

High expression of long noncoding RNA Sox2ot is associated with the aggressive progression and poor outcome of gastric cancer

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Abstract. – OBJECTIVE: The aim of our study was to investigate the expression of long non-coding RNA Sox2ot (Sox2ot) in gastric cancer (GC) patients and its association with clinicopathologic parameters and the prognosis.

PATIENTS AND METHODS: Quantitative real-time polymerase chain reaction was performed to detect Sox2ot expression in 155 GC tissues and paired adjacent normal tissues. The relationships between Sox2ot expression and the clinicopathological features of GC patients were analyzed. Furthermore, overall survival (OS) and disease-free survival (DFS) were evaluated using the Kaplan-Meier method, and multivariate analysis was performed using the Cox proportional hazard analysis.

RESULTS: Sox2ot expression levels were decreased in cancerous tissues compared to their corresponding non-cancerous controls ($p < 0.01$). Sox2ot expression was associated with T stage, distant metastasis and differentiation ($p = 0.009, 0.034$ and 0.001 , respectively). In addition, patients with high Sox2ot expression tended to have poorer OS and DFS ($p < 0.001$, respectively). Finally, multivariate analysis showed Sox2ot expression was an independent prognostic factor for GC patients.

CONCLUSIONS: Our findings showed that overexpressed Sox2ot was correlated with aggressive tumor behavior. Sox2ot may serve as a novel prognostic factor and a potential target to improve the long-term outcome of GC.

Key Words

LncRNA Sox2ot, Prognosis, Gastric cancer.

Introduction

Gastric cancer (GC) remains one of the most frequent malignant diseases, despite its steady decline in incidence worldwide over the last few decades. Overall, gastric cancer is the second leading cause of cancer mortality, based on recent

statistics¹⁻³. Despite great advances in the diagnosis and treatment of gastric cancer, the prognosis of individuals with an advanced stage of disease remains poor and treatment options mainly focus on surgery and chemoradiotherapy⁴. Although some biomarkers have been identified as significant prognostic factors for GC^{5,6}, few of them have been confirmed as independent predictive factors. Thus, identifying new biological markers would be helpful for improving effective targeted therapies.

The rapid development of RNA genomics has highlighted the role of long non-coding RNAs (lncRNAs) in many human diseases, especially in cancers^{7,8}. Accumulating data strongly support the involvement of lncRNAs in cancer. Alteration of the expression or structure of lncRNAs may promote tumor formation, progression, and metastasis^{9,10}. For instance, Li et al¹¹ found that over-expression of lncRNA HOTAIR promoted tumorigenesis via downregulating SETD2 in liver cancer stem cells. Shi et al¹² showed that lncRNA GAS5 is a tumor suppressor in non-small-cell lung carcinoma, and the action of lncRNA GAS5 is mediated by p53-dependent and p53-independent pathways. He et al¹³ showed lncRNA NEAT1 expression was significantly increased in glioma and the elevated expression of lncRNA NEAT1 was associated with poor prognosis and distant metastasis. Tuo et al¹⁴ found that lncRNA UCA1 functioned as a tumor suppressor in breast Cancer by downregulating miR-143. However, to our knowledge, the clinical significance of lncRNA Sox2ot in GC remains unclear.

In the present study, we investigated the Sox2ot expression in GC and adjacent normal tissues. We also examined the relationship between the Sox2ot expression and clinical parameters and overall survival (OS) and disease-free survival (DFS) in GC patients.

Patients and Methods

Patients and Tissue Samples

Human gastric cancer tissue and the adjacent normal tissue samples were obtained during surgery from 155 gastric cancer patients (91 males and 64 females) aged from 37 to 84 years (63.3 ± 6.5). All patients received the same physical examination, blood test and without any therapeutic before sampling. The clinicopathological features, such as age, T stage, tumor size, lymph node metastasis, distant metastasis, differentiation were obtained from the follow-up database and analyzed. Written informed consent was obtained from all patients, and research protocols were approved by the Clinical Research Ethics Committee of Cangzhou Central Hospital, China.

Quantitative Real-time PCR (qRT-PCR)

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was generated with the Primer-Script™ one-step RT-PCR kit (TaKaRa, Dalian, Liaoning, China). The quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the SYBR Select Master Mix (Biosystems, Foster City, CA, USA) on ABI 7900 system (Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. GAPDH was used as an internal control. The $2^{-\Delta\Delta C_t}$ method was employed to calculate the relative expression levels. The primers were designed as follows: lncRNA Sox2ot sense, 5'-GCTCGTGG-CTTAGGAGATTG-3' and reverse, 5'-CTGGCAAA-GCATGAGGAACT-3'; GAPDH sense, 5'-GTCAACG-GATTTGGTCTGTATT-3' and reverse, 5'-AGTCTTC-TGGGTG-GCAGTGAT-3'.

Statistical Analysis

The statistical analysis was performed by the SPSS 17.0 software statistical software package (SPSS Inc., Chicago, IL, USA). Paired Student's *t*-test was conducted to compare Sox2ot expression in paired clinical samples. The chi-square test or Fisher's exact test was appropriately used to determine the association between Sox2ot expression and clinicopathological variables of GC. For survival analysis, Kaplan-Meier curve was generated with the log-rank test. Further multivariate survival analysis was conducted using the Cox regression model. Statistical significance was defined as $p < 0.05$.

Results

Expression of Sox2ot in GC

Sox2ot has been observed to be up-regulated in various types of cancer; however, its expression in GC has not been previously investigated until now. We detected the expression levels of both sequences in 155 pairs of GC and matched adjacent non-cancerous tissues by real-time PCR. We found that Sox2ot expression level in human GC tissues was significantly lower than that in adjacent non-tumorous tissues ($p < 0.01$, Figure 1). Based on the median value of the Sox2ot expression in GC tissues, the patients were divided into two groups: cases with low Sox2ot expression ($N = 75$) and cases with high Sox2ot expression ($N = 80$).

Sox2ot Upregulation Associates with Aggressive Clinicopathological Parameters of Human GC

Table I showed the relationships between Sox2ot expression and the clinicopathological characteristics of patients with GC. The Chi-square test showed that Sox2ot expression was associated with T stage, distant metastasis and differentiation ($p = 0.009$, 0.034 and 0.001 , respectively). No significant difference was observed between Sox2ot expression and patients' age, gender, lymph node metastasis, and tumor size.

Association of High Sox2ot Expression with Prognosis in GC Patients

To determine the prognostic value of the Sox2ot expression in human GC, clinical follow-up was available for all patients. Kaplan-

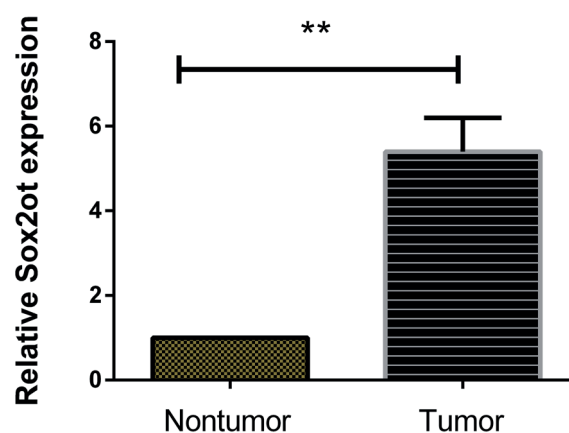


Figure 1. The expression of lncRNA Sox2ot was determined by quantitative real-time PCR in 155 paired human GC tissues and adjacent normal tissues. $**p < 0.01$.

Table I. Correlation between Sox2ot expression and clinicopathological variables of GC cases.

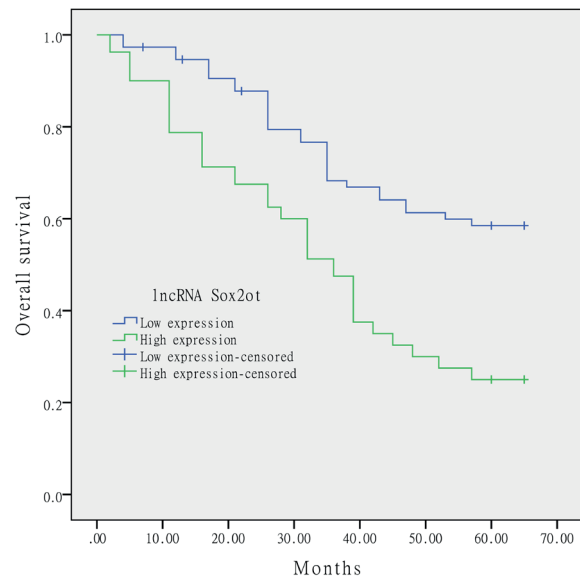
Variables	Cases (n=155)	lncRNA Sox2ot		<i>p</i>
		Low	High	
Gender				0.322
Male	91	41	50	
Female	64	34	30	
Age				0.347
<60	87	45	42	
≥60	68	30	38	
T stage				0.009
T1-T2	72	43	29	
T3-T4	83	32	51	
Lymph node metastasis				0.330
No	58	31	27	
Yes	97	44	53	
Distant metastasis				0.034
No	121	64	57	
Yes	34	11	23	
Differentiation				0.001
well/moderate	82	29	53	
poor	46	46	27	
Tumor size (cm)				0.608
<5	67	34	33	
≥5	88	41	47	

Meier survival analyses showed that GC patients with high Sox2ot expression had a significantly shorter 5-year overall survival (OS) ($p < 0.01$, Figure 2) and 5-year disease-free survival (DFS) ($p < 0.01$, Figure 3) than those with low Sox2ot expression. Sox2ot expression was further found to be an independent prognostic factor by the Cox proportional hazard model (Table II).

Discussion

Effective screening methods for early detection and biomarkers for prognostic prediction could guide therapeutic strategies and reduce the mortality rate of cancer¹⁵. So, finding new molecular targets for its diagnosis, prognosis and treatment has the potential to improve the clinical strategies and outcomes of this disease. The tissue-specific nature of lncRNA revealed that lncRNA might function as sensitive biomarkers¹⁶. Therefore, the association of Sox2ot with GC was explored in this study to find possible treatment targets of GC.

The SOX2 overlapping transcript is a long non-coding RNA gene located in chr3q26.33¹⁷. Previous research has reported the tumor promo-

**Figure 2.** Correlation between expression levels of lncRNA Sox2ot and patients' overall survival. Patients with higher lncRNA Sox2ot expression were closely correlated with poorer overall survival ($p < 0.01$).

ter function of Sox2ot in numerous human malignancies. For instance, Askarian-Amiri et al¹⁸ has shown that Sox2ot overexpression promoted human breast cancer cell growth, clonogenicity, and reduces apoptosis. Hou et al¹⁹ found that over-expression of Sox2ot promoted tumor cell proliferation and metastasis-related traits *in vitro*, supporting an oncogenic role of this lncRNA

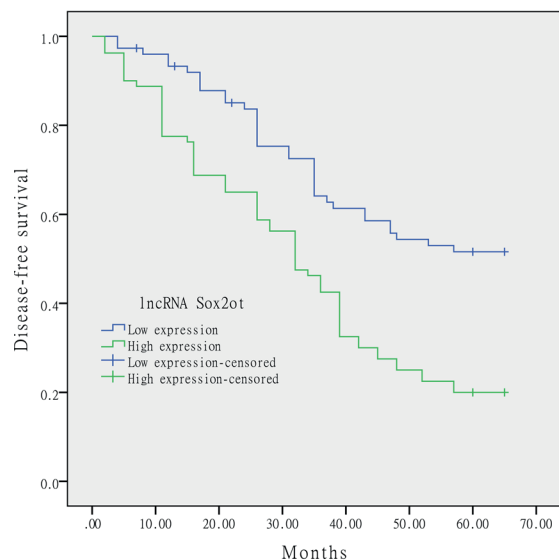
**Figure 3.** Correlation between expression levels of lncRNA Sox2ot and patients' disease-free survival. Patients with higher lncRNA Sox2ot expression were closely correlated with poorer disease-free survival ($p < 0.01$).

Table II. Correlation between Sox2ot expression and clinicopathological variables of GC cases.

Variable	Overall survival			Disease-free survival		
	HR	95%CI	p	HR	95%CI	p
T stage (T3, T4 vs. T1, T2)	2.773	0.842-5.549	0.018	2.038	0.663-4.691	0.032
Distant metastasis (Yes vs. No)	1.831	0.463-2.883	0.184	2.428	0.933-3.238	0.261
Differentiation (poor vs. well/moderate)	3.934	1.934-5.823	0.006	3.128	2.238-5.128	0.011
Sox2ot (high vs. low)	3.241	1.239-6.428	0.018	3.844	1.873-7.332	0.009

in NSCLC progression. Shi et al²⁰ showed that Sox2ot was overexpression in human hepatocellular carcinoma and associated with advanced clinical stage and lymph node metastasis. Furthermore, the 5-year overall survival of high Sox2ot expression group was also significantly shorter than that of low Sox2ot expression group. These results showed that there was a close relationship between Sox2ot and human cancer progression. However, whether Sox2ot played a similar role in GC remained unclear.

In the present study, we found that Sox2ot expression was significantly higher in GC tissues than that in normal gastric tissues. Also, high Sox2ot expression was significantly related to advanced cancer, indicated by T stage and differentiation. To further explore the clinical importance of Sox2ot, we investigated the correlation between Sox2ot expression and the overall survival of the GC patients. Patients with high Sox2ot expression had a shorter overall survival (OS) and disease-free survival (DFS) compared with those with low expression of this lncRNA. Finally, in a multivariate Cox model, our results demonstrated that Sox2ot expression was an independent poor prognostic factor for both 5-year OS and DFS survival, suggesting that Sox2ot is a promising prognostic factor for this disease.

Conclusions

Our results suggested that the Sox2ot could serve as a potential target for developing therapeutic intervention and as a potential prognostic biomarker for human GC. Further studies need to be performed to explore the potential of Sox2ot as a new therapeutic target for GC therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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