European Journal of Cancer (2015) **51**, 157–165

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Clinical impact of herpesvirus entry mediator expression in human hepatocellular carcinoma

Daisuke Hokuto, Masayuki Sho *, Ichiro Yamato, Satoshi Yasuda, Shinsaku Obara, Takeo Nomi, Yoshiyuki Nakajima

Department of Surgery, Nara Medical University, Nara 6348522, Japan

Received 28 May 2014; received in revised form 5 November 2014; accepted 9 November 2014
Available online 25 November 2014

KEYWORDS
HVEM
HCC
TIL
Prognosis
Tumour immunity
TNFRSF14

Abstract ***Background:*** Herpes virus entry mediator (HVEM), also known as tumour necrosis factor receptor (TNFR) superfamily 14, regulates a variety of physiological and pathological responses in both innate and acquired immunity. Although HVEM is also suggested to be a critical regulator in tumours, actual roles in human cancer are largely unknown. This study aimed to clarify clinical importance of HVEM in human hepatocellular carcinoma (HCC).
Patients and methods: We studied HVEM expression in 150 HCC patients to explore its clinical relevance, and we examined tumour infiltrating T cells and local immune status of them.
Results: HVEM was expressed in HCC cells, while no or only limited expression was observed in normal tissues in the liver. Tumour HVEM expression was significantly correlated with age, serum protein induced by vitamin K absence or antagonist-II (PIVKA-II) level, vascular invasion and tumour node metastasis (TNM) stage. Furthermore, tumour HVEM expression significantly correlated with postoperative recurrence and survival. Importantly, multivariate analysis indicated that the HVEM status had an independent prognostic value. Furthermore, HVEM status was inversely correlated with tumour-infiltrating $CD4^+$, $CD8^+$ and $CD45RO^+$ lymphocytes. In addition, it was also associated with reduced expression of perforin, granzyme B and interferon-γ (IFN-γ). Taken together, tumour-expressing HVEM plays a functionally important role in HCC.
Conclusion: Tumour-expressing HVEM plays a critical role in human HCC, possibly through regulating immune evasion. Therefore, targeting HVEM may be a novel promising therapeutic strategy for HCC.

* *Corresponding author at:* Department of Surgery, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan. Tel.: +81 744 29 8863; fax: +81 744 24 6866.
E-mail address: m-sho@naramed-u.ac.jp (M. Sho).

http://dx.doi.org/10.1016/j.ejca.2014.11.004
0959-8049/

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumours worldwide and the third cause of cancer-related death [1]. Despite improved diagnostic and treatment strategies, the postoperative prognosis of HCC remains unsatisfactory mostly due to frequent recurrence [2]. The treatments for HCC include surgical resection, radiofrequency ablation (RFA), transcatheter arterial chemoembolisation (TACE) and radiation. Furthermore, multi-tyrosine kinase inhibitor has recently been approved as a new therapeutic option for advanced HCC [3]. However, none of them is enough to completely prevent tumour formation and recurrence. Clearly, new therapeutic strategies are needed to improve the survival of patients with HCC. One of potential promising strategies may be immunotherapy. In fact, it has been reported that there are a number of tumour-infiltrating T cells in HCC tissue [4,5]. Furthermore, recent studies have revealed that some specific subsets of T cells may be functionally important and also have prognostic value [6,7]. Importantly, a randomised clinical trial has shown that certain adoptive immunotherapy has significantly inhibited postsurgical recurrence in humans [8]. However, on the other hand, clinical efficacy of immunotherapy is very limited to date [9]. This may be, at least in part, due to tumour immune evasion through a variety of mechanisms [10–13].

Herpes virus entry mediator (HVEM), also known as tumour necrosis factor receptor (TNFR) superfamily 14 (TNFRSF14), was identified as a cellular mediator of herpes simplex virus entry [14]. It is expressed on several types of cells, including T cells, B cells, NK cells, dendritic cells, and myeloid cells, as well as non-lymphoid organs including lung, liver and kidney [14,15]. HVEM is a ligand for the immunoglobulin (Ig) superfamily members B-lymphocyte and T-lymphocyte attenuator (BTLA) and CD160, and is also a receptor for the TNF superfamily members LIGHT and LTα [16,17]. HVEM expressing on haematopoietic cells is known to display a dual functional activity for both T-cell activation and inhibition depending on the ligands engaged [16–18]. Previous studies have reported the roles of HVEM pathway in several disease conditions including autoimmune disease, infection and inflammation [18,19]. In contrast, there are relatively few reports on the roles of HVEM in tumours. HVEM on tumour cells has been shown to inhibit cytokine production and proliferation of tumour antigen-specific $CD8^+$ T cells via BTLA *in vitro* [20]. Furthermore, several murine studies have demonstrated that blockade of HVEM/BTLA pathway augments tumour antigen-specific immune responses and inhibits tumour growth [21,22]. Others have also reported that LIGHT mediates tumour cell apoptosis via signalling through tumour-expressed HVEM, leading to the suppression of tumour growth [23,24]. Taken together, the functions of HVEM in tumour environments are likely to be immunosuppressive rather than activating tumour immunity. However, to date, there are very few clinical studies on the HVEM expression on human malignant tumours. Although a few studies have shown HVEM expression and gene polymorphisms in actual human malignancies such as melanoma, breast cancer and haematopoietic cancer, the precise functions of HVEM in human tumours are still largely unknown [20,24–26].

The aim of this study was to clarify clinical significance of HVEM in human HCC. Furthermore, in order to reveal immunological function of HVEM, we investigated the correlation of HVEM expression with several subsets of tumour-infiltrating T cells and local immune status.

2. Patients and methods

2.1. Patients and specimens

We examined 150 patients with HCC who underwent surgery at the Department of Surgery, Nara Medical University, between 2000 and 2012. Follow-up was until death or September 2013. The median follow-up for all patients was 51.8 months, with a range of 6.7–151.7 months. None of the patients received anticancer therapy before surgical resection. Clinicopathological stages were classified according to the International Union against Cancer. For *in vitro* experiments, 49 fresh tissues out of the above 150 resected specimens were rapidly frozen at −80 °C for storage until use. The remainder of each specimen was fixed in 10% phosphate-buffered formalin, embedded in paraffin. Written informed consent was obtained from all patients before treatment, according to our institutional guidelines. This study was approved by the institutional review board.

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues of primary tumour were cut into 5-μm sections, deparaffinised and rehydrated in a graded series of ethanol. Antigen retrieval was carried out by heating tissue sections using a Target Retrieval Solution, pH 9.0 (DAKO, Tokyo, Japan). To block endogenous peroxidase, sections were immersed in 3% solution of hydrogen peroxide in absolute methanol for 5 min at room temperature and washed thrice in fresh PBS, each of 5 min duration. Then the sections were incubated overnight at 4 °C with anti-human HVEM/TNFRSF14 antibody (MAB3561, monoclonal mouse, R&D Systems) diluted 1:20 with Antibody Diluent (DAKO) or anti-human CD45RO (UHL1, monoclonal mouse; DAKO), CD4 (1:40) (4B12, monoclonal mouse; DAKO) and CD8 (C8/144B, monoclonal mouse; DAKO). Sections were washed thrice in PBS, fol-

lowed by incubation with the EnVision detection system (DAKO), according to the instructions of the manufacturer. Sections were counterstained with haematoxylin, dehydrated in ethanol, cleared in xylene and coverslipped.

2.3. Evaluation of immunostaining

Immunohistochemistry for HVEM was evaluated by authorised pathologists who had no knowledge of the patients' clinical status and outcome. At least 1000 tumour cells were scored, and percentage of tumour cells showing positive staining was calculated, as previously reported [26]. Tumour-infiltrating $CD45RO^+$, $CD4^+$ and $CD8^+$ T cells into HCC tissues were evaluated [27]. Each sample was thoroughly evaluated at a magnification of ×200. To count stained cells, five randomly selected areas were counted independently by two investigators without knowledge of clinical information. The median value was calculated for each sample.

2.4. Extraction of total RNAs and real-time reverse transcriptase polymerase chain reaction (PCR) analysis

Total RNA was isolated using RNAspin Mini (GE Healthcare, Tokyo, Japan) and the first-strand cDNA was synthesised from 1 μg RNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, United States of America (USA)), according to the instructions of the manufacturer. Real-time quantitative PCR analysis was carried out using an ABI Prism 7700 sequence detector system (Applied Biosystems). All primer/probe sets were purchased from Applied Biosystems. PCR was carried out using the TaqMan Universal PCR Master Mix (Applied Biosystems) using 1 μl of cDNA in a 20 μl final reaction volume. The PCR thermal cycle conditions were as follows: initial step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The expression level of the housekeeping gene β2-microglobulin was measured as an internal reference with a standard curve to determine the integrity of template RNA for all specimens. The ratio of the mRNA level of each gene was calculated as follows: (absolute copy number of each gene)/(absolute copy number of β2-microglobulin) [26].

2.5. Statistical analysis

Age, serum AFP and PIVKA-II protein induced by vitamin K absence or antagonist-II (PIVKA-II) levels were dichotomised at the median. Statistical significance between two groups of parametric date was evaluated using the Student's t test or the chi-square test as appropriate. Survival curves were estimated using the Kaplan–Meier method, and the significances of differences between survival curves were determined using log-rank test. Multivariate comparisons of survival distributions were made using Cox proportional hazards models. A value of $p < 0.05$ was considered statistically significant in all analyses.

3. Results

3.1. Tumour HVEM expression in human hepatocellular carcinoma

We first examined the expression of HVEM in 150 surgically resected HCC tissues by immunohistochemistry. The expression of HVEM was observed in the cell membrane, cytoplasm, or both of hepatocellular carcinoma cells (Fig. 1). On the other hand, there was no or only limited expression of HVEM in other normal tissues in the liver including normal hepatocyte, sinusoidal cell, bile duct epithelial cell and vascular endothelium.

3.2. Correlation between tumour HVEM expression and clinicopathological characteristics

To investigate the clinical importance of tumour HVEM expression in HCC, we divided 150 cases into high HVEM group (⩾50% of HVEM-positive tumour

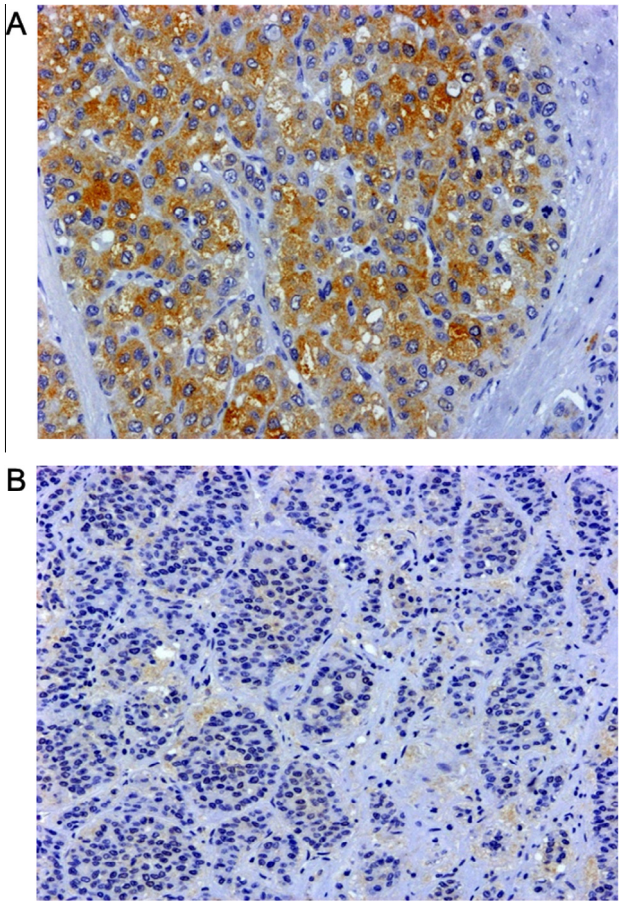

Fig. 1. Representative case of high and low expression of herpes virus entry mediator (HVEM) in surgically resected hepatocellular carcinoma (HCC) tissues. (A) High HVEM expression case (>50% of HCC cells). (B) Low HVEM expression case (<50% of HCC cells).

cells) and low HVEM group (<50% of HVEM-positive tumour cells). Then, we examined correlation between HVEM expression and various clinicopathological characteristics (Table 1). As a result, HVEM expression was significantly correlated with age ($P = 0.024$), presence of vascular invasion ($P = 0.022$), serum PIVKA-II level ($P = 0.009$) and tumour node metastasis (TNM) stage ($P = 0.036$).

3.3. Impact of tumour HVEM expression on postoperative recurrence and survival

Next, we compared postoperative recurrence and survival according to HVEM status (Fig. 1). As a result, high HVEM group had significantly poorer recurrence-free survival (RFS) and overall survival (OS) than low HVEM group (Fig. 2). The median RFS time was 1.3 and 4.9 years, respectively ($P < 0.001$). Moreover, the median OS time was 4.8 and 8.5 years, respectively ($P = 0.002$). Five-year postoperative survival rate of high-HVEM and low-HVEM patients was 47.9% and 78.4%, respectively.

3.4. Prognostic value of tumour HVEM expression in HCC

Furthermore, we examined the prognostic value of tumour HVEM expression in HCC. In univariate analysis, serum PIVKA-II level, tumour size, vascular invasion, TNM stage and tumour HVEM expression were significantly associated with both RFS and OS (Table 2). Multivariate analysis revealed that tumour size ($P = 0.028$) and tumour HVEM expression ($P < 0.001$) were significant factors for RFS (Table 3). In terms of OS, tumour size ($P = 0.003$) and tumour HVEM expression ($P = 0.008$) were turned out to be statistically significant (Table 3).

Table 1
Correration between tumour herpes virus entry mediator (HVEM) expression and clinicopathological characteristics of 150 hepatocellular carcinoma (HCC) patients.

		High herpes virus entry mediator (HVEM) ($n = 66$)	Low HVEM ($n = 84$)	P value
Age at surgery	<70	27	50	0.024
	⩾70	39	34	
Gender	Female	17	17	0.423
	Male	49	67	
Cirrhosis	Absent	41	52	0.978
	Present	25	32	
Viral status	Absent	19	23	0.849
	Present	47	61	
Alcohol consumption	Absent	52	68	0.742
	Present	14	16	
AFP	<15 ng/ml	31	41	0.823
	⩾15 ng/ml	35	43	
PIVKA-II	<150 U/ml	26	51	0.009
	⩾150 U/ml	40	33	
Tumour size	<5 cm	46	64	0.372
	⩾5 cm	20	20	
Tumour number	Single	59	72	0.501
	Multiple	7	12	
Vascular invasion	Absent	23	45	0.022
	Present	43	39	
TNM stage	I	24	45	0.036
	II, III, IV	42	39	
Histological differentiation	Well	19	27	0.658
	Mod, Por	47	57	

3.5. Correlation between tumour HVEM expression and recurrence pattern

In total, 86 recurrences (57.3%) were confirmed until the final follow-up. While intrahepatic recurrence was observed in 78 patients, extrahepatic recurrence was found in 36 patients. The sites of extrahepatic recurrence were the lung in 23, the bone in 12 and others in six. There were significant correlations of HVEM expression with both intrahepatic and extrahepatic recurrence (Table 4). In particular, early recurrence within 2 years after surgery was found more frequently in high HVEM group.

3.6. Association of HVEM expression with tumour-infiltrating T cells and local immune status

To investigate the underlying mechanism of prognostic impact of tumour HVEM expression in HCC, we examined tumour-infiltrating T cells by immunohistochemistry. As a result, we found that the numbers of tumour infiltrating $CD4^+$, $CD8^+$ and $CD45RO^+$ T cells were significantly lower in high HVEM group compared to low HVEM group (Fig. 3). These data suggested that tumour HVEM expression might prevent the infiltration of T cells into HCC tissues. Furthermore, to evaluate local immune status, we examined the expression of perforin, granzyme B and interferon-γ (IFN-γ) in HCC tissue by real time PCR analysis on available 49 frozen tissues. The expressions of perforin, granzyme B as well as IFN-γ were significantly lower in high HVEM group than low HVEM group (Fig. 4). Data indicated that tumour HVEM expression might be correlated with the inhibition of local immune activation. Taken together, HVEM may play a functionally important role through regulating the infiltrations of T cells into HCC.

4. Discussion

HVEM has been shown to possess diverse and unique immunological functions in various physiological and pathological conditions [28]. One of clinically relevant functions of HVEM may be involvement in tumour immune evasion. We have recently reported that HVEM plays a critical role in the evasion of host antitumour immune responses in human oesophageal cancer,

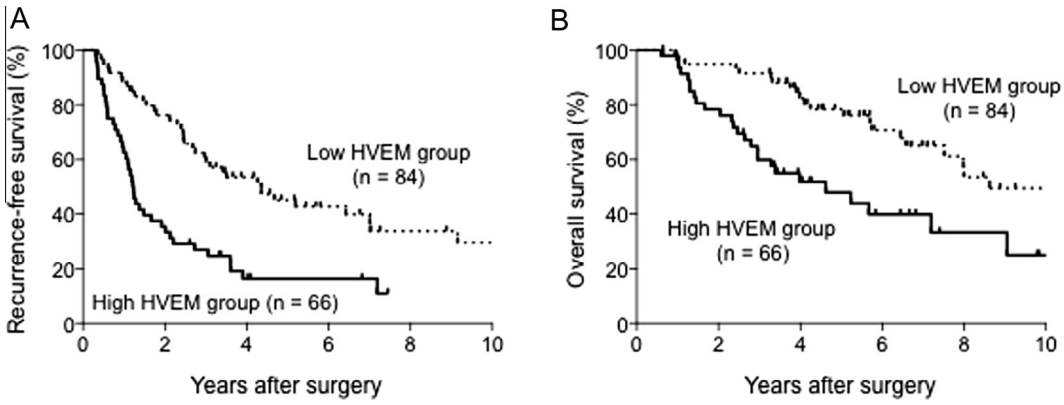

Fig. 2. Tumour herpes virus entry mediator (HVEM) expression significantly correlated with recurrence and prognosis after surgery for hepatocellular carcinoma (HCC). (A) Recurrence-free survival was significantly worse in high HVEM group than in low HVEM group ($P < 0.001$). (B) Overall survival was also poorer in high HVEM group than low HVEM group ($P = 0.002$).

Table 2
Univariate analysis of factors associated with recurrence-free and overall survival.

		Number	Recurrence free survival			Overall survival (OS)		
			Hazard ratio (HR)	95% Confidence interval (CI)	*P*	HR	95% CI	*P*
Age	<70/⩾70	77/73	1.18	0.77–1.88	0.441	0.90	0.52–1.54	0.689
Gender	Male/Female	116/34	0.76	0.45–1.28	0.302	0.56	0.26–1.20	0.133
Cirrhosis	Absent/Present	93/57	0.77	0.50–1.20	0.255	0.75	0.43–1.32	0.198
Viral status	Non/Hep B + C	42/108	1.48	0.89–2.47	0.131	1.21	0.63–2.33	0.571
AFP, ng/ml	<15/⩾15	72/78	1.32	0.86–2.03	0.208	1.30	0.75–2.24	0.346
PIVKA-II, U/ml	<150/⩾150	77/73	1.85	1.21–2.84	0.005	3.10	1.77–5.44	<0.001
Tumour size, cm	<5/⩾5	111/39	2.37	1.49–3.78	<0.001	4.20	2.41–7.33	<0.001
Tumour number	Single/Multiple	131/19	1.69	0.91–3.11	0.095	1.39	0.66–2.95	0.390
Histological differentiation	Well/Mod, Por	46/104	1.22	0.76–1.94	0.410	1.20	0.66–2.18	0.551
Vascular invasion	Absent/Present	68/82	1.59	1.03–2.44	0.034	2.28	1.31–3.98	0.004
Capsule invasion	Absent/Present	44/106	1.18	0.73–1.90	0.490	1.20	0.67–2.17	0.535
TNM stage	I/II–IV	69/81	1.58	1.03–2.42	0.037	2.45	1.41–4.29	0.042
Herpes virus entry mediator (HVEM) expression	Low/High	84/66	2.77	1.79–4.29	<0.001	2.31	1.34–3.97	0.002

Table 3
Multivariate analysis for the predictors of recurrence-free and overall survival.

		Recurrence-free survival			Overall survival (OS)		
		Hazard ratio (HR)	95% Confidence interval (CI)	*P* value	HR	95% CI	*P* value
PIVKA-II, U/ml	<150/⩾150	1.20	0.70–2.05	0.508	1.66	0.84–3.30	0.145
Tumour size, cm	<5/⩾5	1.85	1.07–3.21	0.028	2.76	1.41–5.38	0.003
Vascular invasion	Absent/Present	1.18	0.74–1.90	0.488	1.58	0.89–2.82	0.119
Herpes virus entry mediator (HVEM) expression	Low/High	2.54	1.63–3.96	<0.001	2.09	1.21–3.62	0.008

suggesting that it may be promising therapeutic target [26]. Consistent with our previous report on oesophageal cancer, this study has also shown that there are inverse correlations between tumour-infiltrating T cells and tumour HVEM expression. We also confirmed the local immune suppression of HCC tissue in association with HVEM expression. More importantly, this study revealed that tumour HVEM expression correlated with postoperative recurrence and also had an independent prognostic value for HCC. To date, although several studies have suggested the potential clinical importance and efficacy of immunotherapy in HCC, fundamental immunological mechanisms are largely unknown in tumour biology of HCC [4–8,29,30]. Our previous study has shown that PD-L1, one of potent immune checkpoint inhibitors, might be a predictor of recurrence for HCC patients [13]. This study further suggested that immune evasion through negative regulatory mechanisms, including HVEM and PD-L1, plays a pivotal role in HCC patients. Taken together, immunotherapy

Table 4
Correlation between tumour herpes virus entry mediator (HVEM) expression and recurrence.

		High herpes virus entry mediator (HVEM)	Low HVEM	*P* value
Intrahepatic recurrence	Present	42	36	0.012
	Absent	24	48	
Extrahepatic recurrence	Present	22	14	0.018
	Absent	44	70	
Early recurrence within 2 years	Present	35	16	<0.001
	Absent	31	68	

targeting immune checkpoints may hold a great promise for improving prognosis of HCC patients.

Most cases of HCC are secondary to either a viral hepatitis infection or alcoholic cirrhosis. Interestingly, recent studies have reported that HVEM-BTLA signalling is likely to affect the pathogenesis of HBV-related acute-on-chronic liver failure [31]. Furthermore, HVEM/TNFRSF14 gene expression was also shown to be upregulated in human alcoholic hepatitis [32]. These studies have suggested that HVEM might play

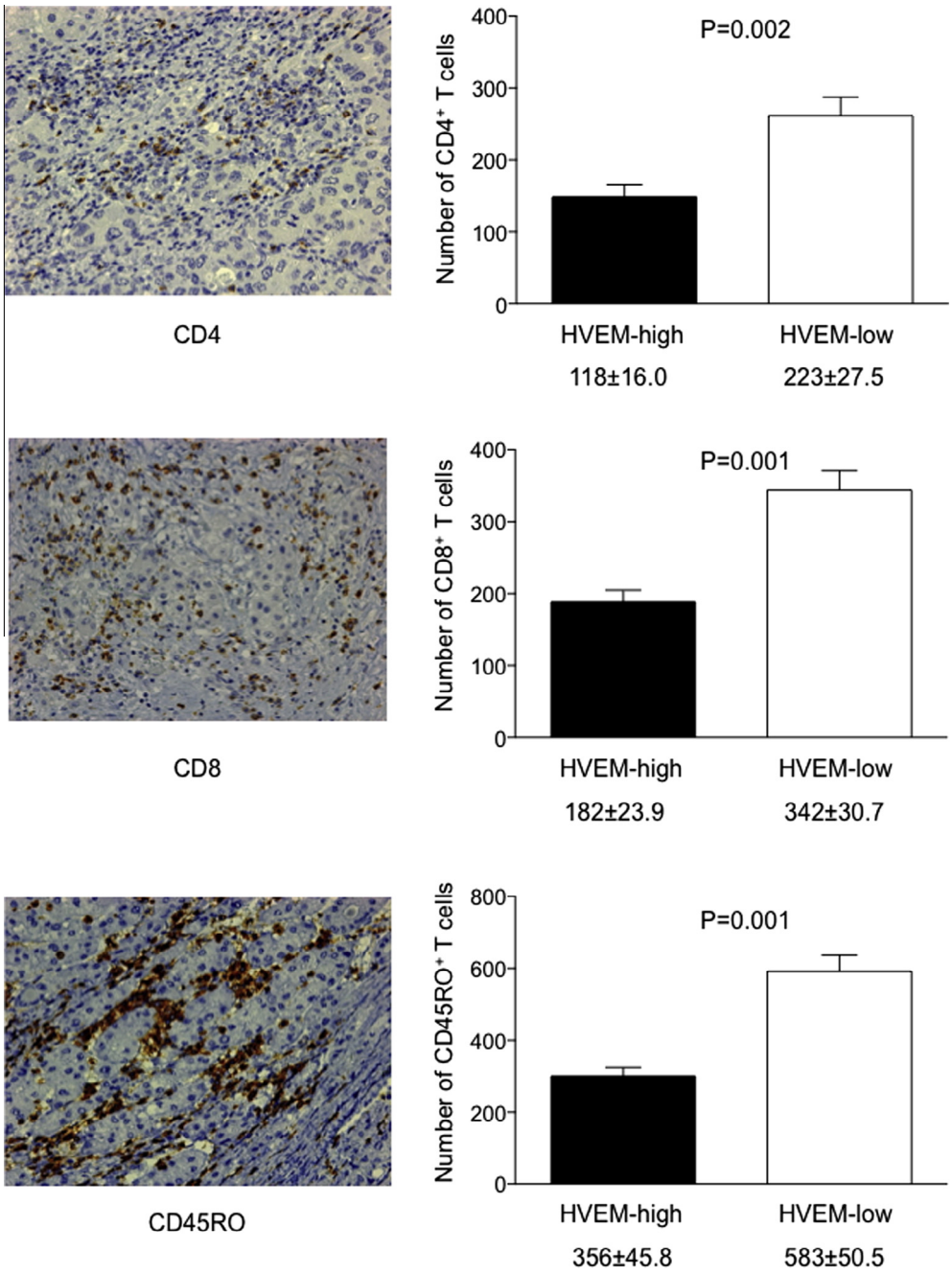

Fig. 3. Correlation between tumour herpes virus entry mediator (HVEM) expression and tumour-infiltrating lymphocytes. Representative images of immunohistochemical staining of tumour-infiltrating T cell subsets are shown on the left. The number of tumour-infiltrating $CD4^+$, $CD8^+$ and $CD45RO^+$ lymphocytes was significantly fewer in HVEM-high tumours compared with HVEM-low tumours ($P = 0.002$, $P = 0.001$ and $P = 0.001$, respectively). (mean ± SEM (the standard error of the mean)).

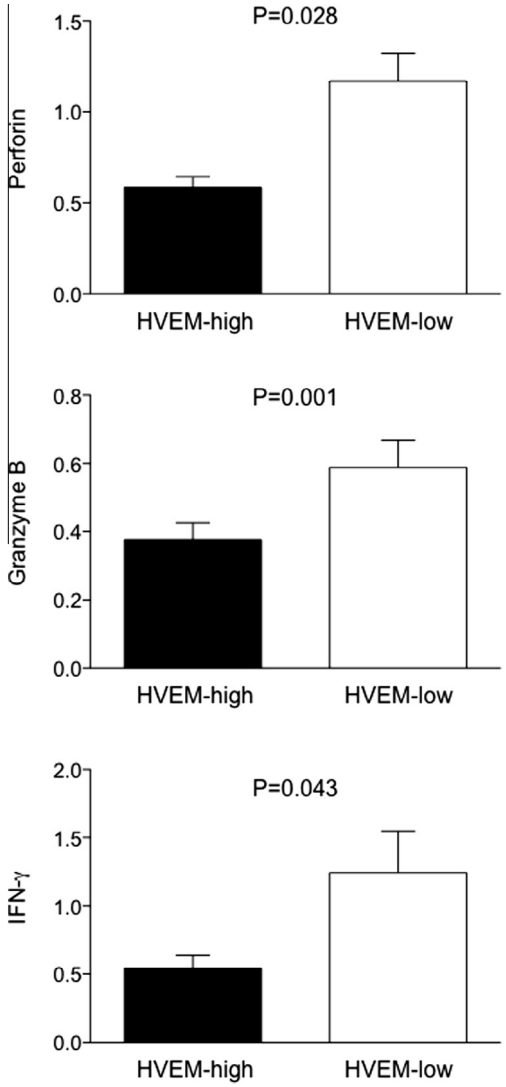

Fig. 4. Correlation of tumour herpes virus entry mediator (HVEM) expression with local immune status in hepatocellular carcinoma (HCC) tissues analysed by real-time polymerase chain reaction (PCR). Expressions of perforin, granzyme B and interferon-γ (IFN-γ) were significantly lower in HVEM-high tumours compared with HVEM-low tumours. ($P = 0.028$, $P = 0.001$ and $P = 0.043$, respectively, $n = 49$). (mean ± SEM (the standard error of the mean)).

some roles in pathogenesis of hepatitis related to either viral infection or alcohol use. Postoperative recurrence of HCC has been suggested to be associated with accompanying active hepatitis [33]. Therefore, the treatment of hepatitis after surgery for HCC may lead to the inhibition of postoperative recurrence and improvement of patient survival. However, to date, several clinical trials to evaluate interferon against hepatitis virus have failed to show reliable efficacy and further suggested the need for more potent antiviral therapy [33,34]. Although neither viral status nor cirrhosis correlated with tumour HVEM expression in this study, targeting HVEM expressed in the liver may lead to not only direct antitumour effect but also the inhibition of active hepatitis. Since our data indicated that HVEM expression was significantly associated with early postoperative recurrence, further studies may be warranted.

A major challenge in HCC treatment is to prevent postoperative recurrence. However, since most current treatments for HCC, including surgery, RFA, TACE and radiation, are theoretically local control of tumours, those are basically ineffective for the prevention of multicentric occurrence of HCC. Immunotherapy may be expected to induce systemic rather than local antitumour effect. In fact, CTLA-4 or PD-1 blockade has been evaluated in clinical trials, and demonstrated to induce significant response and prolong patient survival [35,36]. Our data warrant further study to evaluate clinical efficacy of targeting HVEM as a new immunological checkpoint inhibitor in human malignancies. Especially for HCC, HVEM blockade may be desirable, since it has a dual effect through inhibiting tumours as well as active hepatitis as described above. In addition, we and others have reported that memory T cells expressing CD45RO may be more important for patient survival than other subsets of T cells in various cancer [37–39]. Our data indicated that tumour HVEM expression was inversely correlated with not only $CD4^+$ or $CD8^+$ T cells but also $CD45RO^+$ T cells in HCC. Therefore, HVEM blockade may lead to promote the infiltrations of memory T cells into the tumour, thereby resulting in long-lasting antitumour efficacy in HCC patients.

In clinical setting, the combination therapy of HVEM blockade with some other anticancer treatments including chemotherapy, radiotherapy, antiangiogenesis and blockade of other immunocheck point including PD-1 and CTLA-4 may induce synergistic anticancer effects. In fact, we have shown synergistic effects induced by several combinations of blocking T cell negative pathways with other strategies such as chemotherapy and antiangiogenic treatment [11,12,40]. However, careful evaluation by clinical trials is needed, since blockade of immunocheck point may induce unfavorable effect such as autoimmune diseases.

There are several limitations in this study. The number of samples evaluated for this study is relatively small. In addition, this study was conducted in a single institution. Therefore, a large-scale and multicenter international collaborative study for HVEM is critically important to confirm reproducibility of our data. Furthermore, to our best of knowledge, there is no antibody available for human HVEM blockade in clinical trial. Therefore, further studies not only to generate new antibody but also to clarify its action *in vivo* are needed, since the antibodies to inhibitory receptor pathways may not be always act as expected.

In conclusion, we have shown for the first time that tumour HVEM expression may be associated with the development of HCC. Furthermore, it is inversely correlated with tumour infiltrating T cells and local immune

activation, suggesting that HVEM plays a functionally important role in HCC. Therefore, targeting HVEM may be a novel promising therapeutic strategy for HCC.

Conflict of interest statement

None declared.

Acknowledgement

This work was supported by the following grants: Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (M. Sho, D. Hokuto), Research Grant from the Foundation for Promotion of Cancer Research in Japan; Research Grant from Daiwa Securities Health Foundation; Research Grant from The Japanese Society of Gastroenterology; and Research Grant from Nakayama Cancer Research Institute (M. Sho).

References

[1] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55(2):74–108.
[2] Prendergast GC, Jaffee EM. Cancer immunologists and cancer biologists: why we didn't talk then but need to now. Cancer Res 2007;67(8):3500–4.
[3] Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359(4):378–90.
[4] Yoong KF, McNab G, Hubscher SG, Adams DH. Vascular adhesion protein-1 and ICAM-1 support the adhesion of tumor-infiltrating lymphocytes to tumor endothelium in human hepatocellular carcinoma. J Immunol 1998;160(8):3978–88.
[5] Wada Y, Nakashima O, Kutami R, Yamamoto O, Kojiro M. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. Hepatology 1998;27(2):407–14.
[6] Flecken T, Schmidt N, Hild S, Gostick E, Drognitz O, Zeiser R, et al. Immunodominance and functional alterations of tumor-associated antigen-specific CD8+ T-cell responses in hepatocellular carcinoma. Hepatology 2014;59(4):1415–26.
[7] Fu J, Zhang Z, Zhou L, Qi Z, Xing S, Lv J, et al. Impairment of CD4+ cytotoxic T cells predicts poor survival and high recurrence rates in patients with hepatocellular carcinoma. Hepatology 2013;58(1):139–49.
[8] Takayama T, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, et al. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. Lancet 2000;356(9232):802–7.
[9] Tada F, Abe M, Hirooka M, Ikeda Y, Hiasa Y, Lee Y, et al. Phase I/II study of immunotherapy using tumor antigen-pulsed dendritic cells in patients with hepatocellular carcinoma. Int J Oncol 2012;41(5):1601–9.
[10] Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, et al. Blockade of B7–H1 improves myeloid dendritic cell-mediated antitumor immunity. Nat Med 2003;9(5):562–7.
[11] Nomi T, Sho M, Akahori T, Hamada K, Kubo A, Kanehiro H, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. Clin Cancer Res 2007;13(7):2151–7.
[12] Yamato I, Sho M, Nomi T, Akahori T, Shimada K, Hotta K, et al. Clinical importance of B7–H3 expression in human pancreatic cancer. Br J Cancer 2009;101(10):1709–16.
[13] Gao Q, Wang XY, Qiu SJ, Yamato I, Sho M, Nakajima Y, et al. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. Clin Cancer Res 2009;15(3):971–9.
[14] Montgomery RI, Warner MS, Lum BJ, Spear PG. Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. Cell 1996;87(3):427–36.
[15] Kwon BS, Tan KB, Ni J, Oh KO, Lee ZH, Kim KK, et al. A newly identified member of the tumor necrosis factor receptor superfamily with a wide tissue distribution and involvement in lymphocyte activation. J Biol Chem 1997;272(22):14272–6.
[16] Sedy JR, Gavrieli M, Potter KG, Hurchla MA, Lindsley RC, Hildner K, et al. B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. Nat Immunol 2005;6(1):90–8.
[17] Cai G, Anumanthan A, Brown JA, Greenfield EA, Zhu B, Freeman GJ. CD160 inhibits activation of human CD4+ T cells through interaction with herpesvirus entry mediator. Nat Immunol 2008;9(2):176–85.
[18] Wang Y, Subudhi SK, Anders RA, Lo J, Sun Y, Blink S, et al. The role of herpesvirus entry mediator as a negative regulator of T cell-mediated responses. J Clin Invest 2005;115(3):711–7.
[19] DiMenna L, Latimer B, Parzych E, Haut LH, Topfer K, Abdulla S, et al. Augmentation of primary influenza A virus-specific CD8+ T cell responses in aged mice through blockade of an immunoinhibitory pathway. J Immunol 2010;184(10):5475–84.
[20] Derré L, Rivals JP, Jandus C, Pastor S, Rimoldi D, Romero P, et al. BTLA mediates inhibition of human tumor-specific CD8+ T cells that can be partially reversed by vaccination. J Clin Invest 2010;120(1):157–67.
[21] Lasaro MO, Tatsis N, Hensley SE, Whitbeck JC, Lin SW, Rux JJ, et al. Targeting of antigen to the herpesvirus entry mediator augments primary adaptive immune responses. Nat Med 2008;14(2):205–12.
[22] Cai G, Freeman GJ. The CD160, BTLA, LIGHT/HVEM pathway: a bidirectional switch regulating T-cell activation. Immunol Rev 2009;229(1):244–58.
[23] Harrop JA, McDonnell PC, Brigham-Burke M, Lyn SD, Minton J, Tan KB, et al. Herpesvirus entry mediator ligand (HVEM-L), a novel ligand for HVEM/TR2, stimulates proliferation of T cells and inhibits HT29 cell growth. J Biol Chem 1998;273(42):27548–56.
[24] Pasero C, Barbarat B, Just-Landi S, Bernard A, Aurran-Schleinitz T, Rey J, et al. A role for HVEM, but not lymphotoxin-beta receptor, in LIGHT-induced tumor cell death and chemokine production. Eur J Immunol 2009;39(9):2502–14.
[25] Hobo W, Norde WJ, Schaap N, Fredrix H, Maas F, Schellens K, et al. B and T lymphocyte attenuator mediates inhibition of tumor-reactive CD8+ T cells in patients after allogeneic stem cell transplantation. J Immunol 2012;189(1):39–49.
[26] Migita K, Sho M, Shimada K, Yasuda S, Yamato I, Takayama T, et al. Significant involvement of herpesvirus entry mediator in human esophageal squamous cell carcinoma. Cancer 2014;120(6):808–17.
[27] Ohigashi Y, Sho M, Yamada Y, Tsurui Y, Hamada K, Ikeda N, et al. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res 2005;11(8):2947–53.
[28] Steinberg MW, Cheung TC, Ware CF. The signaling networks of the herpesvirus entry mediator (TNFRSF14) in immune regulation. Immunol Rev 2011;244(1):169–87.
[29] Pedroza-Gonzalez A, Verhoef C, Ijzermans JN, Peppelenbosch MP, Kwekkeboom J, Verheij J, et al. Activated tumor-infiltrating CD4+ regulatory T cells restrain antitumor immunity in patients with primary or metastatic liver cancer. Hepatology 2013;57(1):183–94.
[30] Huang Y, Wang FM, Wang T, Wang YJ, Zhu ZY, Gao YT, et al. Tumor-infiltrating FoxP3+ Tregs and CD8+ T cells affect the

prognosis of hepatocellular carcinoma patients. Digestion 2012;86(4):329–37.
[31] Xu H, Cao D, Guo G, Ruan Z, Wu Y, Chen Y. The intrahepatic expression and distribution of BTLA and its ligand HVEM in patients with HBV-related acute-on-chronic liver failure. Diagn Pathol 2012;7:142.
[32] Seth D, Gorrell MD, Cordoba S, McCaughan GW, Haber PS. Intrahepatic gene expression in human alcoholic hepatitis. J Hepatol 2006;45(2):306–20.
[33] Shen YC, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. J Hepatol 2010;52(6):889–94.
[34] Chen LT, Chen MF, Li LA, Lee PH, Jeng LB, Lin DY, et al. Long-term results of a randomized, observation-controlled, phase III trial of adjuvant interferon Alfa-2b in hepatocellular carcinoma after curative resection. Ann Surg 2012;255(1):8–17.
[35] Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366(26):2443–54.
[36] Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366(26):2455–65.
[37] Enomoto K, Sho M, Wakatsuki K, Takayama T, Matsumoto S, Nakamura S, et al. Prognostic importance of tumour-infiltrating memory T cells in oesophageal squamous cell carcinoma. Clin Exp Immunol 2012;168(2):186–91.
[38] Hotta K, Sho M, Fujimoto K, Shimada K, Yamato I, Anai S, et al. Prognostic significance of CD45RO+ memory T cells in renal cell carcinoma. Br J Cancer 2011;105(8):1191–6.
[39] Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313(5795):1960–4.
[40] Yasuda S, Sho M, Yamato I, Yoshiji H, Wakatsuki K, Nishiwada S, et al. Simultaneous blockade of programmed death 1 and vascular endothelial growth factor receptor 2 (VEGFR2) induces synergistic anti-tumour effect *in vivo*. Clin Exp Immunol 2013;172(3):500–6.