Synergistic upregulation of PD-L1 in tumor cells and CD39 in tumor-infiltrating CD8+ T cells leads to poor prognosis in patients with hepatocellular carcinoma

XI KANG[1*], SINAN ZHAO[2*], SHAN LIN[1,3], JING LI[1,4] and SHUNXIANG WANG[1]

[1]Department of Hepatobiliary Surgery, The Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei 050000, P.R. China; [2]Department of Endocrinology, The Second Hospital of Hebei Medical University, Shijiazhuang, Heibei 050000, P.R. China; [3]Department of General Surgery, Cangzhou Central Hospital, Cangzhou, Hebei 061000, P.R. China; [4]Department of General Surgery, Chengde Central Hospital, Chengde, Hebei 067000, P.R. China

Received October 16, 2023; Accepted May 16, 2024

DOI: 10.3892/ol.2024.14501

Abstract. The immune escape of tumor cells and functional status of tumor-infiltrating T cells may serve pivotal roles in the tumor immune microenvironment and progression of hepatocellular carcinoma (HCC). The present study enrolled 91 patients with HCC and examined programmed cell death ligand 1 (PD-L1) expression in tumor cells and CD39 expression in tumor-infiltrating CD8+ T cells in patient samples using multiplex immunofluorescence assays. The impact of PD-L1 and CD39 expression levels on the prognosis of patients with HCC was investigated utilizing Kaplan-Meier analyses. The individual upregulation of PD-L1 in tumor cells, as well as the individual upregulation of CD39 expression in tumor-infiltrating CD8+ T cells did not significantly affect the prognosis of patients with HCC. However, the simultaneous upregulation of both PD-L1 in tumor cells and CD39 in tumor-infiltrating CD8+ T cells was associated with reduced overall survival in patients with HCC. Therefore, the results of the present study suggested that the interplay between tumor cell immune escape and tumor-infiltrating immune cell functional status within the tumor immune microenvironment may have had a substantial impact on the prognosis of patients with HCC. Mechanistically, increased expression levels of PD-L1 in tumor cells may improve the immune escape capacity of tumors, whilst upregulation of CD39 in tumor-infiltrating T cells may be associated with T cell exhaustion. Therefore, the upregulation of PD-L1 expression in tumor cells, in conjunction with the exhaustion of tumor-infiltrating CD8+ T cells, could serve as a future potential prognostic indicator of patients with HCC.

Correspondence to: Professor Shunxiang Wang, Department of Hepatobiliary Surgery, The Fourth Hospital of Hebei Medical University, 12 Jiankang Road, Shijiazhuang, Hebei 050000, P.R. China
E-mail: gdwangsx@163.com

*Contributed equally

Key words: hepatocellular carcinoma, programmed cell death ligand 1, CD-39, immune microenvironment, prognosis

Introduction

Hepatocellular carcinoma (HCC) is a principal histologic type of liver cancer, which primarily arises in cirrhotic livers when repeated inflammation and fibrinogenesis leads to dysplasia and malignant transformation of the liver (1). China has the highest number of HCC cases worldwide, attributable to a high incidence rate of 18.3 cases per 100,000 people (2). The recognized risk factors for HCC include HBV/HCV infection, alcohol, nonalcoholic fatty liver disease (NAFLD), Aflatoxin B_1 and several metabolic syndromes (2). Traditional treatments for HCC include surgery, local ablation and transarterial chemoembolization (TACE). In recent years, a variety of target drugs and immune checkpoint inhibitors (ICIs) have been used in the treatment of HCC. However, the prognosis of patients with HCC remains poor (3).

There has been a growing emphasis on the investigation of the tumor immune microenvironment of HCC, given the use of ICIs for HCC therapy. The HCC tumor microenvironment consists of a multifaceted and dynamic system that is formed of cancer cells, a complex cytokine milieu, extracellular matrix components, immune cells, physical and chemical characteristics (4). In terms of immune cells, tumor-infiltrating CD8+ T cells are considered to be one of the principal T cell subsets responsible for mediating effective antitumor responses (5). The function of tumor-infiltrating CD8+ T cells have been associated with improved survival outcomes in patients with lung cancer, breast cancer and malignant pleural mesothelioma (6-8). However, T cell exhaustion frequently occurs in the tumor microenvironment due to the prolonged activation of the immune response (9). The exhausted T cells show upregulated inhibitory receptors, decreased effector cytokine production and cytolytic activity, leading to cancer immune evasion (9). CD39 is an enzyme expressed on the cell surface that can attenuate the functionality of effector T cells by hydrolyzing extracellular ATP and impeding effector responses in lymphocytes (10). Previous studies have indicated

that the expression level of CD39 in tumor-infiltrating $CD8^+$ T cells may be associated with the state of T cell exhaustion, with T cells exhibiting high CD39 expression potentially manifesting as 'bystander' T cells (11,12). In contrast to this, programmed cell death ligand 1 (PD-L1), a transmembrane cell surface protein of tumor cells that interacts with programmed cell death protein 1 (PD-1), regulates T cell proliferation, cytokine production and cellular exhaustion by binding to the PD-1 molecule on immune cells, ultimately leading to tumor immune evasion (13-16). Several studies have attempted to elucidate the correlation between PD-L1 expression levels in HCC and patient prognosis. However, these investigations have yielded inconsistent conclusions (17-22). Therefore, it could be suggested that the combined influence of PD-L1 expression levels in tumors and the functionality of tumor-infiltrating $CD8^+$ T cells may be a significant factor affecting the prognosis of patients with HCC.

The present study utilized multiplex immunofluorescence assays to analyze CD39 expression levels in tumor-infiltrating $CD8^+$ T cells and PD-L1 expression levels in HCC tumor cells. A comprehensive examination of the combined influence of immune cell exhaustion and PD-1/PD-L1-mediated tumor immune evasion was conducted to further elucidate their potential as a prognostic factors and therapeutic targets for HCC.

Materials and methods

Patients. The present study evaluated HCC tumor samples collected from 91 patients with HCC treated at Hebei Medical University 4th Hospital (Shijiazhuang, China) between January 2012 to December 2015. The age range of the patients was 34-73 years, with a median age of 57 years. All tissue specimens were obtained from patients who were diagnosed with HCC by a pathologist and subsequently underwent hepatectomy. All patients provided written informed consent for the collection of tissue specimens. The study was approved by the Clinical Research Ethics Committee of Hebei Medical University 4th Hospital (approval no. 2018MEC149; Shijiazhuang, P.R. China). All participants were regularly followed up for 5 years by outpatient clinics and telephone interviews at least every 3 months. Imaging examinations were performed every 3 months within 2 years after surgery, and every 6 months after 2 years. Overall survival (OS) and recurrence-free survival (RFS) were assessed to analyze the survival status of patients with HCC.

Inclusion and exclusion criteria. The inclusion criteria of patient enrollment were patients with a diagnosis of primary HCC by histopathological examination and who had received no systematic therapy, including chemotherapy, targeted therapy or immunotherapy, prior to radical resection of the tumor. The exclusion criteria were patients who had other types of cancer and had a past history of systematic therapy before surgery.

Multiplex immunofluorescence assay. Samples from patients were immersed in 10% neutral buffered formalin for fixation for 3 days at room temperature. Slides of the tissue microarray were cut into 4-μm slides from paraffin embedded samples. Slides were heated in an oven at 63˚C for 1 h, dewaxed in xylene and a series of graded ethanol (100, 95, 85 and 75%), boiled in citrate buffer (100˚C for 3 min), and cooled down to room temperature for 15-20 min. To block non-specific peroxidase reactions, 3% hydrogen peroxide was used for 20 min at room temperature. After cooling and washing with PBS (3 times, 5 min each), the slides were incubated with the following primary antibodies at 4˚C overnight: Anti-PD-L1 (cat. no. ab205921; Abcam), anti-CD39 (1:2,000; cat. no. ab223842; Abcam), anti-CD8 (cat. no. ab4055; Abcam) and anti-pan-cytokeratin (CK; cat. no. PA125; Suzhou Baidao Medical Technology Co., Ltd.). After rewarming to room temperature, slides were washed by PBS (3 times, 5 min each). HRP-conjugated goat anti-rabbit secondary antibodies (cat. no. GK500705, Dako; Agilent Technologies, Inc.) were subsequently added and incubated for 15 min at room temperature for visualization. Staining with Opal dye solution (1:100; cat. no. NEL820001KT, Akoya Biosciences, Inc.) was performed according to the manufacturer's instructions. The samples were counterstained with DAPI (cat. no. 28718-90-3; MilliporeSigma) for 10 min at room temperature, and imaged using an automatic quantitative pathology imaging system (Vectra Polaris; PerkinElmer, Inc.).

Statistical analysis. The data were analyzed using R (version 4.2.2; Rstudio, Inc.) and GraphPad Prism (version 10; Dotmatics) software. The survival receiver operating characteristic (ROC) method was used to determine the optimal cutoff values for CD39 expression. Kaplan-Meier analysis with the log-rank test was utilized to analyze differences in the survival rates between different groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Baseline patient characteristics. The present study analyzed 91 clinical samples from patients with HCC (Table I). Among the patients, there were 79 male patients (86.8%) and 12 female patients (13.2%). Within the patient cohort, 87 patients had hepatitis B (95.6%), while 89 patients had cirrhosis (97.8%). According to the Barcelona Clinic Liver Cancer staging system (23), 3 patients (3.3%) had stage 0 disease, 58 patients (63.7%) had stage A disease and 30 patients (33%) had stage B or C disease. According to the China Liver Cancer staging system (24), 61 patients (67.1%) had stage Ia or Ib disease, 13 patients (14.3%) had stage IIa or IIb disease and 17 patients (18.6%) had stage IIIa or IIIb disease. Within the 5-year follow-up period, 69 patients (75.8%) experienced tumor recurrence, while 22 patients (24.2%) did not experience tumor recurrence.

Association of PD-L1 and CD39 expression with survival of patients with HCC. In order to investigate the relationship between PD-L1 expression levels in HCC tumor cells and patient prognosis, patients were divided into either $PD\text{-}L1^{tumor+}$ or $PD\text{-}L1^{tumor-}$ groups based on expression levels of PD-L1 calculated using multiplex immunofluorescent assay results (Fig. 1A; Table II). PD-L1 expression levels were selectively quantified within CK^+ areas to evaluate PD-L1 expression levels specifically in tumor cells and to exclude stromal cells

Table I. Baseline characteristics of patients with hepatocellular carcinoma.

Patient characteristic	Patients, n	Percentage of total patients, %
Sex		
Male	79	86.8
Female	12	13.2
Hepatitis		
None	4	4.4
Hepatitis B	87	95.6
Hepatitis C	0	0.0
Cirrhosis		
Yes	89	97.8
No	2	2.2
Tumor number		
Single	81	89.0
Multiple	10	11.0
Vascular invasion		
Yes	16	17.6
No	75	82.4
Barcelona Clinic Liver Cancer stage		
0	3	3.3
A	58	63.7
B	13	14.3
C	17	18.7
China Liver Cancer stage		
Ia	29	31.9
Ib	32	35.2
IIa	8	8.8
IIb	5	5.5
IIIa	16	17.5
IIIb	1	1.1
α-fetoprotein		
≤7.86 (normal)	26	28.6
>7.86 (high)	65	71.4
Recrudescence		
Yes	69	75.8
No	22	24.2

(Fig. S1A). PD-L1^{+} cells ≥1% were defined as PD-L1^{tumor+} representing high expression of PD-L1 and PD-L1^{+} cells <1% were defined as PD-L1^{tumor-} reflecting low expression of PD-L1. OS and RFS were compared between the PD-L1^{tumor+} and PD-L1^{tumor-} groups. Kaplan-Meier survival analysis results demonstrated no significant difference between a median OS of 25.1 months for PD-L1^{tumor+} group (n=56) and 52.4 months for PD-L1^{tumor-} group (n=35; P=0.2172; Fig. 1B). The median RFS was 15.76 months for PD-L1^{tumor+} group and 32.02 months for PD-L1^{tumor-} group. No significant difference in RFS was observed between the PD-L1^{tumor+} and PD-L1^{tumor-} groups (P=0.5877; Fig. S1B).

Table II. Patient grouping information.

Group	Patients, n	Percentage, %
PD-L1^{tumor}		
High expression (+)	56	61.5
Low expression (-)	35	38.5
CD39$^{T\ cell}$		
High expression (+)	32	35.2
Low expression (-)	59	64.8
CD39$^{T\ cell}$/PD-L1^{tumor}		
Co-upregulated	23	25.3
Non-co-upregulated	68	74.7
Co-low-expression	26	28.6

To further investigate the impact of tumor-infiltrating CD8^{+} T cell exhaustion on the prognosis of patients with HCC, patients were divided into CD39$^{T\ cell+}$ or CD39$^{T\ cell-}$ groups reflecting high and low expression of CD39 respectively (Fig. 2A; Table II). Using the survival ROC method, the cut-off value for the CD39$^{T\ cell+}$ group was CD39^{+} T cells/CD39^{-} T cells ≥1.42, and CD39^{+} T cells/CD39^{-} T cells <1.42 for the CD39$^{T\ cell-}$ group (Fig. S2). Similar to aforementioned PD-L1 expression levels, CD39 expression levels on CD8^{+} T cells were selectively quantified within CK^{+} areas (Fig. S3A). Survival analysis demonstrated a median OS of 22.1 months for CD39$^{T\ cell+}$ group (n=32) and 49.1 months for CD39$^{T\ cell-}$ group (n=59). Results demonstrated no statistically significant difference in OS between the CD39$^{T\ cell+}$ and CD39$^{T\ cell-}$ groups (P=0.0840; Fig. 2B). The median RFS was 11.69 months for CD39$^{T\ cell+}$ group and 31.54 months for CD39$^{T\ cell-}$ group and no significant difference in RFS was observed between the two groups (P=0.0584; Fig. S3B).

Based on the findings of the present study, it could be suggested that tumor cell PD-L1 expression levels, as well as CD39 expression levels on CD8^{+} T cells individually, were insufficient to influence the overall prognosis of patients with HCC.

Co-upregulation of PD-L1^{tumor} and CD39$^{T\ cell}$ as a prognostic factor for patients with HCC. In the context of HCC progression, it could be suggested that the co-upregulation of PD-L1 in tumor cells and CD39 in tumor-infiltrating CD8^{+} T cells may synergistically impact the prognosis of patients with HCC. Accordingly, patients were divided into two groups: PD-L1^{tumor}/CD39$^{T\ cell}$ co-upregulated group (PD-L1^{+} cells ≥1% and CD39^{+} T cells/CD39^{-} T cells ≥1.42) and the PD-L1^{tumor}/CD39$^{T\ cell}$ non-co-upregulated group (PD-L1^{+} cells <1% or CD39^{+} T cells/CD39^{-} T cells <1.42) (Fig. 3A; Table II). Survival analysis between these two cohorts demonstrated a median OS of 17.2 months for the co-upregulated group (n=23) and 45.6 months for the non-co-upregulated group (n=68). Kaplan-Meier analysis demonstrated that the OS significantly reduced among patients in the co-upregulated group compared with the non-co-upregulated group (P=0.0376; Fig. 3B). The median RFS was 9.26 months for co-upregulated patients

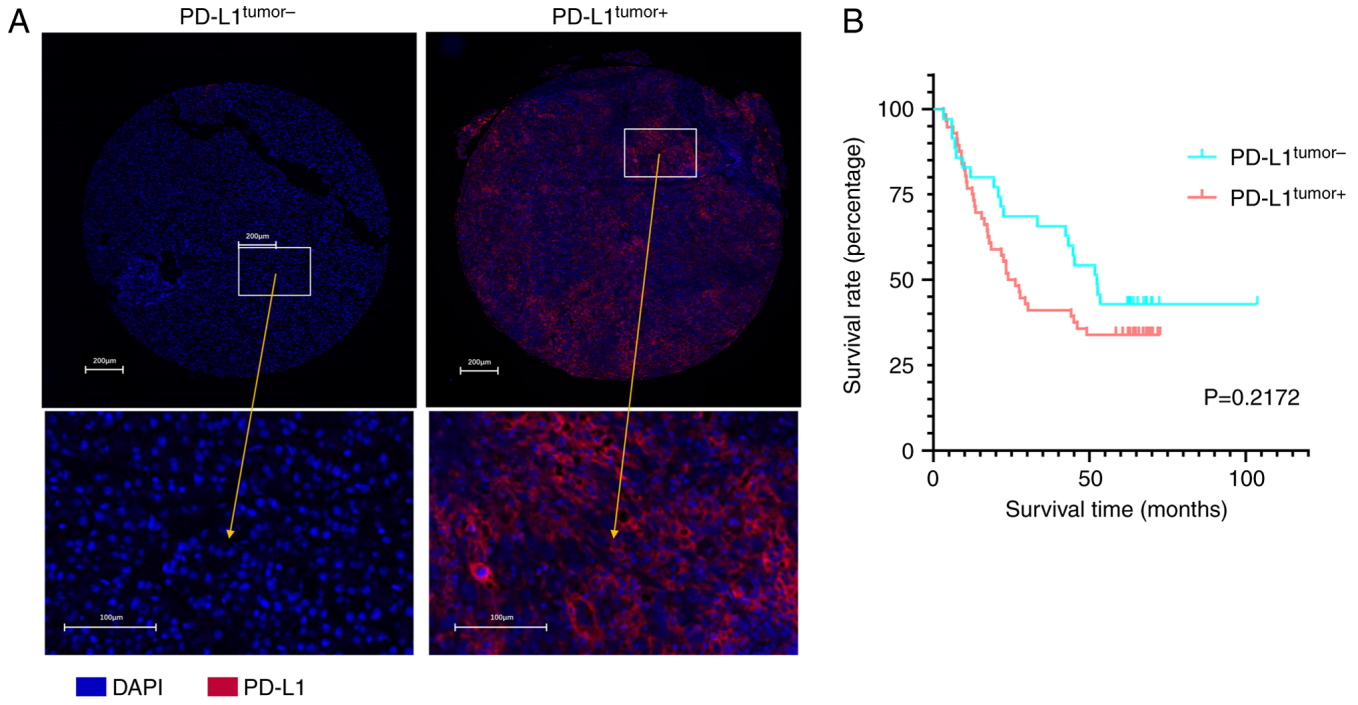

Figure 1. Multiplex immunofluorescence images and survival curves of PD-L1^{tumor-} and PD-L1^{tumor+} groups. (A) Representative multiplex immunofluorescence images of PD-L1^{tumor-} and PD-L1^{tumor+} groups. Patients were divided into two groups according to the expression level of PD-L1 on tumor cells (PD-L1^{tumor-}: PD-L1^{+} cell count <1% in the tumor region; PD-L1^{tumor+}: PD-L1^{+} cell count ≥1% in the tumor region). (B) Kaplan-Meier analysis showed no significant difference in overall survival between PD-L1^{tumor-} and PD-L1^{tumor+} groups (P=0.2172). Scale bar, 200 μm. PD-L1, programmed cell death ligand 1.

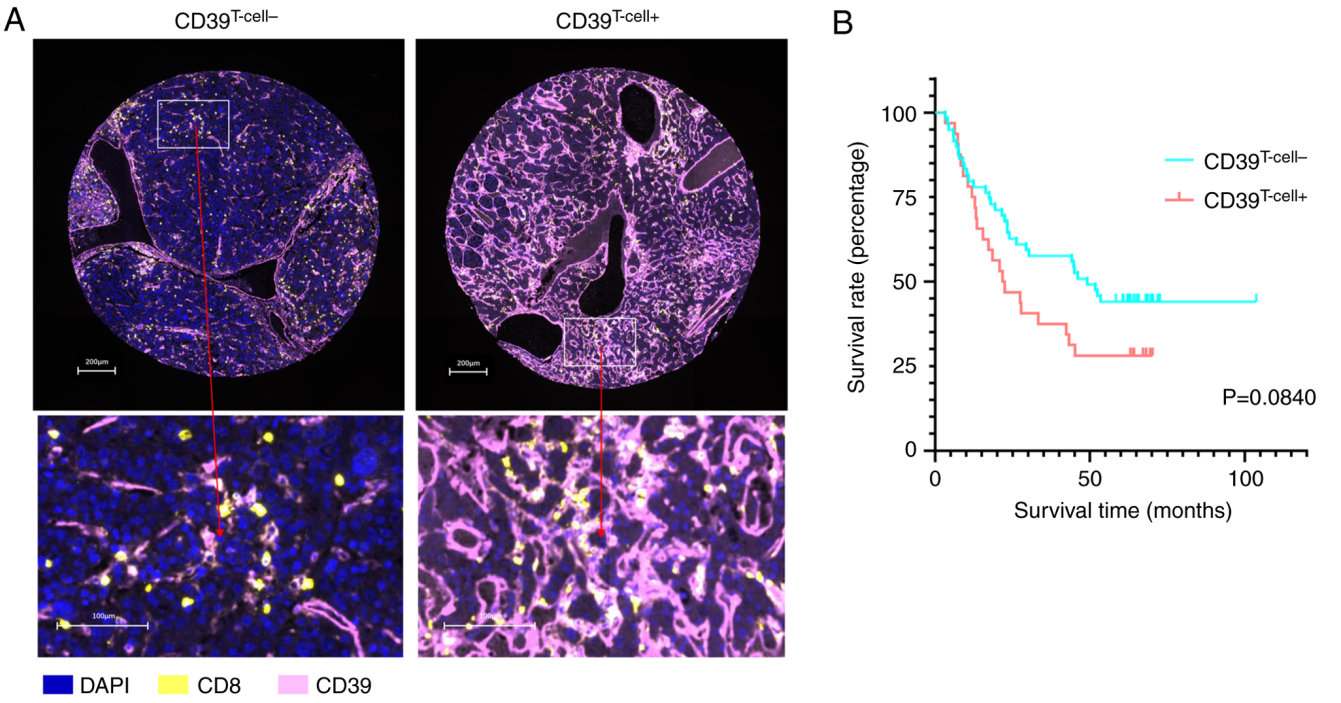

Figure 2. Multiplex immunofluorescence images and survival curves of CD39$^{T\ cell-}$ and CD39$^{T\ cell+}$ groups. (A) Representative multiplex immunofluorescence images of CD39$^{T\ cell-}$ and CD39$^{T\ cell+}$ groups. Patients were divided into two groups according to the expression level of CD39 on tumor-infiltrating CD8^{+} T cells (CD39$^{T\ cell-}$: CD39^{+} T cell/CD39^{-} T cell <1.42; CD39$^{T\ cell+}$: CD39^{+} T cell/CD39^{-} T cell ≥1.42). The optimal cutoff value was determined using the survival receiver operating characteristic method. (B) Kaplan-Meier analysis demonstrated no significant difference in overall survival between CD39$^{T\ cell-}$ and CD39$^{T\ cell+}$ groups (P=0.0840). Scale bar, 200 μm.

and 31.42 months for non-co-upregulated group. The RFS between the co-upregulated and non-co-upregulated groups was statistically significant (P=0.0269; Fig. S4A).

The median OS of the co-upregulated group was 17.2 months and 57.8 months in the PD-L1^{tumor}/CD39$^{T\ cell}$ co-low-expression group (PD-L1^{+} cells <1% and CD39^{+} T cells/CD39^{-} T cells

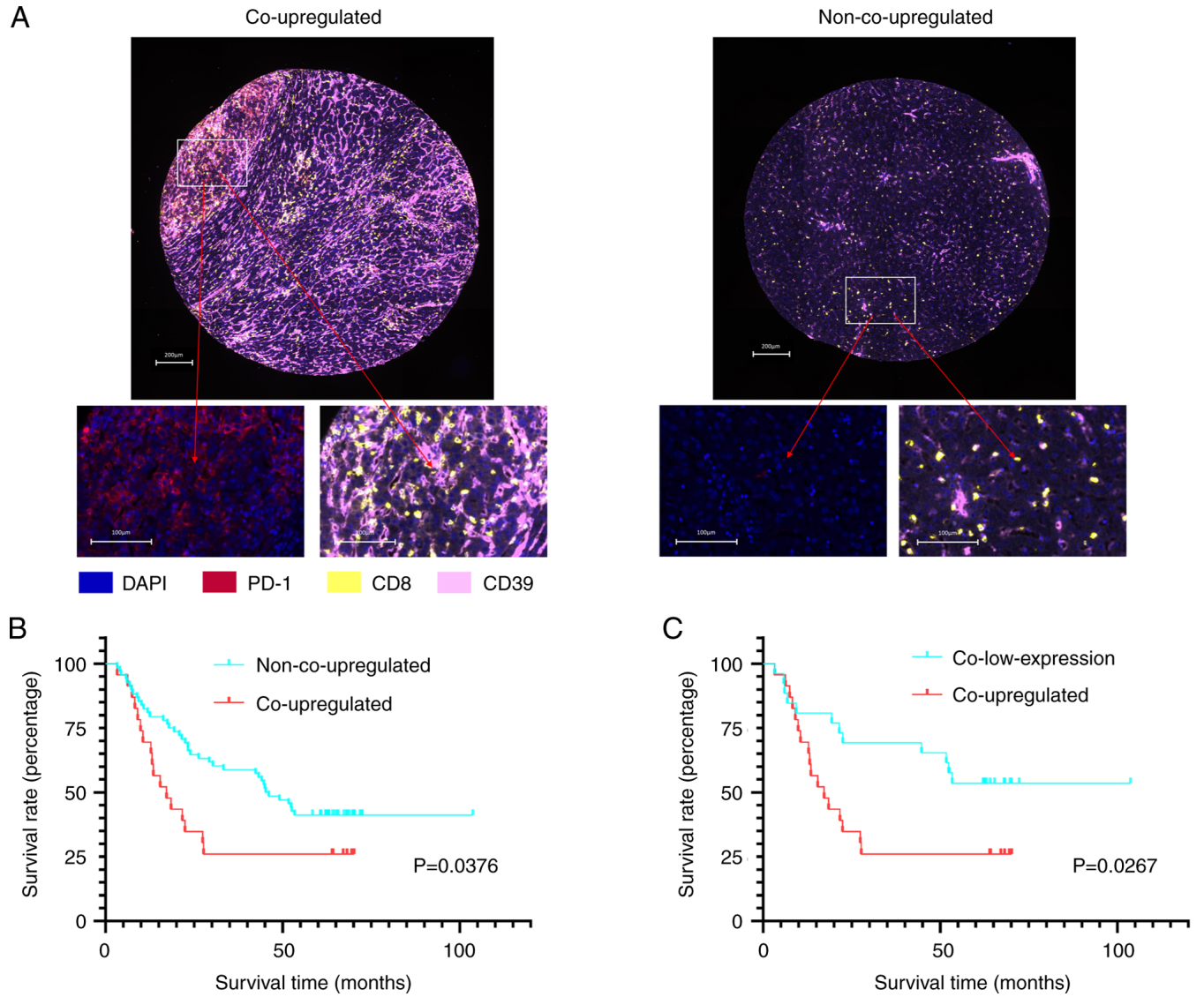

Figure 3. Multiplex immunofluorescence images and survival curves of co-upregulated, non-co-upregulated and co-low-expression groups. (A) Representative multiplex immunofluorescence images of co-upregulated and non-co-upregulated groups. Patients were divided into two groups: The PD-L1^{tumor}/CD39$^{T\ cell}$ co-upregulated (co-upregulated group) and the PD-L1^{tumor}/CD39$^{T\ cell}$ non-co-upregulated (non-co-upregulated) group. The co-upregulated group: PD-L1^{+} cell count ≥1% in the tumor region and CD39^{+} T cell/CD39^{-} T cell ≥1.42. The non-co-upregulated: PD-L1^{tumor-}: PD-L1^{+} cell count <1% in the tumor region or CD39$^{T\ cell-}$: CD39^{+} T cell/CD39^{-} T cell <1.42). (B) Kaplan-Meier analysis demonstrated significantly decreased OS among patients in the co-upregulation group compared with the non-co-upregulated group (P=0.0376). (C) Kaplan-Meier analysis demonstrated that the OS of the co-upregulated subgroup significantly decreased in comparison with the co-low-expression subgroup (P=0.0267). Scale bar, 200 μm. PD-L1, programmed cell death ligand 1; OS; overall survival.

<1.42; n=26). Survival analysis demonstrated that the OS of the co-upregulated group was significantly different compared with the PD-L1^{tumor}/CD39$^{T\ cell}$ co-low-expression group (P=0.0267; Fig. 3C; Table II). The median RFS of the co-upregulated group was 9.26 months and 37.37 months in the co-low-expression group. The median RFS of the co-upregulated group compared with the co-low-expression group was not significantly different (P=0.0864; Fig. S4B).

Additionally, within the PD-L1^{tumor+} group, patients with upregulated CD39 expression (n=33) had a markedly shorter OS (17.2 months) compared with patients with low CD39 expression (n=23; 44.1 months), but there was no significant difference in OS between the two subgroups (P=0.1017; Fig. S4C).

Collectively, the findings of the present study indicated that the co-upregulation of PD-L1^{tumor} and CD39$^{T\ cell}$ expression significantly correlated with poor prognosis in patients with HCC and therefore could potentially serve as an independent prognostic factor for patients with HCC.

Discussion

In previous years, ICIs, such as PD-1 inhibitors, has become an important approach for the systemic treatment of advanced HCC (25). However, the efficacy of systemic therapy for patients with HCC, whether as monotherapy with ICIs or in combination with antiangiogenic agents, remains limited (26). The efficacy of immunotherapy depends on the interaction between immune cells and tumor cells within the tumor immune microenvironment (27). Therefore, it is important to assess the impact of both tumor cells and immune cell states within the tumor microenvironment of HCC on patient prognosis.

The present study employed a multiplex immunofluorescence assay to simultaneously assess PD-L1 expression in tumor cells and CD39 expression in tumor-infiltrating $CD8^+$ T cells. The results indicated that the individual upregulation of PD-L1 in tumor cells or CD39 in tumor-infiltrating $CD8^+$ T cells had no significant impact on the prognosis of patients with HCC. However, patients exhibiting co-upregulation of these two indicators had significantly decreased OS and RFS. To the best of our knowledge, the present study represented the first report of the synergistic impact of tumor cell and tumor-infiltrating $CD8^+$ T cell states within the immune microenvironment of HCC on patient prognosis.

PD-L1 is an immunosuppressive molecule expressed in tumor cells. Inhibiting the activity of immune cells regulates the immune system (28). Increased expression of PD-L1 in tumor cells has been associated with poor overall survival times in certain types of cancers, including breast cancer, colorectal cancer and non-small cell lung cancer (29-31). However, the impact of PD-L1 expression levels on the prognosis of cancer patients is inconsistent in the currently available literature. Meta-analysis in a previous study of HCC indicated that PD-L1 upregulation predicted reduced disease-free survival and progression-free survival, however did not impact OS (22). A further two studies reported that the upregulation of C-type lectin domain family 1 member B or CMTM6 with PD-L1 in tumor cells was associated with poor prognosis of patients with HCC (20,21). Conversely, other studies have suggested that PD-L1 expression on tumor cells does not significantly correlate with the prognosis of patients with HCC or that PD-L1 expression was associated with an improved prognosis of patients with HCC (18,19). These studies did not analyze the synergistic impact of the PD-L1 expression on tumor cells and the function of tumor-infiltrating immune cells. The results in the present study indicated that high PD-L1 expression level in tumor cells was not associated with the prognosis of patients with HCC. However, patients with high PD-L1 expression levels in tumor cells, in conjunction with high CD39 expression levels in tumor-infiltrating $CD8^+$ T cells exhibited a significantly worse prognosis, in terms of OS and RFS.

Tumor-infiltrating $CD8^+$ T cells serve a pivotal role in mediating the antitumor response, but their efficacy can be compromised by T cell exhaustion (5,9). Previous studies have demonstrated that in settings of chronic antigen exposure, such as chronic infections and cancer, T cells progressively lose their effector functions, which is referred to as T cell exhaustion (32). High expression levels of CD39 is considered a hallmark of $CD8^+$ T cell exhaustion (11,12). In the present study, it was suggested that the immune escape induced by the upregulation of PD-L1 in tumor cells may be coupled with the functional exhaustion of tumor-infiltrating $CD8^+$ T cells to result in reduced OS and RFS in patients with HCC. The research findings reported in the present study indicated that the expression levels of PD-L1 in tumor cells and the functional status of immune cells synergistically impact the prognosis of patients with HCC.

However, there were several limitations of the present study. Firstly, as sample size was limited (n=91), it was not possible to include all factors including tumor size, tumor number and vascular invasion into the multivariate survival analysis. Furthermore, the sample sizes of certain subgroups ($PD\text{-}L1^{tumor}/CD39^{T\ cell}$ co-upregulated group and $PD\text{-}L1^{tumor}/CD39^{T\ cell}$ co-low-expression group) were small, which limited the conclusions that could be drawn from the statistical analysis. Furthermore, it was not possible to explore the effect of ICIs as treatment in the patients with HCC included in the present study, as ICIs were not widely used in the treatment of HCC in China during sample collection (January 2012-December 2015). The treatments received by patients were limited to surgical treatment, ablation therapy, interventional therapy and traditional targeted therapy such as tyrosine kinase inhibitors. In order to explore the impact of tumor cells PD-L1 expression and immune cell exhaustion on ICI treatment, future work should focus on the collection of clinical samples from patients with HCC who have received ICI treatment for further investigation.

In conclusion, the findings of the present study demonstrated a significant impact of the interplay between tumor cells and tumor-infiltrating immune cells on the prognosis of patients with HCC. The upregulation of PD-L1 expression in tumor cells, coupled with functional exhaustion of tumor-infiltrating $CD8^+$ T cells reflected by upregulation of CD39 expression, may potentially be associated with poor prognosis in patients with HCC. These findings suggested that the co-upregulation of PD-L1 expression in tumor cells and CD39 expression in tumor-infiltrating $CD8^+$ T cells could possibly serve as a clinical indicator for prognostic assessment of patients with HCC and has the potential to guide immunotherapeutic approaches in the future.

Acknowledgements

Not applicable.

Funding

This work was supported by the Hebei Province Medical Research Project Plan (grant no. 20200092).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XK designed the study, analyzed the data and drafted the manuscript. SZ, SL and JL performed data acquisition, data analysis and edited the manuscript. XK and SL confirm the authenticity of all the raw data. SW designed the study and edited the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the Fourth Hospital of Hebei Medical University Research Ethics Committee (approval no. 2018MEC149; Shijiazhuang, China). All procedures performed were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments

or comparable ethical standards. Written informed consent was obtained from all patients for the retention of diagnostic samples for future experimental use at the time of collection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Block TM, Mehta AS, Fimmel CJ and Jordan R: Molecular viral oncology of hepatocellular carcinoma. Oncogene 33: 5093-5107, 2003.
2. McGlynn KA, Petrick JL and El-Serag HB: Epidemiology of hepatocellular carcinoma. Hepatology 73 (Suppl 1): S4-S13, 2021.
3. Han Z, Yang F, Zhang Y, Wang J, Ni Q, Zhu H, Zhou X, Gao H and Lu J: Prognostic efficacy and prognostic factors of TACE plus TKI with ICIs for the treatment of unresectable hepatocellular carcinoma: A retrospective study. Front Oncol 12: 1029951, 2022.
4. Chew V, Lai L, Pan L, Lim CJ, Li J, Ong R, Chua C, Leong JY, Lim KH, Toh HC, *et al*: Delineation of an immunosuppressive gradient in hepatocellular carcinoma using high-dimensional proteomic and transcriptomic analyses. Proc Natl Acad Sci USA 114: E5900-E5909, 2017.
5. Yu P and Fu YX: Tumor-infiltrating T lymphocytes: Friends or foes? Lab Invest 86: 231-245, 2006.
6. Djenidi F, Adam J, Goubar A, Durgeau A, Meurice G, de Montpréville V, Validire P, Besse B and Mami-Chouaib F: CD8+CD103+ tumor-infiltrating lymphocytes are tumor-specific tissue-resident memory T cells and a prognostic factor for survival in lung cancer patients. J Immunol 194: 3475-3486, 2015.
7. Anraku M, Cunningham KS, Yun Z, Tsao MS, Zhang L, Keshavjee S, Johnston MR and de Perrot M: Impact of tumor-infiltrating T cells on survival in patients with malignant pleural mesothelioma. J Thorac Cardiovasc Surg 135: 823-829, 2008.
8. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, Ellis IO and Green AR: Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol 29: 1949-1955, 2011.
9. Jiang Y, Li Y and Zhu B: T-cell exhaustion in the tumor microenvironment. Cell Death Dis 6: e1792, 2015.
10. Antonioli L, Pacher P, Vizi ES and Haskó G: CD39 and CD73 in immunity and inflammation. Trends Mol Med 19: 355-367, 2013.
11. Canale FP, Ramello MC, Núñez N, Araujo Furlan CL, Bossio SN, Gorosito Serrán M, Tosello Boari J, Del Castillo A, Ledesma M, Sedlik C, *et al*: CD39 Expression defines cell exhaustion in tumor-infiltrating CD8$^+$ T cells. Cancer Res 78: 115-128, 2018.
12. Simoni Y, Becht E, Fehlings M, Loh CY, Koo SL, Teng KWW, Yeong JPS, Nahar R, Zhang T, Kared H, *et al*: Bystander CD8$^+$ T cells are abundant and phenotypically distinct in human tumour infiltrates. Nature 557: 575-579, 2018.
13. Latchman YE, Liang SC, Wu Y, Chernova T, Sobel RA, Klemm M, Kuchroo VK, Freeman GJ and Sharpe AH: PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. Proc Natl Acad Sci USA 101: 10691-10696, 2004.
14. Dong H, Zhu G, Tamada K and Chen L: B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med 5: 1365-1369, 1999.
15. Jiang Y, Chen M, Nie H and Yuan Y: PD-1 and PD-L1 in cancer immunotherapy: Clinical implications and future considerations. Hum Vaccin Immunother 15: 1111-1122, 2019.
16. Dolina JS, Van Braeckel-Budimir N, Thomas GD and Salek-Ardakani S: CD8$^+$ T cell exhaustion in cancer. Front Immunol 12: 715234, 2021.
17. Jung HI, Jeong D, Ji S, Ahn TS, Bae SH, Chin S, Chung JC, Kim HC, Lee MS and Baek MJ: Overexpression of PD-L1 and PD-L2 is associated with poor prognosis in patients with hepatocellular carcinoma. Cancer Res Treat 49: 246-254, 2017.
18. Guo M, Yuan F, Qi F, Sun J, Rao Q, Zhao Z, Huang P, Fang T, Yang B and Xia J: Expression and clinical significance of LAG-3, FGL1, PD-L1 and CD8$^+$T cells in hepatocellular carcinoma using multiplex quantitative analysis. J Transl Med 18: 306, 2020.
19. Huang CY, Wang Y, Luo GY, Han F, Li YQ, Zhou ZG and Xu GL: Relationship between PD-L1 expression and CD8+ T-cell immune responses in hepatocellular carcinoma. J Immunother 40: 323-333, 2017.
20. Yugawa K, Itoh S, Yoshizumi T, Iseda N, Tomiyama T, Morinaga A, Toshima T, Harada N, Kohashi K, Oda Y and Mori M: CMTM6 stabilizes PD-L1 expression and is a new prognostic impact factor in hepatocellular carcinoma. Hepatol Commun 5: 334-348, 2021.
21. Hu K, Wang ZM, Li JN, Zhang S, Xiao ZF and Tao YM: CLEC1B expression and PD-L1 expression predict clinical outcome in hepatocellular carcinoma with tumor hemorrhage. Transl Oncol 11: 552-558, 2018.
22. Wang Q, Liu F and Liu L: Prognostic significance of PD-L1 in solid tumor: An updated meta-analysis. Medicine (Baltimore) 96: e6369, 2017.
23. Reig M, Forner A, Rimola J, Ferrer-Fàbrega J, Burrel M, Garcia-Criado Á, Kelley RK, Galle PR, Mazzaferro V, Salem R, *et al*: BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update. J Hepatol 76: 681-693, 2022.
24. Zhou J, Sun H, Wang Z, Cong W, Zeng M, Zhou W, Bie P, Liu L, Wen T, Kuang M, *et al*: Guidelines for the diagnosis and treatment of primary liver cancer (2022 edition). Liver Cancer 12: 405-444, 2023.
25. Huang A, Yang XR, Chung WY, Dennison AR and Zhou J: Targeted therapy for hepatocellular carcinoma. Signal Transduct Target Ther 5: 146, 2020.
26. Xing R, Gao J, Cui Q and Wang Q: Strategies to improve the antitumor effect of immunotherapy for hepatocellular carcinoma. Front Immunol 12: 783236, 2021.
27. Ribas A and Wolchok JD: Cancer immunotherapy using checkpoint blockade. Science 359: 1350-1355, 2018.
28. Han Y, Liu D and Li L: PD-1/PD-L1 pathway: current researches in cancer. Am J Cancer Res 10: 727-742, 2020.
29. Wang S, Yuan B, Wang Y, Li M, Liu X, Cao J, Li C and Hu J: Clinicopathological and prognostic significance of PD-L1 expression in colorectal cancer: A meta-analysis. Int J Colorectal Dis 36: 117-130, 2021.
30. Brody R, Zhang Y, Ballas M, Siddiqui MK, Gupta P, Barker C, Midha A and Walker J: PD-L1 expression in advanced NSCLC: Insights into risk stratification and treatment selection from a systematic literature review. Lung Cancer 112: 200-215, 2017.
31. Zhang M, Sun H, Zhao S, Wang Y, Pu H, Wang Y and Zhang Q: Expression of PD-L1 and prognosis in breast cancer: A meta-analysis. Oncotarget 8: 31347-31354, 2017.
32. Wherry EJ and Kurachi M: Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol 15: 486-499, 2015.