

Decreased expression of SPINT1-AS1 and SPINT1 mRNA might be independent unfavorable prognostic indicators in esophageal squamous cell carcinoma

This article was published in the following Dove Press journal:
OncoTargets and Therapy

Fang-Fang Shen,^{1,*} Ying Pan,^{1,*} Hai-Jun Yang,² Jun-Kuo Li,² Fang Zhao,² Jing-Fen Su,² Yan-Yan Li,¹ Lin-Qiang Tian,¹ Pan-Ting Yu,¹ Yan-Tian Cao,¹ Yao-Wen Zhang,² Fu-You Zhou,^{1,2}

¹The Key Laboratory for Tumor Translational Medicine, The Third Affiliated Hospital of Xinxiang Medical University, Xinxiang, Henan, People's Republic of China; ²Anyang Key Laboratory for Esophageal Cancer Research, Anyang Tumor Hospital, Anyang, Henan, People's Republic of China

*These authors contributed equally to this work

→ Video abstract



Point your SmartPhone at the code above. If you have a QR code reader the video abstract will appear. Or use: <https://youtu.be/1n97rmu26Yw>

Correspondence: Fu-You Zhou
Anyang Key Laboratory for Esophageal Cancer Research, Anyang Tumor Hospital, Huan Bin North Street of Anyang, Anyang, Henan 455000, People's Republic of China
Tel +86 186 3721 8799
Email ayzhoufuyou@163.com

Purpose: The serine peptidase inhibitor, Kunitz type 1 antisense RNA1 (*SPINT1-AS1*), a long non-coding RNA, has been linked to cancer progression. In this study, we aimed to explore the *SPINT1-AS1* expression in matched esophageal squamous cell carcinoma (ESCC) and normal tissues, and analyze the potential correlations of *SPINT1-AS1* expression with clinicopathological characteristics, in order to evaluate its prognosis and therapeutic value.

Methods: *SPINT1-AS1* expression was detected in 99 cases of matched ESCC and normal tissues samples using the quantitative real-time polymerase chain reaction method.

Results: The expression level (ΔCt) of *SPINT1-AS1* and *SPINT1* mRNA was significantly downregulated in ESCC tissues compared with matched normal tissues ($P=0.0005$; $P=0.0002$, respectively), and there was an obvious positive correlation between *SPINT1-AS1* and *SPINT1* mRNA expression. Clinicopathological characteristics indicated that *SPINT1-AS1* expression was correlated with age and tumor size, while *SPINT1* mRNA expression was correlated with age and gender. The receiver operating characteristic (ROC) curve analysis of the expression level of *SPINT1-AS1* and *SPINT1* mRNA yielded an area under the ROC curve value of 0.638 and 0.625, respectively. The overall survival is shorter in patients with low *SPINT1-AS1* expressed than those with high levels of *SPINT1-AS1* ($P=0.044$), and *SPINT1* mRNA expression level is associated with the OS ($P=0.001$). Univariate and multivariate analysis suggested that *SPINT1-AS1* was an independent prognostic indicator in ESCC.

Conclusions: We found that the expression of *SPINT1-AS1* and *SPINT1* mRNA is downregulated in ESCC tissues, which could contribute to tumor progression. *SPINT1-AS1* and *SPINT1* mRNA may be therapeutic targets and prognosis markers for ESCC.

Keywords: esophageal squamous cell carcinoma, *SPINT1-AS1*, *SPINT1*, prognosis

Introduction

Esophageal cancer (EC) represents the sixth most frequent cause of cancer-related deaths worldwide.^{1,2} Over half of all EC-related deaths occur in China where esophageal squamous cell carcinoma (ESCC) is the predominant histologic subtype, particularly in high-risk populations.^{3,4} Despite the rapid development of medical techniques and the continuous improvement of techniques for early diagnosis of EC, advanced cases still account for approximately 50% of the clinical diagnoses, and the overall five-year survival rate with EC is 15–25%.⁵ Therefore, early detection of tumors provides effective treatments and timely interventions, which may improve the outcomes. Determination of molecular markers of early-stage cancer is urgently required to improve patient prognosis.

Long non-coding RNAs (lncRNAs) are novel families of long (>200 nucleotides), lacking protein-coding ability, which has been discovered to have a critical role in regulating gene expression and oncogenic pathways.^{6–8} Several lncRNAs were recently found to play important roles in modulating ESCC development.^{9,10} Among many of the cancer-associated lncRNAs, the serine peptidase inhibitor, Kunitz type 1 antisense RNA1 (*SPINT1-AS1*), a member of the Kunitz family of serine protease inhibitors, inhibits multiple proteases activity including hepatocyte growth factor (HGF) activator and matriptase.¹¹ Abnormal expression of these serine protease inhibitors has been demonstrated in several types of cancer.^{12–16} However, the role of *SPINT1-AS1* in ESCC remains unintelligible.

In this study, we investigated the expression of *SPINT1-AS1* in matched ESCC and normal tissues by quantitative real-time polymerase chain reaction (qRT-PCR) method. We further analyzed the relationship between *SPINT1-AS1*, its corresponding sense transcript serine peptidase inhibitor, Kunitz type 1 (*SPINT1*) mRNA, and clinicopathological characteristics in ESCC. Our study provides novel insights into the role of *SPINT1-AS1* in ESCC progression, and identifies a potential new target for diagnosis and treatment of ESCC.

Materials and methods

Ethics statement

The study protocol was approved by the ethical review committee of the Third Affiliated Hospital of Xinxiang Medical University and Anyang Tumor Hospital. All the assays were conducted according to Declaration of Helsinki principles. Written informed consent was obtained from all the participants.

Recruitment of patients and controls

A total of 99 cases of matched ESCC and normal tissues samples (3–7 cm away from the tumor margin) were obtained from Anyang Tumor Hospital (Henan, China) ranging from January 2011 to February 2012. All tissues were snap-frozen in liquid nitrogen immediately after surgical resection and then preserved at –80°C until used for total RNA extraction. All the cases were histopathologically confirmed, and there was no prior treatment to those patients. Patient demographic, clinical, and pathological data were recorded. All the tumors were confirmed to contain more than 80% tumor cells by histological

examination of sequential sections. TNM staging was classified according to the American Joint Committee on Cancer (7th edition). All the personal information was concealed to ensure the privacy. The clinicopathological characteristics of the ESCC patients are summarized in Table 1.

All patients enrolled in this study have been followed up at intervals of 1–3 months through outpatient visits or telephone calls. The follow-up data were completed on July 30, 2017. The overall survival (OS) time was defined as the period from diagnosis to death or the last follow-up date.

Table 1 Clinicopathological characteristics of 99 ESCC cases

Variables	N	% of cases
Age (years)		
≤65	57	57.6
>65	42	42.4
Gender		
Female	46	46.5
Male	53	53.5
Family history		
Positive	36	36.4
Negative	63	63.6
Drinking history (years)		
≤10	68	68.7
>10	31	31.3
Smoking history (years)		
≤10	65	65.7
>10	34	34.3
Differentiation		
Well	13	13.1
Moderate	54	54.6
Poor	32	32.3
T classification		
T1–T2	26	26.3
T3	73	73.7
N classification		
N0	52	52.5
N1+ N2+ N3	47	47.5
Tumor size (cm)		
≤4	60	60.6
>4	39	39.4
Location		
Upper	17	17.2
Middle	67	67.7
Low	15	15.1

Abbreviation: ESCC, esophageal squamous cellcarcinoma.

RNA extraction, reverse transcription, and qRT-PCR

The relative expression levels of *SPINT1-AS1* and *SPINT1* mRNA in ESCC tissues and matched normal tissues were determined using qRT-PCR assays. Total RNA from each specimen was extracted using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Inc., Carlsbad, California, USA). RNA concentration was determined by ScanDrop Nuclear Acid Analyzer (Analytikjena, German). The PrimeScript RT Reagent Kit (Invitrogen, Thermo Fisher Scientific, Inc., Carlsbad, California, USA) was used for reverse transcription. The levels of mRNA of *SPINT1* expression and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were determined using the ABI 7500 FAST sequence detection system (Applied Biosystems, Thermo Fisher Scientific, Inc., Carlsbad, California, USA). Triplicates of each target genes and each specimen were used, with *GAPDH* as an internal standard. The manufacturer's instructions for each kit were followed during the whole process. The primer sequences used are listed in Table 2. The relative expression levels of target genes were calculated by using the $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

All of the data were analyzed using SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Chi-squared test was used to evaluate the relationship between *SPINT1-AS1* and *SPINT1* mRNA and clinicopathological characteristics, and independent two-tailed *t*-test was used to analyze the statistical differences in *SPINT1-AS1* and *SPINT1* mRNA expression in ESCC tissues and matched normal tissues. Receiver operating characteristic (ROC)

curve was performed, and the area under the ROC curve (AUC) was calculated to evaluate the diagnostic value of *SPINT1-AS1* and *SPINT1* mRNA in ESCC. Survival analysis was conducted by Kaplan–Meier method and log-rank test. 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 Cox proportional hazards regression. Univariate and multivariate Cox regression analyses were used to assess the prognostic value of biomarkers alone and when adjusted for clinical parameters. The difference was statistically significant when $P < 0.05$.

Results

Expression of *SPINT1-AS1* in ESCC and matched normal tissues

qRT-PCR was done to estimate the transcriptional level of *SPINT1-AS1* in 99 ESCC and matched normal tissues. Results showed that *SPINT1-AS1* expression level (ΔC_t) in ESCC tissues and matched normal tissues is 4.786 ± 0.258 and 3.651 ± 0.209 , respectively. In this study, we found that *SPINT1-AS1* was downregulated in ESCC tissues ($P = 0.0005$, Figure 1A) and was downregulated more than twofold in 60 of the 99 ESCC tissues (Figure 1B).

Relationship between *SPINT1-AS1* and *SPINT1* mRNA

To study the relationship between *SPINT1-AS1* and *SPINT1* mRNA, we assessed the expression of *SPINT1* mRNA in 99 pairs of ESCC tissues and matched normal tissues. As shown in Figure 1C, the expression level of *SPINT1* mRNA (ΔC_t) was significantly downregulated in ESCC tissues (2.279 ± 0.240) compared with matched normal tissues (1.132 ± 0.188) ($P = 0.0002$). By performing the correlation analysis, the Spearman's correlation coefficients showed a statistically positive correlation between *SPINT1-AS1* and *SPINT1* mRNA expression in ESCC tissues ($r = 0.7063$, $P < 0.0001$, Figure 1D).

Relationship between *SPINT1-AS1*, *SPINT1* mRNA, and clinicopathological characteristics in ESCC

To further explore the role of *SPINT1-AS1* and *SPINT1* mRNA in ESCC, the correlations between *SPINT1-AS1*,

Table 2 Primer sequences for reverse transcription quantitative polymerase chain reaction

Gene	Primer sequence (5'–3')
<i>SPINT1-AS1</i>	
Forward	GCCCTGGAGGATGAGAG
Reverse	CAGATGCTGTTGGCTAAAGA
<i>SPINT1</i> mRNA	
Forward	ACCTGTCAGCCACCCAGTT
Reverse	CCACGCAGTGCCCTTTGTCA
<i>GAPDH</i>	
Forward	GTGGAGTCCACTGGCGTCTT
Reverse	GTGCAGGAGGCATTGCTGAT

Table 3 Clinicopathological characteristics and SPINT1-ASI and SPINT1 mRNA expression level of 99 ESCC cases

Variables	N	SPINT1-ASI expression		P value	SPINT1 mRNA expression		P value
		High (n=39)	Low (n=60)		High (n=36)	Low (n=63)	
Age (years)							
≤65	57	16	41	0.007 ^a	15	42	0.015 ^a
>65	42	23	19		21	21	
Gender							
Female	46	14	32	0.089	12	34	0.048 ^a
Male	53	25	28		24	29	
Family history							
Positive	36	13	23	0.613	12	24	0.636
Negative	63	26	37		24	39	
Drinking history (years)							
≤10	68	24	44	0.216	22	46	0.219
>10	31	15	16		14	17	
Smoking history (years)							
≤10	65	23	42	0.259	22	43	0.472
>10	34	16	18		14	20	
Differentiation							
Well	13	7	6	0.477	6	7	0.444
Moderate	54	21	33		21	33	
Poor	32	11	21		9	23	
T classification							
T1–T2	26	11	15	0.723	10	16	0.796
T3	73	28	45		26	47	
N classification							
N0	52	21	31	0.832	18	34	0.704
N1+ N2+ N3	47	18	29		18	29	
Tumor size (cm)							
≤4	60	29	31	0.024 ^a	23	37	0.613
>4	39	10	29		13	26	
Location							
Upper	17	8	9	0.117	8	9	0.323
Middle	67	22	45		21	46	
Low	15	9	6		7	8	

Note: ^aStatistically significant.

Abbreviations: ESCC, esophageal squamous cell carcinoma; SPINT1-ASI, serine peptidase inhibitor, Kunitz type I antisense RNAI; SPINT1, serine peptidase inhibitor, Kunitz type I.

SPINT1 mRNA, expression and the clinicopathological variables of patients with ESCC were assessed as shown in Table 3. Compared with the matched normal tissues, the high *SPINT1-ASI* expression was significantly related to the characters, including age (>65 years old vs ≤65 years old: 59.0% vs 31.7%, $P=0.007$) and tumor size (≤4 cm vs >4 cm: 74.3% vs 51.7%, $P=0.024$). However, there was no significant difference between *SPINT1-ASI* expression and clinicopathological factors, including gender ($P=0.089$), family history

($P=0.613$), drinking history ($P=0.216$), smoking history ($P=0.259$), differentiation ($P=0.477$), T classification ($P=0.723$), N classification ($P=0.832$), and location ($P=0.117$). *SPINT1* mRNA expression was upregulated in 58.3% (21/36) ESCC tissues in >65 years old group, while this percentage was significantly lower in ≤65 years old group of 33.3% (21/63) ($P=0.007$). The same tendency was also observed in male patients (male vs female: 66.7% vs 46.0%, $P=0.048$). There was no significant correlation

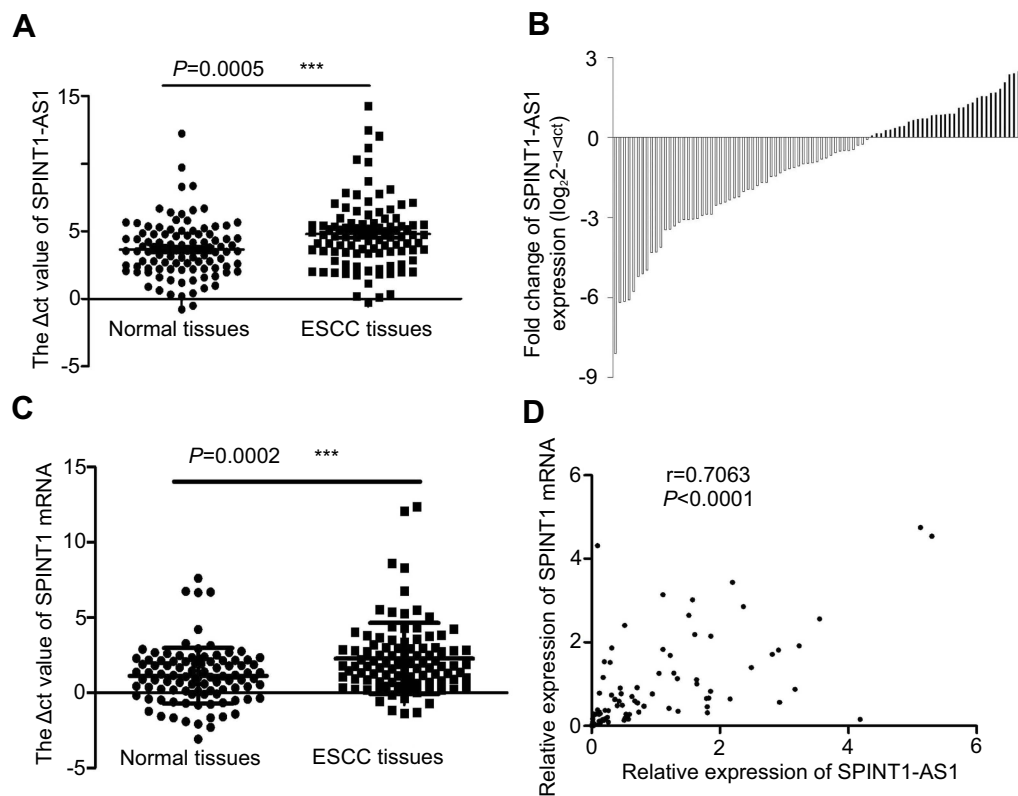


Figure 1 *SPINT1-AS1* and *SPINT1* mRNA expression in ESCC tissues and matched normal tissues. (A) qRT-PCR analysis of *SPINT1-AS1* expression level in 99 ESCC tissues and matched normal tissues ($P=0.0005^{***}$). Data are shown as median with interquartile range. The expression of *SPINT1-AS1* in the tissues was calculated using the ΔCt method. (B) Expression of *SPINT1-AS1* in 99 ESCC tissues. The data are shown as fold change in ESCC tissues normalized to matched normal tissues expression ($\log_2 2^{-\Delta\Delta\text{Ct}}$). (C) qRT-PCR analysis of *SPINT1* mRNA expression level in 99 ESCC tissues and matched normal tissues ($P=0.0002^{***}$). Data are shown as median with interquartile range. The expression of *SPINT1* mRNA in the tissues was calculated using the ΔCt method. (D) The correlation between *SPINT1-AS1* and *SPINT1* mRNA expression levels was analyzed by Spearman's correlation analysis ($r=0.7063$, $P<0.0001$).

Abbreviations: ESCC, esophageal squamous cell carcinoma; *SPINT1-AS1*, serine peptidase inhibitor, Kunitz type I antisense RNA1; *SPINT1*, serine peptidase inhibitor, Kunitz type I.

between *SPINT1* mRNA and clinicopathological factors including family history ($P=0.636$), drinking history ($P=0.219$), smoking history ($P=0.472$), differentiation ($P=0.444$), T classification ($P=0.796$), N classification ($P=0.704$), tumor size ($P=0.613$), and location ($P=0.323$).

The diagnostic value of *SPINT1-AS1* and *SPINT1* mRNA expression in ESCC

The ROC curve analysis illustrated that the expression level of *SPINT1-AS1* and *SPINT1* mRNA was able to distinguish ESCC tissues from normal tissues, with AUC value of 0.638 ($P=0.001$, 95% CI: 0.560–0.716, Figure 2) and 0.625 ($P=0.002$, 95% CI: 0.547–0.703, Figure 2), respectively.

The prognostic values of *SPINT1-AS1* and *SPINT1* mRNA expression in ESCC

To assess the potential role of *SPINT1-AS1* and *SPINT1* mRNA expression on the prognosis of ESCC, the Kaplan–

Meier survival analysis and log-rank tests for OS were conducted on 99 patients who had intact follow-up information. The median survival time for the high *SPINT1-AS1* expression group was 40 months, while it was 35 months for the low *SPINT1-AS1* expression group. As it is shown in Figure 3A, the OS is shorter in *SPINT1-AS1* low-expressed patients than those of high levels of *SPINT1-AS1* ($P=0.044$, HR=0.631, 95% CI: 0.403–0.989). The 5-year OS rate for the high *SPINT1* mRNA expression group was 11.1%, while it was 6% in the compared groups. The results revealed that *SPINT1* mRNA expression level was associated with the OS time ($P=0.001$, HR=0.466; 95% CI: 0.296–0.733, Figure 3B).

SPINT1-AS1 expression and the indicator for survival

The association between *SPINT1-AS1* expression and prognosis of ESCC patients was evaluated by univariate analysis

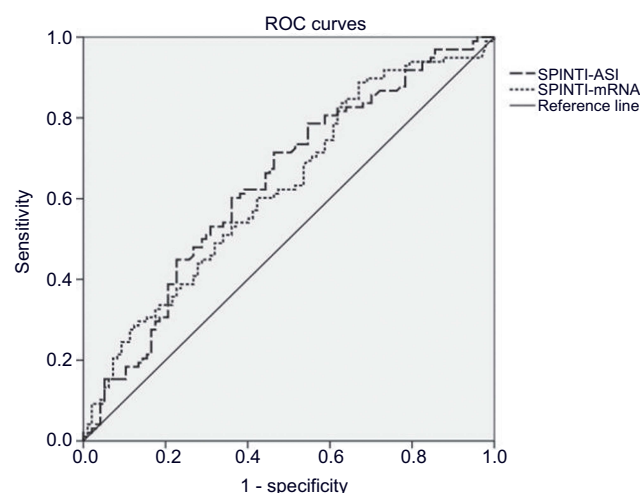


Figure 2 The ROC curve analysis of *SPINT1-ASI* and *SPINT1* mRNA. The expression level of *SPINT1-ASI* and *SPINT1* mRNA was able to distinguish ESCC tissues from normal tissues, with AUC value of 0.638 ($P=0.001$) and 0.625 ($P=0.002$).

Abbreviations: ROC, receiver operating characteristic; AUC, the area under the ROC curve; ESCC, esophageal squamous cell carcinoma; *SPINT1-ASI*, serine peptidase inhibitor, Kunitz type I antisense RNA1; *SPINT1*, serine peptidase inhibitor, Kunitz type I.

and multivariate analysis. As shown in Table 4, the OS of those patients was significantly dependent on family history ($P=0.022$, HR=0.596, 95% CI, 0.382–0.929), differentiation ($P=0.009$), N classification ($P=0.000$, HR=0.279, 95% CI, 0.169–0.461), and *SPINT1-ASI* expression levels ($P=0.006$, HR=0.525, 95% CI, 0.331–0.833). Thus, these factors were enrolled in the multivariate Cox regression analysis. The expression of *SPINT1-ASI* ($P=0.030$, HR=0.598, 95% CI: 0.376–0.953) was an independent indicator for the OS in ESCC, along with family history ($P=0.001$, HR=2.278,

95% CI, 1.420–3.654) and N classification ($P=0.000$, HR=0.279, 95% CI: 0.169–0.461) (Table 4).

Discussion

ESCC is an aggressive tumor with rapid growth rate and high opportunity of regional and distant metastasis, which contributes to the poor prognosis.¹⁷ Therefore, it is urgent to find an effective early biomarker for ESCC so that the tumor can be treated at the early stage. In recent years, a new insight into the pathogenesis of human tumors has emerged with the discovery of lncRNAs. To date, thousands of lncRNAs have been reported to play important roles in ESCC tumorigenesis, progression, and metastasis.^{9,18–20} For example, *LncRNA-NEF* inhibits proliferation, migration, and invasion of ESCC through inactivating wnt/ β -catenin pathway.⁹ *HOTAIR* directly downregulates the expression of *WIF-1* and activates the Wnt/ β -catenin signaling pathway to promote the migration and invasion of ESCC.¹⁴ *LncRNA CASC9* promotes ESCC metastasis through upregulating *LAMC2* expression by interacting with the CREB-binding protein.¹⁹ Upregulation of the long noncoding RNA *PCAT-1* correlates with advanced clinical stage and poor prognosis in ESCC.²⁰ Although the function of these dysregulated lncRNAs in ESCC has previously been reported, the function of *SPINT1-ASI* in ESCC has not been reported. In this study, we performed qRT-PCR analysis of *SPINT1-ASI* and *SPINT1* mRNA to investigate its clinical value on the prognosis of ESCC.

SPINT1-ASI is transcribed from the reverse strand of the *SPINT1* locus, located at 15q15.1, and consists of three

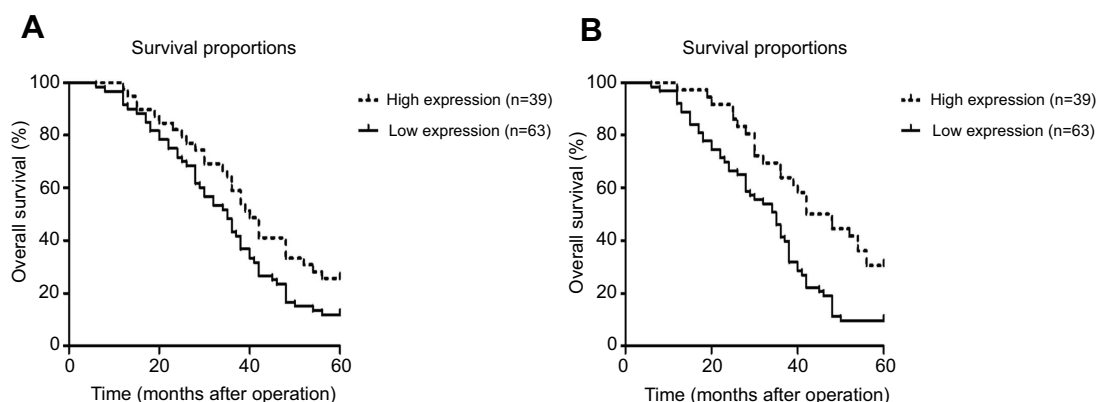


Figure 3 Kaplan-Meier survival curves for 99 ESCC based on *SPINT1-ASI* and *SPINT1* mRNA expression level. The x-axis represents survival time after surgery, and the y-axis represents cumulative OS. (A) Patients with low *SPINT1-ASI* expression had a significantly poorer prognosis (shorter five-year survival rate) than those with high *SPINT1-ASI* expression (imaginary line) ($P=0.044$). (B) Patients with low *SPINT1* mRNA expression had a significantly poorer prognosis (shorter five-year survival rate) than those with high *SPINT1* mRNA expression (imaginary line) ($P=0.001$).

Abbreviations: ESCC, esophageal squamous cell carcinoma; *SPINT1-ASI*, serine peptidase inhibitor, Kunitz type I antisense RNA1; *SPINT1*, serine peptidase inhibitor, Kunitz type I; OS, overall survival.

Table 4 Univariate and multivariate cox regression analysis of overall survival in 99 ESCC cases

Variables	N	Univariate analysis		Multivariate analysis	
		HR(95%CI)	P value	HR(95%CI)	P value
Age (years)					
≤65	57	1		–	
>65	42	0.801(0.510–1.257)	0.335		
Gender					
Female	46	1		–	
Male	53	0.878(0.567–1.359)	0.559		
Family history					0.001 ^a
Positive	36	1		1	
Negative	63	0.596(0.382–0.929)	0.022 ^a	2.278(1.420–3.654)	
Drinking history (years)					
≤10	68	1		–	
>10	31	1.188(0.748–1.886)	0.466		
Smoking history (years)					
≤10	65	1		–	
>10	34	1.164(0.739–1.832)	0.513		
Differentiation					0.242
Well	13	1	0.009 ^a	1	
Moderate	54	0.343(0.161–0.728)	0.005 ^a	0.552(0.252–1.209)	0.137
Poor	32	0.582(0.363–0.933)	0.025 ^a	0.716(0.440–1.164)	0.178
T classification					
T1–T2	26	1		–	
T3	73	1.354(0.811–2.263)	0.247		
N classification					0.000 ^a
N0	52	1	0.000 ^a	1	
N1+ N2+ N3	47	3.556(2.237–5.653)		0.279(0.169–0.461)	
Tumor size (cm)					
≤4	60	1		–	
>4	39	1.098(0.708–1.705)	0.676		
Location					
Upper	17	1	0.534	–	
Middle	67	1.071(0.510–2.250)	0.856		
Low	15	0.800(0.443–1.442)	0.457		
<i>SPINT1-AS1</i> expression					0.030 ^a
High	39	1	0.006 ^a	1	
Low	60	0.525(0.331–0.833)		0.598(0.376–0.953)	

Note: ^aStatistically significant.

Abbreviations: ESCC, esophageal squamous cellcarcinoma; HR, hazard ratio; 95% CI, 95% confidence interval.

exons. The sense transcript *SPINT1* mRNA is transcribed from the forward strand of the same locus and consists of 10 exons. Both play important roles in a number of human malignant tumors development and progression, including cell proliferation, migration, invasion, and metastasis. In this study, we revealed that *SPINT1-AS1* and *SPINT1*

mRNA were downregulated in ESCC compared with matched normal tissues. Also, the *SPINT1-AS1* expression level was positively associated with the expression of *SPINT1* mRNA in ESCC tissues. Moreover, low *SPINT1-AS1* expression was significantly correlated with age, tumor size, and short OS. *SPINT1* mRNA expression was

related to the age, gender, and OS. Hence, *SPINT1-AS1* and *SPINT1 mRNA* might be a potential biomarker for therapeutic evaluation of ESCC.

SPINT1, also known as HGF activator (*HGFA*) inhibitor type 1 (*HAI-1*), is a Kunitz-type serine protease inhibitor which expresses on the surface of most epithelial cells.^{11,21,22} This protease is reported to be increased or decreased in cancer tissue and contribute to cancer progression.²³ In prostate cancer, breast cancer, pancreatic ductal adenocarcinoma, colorectal cancer, and thyroid cancer, it has been reported that the high expression of *HAI-1* is possibly involved in the progression of these cancers.^{16,24–27} However, *HAI-1* showed a remarkable decrease in gastric cancer and endometrial cancer.^{14,15} These seemingly contradictory observations highlighted that the role of *SPINT1* may be organ-specific. In our study, the expression of *SPINT1-AS1* and *SPINT1 mRNA* was observed to be significantly downregulated in ESCC tissues than in matched normal tissues. From the survival analysis, we observed that patients with low levels of *SPINT1-AS1* and *SPINT1 mRNA* expression had significantly shorter OS than those with high levels of *SPINT1-AS1* and *SPINT1 mRNA* expression. The lower *SPINT1* expression might cause deregulated pericellular activities of proteases and promoted the proteolytic degradation of the basement membrane and extracellular matrix, resulting in a more invasive phenotype. Therefore, decreased expression of *SPINT1-AS1* and *SPINT1 mRNA* might be unfavorable prognostic indicators in ESCC. Whether the *SPINT1* expression is critical to ESCC development or progression needs to be further investigated.

We reported that the mRNA level of *SPINT1* is decreased significantly in ESCC tissues. A decrease in *SPINT1 mRNA* expression would allow for a more potent influence from lncRNA, because a lower level of *SPINT1* would be processed. Our results revealed that *SPINT1-AS1* and *SPINT1 mRNA* expression were significantly higher in long OS compared with the poor OS, suggesting that *SPINT1-AS1* may have other functions independent of its mRNA accelerative activity. It does regulate *SPINT1* activity but also plays roles in tissue injury and a possible role at the invasion front in cancer.

However, certain limitations in our study should be addressed as follows. A limitation of this study is that we did not measure the serum levels of *SPINT1* in our patients. A limited number of patients (99 cases) might result in bias in the final results. Besides, the molecular mechanisms underlying the altered expression of *SPINT1-*

AS1 in ESCC cell lines and mouse model need to be further addressed.

Conclusions

In conclusion, we found that the expression of *SPINT1-AS1* lncRNA and *SPINT1 mRNA* was downregulated in ESCC tissues which could contribute to tumor progression. This, in turn, impacts patients' postoperative survival time and can serve as an auxiliary index for predicting the prognosis of ESCC patients. It was suggested that both lncRNA and mRNA may be therapeutic targets and prognosis markers for ESCC.

Abbreviation list

EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; lncRNA, long non-coding RNA; *SPINT1-AS1*, serine peptidase inhibitor, Kunitz type 1 antisense RNA1; *SPINT1*, serine peptidase inhibitor, Kunitz type 1; OS, overall survival; ROC, receiver operating characteristic; AUC, the area under the ROC curve; HR, hazard ratio; 95% CI, 95% confidence interval.

Acknowledgments

We thank all the patients and their family members whose contributions made this work possible. We also thank Dr. Bing Su at University at Buffalo, New York, USA for helping with the manuscript preparation. This study was supported by National Natural Science Foundation of China (grant number: U1504814) and the Medical Science and Technology Project (grant number: 2018020377).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136(5):E359–E386. doi:10.1002/ijc.29210
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87–108. doi:10.3322/caac.21262
3. Li JY, Liu BQ, Li GY, Chen ZJ, Sun XI, Rong SD. Atlas of cancer mortality in the People's Republic of China. An aid for cancer control and research. *Int J Epidemiol*. 1981;10(2):127–133. doi:10.1093/ije/10.2.127
4. Li B, Taylor PR, Li JY, et al. Linxian nutrition intervention trials. Design, methods, participant characteristics, and compliance. *Ann Epidemiol*. 1993;3(6):577–585.
5. Lagergren J, Smyth E, Cunningham D, Lagergren P. Oesophageal cancer. *Lancet*. 2017;390(10110):2383–2396. doi:10.1016/S0140-6736(17)31462-9

6. Mitra SA, Mitra AP, Triche TJ. A central role for long non-coding RNA in cancer. *Front Genet.* **2012**;3:17. doi:10.3389/fgene.2012.00017
7. Guttman M, Donaghey J, Carey BW, et al. lincRNAs act in the circuitry controlling pluripotency and differentiation. *Nature.* **2011**;477(7364):295–300. doi:10.1038/nature10398
8. Hung T, Chang HY. Long noncoding RNA in genome regulation: prospects and mechanisms. *RNA Biol.* **2010**;7(5):582–585. doi:10.4161/rna.7.5.13216
9. Zhang J, Hu SL, Qiao CH, et al. LncRNA-NEF inhibits proliferation, migration and invasion of esophageal squamous-cell carcinoma cells by inactivating wnt/beta-catenin pathway. *Eur Rev Med Pharmacol Sci.* **2018**;22(20):6824–6831. doi:10.26355/eurrev_201810_16150
10. Yao J, Huang JX, Lin M, et al. Microarray expression profile analysis of aberrant long non-coding RNAs in esophageal squamous cell carcinoma. *Int J Oncol.* **2016**;48(6):2543–2557. doi:10.3892/ijo.2016.3457
11. Kataoka H, Kawaguchi M. Hepatocyte growth factor activator (HGFA): pathophysiological functions in vivo. *Febs J.* **2010**;277(10):2230–2237. doi:10.1111/j.1742-4658.2010.07640.x
12. Oberst MD, Johnson MD, Dickson RB, et al. Expression of the serine protease matriptase and its inhibitor HAI-1 in epithelial ovarian cancer: correlation with clinical outcome and tumor clinicopathological parameters. *Clin Cancer Res.* **2002**;8(4):1101–1107.
13. Nakamura K, Abarzua F, Hongo A, et al. The role of hepatocyte growth factor activator inhibitor-1 (HAI-1) as a prognostic indicator in cervical cancer. *Int J Oncol.* **2009**;35(2):239–248.
14. Zeng L, Cao J, Zhang X. Expression of serine protease SPC19/matriptase and its inhibitor hepatocyte growth factor activator inhibitor type 1 in normal and malignant tissues of gastrointestinal tract. *World J Gastroenterol.* **2005**;11(39):6202–6207. doi:10.3748/wjg.v11.i39.6202
15. Nakamura K, Hongo A, Kodama J, Hiramatsu Y. The role of hepatocyte growth factor activator inhibitor (HAI)-1 and HAI-2 in endometrial cancer. *Int J Cancer.* **2011**;128(11):2613–2624. doi:10.1002/ijc.25606
16. Li C, Li W, Zhang Y, et al. Increased expression of antisense lncRNA SPINT1-AS1 predicts a poor prognosis in colorectal cancer and is negatively correlated with its sense transcript. *Oncotargets Ther.* **2018**;11:3969–3978. doi:10.2147/OTT.S163883
17. Zhang L, Ma J, Han Y, et al. Targeted therapy in esophageal cancer. *Expert Rev Gastroenterol Hepatol.* **2016**;10(5):595–604. doi:10.1586/17474124.2016.1140036
18. Song W, Zou SB. Prognostic role of lncRNA HOTAIR in esophageal squamous cell carcinoma. *Clin Chim Acta.* **2016**;463:169–173. doi:10.1016/j.cca.2016.10.035
19. Liang Y, Chen X, Wu Y, et al. LncRNA CASC9 promotes esophageal squamous cell carcinoma metastasis through upregulating LAMC2 expression by interacting with the CREB-binding protein. *Cell Death Differ.* **2018**;25(11):1980–1995. doi:10.1038/s41418-018-0084-9
20. Shi WH, Wu QQ, Li SQ, et al. Upregulation of the long noncoding RNA PCAT-1 correlates with advanced clinical stage and poor prognosis in esophageal squamous carcinoma. *Tumour Biol.* **2015**;36(4):2501–2507. doi:10.1007/s13277-014-2863-3
21. Shimomura T, Denda K, Kitamura A, et al. Hepatocyte growth factor activator inhibitor, a novel Kunitz-type serine protease inhibitor. *J Biol Chem.* **1997**;272(10):6370–6376. doi:10.1074/jbc.272.10.6370
22. Kataoka H, Suganuma T, Shimomura T, et al. Distribution of hepatocyte growth factor activator inhibitor type 1 (HAI-1) in human tissues. Cellular surface localization of HAI-1 in simple columnar epithelium and its modulated expression in injured and regenerative tissues. *J Histochem Cytochem.* **1999**;47(5):673–682. doi:10.1177/002215549904700509
23. Tanabe LM, List K. The role of type II transmembrane serine protease-mediated signaling in cancer. *Febs J.* **2017**;284(10):1421–1436. doi:10.1111/febs.13971
24. Saleem M, Adhami VM, Zhong W, et al. A novel biomarker for staging human prostate adenocarcinoma: overexpression of matriptase with concomitant loss of its inhibitor, hepatocyte growth factor activator inhibitor-1. *Cancer Epidemiol Biomarkers Prev.* **2006**;15(2):217–227. doi:10.1158/1055-9965.EPI-05-0737
25. Parr C, Watkins G, Mansel RE, Jiang WG. The hepatocyte growth factor regulatory factors in human breast cancer. *Clin Cancer Res.* **2004**;10(1 Pt 1):202–211.
26. Sakugawa C, Haruyama Y, Tanaka H, Fukushima T, Kawaguchi M, Kataoka H. Prognostic significance of hepatocyte growth factor activator inhibitor type 1 (HAI-1) immunoreactivity in pancreatic ductal adenocarcinoma. *BMC Res Notes.* **2017**;10(1):674. doi:10.1186/s13104-017-3014-x
27. Liu CL, Yang PS, Chien MN, Chang YC, Lin CH, Cheng SP. Expression of serine peptidase inhibitor Kunitz type 1 in differentiated thyroid cancer. *Histochem Cell Biol.* **2018**;149(6):635–644. doi:10.1007/s00418-018-1660-2

OncoTargets and Therapy

Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic

agents and protocols on patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/oncotargets-and-therapy-journal>

Dovepress