ORIGINAL

The combination of CD8 and TIM3 expression predicts survival outcomes in hepatocellular carcinoma

Takayuki Noma, Yuma Wada, Masaaki Nishi, Hiroki Teraoku, Shinichiro Yamada, Yu Saito, Tetsuya Ikemoto, Yuji Morine, and Mitsuo Shimada

Department of Surgery, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima University, Tokushima, Japan

Abstract : BACKGROUND : Tumor-infiltrating CD8+ T cells express many immune checkpoint proteins and are currently the focus of therapeutic targeting, although dramatic results have not yet been achieved in hepatocellular carcinoma (HCC). In this study, we performed a statistical analysis of immune checkpoint proteins and prognosis in HCC to evaluate its utility as a biomarker for predicting prognosis. METHODS : We analyzed 474 HCC patients with two comprehensive miRNA profiling datasets (GSE76427 : n=115 and TCGA : n=359) with clinical and transcriptomic data to examine associations between mRNA levels of CD8, TIM3, and PD-1/PD-L1. Each gene was divided into low- and high-expression groups using the tertiles of RNA expression. RESULTS : CD8 and TIM3 expression were associated with overall survival (OS) (P=0.021 and P=0.025, respectively), while PD-1/PD-L1 levels were not associated with OS (P=0.306 and P=0.318, respectively) in TCGA dataset. Among patients with high CD8 expression, low TIM3 expression was associated with better OS (TCGA : P=0.009, Whole patient cohort (TCGA+GSE dataset) : P=0.008). CONCLUSIONS : The combination of CD8 and TIM3 expression which indicates T cell exhaustion was useful biomarker for predicting HCC prognosis than PD-1/PD-L1, suggesting that TIM3 may be a future target for immune checkpoint inhibition. J. Med. Invest. 72 : 354-360, August, 2025

Keywords **:** *Hepatocellular carcinoma, CD8+ T cells, TIM3, Immune checkpoint inhibitor*

INTRODUCTION

Among all cancers, hepatocellular carcinoma (HCC) ranks sixth in terms of incidence worldwide (1), making it the most common primary liver malignancy. The prognosis for hepatocellular carcinoma remains poor, treatments for early-stage HCC include radiofrequency ablation and surgical resection, while for advanced stages, treatments are limited to drug therapies and other options (2, 3).

Immune checkpoint inhibitors (ICIs) are effective against a number of cancers, including malignant melanoma and lung cancer. The discovery of new cancer drugs is also now focused on ICIs, and clinical trials of many ICIs are underway in HCC (4-6). Clinical trials with single-agent nivolumab and pembrolizumab, anti-programmed cell death-1 (PD-1) antibodies, have failed to demonstrate statistically significant efficacy (4, 5), but combination therapy with atezolizumab, an anti-programmed cell death ligand 1 (PD-L1) antibody, and bevacizumab, a vascular endothelial growth factor antibody, has shown efficacy (6) and been approved for the treatment of unresectable advanced HCC. However, ICIs are only effective in a subset of HCC patients, with the response rate to checkpoint inhibitors being approximately 15% to 20% ; additionally, patients who initially respond to ICIs may develop resistance (7). Furthermore, it has been reported that approximately 33% of patients with melanoma who had an initial response to PD-1 inhibitors experienced tumor recurrence (8), suggesting that the improvement of T cell function with ICI may only be transient. One reason for this is that inhibition of a single immune checkpoint does not inhibit other types of immune checkpoints, and therefore may be insufficiently effective (9). The most widely studied biomarkers for anti-PD-1/PD-L1 ICI therapy include PD-L1 expression, tumor mutation burden (TMB), and expression patterns of immune-related genes (10, 11). However, due to the low TMB and PD-L1 expression rates in HCC, there are no biomarkers to accurately predict ICI response or prognosis in HCC (12, 13). Therefore, further studies using ICIs other than anti-PD-1/PD-L1 antibodies are needed.

CD8+ T cells have recently been reported to play an important role in anti-tumor immunity, and high CD8 expression in tumor tissues has been associated with better overall survival (OS) in HCC patients (14). However, when antigen exposure is prolonged in the tumor environment, CD8+ T cells are more likely to differentiate into a stage known as "T cell exhaustion". These exhausted CD8+ T cells lose their ability to produce cytokines (IL2, TNF-α, IFN-γ), and their ability to suppress tumor cells is also reduced (15). It has also been reported that multiple immune checkpoint proteins, including T cell immunoglobulin and mucin domain-containing-3 (TIM3), are expressed on exhausted CD8+ T cells to suppress anti-tumor immunity (16-18) and that their expression levels are positively correlated with the severity of exhaustion (19, 20). As the exhausted T cells showed a decrease of effector cytokine production and cytolytic activity, leading to the tumor progression, TIM3 is one of the most important exhaustion markers. Therefore, in this study, we focused on exhausted T cells and investigated the role of TIM3 and CD8 expression as a prognostic marker using statistical analysis of a comprehensive dataset.

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Address correspondence and reprint requests to Dr. Yuma Wada, MD., PhD., FACS, Department of Surgery, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima University, 3-18-15, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-88-631-9698. E-mail : yumao9isi@gmail.com

MATERIALS AND METHODS

Patient cohort and comprehensive analyses

Both clinical and genomic data were obtained from two public datasets (GSE76427 and the Cancer Genome Atlas [TCGA]), and we analyzed gene expression profiles (RNA sequences). Samples

without gene expression data and survival longer than 3000 days were excluded. In this study, 474 HCC patients with two comprehensive miRNA profiling datasets (GSE76427 : n=115 and TCGA : n=359) were analyzed. CD8, TIM3, PD-1, and PD-L1 expression were assessed for associations with survival. The patient data used in this study are all de-identified.

Statistical analyses

All statistical analyses were performed using JMP Pro 17 (SAS Institute, Cary, NC, USA). Patients were divided into low- and high-expression groups using the tertiles for RNA expression levels of CD8, TIM3, PD-1, and PD-L1 (CD8 and TIM3 : high = upper 1/3 tertile, PD-1 and PD-L1 : high = upper 2/3 tertile). Survival outcomes were analyzed using Kaplan–Meier plots and the log-rank test. A p-value <0.05 was considered statistically significant.

RESULTS

Relationship between the expression of CD8 and immune checkpoint proteins with survival outcomes

To examine the clinical relevance of CD8+ T cell infiltration in HCC, we first examined the association between CD8 expression and survival outcomes. Patients were divided into low and high groups using the tertiles for CD8 expression in the public datasets. There was a significant difference between high CD8 expression and better survival outcomes in TCGA cohort (P=0.021, Fig. 1A). Also, another dataset shows that there was a significant difference between high CD8 expression and better survival outcomes in GSE76427 cohort (P=0.014, Fig. 1B). As the exhausted T cells showed a decrease of effector cytokine production and cytolytic activity, TIM3 is one of the most important exhaustion markers. Then, to investigate the exhaustion of T cells, we focused on the immune checkpoint proteins (TIM3 and PD-1/PD-L1 expression). There was a significant difference between low TIM3 expression and better survival outcomes (TCGA : P=0.025, Fig. 1C ; GSE76427 : P=0.009, Fig. 1D). Interestingly, there were no differences between PD-1 or PD-L1 expressions with survival outcomes (PD-1 TCGA : P=0.306, Fig. 1E ; GSE76427 : P=0.565, Fig. 1F ; PD-L1 : TCGA : P=0.318, Fig. 1G ; GSE76427 : P=0.612, Fig. 1H). In summary, infiltration of CD8+ T cells into the tumor tissue and low TIM3 expression were significantly associated with better survival outcomes in patients with HCC using the comprehensive dates.

Role of the immune checkpoint proteins with survival outcomes among the patients with high CD8 expression

Next, to investigate the role of the relationship between CD8 expression and immune checkpoints, we examined the relationship between TIM3 and PD-1/PD-L1 levels and OS in patients with high CD8 expression (n=239). For TIM3, patients with low TIM3 expression and high CD8 expression had better OS than those with high TIM3 expression (P=0.009, Fig. 2A). Regarding PD-1/PD-L1, no association was found between PD-1 or PD-L1 expression and OS under CD8 high expression (P=0.491 and P=0.772, Fig. 2B, 2C). Univariate and multivariate Cox proportional hazard regression analysis for overall survival revealed that TIM3 levels are most relevant to prognosis (Supplementary Figure). Because CD8 and TIM3 levels are expected to be highly associated with OS, we analyzed the association of the combination of CD8 and TIM3 expression with OS among all HCC patients. High CD8 and low TIM3 expression were associated with improved OS compared with the overall HCC patient population (P=0.008, Fig. 3A). Conversely, low CD8 and high TIM3 expression were associated with significantly worse OS compared with the overall HCC patient population (P<0.001, Fig. 3B).

Additionally, the group with high CD8 and high TIM3 expression did not have significantly different survival outcomes compared with the group with low CD8 expression (P=0.132, Fig. 4). In other words, regardless of CD8-positive cell infiltration in the tumor, if TIM3 was highly expressed and CD8+ T cells were fatigued, there were no differences in OS from patients with low levels of tumor-infiltrating CD8+ T cells. In summary, regardless of PD-1/PD-L1, tumor-infiltrating CD8+ T cells with low TIM3 expression were associated with a good prognosis, and low TIM3 expression was essential for a good prognosis in HCC.

DISCUSSION

CD8+ T cells are the most important component of anti-tumor immunity. CD8+ T cells directly attack tumor cells *via* perforin and granzymes and induce apoptosis in tumor cells *via* cytokines such as IFN-γ, IL-2, and TNF-α. However, CD8+ T cells usually enter a state of "exhaustion" or "dysfunction" in the tumor microenvironment (TME) (21). CD8+ T cells in the TME express high levels of immune checkpoint proteins such as PD-1, TIM3, and CTLA4 and have reduced release of cytokines and cytotoxic potential and increased expression of IL-10, a cytokine that suppresses immunity (22). Additionally, PD-L1 and galentin9, ligands for the immune checkpoints PD-1 and TIM3, respectively, are primarily expressed on HCC tumor cells and CD68-positive tumor-associated macrophages (TAMs) to promote immune escape (19, 23). Clarifying such mechanisms of the TME, in which tumor-associated macrophages and regulatory T cells are heavily involved, may be important for improving the outcome of ICI therapy (24).

Binding of TIM3 to its ligand galectin-9 inhibits the production of IL-2, TNFα, and IFN-γ, inhibiting the immunity of Th17, Th1, and Tc2 cells (25). In HCC tumor immunity, there is a marked increase in TIM3 expression on CD8+ T cells (19, 20, 26), which suppresses anti-tumor immunity by promoting TME formation and inhibiting T cell function (27, 28). In HCC patients, TIM3 expressions on CD8+ T cells and TAMs and serum TIM3 levels are significantly correlated with shorter OS and more advanced tumor stage (17, 26-28). Moreover, T cells with high TIM3 expression are increased among peripheral blood mononuclear cells (PBMCs) and are also increased in patients with recurrent HCC after hepatectomy as well as in those with treatment-resistant HCC (29). These findings suggest that TIM3 may be an important marker to predict OS and treatment resistance or relapse in HCC. Although there is currently no standard therapy targeting TIM3, anti-TIM3 antibodies have shown anti-tumor effects in preclinical studies (19, 20, 29), and early-stage clinical trials with various anti-TIM3 antibodies are currently underway worldwide (30, 31).

Our statistical analysis showed that regardless of PD-1/PD-L1 expression, high CD8 and low TIM3 expression were associated with better OS in HCC (Figs. 1, 2, 3). We found that both low TIM3 and high CD8 expression were essential for a good prognosis in HCC (Fig. 4). This suggests that TIM3 may be a more useful biomarker for predicting HCC prognosis than PD-1/PD-L1, which is currently a therapeutic target.

In this study, we analyzed mRNA expression levels of CD8 and several immune checkpoint molecules using publicly available datasets. While our findings highlight gene expression patterns that may reflect underlying immune activity and potential therapeutic targets, an important limitation is the lack of direct clinical validation. Future studies integrating protein-level analysis, immunohistochemistry, and patient outcome data are needed to confirm the prognostic and predictive value of these

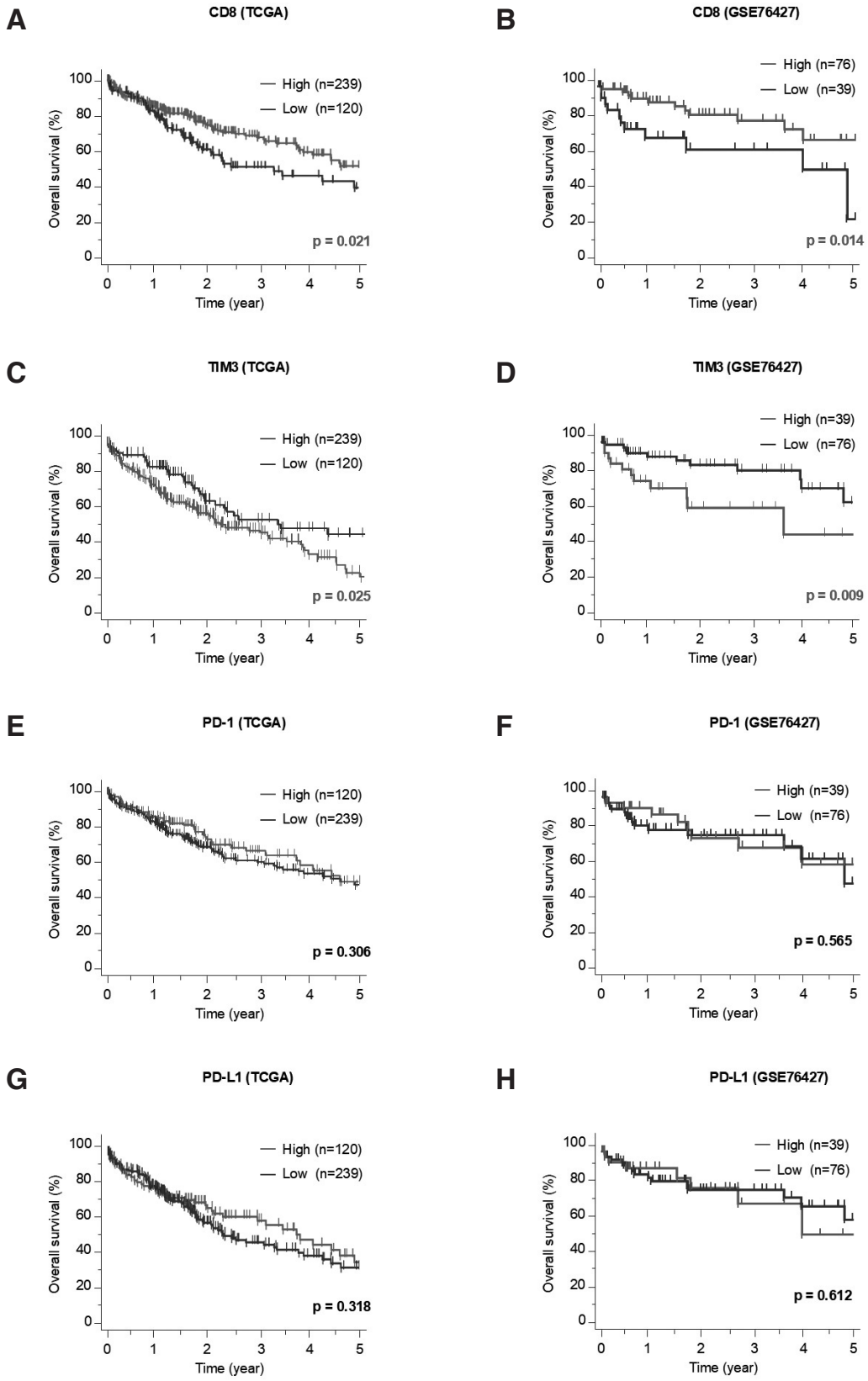

Figure 1. Relationship between the expression of CD8 and other immune checkpoint proteins with OS in patients with hepatocellular carcinoma.
A–B) A comparison of OS between high and low-risk group estimated by the CD8 expression in (A) TCGA : n=359, and (B) GSE76427 : n=115 dataset. C-D) A comparison of OS between high and low-risk group estimated by the TIM3 expression in (C) TCGA : n=359, and (D) GSE76427 : n=115 dataset. E-F) A comparison of OS between high and low-risk group estimated by the PD-1 expression in (E) TCGA : n=359, and (F) GSE76427 : n=115 dataset. G-H) A comparison of OS between high and low-risk group estimated by the PD-L1 expression in (G) TCGA : n=359, and (H) GSE76427 : n=115 dataset. Survival outcomes were analyzed using Kaplan–Meier plots and the log-rank test. A p-value <0.05 was considered statistically significant.

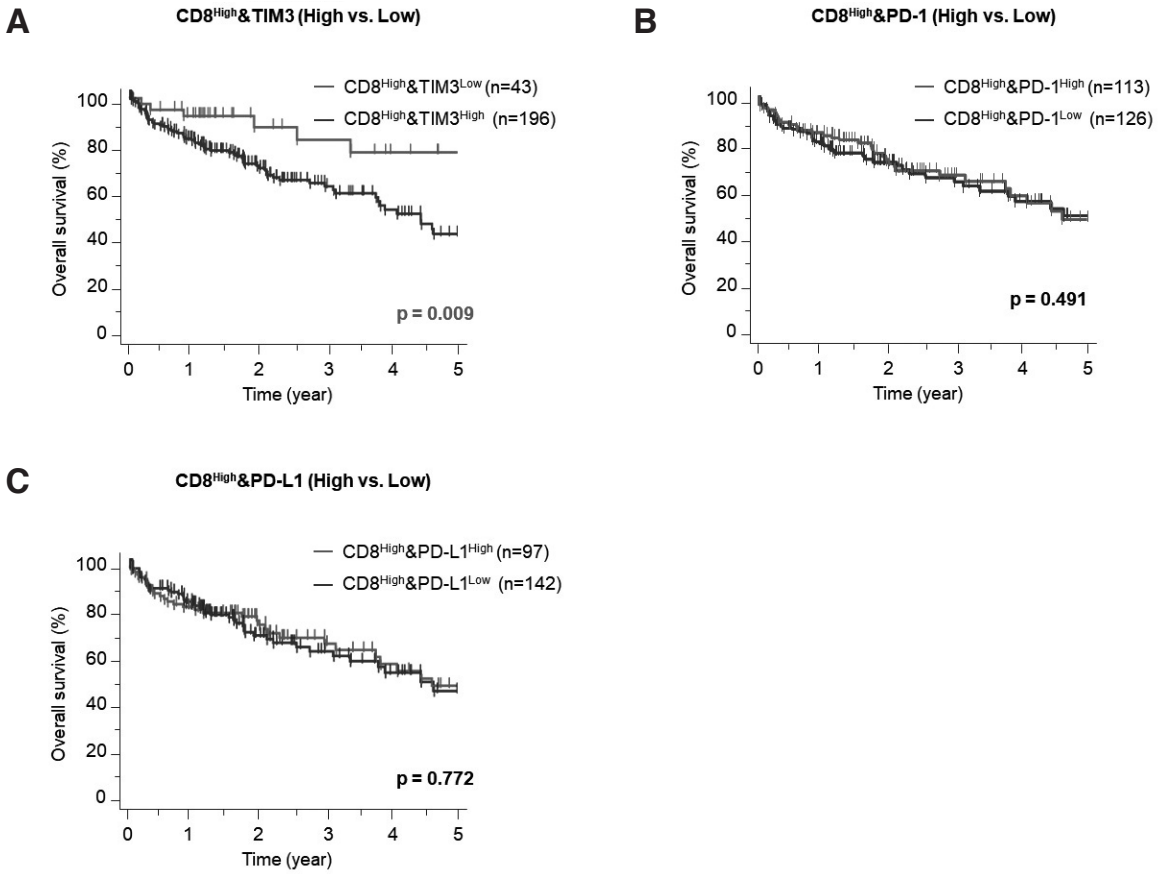

Figure 2. Relationship between the expression of immune checkpoint proteins and OS among the patients with high CD8 expression.
A–C) A comparison of OS between high and low-risk group estimated by the (A) TIM3, (B) PD-1, and (C) PD-L1 expression among the patients with high CD8 expression in TCGA dataset. Survival outcomes were analyzed using Kaplan–Meier plots and the log-rank test. A p-value <0.05 was considered statistically significant.

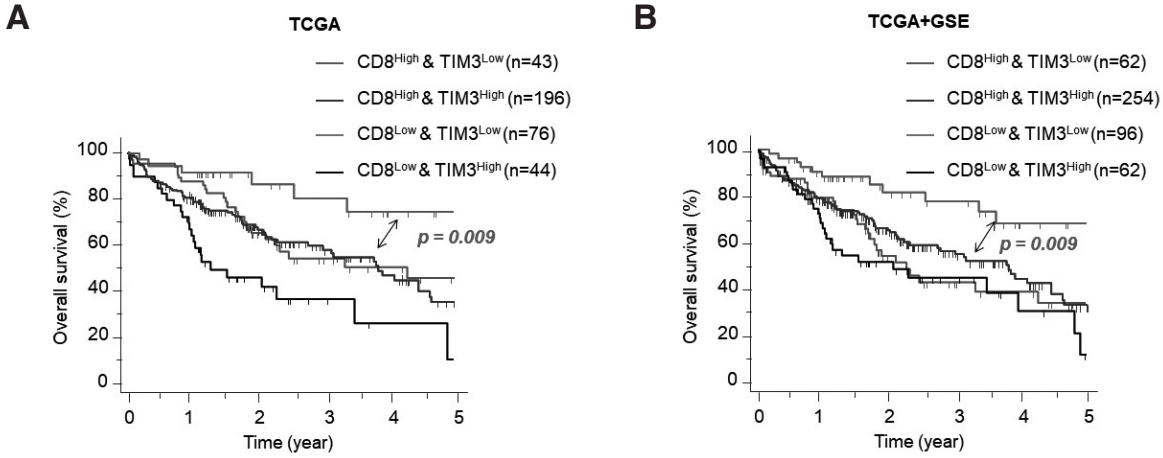

Figure 3. The combination between the CD8 and TIM3 expression with OS in patients with hepatocellular carcinoma.
A–B) A comparison of OS between high and low-risk group combined with CD8 and TIM3 expression in (A) TCGA : n=359, and (B) whole dataset (TCGA+GSE76427 : n=474). Survival outcomes were analyzed using Kaplan–Meier plots and the log-rank test. A p-value <0.05 was considered statistically significant.

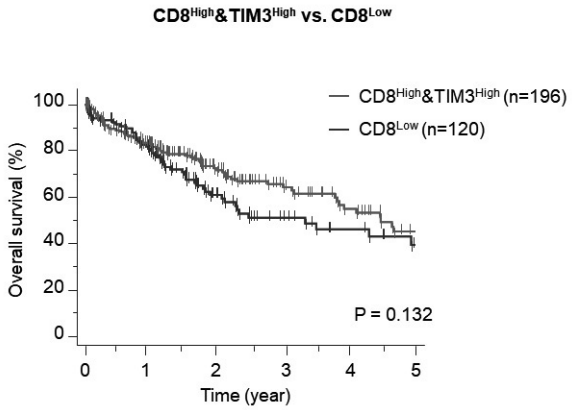

Figure 4. Relationship between the high CD8 and high TIM3 expression compared with low CD8 expression cohort in OS. A comparison of OS between the high CD8 and high TIM3 expression (exhausted CD8+ T cells) compared with low CD8 expression cohort in TCGA dataset. Survival outcomes were analyzed using Kaplan–Meier plots and the log-rank test. A p-value <0.05 was considered statistically significant.

immune markers. In this study, we analyzed mRNA levels of CD8 and immune checkpoint proteins from TGCA dataset and not from single cell RNA sequencing of CD8+ T cells. In the future, we would like to examine the RNA expression levels of CD8+ T cells, where TIM3 data would reflect the actual expression levels of cells and receptors *in vivo*, to examine the relationship between the real expression levels of immune checkpoint proteins in CD8+ T cells with prognosis in patient samples to confirm the results of this study. It has also been reported that blocking PD-1 increases the expression of other immune checkpoint proteins such as TIM3, CTLA-4, and LAG-3 in tumor-infiltrating immune cells (32). Co-expression of PD-1 and TIM3 on CD8+ T cells in the TME has been observed in several tumor types (33, 34), and combined inhibition of both pathways may have synergistic anti-tumor effects (32, 35). We believe that further research into the clinical application of TIM3, which was found to be statistically useful as a prognostic predictor for HCC in this study, and a combined strategy to inhibit the PD-1 and TIM3 pathways in HCC should be explored in the future.

CONCLUSION

Our results show that the patients with high CD8 expression was significantly better OS, reflecting active anti-tumor immunity, while the patients with high TIM3 expression was significantly worse OS, indicating the fatigue of anti-tumor immunity in CD8+ T cells in the patients with HCC. High CD8 and low TIM3 expression (non-exhausted CD8+ T cells) was associated with better OS in HCC than PD-1/PD-L1, suggesting that CD8 and TIM3 are useful biomarkers for predicting HCC prognosis. Restoring exhausted T cells via the suppression of TIM3 is a promising strategy for cancer treatment, which has become a breakthrough in cancer immunotherapy.

DISCLOSURES

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

HUMAN/ANIMAL RIGHTS

All procedures followed were in accordance with the ethical standards of the responsible committee for human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008(5).

INFORMED CONSENT

Informed consent was obtained from all patients to be included in the study.

FUNDING

No funding was received.

AVAILABILITY OF DATA AND MATERIALS

The data generated in the present study are included in the materials and methods section of this article.

AUTHORS' CONTRIBUTIONS

TN, YW, MN and MS conceived the study idea and designed the study. YM, TI, and YM provided administrative support and study materials or patients. TN, YW, MN, HT, SY, YS, TI, YM and MS collected and assembled the data. TN, YW and MS analyzed and interpreted the data. All authors contributed to the writing of the manuscript. MN, HT and SY confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F : Global Cancer Statistics 2020 : GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 71(3) : 209-249, 2021
2. Paradis V : Histopathology of hepatocellular carcinoma. Recent Results Cancer Res 190 : 21-32, 2013
3. Lee SK, Lee SW, Jang JW, Bae SH, Choi JY, Yoon SK : Immunological Markers, Prognostic Factors and Challenges Following Curative Treatments for Hepatocellular Carcinoma. Int J Mol Sci 22(19), 2021
4. Yau T, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, Kudo M, Harding JJ, Merle P, Rosmorduc O, Wyrwicz L, Schott E, Choo SP, Kelley RK, Sieghart W, Assenat E, Zaucha R, Furuse J, Abou-Alfa GK, El-Khoueiry AB, Melero I, Begic D, Chen G, Neely J, Wisniewski T, Tschaika M, Sangro B : Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459) : a randomised, multicentre, open-label, phase 3 trial. Lancet Oncol 23(1) : 77-90, 2022
5. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, Breder V, Edeline J, Chao Y, Ogasawara S, Yau T, Garrido M, Chan SL, Knox J, Daniele B, Ebbinghaus SW, Chen E, Siegel AB, Zhu AX, Cheng AL, investigators K- : Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240 : A Randomized, Double-Blind, Phase III Trial. J Clin Oncol 38(3) : 193-202, 2020
6. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, Li D, Verret W, Xu DZ, Hernandez S, Liu J, Huang C, Mulla S, Wang Y, Lim HY, Zhu AX, Cheng AL, Investigators IM : Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. N Engl J Med 382(20) : 1894-1905, 2020
7. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling THR, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I : Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040) : an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet 389(10088) : 2492-2502, 2017
8. Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, Joshua AM, Patnaik A, Hwu WJ, Weber JS, Gangadhar TC, Hersey P, Dronca R, Joseph RW, Zarour H, Chmielowski B, Lawrence DP, Algazi A, Rizvi NA, Hoffner B, Mateus C, Gergich K, Lindia JA, Giannotti M, Li XN, Ebbinghaus S, Kang SP, Robert C : Association of Pembrolizumab With Tumor Response and Survival Among Patients With Advanced Melanoma. JAMA 315(15) : 1600-1609, 2016
9. Blackburn SD, Shin H, Freeman GJ, Wherry EJ : Selective expansion of a subset of exhausted CD8 T cells by alphaPD-L1 blockade. Proc Natl Acad Sci U S A 105(39) : 15016-15021, 2008
10. Ott PA, Bang YJ, Piha-Paul SA, Razak ARA, Bennouna J, Soria JC, Rugo HS, Cohen RB, O'Neil BH, Mehnert JM, Lopez J, Doi T, van Brummelen EMJ, Cristescu R, Yang P, Emancipator K, Stein K, Ayers M, Joe AK, Lunceford JK : T-Cell-Inflamed Gene-Expression Profile, Programmed Death Ligand 1 Expression, and Tumor Mutational Burden Predict Efficacy in Patients Treated With Pembrolizumab Across 20 Cancers : KEYNOTE-028. J Clin Oncol 37(4) : 318-327, 2019
11. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, Sher X, Liu XQ, Lu H, Nebozhyn M, Zhang C, Lunceford JK, Joe A, Cheng J, Webber AL, Ibrahim N, Plimack ER, Ott PA, Seiwert TY, Ribas A, McClanahan TK, Tomassini JE, Loboda A, Kaufman D : Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science 362(6411), 2018
12. Calderaro J, Rousseau B, Amaddeo G, Mercey M, Charpy C, Costentin C, Luciani A, Zafrani ES, Laurent A, Azoulay D, Lafdil F, Pawlotsky JM : Programmed death ligand 1 expression in hepatocellular carcinoma : Relationship With clinical and pathological features. Hepatology 64(6) : 2038-2046, 2016
13. Dhanasekaran R, Nault JC, Roberts LR, Zucman-Rossi J : Genomic Medicine and Implications for Hepatocellular Carcinoma Prevention and Therapy. Gastroenterology 156(2) : 492-509, 2019
14. Cherkassky L, Oshi M, Abdelfatah E, Wu R, Takabe Y, Yan L, Endo I, Takabe K : An immune-inflamed tumor microenvironment as defined by CD8 score is associated with favorable oncologic outcomes in hepatocellular carcinoma independent of measures of tumor mutational burden. Am J Cancer Res 12(7) : 3099-3110, 2022
15. Khan O, Giles JR, McDonald S, Manne S, Ngiow SF, Patel KP, Werner MT, Huang AC, Alexander KA, Wu JE, Attanasio J, Yan P, George SM, Bengsch B, Staupe RP, Donahue G, Xu W, Amaravadi RK, Xu X, Karakousis GC, Mitchell TC, Schuchter LM, Kaye J, Berger SL, Wherry EJ : TOX transcriptionally and epigenetically programs CD8(+) T cell exhaustion. Nature 571(7764) : 211-218, 2019
16. Grosser R, Cherkassky L, Chintala N, Adusumilli PS : Combination Immunotherapy with CAR T Cells and Checkpoint Blockade for the Treatment of Solid Tumors. Cancer Cell 36(5) : 471-482, 2019
17. Spranger S, Spaapen RM, Zha Y, Williams J, Meng Y, Ha TT, Gajewski TF : Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med 5(200) : 200ra116, 2013
18. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A : PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515(7528) : 568-571, 2014
19. Li H, Wu K, Tao K, Chen L, Zheng Q, Lu X, Liu J, Shi L, Liu C, Wang G, Zou W : Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma. Hepatology 56(4) : 1342-1351, 2012
20. Zhou G, Sprengers D, Boor PPC, Doukas M, Schutz H, Mancham S, Pedroza-Gonzalez A, Polak WG, de Jonge J, Gaspersz M, Dong H, Thielemans K, Pan Q, JNM IJ, Bruno MJ, Kwekkeboom J : Antibodies Against Immune Checkpoint Molecules Restore Functions of Tumor-Infiltrating T Cells in Hepatocellular Carcinomas. Gastroenterology 153(4) : 1107-1119 e1110, 2017
21. Wherry EJ, Kurachi M : Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol 15(8) : 486-499, 2015
22. Ma J, Zheng B, Goswami S, Meng L, Zhang D, Cao C, Li T, Zhu F, Ma L, Zhang Z, Zhang S, Duan M, Chen Q, Gao Q, Zhang X : PD1(Hi) CD8(+) T cells correlate with exhausted signature and poor clinical outcome in hepatocellular carcinoma. J Immunother Cancer 7(1) : 331, 2019
23. Liu CQ, Xu J, Zhou ZG, Jin LL, Yu XJ, Xiao G, Lin J, Zhuang SM, Zhang YJ, Zheng L : Expression patterns of

programmed death ligand 1 correlate with different microenvironments and patient prognosis in hepatocellular carcinoma. Br J Cancer 119(1) : 80-88, 2018
24. Cassetta L, Kitamura T : Targeting Tumor-Associated Macrophages as a Potential Strategy to Enhance the Response to Immune Checkpoint Inhibitors. Front Cell Dev Biol 6 : 38, 2018
25. Kandel S, Adhikary P, Li G, Cheng K : The TIM3/Gal9 signaling pathway : An emerging target for cancer immunotherapy. Cancer Lett 510 : 67-78, 2021
26. Dinney CM, Zhao LD, Conrad CD, Duker JM, Karas RO, Hu Z, Hamilton MA, Gillis TR, Parker TM, Fan B, Advani AH, Poordad FB, Fauceglia PL, Kirsch KM, Munk PT, Ladanyi MP, Bochner BA, Bekelman JA, Grandori CM, Olson JC, Lechan RD, Abou GM, Goodarzi MA : Regulation of HBV-specific CD8(+) T cell-mediated inflammation is diversified in different clinical presentations of HBV infection. J Microbiol 53(10) : 718-724, 2015
27. Huang YH, Zhu C, Kondo Y, Anderson AC, Gandhi A, Russell A, Dougan SK, Petersen BS, Melum E, Pertel T, Clayton KL, Raab M, Chen Q, Beauchemin N, Yazaki PJ, Pyzik M, Ostrowski MA, Glickman JN, Rudd CE, Ploegh HL, Franke A, Petsko GA, Kuchroo VK, Blumberg RS : CEACAM1 regulates TIM-3-mediated tolerance and exhaustion. Nature 517(7534) : 386-390, 2015
28. Sakuishi K, Ngiow SF, Sullivan JM, Teng MW, Kuchroo VK, Smyth MJ, Anderson AC : TIM3(+)FOXP3(+) regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer. Oncoimmunology 2(4) : e23849, 2013
29. Liu F, Zeng G, Zhou S, He X, Sun N, Zhu X, Hu A : Blocking Tim-3 or/and PD-1 reverses dysfunction of tumor-infiltrating lymphocytes in HBV-related hepatocellular carcinoma. Bull Cancer 105(5) : 493-501, 2018
30. Harding JJ, Moreno V, Bang YJ, Hong MH, Patnaik A, Trigo J, Szpurka AM, Yamamoto N, Doi T, Fu S, Calderon B, Velez de Mendizabal N, Calvo E, Yu D, Gandhi L, Liu ZT, Galvao VR, Leow CC, de Miguel MJ : Blocking TIM-3 in Treatment-refractory Advanced Solid Tumors : A Phase Ia/b Study of LY3321367 with or without an Anti-PD-L1 Antibody. Clin Cancer Res 27(8) : 2168-2178, 2021
31. Hollebecque A, Chung HC, de Miguel MJ, Italiano A, Machiels JP, Lin CC, Dhani NC, Peeters M, Moreno V, Su WC, Chow KH, Galvao VR, Carlsen M, Yu D, Szpurka AM, Zhao Y, Schmidt SL, Gandhi L, Xu X, Bang YJ : Safety and Antitumor Activity of alpha-PD-L1 Antibody as Monotherapy or in Combination with alpha-TIM-3 Antibody in Patients with Microsatellite Instability-High/Mismatch Repair-Deficient Tumors. Clin Cancer Res 27(23) : 6393-6404, 2021
32. Koyama S, Akbay EA, Li YY, Herter-Sprie GS, Buczkowski KA, Richards WG, Gandhi L, Redig AJ, Rodig SJ, Asahina H, Jones RE, Kulkarni MM, Kuraguchi M, Palakurthi S, Fecci PE, Johnson BE, Janne PA, Engelman JA, Gangadharan SP, Costa DB, Freeman GJ, Bueno R, Hodi FS, Dranoff G, Wong KK, Hammerman PS : Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun 7 : 10501, 2016
33. Zhou Q, Munger ME, Veenstra RG, Weigel BJ, Hirashima M, Munn DH, Murphy WJ, Azuma M, Anderson AC, Kuchroo VK, Blazar BR : Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. Blood 117(17) : 4501-4510, 2011
34. Li J, Shayan G, Avery L, Jie HB, Gildener-Leapman N, Schmitt N, Lu BF, Kane LP, Ferris RL : Tumor-infiltrating Tim-3(+) T cells proliferate avidly except when PD-1 is co-expressed : Evidence for intracellular cross talk. Oncoimmunology 5(10) : e1200778, 2016
35. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC : Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med 207(10) : 2187-2194, 2010

Factors	Univariate		Multivariate		
	3-year OS (%)	P value	HR	CI	P value
PD-L1 (- / +)	68.6 / 68.2	0.99	0.83	0.49-1.40	0.47
PD-1 (- / +)	66.4 / 67.5	0.80	0.75	0.37-1.51	0.42
TIM-3 (- / +)	83.5 / 63.9	0.02	0.36	0.16-0.79	0.01

Supplementary Figure. Univariate and multivariate Cox proportional hazard regression analysis for overall survival