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The prognostic roles of long non-coding RNA SNHG17 in the patients with gastric cancer

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Abstract. – OBJECTIVE: Dysregulated long non-coding RNAs (lncRNAs) might exert critical roles in pathways associated with gastric cancer (GC) development. Long non-coding RNA (SNHG17), a newly identified lncRNA, has been reported to be dysregulated in several tumors. Our present study aimed to explore the expression level of SNHG17 in patients with GC and investigate the relationship between the SNHG17 level and the prognosis of GC patients.

PATIENTS AND METHODS: The expression levels of SNHG17 were detected by Real-time-quantitative Polymerase Chain Reaction (RT-qPCR). The chi-square test was performed to investigate the associations between SNHG17 expression and the clinical features of GC patients. The prognostic value of SNHG17 was demonstrated using Kaplan-Meier method and multivariate Cox regression analysis.

RESULTS: We showed that SNHG17 was significantly up-regulated in GC tissues compared with adjacent normal gastric tissues ($p<0.01$) and higher expression of SNHG17 was observed in advanced GC tissues. The results by analyzing the clinicopathological features showed that overexpression of SNHG17 expression was associated with advanced TNM stage ($p=0.006$), positively lymph node metastasis ($p=0.006$) and positively distant metastasis $p=0.024$). Moreover, clinical assays revealed that patients with high SNHG17 expression levels had a significantly shorter overall survival (OS) ($p=0.0034$) and progression-free survival (PFS) ($p=0.0002$) than those expressing lower. Finally, multivariate assays showed that SNHG17 was an independent prognostic marker for both OS and PFS of patients with GC.

CONCLUSIONS: Our findings indicated that SNHG17 was a novel molecule involved in GC progression, providing a potential prognostic biomarker for GC patients.

Key Words:
Long noncoding RNA, SNHG17, Gastric cancer, Prognosis.

Introduction

Gastric cancer is the fourth most common cancer and remains the second leading cause of cancer death worldwide[1]. In 2012, it newly occurred in 950,000 people and caused 723,000 deaths[2]. Over the past 20 years, the incidences of GC have decreased worldwide[3]. However, it remains a serious burden for China. Although remarkable improvements of therapeutic strategy for GC have been made, surgery remains the only curative therapy for stomach cancer[4,5]. Unfortunately, the five-year overall survival of GC patients with advanced stages remains very poor due to frequent recurrence and distant metastasis[6]. Up to date, identification of early-stage GC is difficult because it is often asymptotic or misdiagnosed[7]. Therefore, revealing the potential molecular mechanism underlying the progression of GC is essential for scientists to explore reliable novel biomarkers of GC for its early diagnosis and prognosis evaluation.

Long non-coding RNAs (lncRNAs) are a new class of non-protein coding RNAs with a minimum length of 200 nucleotides[8]. Recent studies[9,10] have shown that lncRNAs, initially thought to represent spurious transcriptional noise, may have various biological functions including post-transcriptional regulation, chromatin modification, and several other biological progressions. Interestingly, growing studies[11,12] have revealed that lncRNAs are closely associated with tumor development and progression by acting as tumor suppressors or oncogenes. Up to date, the biological function of lncRNAs and its potential as diagnostic and prognostic biomarkers have attracted increasing attention because of the development of microarrays and high-throughput RNA sequencing tools which can facilitate the identification of dysregulated lncRNAs that play a functional role in tumors[13-15]. Although more and more dysregu-

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lated lncRNAs have been identified to be factors which could influence the prognosis of tumor patients, most lncRNAs remain to be elucidated.

LncRNA SNHG17 (SNHG17), as a newly identified functional lncRNA, has been reported to be abnormally expressed and act as potential regulators in the progression of two tumors, gastric cancer[16], and colorectal cancer[17]. Functionally, it was reported SNHG17 could promote GC cells proliferation and invasion, suggesting that SNHG17 may contribute to the clinical progression of GC. However, the clinical significance of SNHG17 and its expression pattern in GC patients remains unknown. In this study, for the first time, we provided important evidence that detecting SNHG17 expression may help to predict the prognosis of GC patients.

Patients and Methods

Patients and Tissue Samples

Total of 157 fresh GC tissues and matched normal gastric tissues were obtained from GC patients at Linyi Central Hospital, from November 2010 to December 2013. All clinical samples were put into liquid nitrogen and then transferred to -80°C for storage. All samples were diagnosed by two pathologists. None of the patients with GC had received radiotherapy or chemotherapy before surgical resection. The patients' TNM stage was defined according to the AJCC staging system for gastric cancer. A five-years follow-up of the 157 GC patients was carried out. The clinicopathological characteristics and parameters are shown in Table I. This study was approved by the Ethics Committee of Linyi Central Hospital. The informed consent was obtained from the patients before the study.

RNA Isolation and qRT-PCR

Total RNA was extracted from GC tissues and matched normal gastric tissues harvested by the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). One thousand nanograms of total RNA were reverse transcribed in a final volume of 20 µl. The RT-qPCR experiments in this study were performed in a Lightcycler 480 Real-Time PCR system (Roche Diagnostics GmbH, Xuhui, Shanghai, China) using the Prime Script RT reagent Kit (Qiagen, Hilden, Germany). The relative expression of long noncoding RNA SNHG17 was calculated using the CT ($2^{-\Delta\Delta CT}$) method. The expression levels of SNHG17 were normalized to the levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Each detection was performed in triplicate. The primer sequences are shown in Table II.

Statistical Analysis

All data were analyzed SPSS 17.0 using the software package (SPSS Inc., Chicago, IL, USA). The expression level of SNHG17 in tumors was compared with matched normal tissues utilizing the paired sample *t*-test. The chi-square test was used to determine the associations between SNHG17 expression and clinicopathological variables of GC. The clinical survival data were calculated by the Kaplan-Meier method and the log-rank test. The correlations between clinicopathological factors and survival probabilities were evaluated using multivariate analyses. Differences with $p<0.05$ were considered statistically significant.

Results

SNHG17 is Upregulated in GC Tissues

Although the previous study has reported that SNHG17 expression was significantly up-regulated in GC tissues and cell lines, the evidence was limited. In this study, we collected 157 GC tissues, matched normal gastric tissues, and performed RT-PCR to further demonstrate whether SNHG17 was frequently dysregulated in GC. As shown in Figure 1A, we found that the expression of SNHG17 was significantly regulated in GC tissues compared to matched normal gastric tissues ($p<0.01$). Furthermore, we observed that the expression of SNHG17 in GC with high stages (TNM stage III + IV) were significantly higher than those in GC with low stages (TNM stage I + II) (Figure 1B). Thus, our results, together with previous results, indicated that SNHG17 was highly expressed in GC and may contribute to the progression of GC.

Association of SNHG17 Expression With Clinicopathological Parameters of GC Patients

To explore the clinical significance of SNHG17 in the progression of GC, we divided enrolled patients into two groups based on SNHG1 expression value. Then, we performed the Chi-square test and the results showed that high expression of SNHG17 was associated with advanced TNM stage ($p=0.006$), positive lymph node metastasis

Table I. Association between SNHG17 expression and clinicopathological features of human GC.

Clinical features	Total	SNHG17 expression		*p*
		High	Low	
Age (years)				NS
< 60	67	31	36	
≥ 60	90	44	46	
Gender				NS
Male	95	51	44	
Female	62	24	38	
Tumor size (cm)				NS
< 5	96	40	56	
≥ 5	61	35	26	
Differentiation				NS
Well	91	38	53	
Moderate + Poor	66	37	29	
TNM stage				0.006
I + II	93	36	57	
III + IV	64	39	25	
Depth of invasion				NS
T1 + T2	109	47	62	
T3 + T4	48	28	20	
Lymph node metastasis				0.006
No	99	39	60	
Yes	58	36	22	
Distant metastasis				0.024
No	104	44	60	
Yes	53	31	20	

(p=0.006), and distant metastasis (p=0.024) (Table III). However, no significant difference was observed between SNHG17 expression and other clinical features such as age, gender, tumor size, differentiation, and depth of invasion (p>0.05). Our findings revealed that SNHG17 may be involved in the clinical progression of GC.

Correlation of SNHG17 Expression With Prognosis of GC Patients

To further explore the prognostic value of SNHG17 in GC patients, we collected five-year clinical data to evaluate the correlation between SNHG17 expression and clinical outcomes. The Kaplan-Meier method was performed and the results were shown in Figure 2A and 2B. We found that GC patients with high SNHG17 expression level had a shorter OS (p=0.0034) and PFS (p=0.002) than those with low SNHG17 expression level. Then, we performed multivariate analyses to confirm whether SNHG17 could be used as an independent prognostic biomarker for GC patients. We found that high SNHG17 expression was an independent predictor of both OS (HR=3.035, 95% CI: 1.325-4.447, p=0.009) and PFS (HR=3.261, 95% CI: 1.488-5.667, p=0.001). Thus, our results further stated the prognostic value of SNHG17 in GC patients.

Table II. The primers used for qRT-PCR analysis.

Genes (qRT-PCR)	Primer sequence (50 - 30)
SNHG17 (forward)	TGCTTGTAAGGCAGGGTCTC
SNHG17 (reverse)	ACAGCCACTGAAAGCATGTG
GAPDH (forward)	AGAAGGCTGGGGCTCATTTG
GAPDH (reverse)	AGGGGCCATCCACAGTCTTC

Discussion

The global distribution of GC has undergone major changes, with an increase in the number of cases in China and worldwide[18,19]. Predicting the prognosis of GC patients is very important for doctors to design the therapeutic regimen[20]. In the recent years, several clinicopathological factors, such as clinical stage and lymph node metastasis, have been used for the prediction of GC patients[21].

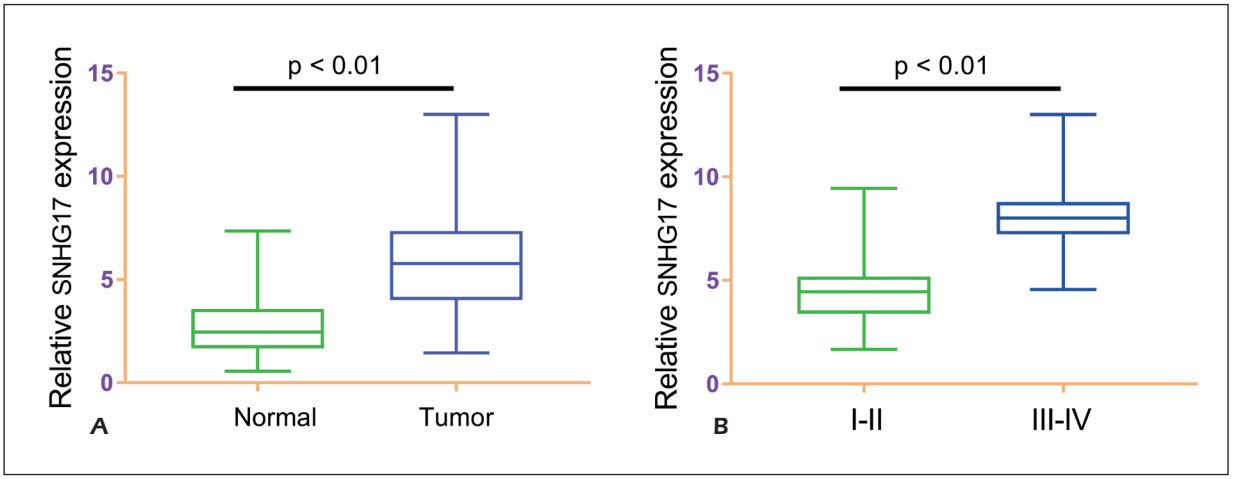

Figure 1. Relative lncRNA ANRIL expression in GC patients. **A**, Relative expression of SNHG17 in GC tissues in comparison with adjacent non-tumor tissues. **B**, Relative expression of SNHG17 in GC patients with TNM stage I + II and III + IV. SNHG17 expression was examined by qRT-PCR and normalized to GAPDH expression.

However, the effectiveness of these factors is limited for many patients. In addition, it has been observed that the development and progression of GC were related to various dysregulated genes[22,23]. Several different biomarkers in tissue, blood, or other body fluids have been reported to have the potential to predict the prognosis of GC patients[24,25]. In spite of extensive research efforts, few GC biomarkers have been successfully integrated into clinical practice. Recently, lncRNAs have been extensively investigated[26,27] for their potential use as potential biomarkers and therapeutic targets for GC patients.

LncRNAs have been reported to be important regulators involved in almost all aspects of gene modulation, such as epigenetic regulation, basal transcription machinery, and post-transcriptional regulation[28,29]. Via such gene regulation, lncRNAs may exert oncogenic activity in a variety of carcinomas. In recent years, several lncRNAs have been identified and well-studied in various tumors, such as lncRNA MALAT1[30], lncRNA PVT1[31], and lncRNA NEAT1[32]. SNHG17, another tumor-related lncRNA, was also reported to be involved in the regulation of two tumors. For instance, Ma et al[17] firstly reported that SNHG17 was highly expressed in colorectal cancer and was correlated with advanced clinical stage and poor prognosis of patients of this disease. In addition, SNHG17 silence cell proliferation by modulating P57 in colorectal cancer. Most recently, Zhang et al[16] showed that SNHG17 was highly expressed in GC and its overexpression promoted GC cell proliferation and metastasis by epigenetically modulation of p15 and p57. Those results highlighted the oncogenic roles of SNHG17 in the progression of GC. However, in

Table III. Multivariate analysis of overall survival and progression-free survival.

Clinicopathological	Overall survival			Progression-free survival		
	HR	95% CI	*p*	HR	95% CI	*p*
Age (years)	1.342	0.645-2.328	0.277	1.166	0.588-2.138	0.321
Gender	0.895	0.664-1.783	0.261	0.937	0.894-1.548	0.231
Tumor size (cm)	1.426	0.832-2.033	0.189	1.327	0.763-2.256	0.147
Differentiation	1.365	0.525-1.987	0.137	1.642	0.678-2.132	0.116
TNM stage	3.325	1.325-5.623	0.001	3.563	1.457-5.778	0.001
Depth of invasion	2.752	0.564-3.211	0.156	2.955	0.784-3.345	0.177
Lymph node metastasis	3.157	1.125-4.372	0.008	3.367	1.349-5.217	0.003
Distant metastasis	3.326	1.327-4.894	0.006	3.643	1.469-5.328	0.001
SNHG17 expression	3.035	1.325-4.447	0.009	3.261	1.488-5.667	0.001

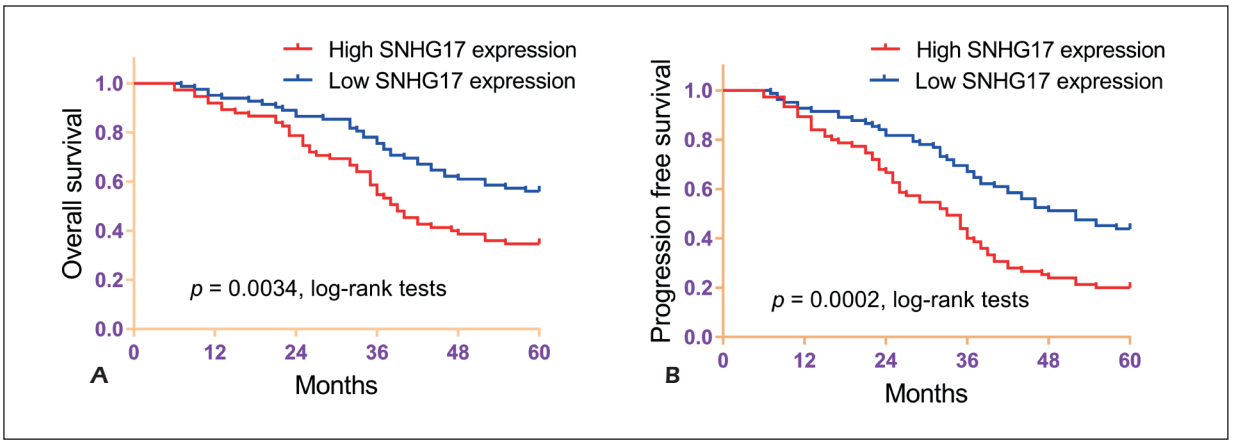

Figure 2. Survival analysis of 157 GC patients by Kaplan-Meier method. Overall survival rate (**A**) and progression-free survival rate (**B**) of GC patients with high SNHG17 were significantly shorter compared to those patients with low SNHG17.

their study, the clinical significance of SNHG17 in GC patients remains largely unclear.

In this study, 157 GC tissues and matched normal gastric tissues were collected and RT-PCR was performed. Our data showed that SNHG17 expression was significantly up-regulated in GC tissues compared to matched normal tissues, which was consistent with previous results. Then, we further explored the association between SNHG17 expression and several clinicopathological characteristics, finding that high SNHG17 expression was significantly associated with advanced TNM stage, positively lymph node metastasis, and positively distant metastasis, suggesting that SNHG17 may contribute to the development of GC. Furthermore, for the first time, we used Kaplan-Meier survival analysis and log-rank tests to explore whether SNHG17 influenced the long-term survival of GC patients; we found that high SNHG17 expression was significantly associated with shorter OS and PFS. Of note, by multivariate Cox analysis, we confirmed that SNHG17 expression was an independent prognostic factor for both OS and PFS of patients with GC.

Conclusions

We demonstrated first-ever evidence that SNHG17 was aberrantly up-regulated in GC, and its high expression was associated with advanced clinical stages and poor prognosis of GC patients. This finding may contribute to a better understanding of SNHG17 as a potential novel prognostic biomarker for GC patients.

Conflict of Interests

The Authors declare that there are no conflicts of interest.

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