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original article

Blocking Tim-3 or/and PD-1 reverses dysfunction of tumor-infiltrating lymphocytes in HBV-related hepatocellular carcinoma

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TIM3 (T cell Ig- and mucin-domain-containing molecule-3)
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Mots clés
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Summary

Background > The immunosuppression of tumor-infiltrating lymphocytes (TILs) is associated with rapid progression of hepatitis B virus-related hepatocellular carcinoma (HBV-HCC). T cell Ig- and mucin-domain-containing molecule-3 (Tim-3) and programmed cell death 1 (PD-1) are important inhibitory molecules expressed on the surface of T cells, but their roles in the function of TILs in HBV-HCC are poorly understood. We aimed to study the roles of these two markers in HBV-HCC.
Methods > Ninety patients with pathologically confirmed HBV-associated HCC were enrolled in our study. Blood samples, paired fresh tumor tissues and adjacent tissues were collected, and isolating peripheral blood mononuclear cells, TILs and adjacent-infiltrating lymphocytes were isolated from these samples. The patients were followed-up to allow survival analysis.
Results > Tim-3 or/and PD-1 was up-regulated expressed on $CD4^+$ and $CD8^+$ TILs in HBV-HCC patients and a higher proportion of TILs expressed PD-1 alone. Tim-3^+ and PD-1^+ TILs greatly decreased secretion of IFN-γ and TNF-α. Expression of Tim-3 and PD-1 on TILs negatively correlated with disease-free survival of HCC patients. Direct blockade of Tim-3 and PD-1 in vitro significantly enhanced TILs proliferation and secretion of IFN-γ and TNF-α.
Conclusion > Expression of Tim-3 and/or PD-1 on TILs impairs their function and correlates negatively with disease-free survival in HBV-HCC. Direct blockade of Tim-3 and PD-1 restores anti-tumor effects of TILs, which suggests a potential target for novel immunotherapy in HBV-HCC.

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Background

Hepatocellular carcinoma (HCC), the second most frequent cause of cancer-related death worldwide, is associated with high mortality, frequent post-surgical recurrence and poor prognosis [1]. Most HCC is a consequence of chronic viral infection with hepatitis B virus (HBV) and/or hepatitis C Virus (HCV), especially HBV in Africa and East Asia [2]. China has the highest prevalence of HCC in the world, and about two-thirds of HCC cases are attributed to HBV [1,2]. Chronic inflammatory responses play a key role in the progression from chronic HBV infection to HCC, and such inflammation persists throughout the disease [3,4]. This inflammation differs from the inflammatory pathology caused by aflatoxin.

Immune cells also play an important role in HCC progression. The tumor microenvironment contains HCC-associated antigen-presenting cells (APCs), regulatory T cells (Tregs), natural killer T cells (NKTs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and tumor-infiltrating lymphocytes (TILs) [5]. TILs, in particular, control tumor progression and prognosis. They are recruited to the tumor microenvironment, where they persist and eventually lose their anti-tumor effect through the up-regulation of PD-1 [6], a member of the CD28 family of costimulatory/coinhibitory molecules. Interaction between PD-1 and B7-H1 contributes to the immunosuppression of T lymphocytes in tumor tissue [7]. Studies of melanomas suggest that PD-1 may be a marker of T-cell exhaustion, in which their proliferation and cytokine secretion are impaired, thereby weakening T cell-mediated anti-tumor immunity [8]. Consistent with this idea, PD-1 is highly expressed on virus-specific exhausted cytotoxic T lymphocytes (CTLs) and regulatory T cells, which are normally required to combat chronic virus infection and other diseases [9,10]. Few studies have examined PD-1 expression on TILs in HCC.

Tim-3 also suppresses the function of T lymphocytes in chronic human immunodeficiency virus (HIV) and HCV infection. Tim-3$^+$ cells are defined as another exhausted T cell population distinct from PD-1$^+$ cells [11,12]. Tim-3 is expressed on TILs in HBV-HCC and it appears to impair TIL function via the Tim-3/galectin-9 signaling pathway [13], but how this affects HCC is unclear.

PD-1 and Tim-3 are surface inhibitory molecules expressed on TILs that have been linked to colon cancer [14], raising the question of whether and how they contribute to HBV-HCC. HBV-HCC is different from other types of cancer because chronic HBV infection precedes the development of the tumor. HBV infection shows different immunological characteristics from HCV infection: intrahepatic HBV- and HCV-specific CD8$^+$ T cells have different costimulatory profiles. Proportions of FoxP3$^+$ and PD-1$^+$ regulatory T cells are higher in HBV-infected liver than in HCV-infected liver [15]. In order to further study the status and function of Tim-3 and PD-1, we analyzed their expression on TILs of HBV-HCC, and investigated how they may work alone or together to impair the anti-tumor function of TILs. Conversely, we wanted to determine whether blockade of the two molecules can reverse TIL dysfunction and thereby promote anti-tumor immunity.

Patients and methods

Patients and cell preparation

A total of 90 patients (*table I*) at the First Affiliated Hospital of Sun Yat-Sen University with pathologically confirmed HBV-HCC were enrolled in this study. Patients were recruited and samples were taken as described in the *supplementary materials*.

Table I
Clinical characteristics of HBV-HCC patients (*n* = 90)

Variable	Value
Age in year, median (range)	49 (25–74)
Gender, male/female	78/12
Cirrhosis, absent/present	20/70
ALT in units/l, median (range)	110 (9–3774)
AFP in ng/ml, ≤ 25/ > 25	7/83
Tumor multiplicity, solitary/multiple	47/43
Tumor size in cm, ≤ 5/ > 5	37/53
Vascular invasion, absent/present	57/33
TNM stage, I–II/III–IV	60/30
Tumor differentiation, I–II/III–IV	63/27

ALT: alanine aminotransferase; AFP: α-fetoprotein; TNM: tumor node metastasis.