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ORIGINAL ARTICLE

THBS2 is a biomarker for AJCC stages and a strong prognostic indicator in colorectal cancer

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Summary

Purpose: *To investigate the expression of thrombospondin 2 (THBS2) in colorectal cancer (CRC) and its relationship with clinicopathological features and prognosis.*

Methods: *THBS2 expression was evaluated with tissue microarrays (TMAs) immunohistochemistry (IHC) staining in 100 CRC samples.*

Results: *High THBS2 expression was found in 73 patients (45 male and 28 female). THBS2 expression was significantly correlated to TNM stages ($p=4.1\times10^{-5}$), T classification (p=0.005), lymph node metastasis ($p=3\times10^{-4}$) and AJCC stages (p=0), while no significant association was found in gender, age, distant metastasis or tumor size. In both univariate and multivariate analyses, THBS2 showed statistically prognostic significance [p<0.001, HR (hazard ratio) = 0.237, 95% CI (0.101-0.557) and p<0.001, HR=0.158, 95% CI (0.062-0.401)]. Kaplan–Meier survival analysis further confirmed that THBS2 expression was significantly correlated with clinical outcomes (p<0.001).*

Conclusions: *All the results indicated THBS2 expression might become a prognostic marker for CRC.*

Key words: *biomarker, colorectal cancer, prognosis, survival, thrombospondin 2*

Introduction

Thrombospondin (THBS2) 2 is a member of a group of functionally related extracellular matrix (ECM) glycoproteins, which can mediate extracellular matrix assembly, cell-to-matrix interactions, degradation of matrix metalloproteinase (MMP)-2 and MMP-9, and inhibition of angiogenesis [1]. Besides angiogenesis, THBS2 has been reported to interact with multiple cell receptors, growth factors and ECM proteins as well as regulate apoptosis, cell proliferation and adhesion [2]. The expression of THBS2 and its prognostic value have been investigated in several cancers. Tokunaga et al. found that THBS2 expressed in patients with colon cancer exhibited a significantly lower risk of hepatic metastases and tumor vascularity compared with the patients whose tumors were THBS2-deficient [3]. Furthermore, De Fraipont et al. demonstrated that THBS2 was significantly correlated with clinical status and outcome, and for most tumors there was an inverse correlation between the THBS2 expression level and the grade of their malignancy [4]. In addition, THBS2 also played a key role in breast cancer [5], malignant ovarian tumors [6] and lung cancer [7].

Colorectal cancer (CRC) is the third most common occurring noncutaneous carcinoma and the third leading cause of cancer-related deaths worldwide [8]. Although surgical techniques and chemotherapeutic options have optimized the treatment of CRC cancer in the last decades, the survival rate

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of CRC seems not to be substantially improved, and approximately 20% of patients die of recurrence and metastasis [9]. The conventional TNM staging system remains the most important indicator for prognosis in CRC and is used for stratification of long-term survival and treatment guidelines [10]. However, TNM system relies on surgical resection, which is not applicable to inoperable patients. Moreover, it could not incorporate molecular data borne from recent technological advances or predict heterogeneous outcomes and responses to therapy with same-stage tumors. Thus, identification of novel prognostic biomarkers in CRC is vital for more effective, targeted therapy and accurate prognostication.

Multiple facets of CRC are currently under investigation for biomarker application [11]. Discerning biological differences between same-stage tumors will complement TNM staging to provide more accurate prognoses. We hypothesized that overexpression of THBS2 may prove to be a useful prognostic indicator in CRC. So, in this study, we attempted to unveil the clinical significance of THBS2 in CRC using a TMA approach.

Methods

Clinicopathological characteristics

A total of 94 patients with primary CRC were enrolled in this study. Detailed clinicopathological characteristics are shown in Table 1. This study was approved by the ethics committee of Baotou Eighth Hospital and signed informed consents were obtained from all participants before study entry.

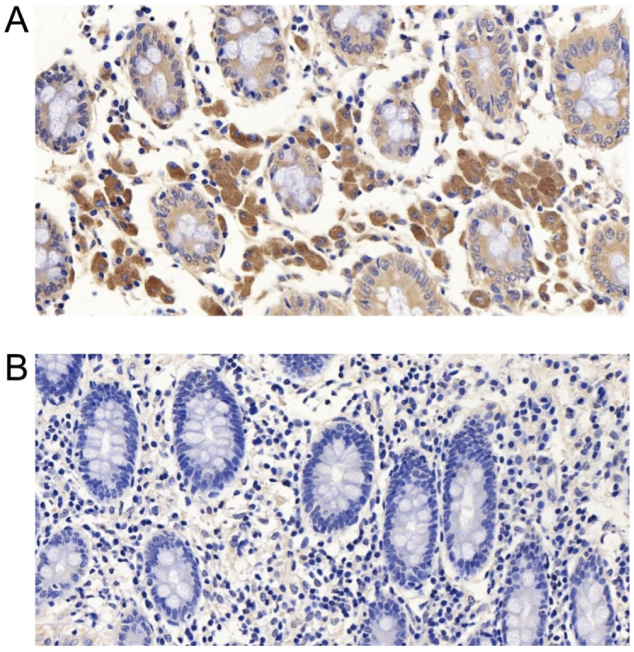

Figure 1. Immunohistochemical tissue microarray staining for THBS2 in a representative colon cancer specimen. **A:** THBS2 with a high H-score. **B:** THBS2 with a low H-score (magnification: 400×).

IHC assessment

For THBS2 staining, a proportional score was given by the estimated percent proportion of positive tumor cells and analysis of multiple regions to assess the average degree of staining within a section. The expression level of THBS2 was assessed by H-score system. The formula for the H-score is: Histoscore = Σ(I×Pi), where I = intensity of staining and Pi = percentage of stained tumor cells, with which a cytoplasmic score ranging from 0 to 300 was produced. The scoring was independently assessed by two assessors who were not aware of the clinical outcomes.

Statistics

For statistical evaluations, the SPSS statistical software (SPSS, Inc., V20.0, Chicago, IL, USA) was used. Chi-square tests were employed for categorical variables while Kaplan–Meier method was used for overall survival curves. Log rank test was used for comparisons between groups. Univariate and multivariate analyses were performed with the Cox's proportional hazards regression model. All tests were 2-tailed with a p value<0.05 signifying statistical significance.

Table 1. Baseline clinicopathological characteristics of 100 patients

Characteristics	*No. of patients n (%)*
Gender	
Male	58 (58)
Female	42 (42)
Age, years	
Range	45-91
Median	69
TNM stage	
I	17 (17)
II	74 (74)
III	9 (9)
T stage	
T1	0
T2	4 (4)
T3	64 (64)
T4	32 (32)
Distant metastasis	
Yes	4 (4)
No	96 (96)
Survival	
Alive	52 (52)
Dead	48 (48)

Results

Clinicopathological features

The clinicopathological features of 94 patients are shown in Table 1. The median patient age at initial surgery was 69 years (range 45-91). Fifty-eight patients were males and 42 females. The mean duration of follow-up was 24 months (range 1-87). Forty-eight patients died during the follow-up period (median 24 months;range 1-87). The grade of tumor differentiation was defined by different staging systems as shown in Table 1.

THBS2 expression pattern

Receiver operating characteristic (ROC) curve distribution analysis was used to determine the

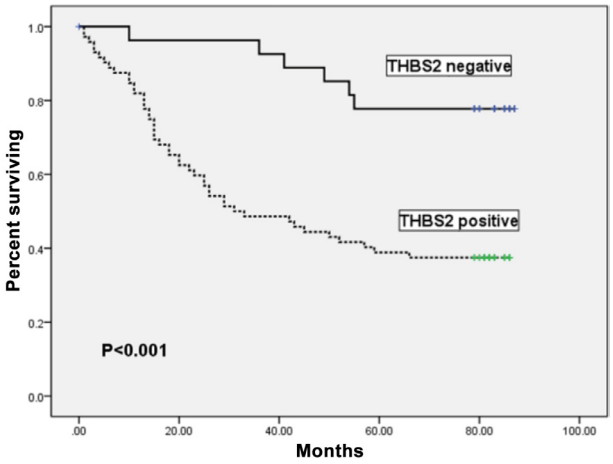

Figure 2. Kaplan–Meier survival analysis with log-rank test for the correlations between different THBS2 expression levels and overall survival.

Table 2. The relationship between THBS2 expression and clinicopathological characteristics

Characteristics	*High THBS2 (>15/300) (n=73)*	*Low THBS2 (≤15/300) (n=27)*	*p value*
Gender			
Male	45/58	13/58	1
Female	28/42	14/42	
Age, years			
<70	41/54	13/54	0.505
≥70	32/46	14/46	
TNM stage			
I	8/17	9/17	4.1×10^{-5}
II	46/74	28/74	
III	9/9	0/9	
T stage			
T2	0/4(25%)	4/4(100%)	0.005
T3	50/64(64.06%)	14/64(35.94%)	
T4	23/32(100%)	9/32(0%)	
Lymph node metastasis			
N0	30/52	22/52	3×10^{-4}
N1	34/36	2/36	
N2	9/12	3/12	
Distant metastasis			
M0	69/95	26/95	1.00
M1	4/5	1/5	
AJCC			
1	0/4	4/4	0.00
2	29/47	18/47	
3	40/44	4/44	
4	4/5	1/5	
Tumor size (cm)			
<3	4/4	0/4	0.324
3-6	39/51	12/51	
6-9	23/33	10/33	
>9	6/12	5/12	

H-scores range: 0-300/300; Optimal Cut-off: 15/300

cutoff of THBS2 expression. Out of a total H-score of 300, the threshold for differentiating between positive and negative immunostaining was set at an H-score of 15. Tumor specimens were categorized as 'low expression' or 'high expression' depending on whether the individual score was 'lower than or equal to' or 'higher than' the respective thresholds. Figure 1 shows the immunohistochemical staining for THBS2 in a representative CRC tissues.

As shown in Table 2, high THBS2 expression was found in 73 patients (45 male and 28 female). High THBS2 expression was significantly correlated to TNM stages ($p=4.1\times10^{-5}$), T stage (p=0.005), lymph node metastasis ($p=3\times10^{-4}$) and AJCC stages (p=0), while no significant association was found in gender, age, distant metastasis or tumor size.

Prognostic significance of THBS2 expression in CRC

To examine the relationship between THBS2 expression and the clinical prognosis of colon cancer patients. As shown in Table 3, tumor size and

Table 3. Univariate and multivariate analysis for important clinicopathological variables

Variables	*No. of cases*	*OS*					
		Univariate analysis			Multivariate analysis		
		p value	HR	95%CI	p value	HR	95%CI
Gender		0.415	0.792	0.453-1.386	0.415	0.792	0.453-1.386
Male	58						
Female	42						
Age, years		0.125	1.534	0.889-2.647	0.125	1.534	0.889-2.647
<70	54						
≥70	46						
TNM stage		0.825	1	-			
I	17	0.804	1.069	0.633-1.803	0.864	0.870	0.175-4.328
II	74				0.824	1.156	0.323-4.144
III	9				-	-	-
T stage					0.560	1	
T2	4	0.498	1.190	0.719-1.969			
T3	64				0.912	3436.711	0.000-1.549E+066
T4	32				0.284	0.714	0.386-1.322
Lymph node metastasis							
N0	52	0.832	0.958	0.644-1.426	0.956	1	-
N1	36				0.919	0.001	0.000-4.073E+059
N2	12				0.758	1.111	0.363-3.400
Distant metastasis							
M0	95						
M1	5	0.227	0.295	0.041-2.135	0.640	1.678	0.192-14.675
AJCC							
1	4	0.608	0.900	0.603-1.345	0.910	1	-
2	47				0.910	4074.708	0.000-1.823E+066
3	44						
4	5						
Tumor size (cm)							
<3	4	**0.005**	0.577	0.393-0.848	0.085	1	-
3-6	51				**0.043**	13.283	1.082-163.054
6-9	33				**0.013**	12.891	1.712-97.098
>9	12				**0.036**	9.069	1.159-70.964
THBS2 expression		**<0.001**	0.237	0.101-0.557	**<0.001**	0.158	0.062-0.401
High	73						
Low	27						

Bold values are statistically significant at p<0.05. HR : hazard ratio

THBS2 expression were statistically significant risk factors for overall survival (OS) in univariate analysis. However, multivariate survival analysis showed that only THBS2 expression was an independent prognostic and risk factor for patient OS ($p<0.001$, HR = 0.158, 95% CI 0.062-0.401). Moreover, Kaplan–Meier overall survival analysis further confirmed that THBS2 expression was significantly correlated with clinical outcomes (Figure 2, $p<0.001$). Patients with higher THBS2 expression displayed a significantly shorter OS (mean±SD 45.81±3.98 months), whereas patients with lower THBS2 expression showed a favorable prognosis (mean±SD 76.74±3.95 months ($p<0.05$). Taken together, our results indicated THBS2 expression was significantly associated with the clinical prognosis of colon cancer patients.

Discussion

In our study, the THBS2 expression with a high H-score was found in 73 patients, with an association to a significant uni- and multivariate indicator of patient survival. We also found that increased THBS2 expression was significantly associated with clinical stages (TNM/AJCC), although it was not significantly associated with gender, age, tumor size or the presence of distant metastasis

Currently, the primary method for identifying prognostic differences among patients with early-stage disease is the TNM system [12,13]. However, varying survival outcomes could exist among patients with a similar pathological disease stage. In general, a molecular marker could provide a basis for more economical and precise information regarding prognosis and appropriate adjuvant therapy for patients with colon cancer. THBS2 has been shown to be a strong prognostic indicator in certain cancer types. Fei et al. found that the expression of THBS2 in plasma of colon cancer patients was higher than in non-cancer controls [14]. However, its role as an independent prognostic marker in colon cancer has not been clearly defined yet.

In the present study we evaluated the significance of THBS2 expression in 100 CRC patients with TMA immunohistochemistry.

The expression of THBS2 and its prognostic value have been investigated in several cancers [5-7]. In our study, THBS2 expression with a high H-score was found in 73 patients, with a significant association in uni- and multivariate analysis of patient OS. We also found that increased THBS2 expression was significantly associated with clinical stages, pathological stages and TNM/AJCC stages, although it was not significantly associated with gender, age, tumor size and the presence of distant metastasis.

Tokunaga et al. first investigated the significance of THBS2 expression in colon cancer [3] and found that increased THBS2 expression was inversely correlated with metastasis of colon cancer, but no significant correlation was noted between THBS2 expression and T stages or lymph node metastasis. However, in a recent study, THBS2 expression was positively correlated with TNM stages, distant metastasis and also lymph node metastasis [15]. In our study, high THBS2 expression was present in 73 patients, and a significant correlation was found with lymph node metastasis, but no correlation with distant metastasis. This interesting finding may be due to the multifunctional role of THBS2 in the differing microenvironments of various cancers. No doubt, more patients are needed to verify the significance of THBS2 expression.

THBS2 expression was positively correlated to tumor TNM stages, a finding similar in several studies [5,15]. Our study also proved that high THBS2 expression was correlated to T classification and AJCC clinical stages. This is the first time to evaluate the potential role of THBS2 in T and AJCC stages. Although the TNM system describes the anatomical extent of malignant neoplasms and projects a stage-derived survival estimate, it likely oversimplifies the assessment of the biological potential of the tumor and the overall risk of recurrence and death. AJCC identifies the potential limitation of pure anatomical staging in colon cancer [16]. Based on our results, it could be suggested that THBS2 might serve as a potential biomarker during the T and AJCC clinical stages evaluation at diagnosis.

In this study, we further demonstrated that high-risk THBS2 was correlated to a short OS, also proved by Wang et al. [17] and Yoshida et al. [18]. Moreover, higher levels of THBS2 in serum from advanced non-small cell lung cancer (NSCLC) patients predicted worse median survival (9 months) compared to 23.7 months of patients with lower expression levels of THBS2. A high tumor THBS2 protein level was also a significant prognostic factor for short OS and poor disease-free survival (DFS) in oral squamous cell carcinoma patients. Overexpression of THBS2 was not only significantly associated with aggressive clinicopathological parameters but was also an independent poor prognostic biomarker predicting shorter disease-specific survival and metastasis-free survival. Overall, these results confirmed that THBS2 could serve as a prognostic indicator for patient overall survival.

Conclusions

In summary, using TMAs, we found that THBS2 expression was significantly correlated to TNM stages, and first proved that THBS2 positively related to T and AJCC stages. High expression of THBS2 was a strong predictor of poor patient overall survival with CRC. Collectively, the results indicate that THBS2 could serve as an indicator for pathological staging and a novel prognostic factor in CRC.

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Conflict of interests

The authors declare no conflict of interests.

References

1. Calabro NE, Kristofik NJ, Kyriakides TR. Thrombospondin-2 and extracellular matrix assembly. Biochim Biophys Acta 2014;1840:2396-2402.
2. Bornstein P, Armstrong LC, Hankenson KD, Kyriakides TR, Yang Z. Thrombospondin 2, a matricellular protein with diverse functions. Matrix Biol 2000;19:557-68.
3. Tokunaga T, Nakamura M, Oshika Y et al. Thrombospondin 2 expression is correlated with inhibition of angiogenesis and metastasis of colon cancer. Br J Cancer 1999;79:354-9.
4. de Fraipont F, Nicholson AC, Feige JJ, Van Meir EG. Thrombospondins and tumor angiogenesis. Trends Mol Med 2001;7:401-7.
5. Perez-Rivas LG, Jerez JM, Fernandez-De SC et al. Serum protein levels following surgery in breast cancer patients: A protein microarray approach. Int J Oncol 2012;41:2200-6.
6. Czekierdowski A, Czekierdowska S, Danilos J et al. Microvessel density and CpG island methylation of the THBS2 gene in malignant ovarian tumors. J Physiol Pharmacol 2008;59 (Suppl 4):53-65.
7. Weng TY, Wang CY, Hung YH, Chen WC, Chen YL, Lai MD. Differential expression pattern of THBS1 and THBS2 in lung cancer: Clinical outcome and a Systematic-Analysis of microarray databases. PLoS One 2016;11:e161007.
8. Antoniou E, Andreatos N, Margonis GA et al. Systemic treatment and primary tumor location in patients with metastatic colorectal cancer. JBUON 2017;22:1447-56.
9. Dienstmann R, Salazar R, Tabernero J. Personalizing colon cancer adjuvant therapy: Selecting optimal treatments for individual patients. J Clin Oncol 2015;33:1787-96.
10. Todorovic V, Cicmil SN, Lakicevic J, Sorat M. Evaluation of safety of bevacizumab as second-line treatment of patients with metastatic colorectal cancer. JBUON 2017;22:1131-6.
11. Gangadhar T, Schilsky RL. Molecular markers to individualize adjuvant therapy for colon cancer. Nat Rev Clin Oncol 2010;7:318-25.
12. Lin CY, Lin CY, Chang IW et al. Low thrombospondin 2 expression is predictive of low tumor regression after neoadjuvant chemoradiotherapy in rectal cancer. Am J Transl Res 2015;7:2423-32.
13. Resnick MB, Routhier J, Konkin T, Sabo E, Pricolo VE. Epidermal growth factor receptor, c-MET, beta-catenin, and p53 expression as prognostic indicators in stage II colon cancer: A tissue microarray study. Clin Cancer Res 2004;10:3069-75.
14. Fei W, Chen L, Chen J et al. RBP4 and THBS2 are serum biomarkers for diagnosis of colorectal cancer. Oncotarget 2017;8:92254-64.
15. Kim H, Watkinson J, Varadan V, Anastassiou D. Multi-cancer computational analysis reveals invasion-associated variant of desmoplastic reaction involving INHBA, THBS2 and COL11A1. BMC Med Genomics 2010;3:51.
16. Thirunavukarasu P, Talati C, Munjal S, Attwood K, Edge SB, Francescutti V. Effect of incorporation of pretreatment serum carcinoembryonic antigen levels into AJCC staging for colon cancer on 5-year survival. JAMA Surg 2015;150:747-55.
17. Wang X, Zhang L, Li H, Sun W, Zhang H, Lai M. THBS2 is a potential prognostic biomarker in colorectal cancer. Sci Rep 2016;6:33366.
18. Yoshida Y, Oshika Y, Fukushima Y et al. Expression of angiostatic factors in colorectal cancer. Int J Oncol 1999;15:1221-5.