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ORIGINAL ARTICLE

Prognostic Value of PD-L1 in Patients with Hepatocellular Carcinoma

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SUMMARY

Background: **The aim of the study is to investigate whether programmed death ligand l (PD-L1) on tumor cells has prognostic significance in patients with hepatocellular carcinoma.**
Methods: **A cohort of 143 patients with hepatocellular carcinoma was enrolled. PD-L1 expression was detected by immunohistochemistry. The association of PD-L1 expression with clinical characteristics and survival was analyzed.**
Results: **PD-L1 positive rate in our study was 13.3% (19/143). None of clinical characteristics, including gender, age, virus infection, AFP, vascular invasion, tumor size, and number, was significantly associated with PD-L1 expression ($p > 0.05$). PD-L1 expression was significantly associated with cirrhosis ($p = 0.016$). PD-L1 expression was not significantly associated with survival (Log-rank $p = 0.076$; HR: 0.363 $p = 0.091$).**
Conclusions: **PD-L1 expression failed to have a markedly significant prognostic association with survival in patients with hepatocellular carcinoma.**
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KEY WORDS

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers, which ranks as the second leading cause of cancer-related death worldwide [1]. Only a minority of patients diagnosed with early HCC are eligible for curative therapies such as surgical resection, liver transplantation, or ablation [2]. The 5-year survival rate is about 70% in patients with early HCC, whereas patients with advanced HCC have a median survival of less than one year [3]. In spite of recent advances in molecular pathogenesis, effective therapeutic options for patients with advanced HCC remain limited.

Recent evidence reveals HCC as an immunogenic tumor [4]. The pathway of programmed cell death protein-1 (PD-1) and programmed death-ligand 1 (PD-L1) plays an important role in tumor microenvironment formation and immune tolerance [5]. Nivolumab, an anti-PD-1 monoclonal antibody to reverse immune tolerance by means of blocking the immune checkpoint in-

teraction between PD-1and PD-L1, has yielded encouraging objective rates and survival in HCC [6]. It is reasonable to speculate that PD-L1 may serve as a valuable biomarker to stratify target population into a subgroup, which is sensitive to PD-1/PD-L1 blockade therapy and benefits from immunotherapy.

However, whether PD-L1 expression on tumor cells can influence prognosis in HCC remains not clear. There are few studies on the association of PD-L1 expression with prognosis in HCC, and their results are inconsistent [7-10]. Moreover, these studies had either a small size or complicated scoring algorithms to define PD-L1 expression status. These available data invariably used immunostaining intensity to define the status of PD-L1 expression on HCC tumor cells, and the results had low reproducibility. To simplify and standardize the criteria for PD-L1 expression, most recently an expert consensus recommends the percentage of positive tumor cells to define the status of PD-L1 expression [11]. Therefore, in this study, we used percentage of positive tumor cells by immunohistochemical staining to dichotomize PD-L1 expression status from a large cohort of patients with HCC. Furthermore, we investigated the association of PD-L1 expression status with clinical characteristics and survival.

MATERIALS AND METHODS

Study population

Our study was approved by the Ethics Committee of Yijishan Hospital. Written informed consent before collecting tissue samples was obtained from all patients. We retrospectively reviewed the medical registry at Yijishan Hospital and identified all patients diagnosed with HCC between July 2011 and September 2017. The eligibility criteria for inclusion were as follows: (1) underwent surgical resection; (2) definite pathologic diagnosis of HCC; (3) HCC treatment-naive before surgery. Patients who died within one month after surgery were excluded.

There were 143 patients, 22 females and 121 males, with a mean age of 57 years (range, 27 to 78 years), who met the above criteria. Clinical characteristics, including age, gender, risk factors (HBV or HCV infection), liver cirrhosis, preoperative serum alpha-fetoprotein (AFP) levels, tumor size and number, vascular invasion, and Child-Pugh classification, were retrieved from patients' medical records and summarized in Table 1. Postoperative treatments and surveillance followed a uniform guideline [3]. Survival time was calculated form the date of surgery to the date of death or last follow-up. During the follow-up, 99 patients were censored and 44 were dead. The median follow-up was 26 months (range, 2 to 87 months).

Immunohistochemical staining

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tumor tissue sections following a standard protocol [11]. PD-L1 expression was detected using rabbit monoclonal antibody (ab 205921, ABCAM, 1:400 dilution ratio). Briefly, 4 μm sections were deparaffinized in xylene and dehydrated in an ethanol series, followed by heat-mediated antigen retrieval with EDTA buffer in an autoclave and deactivation of endogenous peroxidases with 3% H_2O_2. All sections were incubated with anti-PD-L1 monoclonal antibody overnight at 4°C. Subsequently, the sections were rinsed, incubated with secondary antibodies (horseradish peroxidase/Fab polymer conjugated; PV-6000, ZSGB-BIO). Reaction products were visualized with 3,3'-diaminobenzidine (ab64238, ABCAM) and counterstained with hematoxylin. Placenta tissue was used as positive control according to antibody instructions. A tumor cell was considered PD-L1 positive if the cell membrane was stained, regardless of staining intensity [11]. Cytoplasmatic staining was also disregarded [11]. The proportion of PD-L1 positive cells was estimated as a percentage of the total tumor cells. Samples with membranous expression of PD-L1 on ≥ 1% of the total cells were defined as tumors with PD-L1 positivity [6]. The expression of PD-L1 was independently evaluated by two experienced pathologists without knowledge of any clinical information on the samples, and discrepancies in expression level were resolved by a mutual discussion.

Statistical analysis

Categorical data were presented as number (n) or percentage, and any differences between the two groups were analyzed by chi-squared test. Alternatively, Fisher's exact test or continuity correction was used when the chi-square test was violated. Survival was assessed by Kaplan-Meier method and log-rank test. Univariate and multivariate regression analysis for hazard ratios (HR) was performed using the Cox proportional hazards model. All of the statistical tests and p-values were two tailed and p-values of < 0.05 were considered statistically significant. All analyses were performed using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Of the 143 patients enrolled, tumor cells with membranous expression of PD-L1 were found in 28 patients (19.6%) and not in 115 patients (80.4%). Of these 28 patients with positive cell expression, 19 (13.3%) patients showed tumor PD-L1 expression ≥ 1% of total tumor cells and 9 (6.3%) patients showed less than 1% of total tumor cells. Levels of PD-L1 expression were dichotomized as positive (≥ 1%) and negative (< 1%).

Association of PD-L1 expression with clinical characteristics

Association of PD-L1 expression with clinical characteristics is shown in Table 2. PD-L1 expression is significantly associated with cirrhosis ($p = 0.016$). PD-L1

Table 1. Clinical characteristics of patients.

Characteristics	Results
Gender (female/male)	22/121
Age (median, range)	59 (27 - 78 years)
Virus infection (hepatitis B/hepatitis C/negative)	113/2/28
Liver cirrhosis (no/yes)	50/93
AFP (median, range)	131.56 (0.46 - 47,902.00 ng/mL)
Tumor number (single/multiple)	122/21
Tumor size (median, range)	5 (1 - 19 cm)
Child-Pugh classification (A/B/C)	143/0/0
Vascular invasion (no/yes)	38/105
Positive surgical margin (no/yes)	3/140

Table 2. Association of PD-L1 expression with clinical characteristics.

Characteristics	No.	PD-L1 status		p
		Negative	Positive	
Gender				0.958
Female	22	19 (15.3)	3 (15.8)	
Male	121	105 (84.7)	16 (84.2)	
Age				0.780
< 60	72	63 (50.8)	9 (47.4)	
≥ 60	71	61 (49.2)	10 (52.6)	
Cirrhosis				0.016
Negative	50	48 (38.7)	2 (10.5)	
Positive	93	76 (61.3)	17 (89.5)	
Virus infection				0.449
Negative	28	26 (21.0)	2 (10.5)	
Positive	115	98 (79.0)	17 (89.5)	
AFP				0.216
< 200	79	71 (57.3)	8 (42.1)	
≥ 200	64	53 (42.7)	11 (57.9)	
Tumor size				0.216
< 5cm	64	53 (42.7)	11 (57.9)	
≥ 5cm	79	71 (57.3)	8 (42.1)	
Tumor number				0.111
Single	122	103 (83.1)	19 (100.0)	
Multiple	21	21 (16.9)	0 (0.0)	
Vascular invasion				0.089
Negative	105	88 (71)	17 (89.5)	
Positive	38	36 (29)	2 (10.5)	

Table 3. Univariate and multivariate analysis of prognostic factors.

Characteristics	Univariate			Multivariate		
	Hazard Ratio	95% CI	p	Hazard Ratio	95% CI	p
Gender			0.201			0.203
Female	1			1		
Male	1.957	0.700 - 5.474		1.959	0.695 - 5.522	
Age			0.447			0.453
< 60	1			1		
≥ 60	1.265	0.690 - 2.320		1.278	0.673 - 2.424	
Cirrhosis			0.758			0.877
Negative	1			1		
Positive	1.103	0.591 - 2.058		0.946	0.468 - 1.913	
Virus infection			0.557			0.561
Negative	1			1		
Positive	1.258	0.585 - 2.708		1.257	0.582 - 2.717	
AFP			0.567			0.592
< 200	1			1		
≥ 200	1.189	0.658 - 2.149		1.198	0.619 - 2.315	
Tumor size			0.016			0.071
< 5cm	1			1		
≥ 5cm	2.185	1.156 - 4.130		1.820	0.950 - 3.486	
Tumor number			0.169			0.986
Single	1			1		
Multiple	1.676	0.804 - 3.494		1.007	0.468 - 2.169	
Vascular invasion			0.000			0.000
Negative	1			1		
Positive	3.569	1.910 - 6.771		3.179	1.670 - 6.050	
PD-L1 status			0.091			0.122
Negative	1			1		
Positive	0.363	0.112 - 1.174		0.395	0.122 - 1.281	

Abbreviation: CI - confidence interval.

positivity had a trend with a higher rate of negative vascular invasion (p = 0.089, borderline significant). The remaining clinical characteristics, including gender, age, virus infection, AFP, tumor size and number, were not significantly associated with PD-L1 expression.

Association of PD-L1 expression with survival

During the follow-up, 3 of 19 patients (15.8%) with PD-L1 positivity died, whereas 41 of 124 patients (36.1%) with PD-L1 negativity died. As shown in Figure 1, PD-L1 expression was not significantly associated with survival, although there was a trend that patients with PD-L1 positivity had a better survival than those with PD-L1 negativity (p = 0.076 borderline significant). Hazard ratios were assessed by the Cox proportional hazards model are shown in Table 3. In univariate analysis, tumor size ≥ 5 cm and vascular invasion were significantly associated with worse survival. Tumor PD-L1 positivity was borderline significantly associated with a better survival (HR 0.363, 95% CI 0.112 - 1.174, p = 0.091). In multivariate analysis, only vascular invasion was an independent and significant prognostic factor.

DISCUSSION

During the last decades, only modest improvement in survival has been made in advanced HCC [12]. The effective therapy options for advanced HCC are limited to multikinase inhibitors, such as sorafenib and regorafenib. The median survival for patients with advanced HCC remains less than one year [3]. It is necessary to

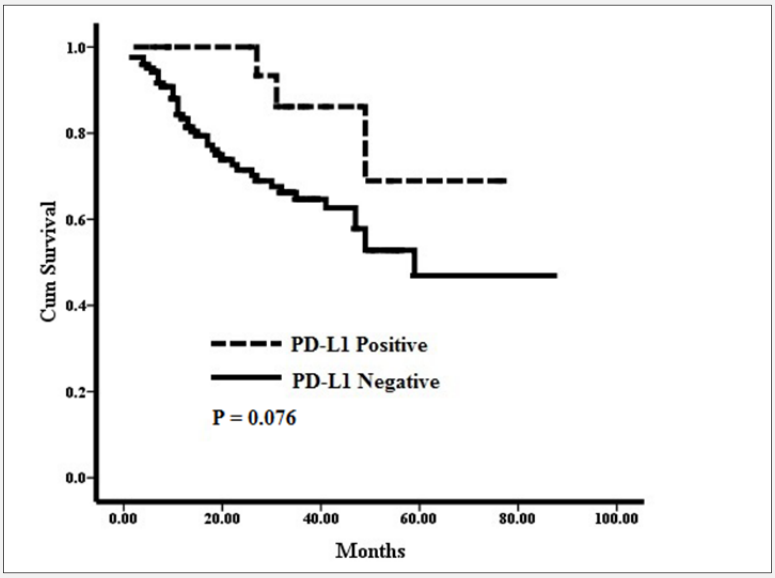

Figure 1. Overall survival for PD-L1 expression analyzed by Kaplan-Meier method and log-rank test.

explore valuable prognostic factors, which help stratify patients to tailor therapeutic strategies [13]. Although extensive research has been done to explore prognostic factors in HCC, reliable biomarkers predictive of survival in HCC are still rare.

Emerging evidence indicates HCC as an immunogenic tumor, which can evade attack from the immune system by availing itself of inhibitory checkpoint pathways [5]. Among the most important of these checkpoints is the PD-1 and PD-L1 pathway, where PD-L1 expressed by tumor cells interacts with PD-1 anchoring on T cells, in turn giving rise to functional exhaustion of T cells and immune tolerance [14]. Although PD-L1 expression in HCC was indicative of high aggressiveness [15], it is yet unclear whether PD-L1 expression has prognostic significance in HCC.

In this present study, patients with resectable HCC of a large scale were enrolled to investigate the association of PD-L1 expression with survival. Immunohistochemical procedure in our study followed a standard protocol, and assessment of PD-L1 expression in our study abided by the de-facto consensus [16], which defines a tumor cell as PD-L1 positive if the cell membrane is stained. PD-L1 expression level is calculated by the percentage of positive cells among total tumor cells, regardless of staining intensity or cytoplasmatic staining [11].

Our study showed that PD-L1 expression failed to serve as a significant prognostic factor in HCC, although there was a trend that PD-L1 positivity was associated with a more favorable survival (HR 0.395, 95% 0.122-1.281). The result of our study is consistent with previous studies [17,18]. Of note, in terms of immunostaining intensity, two studies showed that PD-L1 positivity was significantly associated with a worse survival [7,8], whereas it was also observed that PD-L1 positivity was significantly associated with a better survival [10].

The inconsistency among these studies may be attributable to several factors. First, the definition of tumor PD-L1 positivity is conflicting. Previous studies utilized a scoring algorithm according to immunohistochemical staining intensity and/or density [7-10], which has been dispensed with by the recent consensus. To simplify and standardize the criteria for PD-L1 expression, an expert meeting in the United Kingdom recommended the use of a percentage cutoff [11]. Our present study utilized the cutoff value of ≥ 1% tumor positive cells. This cutoff value was also used in the milestone trial of CheckMate 040 [6], which demonstrated that patients with advanced HCC can benefit from anti-PD-1 immunotherapy, that is, Nivolumab. Second, the detecting protocol and antibodies vary depending on different studies, which could influence the results of immunohistochemical staining. To some extent, it may also account for different PD-L1 positive rates in these studies. The PD-L1 positive rate in our study was 13.3%, which was slightly lower than the rate of 20% in the trial of CheckMate 040 [6]. In addition, sample size is relatively small in two previous studies, which enrolled no more than eighty patients [8,9]. A small scale in sample size could be more susceptible to a risk of error in statistical analysis. Finally, tumor heterogeneity and dynamic nature of

PD-L1 expression might also contribute to discrepancies among these results [19].

With regard to clinical characteristics, our present study showed that none of the clinical characteristics, including gender, age, virus infection, AFP, vascular invasion, tumor size and number, was significantly associated with PD-L1 expression. Of note, PD-L1 expression was significantly associated with cirrhosis in our study. It has been reported that genetic variations of PD-1 predisposed patients with chronic HBV infection to cirrhosis [20]. Whether an association of tumor PD-L1 positivity with cirrhosis is a causal relationship needs to be further investigated.

CONCLUSION

In conclusion, our study showed that PD-L1 expression failed to serve as a significant prognostic factor in HCC. Noteworthy, the conclusion of our study must be interpreted with caution. First, our study is potentially limited by its retrospective nature and many censored patients for survival analysis. Second, the value of 1% was used as dichotomized threshold. PD-L1 expression is a continuous variable, and a simple definition of PD-L1 status as positive or negative may not unveil more prognostic information.

Declaration of Interest:
None declared.

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