scientific reports

Check for updates

OPEN

Expression and relationship of PD-L1, CD24, and CD47 in hepatitis B virus associated hepatocellular carcinoma

Aiping Lin[1,7], Mingxia Wang[2,7], Zhihui Wang[3,7], Juan Lin[4], Zhihui Lin[1], Shaowei Lin[5], Shiyun Lu[1], Hong Lin[1], Haijun Tang[6✉] & Xueping Huang[1✉]

Immune checkpoint inhibitor (ICI) therapy is the new standard treatment for advanced or metastatic hepatocellular carcinoma (HCC); however, many patients still fail to respond. This study explored the expression and prognosis of programmed death ligand 1 (PD-L1), cluster of differentiation 24 (CD24), and cluster of differentiation 47 (CD47) in patients with hepatitis B virus-associated HCC (HBV-associated HCC). We analyzed sequencing data from the Cancer Genome Atlas (TCGA) and investigated the expression of PD-L1, CD24, and CD47 in HBV-associated HCC patients by immunohistochemistry and their relationship with prognosis and clinicopathological factors. HCC data from the TCGA database show that PD-L1 was substantially correlated with various immune cells. In 67 patients with HBV-associated HCC, high PD-L1 and CD24 expression levels were related to poor overall survival (OS) and progression-free survival (PFS). PD-L1 expression was significantly associated with the staging of HBV-associated HCC ($p = 0.011$) and Ki67 expression ($p = 0.024$). Correlation analysis between variables reveals that PD-L1 was significantly positively correlated with CD24 and CD47. High expression of PD-L1 and CD24 are risk factors for poor prognosis in HBV-associated HCC patients following curative resection. PD-L1 is significantly correlated with CD24 and CD47.

Keywords HBV-associated hepatocellular carcinoma, Immune checkpoint inhibitors, PD-L1, CD24, CD47

Liver cancer is the fourth most common malignant tumor in China and the second leading cause of cancer-related death, after lung cancer. Approximately 90% of liver cancers are hepatocellular carcinoma (HCC)[1,2]. HCC is a typical inflammatory tumor, and one of the primary triggers of HCC is the chronic inflammation induced by hepatitis B virus (HBV) infection[2]. Despite the availability of effective vaccines and antiviral treatments for HBV, HBV-associated HCC remains one of the leading causes of death worldwide, especially in Asia and Africa[3]. Unfortunately, over 70% of HBV-associated HCC cases are diagnosed at an advanced stage, leading to limited treatment options and poor prognosis[4]. Prognostic indicators for post-operative HBV-associated HCC are still not fully understood.

As an immune-tolerant organ, the liver contains immune cells that make up 20% of the total liver cell population, creating a complex immune-tolerant microenvironment within the liver tissue[5]. Immune cells facilitate the growth of tumor cells throughout malignant tumorigenesis and progression[6]. Immune checkpoint inhibitors (ICIs), such as antibodies for programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1), have become the hotspot of HCC immunotherapy[7]. The treatment paradigm for advanced HCC has shifted from monotherapy with tyrosine kinase inhibitors, exemplified by sorafenib, to a combination of targeted therapy and PD-1/PD-L1 inhibitors, known as "targeted-immunotherapy" combination treatment[8]. Although PD-1/PD-L1

[1]Department of Gastroenterology, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, Fuzhou University Affiliated Provincial Hospital, Fuzhou 350001, Fujian, China. [2]Department of Hepatology, Mengchao Hepatobiliary Hospital of Fujian Medical University, Fuzhou 350025, Fujian, China. [3]Department of Thoracic Oncology, Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai 519000, Guangdong, China. [4]Department of Pathology, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, Fuzhou University Affiliated Provincial Hospital, Fuzhou 350001, Fujian, China. [5]School of Public Health, Fujian Medical University, Fuzhou 350108, Fujian, China. [6]Center for Experimental Research in Clinical Medicine, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, Fuzhou University Affiliated Provincial Hospital, Fuzhou 350001, China. [7]Aiping Lin, Mingxia Wang and Zhihui Wang contributed equally to this work. ✉email: tanghaijun16@163.com; hxuep@mail2.sysu.edu.cn

inhibitors, representative of ICIs, have achieved breakthroughs as first- and second-line treatments of advanced liver cancer, they are limited by their efficacy rates of only approximately 10-30% and issues of low objective response rates and drug resistance[9]. Therefore, exploring new therapeutic targets and combination treatment strategies is necessary.

Cluster of differentiation 24 (CD24) serves as a costimulatory molecule for T lymphocyte responses and a regulator of autoimmunity[10]. CD24 drives the onset and development of tumors as an "oncogene," promoting cell migration, invasion, and proliferation. It is a factor associated with poor prognosis in malignant tumors such as HCC[10,11]. Cluster of differentiation 47 (CD47) is a glycoprotein widely expressed in the immunoglobulin superfamily. High CD47 expression is observed in various human malignant tumors and is positively correlated with poor prognosis across different types of cancer[12]. This suggests that CD47 could be an attractive new target for cancer treatment. In recent years, the efficacy of anti-CD47 antibody therapy in treating various malignant tumors has garnered significant attention.

This study aims to explore the expression of PD-L1, CD24, and CD47 in HBV-associated HCC and their effect on patient prognosis.

Results

### Identification and analysis of differentially expressed mRNAs of HCC

We first retrieved the transcriptome profiling data of patients with HCC from the Cancer Genome Atlas (TCGA) database. This study included 50 normal and 374 tumor samples. The data were analyzed using R software to study the differential expression of mRNAs. A total of 59,427 genes were identified; among which 1,471 were recognized as differentially expressed mRNAs, with 452 upregulated and 1,019 downregulated (Fig. 1).

### Enrichment analysis of signaling pathways and immune infiltration in HCC

These differentially expressed genes were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. The GO enrichment results were concentrated in complement activation, immunoglobulin complex, and antigen binding. The KEGG enrichment results were focused on chemical carcinogenesis-DNA adducts and retinol metabolism. Correlation analysis was performed between essential genes and immune infiltration, revealing a significant correlation between PD-L1 and multiple immune cells (Fig. 2).

### Prognostic of differentially expressed PD-L1, CD24, CD47 in HCC tissues

We studied the prognostic impact of differentially expressed PD-L1, CD24, and CD47 in the tumor tissues of patients with HCC whose data were sourced from the TCGA database. Kaplan-Meier survival analysis and log-rank test were used to compare the survival rates of patients in the high expression group ($N = 188$) and low expression group ($N = 189$). Kaplan-Meier analysis indicated that the patients exhibited different overall survival (OS) based on varying expression levels of PD-L1, CD24, and CD47 (Fig. 3).
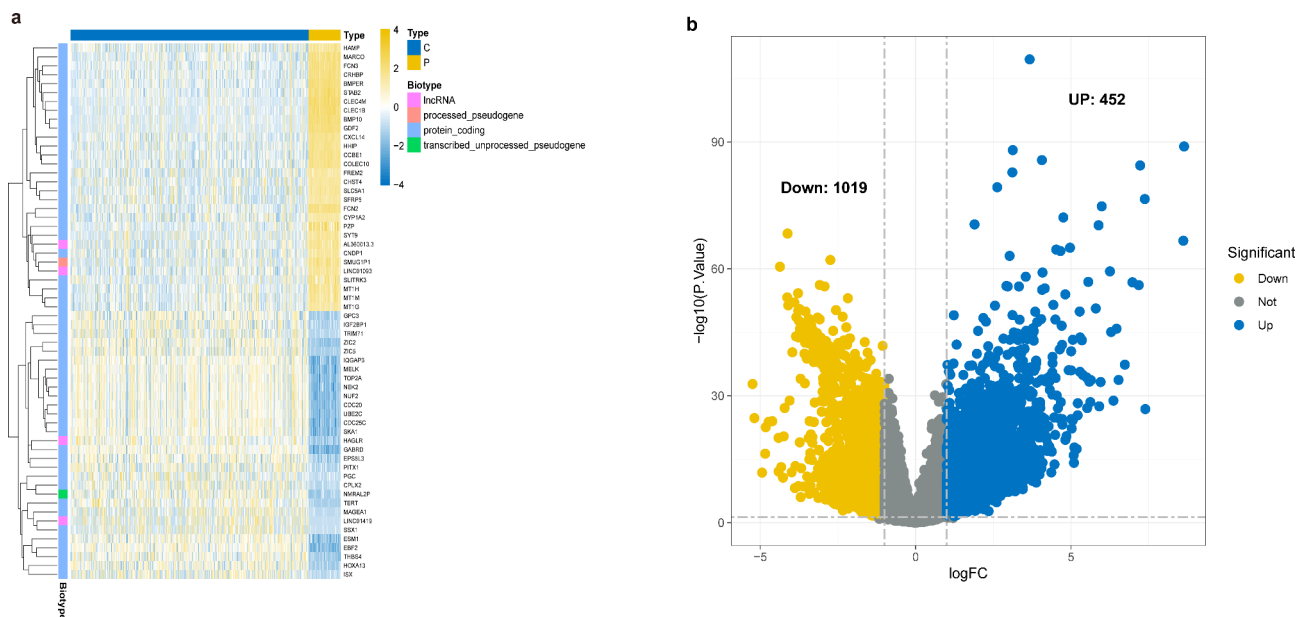
Fig. 1. Differentially expressed genes between HCC and adjacent ($N = 424$). (**a**). Heatmap demonstrating the differentially expressed genes between HCC and adjacent tissues. (**b**). Volcanoes indicate the 452 upregulated genes and 1019 down-regulated genes in HCC compared with adjacent tissues.
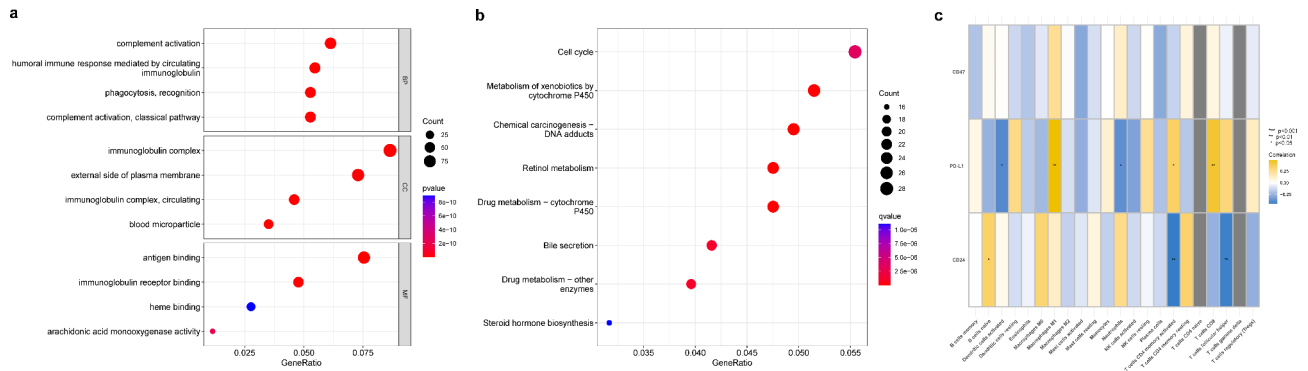
Fig. 2. Enrichment and immune infiltration analysis in HCC. (**a**). GO enrichment analyses ($N=1471$). (**b**). KEGG enrichment analyses ($N=1471$). Pathway analysis was performed using the KEGG database (www.kegg.jp/kegg/kegg1.html). (**c**). Correlation analysis between key genes and immune infiltration ($N=424$).
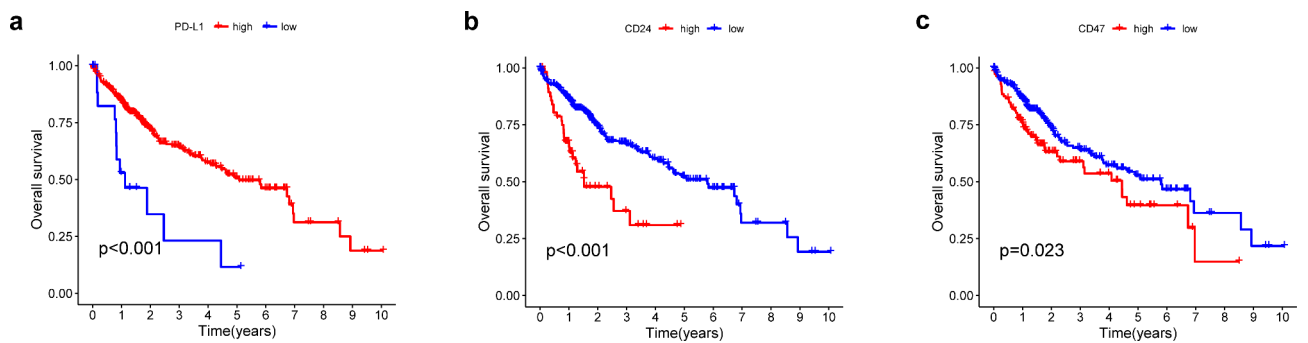
Fig. 3. Kaplan-Meier survival analysis comparing overall survival between the high-expression group ($N=188$) and the low-expression group ($N=189$) for PD-L1, CD24, and CD47. *OS* Overall survival.

Patient characteristics of HBV-associated HCC
Considering the limited data in the TCGA database, we attempted to evaluate the role of biomarkers in HBV-associated HCC using data from other patients. Sixty-seven patients with HBV-associated HCC were included; among them, 55 were male (82.09%), and 12 were female (17.91%), with an average age of 55.22 ± 10.81 years. All the patients received surgical treatment. The patients' detailed characteristics are shown in Table 1.

Survival analysis for HBV-associated HCC
Among the 67 patients, the longest OS was 82 months, the shortest OS was 1 month, and the average OS was 43.24 ± 22.17 months. The longest progression-free survival (PFS) was 82 months, the shortest PFS was 1 month, and the average PFS was 32.32 ± 24.25 months. The median follow-up time was 44 months (95% CI: 38.692–49.308 months), the median OS was 61 months (95% CI: 55.067–66.933 months), and the median PFS was 41 months (95% CI: 20.535–61.465 months). The cumulative 1-year survival rate post-surgery was 86.57%, the 2-year survival rate was 80.60%, and the 5-year survival rate was 29.85%. The 1-, 2-, and 3-year progression-free rates post-surgery were 71.64%, 61.94%, and 16.42%, respectively (Fig. 4).

PD-L1, CD24, and CD47 in the prognostic analysis of HBV-associated HCC
Univariate survival analysis was conducted on PD-L1, CD24, and CD47 in relation to the OS and PFS of patients with liver cancer. For OS and PFS, the prognosis was significantly better in the low PD-L1 expression group than in the high expression group (OS, $P=0.013$; PFS, $P=0.015$). Similarly, the prognosis for the low CD24 expression group was better than that for the high expression group (OS, $P=0.023$; PFS, $P=0.011$). Analysis of prognosis between the high and low CD47 expression groups showed that the survival curves intersected between the groups, and no statistically significant difference was observed (OS, $P=0.3$; PFS, $P=0.39$) (Fig. 5).

### Analysis of prognostic factors in HBV-associated HCC
The prognostic factors influencing OS and PFS were analyzed using Cox univariate and multivariate regression models. Univariate analysis identified several factors significantly associated with OS and PFS, including smoking, TNM stage, number of tumors, PD-L1 expression, CD24 expression, and maximum diameter of the primary tumor (see Supplementary Table S1). Multivariate analysis further adjusted for confounding variables and revealed that smoking, maximum tumor diameter, and PD-L1 expression were independent predictors of OS and PFS ($P<0.05$) (Table 2).

Characteristics		Categorical variables (percentage)	Characteristics	Continuous variables (mean ± standard)
Gender	Male	55(82.09%)	Age (years)	55.22 ± 10.81
	Female	12(17.91%)	AFP (ng/mL)	3192.06 ± 10962.96
Smoking	Yes	48(71.64%)	CEA (ng/mL)	2.91 ± 1.59
	No	19(28.36%)		
Drinking	Yes	13(19.40%)	CA199 (U/mL)	32.74 ± 38.64
	No	54(80.60%)		
HBeAg	Negative	51(76.12%)	ALT (U/L)	69.15 ± 85.77
	Positive	16(23.88%)		
Child-Pugh grade	A	63(94.03%)	AST (U/L)	63.49 ± 66.20
	B	4(5.97%)		
Number of tumors	1	57(85.07%)	ALB (g/L)	41.66 ± 5.18
	2	7(10.45%)	TBIL (umol/L)	14.33 ± 9.10
	> 2	3(4.48%)	DBIL (umol/L)	6.73 ± 5.76
Vascular invasion	No	56(83.58%)		
	Yes	11(16.42%)	IBIL (umol/L)	7.60 ± 4.53
Invasion of the liver capsule	No	13(19.40%)	RGT (μ/L)	110.36 ± 138.92
	Yes	54(80.60%)	AKP (U/L)	107.49 ± 72.35
Histopathological grading	Low	13(19.40%)	PT (sec)	13.29 ± 1.40
	Intermediate	49(73.13%)	WBC (×10^9/L)	6.73 ± 2.77
	High	5(7.46%)		
TNM stage	I	33(49.25%)	PLT (×10^9/L)	212.46 ± 155.53
	II	22(32.84%)		
	III	11(16.42%)	Hb (g/L)	141.58 ± 17.33
	IV	1(1.49%)		
Postoperative treatment modalities	RFA	2(2.99%)	Maximum diameter of primary tumor (cm)	7.16 ± 4.67
	TACE	30(44.78%)		
	N/A	35(52.24%)		

Table 1. Patient characteristics. *RFA* radiofrequency ablation, *TACE* transcatheter arterial chemoembolization.
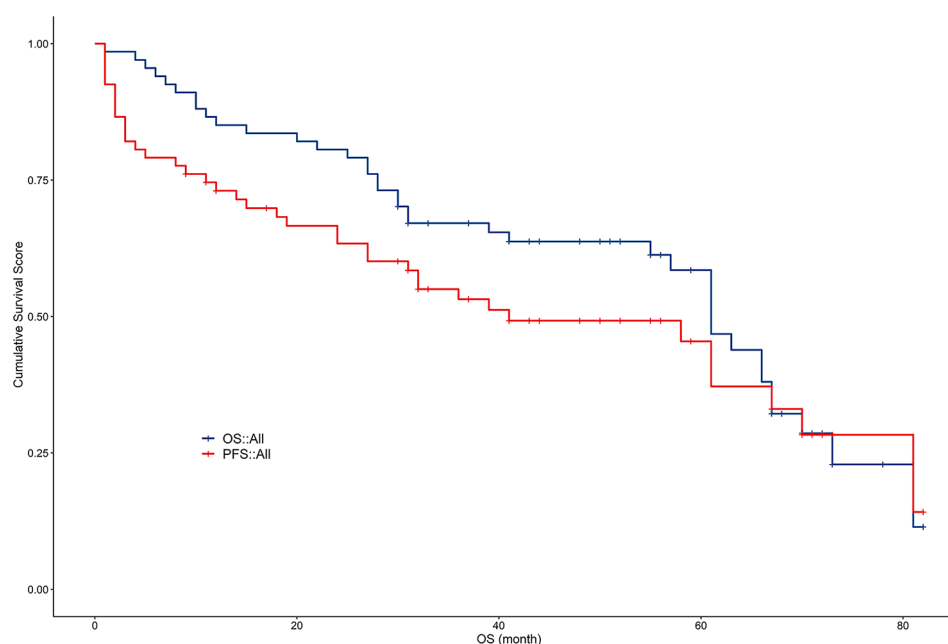
Fig. 4. Survival Analysis Curve ($N = 67$). (**a**). OS survival curve. (**b**). PFS survival curve. *OS* overall survival, *PFS* progression-free survival.
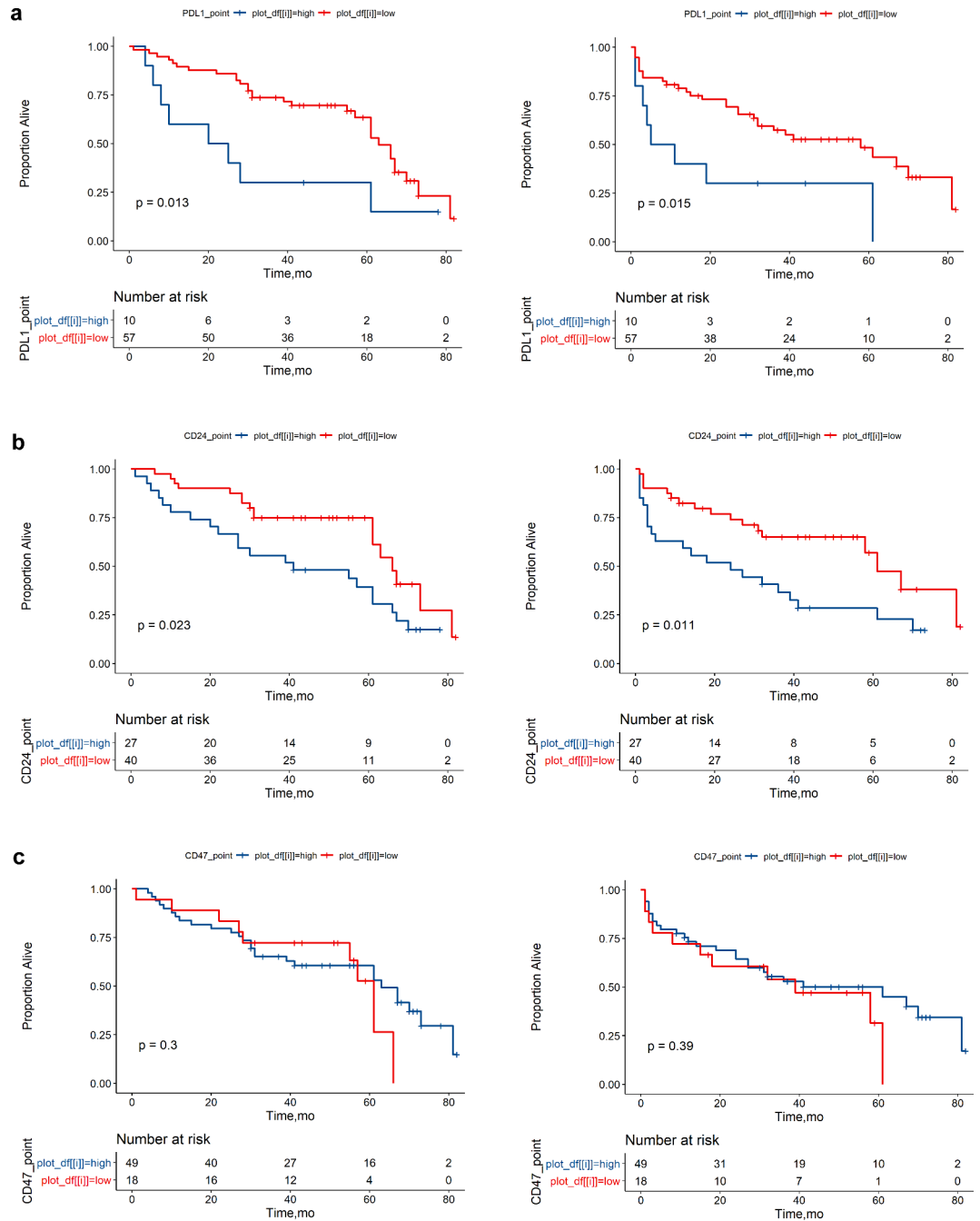
Fig. 5. The OS and PFS of HBV-associated HCC between high expression and low expression of PD-L1, CD24, and CD47 ($N = 67$). (**a**). PD-L1 OS survival curve and PFS survival curve. (**b**). CD24 OS survival curve and PFS survival curve. (**c**). CD47 OS survival curve and PFS survival curve. *OS* overall survival, *PFS* progression-free survival.

Relationship of PD-L1, CD24, and CD47 expression and clinicopathological factors

As shown in Table 3, PD-L1 expression was statistically associated with the staging of HBV-associated HCC ($p = 0.003$), tumor histopathological grading ($p = 0.025$), and Ki67 expression levels ($p = 0.024$). No statistically significant difference was found in tumor number, tumor size, cytokeratin 19, cytokeratin 18, cluster of differentiation 34 (CD34), glypican proteoglycan 3, and hepatocyte paraffin 1 ($P > 0.05$). Further Spearman correlation analysis revealed that PD-L1 was positively correlated with HCC staging and Ki67 expression. CD24 expression was significantly positively correlated with tumor staging ($p = 0.048$) but not with other clinicopathological factors (Table 4). In the analysis of CD47, statistical difference was found only with CD34, but the P-value was not statistically significant in the Spearman correlation coefficient analysis ($P > 0.05$) (see Supplementary Table S2).

Factors	P (OS)	HR (OS)	95%CI (OS)	P (PFS)	HR (PFS)	95%CI (PFS)
Smoking	0.04	2.062	1.035–4.11	0.014	2.433	1.201–4.931
TNM stage	0.665			0.177		
Histopathological grading	0.247			0.369		
Number of tumors	0.848			0.543		
Maximum diameter of primary tumor(cm)	0.008	1.091	1.023–1.163	0.008	1.089	1.023–1.159
CD24	0.365			0.13		
PD-L1	0.016	2.77	1.212–6.331	0.042	2.408	1.031–5.621

Table 2. Multivariate analysis of prognostic factors in HBV-associated HCC. *OS* overall survival, *PFS* progression-free survival.

Factors	N	PD-L1		P	Spearman correlation coefficient
		Low expression (n = 57)	High expression (n = 10)		
TNM stage (%)				0.003	0.260*
I	33	32(56.1)	1 (10.0)		
II	22	18 (31.6)	4 (40.0)		
III	11	7 (12.3)	4 (40.0)		
IV	1	0 (0.0)	1 (10.0)		
Histopathological grading (%)				0.025	0.214
Low	13	8 (14.0)	5 (50.0)		
Intermediate	49	44 (77.2)	5 (50.0)		
High	5	5 (8.8)	0 (0.0)		
Number of tumors (%)				0.343	
1	57	50 (87.7)	7 (70.0)		
2	7	5 (8.8)	2 (20.0)		
>2	3	2 (3.5)	1 (10.0)		
Maximum diameter of primary tumor (cm) (mean ± SD)	67	6.95 ± 4.32	8.34 ± 6.49	0.389	
CK19 (mean ± SD)	67	0.46 ± 1.74	0.60 ± 1.265	0.804	
CK18 (mean ± SD)	67	5.23 ± 4.09	4.10 ± 4.38	0.429	
CD34 (mean ± SD)	67	2.74 ± 3.60	3.70 ± 3.95	0.444	
Glypican-3 (mean ± SD)	67	3.77 ± 3.83	5.00 ± 4.37	0.363	
Hepar-1 (mean ± SD)	67	2.39 ± 3.53	0.50 ± 1.27	0.102	
Ki67(%) (mean ± SD)	67	24.91 ± 20.41	41.50 ± 21.56	0.024	0.262*

Table 3. Correlation between PD-L1 expression and clinicopathological factors in HBV-associated HCC. '*'indicates a significant correlation at a two-sided confidence level of 0.05.

Correlation analysis of PD-L1, CD24, and CD47

The results of correlation analysis between different variables, including PD-L1, CD24, and CD47 in patients with HBV-associated HCC, are shown in the scatter plot (Fig. 6). PD-L1 was positively correlated with CD24 and CD47 ($r = 0.31$, $r = 0.41$). CD47 was positively correlated with sialic acid-binding Ig-like lectin 10 (Siglec 10) and Child-Pugh grade ($r = 0.21$, $r = 0.23$). PD-L1 also showed positive correlations with Siglec 10 ($r = 0.42$), vascular invasion ($r = 0.19$), TNM staging ($r = 0.20$), and number of tumors ($r = 0.17$). Furthermore, CD24 was positively correlated with Siglec 10 ($r = 0.24$).

Discussion

This study aimed to unravel the complex interplay among PD-L1, CD24, and CD47 in HBV-associated HCC and their collective impact on patient prognosis. The findings highlighted the elevated expression of CD24 and PD-L1 as potential harbingers of adverse outcomes in HBV-associated HCC, corroborating their roles in tumor progression and immune evasion mechanisms.

Although surgical resection is a potentially curative treatment option for HCC, the high recurrence rate of HCC post-surgery remains a significant threat and leads to low survival rates among patients, especially those with HBV-associated HCC[13]. PD-L1 expression is elevated in the liver tissues of chronic HBV carriers

| Factors | N | CD24 | | P | Spearman correlation coefficient |
		Low expression ($n=40$)	High expression ($n=27$)		
TNM stage (%)				0.048	0.265[*]
I	33	22 (55.0)	11 (40.7)		
II	22	15 (37.5)	7 (25.9)		
III	11	3 (7.5)	8 (29.6)		
IV	1	0 (0.0)	1 (3.7)		
Histopathological grading (%)				0.988	
Low	13	8 (20.0)	5 (18.5)		
Intermediate	49	29 (72.5)	20 (74.1)		
High	5	3 (7.5)	2 (7.4)		
Number of tumors (%)				0.093	
1	57	36 (90.0)	21 (77.8)		
2	7	4 (10.0)	3 (11.1)		
>2	3	0 (0.0)	3 (11.1)		
Maximum diameter of primary tumor (cm) (mean ± SD)	67	6.49 ± 4.20	8.14 ± 5.22	0.156	
CK19 (mean ± SD)	67	0.40 ± 1.55	0.59 ± 1.87	0.647	
CK18 (mean ± SD)	67	4.33 ± 4.12	6.15 ± 3.95	0.075	
CD34 (mean ± SD)	67	2.25 ± 3.31	3.81 ± 3.95	0.084	
Glypican-3 (mean ± SD)	67	3.93 ± 3.87	4.00 ± 4.03	0.939	
Hepar-1 (mean ± SD)	67	2.57 ± 3.40	1.41 ± 3.24	0.164	
Ki67(%) (mean ± SD)	67	26.68 ± 20.61	28.44 ± 23.25	0.744	

Table 4. Correlation between CD24 expression and clinicopathological factors in HBV-associated HCC. '*' indicates a significant correlation at a two-sided confidence level of 0.05.

and the tumor tissues of patients with HBV-related HCC. This finding suggests that the PD-1/PD-L1 pathway may be involved in suppressing the host's antiviral and antitumor immune responses, thereby facilitating the progression of HBV-associated HCC[14,15]. To protect themselves, tumor cells continuously express some immune checkpoint molecules at high levels on their surface, thereby inhibiting the function of T-cells[16]. This mechanism helps avoid attacks from immune cells, resulting in immune escape[17]. PD-L1 is one of these immune checkpoints. By binding to the PD-1 expressed in them, tumor cells can inhibit the function of immune cells and eventually evade detection[18]. However, current data on the prognostic role of PD-L1 in HCC are inconsistent[19]. Some studies found that PD-L1 does not have a significant prognostic effect on patients with liver cancer after therapeutic liver resection[20]. In the present work, the group with low PD-L1 expression showed a significantly better prognosis than the high expression group. Moreover, PD-L1 expression was significantly associated with the staging of HBV-associated HCC, tumor differentiation degree, and Ki67 expression. A correlation was found between PD-L1 and adverse clinicopathological factors in HBV-associated HCC, suggesting that PD-L1 can serve as a relevant risk factor in the prognosis evaluation of HBV-associated HCC.

The PD-1/PD-L1 pathway plays a crucial role in the immunotherapy of HCC, where inhibitors have achieved breakthroughs in treatment and offer hope to patients[21,22]. However, only a subset of patients respond to these treatments[23]. During chronic HBV infection, these PD1 + T cells become exhausted, leading to a reduced tumor response during anti-PD1 immunotherapy[24]. Furthermore, many responders develop resistance after an initial response, leading to tumor recurrence and treatment failure in patients with HCC[25]. Therefore, on the basis of current clinical outcomes, PD-1/PD-L1 antibodies may be suitable for combination therapy[26]. CD24 is overexpressed in various human malignancies, including in gastrointestinal tumors, HCC tissues, and highly metastatic HCC cell lines[11,27,28]. Kim et al.[29] highlighted its role as a critical predictor for distant metastasis in patients undergoing curative surgery and subsequent adjuvant therapy, particularly in those with node-positive extrahepatic bile duct cancer. Yang et al.[11] also revealed that CD24 predicts OS and PFS in HCC. The expression of CD24 is not associated with α-fetoprotein, tumor-node-metastasis staging, or Edmondson grading but is related to poor prognosis. Our study found that CD24 was significantly correlated with OS and PFS in HBV-associated HCC. It was also significantly related to the staging of HBV-associated HCC. This suggests that CD24 expression may have potential prognostic value in HBV-associated HCC and is associated with specific adverse clinical and pathological features. Moreover, the positive correlation between CD24 and PD-L1 suggests that combined targeted therapy against CD24 and PD-L1 antibodies could hold value in treating HBV-associated HCC.

The CD47/SIRPα axis was identified as the first tumor phagocytosis-related checkpoint, also known as the macrophage "do not eat me" signal[30]. Whether CD47 can serve as an independent risk factor for the prognosis
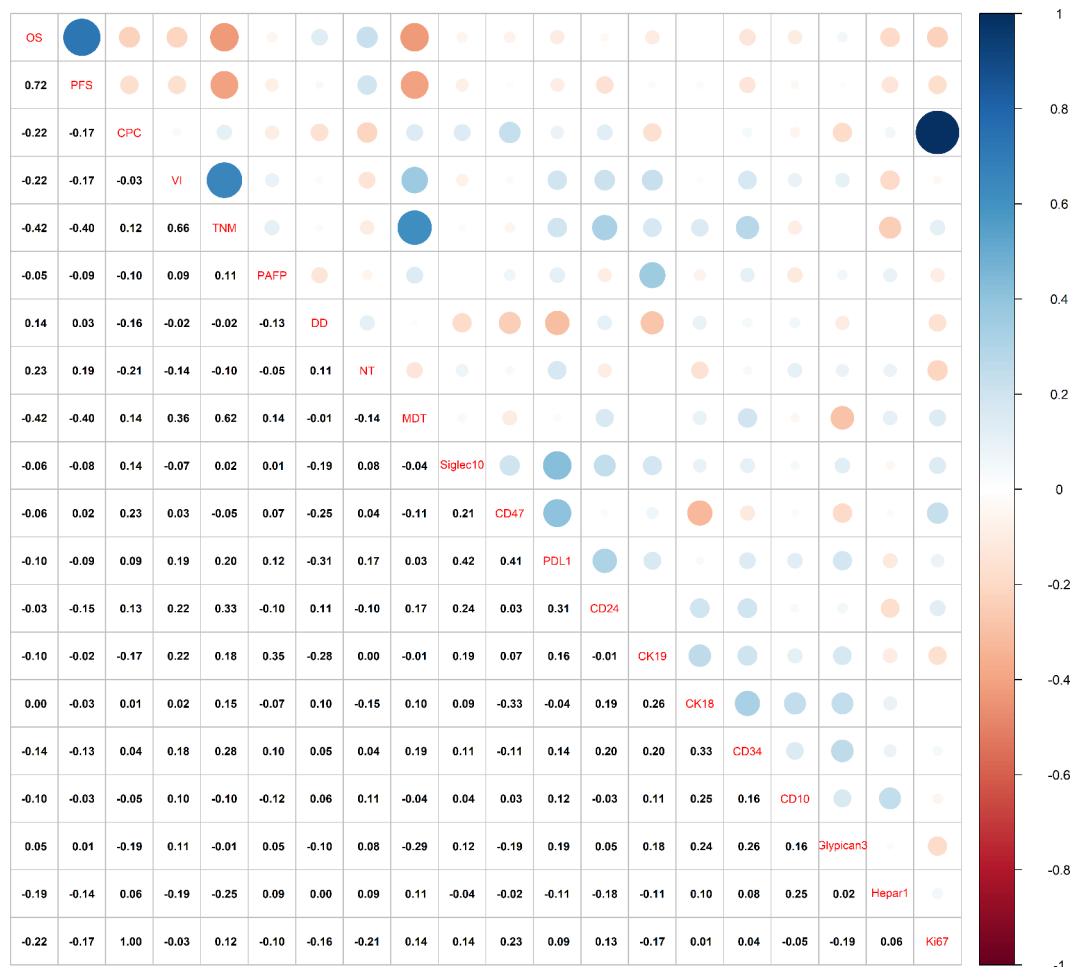
Fig. 6. Correlation Analysis between Different Variables in HBV-associated HCC patients ($N=67$). *OS* overall survival, *PFS* progression-free survival, *CPC* child-Pugh grade; *VI* vascular invasion, *PAFP* preoperative AFP, *DD* degree of differentiation, *NT* number of tumors, *MDT* maximum diameter of tumor, *Siglec10* sialic acid-binding immunoglobulin-like lectin 10, *Hepar1* hepatocyte paraffin 1.

of HCC remains uncertain. A recent study from South Korea analyzing survival outcomes based on CD47 expression in 166 HCC tissue samples found that CD47 expression was not related to PFS or OS in patients with HCC. However, CD47 expression was found to be related to PFS in patients with HCC who underwent surgical resection without any adjuvant treatment[31]. In our cohort, CD47 expression was not directly related to OS and PFS, indicating the multifaceted nature of immune evasion strategies adopted by tumor cells. This finding suggests that the impact of CD47 on patient prognosis could be modulated by other factors or signaling pathways within the tumor microenvironment. Its significant relationship with CD47 and PD-L1 expression implies a complex immune regulatory network. A combination therapy targeting CD47 and PD-L1 could be a novel and promising treatment strategy for HBV-associated HCC[32].

Multivariate Cox regression analysis revealed smoking, maximum diameter of tumor (MDT), and PD-L1 expression as independent prognostic factors for HBV-associated HCC. Approximately 13% of HCC cases worldwide are associated with smoking, and its interaction with chronic viral hepatitis increases the risk of HCC[33]. HBV and hepatitis C virus primarily act as promoters through chronic inflammation, chronic hepatitis, and liver cirrhosis-induced cell proliferation. Introduced with the Milan criteria, a 5 cm MDT could serve as a threshold for liver transplant in cases of single HCC lesions, beyond which recurrence rates increase[34]. With the increase in MDT, the median serum AFP levels and the percentage of patients with portal vein thrombosis and multifocal lesions also increase, suggesting that the aggressiveness of liver cancer may worsen or evolve with tumor growth[35]. Therefore, intrinsic tumor characteristics and extrinsic factors, such as lifestyle habits, must be considered in the management and prognostic prediction of HCC. For treatment, targeted therapy must be integrated with lifestyle and environmental changes.

Our research also has some limitations. (1) This study involved 67 patients with HBV-associated HCC, which, while informative, represented a relatively small cohort. It also limited the generalizability of the findings across the broad population of patients with HBV-associated HCC, especially when considering the diverse genetic and environmental factors influencing the development and progression of this disease. (2) Our findings need to be validated in a randomized controlled trial. (3) The clinical efficacy of combining anti-CD24 and anti-

CD47 with anti-PD1 must be evaluated, and (4) the complex immune characteristics of HBV-associated HCC require thorough elucidation.

Our study indicates that PD-L1 and CD24 can serve as predictors of poor prognosis in patients with HBV-associated HCC following curative resection, with PD-L1 identified as an independent prognostic risk factor. PD-L1 and CD24 expression levels are associated with certain adverse clinical pathological features of HBV-associated HCC. Moreover, PD-L1 is significantly positively correlated with CD24 and CD47, providing a basis for combined targeted therapy against immune checkpoints in clinical practice.

Materials and methods

Differential expression gene analysis of HCC and paracancerous tissues from the TCGA dataset

The Limma package (version 3.40.2) in R software was used to analyze the differential expression of mRNAs. Adjusted P-values were applied to correct for false positives in TCGA. A threshold of "Adjusted P-value < 0.05 and Log2 (fold change) > 1 or Log2 (fold change) < -1" was used to identify differentially expressed mRNAs. To confirm the underlying functions of potential targets, a functional enrichment analysis was performed. To investigate the carcinogenic roles of mRNAs, the ClusterProfiler package (version: 3.18.0) in R was employed to analyze GO terms and KEGG pathways.

Kaplan-meier analysis of gene features in the TCGA dataset using the survival and survminer packages in R

This study included 374 cases of HCC from the TCGA dataset. The overall survival times of differentially expressed PD-L1, CD24, and CD47 were compared using Kaplan-Meier analysis.

Immune infiltration analysis using CIBERSORT

CIBERSORT was used to analyze immune cell infiltration in HCC, with data processed using R version 4.0.3 (R Foundation for Statistical Computing, 2020) and visualized using the ggplot2 and pheatmap packages.

Patients and tumor samples

The study included 67 consecutive patients with HBV-associated HCC who underwent curative resection at the Fujian Provincial Hospital between January 2014 and January 2018. Patients included in the study were diagnosed with HCC based on postoperative pathology and tumor histological type, in accordance with the 2010 WHO classification criteria[36]. The specific inclusion criteria were as follows: (i) infection with HBV; (ii) pathological confirmation of HCC; (iii) liver function classified as Child-Pugh grade A or B; (iv) no prior anticancer treatment before surgery; (v) generally good overall health, without significant lesions in vital organs such as the heart, lungs, or kidneys. All methods and procedures in this study were conducted in accordance with Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki. This study was approved by the Medical Ethics Committee of Fujian Provincial Hospital (Approval No. K2021-02-013), and informed consent was obtained from all patients.

Immunohistochemistry and quantification of PD-L1, CD24 and CD47 density

Formalin-fixed, paraffin-embedded Sect. (4 μm thick) from the tumor core and adjacent normal tissues of HBV-associated HCC were dewaxed and rehydrated. The sections were incubated overnight at 4 °C with rabbit SIGLEC10-C-terminal polyclonal antibody (ab198724; Abcam, USA), rabbit anti-human PD-L1 polyclonal antibody (ab58810; Abcam, USA), and SIRP-α1 polyclonal antibody (Cat# YT4301, China). Subsequently, the sections were serially washed and incubated with secondary antibodies. The stained sections were evaluated microscopically by pathologists using a double-blind method, ensuring they were unaware of the patients' clinical data and outcomes. Staining intensity was scored as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive cells was scored as 0 (0%), 1 (1–10%), 2 (11–50%), and 3 (> 50%). The final score for each sample was calculated by multiplying the staining intensity score by the percentage score. Groups were defined as high expression when PD-L1 scores exceeded 3, CD24 scores exceeded 5, and CD47 scores were greater than 0; otherwise, they were classified as low expression. The staining intensity is shown in Supplementary Fig. S1–S3 online.

Follow-up and survival outcomes in patients with hbv-associated HCC

Postoperative follow-up was conducted through telephone interviews or outpatient visits. During the first two years, evaluations were performed every three months. From 2 to 5 years post-surgery, follow-ups were conducted every six months and annually thereafter. Patients' survival status, serum AFP levels, and imaging results (abdominal CT and/or MRI) were recorded. OS was defined as the interval from surgery to death or the last follow-up, while PFS was defined as the time from surgery to tumor recurrence, death, or the last follow-up. The endpoint of follow-up was either death or the completion of the follow-up period.

Statistical analysis

Quantitative data were expressed as mean ± standard deviation (x ± s), while qualitative data were summarized as counts (n) and percentages (%). The survival package in R was used to determine the optimal cutoff points for PD-L1, CD24, and CD47 based on survival time. Samples were then categorized into high and low expression groups accordingly. Kaplan-Meier survival curves were generated to evaluate survival differences, and the log-rank test was applied for group comparisons. Correlations between these markers, clinicopathological parameters, and among variables were analyzed using SPSS 25.0 software, with the χ^2 test, Fisher's exact test, and T-test. A P-value < 0.05 was considered statistically significant.

Data availability
The data that support the plots within this paper and other findings of this study are available from the corresponding author upon reasonable request.

References
1. Zheng, R. et al. Liver cancer incidence and mortality in China: Temporal trends and projections to 2030. *Chin. J. Cancer Res.* **30**, 571–579 (2018).
2. Villanueva, A. & Hepatocellular carcinoma *N Engl. J. Med.* **380**, 1450–1462 (2019).
3. Bray, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **68**, 394–424 (2018).
4. Singal, A. G., Lampertico, P. & Nahon, P. Epidemiology and surveillance for hepatocellular carcinoma: New trends. *J. Hepatol.* **72**, 250–261 (2020).
5. Gajewski, T. F., Schreiber, H. & Fu, Y. X. Innate and adaptive immune cells in the tumor microenvironment. *Nat. Immunol.* **14**, 1014–1022 (2013).
6. Hinshaw, D. C. & Shevde, L. A. The tumor microenvironment innately modulates cancer progression. *Cancer Res.* **79**, 4557–4566 (2019).
7. Abd, E. et al. Immune checkpoint inhibitors for unresectable hepatocellular carcinoma. *Vaccines* **8**, 616 (2020).
8. Llovet, J. M. et al. Immunotherapies for hepatocellular carcinoma. *Nat. Rev. Clin. Oncol.* **19**, 151–172 (2022).
9. Sangro, B., Sarobe, P., Hervás-Stubbs, S. & Melero I. Advances in immunotherapy for hepatocellular carcinoma. *Nat. Rev. Gastroenterol. Hepatol.* **18**, 525–543 (2021).
10. Altevogt, P., Sammar, M., Hüser, L. & Kristiansen, G. Novel insights into the function of CD24: A driving force in cancer. *Int. J. Cancer.* **148**, 546–559 (2021).
11. Yang, X. R. et al. CD24 is a novel predictor for poor prognosis of hepatocellular carcinoma after surgery. *Clin. Cancer Res.* **15**, 5518–5527 (2009).
12. Veillette, A. & Chen, J. SIRPα-CD47 immune checkpoint blockade in anticancer therapy. *Trends Immunol.* **39**, 173–184 (2018).
13. Tung-Ping Poon, R., Fan, S. T. & Wong, J. Risk factors, prevention, and management of postoperative recurrence after resection of hepatocellular carcinoma. *Ann. Surg.* **232**, 10–24 (2000).
14. Chen, J. et al. Intrahepatic levels of PD-1/PD-L correlate with liver inflammation in chronic hepatitis B. *Inflamm. Res.* **60**, 47–53 (2011).
15. Han, X. et al. Pre-treatment serum levels of soluble programmed cell death-ligand 1 predict prognosis in patients with hepatitis B-related hepatocellular carcinoma. *J. Cancer Res. Clin. Oncol.* **145**, 303–312 (2019).
16. Hepatitis, B. virus X protein-mediated upregulation of miR-221 activates the CXCL12-CXCR4 axis to promote NKT cells in HBV-related hepatocellular carcinoma. *Biocell* **47**, 1537–1548 (2023).
17. Liu, L. et al. Dynamic toxicity landscape of immunotherapy for solid tumors across treatment lines. *J. Natl. Cancer Cent.* **3**, 186–196 (2023).
18. Butte, M. J., Keir, M. E., Phamduy, T. B., Sharpe, A. H. & Freeman, G. J. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity* **27**, 111–122 (2007).
19. Yang, B. et al. Advance in yumor immunotherapy: establishing a new paradigm for oncological treatment. *Translational Surg. Oncol.* **1**, 30–43 (2023).
20. Liu, G. M., Li, X. G. & Zhang, Y. M. Prognostic role of PD-L1 for HCC patients after potentially curative resection: a meta-analysis. *Cancer Cell. Int.* **19**, 22 (2019).
21. Yi, M. et al. Combination strategies with PD-1/PD-L1 blockade: current advances and future directions. *Mol. Cancer.* **21**, 28 (2022).
22. A novel prognostic. target-gene signature and nomogram based on an integrated bioinformatics analysis in hepatocellular carcinoma. *Biocell* **46**, 1261–1288 (2022).
23. Kudo, M. Immune checkpoint inhibition in hepatocellular carcinoma: basics and ongoing clinical trials. *Oncology* **92** (Suppl 1), 50–62 (2017).
24. Lu, J. C. et al. Distinct PD-L1/PD1 Profiles and clinical implications in intrahepatic cholangiocarcinoma patients with different risk factors. *Theranostics* **9**, 4678–4687 (2019).
25. Chen, D. S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **39**, 1–10 (2013).
26. Li, Q., Han, J., Yang, Y. & Chen, Y. PD-1/PD-L1 checkpoint inhibitors in advanced hepatocellular carcinoma immunotherapy. *Front. Immunol.* **13**, 1070961 (2022).
27. Ni, Y. H., Zhao, X. & Wang, W. CD24, A review of its role in tumor diagnosis, progression and therapy. *Curr. Gene Ther.* **20**, 109–126 (2020).
28. Yao, C. et al. Angiogenesis in hepatocellular carcinoma: mechanisms and anti-angiogenic therapies. *Cancer Biology Med.* **20**, 25 (2023).
29. Kim, H. J. et al. Different prognostic significance of CD24 and CD44 expression in breast cancer according to hormone receptor status. *Breast* **20**, 78–85 (2011).
30. Oldenborg, P. A., Gresham, H. D. & Lindberg, F. P. Cd47-signal regulatory protein α (Sirpα) regulates Fcγ and complement receptor–mediated phagocytosis. *J. Exp. Med.* **193**, 855–862 (2001).
31. Kim, H., Bang, S., Jee, S., Paik, S. S. & Jang, K. Clinicopathological significance of CD47 expression in hepatocellular carcinoma. *J. Clin. Pathol.* **74**, 111–115 (2021).
32. Dai, X. et al. Immunotherapy for targeting cancer stem cells in hepatocellular carcinoma. *Theranostics* **11**, 3489–3501 (2021).
33. Chuang, S. C. et al. Interaction between cigarette smoking and hepatitis B and C virus infection on the risk of liver cancer: a meta-analysis. *Cancer Epidemiol. Biomarkers Prev.* **19**, 1261–1268 (2010).
34. Mazzaferro, V. et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl. J. Med.* **334**, 693–699 (1996).
35. Carr, B. I. et al. Changes in hepatocellular carcinoma aggressiveness characteristics with an increase in tumor diameter. *Int. J. Biol. Markers.* **36**, 54–61 (2021).
36. Jf, F. [WHO Classification of digestive tumors: the fourth edition]. *Ann. Pathol.* **31**, (2011).

Author contributions
All authors contributed to the study's conception and design. H. X. and T. H. conceived, designed, and supervised the study. L. A. , W. M. and W. Z. performed the experiments, analyzed data, authored and reviewed the article. L. J. , L. Z. , L. S. , L. S. and L. H. collected data, prepared figures and tables. All authors reviewed and

approved the final manuscript.

Declaration

Competing interests

The authors declare no competing interests.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Fujian Provincial Hospital (Approval No. K2021-02-013).

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-83145-5.

Correspondence and requests for materials should be addressed to H.T. or X.H.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.