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Original Article

ORIGINAL ARTICLE

Programmed death-ligand 1 expression in the tumour stroma of colorectal liver oligometastases and its association with prognosis after liver resection

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Abstract

Background The clinical value of programmed death-ligand 1 (PD-L1) expression in colorectal liver oligometastases (CLOs) remains undefined. This study aimed to detect PD-L1 in the microenvironment of CLOs and determine its association with patient prognosis.

Methods We collected 126 liver-resection specimens from CLO patients who underwent curative liver resection between June 1999 and December 2016. Immunohistochemistry (IHC) was performed to assess PD-L1 expression in paraffin-embedded specimens. Overall survival (OS) and recurrence-free survival (RFS) were analysed using the Kaplan–Meier method and log-rank test.

Results PD-L1 was mainly expressed in the stroma of liver oligometastases. Patients with high PD-L1 expression had a higher proportion of clinical-risk scores (CRSs) of 2–4 (67.7% vs 40.4%; $P = 0.004$). With a median 58-month follow-up, patients with high PD-L1 expression had a significantly lower 3-year OS rate (65.5% vs 92.7%; $P = 0.001$) and 3-year RFS rate (34.7% vs 83.8%; $P < 0.001$) than patients with low PD-L1 expression. Multivariate Cox analysis demonstrated that high PD-L1 expression (hazard ratio [HR] = 3.581; 95% confidence interval [CI] 2.301–9.972; $P = 0.015$), CRS 2–4 (HR = 6.960; 95% CI 1.135–42.689; $P = 0.036$) and increased preoperative CA19-9 (HR = 2.843; 95% CI 1.229–6.576; $P = 0.015$) were independent risk factors for OS. High PD-L1 expression (HR = 4.815; 95% CI 2.139–10.837; $P < 0.001$) and lymph-node metastasis (HR = 2.115; 95% CI 1.041–4.297; $P = 0.038$) were independent risk factors for RFS.

Conclusion This study found that PD-L1 was commonly expressed in the tumour stroma of CLOs and high PD-L1 expression was associated with poor prognosis.

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Key words: PD-L1; colorectal liver oligometastases; expression; prognosis

Introduction

Colorectal cancer (CRC) ranks third in terms of incidence and second in mortality among malignancies, and it is estimated that 147,950 individuals will be newly diagnosed with CRC in the USA in 2020 [1]. Moreover, there will be an estimated 53,200 CRC deaths in 2020, accounting for approximately 1 in 10 cancer cases and tumour-related deaths [1, 2]. The major cause of death in patients with CRC is distant metastasis, with liver metastasis representing the most common metastatic pattern [3]. Colorectal liver metastasis (CRLM) is diagnosed in almost 26.5% of patients within 5 years of primary cancer diagnosis [4]. Liver resection is the mainstay of curative treatment for CRLM; nevertheless, more than half of individuals who underwent curative liver resection experienced disease recurrence [5, 6]. There are various risk clinicopathological factors associated with poor prognosis after hepatectomy for CRLM, such as advanced T category of the primary tumour, moderate–poor tumour differentiation, positive and narrow resection margins, and high preoperative carcinoembryonic antigen (CEA) levels [7–10]. However, CRLM was recognized as a heterogeneous disease [11] and oligometastatic disease was recently highlighted by the latest version of the European Society for Medical Oncology Guidelines for the management of patients with metastatic colorectal cancer [12]. According to the previous definition, patients with colorectal liver oligometastases (CLOs) presented no more than five liver metastases confined to the liver, which also represented a disease state that existed in a transitional zone between localized and widespread systemic diseases, indicating a genuine potential for curable resection [13, 14]. Our previous study reported that CLO showed a 5-year overall survival (OS) rate of 45.9% after liver resection, with a 57.3% total-recurrence rate and a 16.0% early-recurrence rate [15]. Therefore, the management of CLO is challenging and exploring novel biomarkers to identify various prognostic risk subgroups to guide individual treatment is urgently needed.

Growing evidence indicates that the host immune response against CRC has a crucial function in tumour progression [16, 17]. As a new molecular targeted therapy, immune checkpoint blockade has attracted extensive attention in the treatment of various malignancies, including CRC [18]. Immunotherapy targeting programmed cell death 1 (PD-1) and programmed cell death-ligand (PD-L1) have recently been shown to improve prognosis in many cancers [19, 20]. PD-1 is mainly expressed on T-cells and regulates their activity, while PD-L1 is overexpressed in various tumour tissues, including melanoma, non-small-cell lung cancer, breast cancer, renal cancer, and gastric cancer. The interaction of PD-1 and PD-L1 weakens T-cell activity, leading to a decreased immune response to cancer cells [21]. Moreover, PD-L1 is also expressed in the tumour stroma [22, 23]. The tumour microenvironment is a site consisting of non-tumour cells (immune cells, fibroblasts, and endothelial cells) recruited to 'prepare the soil' for the arrival and growth of tumour cells [24, 25]. However, the characteristic of PD-L1 expression in the tumour stroma of liver oligometastasis has remained undefined. Therefore, the aim of this study was to investigate PD-L1 expression in CLO and confirm its prognostic value for CLO patients after liver resection.

Patients and methods

Clinical samples

The present study included 126 patients with CLO who underwent resection between 1999 and 2016 at Sun Yat-sen University Cancer Center (Guangzhou, China). The eligibility criteria were as follows: (i) histologically confirmed as colorectal adenocarcinoma; (ii) radiologically diagnosed colorectal single liver metastasis; (iii) curative resection for both primary colorectal tumour and liver metastases; and (4) presence of adequate metastatic specimens for analysis. The exclusion criteria were as follows: (i) preoperative extra-hepatic metastases and (ii) a history of prior liver resection. Demographic and clinicopathological characteristics were retrieved from medical records and follow-up data were collected from the hospital's tracking system. The treatment strategy and operability of liver metastases in each patient were determined based on the final consensus of a multidisciplinary team. This study was conducted with the approval of the Institute Research Ethics Committee of Sun Yat-sen University Cancer Center (approval number GZR2019-088).

Immunohistochemical staining

The liver-metastasis specimens were formalin-fixed, paraffin-embedded, and prepared for immunohistochemistry (IHC) according to standard procedures. The paraffin-embedded samples were subsequently continuously sliced into 4-μm-thick sections, which were dewaxed in xylene, rehydrated, and rinsed in graded ethanol solutions. Antigen retrieval was performed by heating at 100°C for 5 min in ethylene diamine tetraacetic acid solution (1 mmol/L, pH 8.0). The sections were then immersed in 0.3% hydrogen peroxide solution for 10 min and rinsed with phosphate buffered saline (PBS) for 5 min. The sections were incubated with 3% bovine serum albumin blocking buffer for 30 min at room temperature. All sections were incubated with a primary anti-PD-L1 antibody (1:300 dilution, ab58810; Abcam, Cambridge, UK) at 4°C overnight. After washing with 1× PBS, the sections were treated with anti-rabbit secondary antibodies (Zhongshan Golden Bridge Biotechnology, Beijing, China) at 37.5°C for 30 min. Finally, the visualized staining was carried out with 3,3'-diaminobenzidine tetrahydrochloride (DAB; Dako, Glostrup, Denmark).

Immunohistochemistry (IHC) scoring

IHC scores for PD-L1 expression were determined based on the percentage of positively stained stromal cells as previously described: 0, <1% positively stained cells; 1, 1%–24% positively stained cells; 2, 25%–49% positively stained cells; 3, 50%–74% positively stained cells; and 4, 75%–100% positively stained cells. Two independent investigators blindly graded all specimens. The cut-off IHC score for liver metastasis was determined as the median value of the IHC scores. High PD-L1 expression was defined as an IHC score exceeding the cut-off value.

Clinical-risk score (CRS)

We assessed the post-operative recurrence risk according to the Memorial Sloan-Kettering Cancer Center clinical-risk score (MSKCC-CRS) [26]. The five parameters of the CRS include positive primary tumour lymph nodes, simultaneous or

heterogeneous metastasis <12 months since the diagnosis of the primary tumour, the number of liver metastases >1, preoperative CEA level >200 ng/mL, and a maximum diameter of the liver metastases >5 cm, with 1 point for each item. Considering that the patients included in the present study had only one liver metastatic lesion, the value of the CRS in this sample ranged from 0 to 4. We classified patients into a low-CRS group (CRS 0–1) and a high-CRS group (CRS 2–4).

Follow-up

The patients were monitored through subsequent visits every 3 months for the first 2 years and semi-annually for 5 years after liver resection. All patients were followed up by regular clinical diagnostic examinations, including analysis of serum CEA and carbohydrate antigen (CA) 19–9 (CA19-9) levels, radiography, ultrasonography, and computed tomography. OS was defined as the time interval from the date of liver-metastases resection to death from any cause or the last follow-up. Recurrence-free survival (RFS) was defined as the time interval from the date of liver-metastases resection to disease recurrence, death, or the last follow-up. Random censoring was applied to patients without recurrence or death at the last follow-up. The final follow-up visit occurred in February 2020.

Statistical analysis

Statistical analyses were performed with the SPSS 25.0 software (IBM, Chicago, IL, USA) and GraphPad Prism 7 software (GraphPad Software, Inc., San Diego, CA, USA). Categorical variables are presented as percentages and compared by the chi-square (χ^2) test, Fisher's exact test, or nonparametric Spearman's correlation test. Continuous variables are presented as the median (range). The Kaplan–Meier method was used to estimate survival rates and group differences were assessed by the log-rank test. Multivariate Cox proportional-hazards analysis was performed for variables with $P < 0.10$ in the univariate analysis. $P < 0.05$ was considered statistically significant. Hazard ratios (HRs) and 95% confidence intervals (CIs) were subsequently calculated.

Results

Patient characteristics

A total of 126 patients were included in the present study. These patients' characteristics are shown in Table 1. The median patient age was 58 years (range, 25–78 years), with 59.5% (75/126) of patients being males and 40.5% (51/126) of patients being females. With regard to available CRS in 109 patients, 48 (44.0%) were classified into the low-CRS group (CRS 0–1) and 61 (56.0%) were classified into the high-CRS group (CRS 2–4). For the 115 patients receiving perioperative treatment, 32 (25.4%) received preoperative chemotherapy before liver resection, whereas 86 (68.3%) received adjuvant chemotherapy after liver resection.

Associations of PD-L1 expression with clinicopathological characteristics

Different levels of PD-L1 expression were clearly shown in the stroma of the liver metastases (Figure 1). The IHC score for PD-L1 was 0 in 9.5% (12/126) of patients, 1 in 35.7% (45/126) of patients, 3 in 18.3% (23/126) of patients, and 4 in 36.5% (46/126) of patients. The PD-L1 IHC score cut-off value was determined according to a median score of 3. A total of 69 patients with IHC scores ≥3 were classified into the high-PD-L1-expression group, while 57 patients with IHC scores <3 constituted the low-PD-L1-expression group. We then evaluated the associations of PD-L1 expression in tumour tissues with clinicopathological variables including sex, age, primary tumour location, tumour differentiation, preoperative CEA and CA19-9 levels, pathological tumour-node-metastasis (TNM) stage, CRS, and metastatic tumour size. As shown in Table 2, patients with high PD-L1 expression presented a significantly higher proportion of CRS 2–4 than those with low PD-L1 expression (67.7% vs 40.4%; $P = 0.004$).

Table 1. Associations of clinicopathological characteristics with programmed death-ligand 1 expression in 126 patients with colorectal liver oligometastases

Variable	No. of patients (%)
Sex	
Male	75 (59.5)
Female	51 (40.5)
Age (years)	
<60	77 (61.1)
≥60	49 (38.9)
Primary tumour location	
Right-sided colon	33 (26.2)
Left-sided colon	47 (37.3)
Rectum	46 (36.5)
T category[a]	
T1	1 (0.9)
T2	6 (5.3)
T3	71 (62.3)
T4	36 (31.6)
N category[b]	
0	41 (36.3)
1	50 (44.2)
2	22 (19.5)
Primary tumour differentiation	
Poor	34 (27.0)
Well to moderate	92 (73.0)
Neoadjuvant chemotherapy	
No	94 (74.6)
Yes	32 (25.4)
Size of liver metastases	
<3 cm	89 (70.6)
≥3 cm	37 (29.4)
Adjuvant chemotherapy	
Yes	86 (68.3)
No	40 (31.7)
CRS[c]	
0–1	48 (44.0)
2–4	61 (56.0)
Preoperative CEA (ng/mL)[d]	
Normal (<5)	40 (46.5)
Advanced (≥5)	46 (53.5)
Preoperative CA19-9 (U/mL)[e]	
Normal (<35)	55 (65.5)
Advanced (≥35)	29 (34.5)

CEA, carcinoembryonic antigen; CA19–9, carbohydrate antigen 19–9; TNM stage, tumour-node-metastasis classification; CRS, clinical-risk score.
[a]The T-category data were available for 114 patients.
[b]The N-category data were available for 113 patients.
[c]The CRS-score data were available for 109 patients.
[d]The preoperative-CEA-level data were available for 86 patients.
[e]The preoperative-CA19–9-level data were available for 84 patients.

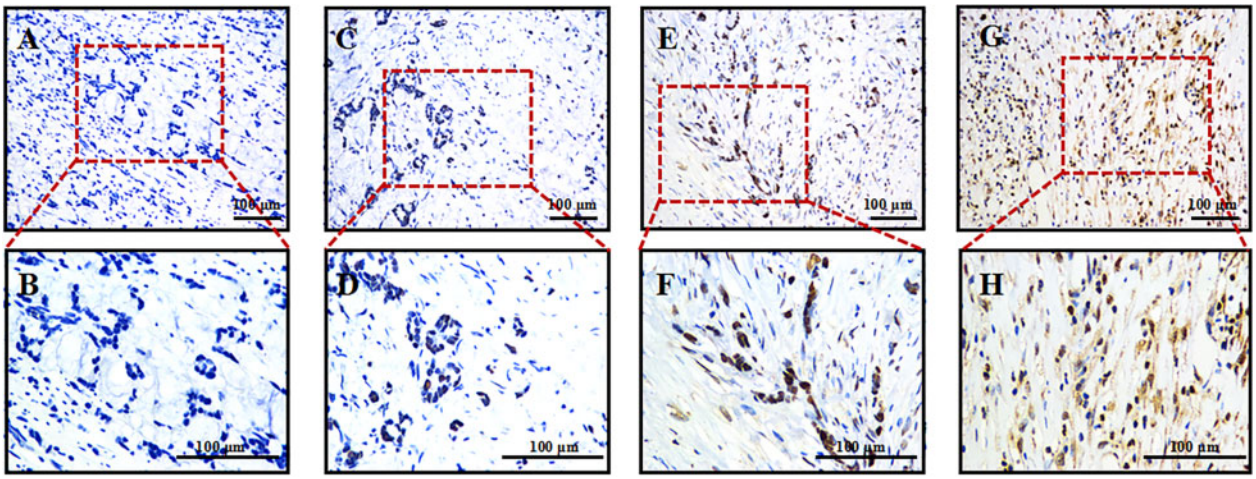

Figure 1. Immunocytochemical staining for programmed death-ligand 1 (PD-L1) detection in the stroma of liver oligometastasis. (A) No expression of PD-L1 indicated an immunohistochemistry (IHC) score of 0 (200×). (C) Weak expression of PD-L1 showing an IHC score of 1 (200×). (E). Medium expression of PD-L1 as an IHC score of 3 (200×). (G) Strong expression of PD-L1 as an IHC score of 4 (200×). (B), (D), (F), and (H) Higher magnification (400×) of the areas in boxes in (A), (C), (E), and (G), respectively

Association of PD-L1 expression with prognosis

At the median follow-up of 58 months (range, 2–153 months), 62 patients (49.2%) experienced tumour recurrence, including 46.8% (29/62) of patients with intra-hepatic recurrence, 8.1% (5/62) of patients with lung metastases, 11.2% (7/62) of patients with abdominal pelvic metastases, and 16.1% (10/62) of patients with multiple organ metastases. As a result, 34 patients (27.0%) died of tumour progression. The 3-year OS rate was 78.9% and the 3-year RFS rate was 56.6%. The 3-year OS rate was significantly lower in the high-PD-L1-expression group than in the low-PD-L1-expression group (65.5% vs 92.7%; $P = 0.001$; Figure 2A). In addition, the 3-year RFS rate was also significantly lower in patients with high PD-L1 expression than in patients with low PD-L1 expression (34.7% vs 83.8%; $P < 0.001$; Figure 2B). The 3-year cumulative occurrence rate of intra-hepatic metastasis was higher in the high-PD-L1-expression group than in the low-PD-L1-expression group (32.1% vs 14.0%; $P = 0.003$; Figure 2C). The 3-year cumulative occurrence rate of extra-hepatic metastasis was also higher in the high-PD-L1-expression group than in the low-PD-L1-expression group (35.2% vs 4.9%; $P < 0.001$; Figure 2D).

Univariate and multivariate analyses of survival outcomes

Univariate analysis revealed that N category 1–2 (HR = 3.804, 95% CI 1.124–12.856; $P = 0.032$), CRS as 2–4 (HR = 2.669; 95% CI 1.127–6.320; $P = 0.026$), and high PD-L1 expression in liver metastases (HR = 4.373, 95% CI 1.887–10.135; $P < 0.001$) were significantly associated with a worse OS (Table 3). Multivariate analysis identified high PD-L1 expression (HR = 3.581, 95% CI 1.286–9.972; $P = 0.015$), elevated preoperative CA19-9 (HR = 2.843, 95% CI 1.229–6.576; $P = 0.015$), and CRS 2–4 (HR = 6.960, 95% CI 1.135–42.689; $P = 0.036$) as independent prognostic factors for a worse OS (Table 3). Additionally, the univariate analysis revealed that N category 1–2 (HR = 2.334, 95% CI 1.152–4.729; $P = 0.019$), CRS 2–4 (HR = 2.221; 95% CI 1.243–3.931; $P = 0.007$), and high PD-L1 expression in liver oligometastases (HR = 5.400, 95% CI 2.864–10.180; $P < 0.001$) were significantly associated with a worse RFS (Table 4). Multivariate analysis indicated that high PD-L1 expression (HR = 4.815, 95% CI 2.139–10.837; $P < 0.001$) and N category 1–2 (HR = 2.115, 95% CI 1.041–4.297; $P = 0.038$) were independent risk prognostic factors for RFS (Table 4).

Discussion

In this study, we assessed PD-L1 expression by using IHC staining of liver metastases from CLO patients. The results revealed that patients with high PD-L1 expression likely presented a higher CRS. Although the prognostic significance of PD-L1 in CRC has been investigated, our current study adds two new and innovative points to the current understanding of CRC. Unlike previous studies that focused solely on the expression of PD-L1 in colorectal primary tumours, our data revealed the widespread expression of PD-L1 in liver-oligometastasis tissues. Another innovation was the discovery of a specific prognostic biomarker for CLO. Our results indicate that patients with high PD-L1 expression in the stroma of CLOs have a significantly lower OS and RFS than those with low PD-L1 expression in the stroma of CLOs, and high PD-L1 expression was an independent predictor of OS and RFS. These findings suggest that PD-L1 expression may serve as a valuable prognostic factor for CLO patients.

Unlike our study results, previous studies have showed that high PD-L1 expression in CRC was associated with improved prognosis [27, 28]. Droeser *et al.* [27] reported that PD-L1 expression was associated with improved survival in mismatch repair (MMR)-proficient CRC. Another study investigated the prognostic value of PD-L1 in colorectal tumour cells and PD-1 in tumour-infiltrating lymphocytes (TILs) in CRC, and found that higher PD-1 and PD-L1 levels were associated with improved OS ($P = 0.032$ and $P = 0.002$, respectively) [28]. The authors explained the positive prognostic impact of TILs expressing PD-1 as a compensatory upregulation. These data reflect the complex tumour–host immune relationship. The discrepancies may result from varying cohort sizes, different antibodies, or different IHC methodologies. In addition, PD-L1 staining is very heterogeneous in a given metastasis [29]. Investigators have also found that the association between PD-L1 and prognosis differs among tumour types [30–33].

Table 2. Associations of clinicopathological characteristics with programmed death-ligand 1 expression in 126 patients with colorectal liver oligometastases

Variable	Low PD-L1 expression ($n = 57$)	High PD-L1 expression ($n = 69$)	P-value
Sex			
Male	34 (59.6)	41 (59.4)	0.979
Female	23 (40.4)	28 (40.6)	
Age (years)			
<60	35 (61.4)	42 (60.9)	0.951
≥60	22 (38.6)	27 (39.1)	
Tumour location			
Right-sided colon	15 (26.3)	18 (26.1)	0.761
Left-sided colon	23 (40.4)	24 (34.8)	
Rectum	19 (33.3)	27 (39.1)	
Size of liver metastases			
<3 cm	43 (75.4)	46 (66.7)	0.282
≥3 cm	14 (24.6)	23 (33.3)	
Neoadjuvant chemotherapy			
Yes	10 (17.5)	22 (31.9)	0.066
No	47 (82.5)	47 (68.1)	
Primary tumour differentiation			
Poor	12 (21.1)	22 (31.9)	0.173
Well to moderate	45 (78.9)	47 (68.1)	
T category[a]			
T1–T3	38 (77.6)	40 (61.5)	0.103
T4	11 (22.4)	25 (38.5)	
N category[b]			
N0	21 (43.8)	20 (30.8)	
N1–2	27 (56.2)	45 (69.2)	0.171
CRS[c]			
0–1	28 (59.6)	20 (32.3)	0.004
2–4	19 (40.4)	42 (67.7)	
Adjuvant chemotherapy			
Yes	35 (61.4)	51 (73.9)	0.133
No	22 (38.6)	18 (26.1)	
Preoperative CEA (ng/mL)[d]			
Normal (<5)	9 (26.5)	13 (25.0)	0.879
Advanced (≥5)	25 (73.5)	39 (75.0)	
Preoperative CA19-9 (U/mL)[e]			
Normal (<35)	20 (60.6)	35 (68.6)	0.450
Advanced (≥35)	13 (39.4)	16 (31.4)	

All values are presented as numbers of cases followed by percentages in parentheses.
CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; TNM stage, tumour-node-metastasis classification; CRS, clinical-risk score.
[a]The T-category data were available for 114 patients.
[b]The N-category data were available for 113 patients.
[c]The CRS-score data were available for 109 patients.
[a]dThe preoperative-CEA-level data were available for 86 patients.
[e]The preoperative-CA19-9-level data were available for 84 patients.

PD-L1 is expressed in different cell types and acts as a ligand of PD-1 and B7-1 [34]. PD-L1 can induce T-cell dysfunction and tolerance through various mechanisms, including induction of T-cell apoptosis and exhaustion [35], IL-10 upregulation [36], and alteration of Treg functions [37]. Moreover, a previous study assessing ovarian cancer suggested that PD-L1 inhibits the intra-tumour migration of $CD8^+$ T-cells [38]. Previous studies have demonstrated that PD-L1 overexpression is associated with poorer prognosis in various cancer types including solid tumours and haematological malignancy [39, 40]. This finding suggests that variable PD-L1 levels and the precise locations of various immune-cell populations might potentially reflect different functions in tumour immune suppression. PD-L1 not only acts by interacting with PD-1 and modifying T-cell receptor or B-cell receptor signals, but also transmits signals to cells expressing PD-L1 [35, 41]. The bidirectional signalling of PD-1 and PD-L1 may help to clarify some of the contradictory results in studies analysing the PD-1–PD-L1 pathway.

Based on our findings, we suggest that PD-L1 expression can be used to stratify CLO patients to predict prognosis. In the present study, we found that PD-L1 was positively associated with high CRS (as 2–4) and showed a high occurrence rate in intrahepatic and extra-hepatic metastases, which indicates that tumours with high PD-L1 expression may present poor biological behaviours. For these patients, more aggressive postoperative chemotherapy should be given; even targeted treatment and more frequent follow-up examinations should be conducted. Based on these results, detecting the expression of PD-L1 could help us to personalize treatment to provide patients with optimal survival benefits and quality of life. Immunotherapeutic drugs targeting T-cell immune checkpoints such as PD-1, PD-L1, and cytotoxic T-lymphocyte-associated

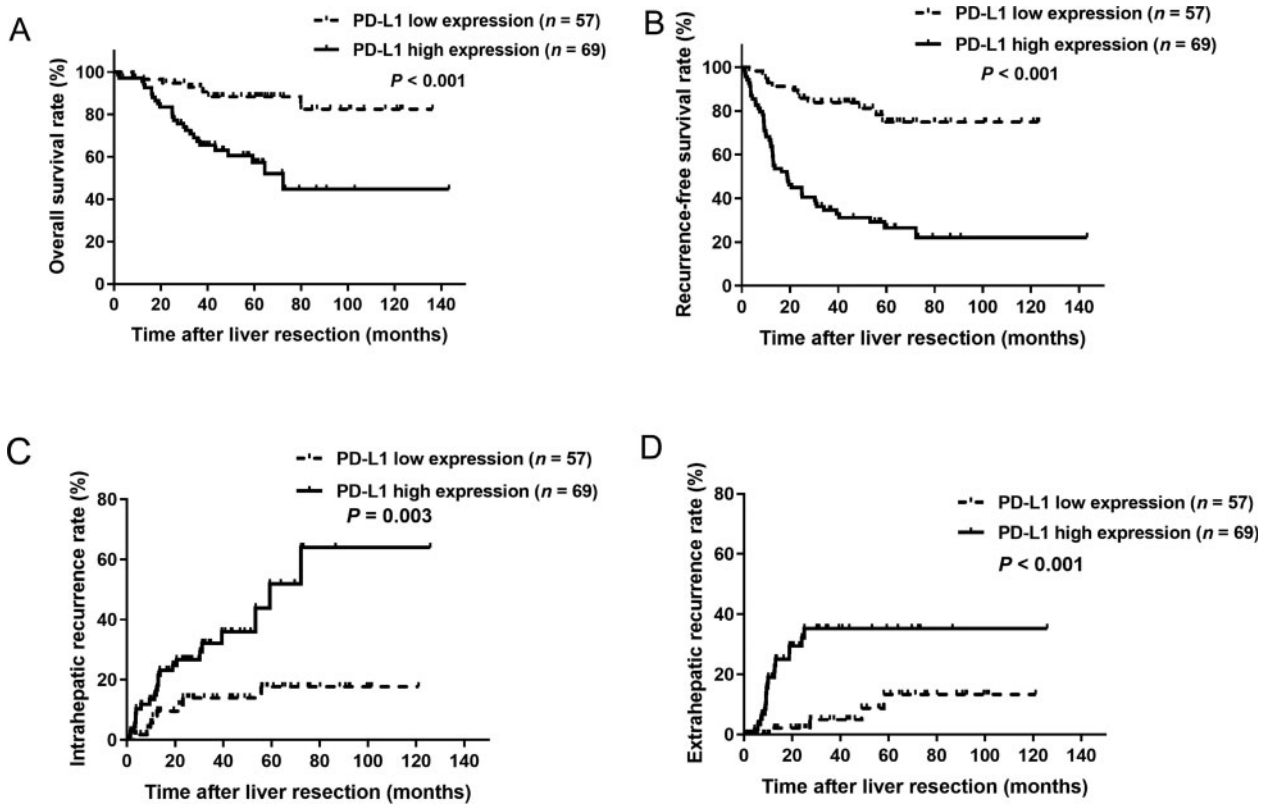

Figure 2. Kaplan–Meier survival curves grouped by high and low programmed death-ligand 1 (PD-L1) expression levels in the tumour stroma of liver oligometastases. (A) Overall survival (OS) of all patients. (B) Recurrence-free survival (DFS) of all patients. (C) Cumulative incidence of intra-hepatic metastasis. (D) Cumulative incidence of extra-hepatic metastasis

Table 3. Univariate and multivariate Cox-regression analyses of prognostic predictors of overall survival in patients

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
PD-L1 expression (high vs low)	4.373 (1.887–10.135)	< 0.001	3.581 (2.301–9.972)	0.015
Sex (male vs female)	1.019 (0.514–2.020)	0.957		
Age (≥60 vs <60 years)	1.320 (0.671–2.599)	0.421		
Tumour differentiation (poor vs well to moderate)	1.127 (0.510–2.490)	0.767		
T category (T4 vs T1–3)	1.626 (0.827–3.198)	0.159		
N category (N1–2 vs N0)	3.804 (1.124–12.856)	0.032		
Size of liver metastasis (≥3 vs <3 cm)	1.800 (0.908–3.568)	0.092		
CRS (2–4 vs 0–1)	2.669 (1.127–6.320)	0.026	6.960 (1.135–42.689)	0.036
Location of primary tumour (right-sided colon vs left-sided colon and rectum)	1.267 (0.573–2.801)	0.558		
Neoadjuvant chemotherapy (yes vs no)	1.274 (0.593–2.739)	0.535		
Adjuvant chemotherapy (yes vs no)	1.635 (0.709–3.774)	0.249		
Preoperative CEA (≥5 vs <5 ng/mL)	1.105 (0.403–2.555)	0.975		
Preoperative CA19-9 (≥35 vs <35 U/mL)	2.022 (0.913–4.476)	0.083	2.843 (1.229–6.576)	0.015

HR, hazard ratio; CI, confidence interval; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; CRS, clinical-risk score.

antigen 4 (CTLA-4) have been investigated as potential treatments for cancer [21, 42, 43]. These immunotherapeutic drugs have shown good clinical efficacy in a variety of cancers, including non-small-cell lung cancer, melanoma, and renal-cell carcinoma [44]. Therefore, immunotherapy targeting PD-L1 may be a useful adjuvant treatment option for CLO patients with high PD-L1 expression.

This retrospective study had several limitations. First, our study evaluated only the associations of PD-L1 expression with clinicopathological characteristics and patient prognosis. We failed to analyse the association of PD-L1 expression with molecular features and other immune markers in the present study. Second, there were no comparisons of different anti-PD-L1 clones in our study. A previously reported study showed that

Table 4. Univariate and multivariate Cox-regression analyses of prognostic factors of recurrence-free survival in patients

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
PD-L1 expression (high vs low)	5.400 (2.864–10.180)	<0.001	4.815 (2.139–10.837)	<0.001
Sex (male vs female)	1.059 (0.591–1.631)	0.994		
Age (≥60 vs <60 years)	1.125 (0.664–1.862)	0.684		
Tumour differentiation (poor vs well to moderate)	1.192 (0.688–2.064)	0.531		
T category (T4 vs T1–3)	1.244 (0.748–2.069)	0.400		
N category (N1–2 vs N0)	2.334 (1.152–4.729)	0.019	2.115 (1.041–4.297)	0.038
Size of liver metastasis (≥3 vs <3 cm)	1.409 (0.832–2.385)	0.202		
CRS (2–4 vs 0–1)	2.221 (1.243–3.931)	0.007		
Location of the primary tumour (right-sided colon vs left-sided colon and rectum)	1.220 (0.690–2.155)	0.494		
Neoadjuvant chemotherapy (yes vs no)	2.416 (1.428–4.089)	0.001		
Adjuvant chemotherapy received (yes vs no)	1.722 (0.947–3.129)	0.075		
Preoperative CEA (≥5 vs <5 ng/mL)	1.244 (0.651–2.375)	0.509		
Preoperative CA19-9 (≥35 vs <35 U/mL)	1.332 (0.723–2.456)	0.358		

HR, hazard ratio; CI, confidence interval; CEA, carcinoembryonic antigen; CRS, clinical-risk score.

the lack of technical homogeneity is a major issue when attempting to compare the results of different CRC-dedicated studies analysing PD-L1 expression and the tumour immune microenvironment [45]. Third, the present study was conducted using a retrospective method with a limited volume size of CLO patients from a single centre. Therefore, large-scale, prospective studies are warranted to confirm the present findings.

In conclusion, we found that PD-L1 was highly expressed in the tumour microenvironment in patients with CLO and that PD-L1 expression was associated with tumour progression and poor prognosis. The above method is simple and provides a new tool for the detection of PD-L1 expression, which may help to identify patients who may benefit from anti-PD-L1/PDL1 immunotherapy. However, the relationship of PD-L1 expression with CLOs and the underlying mechanism remain unclear and require further investigation.

Authors' contributions

J.H.P., Y.T., and Z.H.L. were responsible for the study design. J.H.P., Y.X.Z., B.J.L., and Z.Z.P. were responsible for data collection. L.Z. and Q.J.O. performed the immunohistochemistry. J.H.P. and Y.T. performed the data analysis. J.H.P., Y.T. and Y.X.Z. drafted the manuscript. L.Z. and Z.H.L. were responsible for the supervision. All authors have read and approved the final manuscript.

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Conflicts of interest

The authors declared that they have no conflicts of interest to this work.

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