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Increased expression of long non-coding RNA SBDSP1 correlates with poor survival in colorectal cancer

H.-B. ZHOU[1], Q. LI[2], M. LIU[3], Y.-Q. CAO[4], J.-Y. XU[5]

[1]Department of General Surgery, Chinese PLA 456th Hospital, Jinan, Shandong, China
[2]Department of Orthopedics, Jiyang People's Hospital, Jinan, Shandong, China
[3]Department of General Surgery, Jiyang People's Hospital, Jinan, Shandong, China
[4]Department of Anus and Intestine Surgery, Taian City Central Hospital, Taian, Shandong, China
[5]Department of General Surgery, Weifang People's Hospital, Weifang, Shandong, China

Abstract. – OBJECTIVE: Long non-coding RNA SBDSP1 (SBDSP1) had been reported to be increased in colorectal cancer (CRC), but its effects on prognosis were still unclear. The aim of this study was to investigate the clinical significance and prognostic value of the SBDSP1 expression in CRC.

PATIENTS AND METHODS: The expression levels of SBDSP1 in CRC tissues and adjacent normal tissues were detected using quantitative Real-time PCR (qRT-PCR). The association between SBDSP1 expression and clinicopathological factors as well as survival rates were analyzed. To determine the prognostic value of SBDSP1, univariate and multivariate analysis were performed using the Cox proportional hazard analysis.

RESULTS: The relative expression levels of SBDSP1 were significantly higher in CRC tissues than in the matched normal colon tissues ($p < 0.01$). SBDSP1 expression was significantly associated with differentiation ($p = 0.002$), depth of invasion ($p = 0.007$) and TNM stage ($p = 0.004$). Additionally, patients with higher SBDSP1 expression have significantly shorter disease-free survival ($p = 0.006$) and overall survival ($p = 0.001$) compared to those with lower expression. Moreover, univariate and multivariate analysis identified high SBDSP1 expression as an unfavorable prognostic factor for both overall survival and disease-free survival.

CONCLUSIONS: The present study provides the first evidence that SBDSP1 may be a useful indicator of prognosis in patients with CRC.

Key Words:
Long non-coding RNA, SBDSP1, Prognosis.

Introduction

Colorectal cancer (CRC) is a frequent malignancy and one of the leading causes of cancer-related death with millions of new cases worldwide each year[1]. The patients suffering from CRC can be cured if detected at an early stage. Unfortunately, many CRC patients are diagnosed at advanced stage[2,3]. In recent years, significant advances have been made in targeted therapies of CRC[4]. However, prognosis of advanced CRC and postoperative recurrence of CRC remains poor. Thus, it is urgent and necessary to identify new biomarkers, which are able to help in the early diagnosis and prognosis prediction for CRC.

Long non-coding RNAs (lncRNAs) are defined as noncoding transcripts with over 200 nucleotides in length and do not have protein-coding function[5]. Growing studies demonstrate that lncRNAs play vital regulatory roles in a wide range of biological processes, such as cell growth, cell cycle, apoptosis and motility[6-8]. More and more evidence showed that lncRNAs play a critical role in tumorigenesis and can regulate gene expression by various mechanisms[9,10]. Indeed, lncRNAs function as tumor suppressors or oncogenes according to the targeting genes[11]. Considering the important role of lncRNAs in development and progression of various tumors, lots of researchers suggest that lncRNAs may have the potential to serve as prognostic markers and therapeutic targets. Although more and more lncRNAs have been identified as important regulator factor in several tumors, the expressions and functions of lncRNAs in CRC are largely unknown.

LncRNA SBDSP1 (SBDSP1) is a novel lncRNA, which was located on 7q11.23. To our best knowledge, only Shi et al[12] showed that SBDSP1 may serve as a tumor promoter in CRC. However, the clinical relevance of SBDSP1 in CRC has not

Corresponding Author: Jia-you Xu, MD; e-mail: xuj1959nn@yeah.net

been investigated. Thus, our present work aimed to explore the expression and prognostic value of SBDSP1 in patients with CRC.

Patients and Methods

Patient Specimens

Fresh CRC tissues and matched normal colon tissues were collected from 171 patients who underwent surgery between July 2007 and June 2011 in Chinese PLA 456th Hospital. The colorectal cancer diagnosis was confirmed by an experienced pathologist. No patient received chemotherapy or radiotherapy treatment prior to surgery. Tissue specimens were conserved in liquid nitrogen for qRT-PCR until use. The patients' clinical information, such as age, gender, tumor location, differentiation and depth of invasion, was collected and stored in a database. The characteristics of these patients are shown in Table I. A complete follow-up was available for at least 5 years. The study was approved by the Medical Ethics Committee of Chinese PLA 456th Hospital. Informed consent was obtained from all patients.

Quantitative Real-time PCR (qRT-PCR)

Total RNA was isolated with TRizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was reverse transcribed into cDNA using the Prime-Script™ one step RT-PCR kit (TaKaRa, Dalian, China). The quantitative Real-time polymerase chain reaction (qRT-PCR) was executed by using SYBR Green PCR kit (TaKaRa, Dalian, China), in accordance with manufacturer's instructions. GAPDH expressions were used to normalize the results for the qRT-PCR. The relative expression was evaluated by using the comparative cycle threshold method ($2^{-\Delta\Delta CT}$). Primers used in qRT-PCR were as follows: SBDSP1 forward, 5'-GCTTCTGGGTCTTTGAACAGCC-3' and SBDSP1 reverse, 5'-CTTGGCAACCTGACCTTAGGAAAC-3'; GAPDH, forward 5'-GTCAACGGATTTGGTCTGTATT-3' and GAPDH reverse: 5'- AGTCTTCTGGGTGGCAGTGAT-3'.

Statistical Analysis

Statistical analysis was performed using SPSS version 13.0 software (SPSS Inc, Chicago, IL, USA). All quantified data were presented as mean ±SD. Student's *t*-test was applied for comparison of the two groups. The X^2 test was appropriately used to determine the association between SBDSP1 expression and clinicopathological variables of CRC. Overall survival time (OS) is the time from surgery until the date of death or last follow-up. Disease-free survival (DFS) was defined as the time from surgery to regional relapse or distant metastasis. OS and PFS were estimated by using Kaplan-Meier method. The effect of different factors on patient survival was performed by univariate and multivariate analysis. *p*-values < 0.05 were considered to be statistically significant.

Results

SBDSP1 was Overexpressed in CRC Tissues

We firstly detected the expression levels of SBDSP1 in CRC tissues and matched normal tissues by qRT-PCR. As shown in Figure 1, SBDSP1 expression was shown to be remarkably increased in CRC tissues in comparison with matched normal colon tissues ($p < 0.01$).

Relationship Between SBDSP1 Expression and clinicopathological Characteristics

Next, we analyzed the relationship between the expression level of SBDSP1 and clinicopathological features in CRC patients. The median value (4.21) of SBDSP1 expression was used as a cutoff point to classified 171 CRC patients into SBDSP1-low (n = 84) and SBDSP1-high (n = 87) expression groups. As shown in Table I, we found that SBDSP1 expression was significantly associated with differentiation ($p = 0.002$), depth of invasion ($p = 0.007$) and TNM stage ($p = 0.004$).

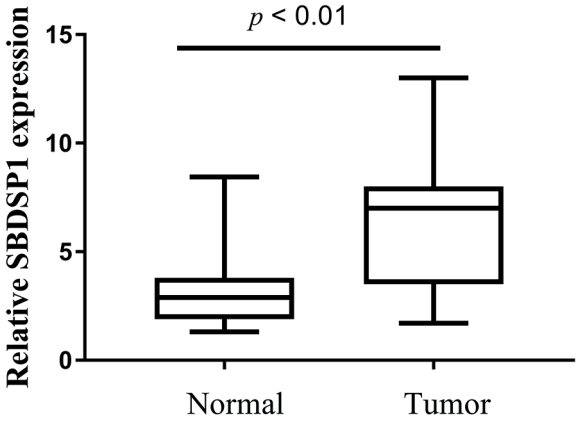

Figure 1. qRT-PCR analysis of SBDSP1 expression in human CRC tissues and matched normal colon tissues from 171 patients with CRC.

Table I. Correlation between the clinicopathologic characteristics and expression of SBDSP1 protein in CRC.

Clinicopathological features	No. of cases	SBDSP1 expression		*p*-value
		Low	High	
Age				NS
<60	79	39	40	
≥60	92	45	47	
Gender				NS
Male	74	31	43	
Female	97	53	44	
Tumor location				NS
Colon	86	47	39	
Rectum	85	37	48	
Tumor size				NS
<5 cm	113	57	56	
≥5 cm	58	27	31	
Histology/differentiation				0.002
Well + Moderate	122	69	53	
Poor	49	15	34	
Depth of invasion				0.007
T1 + T2	109	62	47	
T3 + T4	62	22	40	
TNM stage				0.004
I-II	119	67	52	
III	52	17	35	

However, there were no significant associations of SBDSP1 with patients' age, gender, tumor location or tumor size (All $p > 0.05$, Table I).

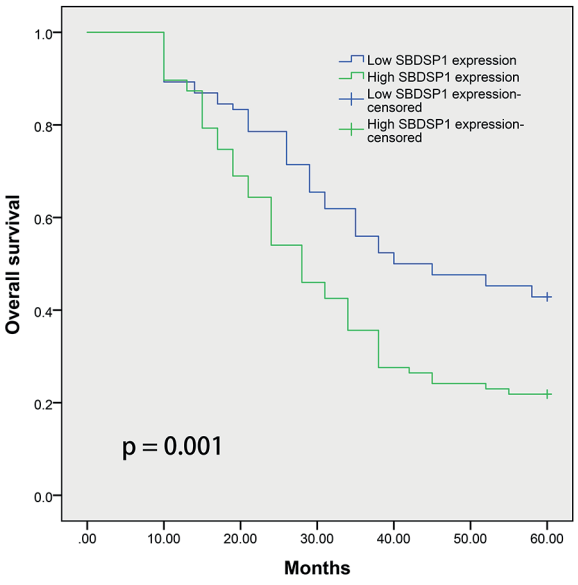

Figure 2. Kaplan-Meier curves for survival time in patients with CRC divided according to SBDSP1 expression: The CRC patients in the high SBDSP1 expression group had significantly lower 5-year OS rate than those in the low SBDSP1 expression group ($p = 0.001$).

Correlation of SBDSP1 Expression with prognosis of CRC Patients

In order to explore the effect of SBDSP1 in prognosis of CRC patients, DFS and OS were assessed by Kaplan-Meier survival analysis. As shown in Figure 2 and 3, our results indicated that patients with higher SBDSP1 expression have significantly shorter DFS ($p = 0.006$) and OS ($p = 0.001$) compared to those with lower expression. To further confirm the prognostic value of SBDSP1 in CRC patients, a univariate and multivariate analysis were performed using Cox regression analysis. The results of univariate analysis indicated that low expression of SBDSP1 (HR: 2.673, $p = 0$ .006), differentiation (HR: 3.421, $p = 0.001$), depth of invasion (HR: 2.893, $p = 0.004$) and TNM stage (HR: 3.138, $p = 0.003$) were significant predictive factors for poor outcome (Table II). Similar findings were observed between DFS and SBDSP1 expression. Moreover, the results of multivariate analysis showed that SBDSP1 expression level was independently associated with the DFS ($p = 0.018$) and OS ($p = 0.009$, Table II). Thus, our findings demonstrated that SBDSP1 expression has an obvious correlation with poor prognosis of CRC patients.

Table II. Prognostic factors for OS and DFS by univariate and multivariate analysis.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Overall survival				
Age	1.412 (0.682-1.944)	0.317	-	-
Gender	1.742 (0.591-2.632)	0.263	-	-
Tumor location	1.312 (0.781-1.933)	0.531	-	-
Tumor size	2.139 (0.891-2.819)	0.327	-	-
Histology/differentiation	3.421 (1.582-6.392)	0.001	3.013 (1.231-5.671)	0.004
Depth of invasion	2.893 (1.239-5.883)	0.004	2.361 (1.031-4.872)	0.009
TNM stage	3.138 (1.382-7.923)	0.003	2.677 (1.138-6.231)	0.006
SBDSP1 expression	2.673 (1.562-5.632)	0.006	2.137 (1.238-4.672)	0.009
Disease-free survival				
Age	1.513 (0.721-2.132)	0.293	-	-
Gender	1.326 (0.623-1.893)	0.367	-	-
Tumor location	1.529 (0.892-2.387)	0.489	-	-
Tumor size	1.893 (0.787-2.652)	0.278	-	-
Histology/differentiation	3.068 (1.328-5.899)	0.004	2.783 (1.193-5.023)	0.008
Depth of invasion	2.763 (1.123-5.123)	0.008	2.183 (1.041-4.238)	0.011
TNM stage	2.893 (1.213-6.939)	0.007	2.423 (1.038-6.132)	0.013
SBDSP1 expression	2.523 (1.429-5.013)	0.009	2.132 (1.123-4.239)	0.018

Discussion

In China, the incidence and mortality rates of CRC were 23.03/100,000 and 11.11/100,000[13]. A multi-step process including both genetic and epigenetic changes is involved in the process of CRC[14,15]. Most of patients with CRC were diagnosed at an advanced stage due to asymptomatic symptoms and lack of effective screening methods[16]. Despite advances in surgical techniques and incorporation of new therapeutic approaches, the 5-year survival rate is only at 25% in CRC patients diagnosed at an advanced stage[17]. Thus, identification of novel biomarkers is required for the early detection and treatment of CRC.

Recently, lncRNAs, as a novel group of non-protein coding molecules, have been shown to be involved in the development and progression of CRC[18]. For instance, Yang et al[19] found that HULC, a well-studied lncRNA, was found to be highly expressed in CRC and it promoted CRC cells proliferation, migration and invasion by suppressing NKD2 expression. Yang et al[20] reported that down-regulation of lncRNA Loc554202 was observed in CRC tissues and patients with low expression of lncRNA Loc554202 have significantly poorer outcome than those with high expression. Wang et al[21] revealed that up-regulated lncRNA NNT-AS1 contributed to progression of CRC through influencing EMT and MAPK/Erk signaling pathway. Recently, a novel lncRNA, SBDSP1, was found to be lowly expressed in CRC tissues. In addition, downregulation of SBDSP1 significantly inhibited CRC cell proliferation and metastasis by suppression of Akt, ERK1/2, and STAT3

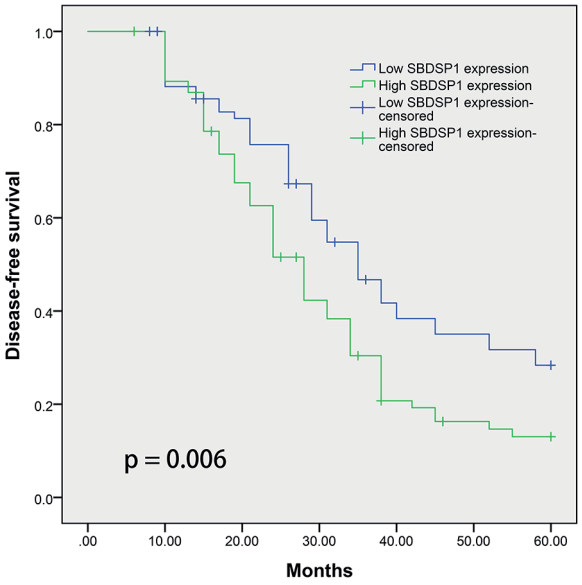

Figure 3. Kaplan-Meier curves for survival time in patients with CRC divided according to SBDSP1 expression: The CRC patients in the high SBDSP1 expression group had significantly lower 5-year DFS rate than those in the low SBDSP1 expression group ($p = 0.006$).

phosphorylation[12]. Those findings indicated that SBDSP1 may serve as a tumor promoter in CRC. However, whether SBDSP1 is associated with the prognosis of CRC patients remains unknown.

In the present work, we found that the relative expression levels of SBDSP1 were significantly higher in CRC tissues than in the matched normal colon tissues by RT-PCR. We also found that increased SBDSP1 expression was significantly correlated with aggressive clinicopathological features. Furthermore, the OS and DFS of low SBDSP1 expression group were significantly shorter than that of high SBDSP1 expression group. Finally, univariate and multivariate Cox proportional hazards regression model analysis indicated that high SBDSP1 expression as an unfavorable prognostic factor for both OS and DFS.

Conclusions

Our study is the first to demonstrate that higher SBDSP1 can predict poor prognosis for patients with CRC. It seems that SBDSP1 is a potential prognostic predictor in CRC.

Conflict of interest

The authors declare no conflicts of interest.

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