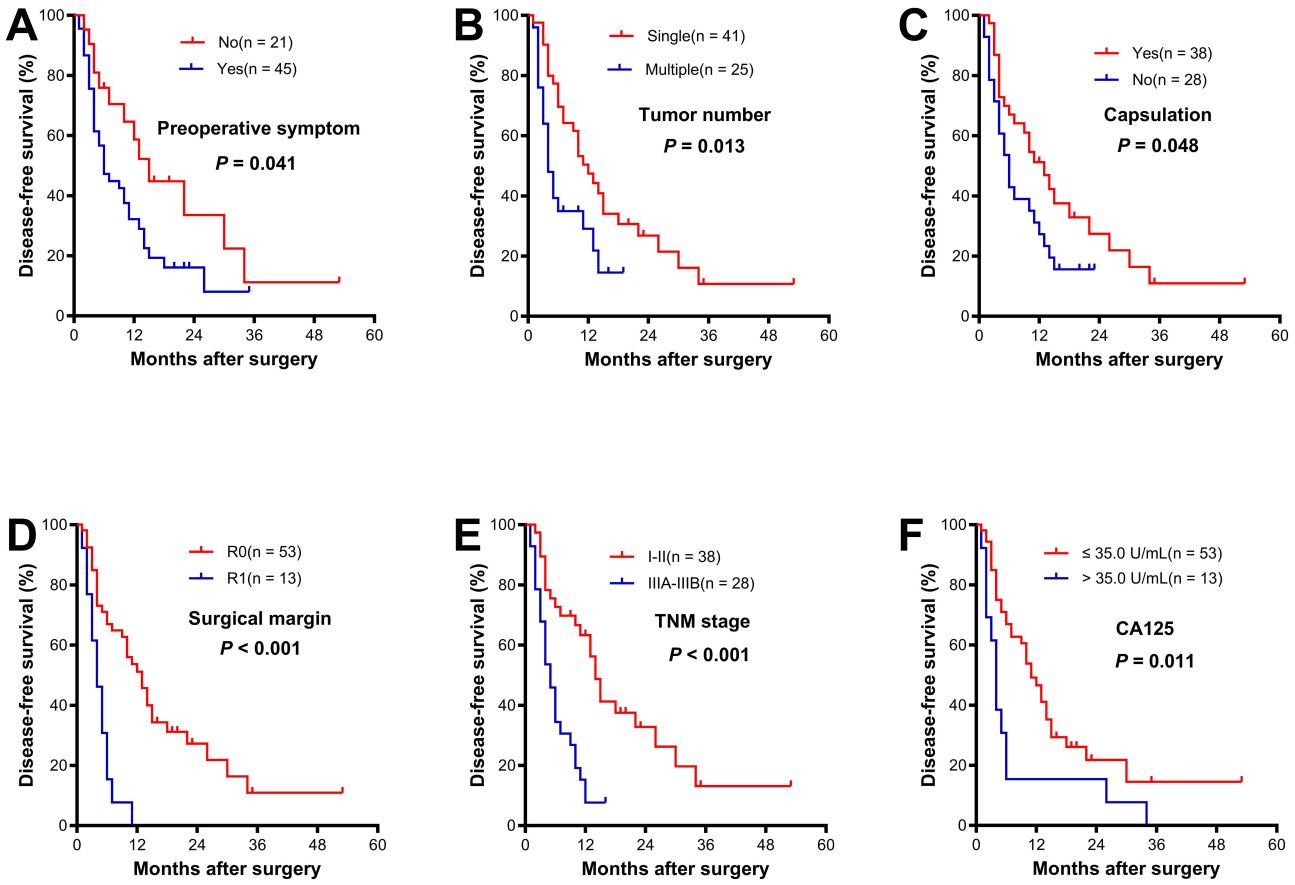

Figure 6 Kaplan–Meier univariate survival analysis of disease-free survival (DFS) in patients with ICC. Preoperative symptoms (**A**) tumor number (**B**) capsulation (**C**) surgical margin (**D**) TNM stage (**E**) preoperative CA125 level (**F**) were prognostic factors of disease-free survival in ICC (all $P < 0.05$).

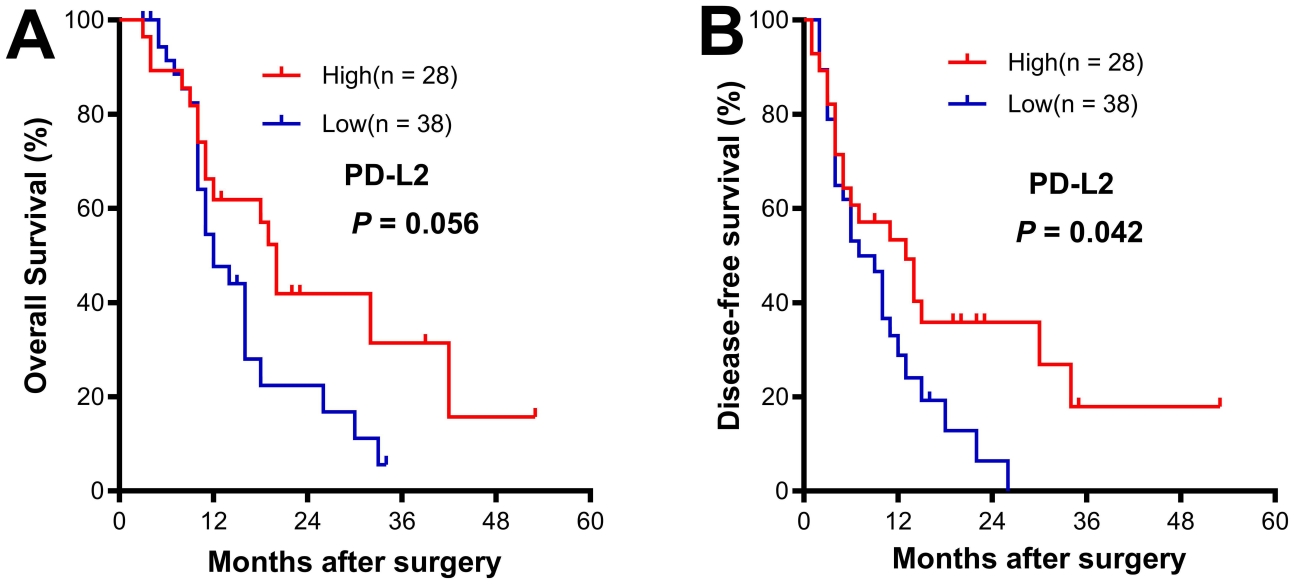

Figure 7 The relationship between PD-L2 expression and prognosis of ICC patients. (**A**) The expression of PD-L2 may be associated with the overall survival prognosis of ICC patients, but there is no statistical significance ($P = 0.056$). (**B**) PD-L2 was prognostic factors of disease- free survival in patients with ICC ($P = 0.042$).

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Table 3 Survival Analysis of Prognostic Factors Associated with ICC (n=66)

Category	Overall Survival (OS)			Disease-Free Survival (DFS)		
	Univariate *P*	Multivariate		Univariate *P*	Multivariate	
		HR (95% CI)	*P*		HR (95% CI)	*p*
Age (>59 vs ≤59 years)	0.830		NA	0.829		NA
Sex (Female vs male)	0.252		NA	0.838		NA
HBsAg (Negative vs Positive)	0.136		NA	0.549		NA
History of cholelithiasis (NO vs YES)	0.367		NA	0.479		NA
Preoperative symptom (NO vs YES)	0.015		NS	0.041	1.755 (0.903–3.411)	0.097
Postoperative complications (NO vs YES)	0.507		NA	0.263		NA
Perioperative blood transfusion (NO vs YES)	0.205		NA	0.075		NS
Cirrhosis (NO vs YES)	0.988		NA	0.664		NA
Tumor Differentiation (W+M vs P)	0.012	1.950 (1.040–3.656)	0.037	0.050		NS
Capsulation (NO vs YES)	0.003		NS	0.048		NS
Surgical margin (R1 vs R0)	<0.001		NS	<0.001	0.280 (0.136–0.575)	0.001
TNM (I+II vs IIIA+ IIIB)	<0.001	4.745 (2.299–9.796)	<0.001	<0.001		NS
NLR (≤2.350 vs > 2.350)	0.836		NA	0.587		NA
r-GT (U/L) (≤50.0 vs >50.0)	0.374		NA	0.575		NA
CEA (ng/mL) (≤5 vs >5)	0.751		NA	0.409		NA
CA125 (IU/mL) (≤35 vs >35)	0.034		NS	0.011		NS
CA19-9 (IU/mL) (≤35 vs >35)	0.895		NA	0.921		NA
PD-L2 (Low vs High)	0.056		NS	< 0.001	0.506 (0.273–0.939)	0.031

Abbreviations: ICC, Intrahepatic cholangiocarcinoma; HBsAg, hepatitis B surface antigen; W+M, well + moderated differentiation; P, poor differentiation; TNM, Tumor Node Metastasis; NLR, Neutrophil-Lymphocyte Ratio; r-GT, serum γ-glutamine transferase; CEA, Carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA19-9, carbohydrate antigen 19–9; PD-L2, Programmed Death Ligand 2; NA, not applicable; NS, not significant.

expression were more likely to have better clinical OS outcomes than those with low PD-L2 expression. Thus, PD-L2 may represent a potential target of immune checkpoint blockade (ICB) for ICC.

High PD-L2 expression may also have a synergistic role with PD-L1 in mediating local immune tolerance.[31] In salivary gland tumors, increased PD-L2 expression is associated with disease recurrence.[32] Positive PD-L2 expression is associated with aggressive biological characteristics of lung adenocarcinoma, such as high-grade tumors, advanced tumor stage, and gene mutation (EGFR), and PD-L2 overexpression is a significant prognostic factor for OS but not for DFS/RFS/PFS.[29,33,34] However, different studies have shown that some malignant tumors with high PD-L2 expression have better prognoses and may relapse later. Studies have shown that in patients with lung squamous cell carcinoma undergoing surgical resection, PD-L2-positive patients have a better prognosis than do PD-L2-negative patients, and the postoperative survival time of PD-L2-positive patients is significantly longer.[35] In addition, a series of early or retrospective studies of patients with metastatic lung adenocarcinoma revealed that PD-L2-positive patients have longer progression-free survival.[36] Among various types of tumors, significant heterogeneity exists in the relationship between PD-L2 expression and prognosis, which may be attributed to the unique immunosuppressive roles played by PD-L2 in different malignancies. PD-L2 may synergize with PD-L1 to mediate local immune tolerance in the tumor microenvironment. Its high expression in early ICC indicates its role in delaying recurrence, possibly by regulating T cell depletion or apoptosis pathways. In clinical practice, PD-L2 can serve as a biomarker for ICB treatment eligibility and is worth conducting trials that combine PD-1/PD-L2 blockade.

This study also has certain limitations. First, the study focused exclusively on PD-L2 expression in ICC, without concurrent examination of PD-L1/PD-1 expression; hence, the interrelationship between these three factors cannot be assessed. Second, we did not investigate whether PD-L2 has an effect on malignant phenotypes, such as ICC cell proliferation, migration and invasion. Finally, the data for this study were obtained from a single center in China. In addition, the number of cases was relatively small, and its statistical effectiveness was relatively limited. Our future work will simultaneously analyze the expression of PD-L1/PD-1 and explore the functional role of PD-L2 through in vitro knockout models to establish causal relationships, and also it is necessary to conduct large-scale multicenter clinical studies on the role of PD-L2 in ICC.

Conclusion

PD-L2 is predominantly highly expressed in patients with early-stage ICC. PD-L2 is an independent prognostic predictor of DFS in ICC patients after hepatectomy, and ICC patients with high PD-L2 expression have later recurrence.

Data Sharing Statement

Under reasonable requirements, the data and materials in this article can be obtained by contacting the main corresponding author, Dr. Liu Lingyun, allllyy@163.com.

Ethics Approval and Informed Consent

All patient data were anonymized to ensure confidentiality and the study followed the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of the Affiliated Hospital of Guilin Medical University (Medical Ethics Approval Number: 2022YJSLL-13). Written informed consent was waived because of the retrospective nature of this study.

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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 declare no conflicts of interest in this work.

References

1. Becht R, Wasilewicz MP. New options for systemic therapies in intrahepatic cholangiocarcinoma (iCCA). *Medicina*. 2023;59(6):1174. doi:10.3390/medicina59061174
2. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin*. 2024;74(1):12–49. doi:10.3322/caac.21820
3. Beal EW, Tumin D, Moris D, et al. Cohort contributions to trends in the incidence and mortality of intrahepatic cholangiocarcinoma. *Hepatobiliary Surg Nutr*. 2018;7(4):270–276. doi:10.21037/hbsn.2018.03.16
4. Njei B. Changing pattern of epidemiology in intrahepatic cholangiocarcinoma. *Hepatology*. 2014;60(3):1107–1108. doi:10.1002/hep.26958
5. Koshiol J, Yu B, Kabadi SM, Baria K, Shroff RT. Epidemiologic patterns of biliary tract cancer in the United States: 2001-2015. *BMC Cancer*. 2022;22(1):1178. doi:10.1186/s12885-022-10286-z
6. Ruff SM, Pawlik TM. Clinical management of intrahepatic cholangiocarcinoma: surgical approaches and systemic therapies. *Front Oncol*. 2024;14:1321683. doi:10.3389/fonc.2024.1321683
7. El-Diwany R, Pawlik TM, Ejaz A. Intrahepatic cholangiocarcinoma. *Surg Oncol Clin N Am*. 2019;28(4):587–599. doi:10.1016/j.soc.2019.06.002
8. Moris D, Palta M, Kim C, Allen PJ, Morse MA, Lidsky ME. Advances in the treatment of intrahepatic cholangiocarcinoma: an overview of the current and future therapeutic landscape for clinicians. *CA Cancer J Clin*. 2023;73(2):198–222. doi:10.3322/caac.21759
9. Hogdall D, O'Rourke CJ, Taranta A, Oliveira DV, Andersen JB. Molecular pathogenesis and current therapy in intrahepatic cholangiocarcinoma. *Dig Dis*. 2016;34(4):440–451. doi:10.1159/000444562
10. Bardhan K, Anagnostou T, Boussiotis VA. The PD1:PD-L1/2 pathway from discovery to clinical implementation. *Front Immunol*. 2016;7:550. doi:10.3389/fimmu.2016.00550
11. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12(4):252–264. doi:10.1038/nrc3239
12. Ren T, Zheng B, Huang Y, et al. Osteosarcoma cell intrinsic PD-L2 signals promote invasion and metastasis via the RhoA-ROCK-LIMK2 and autophagy pathways. *Cell Death Dis*. 2019;10(4):261. doi:10.1038/s41419-019-1497-1

13. Espinosa-Carrasco G, Chiu E, Scrivo A, et al. Intratumoral immune triads are required for immunotherapy-mediated elimination of solid tumors. *Cancer Cell*. 2024;42(7):1202–1216e1208. doi:10.1016/j.ccell.2024.05.025
14. Takamori S, Takada K, Azuma K, et al. Prognostic impact of programmed death-ligand 2 expression in primary lung adenocarcinoma patients. *Ann Surg Oncol*. 2019;26(6):1916–1924. doi:10.1245/s10434-019-07231-z
15. Okadome K, Baba Y, Nomoto D, et al. Prognostic and clinical impact of PD-L2 and PD-L1 expression in a cohort of 437 oesophageal cancers. *Br J Cancer*. 2020;122(10):1535–1543. doi:10.1038/s41416-020-0811-0
16. Gao Y, Li S, Xu D, et al. Prognostic value of programmed death-1, programmed death-ligand 1, programmed death-ligand 2 expression, and CD8 (+) T cell density in primary tumors and metastatic lymph nodes from patients with stage T1-4N+M0 gastric adenocarcinoma. *Chin J Cancer*. 2017;36(1):61. doi:10.1186/s40880-017-0226-3
17. Jung HI, Jeong D, Ji S, et al. Overexpression of PD-L1 and PD-L2 is associated with poor prognosis in patients with hepatocellular carcinoma. *Cancer Res Treat*. 2017;49(1):246–254. doi:10.4143/crt.2016.066
18. Zhang Y, Xu J, Hua J, et al. A PD-L2-based immune marker signature helps to predict survival in resected pancreatic ductal adenocarcinoma. *J Immunother Cancer*. 2019;7(1):2–33. doi:10.1186/s40425-019-0703-0
19. Yang Y, Wang X, Bai Y, et al. Programmed death-ligand 2 (PD-L2) expression in bladder cancer. *Urol Oncol*. 2020;38(6):e609–603e615. doi:10.1016/j.urolonc.2020.01.001
20. Wang H, Yao H, Li C, et al. PD-L2 expression in colorectal cancer: independent prognostic effect and targetability by deglycosylation. *Oncoimmunology*. 2017;6(7):e1327494. doi:10.1080/2162402X.2017.1327494
21. Ruzzenente A, Conci S, Valdegamberi A, Pedrazzani C, Guglielmi A. Role of surgery in the treatment of intrahepatic cholangiocarcinoma. *Eur Rev Med Pharmacol Sci*. 2015;19(15):2892–2900.
22. Malik AK, Davidson BR, Manas DM. Surgical management, including the role of transplantation, for intrahepatic and peri-hilar cholangiocarcinoma. *Eur J Surg Oncol*. 2024;108248. doi:10.1016/j.ejso.2024.108248
23. Latchman Y, Wood CR, Chernova T, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol*. 2001;2(3):261–268. doi:10.1038/85330
24. Liang SC, Latchman YE, Buhlmann JE, et al. Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. *Eur J Immunol*. 2003;33(10):2706–2716. doi:10.1002/eji.200324228
25. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366 (26):2455–2465. doi:10.1056/NEJMoa1200694
26. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366 (26):2443–2454. doi:10.1056/NEJMoa1200690
27. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014;515(7528):563–567. doi:10.1038/nature14011
28. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature*. 2014;515 (7528):568–571. doi:10.1038/nature13954
29. Takamochi K, Hara K, Hayashi T, et al. Clinical relevance of PD-L2 expression in surgically resected lung adenocarcinoma. *Lung Cancer*. 2022;168:50–58. doi:10.1016/j.lungcan.2022.04.011
30. Youngnak P, Kozono Y, Kozono H, et al. Differential binding properties of B7-H1 and B7-DC to programmed death-1. *Biochem Biophys Res Commun*. 2003;307(3):672–677. doi:10.1016/s0006-291x(03)01257-9
31. Weber M, Wehrhan F, Baran C, et al. Prognostic significance of PD-L2 expression in patients with oral squamous cell carcinoma-A comparison to the PD-L1 expression profile. *Cancer Med*. 2019;8(3):1124–1134. doi:10.1002/cam4.1929
32. Chang H, Kim JS, Choi YJ, et al. Overexpression of PD-L2 is associated with shorter relapse-free survival in patients with malignant salivary gland tumors. *Onco Targets Ther*. 2017;10:2983–2992. doi:10.2147/OTT.S134589
33. Shinchi Y, Komohara Y, Yonemitsu K, et al. Accurate expression of PD-L1/L2 in lung adenocarcinoma cells: a retrospective study by double immunohistochemistry. *Cancer Sci*. 2019;110(9):2711–2721. doi:10.1111/cas.14128
34. Song W, Wu Y, Wang Y, Che G. Prognostic value of PD-L2 in lung adenocarcinoma. *Asian J Surg*. 2023;46(6):2452–2453. doi:10.1016/j.asjsur.2022.12.058
35. Matsubara T, Takada K, Azuma K, et al. A clinicopathological and prognostic analysis of PD-L2 expression in surgically resected primary lung squamous cell carcinoma. *Ann Surg Oncol*. 2019;26(6):1925–1933. doi:10.1245/s10434-019-07257-3
36. Matsubara E, Shinchi Y, Komohara Y, et al. PD-L2 overexpression on tumor-associated macrophages is one of the predictors for better prognosis in lung adenocarcinoma. *Med Mol Morphol*. 2023;56(4):250–256. doi:10.1007/s00795-023-00361-0