

Original Article

Up-regulated expression of long non-coding RNA ZFAS1 associates with aggressive tumor progression and poor prognosis in gastric cancer patients

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Abstract: Emerging evidences showed that long non-coding RNAs (lncRNAs) play critical roles in tumor progression. Although up-regulation of lncRNA ZFAS1 was been found in several types of tumors, its role in gastric cancer (GC) remains unclear. The aim of this study was to identify the expression of lncRNA ZFAS1 in gastric cancer. Expression of ZFAS1 was detected in 104 pairs of tumor and adjacent non-tumor tissues from GC patients using quantitative real-time PCR (qRT-PCR). In the present study, we found that the expression level of ZFAS1 was increased in GC tissues compared with adjacent non-tumor tissues. Moreover, clinicopathological analysis revealed that high ZFAS1 expression was positively associated with depth of invasion, lymph node metastasis and TNM stage in GC patients. Kaplan-Meier analysis showed that up-regulated expression of ZFAS1 contributed to poor overall survival of GC patients. A multivariate survival analysis demonstrated that ZFAS1 could be an independent unfavorable prognostic factor in GC patients. Those finding suggested that lncRNA ZFAS1 was significantly increased in GC and could represent a new biomarker of poor prognosis and a potential therapeutic target for GC intervention.

Keywords: Long non-coding RNAs, ZFAS1, gastric cancer, overall survival, prognosis

Introduction

Gastric cancer (GC) is one of the most prevalent types of cancer, and it is the second leading cause of cancer related death worldwide [1]. Despite efforts using diagnostic techniques and patient management, the 5-year overall survival rate remains less than 25% [2]. Because most patients present with unresectable or metastatic disease at the time of diagnosis [3]. Therefore, it is critical for reducing the mortality to identify novel biomarkers for early diagnosis and prognosis evaluation in GC patients.

Long non-coding RNAs (lncRNAs), which are more than 200 nucleotides in length and unable to be translated into proteins, have been detected with the progression of whole-genome sequencing technology [4, 5]. Multiple lines of evidence revealed that lncRNAs could play important roles in cellular development, differentiation, and many other biological processes [6]. A lot of studies showed that the dysregulation of lncRNAs could play functional

roles in tumor progression. For example, Hirata et al. showed that lncRNA MALAT1 promoted aggressive renal cell carcinoma through Ezh2 and interacted with miR-205 [7]. Hu et al. found that lncRNA GAS5 suppressed the migration and invasion of hepatocellular carcinoma cells via miR-21 [8]. Ke et al. suggested that decreased expression of lncRNA HOTAIR could inhibit malignant biological behaviors of human glioma cells via modulation of miR-326 [9]. These studies indicated that lncRNAs play critical roles in human tumor progression.

In the present study, we evaluated the expression level of ZFAS1 in GC tissues and adjacent non-tumor tissues. Then, we investigated the association of ZFAS1 with clinicopathological features and overall survival of the patients and determined its prognostic role in GC. Our findings suggested that ZFAS1 was increased in GC tissues and could be regarded as a novel diagnostic biomarker and prognosis indicator in GC patients.

LncRNA ZFAS1 expression in gastric cancer

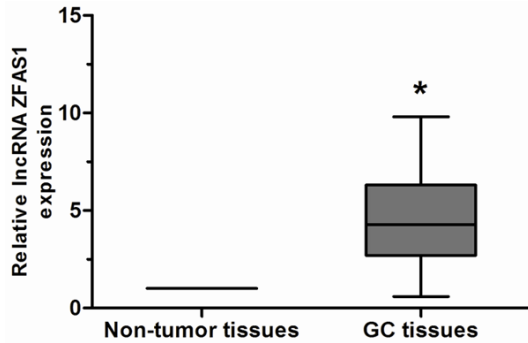


Figure 1. LncRNA ZFAS1 expression in 104 pairs of GC tissues and adjacent non-tumor tissues detected by qRT-PCR analysis. ZFAS1 expression was significantly increased in GC tissues when compared with adjacent non-tumor tissues. * $P < 0.05$.

Table 1. Association of lncRNA ZFAS1 expression with clinicopathological features in GC patients

Clinicopathological features	Total	LncRNA ZFAS1 expression		P value
		Low	High	
Age (years)				0.550
<60	43	23	20	
≥60	61	29	32	
Gender				0.687
Male	64	33	31	
Female	40	19	21	
Tumor size (cm)				0.169
<5	55	31	24	
≥5	49	21	28	
Differentiation				0.216
Well	36	15	21	
Moderate + Poor	68	37	31	
Depth of invasion				0.001
T1 + T2	43	30	13	
T3 + T4	61	22	39	
Lymph node metastasis				0.000
No	71	44	27	
Yes	33	8	25	
TNM stage				0.000
I + II	47	38	9	
III + IV	57	14	43	

Materials and methods

Patients and specimens

A total of 104 GC tissues and their adjacent non-tumor tissues were obtained from the Department of General Surgery, Huaihe Hos-

pital of Henan University between 2008 and 2010, and were diagnosed with gastric cancer based on histopathological evaluation. No patients received neoadjuvant chemotherapy or radiotherapy before surgery. All specimens were immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction. The study was approved by the Research Ethics Committee of Huaihe Hospital of Henan University. Informed consents were obtained from all patients.

Quantitative real-time PCR

Total RNA was extracted from GC tissues and adjacent non-tumor tissues with RNAiso Plus (Takara). The isolated total RNA was reverse transcribed using the PrimeScript RT Master Mix (Takara) according to manufacturer instructions. The sequence-specific forward and reverse primers sequences for ZFAS1 were 5'-TCTGACCAACGGCTCTTAGAC-3' and 5'-GTGCCATAGTTGACCAGAGTC-3' respectively. Forward and reverse primers sequences for GAPDH were 5'-AGAAGGCTGGGGCTCATTTG-3' and 5'-AGGGGCCATCCACAGTCTTC-3' respectively. qPCR was performed using SYBR Premix Ex Taq™ II (Takara) on a Light Cycler (Roch). Relative quantification of lncRNA ZFAS1 expression was calculated by using the $2^{-\Delta\Delta\text{Ct}}$ method. Each experiment was performed in triplicate.

Statistics analyses

All computations were carried out using the software of SPSS version 18.0 for windows. Data were expressed as means \pm standard deviation (SD). Statistical significance was tested by a Student's t-test or a Chi-square test as appropriate. Survival analysis was performed using the Kaplan-Meier method, and the log-rank test was used to compare the differences between patient groups. The Cox proportional hazards model for multivariate survival analysis was used to assess predictors related to survival. Differences were considered statistically significant when P was less than 0.05.

Results

Expression of lncRNA ZFAS1 is up-regulated in GC tissues

In order to explore the role of ZFAS1 in GC progression, we performed qRT-PCR to detect the expression level of ZFAS1 in 104 pairs of GC

LncRNA ZFAS1 expression in gastric cancer

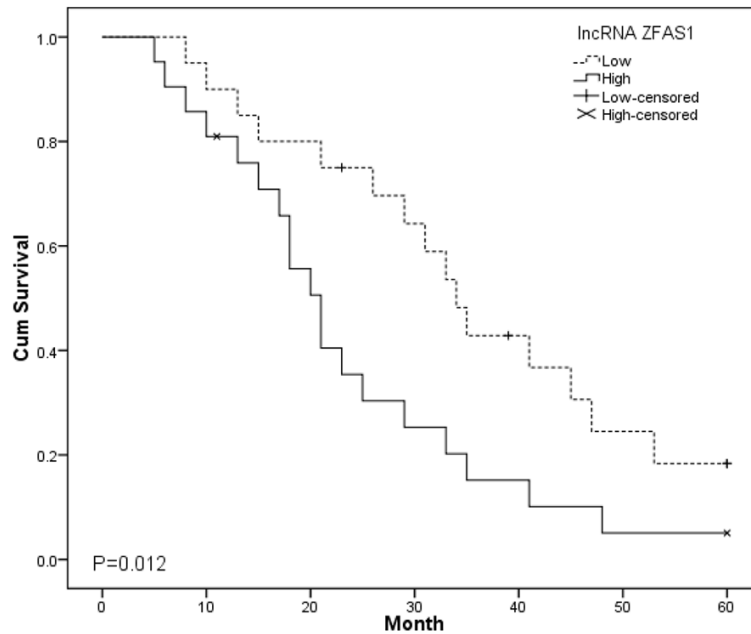


Figure 2. Kaplan-Meier curves for GC patients according to the expression of lncRNA ZFAS1. The group with high ZFAS1 expression exhibited a poorer overall survival compared with the group with low ZFAS1 expression ($P < 0.05$, log-rank test).

tissues and adjacent non-tumor tissues. qRT-PCR showed that the expression of ZFAS1 was significantly up-regulated in GC tissues compared to adjacent non-tumor tissues (**Figure 1**, $P < 0.05$). These findings indicated that abnormal ZFAS1 expression may be related to gastric cancer progression.

Association between clinicopathological features and lncRNA ZFAS1 expression in GC patients

We further analyzed the association between the expression of ZFAS1 and clinicopathological features of GC patients. 104 GC patients were classified into the low expression group ($n=52$) and the high expression group ($n=52$) according to the median ratio of relative ZFAS1 expression in tumor tissues. The association between clinicopathological features and ZFAS1 expression levels in GC patients was summarized in **Table 1**. Our data showed that high expression of ZFAS1 was positively correlated with deeper depth of invasion, lymph node metastasis, and advanced TNM stage in GC patients ($P < 0.05$). However, ZFAS1 expression level was not associated with other clinicopathological features such as age, gender, tumor size, and differentiation in GC patients ($P > 0.05$).

Association of lncRNA ZFAS1 expression with GC patients' survival

In order to identify the prognostic value of ZFAS1 expression for GC, we explored the association between the levels of ZFAS1 expression and overall survival through Kaplan-Meier analysis and log-rank test. Our data showed that the overall survival of GC patients with high ZFAS1 expression was significantly poorer compared to those patients with low ZFAS1 expression (**Figure 2**, $P < 0.05$). Univariate analysis showed that depth of invasion, lymph node metastasis, TNM stage, and ZFAS1 expression were significantly correlated with overall survival of GC patients (**Table 2**, $P < 0.05$). To evaluate the prognostic value of ZFAS1

expression, variables with a value of $P < 0.05$ were selected for multivariate analysis. Our results revealed that depth of invasion, lymph node metastasis, TNM stage, and ZFAS1 expression were independent prognostic factors for overall survival of GC patients (**Table 2**, $P < 0.05$). Taken together, these data suggested that high ZFAS1 expression level was an independent risk factor for GC patients.

Discussion

GC is a highly heterogeneous disease. Mainstream tumorigenic processes involved in GC are characterized by phenotypic multistep progression cascades [10]. The reliable identification of GC progression-specific targets has huge implications for its prevention and treatment [11, 12]. However, identification of the molecular mechanisms underlying tumorigenesis still remains a challenge.

LncRNA dysregulation contributes to a range of biological functions and provides a cellular growth advantage, resulting in progressive and uncontrolled tumor growth [13]. Effective control of both cell proliferation and invasion is critical to the prevention of oncogenesis and successful cancer therapy [14]. Therefore,

LncRNA ZFAS1 expression in gastric cancer

Table 2. Univariate and multivariate analysis of overall survival in GC patients

Clinicopathological features	Univariate analysis			Multivariate analysis		
	Hazard Ratio	95% CI	P	Hazard Ratio	95% CI	P
Age (years) ≥60 vs <60	0.873	0.514-1.824	0.274			
Gender Male vs Female	1.134	0.638-2.149	0.316			
Tumor size ≥5 cm vs <5 cm	2.214	0.776-3.817	0.178			
Differentiation Moderate + Poor vs Well	2.843	0.713-4.219	0.203			
Depth of invasion T3 + T4 vs T1 + T2	2.158	0.823-4.236	0.019	1.938	0.747-3.926	0.013
Lymph node metastasis Yes vs No	3.721	1.186-7.022	0.017	3.347	1.071-6.758	0.007
TNM stage III + IV vs I + II	3.381	1.253-6.915	0.014	3.074	1.185-5.831	0.004
LncRNA ZFAS1 High vs Low	2.953	1.318-7.043	0.002	2.573	1.251-6.836	0.003

identification of GC associated lncRNAs and investigation of their clinical significance may provide a missing piece of the well known oncogenic and tumor suppressor network puzzle. Recently, lots of studies showed that lncRNAs were dysregulated in multiple cancers including GC. For example, Zhou et al. suggested that down-regulation of lncRNA LET correlated with clinical progression and unfavorable prognosis in GC [15]. Fei et al. reported that decreased lncRNA LINC00982 could be identified as a poor prognostic biomarker in GC and regulate cell proliferation [16]. Li et al. found that up-regulated expression of lncRNA BANCR was associated with clinical progression and poor prognosis in GC [17]. Kong et al. revealed that increased expression of lncRNA PVT1 indicated a poor prognosis of GC and promoted cell proliferation through epigenetically regulating p15 and p16 [18]. However, little is known about the clinical significance of ZFAS1 in GC patients.

In the present study, we explored the expression of lncRNA ZFAS1 in GC, our results showed that the expression levels of ZFAS1 in GC tissues were significantly higher than those in adjacent non-tumor tissues. High ZFAS1 expression was associated with deeper depth of invasion, lymph node metastasis and advanced TNM stage in GC patients. Then, Kaplan-Meier analysis showed that patients with high ZFAS1 expression were associated with shorter over-

all survival. Finally, Univariate and multivariate analyses revealed that increased expression of ZFAS1 was an independent prognostic biomarker for shorter overall survival of GC patients.

Previous studies showed that ZFAS1 was dysregulated in types of cancers and play important roles in tumor progression. For example, Askarian-Amiri et al. showed that ZFAS1 was highly expressed in the mammary gland and down-regulated in breast tumors. Furthermore, they demonstrated that decreased expression of ZFAS1 in mammary epithelial cells resulted in a significant increased in proliferation and metabolic activity, suggesting that ZFAS1 act as a putative tumor suppressor gene in breast cancer [19]. However, Li et al. found that ZFAS1 was increased and associated with intrahepatic and extrahepatic metastasis and poor prognosis of hepatocellular carcinoma. Furthermore, they showed that ZFAS1 could function as an oncogene in hepatocellular carcinoma progression by binding miR-150 and abrogating its tumor suppressive function in this setting [20]. Thorenor et al. showed that ZFAS1 was increased in colorectal cancer (CRC) and promoted the proliferation of CRC cells in vitro [21]. Our finding expanded the tumor oncogenic role of ZFAS1 in GC progression.

In conclusion, this is the first study demonstrated that the increased expression of lncRNA

ZFAS1 was associated with advanced clinical features and poor prognosis of GC patients, indicating that increased expression of ZFAS1 could act as an unfavorable prognostic biomarker in GC patients. However, further studies are needed to investigate the precise molecular mechanism of ZFAS1 in the development and progression of GC.

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Disclosure of conflict of interest

None.

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