Role of thrombospondin-1 expression in colorectal liver metastasis and its molecular mechanism

Hiroki Teraoku, Yuji Morine, Tetsuya Ikemoto, Yu Saito, Shinichiro Yamada, Masato Yoshikawa, Chie Takasu, Jun Higashijima, Satoru Imura and Mitsuo Shimada.

Department of Surgery, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima 770-8503, Japan

Subtitle: THBS1 expression in CRLM

Type of paper: Original article

Key words: Thrombospondin-1, colorectal liver metastasis, immunohistochemistry

Words count of the abstract: 199 words

Words count of the text: 3,011 words

Words count of the figures: 83 words

Number of figures and tables: 3 figures and 4 tables

Conflict of Interest

The authors declare that they have no competing interests.

Address for correspondence:

Mitsuo Shimada, M.D, Department of Surgery, Institute of Biomedical Sciences, Tokushima University Graduate School

3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan.

Tel: +81-88-633-9276

Fax: +81-88-631-9698

E-mail: **mitsuo.shimada@tokushima-u.ac.jp**

ABSTRACT:

Background/Purpose

Thrombospondin-1 (THBS-1), a glycoprotein, is an endogenous inhibitor of angiogenesis and tumor growth. In this study, we investigated the clinical role and mechanism of THBS-1 expression in colorectal liver metastases, focusing on the relationships between its expression and tumor growth, epithelial–mesenchymal transition (EMT), and expression of other relevant molecules.

Methods

Ninety-four patients who initially underwent curative hepatic resection were enrolled in this study and correlations between expression of THBS-1 (THBS-1 high [n=35] and THBS-1 low [n=59]) and tumor growth, Ki-67 labelling index (Ki-67 LI), expression of other relevant molecules, and microvessel density (MVD) investigated.

Results

THBS1 low expression correlated with more advanced grade of liver and lymph node metastases and significantly worse overall survival than strong THBS1 expression (3-year survival: 96.7% vs. 65.4%, p<0.01). Multivariate analysis identified THBS1 low expression as an independent prognostic factor (HR=2.82, 95% CI=1.21–7.71, p=0.01). THBS-1 low expression correlated positively with high Ki-67 LI (p<0.05) and inversely with E-cadherin (p<0.05) and hypoxia inducible factor-1α (HIF-1α) expression (p<0.05); THBS-1 expression and MVD were not significantly correlated.

Conclusions

Low THBS-1 expression may be an independent poor prognostic factor that affects tumor growth and EMT acquisition. Additionally, THBS-1 may be regulated by the HIF-1 pathway.

INTRODUCTION:

Colorectal cancer, one of the most commonly diagnosed cancers worldwide in both male and female subjects, has high morbidity and mortality [1]. Liver metastasis is one of the most significant prognostic factors in patients with colorectal cancer, 15% to 25% of whom have liver metastases (CRLM) at diagnosis [2, 3]. Furthermore, CRLM occurs in 25% to 50% of cases within 3 years of resection of a primary colorectal tumor [4-6]. Hence, because of their systemic nature and resistance to therapeutic agents, metastatic rather than primary tumors are the major contributor to patient death [7, 8]. It is therefore important to identify prognostic biomarkers, promising molecular targets, and the mechanisms of the malignant behavior of CRLM.

The thrombospondin family of high molecular weight glycoproteins has five members (thrombospondin-1, -2, -3, -4, and -5) [9]. Thrombospondin-1 (THBS-1) is known to be expressed during the tissue remodeling associated with wound healing and tumor progression [9, 10]. Low THBS-1 expression is reportedly correlated with a poor prognosis in cervical [11], lung [12], and breast cancer [13]. In terms of the mechanisms of action of THBS-1, several reviews have discussed the role of THBS-1 in inhibiting angiogenesis and tumor growth [9, 14, 15]. According to these reports, THBS-1 inhibits tumor growth via activating transforming growth factor-β (TGF-β) [16-18] and inhibits angiogenesis through direct effects on endothelial cells and indirect effects on vascular endothelial cell growth factor (VEGF) [19]. As to other mechanisms of THBS-1 in cancer, a correlation with epithelial-mesenchymal transition (EMT) has reported in pancreatic carcinoma [20].

Hypoxia-inducible factor-1 (HIF-1), a transcription factor that responds to changes in available oxygen in the cellular environment, is considered the master transcriptional regulator of cellular and developmental responses to hypoxia [21, 22]. HIF-1 induces transcription of genes involved in angiogenesis, cell proliferation, and survival [23]. This understandings indicate HIF-1 possibly regulate angiogenesis or cell

proliferation through THBS-1 pathway. It was recently reported that HIF-1α may regulate THBS-1 in renal carcinoma [24] and colon cancer cell lines [25]. However, no studies have simultaneously investigated all of these molecular mechanisms and how THBS-1 is regulated remains unclear. Furthermore, the relationship between THBS-1 and HIF-1 expression has not been elucidated, especially not in clinical settings.

To identify the clinical biomarker contributing to patient prognosis in CRLM, we focused on THBS-1 in this study. The aims of this study were to evaluate the clinical role of THBS-1 expression in colorectal liver metastasis and to investigate the relationships of its molecular mechanism with tumor growth, EMT, and HIF-1 expression.

METHODS:

Patients and specimens

Ninety-four patients who initially underwent curative hepatic resection at Tokushima University from 1994 to 2013 were enrolled in this study retrospectively. Patients who underwent non-curative surgical treatment or preoperative chemotherapy or both were excluded. Fifty-one patients underwent synchronous resection of a primary colorectal cancer and liver metastases; the remaining 43 underwent metachronous hepatic resection. Regarding the primary region, 55 patients had colon and 39 rectal cancers (Table 1). Regarding the extent of liver metastasis, H-number and grade were classified according to the Japanese Classification of Colorectal Carcinoma, Second English Edition [26]. 64 patients received adjuvant chemotherapy after hepatectomy; 20 was received oxaliplatin – based chemotherapy, 14 was irinotecan – based chemotherapy and 30 was others. Among 59 relapsed patients after initial hepatectomy, 24 patients received another chemotherapy treatment regimen, 17 patients received repeat hepatectomy against the hepatic recurrence and 18 patients was observed without any treatment. Furthermore, 60 patients were also assessed in primary colorectal cancer.

Operative specimens were embedded in paraffin and stained with hematoxylin and eosin for histopathological diagnosis by the Pathology Department of Tokushima University Hospital. This study was approved by the Research Ethics Committee of Tokushima University Hospital and written informed consent was obtained from all patients.

Immunohistochemistry Analysis

Immunohistochemistry was performed according to the protocol used in our department; this has previously been reported by Ishikawa et al. [27]. Tissue specimens were fixed in 10% formaldehyde, embedded in paraffin, and cut into 4 μm-thick sections that were deparaffinized with xylene, dehydrated with ethanol, and then treated with 0.03% hydrogen peroxide in methanol for 10 minutes. For antigen retrieval, the sections were heated in 10 mM citrate buffer (pH 6) in a microwave. After cooling the sections at room temperature and washing three times for 5 minutes in phosphate-buffered saline, the sections were incubated with 1% goat serum to block nonspecific reactions. Next, the sections were incubated with primary anti-bodies against THBS-1 (anti-THBS-1 antibody, dilution 1:50, LS-C87484; LSBio, Seattle, WA, USA), Ki-67 (anti-human Ki-67 antigen clone MIB-1, dilution 1:100, M7240; Dako, Tokyo, Japan), E-cadherin (anti-E-cadherin antibody, dilution 1:200, M3612; Dako), CD34 (anti-CD34 antibody, dilution 1:200, M7165; Dako), CD105 (anti-CD105 antibody, dilution 1:100, M3527; Dako), or HIF-1α (anti-HIF-1α antibody, dilution 1:100, HPA001275; Sigma, St Louis, MO, USA) for 60 min at room temperature. After washing three times for 5 minutes in phosphate-buffered saline, the sections were subjected to a Dako REAL EnVision/HRP detection system (Dako, Tokyo, Japan) for 60 minutes at room temperature. The sections were then washed three times for 5 minutes in phosphate-buffered saline, the peroxidase reaction developed with 3, 30-diaminobenzidine (Sigma-Aldrich), and the sections counterstained with 10%

Mayer's hematoxylin. The sections were then dehydrated, treated with xylene, and enclosed.

Evaluation of immunostaining

All immunostaining was evaluated by a pathologist who was blinded to clinical information. THBS-1 expression was evaluated by scoring staining intensity (0, negative; 1, weak; 2, moderate; 3, intense) and stained area (0, 0–10%; 1, 10%–25%; 2, 25%–50%; 3, ≥50%) [28]. A cut-off value for THBS-1 total score was set at 3 and the subjects divided into two groups accordingly: THBS-1 high (total score ≥3, n=35; 37.2%) and THBS-1 low (total score <3, n=59; 62.8%). Ki-67 LI was scored by counting cells with positive nuclei in at least five fields with more than 500 tumor cells per high-power field (HPF) [29-31]. The percentage of positive cells was then used to express Ki-67 LI and the subjects divided into two groups according to the median value (41%). E-cadherin expression was scored as either membranous staining positive (+) or negative (−) [32]. MVD was evaluated as previously reported [33-35] by calculating the average number of positive staining cells in three to five hot spot fields per HPF for each tumor section and the subjects divided into two groups by the median value. HIF-1α expression was evaluated by scoring the staining intensity (0, negative; 1, low; 2, medium; 3, high) and extent of staining (0, 0%; 1, 1%–25%; 2, 26%–50%; 3, ≥51%) [36].

Statistical analysis

All statistical analyses were performed using JMP 11 (SAS Institute, Cary, NC, USA). The χ^2 test was used to compare values between the two groups and the relationships between THBS-1 expression and that of other molecules. Overall survival and disease-free survival curves were generated using the Kaplan–Meier method and differences were compared using the log-rank test. Multivariate analysis was carried out

based on the Cox proportional hazard regression model. For all statistical analyses, a p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS:

THBS-1 expression in colorectal liver metastases

THBS-1 in cancer cells' cytoplasm was stained by immunohistochemistry in 94 patients with colorectal liver metastases. Representative examples of the staining of THBS-1 are shown in Fig. 1A-D. Correlations between THBS-1 expression and clinicopathological factors are shown in Table 1. THBS1 low was significantly correlated with more advanced grade (Grades B/C) (p=0.01) and presence of lymph nodes metastases (p=0.02). We also investigated THBS-1 expression in primary site (n=60), and THBS-1 expression tended to have positive correlation with the metastatic site (p=0.07, Table 1).

Correlation between THBS-1 expression and Ki-67, E-cadherin, MVD, and HIF-1α

To investigate the molecular mechanism of the THBS-1 pathway, other molecules, namely Ki-67, E-cadherin, MVD, and HIF-1α, were stained in the same liver specimens. Representative examples of staining are shown in Fig. 2. Staining was evaluated and categorized as described in the Methods section. Table 2 shows the correlations between THBS-1 expression and that of other molecules. THBS-1 expression inversely correlated with Ki-67 LI (p<0.05), and HIF-1α expression (p<0.05), and positively correlated with E-cadherin expression (p<0.05). However, there was no significant correlation between THBS-1 expression and MVD for either CD34 or CD105.

THBS-1 low expression is an independent prognostic factor

The THBS1 low group had a significantly worse overall survival than the THBS1 high group, their 3-year survival rates being 65.4% and 96.7%, respectively (p<0.01)

(Fig. 3A). The THBS-1 low group also tended to have a poorer disease-free survival than the THBS-1 high group (p=0.06) (Fig. 3B). Mean follow-up period was 4.76 years (range, 0.67–13.99 years) and 3.71 years (range, 0.39-13.39 years) after hepatectomy.

Univariate analysis in overall survival identified Grade B/C (3-year survival rate; 87.5% vs 67.8%, p=0.03) as poor prognostic factors in addition to low expression of THBS1 (Table 3). Furthermore, we also investigated whether other molecules were significant prognostic factors or not. In univariate analysis, Ki-67 LI high (cut-off value; 41%) was identified as a significant prognostic factor (p=0.02), and E-cadherin low expression tended to have a poorer overall survival (p=0.06), however, HIF-1α expression was not a prognostic factor (p=0.30). Multivariate analysis (proportional hazard model) of those prognostic factors identified THBS1 low expression as the only independent prognostic factor (HR=2.61, 95% CI; 1.00-8.16, p<0.05) (Table 3).

There were no significant correlations between recurrence pattern and THBS-1 expression group; however, peritoneal recurrence was observed only in the THBS-1 low group (Table 4). In addition, repeat hepatectomy for liver recurrence was performed significantly less frequently in the THBS-1 low group (Table 1).

DISCUSSION

In this study, we investigated THBS-1 expression in 94 patients with CRLM. THBS1 low expression correlated significantly with more advanced grade (Grades B/C), presence of lymph nodes metastases, and poor prognosis, being an independent prognostic factor in patients with CRLM. Our findings are consistent with previous reports about other cancers, such as cervical, lung, and breast cancer [11-13]. Moreover, we identified correlations between Ki-67 LI and HIF-1α and E-cadherin expression and THBS-1 expression. Although we found no significant correlations between disease-free survival and recurrence pattern and strength of THBS-1 expression, peritoneal recurrence occurred only in the THBS-1 low group. Repeat hepatectomy for

liver recurrence was performed significantly less frequently in the THBS-1 low group (data not shown). Those findings indicate that THBS-1 low expression is associated with more out-of-control recurrence and a poorer prognosis than strong THBS-1 expression.

THBS-1, a member of the thrombospondin family, was first isolated from platelets that had been stimulated with thrombin in 1971 [37]. THBS-1 is 450-kD large glycoprotein and has multiple domains comprising a heparin domain, procollagen-like domain, type 1, 2, and 3 repeats and a cell-binding domain [38]. For this reason, THBS-1 interacts with numerous receptors and proteases involved in cell adhesion, angiogenesis, and cell growth [38, 39]. In cancers, THBS-1 reportedly functions as a natural inhibitor of neovascularization and tumorigenesis [9, 10]. Our finding that THBS-1 low expression is correlated with a poor prognosis is consistent with previous reports [11-13]. On the other hand, it has also been reported that strong THBS-1 expression is correlated with a poor prognosis in melanoma [40], intraductal papillary mucinous neoplasm [41], pancreatic carcinoma [42], and colorectal liver metastasis [43]. However, in those studies, THBS-1 expression was evaluated not in the cytoplasm but in the stroma. Because THBS-1 is a large glycoprotein with multiple domains, the significance of its expression may vary according to its location.

In this study, we examined the downstream signaling of THBS-1 focusing on tumor growth, angiogenesis, and EMT. However, THBS-1 expression did not correlate with MVD as indicated by CD34 and CD105 antibodies, which are markers of vascular endothelial cells. CD34 is a common marker of MVD, whereas CD105 is reportedly a novel vascular marker that can detect immature, but not mature, vessels [36]. Other molecules such as vascular endothelial growth factor (VEGF) may be correlated with THBS-1 expression. Regarding tumor growth, THBS-1 expression was inversely correlated with Ki-67 LI. THBS-1 inhibits tumor growth via activating TGF-β [16-18]. Although we did not assess TGF-β expression in this study, Ki-67 protein is present

during all active phases of the cell cycle and is well known as an excellent biomarker for cellular proliferation [44]. Therefore, THBS-1 may regulate tumor growth. There are few reports of correlations between THBS-1 and EMT. Nakajima et al. reported that fibroblast growth factor-2, which is inhibited by THBS-1, induces EMT in pancreatic carcinoma [20]. Our finding that THBS-1 expression correlates with E-cadherin expression is consistent with this report and suggests that THBS-1 regulates EMT. Our findings suggest that THBS-1 low expression is associated with a poor prognosis through induction of tumor growth and EMT.

HIF-1 is overexpressed in many human cancers [45]. Strong expression of HIF-1α in a number of cancers has been associated with aggressive tumor progression, suggesting that it is a predictive and prognostic marker for resistance to radiation treatment, chemotherapy, and increased mortality [46]. Recently, HIF-1 was reported to be one of the regulators of THBS-1 [24, 25]; however, few studies have explored their relationships. Our finding that THBS-1 expression is inversely correlated with HIF-1α expression indicates that THBS-1 may be regulated by HIF-1α in CRLM.

A relationship between microRNA (miR) and various cancers was recently reported by a number of research groups. Two types of miRs are reportedly involved in cancer; one as an oncogene and the other as a tumor suppressor gene. Some microRNAs reportedly regulate THBS-1 [47-50] and HIF-1α [51, 52]; in particular, miR-21 and miR-182 are reportedly correlated with both THBS-1 and HIF-1 [48, 50-52]. Therefore, miR-21 and miR-182 may exist upstream of THBS-1 or HIF-1 signaling pathway and regulate both HIF-1 and THBS-1 expression.

In this study, we demonstrated that THBS-1 low expression is an independent prognostic factor in CRLM. We showed that THBS-1 may inhibit tumor growth and EMT downstream of it and that THBS-1 is possibly regulated by HIF-1α in CRLM. Limitations of this study include that we identified correlations between THBS-1 expression and other factors by immunohistochemistry means, which are not

quantitative. To more precisely demonstrate its regulatory mechanisms, additional experiments such as knock-down experiments *in vivo* are necessary.

In conclusion, THBS-1 low expression may be an independent poor prognostic factor that affects malignant behavior, including tumor growth and EMT acquisition. Additionally, THBS-1 may be regulated by the HIF-1 pathway.

Discussion at the plenary session of 28th Congress of the Japanese Society of Hepato-Biliary-Pancreatic Surgery

Comments by H. Yoshidome

The present data showed that THBS-1 was not associated with angiogenesis. Although HIF-1α is well known to regulate several molecules associated with angiogenesis such as VEGF, the authors should discuss the more possible mechanism of regulation of HIF-1α to THBS-1.

Answer by H. Teraoku

Of course, HIF-1α is known to regulate angiogenesis, and VEGF is one of the typical target of HIF-1α. Therefore, we expected to correlate between VEGF and THBS-1 or HIF-1α expression. However, we could not stain well although some anti-VEGF primary antibodies were used (DAKO; M7273; 1:50, SANTA CRUZ; sc-152; 1:50). In relation to the MVD as the actual evaluation of angiogenesis, there was no correlation with either THBS-1 or HIF-1α though we tried more than five times with several methods for counting microvessels. Regarding the correlation between THBS-1 and HIF-1α, there are few reports. In this study, there was the correlation between THBS-1 and HIF-1α, however, it is still unclear whether HIF-1 truly regulate THBS-1 on upstream. Therefore, it is necessary to investigate its correlation and other possible mechanism for regulation of these molecules *in vitro* and *in vivo*.

Comments by M. Kido

Why is there high THBS-1 expression low in metastatic site though THBS-1 expression in the primary site?

Answer by H. Teraoku

In this study, we concluded that THBS-1 may be regulated by HIF-1α pathway in CRLM. It is reported that THBS-1 has multiple domains and interacts with numerous receptors and proteases involved in cell adhesion, angiogenesis, and cell growth. For this reason, THBS-1 expression may be regulated by several molecules even in metastatic site. Therefore, it is considered that THBS-1 expression in metastatic site may be up-regulated by other cytokines, and there are some patients with THBS-1 high expression in liver metastatic site.

Comments by K. Takeda

For THBS-1 low group, what kind of intervention is necessary?

Answer by H. Teraoku

In this study, THBS-1 low expression was the only prognostic factor in overall survival. Also, in disease-free survival, THBS-1 low expression tended to be a poor prognostic factor, and it was suspected that THBS-1 low expression group occurred more uncontrollable recurrence. Hence, some kind of intervention should be necessary in patients with THBS-1 low expression. Regarding the induction of THBS-1, several phaseⅡtrials with ABT-510 which is THBS-1 analog have already reported and these trials unexpectedly failed to reach its primary endpoint. However, it showed the decrease of VEGF expression in some patients. In the future, so that THBS-1 could be a new therapeutic agent for cancers, the combination of other molecular targeted agent may be necessary. At least, the immediate adjuvant chemotherapy after hepatectomy should be introduced in patients with THBS-1 low expression.

Comments by T. Kamiyama

The authors showed that THBS-1 is possibly regulated by HIF-1α in CRLM and THBS-1 was related to overall survival. Why HIF-1α which regulated THBS-1 was not significant prognostic factor by univariate analysis?

Answer by H. Teraoku

THBS-1 low expression was the only prognostic factor in overall survival and could be a poor prognostic factor in disease-free survival. THBS-1 low expression occurred the more massive recurrence and the less introduced repeat hepatectomy. Hence, loss of THBS-1 expresses the tumor malignancy including uncontrollable recurrent pattern, therefore this molecule could be a reliable predictor for tumor malignancy. On the other hand, HIF-1α was not identified as a prognostic factor in overall survival, however, HIF-1α high expression was a strong prognostic factor in disease free survival ($p < 0.01$, data not shown). It is suggested that HIF-1α does not reflect the degree of recurrence compared to THBS-1, though HIF-1α high expression is a prognostic factor in disease-free survival. Therefore, HIF-1α was not identified as a prognostic factor in overall survival in this study.

ACKNOWLEDGEMENT:

CONFLICT OF INTEREST:

The authors declare that they have no competing interests.

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Figure 1: Representative immunohistochemical staining of THBS-1 in CRLM

Representative staining of THBS-1; (a) score 6, (b) score 4, (c) score 2 and (d) score 0.

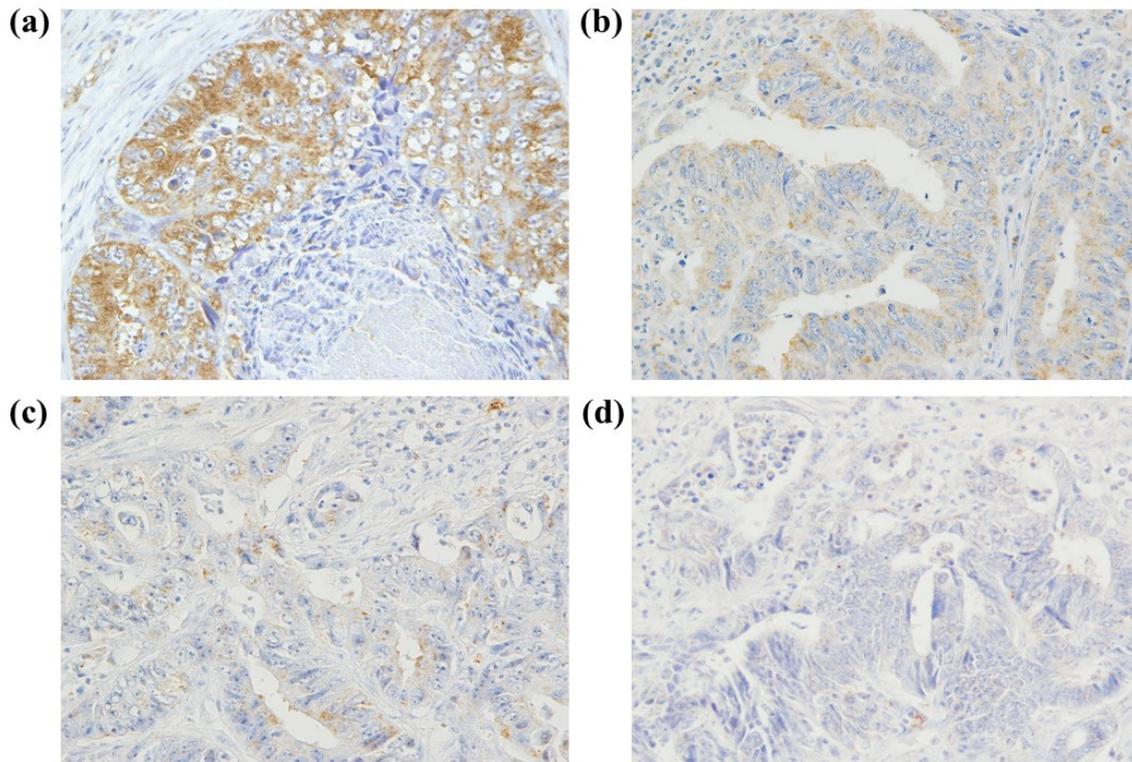THBS-1 expression was assessed in the cytoplasm of cancer cells.

Figure 2: Correlation with possible molecules of THBS-1 pathway.

Representative examples of staining for Ki67, E-cadherin, CD34, CD105, and HIF-1α.
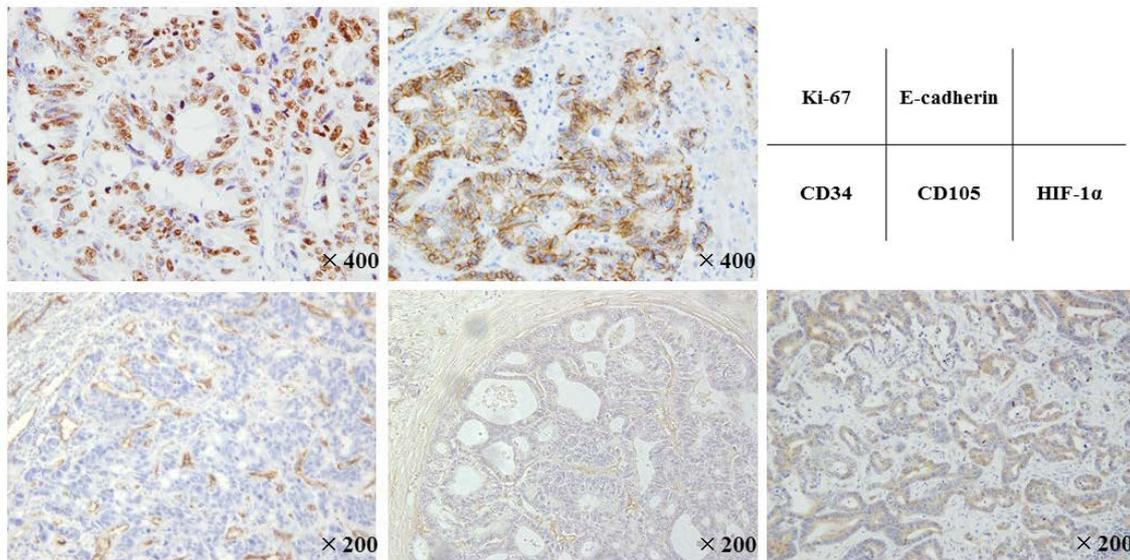
Figure 3: Kaplan–Meier curves according to THBS-1 expression in CRLM.

(a) Overall survival curves according to THBS-1 expression.

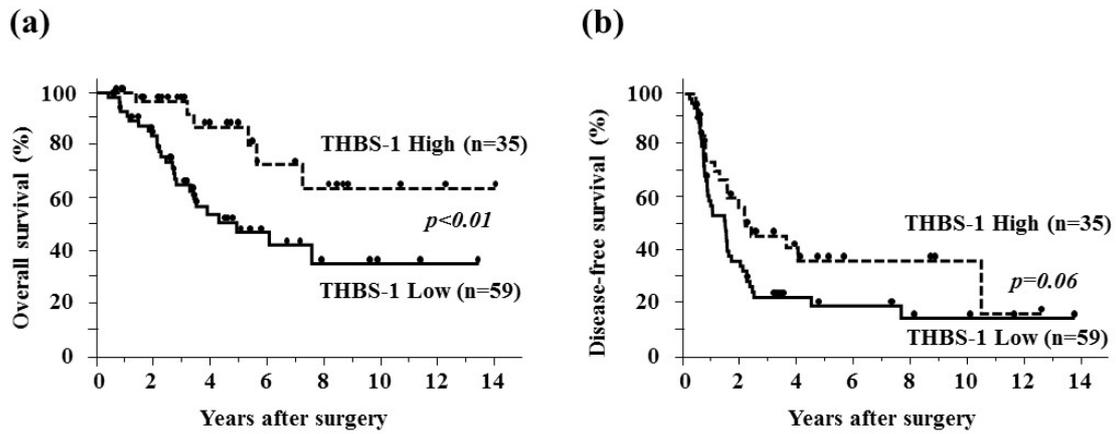**(b)** Disease-free survival curves according to THBS-1 expression.

Table 1. Clinicopathological factors according to THBS-1 expression

Factors		THBS-1 low (n=59)	THBS-1 high (n=35)	*P* value
Metastatic site				
Age	<65 / ≧65	26 / 33	17 / 18	0.67
Sex	Male / Female	24 / 35	9 / 26	0.13
Metastasis period	Synchro. / Metachro.	32 / 27	19 / 16	0.90
Size of tumor (cm)	<5 / ≧5	42 / 17	28 / 7	0.42
Number of tumors	<3 / ≧3	35 / 24	17 / 18	0.27
H classification	1 / 2,3	38 / 21	24 / 11	0.75
Grade classification	A / B,C	23 / 36	24 / 11	0.01
CEA (ng/ml)	<5 / ≧5	10 / 34	9 /19	0.37
CA19-9 (U/ml)	<37 / ≧37	28 / 16	18 / 10	0.95
Primary site				
Location	Colon / Rectum	36 / 23	19 / 16	0.52
Differentiation	Well / Others	20 / 39	10 / 25	0.86
LN metastasis	Negative / Positive	19 / 40	20 / 15	0.02
THBS-1 (primary site)	Negative / Positive	30 / 4	18 / 8	0.07
Treatment for the recurrence	Hx ± Chemo. / Chemo. only / BSC	9 / 17 / 15	8 / 7 / 3	0.14

CEA: carcinoembryonic antigen, CA19-9: carbohydrate antigen 19-9, LN: lymph node,

synchro.: synchronous metachro.: metachronous, Hx: hepatectomy, Chemo.:

chemotherapy, BSC: best supportive care

Table 2. Correlation between THBS-1 expression and that of other molecules

Parameters		THBS-1 low (n=59)	THBS-1 high (n=35)	P value
Ki-67 LI	Low / High	24 / 35	22 / 13	0.04
E-cadherin	Low / High	25 / 34	8 / 27	0.05
MVD (CD34)	Low / High	35 / 24	20 / 15	0.84
MVD (CD105)	Low / High	29 / 30	19 / 16	0.63
HIF-1α	Low / High	12 / 47	14 / 21	0.04

Ki-67 LI: Ki-67 Labelling Index, MVD: microvessel density

Table 3. Prognostic factors in patients with CRLM

Factors		3-year OS rate (%)	Univariate *P* value	Multivariate HR (95% CI)	*P* value
Metastatic site					
Age	<65 / ≧65	81.1 / 72.4	0.88		
Sex	Male / Female	76.1 / 81.4	0.83		
Metastasis period	Synchro. / Metachro.	76.0 / 80.3	0.36		
Size of tumor (cm)	<5 / ≧5	80.7 / 66.5	0.44		
Number of tumors	<3 / ≧3	77.2 / 80.7	0.17		
H classification	1 / 2,3	80.6 / 72.7	0.26		
Grade classification	A / B,C	87.5 / 67.8	0.03	1.70 (0.77-3.95)	0.19
CEA (ng/ml)	<5 / ≧5	93.7 / 78.3	0.13		
CA19-9 (U/ml)	<37 / ≧37	82.4 / 83.0	0.38		
THBS-1	Low / High	65.4 / 96.7	0.01	2.61 (1.00-8.16)	<0.05
Ki-67 LI	Low / High	89.6 / 66.2	0.02	1.89 (0.87-4.32)	0.11
E-cadherin	Low / High	59.0 / 86.2	0.06	1.74 (0.81-3.78)	0.16
MVD (CD34)	Low / High	77.9 / 77.0	0.89		
MVD (CD105)	Low / High	69.8 / 82.5	0.41		
HIF-1α	Low / High	91.7 / 71.0	0.30		
Primary site					
Location	Colon / Rectum	76.2 / 80.9	0.35		
Differentiation	Well / Others	78.3 / 75.7	0.91		
LN metastasis	Negative / Positive	82.3 / 74.5	0.44		
THBS-1 (primary site)	Negative / Positive	70.3 / 74.6	0.37		

CEA: carcinoembryonic antigen, CA19-9: carbohydrate antigen 19-9, LN: lymph node,

synchro.: synchronous, metachro.: metachronous

Table 4. Correlation between THBS-1 expression and recurrence site

	THBS-1 low (n=59)	THBS-1 high (n=35)	*P* value
Recurrence	41 (69.7%)	18 (51.4%)	0.08
Sites of recurrence			
Liver	28 (68.3%)	13 (72.2%)	0.76
Lung	17 (41.5)	9 (50.0%)	0.54
Lymph nodes	5 (12.2%)	1 (5.6%)	0.41
Peritoneum	2 (4.9%)	0 (0.0%)	0.22