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Significance of herpesvirus entry mediator expression in human colorectal liver metastasis

Running title: *HVEM expression in human CRLM*

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Synopsis

This is the first report clarifying HVEM expression in human CRLM. High HVEM expression was an independent poor prognostic factor for OS after liver resection. CD8+, CD45RO+ tumor infiltrating T cells were significantly lower in the high HVEM expressed CRLM.

Abstract

Background. Herpesvirus entry mediator (HVEM) has been suggested to play various roles in cancer biology. We have reported that HVEM expression in tumor cells is associated with a reduction in the number of tumor-infiltrating lymphocytes and a poor prognosis after surgical resection in various human gastrointestinal cancers. The aim of this study was to clarify the clinical significance of HVEM expression in human colorectal liver metastasis (CRLM).

Methods. We examined the cases of 104 patients with CRLM who underwent curative liver resection at Nara Medical University between 2000 and 2014. The median duration of the follow-up period was 50.2 months. Immunohistochemical staining was performed using antibodies against HVEM, CD4, CD8, and CD45RO.

Results. High HVEM expression was observed in 49 patients (47.1%) with CRLM. HVEM expression was not associated with age, gender, the administration of preoperative chemotherapy, tumor size, the number of tumors, or histological differentiation. The high HVEM group exhibited significantly worse overall survival (OS) than the low HVEM group (P=0.002). Multivariate analysis revealed that high HVEM expression in CRLM is an independent poor prognostic factor for OS (HR: 3.35, 95%CI: 1.41-7.93, P=0.006) as is being aged ≥70 and having ≥5 tumors. The numbers

of tumor-infiltrating CD8+ and CD45RO+ T cells were significantly lower in the high HVEM group than in the low HVEM group. High HVEM expression in primary colorectal cancer was significantly associated with synchronous CRLM, but not metachronous CRLM.

Conclusions. Tumor HVEM expression might play a critical role in CRLM.

Background

Colorectal cancer (CRC) is the third most common cause of cancer-related death, and the number of patients with CRC is increasing worldwide [1]. Colorectal liver metastases (CRLM) have also become more common, which has had a marked effect on the prognosis of CRC [2–4]. Liver resection represents the only chance of a cure for patients with CRLM. Despite improvements in surgical techniques and the introduction of new chemotherapy regimens and molecular-targeted therapy, the 5-year survival rates of patients with CRLM after hepatic resection reportedly range from 33 to 61%. Therefore, novel approaches still need to be developed to improve the prognosis of patients with CRLM. One potentially promising strategy is immunotherapy. Tumor-infiltrating lymphocytes (TIL) are considered to contribute to primary host immune responses against several types of malignant tumors [5, 6]. As for CRC, it was reported that the presence of T-cell infiltrates in primary colon tumors was associated with better overall survival (OS) [7, 8]. Furthermore, an association between an increased number of TIL in CRLM and improved OS has been reported [9, 10]. However, as tumors have a variety of mechanisms for evading immune responses, the clinical efficacy of immunotherapy against CRC is very limited [11, 12]. Recently, immunotherapy has changed markedly with the introduction of checkpoint inhibitors,

such as anti-programmed cell death 1 (PD-1) and anti-programmed cell death ligand 1 (PD-L1) agents [13]. Regarding CRC, microsatellite instability-high CRC appeared to respond to checkpoint blockade with anti-PD-1 or anti-PD-L1 agents [12]. However, microsatellite-stable CRC was much less responsive to anti-PD-1 and anti-PD-L1 agents. Furthermore, anti-PD-1 antibodies exhibited very limited antitumor activity in patients with advanced colorectal carcinoma [14]. Thus, further immunotherapy agents are needed to improve the prognosis of patients with CRC.

Herpesvirus entry mediator (HVEM), which is also known as tumor necrosis factor receptor superfamily 14 (TNFRSF14), was identified as a cellular mediator of herpes simplex virus entry [15]. It is expressed on several types of cells, including T cells, B cells, natural killer cells, dendritic cells, and myeloid cells, as well as in non-lymphoid organs, including the lungs, liver, and kidneys [15, 16]. HVEM ligands belong to two distinct families: TNF-related cytokines, such as lymphotoxin-related inducible ligand that competes for glycoprotein D binding to herpesvirus entry mediator on T cells (LIGHT) and lymphotoxin-α, and Ig-related membrane proteins, such as B and T lymphocyte attenuator (BTLA) and cluster of differentiation 160 (CD160) [17, 18]. Previous studies have reported that the HVEM pathway plays roles in several types of disease, including autoimmune disease,

infections, and inflammation [19, 20]. Recently, HVEM has been suggested to play various roles in cancer biology [21, 22]. We have reported that HVEM expression in tumor cells was associated with reductions in the number of TIL and a poor prognosis after surgical resection in human esophageal squamous cell carcinoma, hepatocellular carcinoma, and primary colorectal cancer [23–25]. However, the significance of HVEM expression in human CRLM is still largely unknown. Furthermore, the association between HVEM expression in primary CRC and CRLM has not been studied yet. The aim of this study was to clarify the clinical significance of HVEM expression in human CRLM.

Methods

Patients

We examined the case of 104 patients with CRLM who underwent curative liver resection at the Department of Surgery of Nara Medical University between 2000 and 2014. The patients were followed-up until death or June 2018. The median duration of the follow-up period was 50.2 months (range: 5.5–121.4 months). The clinicopathological stage was classified according to the International Union against Cancer system. The remainder of each specimen was fixed in 10% phosphate-buffered

formalin and embedded in paraffin. Written informed consent was obtained from all patients before treatment, according to our institutional guidelines. The study protocol was approved by the institutional review board (approval number: 1531).

Immunohistochemistry

Formalin-fixed, paraffin-embedded CRLM tissue samples were cut into 5-μm sections, deparaffinized, and rehydrated in a graded series of ethanol. Antigen retrieval was carried out by heating the tissue sections using a target retrieval solution (pH 9.0) (DAKO, Tokyo, Japan). To block endogenous peroxidase activity, the sections were immersed in a 3% solution of hydrogen peroxide in absolute methanol for 5 min at room temperature, before being washed three times in fresh phosphate-buffered saline (PBS), for 5 min each time. 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), anti-human CD4 (1:40) (4B12, monoclonal mouse; DAKO), and anti-human CD8 (C8/144B, monoclonal mouse; DAKO) antibodies. The sections were washed three times in PBS, before being incubated with the EnVision detection system (DAKO), according to the manufacturer's instructions. The sections

were then counterstained with hematoxylin, dehydrated in ethanol, cleared in xylene, and coverslipped.

Evaluation of immunostaining

The immunohistochemical staining of HVEM was evaluated according to the intensity of the staining and the percentage of positively stained tumor cells in a blinded manner. Five fields were randomly selected and evaluated by authorized pathologists, who had no knowledge of the patients' clinical status or outcomes. At least 1000 tumor cells were scored in each sample, and the percentage of tumor cells that were positively stained and the staining intensity were recorded. The staining intensity was classified into four groups: none: 0 points, weak: 1 point, intermediate: 2 points, and strong: 3 points. The percentage of positively stained tumor cells was classified into four groups as follows: 0–25%: 1 point, 26–50%: 2 points, 51–75%: 3 points, and 76–100%: 4 points. We then evaluated HVEM expression in each tissue according to the total score by adding the scores for each parameter together (total score: 1–7). Specimens with total scores of 1–5 were classified as having low HVEM expression, and those with total scores of 6–7 were classified as having high HVEM expression. Immunohistochemical staining of CD4+, CD8+, and CD45RO+ T-cells was used to count the number of TIL, as described previously [24, 25].

Statistical analysis

The significance of differences in HVEM expression according to various clinicopathological variables was assessed using the Student's *t*-test, Chi-square test, or Fisher's exact test, as appropriate. The Kaplan-Meier method was used to estimate the probability of survival, and significance was assessed using the log-rank test. Univariate and multivariate analyses were performed using the Cox proportional hazards model to identify significant prognostic predictors. P-values of <0.05 were considered statistically significant in all analyses.

Results

HVEM expression in human CRLM and its relationships with clinicopathological factors

We first examined the expression of HVEM in 104 surgically resected CRLM tissue samples via immunohistochemistry. HVEM was detected in the cell membrane, cytoplasm, or both in the CRLM cells. On the other hand, no or only limited HVEM expression was seen in the normal liver tissues, including normal hepatocytes, sinusoidal cells, bile duct epithelial cells, and the vascular endothelium. To investigate the clinical importance of tumor HVEM expression in CRLM, we divided the 104 cases

into a high HVEM group (n=49) and a low HVEM group (n=55) **(Figs. 1A/B)**. Then, we examined the associations between HVEM expression and various clinicopathological characteristics **(Table 1)**. As a result, HVEM expression was not found to be associated with age, gender, the administration of preoperative chemotherapy, tumor size, the number of tumors, histological differentiation, or tumor sidedness of primary CRC. Regarding RAS mutation, 23.8% of the high HVEM group had RAS mutation, while 22.7% in the low HVEM group (P=0.933).

Impact of tumor HVEM expression on postoperative recurrence and survival

Next, we compared postoperative recurrence and survival according to HVEM status. As a result, we found that the high HVEM group exhibited significantly worse OS than the low HVEM group, while recurrence-free survival (RFS) did not differ significantly between the groups **(Figs. 2A/B)**. The median OS time was 3.1 and 9.6 years in the high and low HVEM groups, respectively (P=0.002). The 5-year postoperative survival rates of the high and low HVEM patients were 35.8% and 66.4%, respectively.

Prognostic value of tumor HVEM expression in CRLM

Furthermore, we examined the prognostic value of HVEM expression in

CRLM. In the univariate analyses, being aged ≥70 years old (P=0.010), the presence of extrahepatic metastasis (P=0.023), having ≥5 tumors (P=0.008), a preoperative carcinoembryonic antigen (CEA) level of ≥20 ng/ml (P=0.002), a preoperative carbohydrate antigen 19-9 (CA19-9) level of ≥100 U/ml (P=0.026), a primary colorectal cancer N factor of N2–3 (P=0.036), and high HVEM expression (P=0.002) were identified as significant prognostic factors for OS **(Table 2)**. In the multivariate analysis, being aged ≥70 years old (P=0.007), having ≥5 tumors (P=0.047), and high HVEM expression (P=0.002) were identified as significant prognostic factors for OS.

Associations between HVEM expression in CRLM and the recurrence pattern or survival after recurrence

There were 41 patients with recurrence in the high HVEM group and 39 patients in the low HVEM group. The initial site of recurrence in the high HVEM group was the remnant liver alone in 17 patients (41.5%), distant sites alone in 8 patients (19.5%), and the remnant liver and distant sites in 16 patients (39.0%), while in the low HVEM group, the remnant liver alone in 26 patients (66.6%), distant sites alone in 5 patients (12.8%), and the remnant liver and distant sites in 8 patients (20.5%). The proportion of liver-limited recurrence was significantly more often in the low HVEM

group than in the high HVEM group (P=0.031). Repeat hepatectomy was performed in 15 of 41 patients (36.6%) in the high HVEM group, which was a significantly fewer than in the low HVEM group (23/39, 59.0%) (P=0.045). The median survival time of the patients who were treated with chemotherapy alone after being diagnosed with recurrence was 18.7 months in the high HVEM group, while it was 44.5 months in the low HVEM group (P=0.033).

Association between HVEM expression and tumor-infiltrating T cells

To investigate the mechanism underlying the prognostic impact of HVEM expression in CRLM, we examined the numbers of TIL in the CRLM using immunohistochemistry (**Figs. 1D/E/F**). As a result, we found that the numbers of tumor infiltrating CD8+ and CD45RO+ T cells in the CRLM were significantly lower in the high HVEM group than in the low HVEM group, while the numbers of CD4+ T cells in the CRLM did not differ significantly between the groups (**Figs. 1G/H/I**).

Relationship between HVEM expression in CRLM and primary CRC

Finally, we examined the relationship between HVEM expression in CRLM and HVEM expression in primary CRC in the same patients. Primary CRC specimens

were obtained from 59 patients. Of these, 28 primary CRC (47.5%) exhibited high HVEM expression, while 31 (52.5%) displayed low HVEM expression (**Fig. 1C**). The concordance of HVEM expression in the primary and liver metastasis among synchronous cancers was 77.8% (28/36) compared to 43.5% (10/23) for metachronous cancers (p=0.007) (**Fig. 3A**). High HVEM expression was observed in the primary CRC in 20 patients (55.6%) with synchronous CRLM and 8 patients (22.2%) with metachronous CRLM (P=0.037). Among these cases, high HVEM expression was also detected in the CRLM in 16 patients (80.0%) with synchronous CRLM and 3 patients (37.5%) with metachronous CRLM (P=0.044). The prognosis of the patients in which high HVEM expression was detected in both the CRLM and primary CRC was significantly worse than that of the patients in which both the CRLM and CRC displayed low HVEM expression (P=0.017) (**Figs. 3B/C**).

Discussion

In this study, high HVEM expression was observed in 47.1% of patients with CRLM. High HVEM expression was not significantly associated with age, a history of chemotherapy, tumor size, the number of tumors, nodal or metastatic status, or histological differentiation. However, in the multivariate analysis high CRLM HVEM

expression, being aged ≥70, and having ≥5 tumors were revealed to be independent poor prognostic factors in patients with CRLM. These findings suggest that HVEM status might play a critical role in the prognosis of CRLM independently of conventional TNM factors. To investigate the underlying mechanism responsible for the prognostic impact of CRLM HVEM expression, we examined the numbers of TIL present in the CRLM via immunohistochemistry. As a result, we found that the numbers of tumor-infiltrating CD45RO+ and CD8+ T cells were significantly lower in the high HVEM group than in the low HVEM group. CD45RO+ TIL are considered to be memory T cells, which can survive for many months or years and are critically important for host tumor immunity [26, 27]. CD8+ TIL also plays an important role in the host immune defense against tumor progression in several organs [6, 28–31]. We have recently reported that HVEM plays a critical role in the evasion of host antitumor immune responses in a variety of human malignancies, including esophageal cancer, hepatocellular carcinoma, and primary CRC [23–25]. Consistent with the findings of our previous studies, the present study detected an inverse correlation between the number of TIL and tumor HVEM expression. To the best of our knowledge, this is the first study to investigate HVEM expression in CRLM. A few investigators have examined TIL in human CRLM in previous studies. The tumor-selective activation and

cytotoxic activity of CD8+ T cells in human CRLM was first reported by Wagner et al. in 2008 [32]. An association between a high TIL density in CRLM and improved prognosis after liver resection was first reported by Halama et al. [9]. Nakagawa et al. found that low numbers of infiltrating peritumoral regulatory T cells was associated with a poor prognosis after liver resection for CRLM [10]. Although these studies suggested that TIL in CRLM affect patients' prognosis after liver resection, the underlying mechanisms responsible for these effects are largely unknown. Our findings suggest that tumor HVEM expression might inhibit the infiltration of TIL into CRLM, and so might play a critical role in the prognosis of patients with CRLM.

In the current study, the high HVEM group exhibited significantly worse OS than the low HVEM group, while RFS did not differ significantly between the two groups. Although the precise mechanisms underlying these findings remain unclear, one possible explanation might be associated with the frequency of repeat hepatectomy. Some recurrent CRLM can be cured by repeat hepatectomy [33]. In fact, the frequency of repeat hepatectomy was significantly lower in the high HVEM group than the low HVEM group (36.6% vs. 59.0%, P=0.045). Repeat hepatectomy is generally adapted for liver-limited recurrence. In this study, the proportion of liver-limited recurrence was 41.5% in the high HVEM group, while 66.6% in the low HVEM group (P=0.031).

Differences in tumor immunity associated with HVEM expression might also have contributed to this finding. The worse OS of the high HVEM group might also have been influenced by the response to chemotherapy. Halama et al. reported that the reduced infiltration of immune cells into CRLM was associated with a worse response to chemotherapy [9]. Since fewer TILs were detected in the high HVEM group than in the low HVEM group, the response to chemotherapy might have been unfavorable in the high HVEM group. In fact, the survival time after recurrence of the patients who were treated with chemotherapy alone was significantly shorter in the high HVEM group. Therefore, HVEM expression in CRLM might play a critical prognostic role through a variety of tumor immunity-related mechanisms.

Furthermore, we also examined the relationship between the expression of HVEM in primary CRC and HVEM expression in CRLM. Interestingly, high HVEM expression in the primary CRC was significantly more common among the patients with synchronous CRLM than among those with metachronous CRLM. Moreover, the frequency of high CRLM HVEM expression was significantly higher among the patients with synchronous CRLM. In general, synchronous metastasis is considered to be a poor prognostic factor (compared with metachronous metastasis) in patients that undergo liver resection for CRLM [34]. The fact that synchronous CRLM are more

likely to exhibit high HVEM expression might contribute to their worse prognosis. Several studies have shown that the gene expression of metachronous CRLM is different from those of primary CRC and synchronous CRLM [35, 36]. Since metachronous CRLM usually develop under long-term host immunity, the expression of HVEM in metachronous CRLM might change over time. Further fundamental studies are required to clarify the underlying mechanisms responsible for CRLM and the role of tumor HVEM expression.

This study has certain limitations. First, the number of samples evaluated for this study is relatively small. Second, the patients analyzed in this study were treated in relatively long period. Third, the study did not include all patients treated in this study period, mainly due to the availability of tumor samples for research purposes. Therefore, further large-scale studies are needed to verify our current findings.

In conclusion, we suggest that tumor HVEM expression might play a critical role in CRLM. Our findings indicate that not only could HVEM expression be a useful prognostic marker, but that it might also have potential as a novel immunotherapeutic target for the treatment of CRLM.

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Figure legends

Figure 1

(A/B) Representative cases of high and low herpesvirus entry mediator (HVEM) expression in surgically resected colorectal liver metastasis (CRLM): High HVEM expression was detected in A, and low HVEM expression was seen in B. (C) Representative case of high HVEM expression in surgically resected colorectal cancer (CRC), (D/E/F) Representative images of immunohistochemical staining of tumor-infiltrating lymphocytes (TIL), D: CD8+ lymphocytes, E: CD4+ lymphocytes, F: CD45RO+ lymphocytes, (G/H/I) The relationships between tumor HVEM expression and the numbers of TIL in CRLM: The numbers of tumor-infiltrating CD8+ and CD45RO+ lymphocytes were significantly lower in the tumors that exhibited high HVEM expression than in those that displayed low HVEM expression ($P<0.002$ and $P=0.001$, respectively) (mean±standard error of the mean [SEM]).

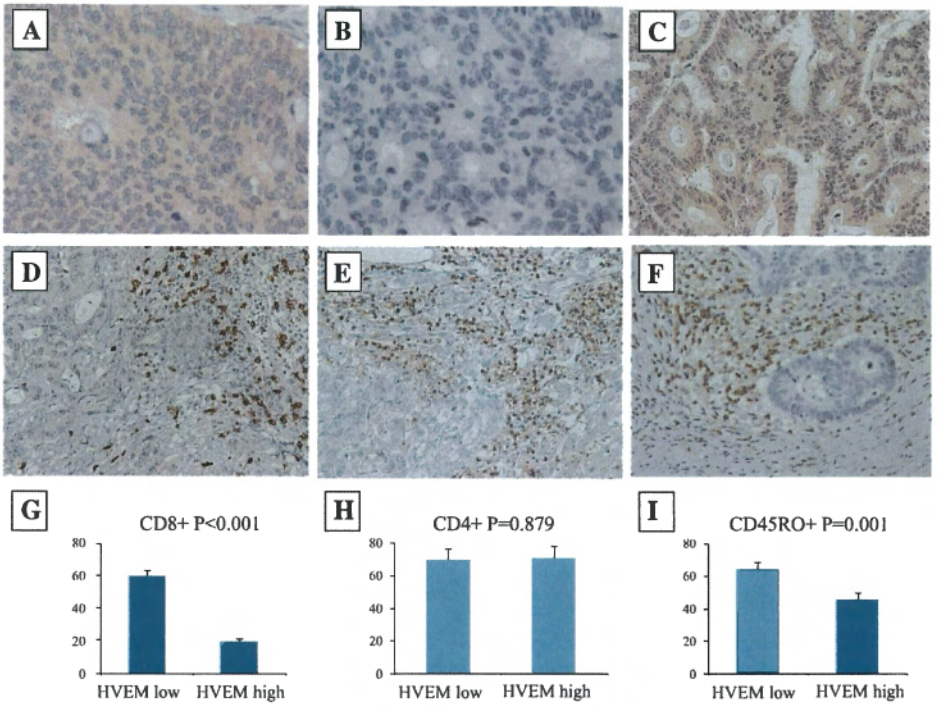
Figure 2

Relationship between tumor herpesvirus entry mediator (HVEM) expression in colorectal liver metastasis (CRLM) and prognosis after surgical resection for CRLM: (A) Recurrence-free survival did not differ significantly between the groups ($P=0.106$), (B) Overall survival was worse in the high HVEM group than in the low HVEM group ($P=0.002$).

Figure 3

(A) Relationships between tumor herpesvirus entry mediator (HVEM) expression in colorectal liver metastasis (CRLM) and primary colorectal cancer (CRC) and prognosis

after surgical resection for CRLM: A high HVEM expression level in the primary CRC was significantly associated with synchronous CRLM (P=0.001). On the other hand, the HVEM expression level of the primary CRC was not significantly associated with metachronous CRLM (P=0.469). (B) Recurrence-free survival did not differ significantly between the patients with high CRLM and CRC HVEM expression levels and the patients with low CRLM and CRC HVEM expression levels (P=0.277). (C) Overall survival was significantly worse in the patients with high CRLM and CRC HVEM expression levels than in the patients with low CRLM and CRC HVEM expression levels (P=0.017).



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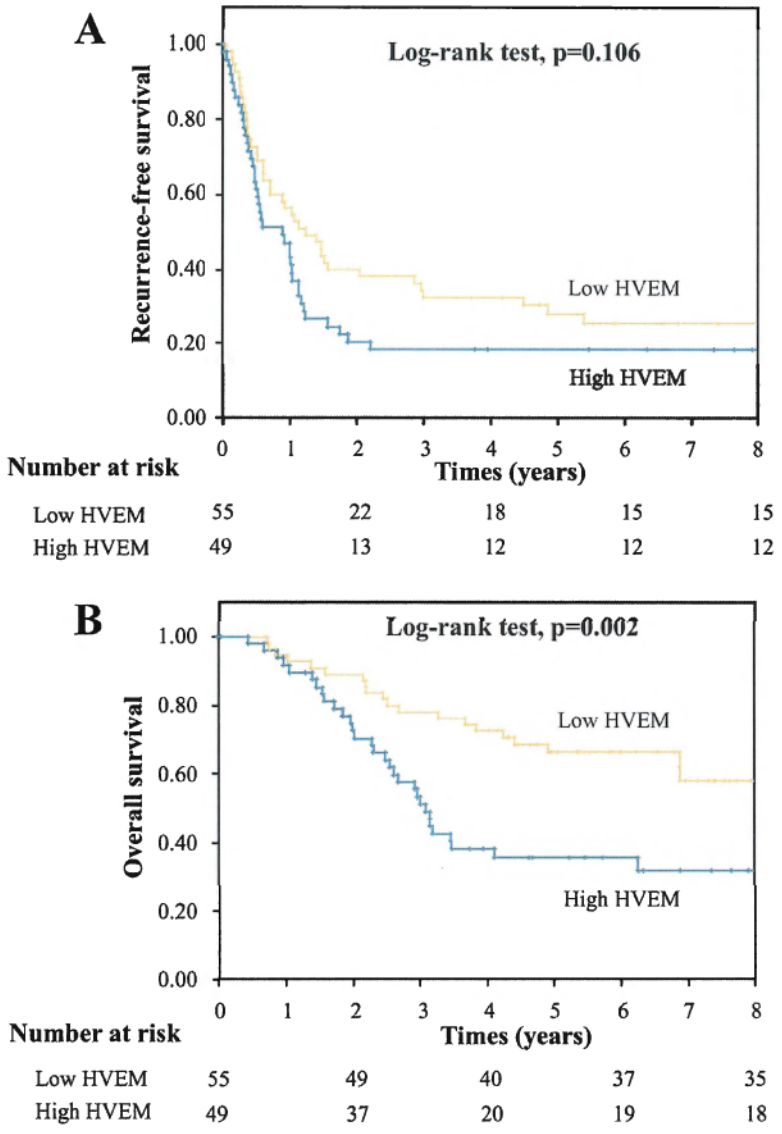

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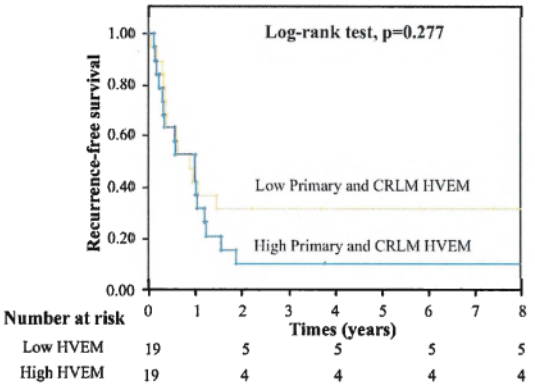
72x104mm (300 x 300 DPI)

A

		Synchronous CRLM (n=36)			Metachronous CRLM (n=23)	
		CRLM			CRLM	
		High HVEM	Low HVEM		High HVEM	Low HVEM
Primary CRC	High HVEM	16	4	High HVEM	3	5
	Low HVEM	4	12	Low HVEM	8	7
		P=0.001			P=0.469	

B



C

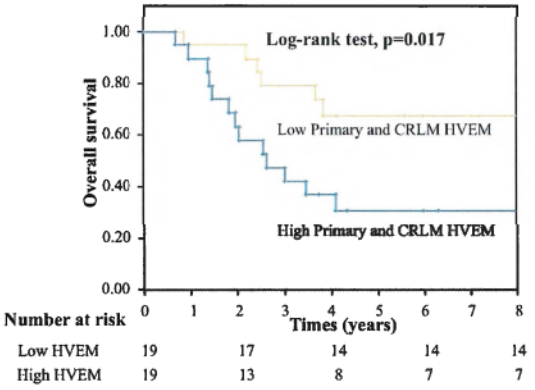


Figure 3

(A) Relationships between tumor herpesvirus entry mediator (HVEM) expression in colorectal liver metastasis (CRLM) and primary colorectal cancer (CRC) and prognosis after surgical resection for CRLM: A high HVEM expression level in the primary CRC was significantly associated with synchronous CRLM (P=0.001). On the other hand, the HVEM expression level of the primary CRC was not significantly associated with metachronous CRLM (P=0.469). (B) Recurrence-free survival did not differ significantly between the patients with high CRLM and CRC HVEM expression levels and the patients with low CRLM and CRC HVEM expression levels (P=0.277). (C) Overall survival was significantly worse in the patients with high CRLM and CRC HVEM expression levels than in the patients with low CRLM and CRC HVEM expression levels (P=0.017).

72x116mm (300 x 300 DPI)

TABLE 1. Associations between tumor herpes virus entry mediator (HVEM) expression and various clinicopathological characteristics

	High HVEM expression (n=49)	Low HVEM expression (n=55)	P-value
Age, median (range)	64 (35–82)	63 (38–82)	0.589
Gender			
Male	27 (55.1%)	33 (60.0%)	0.614
Female	22 (44.9%)	22 (40.0%)	
Timing of liver metastasis			
Synchronous	26 (53.1%)	24 (43.6%)	0.337
Metachronous	23 (46.9%)	31 (56.4%)	
Preoperative chemotherapy			
Absent	33 (67.3%)	38 (69.1%)	0.849
Present	16 (32.7%)	17 (30.9%)	
Adjuvant chemotherapy			
Absent	22 (44.9%)	20 (36.4%)	0.376
Present	27 (55.1%)	35 (63.6%)	
Extrahepatic metastasis			
Absent	41 (83.7%)	41 (74.5%)	0.370
Present	8 (16.3%)	14 (25.5%)	
Maximum tumor size (cm)			
<5	39 (79.6%)	43 (78.2%)	0.860

≥5	10 (20.4%)		12 (21.8%)		
Tumor number					
<5	36 (73.5%)		46 (83.6%)		0.305
≥5	13 (26.5%)		9 (16.4%)		
Preoperative CEA level (ng/ml)					
<20	31 (63.3%)		36 (65.5%)		0.816
≥20	18 (36.7%)		19 (34.5%)		
Preoperative CA19-9 level (U/ml)					
<100	42 (85.7%)		48 (87.3%)		0.956
≥100	7 (14.3%)		7 (12.7%)		
Location of primary colorectal cancer					
Colon	29 (59.2%)		37 (67.3%)		0.392
Rectum	20 (40.8%)		18 (32.7%)		
T factor of primary colorectal cancer (UICC 7th)					
T1–3	33 (67.3%)		37 (67.3%)		0.994
T4	16 (32.7%)		18 (32.7%)		
N factor of primary colorectal cancer (UICC 7th)					
N0–1	34 (69.4%)		42 (76.4%)		0.423
N2–3	15 (30.6%)		13 (23.6%)		
Histological differentiation of primary colorectal cancer					
Well	18 (24.0%)		28 (40.9%)		0.146
Other	31 (76.0%)		27 (59.0%)		

Tumor location of primary colorectal cancer				
Right side	11 (40.7%)		16 (59.3%)	0.441
Left side	38 (49.3%)		39 (50.7%)	
CRLM: colorectal liver metastasis, *CEA*: carcinoembryonic antigen, *CA19-9*: carbohydrate antigen 19-9,				
UICC: Union for International Cancer Control				

TABLE 2 Univariate and multivariate analysis of factors associated with overall survival after liver resection for CRLM

			Univariate analysis			Multivariate analysis		
		number	HR	95%CI	*P value*	HR	95%CI	*P value*
Age	<70 / ≥70	71/33	2.09	1.19-3.64	0.010	3.21	1.38-7.52	0.007
Gender	Female / Male	44/60	1.31	0.74-2.29	0.344			
Timing of liver metastasis	Synchronous / Metachronous	50/54	1.22	0.70-2.10	0.476			
preoperative chemotherapy	Absent / Present	71/33	1.68	0.96-2.96	0.069			
adjuvant chemotherapy	Absent / Present	42/62	0.58	0.33-1.00	0.051			
extrahepatic metastasis	Absent / Present	82/22	1.99	1.10-3.60	0.023	2.34	0.98-5.59	0.055
Maximum tumor size, cm	<5 / ≥5	91/13	0.49	0.29-1.82	0.105			
Tumor number	<5 / ≥5	82/22	2.28	1.24-4.18	0.008	2.60	1.01-6.67	0.047

CEA, ng/ml	<20 / ≥20	67/37	2.35	1.36-4.06	0.002		1.55	0.70-3.41	0.281
CA19-9, U/ml	<100 / ≥100	90/14	2.79	1.13-6.87	0.026		2.63	0.82-8.46	0.104
Location of primary colorectal cancer	Colon / Rectum	66/38	1.07	0.61-1.89	0.809				
T factor of primary colorectal canccer (UICC 7th)	T1-3 / T4	70/34	1.01	0.56-1.80	0.986				
N factor of primary colorectal cancer (UICC 7th)	N0-1 / N2-3	76/28	1.86	1.04-3.34	0.036		1.87	0.74-4.70	0.186
Histological differentiation of primary colorectal cancer	Well / Other	41/63	1.44	0.81-2.57	0.211				
Herpes virus entry mediator (HVEM) expression	High / Low	55/49	2.34	1.34-4.10	0.002		3.35	1.41-7.93	0.006
CRLM colorectal liver metastasis, *CEA* carcinoembryonic antigen, *CA19-9* carbohydrate antigen 19-9, *UICC* Union for International Cancer Control									