PD-L1 expression in liver metastasis: its clinical significance and discordance with primary tumor in colorectal cancer

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Research

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Abstract

Background: The outcomes of immune checkpoint inhibitors of patients with cancer liver metastases are poor, which may be related to a different tumor microenvironment from primary cancers. This study was aimed to analyze the PD-L1 expression and the immune microenvironment status in liver metastatic diseases and compare the differences of PD-L1 expression between primary tumors and liver metastases of colorectal cancer.

Methods: 74 cases of pathologically confirmed colorectal cancer liver metastasis underwent resection from our hospital were included. Tissue microarrays were used for the interpretation of PD-L1 expression, cluster of differentiation 4 (CD4) and CD8 density by immunohistochemistry. Then, we evaluated the disparity between primary tumor and liver metastasis in PD-L1, CD4 and CD8 density and analyzed the factors associated with obvious disparity.

Results: A total of 74 paired colorectal cancer with liver metastases were included in this study. The expression of PD-L1 in liver metastases was positively related to the density of CD4 and CD8. The expression of PD-L1 was higher in liver metastases than in primary tumors in certain subgroups of colorectal cancer, including the patients with concurrent liver metastases (n=63, p=0.05), receiving concurrent resection of primary and metastatic tumors (n=56, p=0.04). The two subgroups generally reflected those without inconsistent external influencing factors, such as treatment and temporal variation, between primary tumors and liver metastases. In these subgroups, the intrinsic differences of microenvironment between primary tumors and liver metastases could be identified, if any. Furthermore, tumor differentiation (moderate vs. poor: *OR*=0.23, 95% *CI*: 0.03-0.99, p=0.05)) were demonstrated to be associated with obvious discordance of PD-L1 expression between primary tumors and hepatic metastases.

Conclusions: The expression of PD-L1 in the hepatic metastases was higher than in the primary tumors in subgroups reflecting intrinsic microenvironment differences between primary and metastatic tumors. Obvious discordance of PD-L1 expression between primary tumor and liver metastasis was closely related to the tumor differentiation.

Background

In recent years, the use of immune checkpoint inhibitors offered new hopes for cancer treatment..[1] With the use of PD-1/PD-L1 inhibitors in various cancers, some organ-specific impact of response was identified in various cancers.[2, 3] Notably, the immunotherapy-related studies of various cancers with liver metastases had shown unsatisfactory results. [4, 5] It had also been shown that the patients with liver metastases benefited less from immunotherapy and may be more likely to develop new metastatic lesions compared to other metastatic lesions such as lymph node metastases and lung metastases.[3] Some researchers thought this phenomenon was associated with the unique tumor microenvironment in liver metastases.

It has been shown that high expression of PD-L1 in tumor was associated with poor prognosis[6–8] and that PD-L1-positive patients with lung cancer or esophageal cancer had a higher response rate to immunotherapy.[9, 10] The heterogeneity of PD-L1 expression may affect its prognostic and predictive accuracy. For instance, MASUGI et al. showed widespread heterogeneity of PD-L1 expression between centers and peripheral parts of primary tumors in colorectal cancer.[11] Studies on non-small cell lung cancer, endometrial cancer, gastric cancer and breast cancer showed that PD-L1 expressions were higher in metastases than in primary tumors .[9, 12–14] It is evident that exploring the differences in the immune microenvironment between the primary tumors and liver metastases is a key point to understand the reasons for the different immunotherapy responses.

Colorectal cancer (CRC) is a very tricky malignancy worldwide.[15] [16] Autopsy suggested liver metastases in about 50% of colorectal cancer patients.[17] Unfortunately, in patients with liver metastases, the efficacy of regorafenib monotherapy or its combination with immunotherapy was undesirable.[18, 19] Previous studies have found the heterogeneity of lymphocyte type, lymphocyte number, KRAS status between primary tumors and liver metastases of colorectal cancer, which suggested that there may be different in the microenvironment between primary tumors and liver metastases.[20, 21]

The current study aim to explore the status of the PD-L1 expression difference between in primary tumors and in liver metastases of CRC to find those influence factors to evaluate the PD-L1 expression status accurately.

Materials And Methods

Study group

A total of 94 cases of pathologically confirmed colorectal cancer with liver metastasis underwent surgical resection in Sun Yat-sen University Cancer Center were screened for this study. The clinical information about patient's age, sex, time of diagnosis, time of resection of metastases, location of primary tumor, metastasis pattern, tumor stage, tumor differentiation, situation of preoperative radiotherapy, situation of adjuvant treatment after resection of primary tumor and survival time, etc were collected. The TNM staging method of the 8th edition of AJCC was used for tumor staging.[22] A total of 74 patients were included in the final analysis by excluding 20 patients with incomplete clinical information.

Tissue microarray (TMA) construction

An experienced pathologist was responsible for reviewing H&E-stained slides and marking the areas with abundant tumor cells to guide core selection. The donor tissue block was 4 mm thick and the recipient block was cast by melting conventional paraffin waxes in molds to make blank blocks. Then transferred the donor tissue block into the recipient wax wells prepared with a 0.6 mm perforated needle. Placed the wax block in an incubator at 37 °C for 10 min so that the tissue core in the block and the wall of the pore are closely integrated. The wax block was then frozen on ice and sliced continuously to a thickness of 4 μm. It was used for immunohistochemistry (IHC) of the expression of PD-L1, cluster of differentiation 4 (CD4) and CD8 in CRC primary tumors and liver metastases.

Immunohistochemistry

After finishing making the tissue microarray blocks, baked the blocks in a 65 °C oven for 60 minutes, then let them cool to room temperature. The endogenous peroxidase was blocked by incubating with 3% hydrogen peroxide for 10 min after dewaxing and rehydration. Incubated primary antibodies (Rabbit antibodies for PD-L1 (SP142, spring bioscience), CD4(ZA-0508, ZSGBBIO), CD8(ZA-0519, ZSGB-BIO)) overnight at 4 °C in a refrigerator, washed with buffer and added the HRP RABBIT/MOUSE secondary antibody (K5007, 20029103, Dako) successively, then incubated at room temperature for 30 minutes. Then generally diaminobenzidine tetrahydro-chloride (DAB, K5007, 20019193, Dako) stained, haematoxylin slightly stained for 1 minute after color rendering was terminated.

Evaluation of PD-L1 expression, CD4 and CD8 density

The results were interpreted by an experienced pathologist who read the images under an microscope and made comprehensive judgments. To determine the expression of PD-L1, the immunostaining of both tumor cells and tumor-infiltrating immune cells should be considered, while the density of CD4 and CD8 should be determined only for the staining of tumor-infiltrating immune cells. The Combined Positive Score(CPS) was used to evaluate PD-L1 expression levels. CPS ≥ 1 was defined as “PD-L1 expression positive” while CPS 1 was defined as “PD-L1 expression negative”;As for CD4 and CD8 density, the proportion of cells < 10% was defined as “absent or low” while ≥ 10% was defined as “high”. To further identify the drivers of PD-L1 expression differences between primary tumor and liver metastases, the difference in CPS values of PD-L1 between primary and hepatic metastases of ≥ 5 was defined as "obvious discordance".

Statistical analysis

The Statistical analysis software using in this study was SPSS for Windows V.13.0 (SPSS Inc., Chicago, IL, USA). The statistical methods for the relationship between PD-L1 expression and clinicopathological characteristics, CD4 density and CD8 density were chi-square test or Kruskal-Wallis H test. Consistency of PD-L1 expression in CRC was assessed using the chi-square test. Mann-Whitney U rank-sum test for the analysis of TNM staging of ordinal data. Comparisons of PD-L1 expression, CD4, and CD8 density in primary tumors versus metastases were performed with paired T test. Logistic regression analysis was used for the analysis of factors associated with obvious discordance of PD-L1 expression between primary tumors and liver metastases.

Results

Patient baseline characteristics

A total of 74 patients with CRC liver metastases were included in this study, 50 (67.6%) of whom were male and 24(32.4%) were female, with a median age of 56 years old ranging from 31 to 76 years old. The primary tumors of CRC was located in the proximal colon in 21 cases (28.4%), the distal colon in 19 cases (25.7%) and the rectum 34 cases (45.9%). 63 patients (85.1%) had concurrent metastases and 18 (24.3%) had metachronous metastases, of which 66 (89.2%) had hepatic metastases only, and 8 cases (10.8%) had concomitant extrahepatic metastases. In terms of the tumor differentiation, 9 cases (12.2%) were poor differentiators and 65 cases (87.8%) were moderate differentiators. 56 cases (75.5%) received concurrent resection of primary and metastatic liver tumors, and the remaining 18 cases (24.3%) received metachronous resection. Detailed information were shown in Supplementary Table 1.

Clinicopathological factors associated with PD-L1 expression in liver metastases

According to Table 1, 41 out of 74 patients (55%) were positive for PD-L1 expression in liver metastases; The rate of PD-L1 positivity in rectal cancer hepatic metastases was higher than in colon cancer hepatic metastases (positivity rate :70.6% vs. 42.5%); The expression of PD-L1 in liver metastases was related to the density of CD4 and CD8 which were higher in PD-L1 positive patients (Fig. 1). 64.3% of PD-L1-positive patients have “high” CD4 density, while there was 35.7% in PD-L1-negative patients ($p = 0.05$); As for the density of CD8, 94.4% for PD-L1-positive patients was higher than 5.6% for PD-L1-negative patients (p 0.001). The patient's age, sex, whether there was concurrent metastasis and concurrent resection, TNM staging, tumor differentiation, and whether there was extrahepatic metastasis were not significantly correlated with the expression of PD-L1 in liver metastases.

Table 1
Clinicopathological factors associated with PD-L1 expression in liver metastasis.

	PD-L1 expression[a], N.(%)		*P* value
	Negative	Positive	
Year of diagnosis			0.36
~ 2009	18 (50.0)	18 (50.0)	
2010~	15 (39.5)	23 (60.5)	
Age (years, median 56)			0.85
< 56	16 (45.7)	19 (54.3)	
≥ 56	17 (43.6)	22 (56.4)	
Gender			0.52
Male	21 (42.0)	29 (58.0)	
Female	12 (50.0)	12 (50.0)	
Primary tumor site			0.02*
Colon	23 (57.5)	17 (42.5)	
Rectum	10 (29.4)	24 (70.6)	
Metastatic time model			0.95
Concurrent	28 (44.4)	35 (55.6)	
Metachronous	5 (45.5)	6 (54.5)	
Resection time model			0.60
Concurrent	24 (42.9)	32 (57.1)	
Metachronous	9 (50.0)	9 (50.0)	
T category (AJCC TNM 8th)			0.31
T1 + T2	1 (16.7)	5 (83.3)	
T3	5 (45.5)	6 (54.5)	
T4	27 (47.4)	30 (52.6)	
N category (AJCC TNM 8th)			0.99
N0	6 (37.5)	10 (62.5)	
N1	10 (52.6)	9 (47.4)	
N2	11 (40.7)	16 (59.3)	
Nx	6 (50.0)	6 (50.0)	

Abbreviations: PD-L1, programmed death ligand 1; N, number; AJCC, American Joint Committee on Cancer; TNM, tumor-node-metastasis; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8.

[a] Definition of PD-L1 expression: negative (CPS < 1), positive (CPS ≥ 1). CPS, combined positive score.[b] Definition of CD4, CD8 density: absent or low (< 10%), high (≥ 10%).

	PD-L1 expression[a], N.(%)		*P* value
Tumor differentiation			0.16
Poor	6 (66.7)	3 (33.3)	
moderate	27 (41.5)	38 (58.5)	
Extra-hepatic metastasis			
No	28 (42.4)	38 (57.6)	
Yes	5 (62.5)	3 (37.5)	
CD4 density[b] in liver metastatic tumor			0.05*
Absent and low	18 (56.3)	13 (43.8)	
High	15 (35.7)	28 (64.3)	
CD8 density[b] in liver metastatic tumor			< 0.001*
Absent and low	32 (57.1)	24 (42.9)	
High	1 (5.6)	17 (94.4)	

Abbreviations: PD-L1, programmed death ligand 1; N, number; AJCC, American Joint Committee on Cancer; TNM, tumor-node-metastasis; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8.

[a] Definition of PD-L1 expression: negative (CPS < 1), positive (CPS ≥ 1). CPS, combined positive score.[b] Definition of CD4, CD8 density: absent or low (< 10%), high (≥ 10%).

Comparisons of PD-L1 expression, CD4 and CD8 density between primary tumors and liver metastases

The results (Table 2) showed that in terms of PD-L1 expression, although there was no significant difference in general PD-L1 expression between liver metastases and primary tumors (median: 1.0 vs. 0.5, $p = 0.10$), the CPS of PD-L1 was higher in patients with concurrent liver metastases ($p = 0.05$), concurrent resection of primary tumor and liver metastases ($p = 0.04$, Fig. 2), the difference was statistically significant. The difference in PD-L1 expression between hepatic metastases and primary tumor was not statistically significant in patients who underwent chemo/radiotherapy for primary tumor.

Table 2
Comparisons of PD-L1 expression, CD4 and CD8 density between primary tumor and liver metastasis.

groups	PD-L1 CPS (median)		*P* value	CD4 density (%, median)		*P* value	CD8 density (%, median)		*P* value
	Primary tumor	Liver metastasis		Primary tumor	Liver metastasis		Primary tumor	Liver metastasis	
General	0.5	1.0	0.10	7.0	12.0	0.003*	4.5	3.5	0.63
Pre-operative che-mo/radiotherapy for primary tumor									
No	1.0	1.0	0.09	7.5	12.5	0.01*	4.0	5.0	0.78
Yes	0.0	0.0	0.31	5.5	7.5	0.28	5.0	1.5	0.03*
Tumor differentiation									
Poor	0.0	0.0	0.63	5.5	3.0	0.68	10.0	1.5	0.07
moderate	0.5	1.0	0.052	7.5	15.0	0.003*	3.0	4.0	0.70
Primary tumor site									
Colon	0.0	0.0	0.27	5.3	10.0	0.03*	2.0	2.5	0.47
Rectum	1.5	1.3	0.20	10.0	17.5	0.04*	5.0	5.0	0.95
Metastatic time model									
Concurrent	0.0	1.0	0.05*	7.5	12.5	0.01*	5.0	3.5	0.95
Metachronous	1.5	1.0	0.22	3.0	5.0	0.22	2.0	4.0	0.32
Resection time model									
Concurrent	0.0	1.0	0.04*	8.8	15.0	0.01*	5.0	3.5	0.81
Metachronous	1.3	0.8	0.13	5.0	5.8	0.23	3.3	3.3	0.12

Abbreviations: PD-L1, programmed death ligand 1; CPS, combined positive score; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8.

Overall, there were more CD4 + cells in liver metastases than in primary tumors (median of density: 12.0% vs. 7.0%, $p = 0.003$). In the subgroup analysis, the density of CD4 in hepatic metastases was significantly higher than the primary tumors in patients with no radiotherapy before primary tumor resection ($p = 0.01$), concurrent liver metastases ($p = 0.01$), concurrent resection of primary tumors and hepatic metastases ($p = 0.01$).

In terms of CD8 density, under most circumstances, there was no significant difference between hepatic metastases and primary tumors. The density of CD8 in liver metastases was significantly lower than primary tumors in those underwent chemo/radiotherapy before primary tumors resected (median: 1.5% vs. 5.0%, $p = 0.03$).

Logistic regression analysis for factors associated with an obvious discordance of PD-L1 expression between primary tumors and liver metastatic tumors

Detailed data on the obvious discordance of PD-L1 expression between liver metastatic tumors and primary tumors were shown in Supplementary Table 2. The results of the logistic regression analysis of the relevant factors were shown in Table 3. The results showed that tumor differentiation (moderate vs. poor: *OR* = 0.23, 95% *CI*: 0.03–0.99, *p* = 0.05), discordance of the density of CD8 between primary tumors and hepatic metastases (yes vs. no: *OR* = 8.95, 95% *CI*: 2.06-39.00, *p* = 0.004) were associated with obvious discordance of PD-L1 expression between primary tumors and hepatic metastases. Whether the liver metastases were concurrent, and whether the primary tumors were resected concurrently with the liver metastases were not associated with the obvious discordance.

Table 3
Logistic regression analysis for factors associated with an obvious discordance of PD-L1 expression between primary tumors and liver metastatic tumors.

	Obvious discordance of PD-L1 expression[a], N. (%)		Univariate analysis		*P*-value	Multivariate analysis		*P*-value
	No (n = 60)	Yes (n = 14)	*OR*	95% *CI*		*AOR*	95% *CI*	
Year of diagnosis					0.29			
~ 2009	31 (86.1)	5 (13.9)	1	Reference				
2010~	29 (76.3)	9 (23.7)	1.92	0.58–6.42				
Age (years, median 56)					0.42			
< 56	27 (77.1)	8 (22.9)	1	Reference				
≥ 56	33 (84.6)	6 (15.4)	0.61	0.19–1.99				
Gender					0.12			
Male	38 (76.0)	12 (24.0)	1	Reference				
Female	22 (91.7)	2 (8.3)	0.29	0.06–1.41				
Primary tumor site					0.35			
Colon	34 (85.0)	6 (15.0)	1	Reference				
Rectum	26 (76.5)	8 (23.5)	1.74	0.54–5.65				
Metastatic time model					0.38			
Concurrent	50 (79.4)	13 (20.6)	1	Reference				
Metachronous	10 (90.0)	1 (9.1)	0.39	0.05–3.28				
Resection time model					0.34			
Concurrent	44 (78.6)	12 (21.4)	1	Reference				
Metachronous	16 (88.9)	2 (11.1)	0.46	0.09–2.28				
T category (AJCC TNM 8th)					0.79			
T1 + T2	4 (66.7)	2 (33.3)	1	Reference				
T3	11 (100.0)	0 (0.0)	< 0.001	< 0.001-NA	1.00			
T4	45 (78.9)	14 (18.9)	0.53	0.09–3.27	0.50			

Abbreviations: N, number; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio; PD-L1, programmed death ligand 1; AJCC, American Joint Committee on Cancer; TNM, tumor- node-metastasis; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8.

[a] Defined as a CPS score gap between primary tumor and liver metastatic tumor ≥ 5.

	Obvious discordance of PD-L1 expression[a], N. (%)		Univariate analysis		*P*-value	Multivariate analysis		*P*-value
	No (n = 60)	Yes (n = 14)	*OR*	95% *CI*		*AOR*	95% *CI*	
N category (AJCC TNM 8th)					0.19			
N0	10 (62.5)	6 (37.5)	1	Reference				
N1	17 (89.5)	2 (10.5)	0.20	0.03–1.16	0.07			
N2	22 (81.5)	5 (18.5)	0.38	0.09–1.54	0.18			
Nx	11 (91.7)	1 (8.3)	0.15	0.02–1.49	0.11			
Tumor differentiation					0.05*			0.05*
Poor	5 (55.6)	4 (44.4)	1	Reference		1	Reference	
moderate	55 (84.6)	10 (15.4)	0.23	0.05-1.00		0.18	0.03–0.99	
Extra-hepatic metastasis					0.63			
No	53 (80.3)	13 (19.7)	1	Reference				
Yes	7 (87.5)	1 (12.5)	0.58	0.07–5.16				
Pre-operative chemo/radiotherapy for primary tumor					0.28			
No	48 (78.7)	13 (21.3)	1	Reference				
Yes	12 (92.3)	1 (7.7)	0.31	0.04–2.59				
Discordance of CD4 density between primary and metastatic tumor					0.86			
No	40 (81.6)	9 (18.4)	1	Reference				
Yes	20 (80.0)	5 (20.0)	1.11	0.32–3.76				
Discordance of CD8 density between primary and metastatic tumor					0.003*			0.004*
No	41 (93.2)	3 (6.8)	1	Reference		1	Reference	
Yes	19 (63.3)	11 (36.7)	7.91	1.98–31.69		8.95	2.06-39.00	

Abbreviations: N, number; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio; PD-L1, programmed death ligand 1; AJCC, American Joint Committee on Cancer; TNM, tumor- node-metastasis; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8.

[a] Defined as a CPS score gap between primary tumor and liver metastatic tumor ≥ 5.

Discussion

The results of current studies of immunotherapy for liver metastases from various tumors remain unsatisfactory.[4] In CRC, EPOC1603 clinical trial of regorafenib plus nivolumab therapy showed promising effect with an ORR of 36% in treatment-refractory CRC, but relatively unsatisfactory outcomes in the subgroup of patients with liver metastases.[18] Meanwhile, phase IIIb CONSIGN study showed that regorafenib significantly improved survival in treatment-refractory CRC, but liver metastases was a significant adverse factor in the progression-free survival (PFS) of regorafenib for metastatic CRC.[19] The poor outcome of patients with liver metastases suggested a possibly distinct microenvironment in liver metastases. Previous researches have provided several possible explanations for this phenomenon. The results of TUMEH's study showed that CD8 + T cells, which are important effector cells for anti-PD-1/PD-L1 therapy, were significantly reduced at the margin of metastases lesions in patients with liver metastases compared to patients without liver metastases. [23] Researchers believed this may be related to the liver tolerance. Liver tolerance mechanisms considered that, the liver, as an important immune organ, is exposed to a large quantity of antigens from the gastrointestinal tract and the portal system.[24, 25] On the one hand, there are a large number of immune cells in the liver that can activate the immune response against pathogens that may harm the organism rapidly, and on the other hand, the liver needs to suppress the overreaction of the immune system through certain mechanisms in order to maintain the stability of the internal environment.[24, 25] This tolerance may be related to incomplete CD8 + T-cell activation or effector T-cell inactivation, CD4 + T-cell inactivation and regulatory T-cell activation induced by Kupffer cells.[26] In our study, a higher PD-L1 level in liver metastases suggested that PD-L1 expression might also be involved in liver tolerance. However, combining the relative resistance to PD-1/PD-L1 inhibitors in patients with liver metastasis, we proposed that the ability of PD-1/PD-L1 inhibitors to enhance the immune system's response may be partially counteracted by other mechanisms of liver tolerance, thus compromising the effectiveness of treatment.[27]

In the present study, we found that PD-L1 expression was higher in certain subgroups of metastases, which included patients who did not receive chemotherapy during the interval of resection of primary tumor and metastasis, patients with concurrent liver metastasis and patients with concurrent resection of primary tumor and liver metastasis. All those subgroups are patients whose primary tumors and liver metastases were not impacted by inconsistent external influencing factors, especially treatment and temporal variation. And these subgroups were suitable for analysis of the intrinsic differences of microenvironment between primary tumors and liver metastases. Our results suggested that liver metastases had higher expression of PD-L1 than primary tumors and were in a stronger immunosuppressive status in the absence of external factors such as chemotherapy and temporal variation. This heterogeneity in PD-L1 expression is consistent with the results of WANG et al. on 22 patients with CRC with metastasis whose metastatic lesion had higher PD-L1 expression than their primary tumor.[28] Meanwhile, researches on non-small cell lung cancer, endometrial cancer, breast cancer, etc., had similar results.[9, 12, 14]

We also found that the proportion of CD8 + cells was higher in primary tumors than in metastatic lesion when the patients had received radiotherapy or chemotherapy before resection of primary tumor, suggesting that radiotherapy or chemotherapy may enhance the infiltration of CD8 + cells in primary tumor. At the same time, we found that the level of PD-L1 expression in rectal cancer liver metastases was higher than that in colon cancer liver metastases, which might be related to the fact that some patients with rectal cancer had undergone chemoradiotherapy. HUANG et al. also showed that neoadjuvant chemotherapy with decitabine promoted expression of immune-related gene and proliferation of TILs.[29] However, whether radiotherapy/chemotherapy improved CD8 + T cells primarily and whether it could improve the response rates to immunotherapy remains to be researched in more trials.

We further analyzed the factors related to the discordance in PD-L1 expression using CPS difference $\geq$ 5 as the boundary. The results showed that only the tumor differentiation and the discordance in the density of CD8 between primary and hepatic metastatic lesions were associated with this discordance. Literature reported that poorly differentiated tumors may contain more cancer stem cells (CSC), a type of cell with specific biological properties such as self-renewal and differentiation potential.[30] The CSC model has long been considered as an important mechanism leading to phenotypic and functional heterogeneity and generating tumor diversity, tumors with more CSCs can evolve into stronger heterogeneous tumors. In terms of spatial and temporal heterogeneity of the tumor, there was heterogeneity in the microenvironment in different regions of the

same tumor, mainly in terms of differences in oxygen availability, acidity, nutrient availability, and lymphocyte infiltration within the tumor.[31, 32] Poorly differentiated tumors have been shown to have higher microvascular and microlymphatic densities than well-differentiated tumors.[33, 34] These heterogeneities were likely to influence the tumor PD-L1 expression leading to the obvious discordance between primary tumors and metastatic lesions observed in this study. In combination with the increased density of CD8 in the PD-L1-positive group in the hepatic metastases, we considered that the discordance of CD8 between the primary tumor and hepatic metastases should be a concomitant state of differential PD-L1 expression, and that the tumor differentiation closely related to tumor heterogeneity may be the intrinsic driver of this discordance. PD-L1 expression had been proposed to be a biomarker for benefit from PD-1/PD-L1 inhibitors in several cancers. While its inter-tumoral spatially heterogeneous expression had been recognized and affected its reliability.[9, 13] The present study suggested that PD-L1 expression of patients with poor tumor differentiation may more likely need to be tested for both primary tumors and metastatic lesions.

As the main tumor-infiltrating lymphocytes, CD8 + T cell was the reaction center for the alternative mechanism, and the key cell for immunotherapy such as anti-PD-L1 therapy. Our results showed an increased number of CD8 + infiltrating cells in PD-L1-positive individuals with liver metastases. However, ZHOU et al. studied 44 CRC patients with liver metastases and found that most of the immune cells in liver metastases were CD33 + inhibitory immune cells, and most of the CD8 + cells were not CD8 + T cells.[20] Moreover, large numbers of suppressive immune cells promote aggregation of myeloid-derived suppressor cells (MDSCs) in hepatic metastases, which can inhibit the proliferation of actived T cell but promote proliferation of suppressor T cell, and also promote tumor angiogenesis, invasion, and metastasis, which may reduce the efficacy of immunotherapy.[35] In addition, TOOR et al. suggested that CD4 + lymphocytes in CRC tumor tissues are predominantly regulatory T cells (TREG) which can promote more expression of immune checkpoints including cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), T cell immunoglobulin and mucin domain-3 (TIM-3), and lymphocyte-activated gene 3 (LAG-3), then further promote immune escape in cancer cells.[36] SHITARA et al. also found that TREG could inhibit the anti-tumor immunity effect of cells by inhibiting the effect of antigen-producing cells and secreting inhibitory cytokines such as TGF-B, IL-10 and IL-35 to inhibit the function of effector T cells or promote apoptosis of effector T cells.[37] In this study, 58% (43/74) of lymphocytes had “high” CD4 expression in liver metastatic lesions and 64.3% in PD-L1-positive group; Table 3 also showed that the proportion of CD4 + lymphocytes in liver metastatic lesions was significantly higher than in primary foci (12.0% vs. 7.0%). Whether the majority of CD4 + lymphocytes in liver metastases are TREG and thus affect the efficacy of immunotherapy requires further subgroup analysis of CD4 + cells in the future.

Although this study has the highest number of cases included among studies analyzing the difference in PD-L1 expression between primary tumors and liver metastases of CRC, it still has some limitations. Firstly, this was a retrospective study, and the cases included were surgically resected with few patients having to be excluded due to lack of clinical information, inevitably leading to selection bias. The patients we selected were largely resected for liver metastases, which were generally small and had a low tumor load, and this may cause the results of this study cannot reflect the circumstance of larger metastases or greater tumor burden. In addition, we did not further study the subgroups of CD4 + and CD8 + cells in the tumor immune microenvironment and failed to further explore the detailed mechanisms of immunosuppression in liver metastases. More detailed studies about the expression of immune checkpoints in the primary center and peripheral parts of the tumors, including comparison of immune cell subgroups, are still needed to be conducted in the future.

Conclusion

In conclusion, in the present study, we not only elucidated the expression pattern of PD-L1 in CRC between primary tumors and hepatic metastases, but also identified tumor differentiation as a causal factor for the obvious discordance of PD-L1 expression between primary tumors and hepatic metastases.

List Of Abbreviations

Abbreviation	Full name
PD-L1	Programmed cell death ligand-1
CRC	Colorectal cancer
TMA	Tissue microarray
IHC	Immunohistochemistry
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CPS	Combined Positive Score
CSC	Cancer stem cells

Declarations

Ethics approval and consent to participate: The study followed the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of Sun Yat-sen University Cancer Center. Signed informed consent was obtained from each patient for their clinical and pathological materials to be used in scientific research.

Consent for publication: Not applicable.

Availability of data and material: All data generated or analysed during this study are included in this published article and its supplementary information files. Other data that were not relevant for the results presented here are available from the corresponding author Dr. Xu on reasonable request.

Competing interests: None declare.

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Authors' contributions: WXL, LX, and LJN for acquisition of data, analysis and interpretation of data, statistical analysis and drafting of the manuscript; SH, WY, CDL, and WFH for technical and material support; WXL, LX, WFH, and XRH for study concept and design, analysis and interpretation of data, drafting of the manuscript, obtained funding and study supervision. All authors read and approved the final manuscript.

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Figures

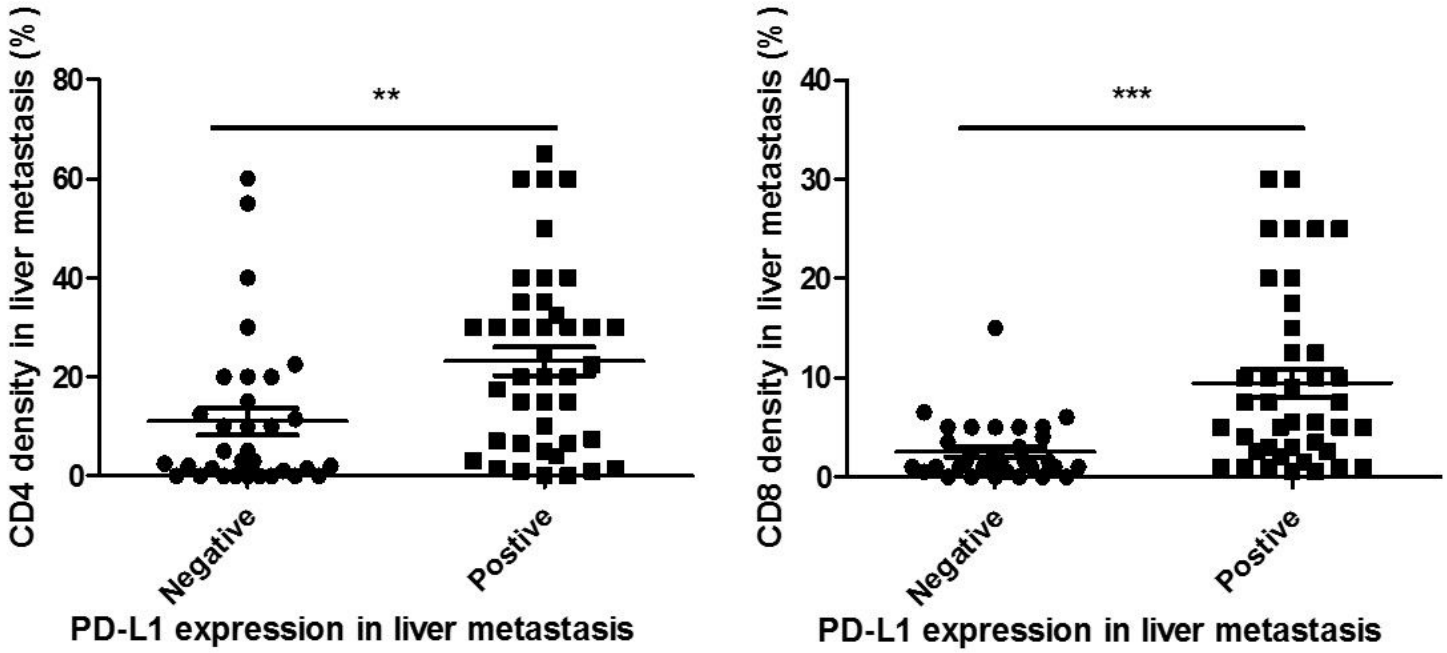

Figure 1

Association of PD-L1 expression with CD4 density and CD8 density in liver metastasis in CRC. Positive PD-L1 expression in liver metastasis is significantly related with higher levels of CD4 density and CD8 density in liver metastasis in CRC.

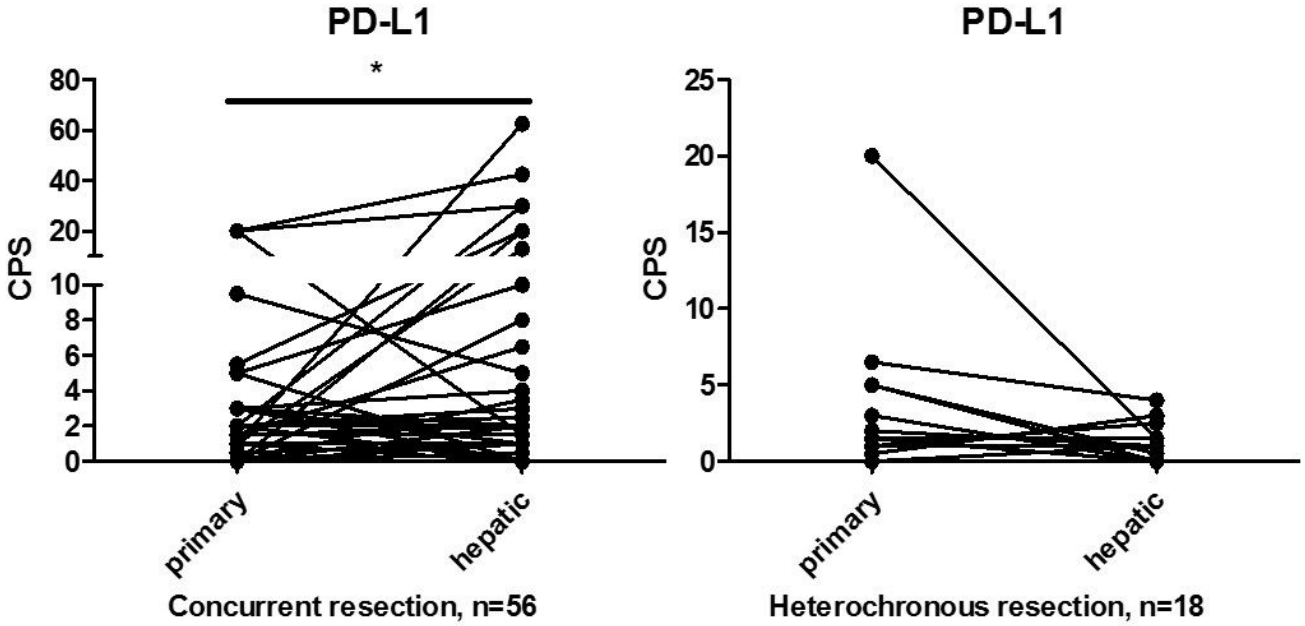

Figure 2

Comparisons of PD-L1 expression between primary tumors and liver metastasis in subgroups of CRC. PD-L1 expression (assessed using CPS scoring) was significantly higher in liver metastasis com-pared with primary colorectal tumors in the patients with concurrent resection of primary and metastatic tumors (n=56) subgroup, but not in metachronous resection subgroup.

Supplementary Files

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- Supplementarytables.docx