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ORIGINAL RESEARCH

Increased expression of antisense lncRNA *SPINT1-AS1* predicts a poor prognosis in colorectal cancer and is negatively correlated with its sense transcript

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Purpose: Colorectal cancer (CRC) is a leading cause of cancer-associated mortality worldwide. Natural antisense transcripts (NATs) are pervasively expressed in human genome and have been confirmed to contribute to cancer progression. In our study, we aimed to investigate the expression and clinical pertinence of serine peptidase inhibitor, Kunitz type 1 antisense RNA1 (*SPINT1-AS1*) in CRC.

Materials and methods: The expression levels of *SPINT1-AS1* and the corresponding sense transcript *SPINT1 mRNA* were analyzed in 150 pairs of CRC tissues and adjacent normal (AN) tissues, along with 45 pairs of preoperative and postoperative serum exosome samples by the strand-specific real-time quantitative polymerase chain reaction.

Results: Compared with AN tissues, the expression of *SPINT1-AS1* was increased ($P<0.001$, 3.771 vs 0.980) in CRC tissues, while *SPINT1 mRNA* expression was decreased in CRC ($P<0.001$, 0.927 vs 1.165), and there was an obviously negative correlation between *SPINT1-AS1* expression and its sense transcript ($r=-0.701$, $P<0.001$). *SPINT1-AS1* yielded an area under the receiver operating characteristic curve value of 0.865 (95% confidence interval, 0.821–0.902) for discriminating CRC tissues from AN tissues. Moreover, high *SPINT1-AS1* expression was correlated with regional lymph node metastasis ($P<0.001$), distant metastasis ($P<0.001$), and shorter relapse-free survival (RFS) time ($P<0.001$), and Cox regression analysis indicated that *SPINT1-AS1* was an independent prognostic factor for RFS. Meanwhile, significant reduction of *SPINT1-AS1* expression level ($P=0.001$) was observed in CRC serum exosomes after surgical resection.

Conclusion: *SPINT1-AS1* is upregulated in CRC tissues and plays an essential role in CRC progression and prognosis. Thereby, *SPINT1-AS1* may serve as a candidate prognostic biomarker and molecular therapy target for CRC.

Keywords: colorectal cancer, natural antisense transcripts, *SPINT1-AS1*, *SPINT1 mRNA*, prognosis

Introduction

Colorectal cancer (CRC) is the third-ranking cancer among males and the second among females worldwide, with an estimated 1.4 million new cases and 693,900 deaths per year.[1] Despite the great progress made in CRC screening and therapeutic methods, the 5-year survival rate of CRC patients remains unsatisfactory. Until now, multiple genetic and epigenetic alterations have been confirmed to exert critical functions in CRC tumorigenesis and progression,[2] whereas they are still underutilized for precise

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prediction of CRC course. Accordingly, it is of imminent importance to explore the appropriate molecular markers associated with clinical outcome and identify effective molecular targets for gene therapies.

Natural antisense transcripts (NATs) are transcribed from the DNA strands complementary to that of their endogenous sense counterparts.[3] NATs, with limited protein-coding ability, exist ubiquitously in mammalian genome.[4] They can regulate the expression of complementary sense transcripts through various mechanisms, such as epigenetic regulation of transcription, pre-mRNA alternative splicing, mRNA transport, mRNA stability, translation, generation of endogenous siRNA, and masking miRNA-binding sites.[5] A growing body of research shows that NATs participate in multiple important biological processes, such as neurogenesis,[6] female X-chromosome inactivation,[7,8] and circadian clock function.[9] Recently, NATs have been found to play important roles in the occurrence and development of various types of cancers. NAT *KRT7-AS* promotes gastric cancer cell proliferation and migration through stabilizing *KRT7 mRNA* by forming an RNA–RNA duplex.[10] *LCT13*, antisense to *TFPI-2*, is associated with deposition of repressive histone marks and then reduces expression of in breast and colon cancer cell lines.[11] Moreover, high gene locus-specific combinations of sense/antisense (S/AS) pairs make NATs promising precise therapeutic targets.[12,13] However, among the enormous amount of non-coding RNAs (ncRNAs), NATs are less studied compared with other types of ncRNA, especially another subclass of long non-coding RNA (lncRNA), long-intergenic non-coding RNAs (lincRNAs);[12,13] hence, the functional roles of the vast majority of NATs in CRC remain to be explored.

In the current study, we focused on one of the NATs termed serine peptidase inhibitor, Kunitz type 1 antisense RNA1 (*SPINT1-AS1*). Until now, the status of *SPINT1-AS1* in CRC has not been explored; thus, we evaluated the expression of *SPINT1-AS1* and analyzed the relationship with its corresponding sense transcript serine peptidase inhibitor, Kunitz type 1 (*SPINT1*) mRNA. The fact that SPINT1 has anti-cancer properties through its inhibitory effects on cell proliferation, migration, invasion, and decreased expression has been confirmed in breast cancer,[14] ovarian cancer,[15] cervical cancer,[16] prostate cancer,[17] gastric cancer,[18] pancreatic cancer,[19] and endometrial cancer.[20] The same trend held true in CRC; the expression of SPINT1 obviously decreased in cancer cells compared with adjacent normal (AN) or adenoma cells.[18,21] It has also been proved in mouse models that SPINT1 has a critical role as a suppressor of tumorigenesis in the intestinal tract.[22] Then, we investigated the relationship between *SPINT1-AS1* and clinicopathological characteristics and further confirmed whether its expression can be used for prognostic and therapeutic evaluation in CRC.

Materials and methods

Patients and sample collection

Written informed consent was obtained from all patients, and ethical approval for the study was obtained from the Ethics Committee of Qilu Hospital, Shandong University (protocol number: KYLL-2013-081). A total of 150 pairs of CRC tissues and AN tissues from patients who underwent radical surgical cancer resection were collected in this study between June 2009 and April 2011. CRC diagnosis was confirmed by pathological method, and noncancerous tissues were at least 5 cm away from the tumor margin. All tissues were snap-frozen in liquid nitrogen immediately after surgical resection and then preserved at −80°C until used for total RNA extraction. Venous blood samples were collected before and 7 days after surgical treatment from another 45 CRC patients. All blood samples were centrifuged at 3,000 rpm for 10 min to collect serum, followed by 110,000 rpm for 10 min to remove the remaining cell debris, and finally stored at −80°C until exosome extraction. Patients who received radiotherapy and chemotherapy before surgical treatment were excluded. The postoperative pathological staging was defined according to the tumor-node-metastasis grading system of the American Joint Committee on Cancer.

All patients enrolled in this study have been followed up at intervals of 3 months in the first 2 years and at 6 months' interval up to the 5th year, and the follow-up was completed in July 30, 2016. Post-surgery follow-up was implemented through imageological examination, colonoscopy, pathological examination, and/or telephone follow-up. The overall survival (OS) time was defined as the period from diagnosis to death or the last follow-up date. The relapse-free survival (RFS) time was defined as the period from diagnosis to the date of relapse or the last follow-up date if no relapse.

RNA extraction and strand-specific reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNAs of the tissue samples were isolated by using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with standard methodology. Exosomes were isolated by using ExoQuick Exosome Precipitation Solution (SBI, Mountain View, CA, USA), and exosomal RNA was extracted by using miRNeasy Mini Kit (Qiagen NV, Venlo, the Netherlands). Total RNAs from the tissue samples or exosomes were denatured at 65°C for 5 min and

then reversely transcribed into cDNA with specific primers by using a TaKaRa Reverse Transcription Kit (TaKaRa, Dalian, China). DNase I was applied to treat total RNAs to remove potential chromosomal DNA contamination before reverse transcription. The conditions for reverse transcription were 37°C for 15 min and 85°C for 5 s. Then, qPCRs were performed for expression analysis on 10-fold diluted first-strand cDNA by using Power SYBR Green (TaKaRa). The amplification was performed as follows: 95°C for 30 s, 40 cycles of 95°C for 5 s, and 60°C for 34 s, and melting curve analysis. The expression of individual genes was normalized to the expression of two reference genes, *GAPDH* and *UBC*. The primer sequences used were listed in Table 1. The relative expressions of target genes were calculated by using the comparative delta-delta CT method ($2^{-\Delta\Delta Ct}$).

Dual luciferase reporter assay

Luciferase reporter gene vectors were constructed with *SPINT1 mRNA* 5′UTR sequences or mutant 5′UTR sequences in pGL3 plasmids (RiboBio, Guangzhou, China). The *SPINT1-AS1* sequence was subcloned into the pcDNA3.1 vector (RiboBio), and an empty pcDNA3.1 vector was used as a control. SW480 cells were co-transfected with wide-type (or mutant-type) pGL3 plasmid and pcDNA3.1 plasmid containing *SPINT1-AS1* sequence (or control pcDNA3 plasmid) by using Lipofectamine 2000 (Thermo Fisher Scientific). The activities of firefly luciferase and Renilla luciferase were measured, and firefly luciferase was standardized to the value of Renilla luciferase.

Statistical analysis

The data were analyzed by using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Distribution of the data was determined by the Kolmogorov–Smirnov test. Data are presented as median (interquartile range). Expression differences between groups were analyzed by using Mann–Whitney *U*-test or Kruskal–Wallis test. Receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) were employed to illustrate the performance of *SPINT1-AS1* to discriminate CRC tissues from AN tissues. MedCalc 9.3.9.0 (MedCalc, Mariakerke, Belgium) was used to construct the ROC curve. OS and RFS curves were plotted by using the Kaplan–Meier method with the log-rank test performed to evaluate the statistical difference between the curves. Spearman's correlation analysis was used to investigate the correlation between the expression of *SPINT1-AS1* and *SPINT1 mRNA*. The hazard ratio (HR) and the 95% confidence interval (95% CI) were calculated by using univariate Cox proportional hazards regression. The final multivariate Cox regression was conducted with all covariates controlled. A P-value of <0.05 was considered statistically significant for all analyses.

Table 1 Primer sequences for reverse transcription quantitative polymerase chain reaction

Gene	Primer sequence (5′–3′)
SPINT1-AS1	
Reverse transcription	TCAAGGTGTCAGCCAGCACT
Forward	GCCCTGGAGGATGAGAG
Reverse	CAGATGCTGTTGGCTAAAGA
SPINT1 mRNA	
Reverse transcription	CCGTAACAACCACCATAGGT
Forward	ACCTGTCAGCCCACCCAGTT
Reverse	CCACGCAGTGCCCTTTGTCA
GAPDH	
Forward	TGCACCACCAACTGCTTAGC
Reverse	GGCATGGACTGTGGTCATGAG
UBC	
Forward	AGTAGTCCCTTCTCGGCGAT
Reverse	GACGATCACAGCGATCCACA

Results

Expression of *SPINT1-AS1* in CRC tissues

The result of lncRNA microarray analysis suggested that certain NATs were dysregulated in CRC tissues compared with AN tissues. Among these differentially expressed transcripts, *SPINT1-AS1* was one of the upregulated NATs demonstrated by human genome-wide lncRNA microarray analysis performed on six pairs of CRC tissues and AN tissues (Figure 1A). By performing strand-specific RT-qPCR on these six pairs of tissues, a consistent result was presented, and the expression level of *SPINT1-AS1* was increased in CRC tissues compared with AN tissues (P=0.015, Figure 1B). We further detected *SPINT1-AS1* expression in 150 pairs of CRC tissues and AN tissues. As shown in Figure 1C, the expression level of *SPINT1-AS1* was significantly upregulated in CRC tissues compared with AN tissues ($P<0.001$, Figure 1C) and was upregulated more than twofold in 116 of the 150 CRC tissues (Figure 1D). The ROC curve analysis illustrated that the expression level of *SPINT1-AS1* was able to distinguish CRC tissues from AN tissues, with an AUC value of 0.865 (95% CI, 0.821–0.902, Figure 1E).

Correlation between *SPINT1-AS1* and its sense transcript

Information from the National Center for Biotechnology Information Gene database (http://www.ncbi.nlm.nih.gov/gene) shows *SPINT1-AS1* and *SPINT1 mRNA* overlap at the 5′UTR in a head-to-head manner (Figure 2A). To study the

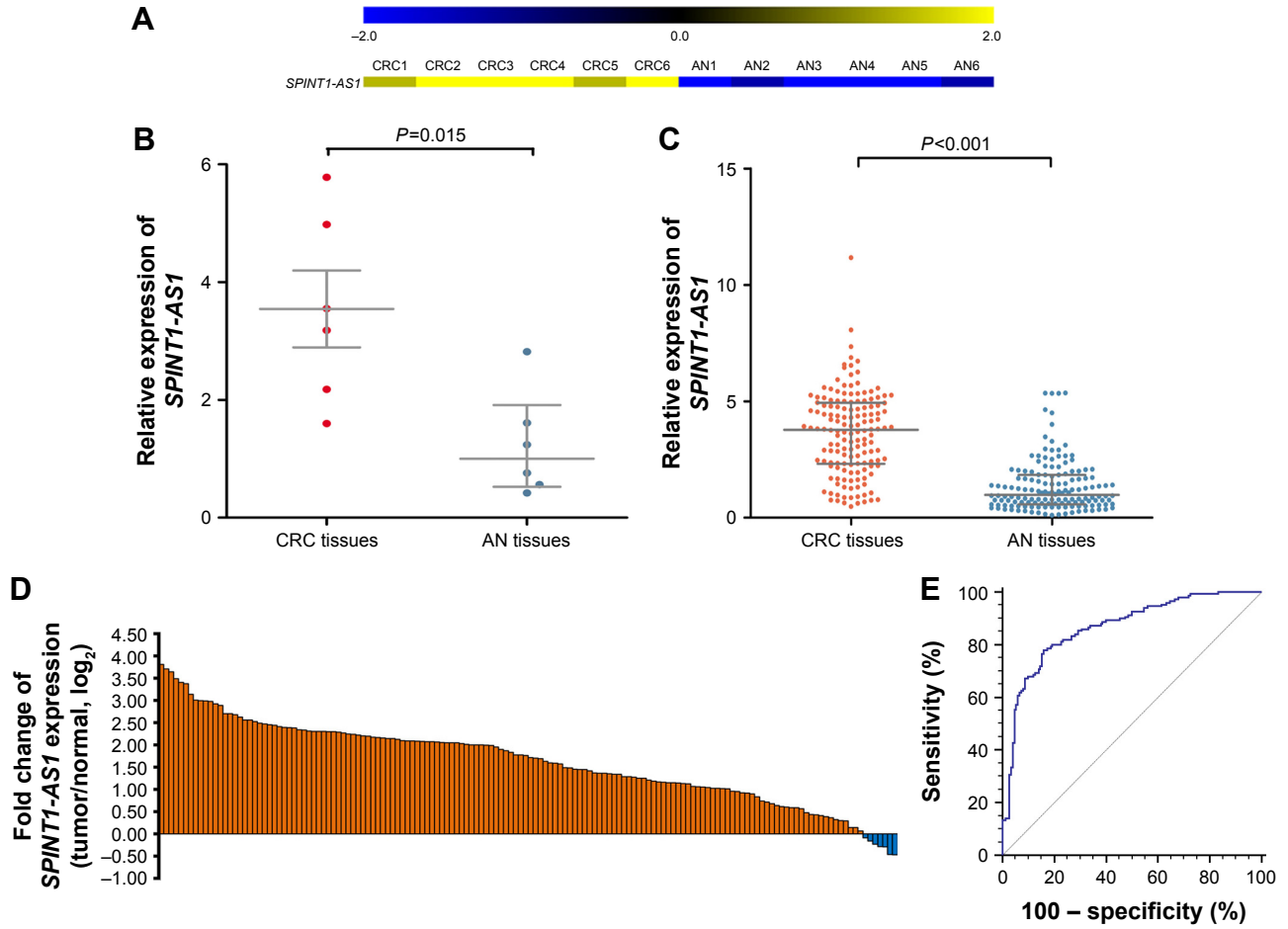

Figure 1 *SPINT1-AS1* was up-regulated in CRC tissues.

Notes: (**A**) Heat map of lncRNA microarray data showing expression level of *SPINT1-AS1* in CRC tissues (n=6) and matched AN tissues (n=6). (**B**) Strand-specific RT-qPCR analysis of *SPINT1-AS1* expression level in six paired CRC tissues and AN tissues used for lncRNA microarray analysis. *P*=0.015 by Wilcoxon signed-rank test. Data are shown as median with interquartile range. (**C**) Strand-specific RT-qPCR analysis of *SPINT1-AS1* expression level in 150 paired CRC tissues and AN tissues. *P*<0.001 by Wilcoxon signed-rank test. Data are shown as median with interquartile range. (**D**) Expression of *SPINT1-AS1* in 150 CRC tissues. The data are shown as $\log_2$-fold change in tumor tissues normalized to AN tissues expression. (**E**) ROC curves for discriminating CRC tissues from AN tissues.

Abbreviations: lncRNA, long non-coding RNA; *SPINT1-AS1*, serine peptidase inhibitor, Kunitz type 1 antisense RNA1; CRC, colorectal cancer; AN, adjacent normal tissues; RT-qPCR, real-time quantitative polymerase chain reaction; ROC, receiver operating characteristic.

relationship between *SPINT1-AS1* and *SPINT1 mRNA*, we also assessed the expression of *SPINT1 mRNA* in the same 150 pairs of CRC tissues and AN tissues. *SPINT1 mRNA* expression level was significantly downregulated in CRC tissues compared with AN tissues ($P<0.001$, Figure 2B). Correlation analysis was performed, and the Spearman's correlation coefficients showed a statistically negative correlation between *SPINT1-AS1* and *SPINT1 mRNA* expression in CRC tissue samples ($r=-0.701$, $P<0.001$, Figure 2C). To test whether the regulation between *SPINT1-AS1* and *SPINT1 mRNA* was mediated through their overlapping 5′UTR, we performed dual luciferase reporter assay, and the result showed that *SPINT1-AS1* overexpression decreased the overlapping region of luciferase activity ($P=0.020$), while mutant overlapping sequence (Figure 2D) could abrogate this effect (Figure 2E).

Relationship between *SPINT1-AS1* expression and clinicopathological characteristics in CRC

To determine the correlation between *SPINT1-AS1* and the progression of CRC, we analyzed whether *SPINT1-AS1* expression was associated with clinicopathological features of CRC patients. As presented in Table 2, high *SPINT1-AS1* expression level was significantly correlated with adverse clinicopathological characteristics of CRC, including regional lymph node metastasis ($P<0.001$, Mann–Whitney *U*-test) and distant metastasis ($P<0.001$, Mann–Whitney *U*-test). However, no significant relevance was found between *SPINT1-AS1* expression level and other clinicopathological factors such as age ($P=0.747$, Mann–Whitney *U*-test), gender ($P=0.218$, Mann–Whitney *U*-test),

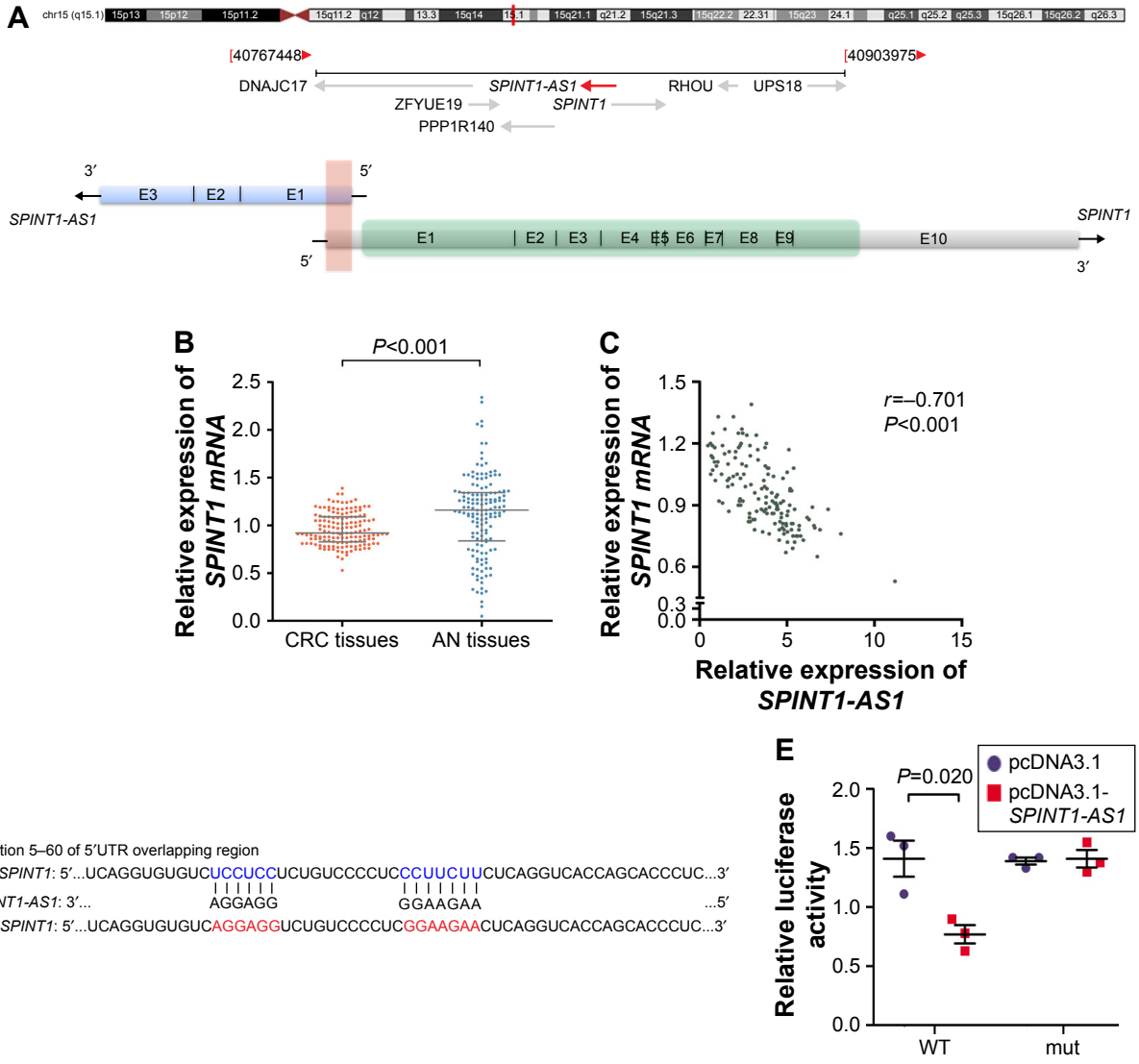

Figure 2 The correlation between *SPINT1-AS1* and *SPINT1 mRNA*.

Notes: (**A**) Upper chart of this panel shows genomic locus of the *SPINT1* indicated on the UCSC site. Lower chart of this panel is a schematic representation of *SPINT1-AS1* and *SPINT1 mRNA*. "E" represents exons. Red shadow represents overlapping region of *SPINT1-AS1* and *SPINT1 mRNA*. Green shadow represents CDS region of *SPINT1 mRNA*. Black arrows show the direction of transcription. The schema is in scale. (**B**) Strand-specific RT-qPCR analysis of *SPINT1 mRNA* expression level in 150 paired CRC tissues and AN tissues. $P<0.001$ by Wilcoxon signed-rank test. Data are shown as median with interquartile range. (**C**) The correlation between *SPINT1-AS1* and *SPINT1 mRNA* expression levels was analyzed by Spearman's rank correlation analysis. (**D**) Schematic of wide-type (WT) and mutant (mut) overlapping 5′UTR sequences used in dual luciferase reporter assay. (**E**) Luciferase activity in SW480 co-transfected with luciferase reporters containing WT or mut 5′UTR overlapping sequence and pcDNA3.1-*SPINT1-AS1* or negative control. Data are presented as the relative ratio of firefly luciferase activity to Renilla luciferase activity. $P=0.020$.

Abbreviations: *SPINT1-AS1*, serine peptidase inhibitor, Kunitz type 1 antisense RNA1; CRC, colorectal cancer; RT-qPCR, real-time quantitative polymerase chain reaction; UCSC, University of California Santa Cruz; CDS, coding sequence.

tumor location ($P=0.959$, Mann–Whitney *U*-test), tumor differentiation ($P=0.647$, Kruskal–Wallis test), T stage ($P=0.430$, Mann–Whitney *U*-test), and tumor size ($P=0.410$, Mann–Whitney *U*-test).

The prognostic value of *SPINT1-AS1* expression for CRC patients

To determine the prognostic value of *SPINT1-AS1*, survival analysis was performed in the 150 CRC patients by using the Kaplan–Meier method along with log-rank test. According to the median value of *SPINT1-AS1* expression level, patients with CRC were categorized into high-expression group and low-expression group. The results revealed that *SPINT1-AS1* expression level had little to do with the OS time ($P=0.152$, HR =1.717; 95% CI, 0.811–3.637, Figure 3A), whereas high *SPINT1-AS1* expression level was a significant prognostic factor for a shorter RFS time ($P<0.001$, HR =4.325; 95% CI, 2.204–8.489, Figure 3B). We further estimated the significance of several clinical parameters that might influence RFS in the subjects, and the univariate Cox proportional hazards

Table 2 Relationship between *SPINT1-AS1* expression and clinicopathological characteristics in colorectal cancer patients

Characteristics	n	*SPINT1-AS1* expression	*P*-value
Age (years)			0.747[a]
<60	64	3.695 (2.293–4.643)	
≥60	86	3.810 (2.295–5.038)	
Gender			0.218[a]
Male	97	3.680 (2.105–4.870)	
Female	53	3.890 (2.640–5.010)	
Tumor location			0.959[a]
Rectum	90	3.780 (2.345–4.845)	
Colon	60	3.695 (2.228–4.950)	
Tumor differentiation			0.647[b]
Poor	36	3.905 (2.193–4.760)	
Moderate	98	3.795 (2.273–4.990)	
Well	16	3.295 (2.438–4.388)	
T stage			0.430[a]
T1–T2	37	3.070 (2.275–5.115)	
T3–T4	113	3.810 (2.295–4.820)	
Regional lymph node metastasis			<0.001[a]
Negative	66	2.640 (1.918–3.920)	
Positive	84	4.365 (3.353–5.113)	
Distant metastasis			<0.001[a]
No	132	3.365 (2.160–4.660)	
Yes	18	5.150 (4.433–5.630)	
Tumor size			0.410[a]
<5 cm	69	3.730 (2.105–5.150)	
≥5 cm	81	3.780 (2.320–4.710)	

Notes: Data presented as median (25%–75% interquartile range). [a]Mann–Whitney *U*-test, [b]Kruskal–Wallis test.
Abbreviation: *SPINT1-AS1*, serine peptidase inhibitor, Kunitz type 1 antisense RNA1.

regression model analysis showed that decreased RFS was correlated with advanced T stage classification (HR =2.259; 95% CI, 1.013–5.036; *P*=0.046), regional lymph node metastasis (HR =3.657; 95% CI, 1.820–7.348; *P*<0.001), distant metastasis (HR =5.606; 95% CI, 3.010–10.439; *P*<0.001), and high expression level of *SPINT1-AS1* (HR =4.355; 95% CI, 2.219–8.549; *P*<0.001). Further multivariate Cox analysis revealed that the expression level of *SPINT1-AS1* (HR =2.519; 95% CI, 1.180–5.378; *P*=0.017), along with distant metastasis (HR =2.770; 95% CI, 1.398–5.489; *P*=0.003) could act as independent predictors of RFS (Table 3).

SPINT1-AS1 expression level in CRC serum exosomes

To further consolidate the vital function of this S/AS pair, *SPINT1-AS1* and *SPINT1 mRNA* expression levels in serum exosomes were measured in 45 pairs of preoperative and postoperative serum samples. Decreased *SPINT1-AS1* expression was observed after surgery (*P*=0.001, Figure 4A), while *SPINT1 mRNA* expression showed no comparable change (*P*=0.062, Figure 4B). These results suggested a promising role of *SPINT1-AS1* in CRC monitoring and prompted us to focus on *SPINT1-AS1* in the follow-up study.

Discussion

In this study, NAT, *SPINT1-AS1* was first found in CRC patients by genome-wide lncRNA microarray and strand-specific RT-qPCR methods. And, its expression was

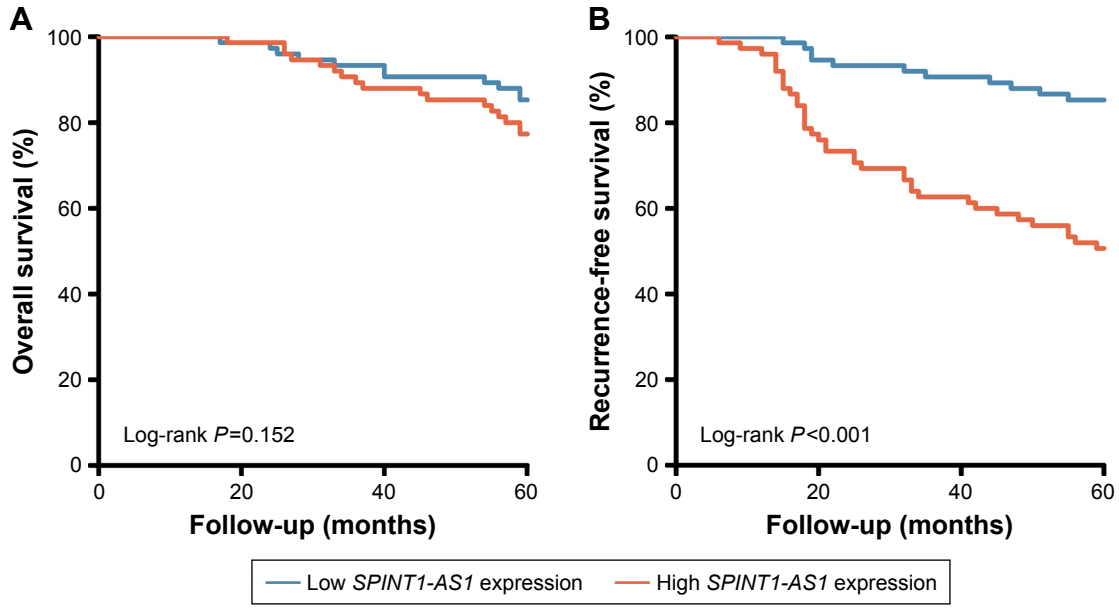

Figure 3 Kaplan–Meier curves for (**A**) overall survival and (**B**) recurrence-free survival according to relative *SPINT1-AS1* expression level in CRC tissues.
Abbreviations: *SPINT1-AS1*, serine peptidase inhibitor, Kunitz type 1 antisense RNA1; CRC, colorectal cancer.

Table 3 Univariate and multivariate analysis of factors associated with recurrence-free survival

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age	0.929 (0.525–1.644)	0.801		
Sex	1.346 (0.758–2.389)	0.311		
Tumor location	0.823 (0.465–1.456)	0.504		
Tumor differentiation	0.998 (0.614–1.625)	0.995		
T stage	2.259 (1.013–5.036)	0.046	2.168 (0.970–4.842)	0.059
Regional lymph node metastasis	3.657 (1.820–7.348)	<0.001	1.969 (0.905–4.284)	0.088
Distant metastasis	5.606 (3.010–10.439)	<0.001	2.770 (1.398–5.489)	0.003
Tumor size	0.599 (0.338–1.059)	0.078		
High *SPINT1-AS1* expression	4.355 (2.219–8.549)	<0.001	2.519 (1.180–5.378)	0.017

Abbreviations: *SPINT1-AS1*, serine peptidase inhibitor, Kunitz type 1 antisense RNA1; HR, hazard ratio; CI, confidence interval.

negatively correlated with its sense transcript expression. Based on the analysis of a large number of specimens, *SPINT1-AS1* showed an increased expression in CRC tissues and had a function to differentiate CRC from AN tissues. Moreover, high *SPINT1-AS1* expression level was significantly correlated with regional lymph node metastasis, distant metastasis, and short RFS. Meanwhile, exosomal *SPINT1-AS1* in serum of CRC patients might be used as a noninvasive biomarker for therapeutic evaluation.

Recently, growing evidences suggests that dysregulation of lncRNAs is involved in the complexity of the CRC process by influencing various aspects of cell biology, including proliferation, differentiation, apoptosis, angiogenesis, epithelial–mesenchymal transition, invasion, and metastasis.[23,24] Certain lncRNAs are uniquely expressed in CRC, and their expression levels are associated with CRC state; therefore, lncRNAs have potential clinical application as biomarkers for diagnosis, prognosis, or as therapeutic targets.[23,24] For these reasons, intensive investigations in recent years have focused on the role of lncRNAs in the pathogenesis of CRC. In our study, strand-specific RT-qPCR performed with specific reverse transcription primers was implemented to eliminate detection interference caused by complementary strands. The results of the quantitative studies showed that *SPINT1-AS1* expression was significantly increased in CRC tissues compared with AN tissues while *SPINT1 mRNA* expression was downregulated.

Over the past few years, mounting evidence pointed to the significance of NATs in regulating gene expression at multiple levels, including epigenetic modifications and transcriptional and post-transcriptional regulation. *BDNF-AS* serves a critical role in the guidance, introduction, and maintenance of H3K27met3 at the *BDNF* locus by recruiting polycomb repressive complex 2 to the *BDNF* promoter region.[25] *Lrp1-AS* directly combines with high mobility group box 2 (Hmgb2) and blocks the function of Hmgb2 to enhance Srebp1a-

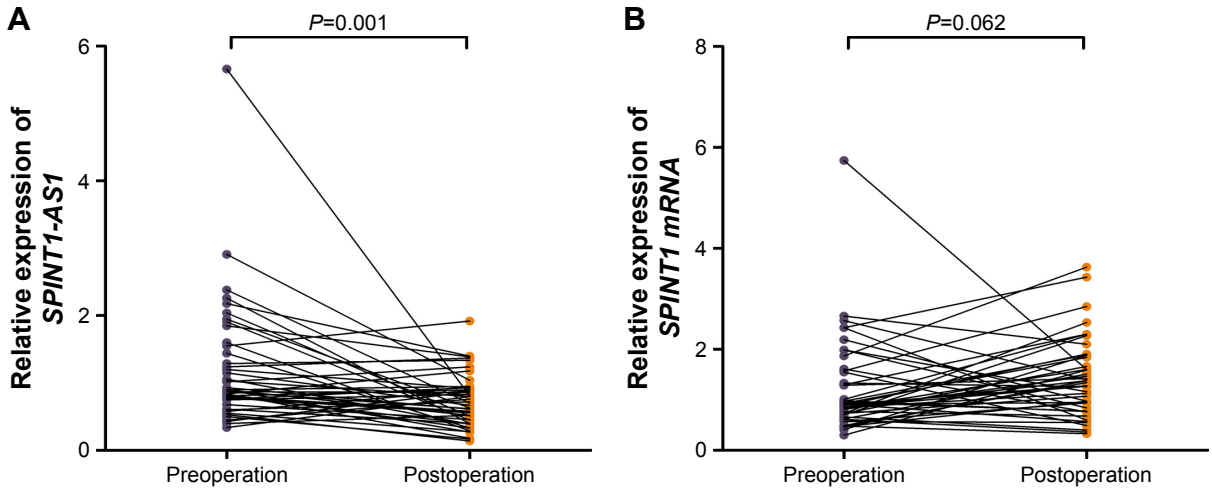

Figure 4 Strand-specific RT-qPCR analysis of (**A**) *SPINT1-AS1*, *P*=0.001 by Wilcoxon signed-rank test and (**B**) *SPINT1 mRNA*, *P*=0.062 by Wilcoxon signed-rank test. Expression in 45 pairs of preoperative and postoperative serum exosome samples.
Abbreviations: *SPINT1-AS1*, serine peptidase inhibitor, Kunitz type 1 antisense RNA1; RT-qPCR, real-time quantitative polymerase chain reaction.

dependent transcription of *Lrp1*.[26] *FOXM1-AS* regulates *FOXM1* post-transcriptionally by forming RNA hybridization to increase *FOXM1 pre-mRNA* stability. *FOXM1-AS* elevates *FOXM1* expression via carrying ALKBH5 to weaken N6-methyladenosine (m6A) RNA modification of *FOXM1 pre-mRNA*.[27] Based on the site of biogenesis, NATs are separated into cis-NATs or trans-NATs. Cis-NATs are produced from opposite DNA strands on the same genomic loci as their sense counterparts, whereas trans-NATs are transcripts originating from different genomic loci.[28,29] Cis-NATs prevailingly form long perfect complementary regions with a specific sense transcript from the same generation locus, while trans-NATs usually form short imperfect matches with several sense transcripts from different genomic loci.[28] Cis-NAT pairs are further classified as head-to-head (5′-regions overlap), tail-to-tail (3′-regions overlap), embedded (one transcript is fully contained within the other), or intronic pairs.[30] *SPINT1-AS1* is transcribed from the reverse strand of the *SPINT1* locus, located at 15q15.1, and consists of three exons. The sense transcript *SPINT1 mRNA* is transcribed from the forward strand of the same locus and consists of 10 exons. The 5′UTR of *SPINT1-AS1* directly overlaps the 5′UTR of *SPINT1 mRNA* by 80 bp. In our study, a negative correlation between *SPINT1-AS1* and *SPINT1 mRNA* expression in CRC tissues was observed. Dual luciferase reporter assay showed decreased *SPINT1 mRNA* 5′UTR luciferase activity in *SPINT1-AS1* overexpression cells. These phenomena may indicate a regulatory role of *SPINT1-AS1* to *SPINT1 mRNA*.

Serine peptidase inhibitor, Kunitz type 1 (SPINT1), also known as hepatocyte growth factor (HGF) activator (HGFA) inhibitor type 1 (HAI-1), encoded by the *SPINT1* gene is a specific cellular inhibitor of active HGFA.[31,32] HGFA is the most potent proteinase to activate HGF which is secreted in an inactive single-chain precursor form.[33] The activation step of HGF is a critical limiting step in the HGF-induced signaling pathway.[34] To date, a growing amount of evidence has accumulated in favor of the notion that HGF gets involved in the invasive growth of tumor cells through a sequence of steps, including dissociation of the tumor cells from their neighbors, migration through the extracellular matrix, and proliferation.[33] The HGFA/HAI-1 ratio is of great significance to the regulation of HGF activity, and the unbalanced ratio is related to tumorigenesis.[35] Clinical implications of *SPINT1-AS1* in CRC were demonstrated in our study. *SPINT1-AS1* might participate in tumorigenesis and progression of CRC, as *SPINT1-AS1* expression level in CRC tissues was significantly related to regional lymph node metastasis and distant metastasis. According to the Kaplan–Meier curve, patients with high *SPINT1-AS1* expression level had a markedly higher tumor recurrence rate than those with low level. Moreover, Cox proportional hazards regression model indicated that elevated *SPINT1-AS1* level was an independent prognostic factor of CRC.

It is of interest to note that besides differential expression of *SPINT1-AS1* between CRC tissues and AN tissues, dynamic expression was also observed in pre- and postoperative serum exosomes. *SPINT1-AS1* expression level significantly declined after surgical resection. This observation positions *SPINT1-AS1* as a potential noninvasive biomarker for monitoring changes in the course of CRC treatment.

In the current study, several obstacles are unclear. Although *SPINT1-AS1* expression level is not closely tied with OS, we are not sure if this is due to the limited follow-up time. Whether *SPINT1-AS1* could serve as a noninvasive biomarker for early diagnosis needs more empirical evidence for further confirmation. CRC screening can greatly reduce CRC-related mortality. Improvement of screening methods has become a main point of concern, such as fecal occult blood testing has been replaced by fecal immunochemical testing (FIT) to increase sensitivity[36,37] and FIT combined with microbiome constituents may improve the screening results.[38] In our future study, we could measure *SPINT1-AS1* level in feces of CRC patients and controls and analyze the microbial diversity and composition to explore the correlation between *SPINT1-AS1* level and the dysbiosis of gut microbiota. Although the regulative role of *SPINT1-AS1* on *SPINT1 mRNA* expression level has been confirmed in our study, whether *SPINT1-AS1* could regulate the alternative splicing of *SPINT1 mRNA* warrants further study. Previous study has demonstrated that antisense lncRNA *TPM1-AS* could regulate the alternative splicing of *TPM1* through an interaction with RNA-binding mofit protein 4 (RBM4).[39] The online RNA-binding protein (RBP) database RBPDB predicts that both *SPINT1-AS1* and *SPINT1 pre-mRNA* have binding sites of RBM4. Thus, there is a possibility that *SPINT1-AS1* regulates the process of CRC by changing the expression level and influencing the alternative splicing of *SPINT1 mRNA* simultaneously.

Conclusion

Our study evaluated the expression pattern of *SPINT1-AS1* in the context of CRC and demonstrated that upregulated *SPINT1-AS1* expression was a common event in CRC. High expression of *SPINT1-AS1* is associated with poor clinical outcome and *SPINT1-AS1* is a promising biomarker for the prognostic prediction and treatment monitoring of CRC patients.

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Disclosure

The authors report no conflicts of interest in this work.

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