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Up-Regulated Expression of *SPRY4-IT1* Predicts Poor Prognosis in Colorectal Cancer

Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Long non-coding RNA SPRY4 intronic transcript 1 (lncRNA *SPRY4-IT1*) has been reported to be associated with the progression of several cancers, but its expression level in colorectal cancer (CRC) has rarely been reported. The purpose of this study was to estimate the clinical significance of *SPRY4-IT1* in CRC.

Material/Methods: The relative expression levels of *SPRY4-IT1* were detected by quantitative real-time polymerase chain reaction (qRT-PCR) in diseased tissues and the adjacent normal tissues of 106 CRC patients. Chi-square method was used to evaluate the association between *SPRY4-IT1* expression and the clinical features. Additionally, we assessed the overall survival at different expression levels of *SPRY4-IT1* using Kaplan-Meier method. The prognostic significance of *SPRY4-IT1* was estimated by Cox regression analysis.

Results: Up-regulated level of *SPRY4-IT1* was detected in pathologic tissues of CRC patients compared with adjacent normal tissues (P=0.000). The relative expression of *SPRY4-IT1* was associated with the tumor size, the depth of invasion, lymph node invasion, distant invasion, and tumor stage (P<0.05). Patients with high expression of *SPRY4-IT1* had poor overall survival compared with those with high level (39.3 *vs.* 49.3 months, log-rank test, P=0.016). Cox regression analysis showed that *SPRY4-IT1* could act as an independent prognostic factor in CRC (HR=2.341, 95% CI=1.136–4.826, P=0.021).

Conclusions: *SPRY4-IT1* might be associated with tumorigenesis and progression of CRC, and it may be a promising biomarker for prognosis in patients with CRC.

MeSH Keywords: Colorectal Neoplasms • Lynch Syndrome II • Prognosis

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Background

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers in the world, with over 600,000 deaths per year [1,2]. Unfortunately, the morbidity of CRC has been rapidly rising in Asian countries in recent years [3]. However, the multiple known carcinogenic factors and complex genetic backgrounds make it difficult to estimate the key factor in CRC progression [4]. At the present time, the basic prognostic biomarker in CRC is the clinicopathologic tumor staging, based on the tumor-node-metastasis (TNM) system. Nevertheless, the TNM stage is not an ideal biomarker for CRC outcomes. Patients at the same TNM stage may have different progressions and clinical outcomes due to their various genetic and epigenetic backgrounds [5,6]. Therefore, it is necessary to identify sensitive and specific molecular biomarkers for CRC clinical outcomes.

Long non-coding RNAs (LncRNAs) transcribed by RNA polymerase II lack open reading frames (ORF) longer than 200 nucleotides [7]. Although the functions of most lncRNAs are unknown, more and more lncRNAs are characterized and many of them are reported to regulate gene expression in the development and differentiation of diseases [8–11]. LncRNAs also have been reported to influence the development of human cancers. For example, lncRNA *CCHE1* promotes cervical cancer cell proliferation [12], *HNF1A-AS1* regulates proliferation and metastasis in lung adenocarcinoma [13], and *MALAT1* is associated with poor prognosis of glioma [14]. *SPRY4-IT1* is significantly increased in plasma samples of NSCLC patients and can act as a biomarker in NSCLC [15]. *TRPM2-AS* can act as a novel biomarker and therapeutic target in prostate cancer [16]. In this study, we focused on lncRNA SPRY4 intronic transcript 1 (*SPRY4-IT1*), which is located within an intron of the *SPRY4* gene. *SPRY4-IT1* was previously reported to be up-regulated in melanoma, gastric cancer, breast cancer, and esophageal squamous cell carcinoma [17–20]. However, the effect of *SPRY4-IT1* in CRC prognosis is unknown.

In the present study, we applied different methods in analyzing the association between *SPRY4-IT1* expression and clinical features, aiming to determine the clinical influences of *SPRY4-IT1* in CRC patients and to discover a reliable predictor for CRC.

Material and Methods

Patients and clinical features collection

In this study, 106 CRC patients confirmed by pathological and clinical diagnoses at the PLA General Hospital were enrolled from October 2008 to January 2014. This study was approved by the Ethics Committee of PLA General Hospital, and written consent was obtained from all the patients. Tumor and adjacent normal tissues were obtained from the CRC patients before they received any chemotherapy or radiotherapy. All the tissue samples were stored in liquid nitrogen until they were utilized.

In order to observe the results of the surgery, follow-up was performed every 3 months in the first 2 years and then every 6 months until the end of the study. All the patients were enrolled in the surgery. Overall survival was used to estimate the influence of *SPRY4-IT1* on CRC patient prognosis.

RNA extraction

Total RNA was extracted from all the tissues using TRIzol reagent according to the manufacturer's instructions. The extracted RNA was dissolved in diethyl pyrocarbonate (DEPC)-water and then treated by DNase to remove DNA. The concentration of the total RNA was detected by UV absorbance at 260 nm and 280 nm (A260/A280). We used 1% agarose gel electrophoresis to check the quality of the total RNA.

Fluorescence quantitative real-time PCR

Fluorescence quantitative real-time PCR (qRT-PCR) was used to assess the relative expression levels of *SPRY4-IT1* in pathologic and adjacent normal tissues of CRC patients. The complementary DNA (cDNA) temples enrolling in the qRT-PCR were from the PrimeScript RT reagent kit (Takara, China). The qRT-PCR was performed with SYBR Green assay (Takara, China). The expression of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used for normalized control. The data were analyzed by $2^{-\Delta\Delta Ct}$ method. The primers sequences are shown in Table 1.

Table 1. The sequences of primers used in this study.

Name		Sequences
SPRY4-IT1	Forward	5'-ATCCGAAGCGCAGACACAATTCA-3'
	Reverse	5'-CCTCGATGTAGTCTATGTCATAGGA-3'
GAPDH	Forward	5'-AGACTCGCTGATGATCCATGC-3'
	Reverse	5'-AGGTGACCACAGTGTTCTG-3'

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Statistical analysis

Statistical analysis was completed in SPSS 18.0 software. Student's t-test was used to estimate the different expression levels of *SPRY4-IT1* and the data are shown as mean ± standard deviation (SD). The association between the clinical features and *SPRY4-IT* expression was evaluated by chi-square method. Kaplan-Meier method with log-rank test was applied to analyze the overall survival of the CRC patients, and univariate and multivariate Cox regression analysis were used to evaluate the prognostic value of *SPRY4-IT1*. $P<0.05$ was considered statistical significance.

Results

Different expression of *SPRY4-IT1* in CRC tissues and normal tissues

The 106 CRC patients enrolled in this study included 52 men and 54 women with an average age of 55.02 years old. The clinical data of the participators are summarized in Table 2. QRT-PCR was used to evaluate the relative expression of *SPRY4-IT1* in CRC tissues and normal tissues. The results indicated that the relative expression of *SPRY4-IT1* in pathologic tissues was significantly higher than that in the adjacent normal tissues ($P=0.000$, Figure 1).

Relationship between *SPRY4-IT1* expression and clinical characteristics

To evaluate the association between *SPRY4-IT1* expression and clinical features, the CRC patients were divided into high and low expression groups on the basis of their average expression of *SPRY4-IT1*. The chi-square results are shown in Table 2, which shows that the expression levels of *SPRY4-IT1* were associated with the tumor size ($P=0.013$), the depth of invasion ($P=0.004$), lymph node metastasis ($P=0.017$), distant invasion ($P=0.012$), and tumor stage ($P=0.015$). However, there was not significant relationship between the expression levels and sex, age, tumor location, histological differentiation, venous invasion, or nerve invasion ($P>0.05$). The results suggest that the expression level of *SPRY4-IT1* might be associated with the development of the CRC.

Overall survival analysis

Overall survival analysis was conducted by Kaplan-Meier method, showing that the CRC patients with high expression of *SPRY4-IT1* had low overall survival (the average overall survival was 39.3 months), while the low expression patients had an average overall survival of 49.3 months (Figure 2). In other words, differences between the 2 groups were significant (log-rank test, $P=0.016$).

Univariate and multivariate Cox regression analysis for CRC prognosis

In this study, we used Cox regression analysis to estimate the prognostic value of *SPRY4-IT1*. The results of univariate analysis show that the levels of *SPRY4-IT1* were significantly associated with the poor prognosis in CRC patients ($P=0.021$). Multivariate analysis suggests that *SPRY4-IT1* is an independent factor for CRC prognosis (HR=2.341, 95% CI=1.136-4.826, $P=0.021$). The results of univariate and multivariate Cox regression analysis are summarized in Table 3.

Discussion

Despite great progress in early diagnosis, surgical techniques, and chemotherapy, the prognosis of patients with CRC is still unsatisfactory [21]. Prognostic factors are helpful in determining the therapeutic regimen in cancers. Due to the complex genetic background and epigenetic factors, there is still no highly sensitive and specific biomarker for CRC clinical outcomes, so the prognosis of CRC patients is poor [4]. Therefore, finding reliable biomarkers may improve the treatment of CRC.

Recently, many lncRNAs have been reported to play significant regulatory roles in human diseases [22]. Cancer-specific lncRNAs have been proven to contribute in tumor progression and serve as prognostic factors in many types of cancer. Wu et al. reported that lncRNA *UCA1* could be a promising biomarker for early detection and prognosis in gastric cancer [23]. LncRNA *BANCR* was proved to regulate the growth and metastasis of the retinoblastoma cells and act as a prognostic target [24]. Chen et al. demonstrated that lncRNA *HOTTIP* promoted pancreatic cancer cell proliferation, survival, and migration [25]. As a result, the association between CRC and lncRNAs may also provide significant information about the development of cancer and clinical outcomes.

LncRNA *SPRY4-IT1* is a novel lncRNA, which was first reported to be associated with molecular etiology in human melanoma [26]. It is a 687nt unspliced, polyadenylated transcript, localized at chromosome 5q31.3. In this study, we detected the relative expression levels of *SPRY4-IT1* in CRC patients using qRT-PCR (*GAPDH* as normalized control), aiming to estimate the clinical significance of *SPRY4-IT1* in CRC. The results showed that the relative expression of *SPRY4-IT1* was much higher in diseased tissues than in the corresponding adjacent normal tissues and there were significant differences between them. The abnormal expression of *SPRY4-IT1* might be associated with CRC progression. We also analyzed the relationship between the *SPRY4-IT1* expression and the clinical characteristics by chi-square method. The results indicated that the relative expression levels of *SPRY4-IT1* were related to

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Table 2. The clinical features of the CRC patients in this study.

Characteristics	Total number (n)	*SPRY4-IT1* expression		χ^2	*P*
		High (n)	Low (n)		
Gender				0.503	0.478
Men	57	33	24		
Women	59	25	24		
Age				0.022	0.882
≥55	61	33	28		
<55	45	25	20		
Tumor size				6.177	0013
≥5 cm	66	37	19		
<5 cm	50	21	29		
Location				0.412	0.521
Colon	60	29	21		
Rectum	56	29	27		
Histological differentiation				1.211	0.271
Well	67	34	23		
Poor	49	24	25		
The depth of invasion				8.183	0.004
T1+T2					
T3+T4					
Lymph node metastasis				5.665	0.017
Absent	55	24	31		
Present	51	34	17		
Venous invasion				0.101	0.751
Absent	57	32	25		
Present	49	26	23		
Nervous invasion				0.212	0.645
Absent	60	34	26		
Present	56	24	22		
Distant invasion				6.344	0.012
Absent	52	22	30		
Present	54	36	18		
Tumor stage				6.177	0.015
I+II	57	25	32		
III+IV	49	33	16		

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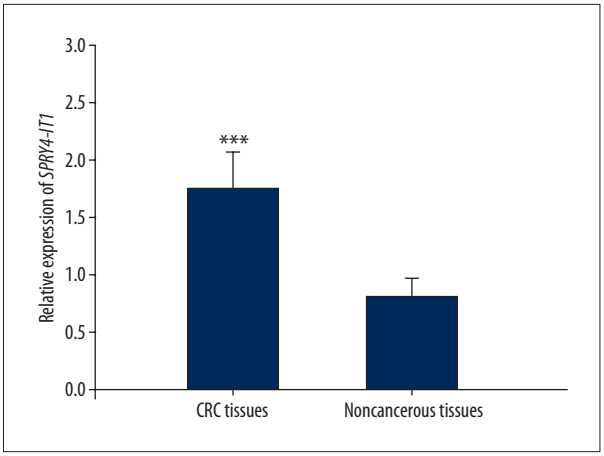

Figure 1. Relative expression of *SPRY4-IT1* in CRC patients. Up-regulated level of *SPRY4-IT1* was detected in CRC tissues compared with adjacent normal tissues (*GAPDH* as normalized control).* Indicated *P*<0.001.

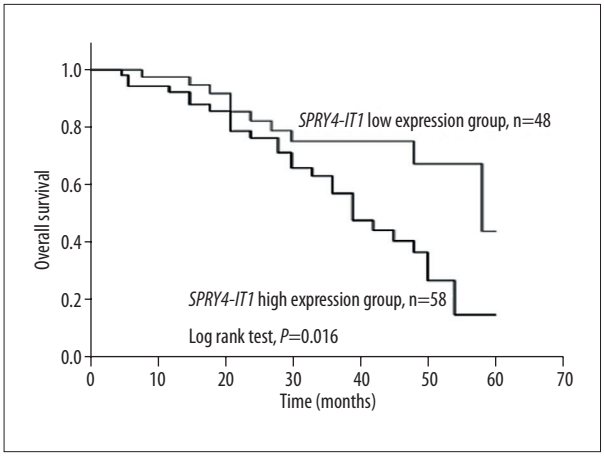

Figure 2. Overall survival analysis for patients with CRC. Patients with low level of *SPRY4-IT1* had better outcomes compared with those with high level (log-rank test, *P*=0.016).

Table 3. Cox regression analysis for prognosis in CRC patients.

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
SPRY4-IT1	2.341	1.136–4.826	0.021	2.341	1.136–4.826	0.021
Gender	1.297	0.677–2.487	0.433	–	–	–
Age	0.897	0.470–1.713	0.742	–	–	–
Tumor size	1.299	0.684–2.467	0.423	–	–	–
Location	0.986	0.521–1.866	0.966	–	–	–
Histological differentiation	1.214	0.637–2.314	0.556	–	–	–
The depth of invasion	0.816	0.425–1.566	0.540	–	–	–
Lymph node invasion	0.949	0.500–1.801	0.872	–	–	–
Venous invasion	0.941	0.493–1.795	0.853	–	–	–
Nervous invasion	0.826	0.435–1.571	0.561	–	–	–
Distant invasion	1.236	0.648–2.357	0.521	–	–	–
Tumor stage	0.825	0.433–1.571	0.557	–	–	–

'–' – Indicated no available data.

the tumor size, the depth of invasion, lymph node invasion, distant invasion, and tumor stage. *SPRY4-IT1* expression was not relevant for sex, age, tumor location, histological differentiation, venous invasion, or nervous invasion. These data suggest that *SPRY4-IT1* may play a role in the tumorigenesis and progression in CRC.

The prognostic value of *SPRY4-IT1* in CRC was also analyzed in this study. Over-expression of *SPRY4-IT1* was correlated with low overall survival and the Cox regression analysis showed that *SPRY4-IT1* could be an independent prognostic biomarker for CRC. Similar results were also found in other types of cancer, and higher expression of *SPRY4-IT1* predicted poor prognosis in many cancers, such as gastric cancer [18], esophageal squamous cell carcinoma [20], clear cell renal cell carcinoma [27], and non-small cell lung cancer [28]. These studies indicated that *SPRY4-IT1* might be useful for providing insights into mechanisms of cancers development.

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Conclusions

This study proves that *SPRY4-IT1* expresses aberrantly in diseased tissues of CRC patients compared with the adjacent normal tissues. Moreover, the expression level is associated with tumor size, the depth of invasion, lymph node invasion, distant invasion, and tumor stage. Additional, *SPRY4-IT1* can act as an independent biomarker for CRC prognosis and overexpression of *SPRY4-IT1* predicts poor prognosis in CRC. These results suggest that *SPRY4-IT1* may be a promising target for CRC therapy.

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