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Original Article

Low thrombospondin 2 expression is predictive of low tumor regression after neoadjuvant chemoradiotherapy in rectal cancer

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Abstract: Background: Neoadjuvant concurrent chemoradiotherapy (CCRT) followed by surgery is the mainstay of treatment for locally advanced rectal cancer. Several heparin-binding associated proteins have been reported to play a critical role in cancer progression. However, the clinical relevancies of such proteins and their associations with CCRT response in rectal cancer have not yet to be fully elucidated. Methods: The analysis of a public transcriptome of rectal cancer indicated that *thrombospondin 2 (THBS2)* is a predictive factor for CCRT response. Immunohistochemical analyses were conducted to evaluate the expression of THBS2 in pretreatment biopsy specimens from rectal cancer patients without distant metastasis. Furthermore, the relationships between THBS2 expression and various clinicopathological factors or survival were analyzed. Results: Low expression of THBS2 was significantly associated with advanced pretreatment tumor (P<0.001) and nodal status (P=0.004), post-treatment tumor (P<0.001) and nodal status (P<0.001), increased vascular invasion (P=0.003), increased perineural invasion (P=0.023) and inferior tumor regression grade (P=0.015). In univariate analysis, low THBS2 expression predicted worse outcomes for disease-free survival, local recurrence-free survival and metastasis-free survival (all P<0.001). In multivariate analysis, low expression of THBS2 still served as a negative prognostic factor for disease-free survival (Hazard ratio=3.057, P=0.002) and metastasis-free survival (Hazard ratio=3.362, P=0.012). Conclusion: Low THBS2 expression was correlated with advanced disease status and low tumor regression after preoperative CCRT and that it acted as an independent negative prognostic factor in rectal cancer. THBS2 may represent a predictive biomarker for CCRT response in rectal cancer.

Keywords: Thrombospondin 2 (THBS2), rectal cancer, chemoradiotherapy, concurrent chemoradiotherapy (CCRT)

Introduction

Because of advancements in economic development and changes in dietary habits, the incidence of colorectal cancer has markedly increased within the past 20 years. Currently, colorectal cancer is the third leading cause of cancer death in males and females in Taiwan (Cancer leading cause of death in Taiwan: http://www.mohw.gov.tw/cht/Ministry/DM2_P.aspx?f_list_no=7&fod_list_no=5313&doc_no=49778). Surgery is the primary treatment for early rectal cancers that have not spread to distant organs. For locally advanced rectal cancers, preoperative concurrent chemoradiotherapy (CCRT) followed by surgical resection is the recommended strategy. Preoperative CCRT may result in cancer shrinkage and render sur-

gery increasingly effective for large tumors. It can also reduce the rate of local recurrence [1-3]. Hence, identifying potential biomarkers that can predict CCRT response is highly valuable in cancer therapy because such biomarkers can facilitate risk stratification and the selection of optimal treatment strategies for patients with rectal cancer who have poor response to neoadjuvant CCRT. Because of the improvements in bioinformatics tools used for analyzing large amounts of gene expression microarray data, the possibility of identifying key biomarkers for accurately predicting CCRT response in rectal cancer is increased.

Previous studies have revealed that several heparin-binding associated proteins, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor receptor (FGFR), play a critical role in cancer cell invasion or progression in colorectal cancer [4, 5]. In the current study, we applied data mining to a public expression profiling dataset of rectal cancer treated with neoadjuvant CCRT (GEO: GSE35452) [6] to search for genes with a molecular function that correlates with that of genes for heparin-binding proteins, and are differentially expressed between the responders and non-responders. We identified *Thrombospondin 2 (THBS2)* as one of the genes considerably downregulated in the non-responders. We observed that in gastric cancer, low THBS2 expression was associated with poor histological gastric cancer grades. *In vitro* analyses of gastric cancer cell lines further demonstrated that high THBS2 expression was associated with a low proliferation rate [7]. Another study also indicated that THBS2 expression was a key inhibitor of angiogenesis and metastasis in colon cancer [8]. Therefore, we focused on *THBS2* as a molecular target to further investigate its role in CCRT response in rectal cancer. *THBS2* belongs to a five-member thrombospondin family comprising *THBS1*, *THBS2*, *THBS3*, *THBS4*, and *THBS5*. THBS2 is a calcium-binding, disulfide-linked homotrimeric glycoprotein that mediates cell adhesion. Studies have shown that this protein exerts inhibitory effects on endothelial cell proliferation and angiogenesis [9, 10].

In this study, we evaluated THBS2 protein expression levels in 172 pairs of cancer tissue and adjacent normal mucosa obtained from patients with rectal cancer receiving neoadjuvant CCRT followed by surgery. We attempted to determine the relationships between THBS2 expression and various clinicopathological characteristics, particularly tumor regression after CCRT. We analyzed the association between THBS2 expression and survival to establish its prognostic significance.

Materials and methods

Analysis of the expression profiles in rectal cancer

In order to identify genes that are differentially expressed between the responders and non-responders to neoadjuvant CCRT, we analyzed the expression profiles of a transcriptomic dataset (GSE35452) composed of 46 rectal cancer patients treated with neoadjuvant CCRT [6]. We used Nexus Expression 3 software (BioDiscovery) to perform comparative analysis of the raw CEL files of GSE35452, and particularly focused on genes associated with heparin binding (GO:0008201). Those genes with P value<0.01 and log 2-transformed expression fold change >±0.1 were selected for further analysis.

Patients and tissue samples

This study included 172 patients with primary rectal adenocarcinoma who underwent neoadjuvant CCRT followed by surgical resection. The study was approved by the Institutional Review Board of Chi Mei Medical Center (IRB10302-014). The pre-treatment tumor samples of rectal cancer were obtained from those with adequate paraffin-embedded tissue blocks and histologically proven primary rectal adenocarcinoma at Chi Mei Medical Center (Tainan, Taiwan) between 1998 and 2004. Patients having initial distant metastasis, screened by chest X-radiography and/or abdominopelvic CT, were excluded. The pre-treatment clinical staging was evaluated by using rectal endoscopic ultrasound (EUS) with or without abdominopelvic CT scan. All of these patients received radiation therapy at a total dose of 45 Gy in 25 fractions over a 5-week period with a 24-h continuous infusion of 5-fluorouracil concurrently before surgery. Adjuvant systemic chemotherapy was performed for those with either positive

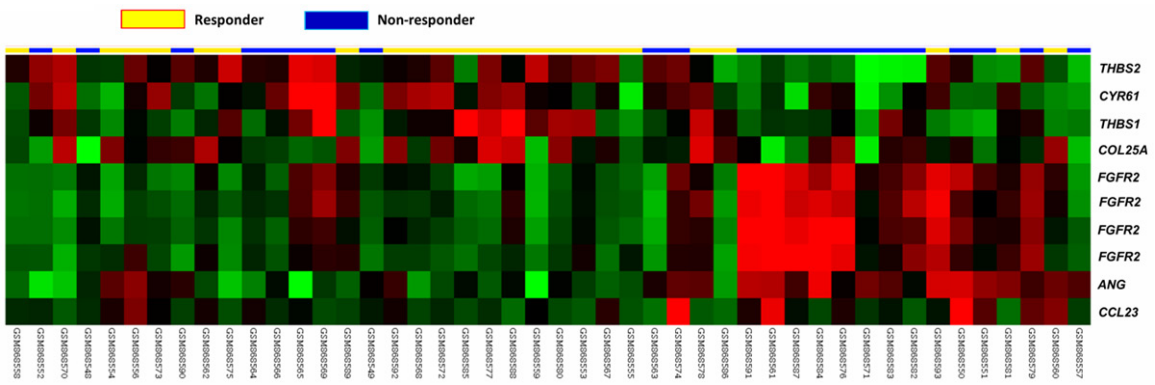

Figure 1. Microarray analysis revealed differential gene expression between the responders and non-responders to neoadjuvant CCRT in rectal cancer. Data mining from a published transcriptomic dataset of rectal cancer with emphasis on genes associated with heparin binding (GO:0008201) demonstrated that THBS2 was one of the significantly downregulated genes in the non-responders. The color-coded scale was illustrated as green for downregulation and red for upregulation, respectively.

nodal status or tumor status of T3 to T4 in the pre-treatment (Pre-Tx) or post-treatment (Post-Tx) status. All patients were under regular follow-up after diagnosis until death or their last appointment.

Immunohistochemistry and histopathological evaluation

Two pathologists (HL He and IW Chang), who were blinded to the patients' information separately evaluated the histopathological features of the tumor specimens. Post-Tx staging was assessed based on the 7th American Joint Committee on Cancer (AJCC) TNM staging system. Tumor regression grade (TRG) was documented as described by Dworak et al [11]. Immunohistochemical study was performed to assess the expression of THBS2. In brief, tissue sections from Pre-Tx rectal tumor biopsies were cut from paraffin-embedded tissue blocks at 3 mm thickness onto precoated glass slides. Slides were then deparaffinized with xylene, rehydrated with ethanol and heated for 7 min by microwave for antigen retrieval in a 10-mM citrate buffer (pH 6). Endogenous peroxidase was blocked by using 3% H_2O_2. Slides were then washed with Tris buffered saline for 15 min and then incubated with a primary monoclonal antibody against THBS2 (1:50, Rabbit polyclonal, Novus Biologicals, CO, U.S). The THBS2 staining was assessed using the H-score by the following equation: H-score = $\Sigma Pi\ (i+1)$, in which i is the intensity of the tumor cells stained (0 to 3+), and Pi is the percentage of stained tumor cells of various intensities. Low expression of THBS2 was defined as having H-scores less than the median of all scored cases.

Statistical analysis

The Chi-square test was used to investigate the relationships between THBS2 expression and various clinicopathological features. For survival analysis, the survival curves, including disease-free survival (DFS), local (pelvic) recurrence-free survival (LRFS), and metastasis-free survival (MeFS), were constructed using the Kaplan-Meier method, and differences in survival were assessed by using the log-rank test. Those parameters demonstrating prognostic significance at univariate test were then enrolled into the Cox regression hazards model for multivariate analysis. For all analyses, differences were considered significant when the associated P value was less than 0.05 under two-sided tests. All statistical analyses were performed with the SPSS 14 software package (SPSS Inc., Chicago, Illinois, U.S).

Results

Downregulation of THBS2 was significantly associated with poor response to neoadjuvant CCRT

In the analysis of expression profiles in the public transcriptome GSE35452, we focused on genes associated with heparin binding (GO:0008201). We found that there were seven genes that were significantly associated with

Table 1. Summary of differentially expressed genes associated with heparin binding (GO:0008201) in relation to response to CCRT in rectal carcinoma

Probe	Comparison log2 ratio	Comparison *p*-value	Gene symbol	Gene name	Biological process	Molecular function
203638_s_at	1.3757	<0.0001	*FGFR2*	Fibroblast growth factor receptor 2 (bacteria-expressed kinase; keratinocyte growth factor receptor; craniofacial dysostosis 1; Crouzon syndrome; Pfeiffer syndrome; Jackson-Weiss syndrome)	Cell growth, protein amino acid phosphorylation	ATP binding, fibroblast growth factor receptor activity, heparin binding, kinase activity, nucleotide binding, protein binding, protein kinase activity, protein-tyrosine kinase activity, receptor activity, transferase activity
208228_s_at	1.1038	0.0001	*FGFR2*	Fibroblast growth factor receptor 2 (bacteria-expressed kinase; keratinocyte growth factor receptor; craniofacial dysostosis 1; Crouzon syndrome; Pfeiffer syndrome; Jackson-Weiss syndrome)	Cell growth, protein amino acid phosphorylation	ATP binding, fibroblast growth factor receptor activity, heparin binding, kinase activity, nucleotide binding, protein binding, protein kinase activity, protein-tyrosine kinase activity, receptor activity, transferase activity
203639_s_at	1.0028	<0.0001	*FGFR2*	Fibroblast growth factor receptor 2 (bacteria-expressed kinase; keratinocyte growth factor receptor; craniofacial dysostosis 1; Crouzon syndrome; Pfeiffer syndrome; Jackson-Weiss syndrome)	Cell growth, protein amino acid phosphorylation	ATP binding, fibroblast growth factor receptor activity, heparin binding, kinase activity, nucleotide binding, protein binding, protein kinase activity, protein-tyrosine kinase activity, receptor activity, transferase activity
205141_at	0.8389	0.0006	*ANG*	Angiogenin; ribonuclease; RNase A family; 5	Actin filament polymerization, activation of phospholipase A2, activation of protein kinase B, angiogenesis, cell communication, cell differentiation, cell migration, diacylglycerol biosynthetic process, homeostatic process, mRNA cleavage, multicellular organismal development, negative regulation of protein biosynthetic process, negative regulation of smooth muscle cell proliferation, ovarian follicle development, phospholipase C activation, placenta development, positive regulation of endothelial cell proliferation, positive regulation of phosphorylation, positive regulation of protein secretion, rRNA transcription, response to hypoxia	DNA binding, actin binding, copper ion binding, endonuclease activity, heparin binding, hydrolase activity, nuclease activity, nucleic acid binding, pancreatic ribonuclease activity, protein binding, rRNA binding, receptor binding, ribonuclease activity
203083_at	-0.8259	0.0041	*THBS2*	Thrombospondin 2	Cell adhesion	Calcium ion binding, heparin binding, protein binding, structural molecule activity
210764_s_at	-0.6505	0.0085	*CYR61*	Cysteine-rich; angiogenic inducer; 61	Anatomical structure morphogenesis, cell adhesion, cell proliferation, chemotaxis, patterning of blood vessels, regulation of cell growth	growth factor binding, heparin binding, insulin-like growth factor binding, protein binding
211401_s_at	0.415	0.0025	*FGFR2*	Fibroblast growth factor receptor 2 (bacteria-expressed kinase; keratinocyte growth factor receptor; craniofacial dysostosis 1; Crouzon syndrome; Pfeiffer syndrome; Jackson-Weiss syndrome)	Cell growth, protein amino acid phosphorylation	ATP binding, fibroblast growth factor receptor activity, heparin binding, kinase activity, nucleotide binding, protein binding, protein kinase activity, protein-tyrosine kinase activity, receptor activity, transferase activity
201107_s_at	-0.2892	0.0088	*THBS1*	Thrombospondin 1	Blood coagulation, cell adhesion, cell motility, inflammatory response, multicellular organismal development, negative regulation of angiogenesis, nervous system development	Calcium ion binding, endopeptidase inhibitor activity, heparin binding, protein binding, signal transducer activity, structural molecule activity
210549_s_at	0.2255	0.0034	*CCL23*	Chemokine (C-C motif) ligand 23	G-protein coupled receptor protein signaling pathway, cell-cell signaling, cellular calcium ion homeostasis, chemotaxis, immune response, inflammatory response, negative regulation of cell proliferation, signal transduction	Chemokine activity, cytokine activity, heparin binding
224389_s_at	-0.12	0.0043	*COL25A1*	Collagen; type XXV; alpha 1	Phosphate transport	Beta-amyloid binding, heparin binding

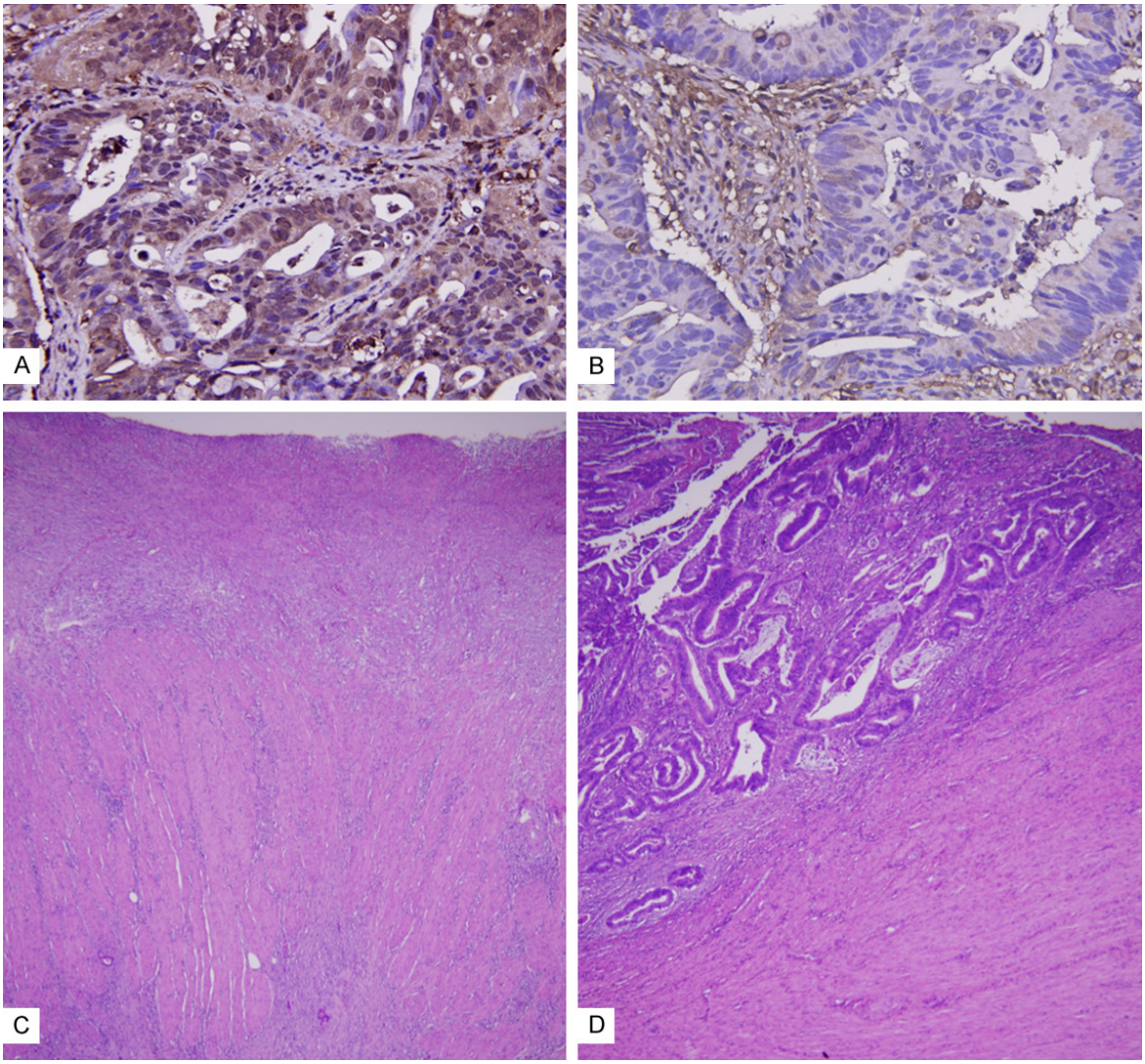

Figure 2. Immunohistochemical expression of THBS2 in rectal cancer. One selected case showing high expression of THBS2 (A) was linked to high degree of tumor regression (C) after CCRT. In the contrary, the case with low THBS2 expression (B) was correlated with low degree of tumor regression (D).

the response to preoperative CCRT, including *FGFR2, ANG, THBS2, CYR61, THBS1, CCL23, and COL25A1* (**Figure 1** and **Table 1**). Among these downregulated genes, *THBS2* exhibited the top-ranking, downregulated fold change (Log2 ratio at -0.8259, $P \leq 0.0041$) (**Table 1**).

THBS2 expression in human rectal cancers and its relationships to clinicopathological variables

The expressions of THBS2 were mainly in the cytoplasm of cancer cells. The H-scores of THBS2 varied widely between tumors (**Figure 2**). The minimum THBS2 score was 105, while the maximum was 325 (median=190). According to the distribution of H-score, we divided rectal cancer patients into two groups by referring to the median score. Each group consisted of 86 patients with either less than or more than/equal to the median score. Relationships between the expression of THBS2 and various clinicopathological parameters were analyzed. The clinicopathological variables were shown in **Table 2**. Low expression of THBS2 was significantly associated with the depth of tumor invasion (T3-T4 versus T1-T2; $P<0.001$ in both Pre-Tx and Post-Tx) and lymph node metastasis ($P=0.004$ in Pre-Tx, $P<0.001$ in Post-Tx) either in the pre-Tx or Post-Tx status. Vascular ($P=0.003$) and perineural invasion ($P=0.023$)

Table 2. Associations and comparisons between THBS2 expression and clinicopathological factors in 172 rectal cancer patients receiving neoadjuvant CCRT.

Parameter		No.	THBS2 Expression		*P*-value
			High Exp.	Low Exp.	
Gender	Male	108	50	58	0.207
	Female	64	36	28	
Age	<70	106	52	54	0.754
	≥70	66	34	32	
Pre-Tx tumor status (Pre-T)	T1-T2	81	53	28	<0.001*
	T3-T4	91	33	58	
Pre-Tx nodal status (Pre-N)	N0	125	71	54	0.004*
	N1-N2	47	15	32	
Post-Tx tumor status (Post-T)	T1-T2	86	58	28	<0.001*
	T3-T4	86	28	58	
Post-Tx nodal status (Post-N)	N0	123	72	51	<0.001*
	N1-N2	49	14	35	
Vascular invasion	Absent	157	84	73	0.003*
	Present	15	2	13	
Perineural invasion	Absent	167	86	81	0.023*
	Present	5	0	5	
Tumor regression grade	Grade 0-1	37	11	26	0.015*
	Grade 2~3	118	64	54	
	Grade 4	17	11	6	

*, Statistically significant.

were also significantly correlated with low expression of THBS2. More importantly, low THBS2 expression was found to be significantly associated with lesser degree of tumor regression (P=0.015). In the group of low THBS2 expression, there were 26 (15.1%) patients with tumor regression grade 0-1, 54 (31.4%) patients with grade 2-3, and 6 (3.5%) patients with grade 4. Conversely, in the group of high THBS2 expression, there were 11 (6.4%) patients with tumor regression grade 0-1, 64 (37.2%) patients with grade 2-3, and 11 (6.4%) patients with grade 4.

Prognostic impact of THBS2 expression in patients with rectal cancer

At univariate level, the advancements of Pre-Tx tumor status, Pre-Tx nodal status, Post-Tx tumor status, Post-Tx nodal status and the presence of vascular invasion were predictive of at least of one of the three endpoints in this study (**Table 3**). Of note, lesser degree of tumor regression was correlated with worse DFS (P<0.0001), LRFS (P=0.0090) and MeFS (P=0.0006). As shown in **Table 3** and **Figure 3**, DFS (P<0.0001; **Figure 3A**), LRFS (P=0.0005; **Figure 3B**) and MeFS (P=0.0002; **Figure 3C**) were significantly worse in patients with low expression of THBS2 than those with high THBS2 expression. A multivariate analysis demonstrated that tumor regression grade and THBS2 expression were independent prognostic factors (**Table 4**). Low THBS2 expression was independently predictive for worse DFS (P=0.002, hazard ratio=3.057) and MeFS (P=0.012, hazard ratio=3.362).

Discussion

Our results indicated that low THBS2 expression was associated with advanced disease status and poor outcome. Moreover, THBS2 expression was determined to be an independent negative prognostic factor in rectal cancer. In particular, low THBS2 expression was predictive of low tumor regression after preoperative CCRT. These findings thus indicated that THBS2 might serve as a tumor suppressor and potential predictive biomarker for CCRT response in rectal cancer.

In the samples from patients with gastric cancer, THBS2 expression levels were negatively correlated with histological grade and microvessel density. Furthermore, THBS2 was a potential prognostic predictor because patients with gastric cancer exhibiting low THBS2 expression were significantly associated with shorter overall survival compared with those having high THBS2 expression. In *in vitro* experiments, THBS2 overexpression was associated with downregulated cell growth and angiogenesis and upregulated apoptosis in gastric adenocarcinoma MKN-45 and SGC-7901 cell lines [7]. Overall, these results confirmed that THBS2 has a pivotal regulatory role in cancer progression. The expression of THBS2 was also affected by epigenetic changes or other regulators. A

Table 3. Univariate log-rank analysis for important clinicopathological variables and THBS2 expression

Parameter		No. of case	DFS		LRFS		MeFS	
			No. of event	*P*-value	No. of event	*P*-value	No. of event	*P*-value
Gender	Male	108	34	0.6344	20	0.2250	17	0.3520
	Female	64	20		7		14	
Age	<70	106	35	0.6999	18	0.6615	20	0.7427
	≥70	66	19		9		11	
Pre-Tx tumor status (Pre-T)	T1-T2	81	20	0.0486*	10	0.2261	11	0.1745
	T3-T4	91	34		17		20	
Pre-Tx nodal status (Pre-N)	N0	125	33	0.0010*	15	0.0070*	19	0.0973
	N1-N2	47	21		12		12	
Post-Tx tumor status (Post-T)	T1-T2	86	15	0.0002*	7	0.0040*	8	0.0033*
	T3-T4	86	39		20		23	
Post-Tx nodal status (Post-N)	N0	123	35	0.2338	16	0.1320	20	0.4634
	N1-N2	49	19		11		11	
Vascular invasion	Absent	157	45	0.0029*	21	0.0028*	27	0.4470
	Present	15	9		6		4	
Perineural invasion	Absent	167	51	0.0647	25	0.0940	30	0.9083
	Present	5	3		2		1	
Tumor regression grade	Grade 0-1	37	23	<0.0001*	10	0.0090*	14	0.0006*
	Grade 2~3	118	30		17		16	
	Grade 4	17	1		0		1	
THBS2 expression	High Exp.	86	11	<0.0001*	6	0.0005*	6	0.0002*
	Low Exp.	86	43		21		25	

DFS, disease-free survival; LRFS, local (pelvic) recurrence-free survival; MeFS, metastasis-free survival; *, statistically significant.

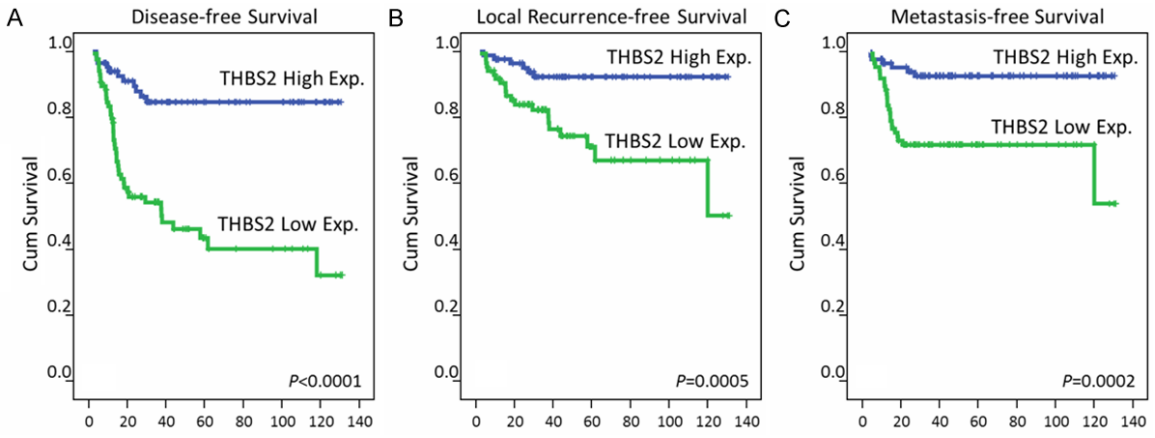

Figure 3. Survival curves of patients treated with neoadjuvant CCRT followed by surgery according to the expression of THBS2. All patients were classified into two groups, low and high expression of THBS2. Low expression of THBS2 was significantly correlated with shorter DFS (A), LRFS (B) and MeFS (C).

previous study analyzed the DNA from cancer cell lines for methylation status in the promoter regions of genes and determined that *THBS2* promoter methylation occurred in a high percentage of primary endometrial adenocarcinomas (33 of 49; 67.3%) [12]. In addition, a recent study revealed that microRNA (MiR-1246) promotes SiHa cervical cancer cell prolifera-

Table 4. Multivariate analysis

Parameter	DFS			LRFS			MeFS		
	H.R.	95% CI	*P*-value	H.R.	95% CI	*P*-value	H.R.	95% CI	*P*-value
Tumor regression grade	2.674	1.582-4.525	<0.001*	2.381	1.133-5.000	0.022*	2.262	1.142-4.484	0.019*
THBS2 expression	3.057	1.492-6.263	0.002*	2.529	0.923-6.931	0.071	3.362	1.306-8.655	0.012*
Vascular invasion	2.146	0.744-2.646	0.051	2.083	0.767-5.655	0.150	-	-	-
Post-Tx tumor status (Post-T)	1.403	0.744-2.646	0.296	1.538	0.603-3.925	0.368	1.533	0.639-3.679	0.338
Pre-Tx nodal status (Pre-N)	1.641	0.873-3.083	0.124	1.828	0.784-4.259	0.162	-	-	-
Pre-Tx tumor status (Pre-T)	1.023	0.551-1.900	0.942	-	-	-	-	-	-

DFS, disease-free survival; LRFS, local (pelvic) recurrence-free survival; MeFS, metastasis-free survival; *, statistically significant.

tion, invasion, and migration by downregulating *THBS2* [13].

A study reported that THBS2 is an antiangiogenic factor in colon cancer [8]. Angiogenesis is a key factor contributing to cancer cell proliferation and metastatic spread because such biological behaviors depend on an adequate supply of oxygen and nutrients and the efficient removal of waste products. Angiogenesis is mediated by the upregulation of angiogenic factors and downregulation of antiangiogenic factors [14]. A previous study analyzed THBS2 and VEGF-189 expression in colon cancer and reported that THBS2 expression was significantly associated with vessel counts and density in the stroma of patients with colon cancer [8]. The colon cancers expressing VEGF-189 but not THBS2 [THBS2(-)/VEGF-189(+)] exhibited significantly increased stromal vessel counts and density, and were associated with poorer prognosis, compared with those expressing THBS2 but not VEGF-198 [THBS2(+)/VEGF-189(-)]. In addition, patients with colon cancers having THBS2 expression had a significantly lower rate of liver metastasis than those without THBS2 expression.

Antiangiogenesis is partially induced by the reorganization of the cytoskeleton and the disassembly of adhesion molecules in endothelial cells. THBS2 is a glycoprotein with a major mediating role in cell-to-cell and cell-to-matrix interactions [15]. The ability of THBS2 to regulate angiogenesis is determined by its specific domain. The NH2-terminal heparin-binding domains of THBS2 can bind to cell-surface heparan sulfate proteoglycans and low-density lipoprotein receptor-related protein 1 (LRP1). LRP1 is a scavenger receptor that functions in the clearance of complexes of THBS and matrix metalloproteinases (MMP) or VEGF from the microenvironment of mesenchymal cells [16-18]. A previous study indicated that the decrease in protease activity leads to an inhibition on the growth of vascular network [19]. Moreover, THBS2 can inhibit angiogenesis through a pro-apoptotic mechanism by interacting with CD36 on endothelial cells [20]. Therefore, elucidating the role of THBS2 in antiangiogenesis can potentially facilitate the development of anticancer therapies by increasing the levels of THBS2 in the tumor microenvironment. Various approaches have been designed to increase THBS expression levels for cancer treatment, such as cell-based gene therapy or the systemic delivery of synthetic peptides that mimic THBS sequences. Streit et al [21] attempted to increase the level of circulating THBS2 by implanting a biodegradable polymer containing THBS2 overexpressing fibroblasts into the peritoneal cavity of nude mice. This approach produced sustained increased levels of THBS2 for more than 5 weeks, and subsequently inhibited tumor growth and angiogenesis of human squamous cell carcinomas, malignant melanomas, and Lewis lung carcinomas. Although certain concerns exist about the safety and side effects of applying the approach to humans, the study findings can potentially facilitate developing an effective adjunctive therapy for human cancers.

Radiotherapy is used to achieve local tumor control in various cancers. Although the efficacy of radiotherapy has markedly increased, a substantial number of patients develop local failures. Poor tumor oxygenation in the tumor microenvironment is partly responsible for the development of radioresistance because oxygen has a critical role in reactions with unstable free radicals and acts as a radiosensitizer during the process of creating radiation-induced DNA double-strand breaks [22, 23]. Because

the vascular network is a key factor affecting the supply of oxygen to the tumor stroma, poor vascular density may result in reduced radiosensitivity. Blocking tumor angiogenesis can induce tumor necrosis and inhibit tumor growth, although the therapeutic efficacy of current antiangiogenic agents is limited because of the rapid development of resistance. The use of angiogenesis inhibitors for tumor control in combination with radiotherapy is thus associated with risks. However, several preclinical and clinical studies have suggested that the combined use of antiangiogenics and radiotherapy has provided new options for cancer therapy because radiotherapy modifies tumor angiogenesis and antiangiogenic agents can potentiate the effects of radiotherapy. The synergistic effects of a combination therapy rely on the dosing and scheduling of both treatment modalities [24, 25]. Gorski et al determined that a combined treatment of anti-VEGF antibody and irradiation suppressed tumor growth in esophageal adenocarcinoma and glioblastoma xenografts to a greater extent than irradiation alone [26]. Therefore, in the future, CCRT combined with THBS-derived peptides may represent a potential treatment modality for rectal cancer. Additional experimental and preclinical studies are required to investigate the efficacy and safety of THBS-derived peptides.

In conclusion, our study results indicated that low THBS2 expression was associated with low tumor regression and an aggressive phenotype, and that was predictive of poor response to neoadjuvant CCRT in rectal cancer. Low THBS2 expression was thus an independent negative prognostic factor for disease-free survival and metastasis-free survival. Understanding the role of THBS2 in rectal cancer and its relationship with CCRT response may potentially facilitate the development of new therapeutic targets.

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Disclosure of conflict of interest

None.

Abbreviations

CCRT, Concurrent chemoradiotherapy; VEGF, vascular endothelial growth factor; FGFR, fibroblast growth factor receptor; THBS2, thrombospondin 2; EUS, endoscopic ultrasound; Pre-Tx, pre-treatment; Post-Tx, post-treatment; AJCC, American Joint Committee on Cancer; TRG, tumor regression grade; DFS, disease-free survival; LRFS, local recurrence-free survival; MeFS, metastasis-free survival; MMP, matrix metalloproteinases; LRP1, low density lipoprotein receptor-related protein 1.

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