International Immunopharmacology 73 (2019) 156–162

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

PRDM1 levels are associated with clinical diseases in chronic HBV infection and survival of patients with HBV-related hepatocellular carcinoma

Na Li[a], Xiude Fan[a], Xiaoyun Wang[a], Huan Deng[a], Kun Zhang[a], Xiaoge Zhang[a], Ye Wang[a], Qunying Han[a], Yi Lv[b,c], Zhengwen Liu[a,c,*]

[a] Department of Infectious Diseases, First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi, People's Republic of China
[b] Department of Hepatobiliary Surgery, First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi, People's Republic of China
[c] Institute of Advanced Surgical Technology and Engineering, Xi'an Jiaotong University, Xi'an 710061, Shaanxi, People's Republic of China

ARTICLE INFO

Keywords:
Hepatitis B virus infection
PRDM1
Hepatocellular carcinoma
Diagnosis
Survival

ABSTRACT

PR domain zinc finger protein 1 (PRDM1)/B lymphocyte-induced maturation protein 1 (BLIMP1) is a transcriptional repressor involved in B and T cell responses which are implicated in chronic hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC). This study investigated the association of PRDM1 with clinical diseases of chronic HBV infection and prognosis of HBV -related HCC patients. Serum PRDM1 levels were determined in 403 patients with chronic HBV infection (171 chronic hepatitis, 119 cirrhosis and 113 HCC), 70 HBV infection resolvers and 96 healthy control individuals. The PRDM1 levels were analyzed with regard to clinical diseases and overall survival of HCC patients. Serum PRDM1 concentrations in patients with chronic HBV infection were significantly elevated compared with infection resolvers and healthy controls. HBV-related HCC patients had the most significantly elevated PRDM1 levels. PRDM1 levels could considerably differentiate HCC from chronic hepatitis [area under receiver operating characteristic curve (AUC) 0.889, $p < 0.001$] or cirrhosis (AUC 0.910, $p < 0.001$). HCC patients with high PRDM1 levels had a poor prognosis (> 300 pg/mL vs. ≤ 300 pg/mL, $p = 0.001$). High PRDM1 levels were independently associated with increased mortality in HCC patients (hazard ratio 2.997, 95% confidence interval 1.103–4.722, $p = 0.003$). Overall, this study demonstrated that PRDM1 levels are associated with the clinical diseases of chronic HBV infection. Highly elevated PRDM1 levels are discriminative of HCC from other clinical diseases and indicative of a poor prognosis of HCC patients. The potential association of PRDM1 levels with disease progression and treatment response warrants further investigation.

1. Introduction

Hepatitis B virus (HBV) infection remains a serious global public health problem. Almost 250 million people worldwide are living with chronic HBV infection which may lead to a variety of liver diseases including chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) [1,2]. The differences in the clinical outcome and natural disease course of HBV infection are determined by virological and immunological factors [3]. Immunologically, both humoral and cellular immune responses are involved. B cell activation is found to be common in chronic hepatitis B [4], the susceptible B lymphocytes to HBV is implicated in the development of immune mediated inflammation of HBV-induced hepatic injury [5], and interlukin (IL)-10-expressing B cells are involved in the suppression of cytotoxic CD4 T cell activity in HBV-induced HCC [6]. T cells also play a primary role in chronic HBV infection [7] and the status of HBV specific CD8 T cell exhaustion and tumor-specific T cell response is closely associated with the outcomes of chronic HBV infection including the development of HCC [8–10].

PR domain zinc finger protein 1 (PRDM1)/B lymphocyte-induced

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; IL-10, interlukin-10; PRDM1, PR domain zinc finger protein 1; BLIMP1, B lymphocyte-induced maturation protein 1; HIV, human immunodeficiency virus; AFP, α-fetoprotein; CT, computerized tomography; MRI, magnetic resonance imaging; ALT, alanine aminotransferase; AST, aspartate transaminase; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; MDD, minimum detectable dose; CV, coefficient of variation; ROC, receiver operating characteristic curve; AUC, area under receiver operating characteristic curve; CI, confidence interval; OR, odds ratio; PD-1, programmed cell death-1; sPD-L1, soluble programmed death-ligand 1

* Corresponding author at: Department of Infectious Diseases, First Affiliated Hospital of Xi'an Jiaotong University, No. 277 Yanta West Road, Xi'an 710061, Shaanxi Province, People's Republic of China.
E-mail address: liuzhengwen113@xjtu.edu.cn (Z. Liu).

https://doi.org/10.1016/j.intimp.2019.05.012
Received 3 March 2019; Received in revised form 29 April 2019; Accepted 8 May 2019
Available online 14 May 2019

maturation protein 1 (BLIMP1) has been well established as a master regulator of plasma cell differentiation and function [11–13]. In fact, PRDM1/BLIMP1 regulates both B cell and T cell differentiation and guides the fate of effector B and T cells [14–17]. PRDM1/BLIMP1 is an essential regulator of T cell homeostasis and plays a critical role in T cell-mediated immunosuppression [14,16,18–21] including the regulation of T cell exhaustion during chronic viral infection and cancer [22–24].

Owing to the critical role in both humoral and cellular immunity, PRDM1 has been implicated in many human diseases, including autoimmune disease, cancer and infectious disease. In autoimmune disease, genetic studies showed that *PRDM1* gene is associated with multiple autoimmune diseases including systemic lupus erythematosus [25], rheumatoid arthritis [26], inflammatory bowel disease [27,28] and systemic sclerosis [29]. In cancer, PRDM1 has been shown to be frequently inactivated in a variety of lymphomas including diffuse large B cell lymphomas, Natural Killer cell lymphoma and anaplastic large T cell lymphoma [30]. Loss of PRDM1 function is associated with the overall poor prognosis of activated B-cell-like diffuse large B-cell lymphoma patients [31]. Decreased expression of PRDM1 has been demonstrated to be correlated with poor prognosis in lung cancer [32], colon cancer [33] and glioma patients [34]. PRDM1 expression is related to the prognosis of multiple myeloma patients [35] and the metastatic status in breast cancer patients [36]. PRDM1 is also involved in infectious disease. For example, PRDM1 has been shown to be involved in the viral replication [37] and the latent viral infection of memory CD4 T cells in human immunodeficiency virus (HIV) infection [38]. Notably, PRDM1 has been revealed to be mechanistically involved in the pathogenesis of Hashimoto thyroiditis associated with human parvovirus B19 infection [39] and the lymphomagenesis of Epstein-Barr virus (EBV) [40].

Given the involvement of B cell and T cell responses in HBV infection and HCC and the role of PRDM1/BLIMP1 in the regulation of immune responses in viral infection and cancers, PRDM1/BLIMP1 is indicated to be potentially implicated in chronic HBV infection and HBV-related liver diseases including HCC. A study in HBV infection showed that PRDM1/BLIMP1 is involved in the partial immune control of HBV infection in the liver by tissue-resident memory T cells [41]. However, the relationship between PRDM1/BLIMP1 and HBV-associated diseases remains largely unknown. We hypothesize that the PRDM1/BLIMP1 levels may be proportionally related to the hierarchy of immune abnormality including T cell dysfunction or exhaustion and thus to the development of different clinical diseases in chronic HBV infection. To this end, the present study examined the serum levels of PRDM1 in HBV chronically infected patients and analyzed the associations with clinical diseases especially the development of HCC and the survival of HCC patients.

2. Materials and methods

2.1. Study population

Four hundred and three patients with chronic HBV infection were recruited from the First Affiliated Hospital of Xi'an Jiaotong University. Chronic HBV infection was diagnosed based on the persistent positivity of hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) or antibody to HBeAg (anti-HBe) and antibody to hepatitis B core antigen (anti-HBc) for > 6 months [42]. Patients with HIV infection, other liver diseases such as hepatitis A, hepatitis C, hepatitis D, hepatitis E, drug-induced liver injury, steatohepatitis, alcoholic hepatitis, autoimmune hepatitis and Wilson's disease, patients with diabetes, hyperthyroidism, severe cardiovascular disease, respiratory disease and renal impairment, and patients under 18 years of age or in pregnancy were excluded. According to the history of HBV infection, serostatus of HBsAg, HBeAg/anti-HBe, and anti-HBc, serum HBV DNA levels, biochemical liver function, α-fetoprotein (AFP) and ultrasonography and/or computerized tomography (CT)/magnetic resonance imaging (MRI) or liver pathology as well as the guidelines [42,43], the patients with chronic HBV infection were diagnosed as chronic hepatitis (n = 171), liver cirrhosis (n = 119), and HCC (n = 113). The 113 HCC patients were followed up for an average of 32.53 ± 22.25 months (median, 28 months; range, 1–75 months). Individuals who spontaneously resolved HBV infection with positivity of serum antibodies to HBsAg (anti-HBs) and anti-HBc, normal liver function, and no other hepatitis B markers were included as HBV infection resolvers (n = 70). Individuals who had no history of hepatitis and no liver and other diseases with normal liver function were enrolled as healthy controls (n = 96).

Fasting blood samples were obtained from each subject. Serum was separated by centrifugation and the serum samples were aliquoted and stored at −80 °C until use. This study was carried out in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. All the subjects included in the study gave informed consent.

2.2. Laboratory tests

Routine laboratory parameters were measured at the central laboratory of the hospital. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) (IU/L), total bilirubin (μmol/L) and albumin (g/L) levels were assayed on the Olympus AU5400 automatic biochemical analyzer (Olympus Corporation, Tokyo, Japan). HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc were detected using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Beijing Wantai Biological Pharmacy, Beijing, China). Serum HBV DNA (IU/mL) levels were determined by HBV Fluorescent Quantitative Polymerase chain reaction (PCR) Detection Kit with TaqMan probes (Da-An Gene Co., Guangzhou, China). Serum AFP (ng/mL) was quantitatively measured using automated Eleceyes (Hoffman-La Roche Ltd., Basel, Switzerland).

2.3. Determination of serum PRDM1 levels

Serum PRDM1 levels were quantitatively determined using a commercially available human PR domain zinc finger protein 1 (PRDM1) ELISA Kit (MyBioSource, San Diego, CA, USA) according to the manufacturer's instructions. The detection range is 25 pg/mL-1600 pg/mL. The minimum detectable dose (MDD) of human PRDM1 is typically < 6.25 pg/mL and no significant cross-reactivity or interference between human PRDM1 and analogues was observed. The intra-assay precision (precision within an assay) has a coefficient of variation (CV)% < 8%. The inter-assay precision (precision between assays) has a CV% < 10%. The persons who performed the determination of PRDM1 levels were blinded to the individuals' characteristics when they read the PRDM1 ELISA.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Comparison of the variables between patients with chronic HBV infection, HBV infection resolvers and healthy controls was performed using Student's t-test or χ^2 test where appropriate. The variables that were not normally distributed were analyzed after logarithmic transformation. Differences of PRDM1 levels between groups were identified using Kruskal-Wallis test for nonparametric comparison. Univariate and multivariate Cox regression was performed to assess factors associated with HCC. Kaplan-Meier analysis was used for survival analysis of patients with HBV-related HCC and the Log-rank test was used to determine statistical significance. The ability of serum PRDM1 for identifying HCC was evaluated by receiver operating characteristic curve (ROC) and the area under ROC curve (AUC). Significances between groups were identified using Student's t-tests.

Differences of AUCs between groups were identified using Kruskal-Wallis test. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Characteristics of the study subjects

The gender (M/F) and age (year) between the 403 patients with chronic HBV infection [283/120 and 40.37 ± 13.68 (18–78), respectively], 70 HBV infection resolvers [45/25 and 41.31 ± 14.09 (18–79), respectively] and the 96 healthy controls [127/69 and 38.58 ± 14.29 (18–76), respectively] had no statistical differences ($p = 0.319$ and $p = 0.230$, respectively).

The clinical diagnosis of the 403 patients with chronic HBV infection included 171 chronic hepatitis, 119 liver cirrhosis, and 113 HCC. Demographic, virologic and biochemical parameters in patients with different clinical diseases of chronic HBV infection are shown in Table S1. The gender, age, HBV DNA, ALT, AST, total bilirubin, and albumin levels between chronic hepatitis, liver cirrhosis, and HCC patients were significantly different (Table S1).

3.2. Serum PRDM1 levels in the study subjects

The levels of serum PRDM1 in patients with chronic HBV infection [169.43 (2.81–750.51) pg/mL] were significantly higher than in HBV infection resolvers [58.97 (4.27–309.45) pg/mL, $p < 0.001$] and healthy controls [28.26 (0.72–351.03) pg/mL, $p < 0.001$, Fig. 1A]. The levels of serum PRDM1 between HBV infection resolvers and healthy controls had no significant difference [$p = 0.393$, Fig. 1A].

The levels of serum PRDM1 in HCC patients [371.01 (2.97–750.51) pg/mL] were significantly higher than in chronic hepatitis [130.95 (2.81–552.64) pg/mL, $p < 0.001$] and cirrhosis patients [96.24 (3.7–499.14) pg/mL, $p < 0.001$, Fig. 1B]. Serum PRDM1 levels between patients with chronic hepatitis and cirrhosis had no significant difference ($p = 0.353$, Fig. 1B).

3.3. Serum PRDM1 levels in differentiating HCC from other liver diseases

The ROC curve was plotted to analyze the ability of serum PRDM1 levels to differentiate HBV-associated HCC from chronic hepatitis, cirrhosis or liver diseases other than HCC (non-HCC). The AUCs of PRDM1 levels for differentiating HCC from chronic hepatitis, cirrhosis or non-HCC were 0.889 ($p < 0.001$), 0.910 ($p < 0.001$) and 0.897 ($p < 0.001$), respectively, with a cutoff value of 296.71 pg/mL, 278.28 pg/mL and 301.8 pg/mL, respectively, a sensitivity of 83.2%, 90.3% and 82.3%, respectively, and a specificity of 80.1%, 75.6% and 81.7%, respectively (Fig. 2, Table 1).

The ability of serum PRDM1 levels for differentiating HCC was compared with AFP levels and the results showed that the AUC of PRDM1 was significantly higher than that of AFP (0.897 vs. 0.834, $p = 0.046$). The combination of PRDM1 and AFP increased the diagnostic ability with an AUC of 0.921 (Fig. 3).

Multivariate regression analysis showed that PRDM1 level, together with gender, age, albumin and AFP, was a significant independent factor associated with HCC in comparison with cirrhosis [$p = 0.006$, OR (95% CI), 2.771(1.106–3.579), Table S2]. Moreover, in comparison to all the patients without HCC (chronic hepatitis and cirrhosis), PRDM1 level, in addition to gender, age, albumin and AFP, was also a significant independent factor associated with HCC [$p = 0.001$, OR (95% CI), 3.627(1.038–4.761), Table S3].

3.4. Association of serum PRDM1 levels with overall survival of HBV-related HCC patients

The 1-year, 3-year and 5-year survival rates of the 113 HCC patients were 79.9%, 39.8%, and 17.7%, respectively. According to the optimal cutoff value of 300 pg/mL, Kaplan-Meier analysis showed that the overall survival time in patients with serum PRDM1 > 300 pg/mL was significantly shorter than those with PRDM1 ≤300 pg/mL ($p = 0.001$, Fig. 4A). HCC patients with AFP > 200 ng/mL also had shorter survival time than those with AFP ≤200 ng/mL ($p = 0.043$, Fig. 4B).

Multivariate analysis showed that higher PRDM1 level, together with total bilirubin, albumin, AFP, Child-pugh grade, tumor size, TNM stage and treatment, was an independent factor associated with overall survival of HBV-related HCC patients (hazard ratio 2.997, 95% confidence interval 1.103–4.722, $p = 0.003$, Table 2].

4. Discussion

This study, for the first time to our knowledge, analyzed the PRDM1 levels in patients with chronic HBV infection of various liver diseases. The results showed that serum PRDM1 levels were significantly elevated in patients with chronic HBV infection in comparison with HBV infection resolvers or healthy controls and the increased PRDM1 levels were associated with the clinical disease. Particularly, patients with HBV-related HCC had significantly higher PRDM1 levels in comparison with other disease conditions. Serum PRDM1 levels were significantly discriminative of HCC from other disease conditions and were associated with the overall survival of HCC patients.

PRDM1/BLIMP1 has been revealed to affect CD8 T cell effector response through repressing CD8 T cell expression of programmed cell death-1 (PD-1) during acute viral infection [44]. PRDM1/BLIMP1 has also been found to be a critical regulator for CD4 T cell exhaustion that is linked to the CD8 T cell exhaustion during chronic toxoplasmosis

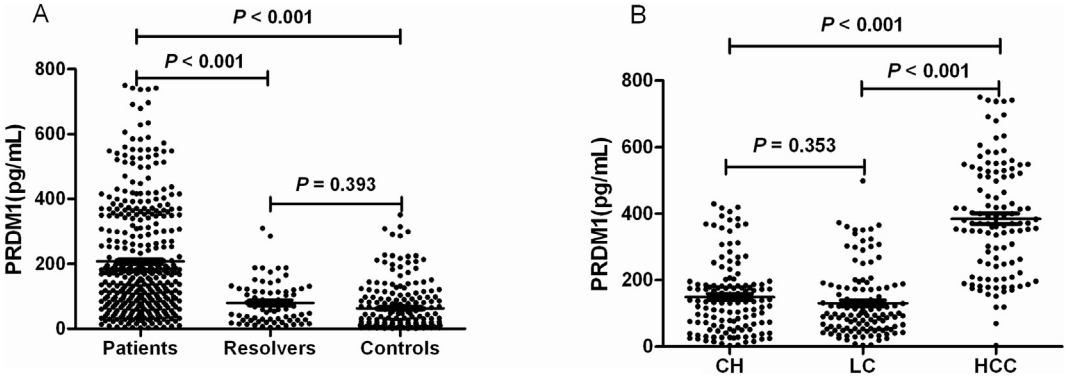

Fig. 1. Serum PR domain zinc finger protein 1 (PRDM1) levels in the study subjects.
A: Serum PRDM1 levels in patients with chronic hepatitis B virus (HBV) infection, HBV infection resolvers and healthy controls. B: Serum PRDM1 levels in chronic HBV patients with chronic hepatitis (CH), liver cirrhosis (LC) and hepatocellular carcinoma (HCC). Data are presented as dot plots with medians and ranges. Differences between groups were identified using Kruskal-Wallis test for nonparametric comparison.

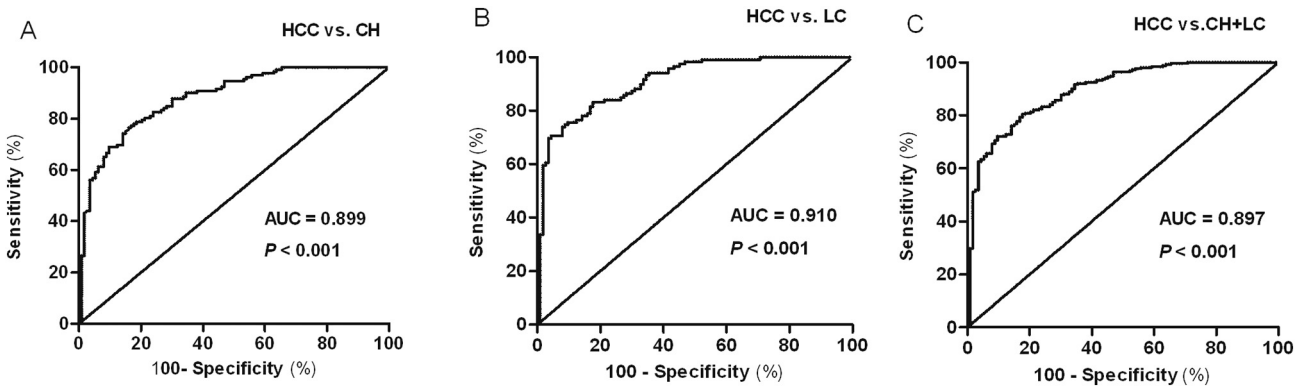

Fig. 2. ROC curves estimating the performance of serum PR domain zinc finger protein 1 (PRDM1) levels in distinguishing hepatocellular carcinoma (HCC) from other disease conditions in chronic hepatitis B virus (HBV) infection.
A: ROC curves of serum PRDM1 levels in distinguishing HCC from chronic hepatitis (CH). B: ROC curves of serum PRDM1 levels in distinguishing HCC from liver cirrhosis (LC). C: ROC curves of serum PRDM1 levels in distinguishing HCC from liver diseases other than HCC (CH and LC). AUC, area under the curve; ROC, receiver operating characteristic. Data are presented as receiver operating characteristic (ROC) curve and the area under ROC curve (AUC). Differences between groups were identified using Student's *t*-tests.

Table 1
Serum PRDM1 levels for differentiating HCC from CH, LC and non-HCC.

	AUC	Cutoff value (pg/mL)	Sensitivity	Specificity	Youden index
HCC vs. CH	0.889	296.71	83.2%	80.1%	0.633
HCC vs. LC	0.910	278.28	90.3%	75.6%	0.659
HCC vs. non-HCC	0.897	301.80	82.3%	81.7%	0.640

PRDM1, PR domain zinc finger protein 1; HCC, hepatocellular carcinoma; CH, chronic hepatitis; LC, liver cirrhosis; non-HCC, liver diseases other than HCC; AUC, area under receiver operating characteristic (ROC) curve.

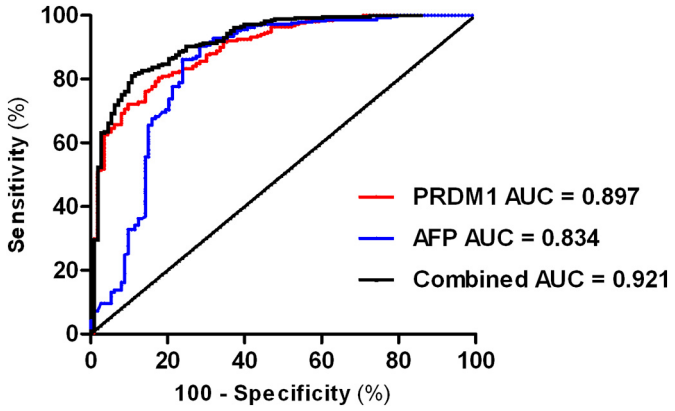

Fig. 3. AUC curves estimating the performance of serum PR domain zinc finger protein 1 (PRDM1) levels, alpha-fetoprotein (AFP) levels and the combination of PRDM1 and AFP in distinguishing hepatocellular carcinoma (HCC) from other disease conditions in chronic hepatitis B virus (HBV) infection.
Data are presented as multivariable receiver operating characteristic (ROC) curve and the area under ROC curve (AUC). Differences between groups were identified using Student's *t*-tests. Differences between AUCs were identified using Kruskal-Wallis test for nonparametric comparison.

[45]. These studies suggest a potential intrinsic link between PRDM1/BLIMP1 and T cell response or exhaustion in both acute and chronic infection. T cell response and exhaustion are prominently featured in chronic HBV infection and HCC [7–9,24]. Therefore, PRDM1/BLIMP1 may play an important role in the regulation of T cell immune response of HBV infection. Actually, PRDM1/BLIMP1 has been shown to participate in the immune response of tissue-resident memory T cells to HBV infection [41]. Yet, despite this, the potential involvement of PRDM1/BLIMP1 in the B cell immune regulation of chronic HBV infection could not be ruled out in view of the critical role of PRDM1/BLIMP1 in humoral immune response [6,11–13]. At least, the elevated PRDM1 levels in chronic HBV infection documented in the present study support the high involvement of PRDM1 in chronic HBV infection and HBV-related liver diseases, especially HCC.

Interestingly, the levels of PRDM1 in HBV infection resolvers were higher than in healthy controls although the difference was not statistically significant. This finding appears to be consistent with the previous study showing that PRDM1 is involved in the partial immune control of HBV infection and the local noncytolytic immunosurveillance of the liver after the resolution of infection [41].

The hierarchy of T cell exhaustion and tumor-specific T cell dysregulation is related to the development of HBV-related HCC [8,9]. PRDM1/BLIMP1 could regulate CD8 T cell effector response by repressing the expression of PD-1 on CD8 T cells during acute viral infection [44], suggesting the interaction between PRDM1/BLIMP1 and PD-1. PD-1 is significantly involved in the CD8 T cell exhaustion in chronic HBV infection [7,24,46] and HBV-related HCC [8,10]. Circulating PD-1 has been shown to be associated with the immune status and HCC risk [47,48]. High levels of the soluble programmed death-ligand 1 (sPD-L1) were also shown to predict a poor outcome in HCC patients [49]. The present study showed that patients with HBV-related HCC had the highest serum PRDM1 levels across the liver diseases associated with chronic HBV infection and higher PRDM1 levels were associated with a poor prognosis of HCC patients. These results add novel information to the role of PRDM1 and its potential interaction with immune suppressive molecules such as PD-1 in the dysregulation of immune response and the subsequent development of HCC during chronic HBV infection.

PRDM1 is thought to be a tumor suppressor [30,33]. However, the present study showed that high serum PRDM1 level was associated with the development of HCC in chronic HBV infection. The explanations for this contradictory phenomenon of PRDM1 remain unclear. However, although the inactivation, loss of function or decreased expression of BLIMP1 is associated with the development of lymphomas [30] and the poor prognosis in B-cell-like diffuse large B-cell lymphoma [31], lung cancer [32], colon cancer [33] and glioma patients [34], high PRDM1 expression has been shown to be related to the poor prognosis of multiple myeloma patients [35] and diffuse large B-cell lymphoma patients [50] and the metastatic status in breast cancer patients [36]. Furthermore, there are different isoforms for PRDM family members and these isoforms are differentially regulated and may play opposite roles in cancer [51]. PRDM1 uses alternative promoter to produce short

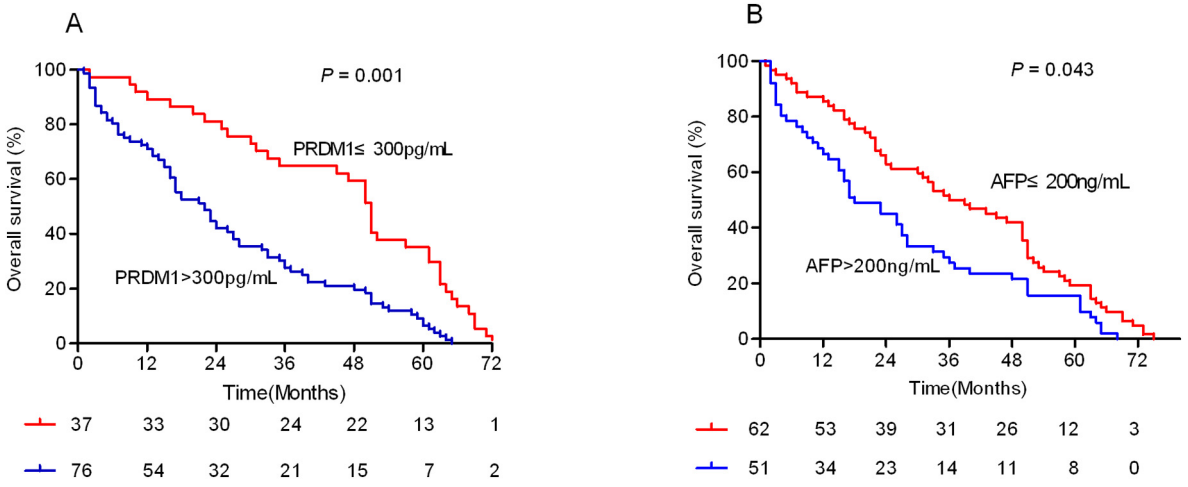

Fig. 4. Kaplan-Meier curves comparing overall survival of hepatocellular carcinoma (HCC) patients according to the serum PR domain zinc finger protein 1 (PRDM1) levels and alpha-fetoprotein (AFP) levels.
A: Kaplan-Meier curves comparing overall survival of HCC patients according to serum PRDM1 levels. The patients were grouped into individuals with high serum PRDM1 concentrations (> 300 pg/mL) and low serum PRDM1 concentrations (≤300 pg/mL). B: Kaplan-Meier curves comparing overall survival of HCC patients according to serum AFP levels. The patients were grouped into individuals with high serum AFP concentrations (> 200 ng/mL) and low serum AFP concentrations (≤200 ng/mL). Differences between groups were determined by the Log-rank test.

isoform lacking the PR domain (PRDM1β). This may lead to the loss of repressor activity associated with the PR domain and confer oncogenic function. Increased levels of PRDM1β have been demonstrated in various cancer cell lines although currently no experimental evidence has been available to support this proposition [51–53]. Presumably, the components of the high serum levels of PRDM1 in HBV-related HCC patients are mainly the truncated protein PRDM1β. Therefore, studies are needed to componently and functionally clarify the highly increased PRDM1 levels in HBV-related HCC patients.

Tumor biopsy and imaging remain the standard of care to establish the diagnosis of HCC. To date no serological diagnostic and prognostic markers have been established in HCC. Therefore, diagnosis and differentiation of HCC from other liver diseases and the prediction of the prognosis of HCC patients in chronic HBV infection are among clinical challenges. Tumoral immune activation or response as diagnostic and predictive markers is a major focus of recent studies. For example, circulating soluble PD-1 [47,48] and sPD-L1 levels [49] have been indicated to be markers for the identification of HCC and the prediction of HCC patients' prognosis. In the present study, PRDM1 levels were shown to be significantly discriminative of HCC from other disease conditions and predictive of the prognosis of HCC patients. The discriminative and predictive ability of PRDM1 seems to be higher than AFP, a well known biomarker of HCC. However, whether PRDM1 may be of potential value in improving HCC diagnosis and monitoring disease progression or treatment response in chronic HBV infection warrants to be further studied. PRDM1/BLIMP1 participates in the regulation of effector CD8 T cell differentiation [54]. In addition, a recent study indicated that the phenotypes, functional states and underlying mechanisms between the CD8 T cell exhaustion in chronic hepatitis B and HCC are somewhat different [10]. Whether PRDM1 plays a role in these differences remains to be investigated.

Targeting immune checkpoint molecules such as PD-1 has been shown to be a promising immunotherapeutic strategy for HCC [55]. Our findings suggest the potential to formulate a strategy to treat HBV-related HCC by targeting PRDM1/BLIMP1 alone or in combination with other immune checkpoint molecules such as PD-1.

This study has some limitations. The number of patients with different clinical diseases including HBV-related HCC is relatively small. The potential correlation between PRDM1 levels and the immune status in particular the CD8 T cell exhaustion has not been investigated. The study did not perform a functional investigation concerning the role of PRDM1/BLIMP1 in chronic HBV infection and HCC. The study also lacks a validation of the findings in additional patient populations. Therefore, further studies are required to confirm and extend our findings, to clarify the role of PRDM1/BLIMP1 in chronic HBV infection and HCC and to explore the underlying mechanisms.

In conclusion, this study showed that serum PRDM1 levels were significantly elevated in patients with chronic HBV infection. Serum PRDM1 levels were considerably discriminative of HCC from other disease conditions and were associated with the overall survival of HCC patients. The predictive value of PRDM1 levels for disease progression and treatment response in chronic HBV infection and HBV-related HCC should be investigated in the future.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.05.012.

Declaration of Competing Interest

The authors declare no potential conflict of interest.

Acknowledgments

We thank Dr. Qianqian Zhu, Dr. Zhu Li and Dr. Guoyu Zhang for their assistance during this study.

Funding

This study was supported by the Science Innovation Foundation of the First Affiliated Hospital of Xi'an Jiaotong University (Youth Project, Grant no. 2016QN-11), the Natural Science Basic Research Foundation of Shaanxi Province (Youth Project, Grant no. 2017JQ8033) and the National Natural Science Foundation of China (Grant no. 81371798). The funders were involved in neither the design and conduction of the study nor the analysis of data.

Ethics

This study was carried out in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. All the subjects included in the study gave informed consent.

Table 2
Univariate and multivariate analysis of parameters associated with overall survival of HBV-related HCC patients.

	No. of patients	Overall survival (%)			Univariate analysis	Multivariate analysis	
		1 year	3 year	5 year	*p*	HR (95% CI)	*p*
Gender					0.252	–	–
Male	98	78.5	41.8	17.3			
Female	15	66.7	26.7	20.0			
Age (year)					0.477	–	–
≤50	64	79.7	46.9	18.8			
> 50	49	73.5	30.6	16.3			
HBV DNA (IU/mL)					0.430	–	–
$\leq 10^4$	35	82.9	45.7	14.3			
$> 10^4$	78	74.3	37.2	19.2			
ALT (IU/L)					0.709	–	–
≤40	36	83.3	44.4	13.9			
> 40	77	74.0	37.7	19.5			
AST (IU/L)					0.072	–	–
≤40	29	93.1	51.7	20.7			
> 40	84	71.4	35.7	16.7			
Tbil (μmol/L)					< 0.001	2.001 (1.172–3.542)	0.023
≤40	78	89.7	50.0	20.5			
> 40	35	48.6	17.1	11.4			
Albumin (g/L)					< 0.001	0.672 (0.167–0.829)	0.011
≤32	50	68.0	44.0	6.0			
> 32	63	84.1	52.4	26.9			
AFP (ng/mL)					0.043	2.521 (1.037–3.127)	0.036
≤200	62	85.5	50.0	20.9			
> 200	51	66.7	27.5	13.7			
Child-pugh grade					0.004	1.793 (1.009–2.362)	0.005
A	73	87.7	42.5	17.8			
B + C	40	57.5	35.0	17.5			
Tumor size (cm)					0.016	1.167 (1.079–1.968)	0.007
≤5	61	78.7	47.5	24.6			
> 5	52	75.0	30.8	9.6			
TNM stage					0.008	3.579 (1.296–4.622)	0.012
I + II	83	80.7	43.4	20.5			
III	30	66.7	30.0	10.0			
PRDM1 (pg/mL)					0.001	2.997 (1.103–4.722)	0.003
≤300	37	89.2	64.9	35.1			
> 300	76	71.1	27.6	9.6			
Treatment					< 0.001	1.617 (1.002–3.048)	0.019
Tumor resection	59	84.7	49.2	22.0			
RF	14	78.6	35.7	21.4			
TACE	22	81.8	31.8	18.2			
SST	18	44.4	11.1	0			

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Tbil, total bilirubin; AFP, alpha-fetoprotein; PRDM1, PR domain zinc finger protein 1. RF, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; SST, supportive and symptomatic treatment.

Availability of data and material

The datasets used are available from the corresponding author on reasonable request.

Authors' contributions

Na Li: Participated in Data collection, Laboratory study, Data analysis and Manuscript preparation. Xiude Fan and Xiaoyun Wang: Participated in Data collection, Laboratory study and Manuscript preparation. Huan Deng: Participated in Data collection and Laboratory study. Kun Zhang: Participated in Data collection and Laboratory study. Xiaoge Zhang: Participated in Data collection and Laboratory study. Ye Wang: Participated in Data collection and Laboratory study. Qunying Han: Participated in Study Conception, Data collection and Data analysis. Yi Lv: Participated in Study Conception, Data analysis and Manuscript preparation. Zhengwen Liu: Participated in the Study Conception and design, Data analysis and Manuscript preparation. All authors read and approved the final manuscript.

References

[1] A. Schweitzer, J. Horn, R.T. Mikolajczyk, G. Krause, J.J. Ott, Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013, Lancet 386 (10003) (2015) 1546–1555, https://doi.org/10.1016/S0140-6736(15)61412-X.
[2] H.B. El-Serag, Hepatocellular carcinoma, N. Engl. J. Med. 365 (12) (2011) 1118–1127, https://doi.org/10.1056/NEJMra1001683.
[3] E.C. Shin, P.S. Sung, S.H. Park, Immune responses and immunopathology in acute and chronic viral hepatitis, Nat. Rev. Immunol. 16 (8) (2016) 509–523, https://doi.org/10.1038/nri.2016.69.
[4] B. Oliviero, A. Cerino, S. Varchetta, E. Paudice, S. Pai, S. Ludovisi, et al., Enhanced B-cell differentiation and reduced proliferative capacity in chronic hepatitis C and chronic hepatitis B virus infections, J. Hepatol. 55 (1) (2011) 53–60, https://doi.org/10.1016/j.jhep.2010.10.016.
[5] A. Mohamadkhani, E. Naderi, M. Sotoudeh, A. Katoonizadeh, G. Montazeri, H. Poustchi, Clinical feature of intrahepatic B-lymphocytes in chronic hepatitis B, Int. J. Inflamm. 2014 (2014) 896864, , https://doi.org/10.1155/2014/896864.
[6] H. Xue, F. Lin, H. Tan, Z.Q. Zhu, Z.Y. Zhang, L. Zhao, Overrepresentation of IL-10-expressing B cells suppresses cytotoxic CD4+ T cell activity in HBV-induced hepatocellular carcinoma, PLoS One 11 (5) (2016) e0154815, , https://doi.org/10.1371/journal.pone.0154815.
[7] M.K. Maini, A.J. Gehring, The role of innate immunity in the immunopathology and treatment of HBV infection, J. Hepatol. 64 (1 Suppl) (2016) S60–S70, https://doi.org/10.1016/j.jhep.2016.01.028.
[8] A.J. Gehring, Z.Z. Ho, A.T. Tan, M.O. Aung, K.H. Lee, K.C. Tan, et al., Profile of tumor antigen-specific CD8 T cells in patients with hepatitis B virus-related hepatocellular carcinoma, Gastroenterology 137 (2) (2009) 682–690, https://doi.org/10.1053/j.gastro.2009.04.045.

[9] C.M. Dinney, L.D. Zhao, C.D. Conrad, J.M. Duker, R.O. Karas, Z. Hu, et al., Regulation of HBV-specific CD8(+) T cell-mediated inflammation is diversified in different clinical presentations of HBV infection, J. Microbiol. 53 (10) (2015) 718–724, https://doi.org/10.1007/s12275-015-5314-y.

[10] X. Wang, Q. He, H. Shen, X.J. Lu, B. Sun, Genetic and phenotypic difference in CD8+ T cell exhaustion between chronic hepatitis B infection and hepatocellular carcinoma, J. Med. Genet. 56 (1) (2019) 18–21, https://doi.org/10.1136/jmedgenet-2018-105267.

[11] M. Shapiro-Shelef, K.I. Lin, L.J. McHeyzer-Williams, J. Liao, M.G. McHeyzer-Williams, K. Calame, Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells, Immunity 19 (4) (2003) 607–620, https://doi.org/10.1016/S1074-7613(03)00267-X.

[12] M. Minnich, H. Tagoh, P. Bönelt, E. Axelsson, M. Fischer, B. Cebolla, et al., Multifunctional role of the transcription factor Blimp-1 in coordinating plasma cell differentiation, Nat. Immunol. 17 (3) (2016) 331–343, https://doi.org/10.1038/ni.3349.

[13] J. Tellier, W. Shi, M. Minnich, Y. Liao, S. Crawford, G.K. Smyth, et al., Blimp-1 controls plasma cell function through the regulation of immunoglobulin secretion and the unfolded protein response, Nat. Immunol. 17 (3) (2016) 323–330, https://doi.org/10.1038/ni.3348.

[14] G.A. Martins, L. Cimmino, M. Shapiro-Shelef, M. Szabolcs, A. Herron, E. Magnusdottir, et al., Transcriptional repressor Blimp-1 regulates T cell homeostasis and function, Nat. Immunol. 7 (5) (2006) 457–465, https://doi.org/10.1038/ni1320.

[15] D. Savitsky, L. Cimmino, T. Kuo, G.A. Martins, K. Calame, Multiple roles for Blimp-1 in B and T lymphocytes, Adv. Exp. Med. Biol. 596 (2007) 9–30, https://doi.org/10.1007/0-387-46530-8_2.

[16] S.L. Nutt, K.A. Fairfax, A. Kallies, BLIMP1 guides the fate of effector B and T cells, Nat. Rev. Immunol. 7 (12) (2007) 923–927, https://doi.org/10.1038/nri2204.

[17] A. Kallies, A. Xin, G.T. Belz, S.L. Nutt, Blimp-1 transcription factor is required for the differentiation of effector CD8(+) T cells and memory responses, Immunity 31 (2) (2009) 283–295, https://doi.org/10.1016/j.immuni.2009.06.021.

[18] A. Kallies, E.D. Hawkins, G.T. Belz, D. Metcalf, M. Hommel, L.M. Corcoran, et al., Transcriptional repressor Blimp-1 is essential for T cell homeostasis and self-tolerance, Nat. Immunol. 7 (5) (2006) 466–474, https://doi.org/10.1038/ni1321.

[19] L.K. Mackay, M. Minnich, N.A. Kragten, Y. Liao, B. Nota, C. Seillet, et al., Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes, Science 352 (6284) (2016) 459–463, https://doi.org/10.1126/science.aad2035.

[20] R. Bankoti, C. Ogawa, T. Nguyen, L. Emadi, M. Couse, S. Salehi, et al., Differential regulation of effector and regulatory T cell function by Blimp1, Sci. Rep. 7 (1) (2017) 12078, , https://doi.org/10.1038/s41598-017-12171-3.

[21] S.L. Nutt, A. Kallies, G.T. Belz, Blimp-1 connects the intrinsic and extrinsic regulation of T cell homeostasis, J. Clin. Immunol. 28 (2) (2008) 97–106, https://doi.org/10.1007/s10875-007-9151-6.

[22] H. Shin, S.D. Blackburn, A.M. Intlekofer, C. Kao, J.M. Angelosanto, S.L. Reiner, et al., A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection, Immunity 31 (2) (2009) 309–320, https://doi.org/10.1016/j.immuni.2009.06.019.

[23] I.A. Parish, H.D. Marshall, M.M. Staron, P.A. Lang, A. Brüstle, J.H. Chen, et al., Chronic viral infection promotes sustained Th1-derived immunoregulatory IL-10 via BLIMP-1, J. Clin. Invest. 124 (8) (2014) 3455–3468, https://doi.org/10.1172/JCI66108.

[24] B. Ye, X. Liu, X. Li, H. Kong, L. Tian, Y. Chen, T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance, Cell Death Dis. 6 (2015) e1694, https://doi.org/10.1038/cddis.2015.42.

[25] V. Gateva, J.K. Sandling, G. Hom, K.E. Taylor, S.A. Chung, X. Sun, et al., A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus, Nat. Genet. 41 (11) (2009) 1228–1233, https://doi.org/10.1038/ng.468.

[26] S. Raychaudhuri, B.P. Thomson, E.F. Remmers, S. Eyre, A. Hinks, C. Guiducci, et al., Genetic variants at CD28, PRDM1 and CD2/CD58 are associated with rheumatoid arthritis risk, Nat. Genet. 41 (12) (2009) 1313–1318, https://doi.org/10.1038/ng.479.

[27] C.A. Anderson, G. Boucher, C.W. Lees, A. Franke, M. D'Amato, K.D. Taylor, et al., Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47, Nat. Genet. 43 (3) (2011) 246–252, https://doi.org/10.1038/ng.764.

[28] D. Ellinghaus, H. Zhang, S. Zeissig, S. Lipinski, A. Till, T. Jiang, et al., Association between variants of PRDM1 and NDP52 and Crohn's disease, based on exome sequencing and functional studies, Gastroenterology 145 (2) (2013) 339–347, https://doi.org/10.1053/j.gastro.2013.04.040.

[29] C. Terao, T. Kawaguchi, P. Dieude, J. Varga, M. Kuwana, M. Hudson, et al., Transethnic meta-analysis identifies GSDMA and PRDM1 as susceptibility genes to systemic sclerosis, Ann. Rheum. Dis. 76 (6) (2017) 1150–1158, https://doi.org/10.1136/annrheumdis-2016-210645.

[30] M. Boi, E. Zucca, G. Inghirami, F. Bertoni, PRDM1/BLIMP1: a tumor suppressor gene in B and T cell lymphomas, Leuk. Lymphoma 56 (5) (2015) 1223–1228, https://doi.org/10.3109/10428194.2014.953155.

[31] Y. Xia, Z.Y. Xu-Monette, A. Tzankov, X. Li, G.C. Manyam, V. Murty, et al., Loss of PRDM1/BLIMP-1 function contributes to poor prognosis of activated B-cell-like diffuse large B-cell lymphoma, Leukemia 31 (3) (2017) 625–636, https://doi.org/10.1038/leu.2016.243.

[32] Z. Zhu, H. Wang, Y. Wei, F. Meng, Z. Liu, Z. Zhang, Downregulation of PRDM1 promotes cellular invasion and lung cancer metastasis, Tumour Biol. 39 (4) (2017) 1010428317695929, , https://doi.org/10.1177/1010428317695929.

[33] C. Liu, C.E. Banister, C.C. Weige, D. Altomare, J.H. Richardson, C.M. Contreras, et al., PRDM1 silences stem cell-related genes and inhibits proliferation of human colon tumor organoids, Proc. Natl. Acad. Sci. U. S. A. 115 (22) (2018) E5066–E5075, https://doi.org/10.1073/pnas.1802902115.

[34] X. Wang, K. Wang, L. Han, A. Zhang, Z. Shi, K. Zhang, et al., PRDM1 is directly targeted by miR-30a-5p and modulates the Wnt/β-catenin pathway in a Dkk1-dependent manner during glioma growth, Cancer Lett. 331 (2) (2013) 211–219, https://doi.org/10.1016/j.canlet.2013.01.005.

[35] G.S. Liu, J.P. Wang, Z.H. Chu, Y.Y. Yu, W. Zhou, Effect of B lymphocyte-induced mature protein-1 expression in bone marrow mononuclear cells on prognosis of patients with multiple myeloma, Zhongguo Shi Yan Xue Ye Xue Za Zhi 25 (5) (2017) 1449–1453, https://doi.org/10.7534/j.issn.1009-2137.2017.05.029.

[36] M. Sciortino, M.D.P. Camacho-Leal, F. Orso, E. Grassi, A. Costamagna, P. Provero, et al., Dysregulation of Blimp1 transcriptional repressor unleashes p130Cas/ErbB2 breast cancer invasion, Sci. Rep. 7 (1) (2017) 1145, https://doi.org/10.1038/s41598-017-01332-z.

[37] A. de Masson, A. Kirilovsky, R. Zoorob, V. Avettand-Fenoel, V. Morin, A. Oudin, et al., Blimp-1 overexpression is associated with low HIV-1 reservoir and transcription levels in central memory CD4+ T cells from elite controllers, AIDS 28 (11) (2014) 1567–1577, https://doi.org/10.1097/QAD.0000000000000295.

[38] K. Kaczmarek Michaels, M. Natarajan, Z. Euler, G. Alter, G. Viglianti, A.J. Henderson, Blimp-1, an intrinsic factor that represses HIV-1 proviral transcription in memory CD4+ T cells, J. Immunol. 194 (7) (2015) 3267–3274, https://doi.org/10.4049/jimmunol.1402581.

[39] L. Wang, W.P. Zhang, L. Yao, W. Zhang, J. Zhu, W.C. Zhang, et al., PRDM1 expression via human parvovirus B19 infection plays a role in the pathogenesis of Hashimoto thyroiditis, Hum. Pathol. 46 (12) (2015) 1913–1921, https://doi.org/10.1016/j.humpath.2015.08.009.

[40] J. Ma, K. Nie, D. Redmond, Y. Liu, O. Elemento, D.M. Knowles, et al., EBV-miR-BHRF1-2 targets PRDM1/Blimp1: potential role in EBV lymphomagenesis, Leukemia 30 (3) (2016) 594–604, https://doi.org/10.1038/leu.2015.285.

[41] L.J. Pallett, J. Davies, E.J. Colbeck, F. Robertson, N. Hansi, N.J.W. Easom, et al., IL-2^{high} tissue-resident T cells in the human liver: sentinels for hepatotropic infection, J. Exp. Med. 214 (6) (2017) 1567–1580, https://doi.org/10.1084/jem.20162115.

[42] N.A. Terrault, A.S.F. Lok, B.J. McMahon, K.M. Chang, J.P. Hwang, M.M. Jonas, et al., Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance, Hepatology 67 (4) (2018) 1560–1599, https://doi.org/10.1002/hep.29800.

[43] European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma, J. Hepatol. 56 (4) (2012) 908–943, https://doi.org/10.1016/j.jhep.2011.12.001.

[44] P. Lu, B.A. Youngblood, J.W. Austin, A.U. Mohammed, R. Butler, R. Ahmed, et al., Blimp-1 represses CD8 T cell expression of PD-1 using a feed-forward transcriptional circuit during acute viral infection, J. Exp. Med. 211 (3) (2014) 515–527, https://doi.org/10.1084/jem.20130208.

[45] S. Hwang, D.A. Cobb, R. Bhadra, B. Youngblood, I.A. Khan, Blimp-1-mediated CD4 T cell exhaustion causes CD8 T cell dysfunction during chronic toxoplasmosis, J. Exp. Med. 213 (9) (2016) 1799–1818, https://doi.org/10.1084/jem.20151995.

[46] B. Bengsch, B. Martin, R. Thimme, Restoration of HBV-specific CD8+ T cell function by PD-1 blockade in inactive carrier patients is linked to T cell differentiation, J. Hepatol. 61 (6) (2014) 1212–1219, https://doi.org/10.1016/j.jhep.2014.07.005.

[47] N. Li, Z. Zhou, F. Li, J. Sang, Q. Han, Y. Lv, et al., Circulating soluble programmed death-1 levels may differentiate immune-tolerant phase from other phases and hepatocellular carcinoma from other clinical diseases in chronic hepatitis B virus infection, Oncotarget 8 (28) (2017) 46020–46033, https://doi.org/10.18632/oncotarget.17546.

[48] H.Y. Cheng, P.J. Kang, Y.H. Chuang, Y.H. Wang, M.C. Jan, C.F. Wu, et al., Circulating programmed death-1 as a marker for sustained high hepatitis B viral load and risk of hepatocellular carcinoma, PLoS One 9 (11) (2014) e95870, , https://doi.org/10.1371/journal.pone.0095870.

[49] F. Finkelmeier, Ö. Canli, A. Tal, T. Pleli, J. Trojan, M. Schmidt, et al., High levels of the soluble programmed death-ligand (sPD-L1) identify hepatocellular carcinoma patients with a poor prognosis, Eur. J. Cancer 59 (2016) 152–159, https://doi.org/10.1016/j.ejca.2016.03.002.

[50] Y. Song, Z. Cao, L. Li, H.T. Zhang, X. Zhang, Blimp-1 protein and Hans classification on prognosis of diffuse large B-cell lymphoma and their interrelation, Chin. J. Cancer 29 (9) (2010) 781–786.

[51] S. Mzoughi, Y.X. Tan, D. Low, E. Guccione, The role of PRDMs in cancer: one family, two sides, Curr. Opin. Genet. Dev. 36 (2016) 83–91, https://doi.org/10.1016/j.gde.2016.03.009.

[52] I. Györy, G. Fejér, N. Ghosh, E. Seto, K.L. Wright, Identification of a functionally impaired positive regulatory domain I binding factor 1 transcription repressor in myeloma cell lines, J. Immunol. 170 (6) (2003) 3125–3133, https://doi.org/10.4049/jimmunol.170.6.3125.

[53] Y.W. Zhang, H.Q. Xie, Y. Chen, B. Jiao, Z.X. Shen, S.J. Chen, et al., Loss of promoter methylation contributes to the expression of functionally impaired PRDM1β isoform in diffuse large B-cell lymphoma, Int. J. Hematol. 92 (3) (2010) 439–444, https://doi.org/10.1007/s12185-010-0689-3 (doi: 10.1007/s12185-010-0689-3).

[54] A. Xin, F. Masson, Y. Liao, S. Preston, T. Guan, R. Gloury, et al., A molecular threshold for effector CD8(+) T cell differentiation controlled by transcription factors Blimp-1 and T-bet, Nat. Immunol. 17 (4) (2016) 422–432, https://doi.org/10.1038/ni.3410.

[55] A.B. El-Khoueiry, B. Sangro, T. Yau, T.S. Crocenzi, M. Kudo, C. Hsu, et al., 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) (2017) 2492–2502, https://doi.org/10.1016/S0140-6736(17)31046-2.