Original Article
LncRNA Sox2ot overexpression serves as a poor prognostic biomarker in gastric cancer

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Abstract: Sox2 overlapping transcript (Sox2ot) serves as an unfavorable prognostic factor in lung cancer and hepatocellular carcinoma, but the clinical significance and biological function of Sox2ot in gastric cancer is still unknown. The objective of our research is to explore the significance of Sox2ot in gastric cancer. Quantitative real-time polymerase chain reaction was performed to determine Sox2ot in gastric cancer tissues and cell lines. Furthermore, the Sox2ot status in one hundred and thirty two gastric cancer samples was detected to analyzed correlations between Sox2ot and clinicopathological features. The biological function of Sox2ot on tumor cell growth and mobility were explored through MTT, colony formation, Transwell migration assay and invasion assay in vitro. In our results, the expression of Sox2ot was overexpressed in gastric cancer tissues and cell lines. High levels of Sox2ot were correlated with malignant status and poor prognosis. Silencing Sox2ot expression effectively inhibited gastric cancer cell growth and motility in vitro. In conclusion, Sox2ot is a potential therapeutic target and a new biomarker for gastric cancer patients.

Keywords: Sox2ot, gastric cancer, lncRNA, biomarker

Introduction

Gastric cancer is still the second leading cause of cancer death worldwide [1]. A total of estimated 10,720 gastric cancer deaths and 24,590 new gastric cancer patients occur based on 2015 American Cancer Statistics [2]. Meanwhile, the mortality of gastric cancer ranked third with the proportion of 14.33% each year, and the morbidity reached to second with 3,621,000 new gastric cancer patients in China [3]. Over half of gastric cancer patients have a relapse after surgical treatment, and most patients at confirmed diagnosis already have advanced stage [4]. In order to increase curative effect and improve prognosis for gastric cancer patients, it is necessary to distinguish high risk group of gastric cancer patients [5]. Up to now, there is still no accurate biomarker to provide early diagnosis and prognosis prediction. Thus, it is very interest to discovery biomarkers which correlate to tumorigenesis and progression in gastric cancer.

Long non-coding RNAs (lncRNAs) are a group of non-protein-coding RNAs that regulate gene expression at the transcriptional or posttranscriptional level [6, 7]. Recently, more and more new lncRNAs are discovered and identified as oncogene or anti-oncogene in gastric cancer, such as H19 [8-11], MEG3 [12, 13], PVT1 [14, 15], BANCR [16] and FER1L4 [17].

Sox2 overlapping transcript (Sox2ot) is mapped to human chromosome 3q26.3 [18, 19] and transcribed in the same orientation as Sox2, which is embedded in anintron of the Sox2ot gene [20]. Sox2ot has been suggested overexpressed in undifferentiated and stem cells and down-regulated in differentiated and mature cells [21]. Meanwhile, the disregulation of Sox2ot expression has been found in a variety of human cancers, such as breast cancer, esophageal cancer, and lung cancer [22]. Up to present, the biological function and clinical significance of Sox2ot in gastric cancer is unclear. In order to identify the expression level and bio-

logical role of Sox2ot in gastric cancer, the expression of Sox2ot in 132 gastric cancer samples were measured and loss-of-function studies of Sox2ot in gastric cancer cell lines were performed.

Materials and methods

Ethics statement

The Research Ethics Committees of Huai'an First People's Hospital and Fifth Hospital of Wuhan approved this protocol and written informed consents were obtained from each patient. The entire study was performed based on the Declaration of Helsinki.

Patients and samples

One hundred and thirty two fresh gastric cancer samples and twenty paired adjacent normal gastric tissue samples were collected, and the pathological information (gender, age, histological type, clinical stage, tumor depth, lymph node metastasis, and distant metastasis) was retrieved from the archives of the Pathology Department of Huai'an First People's Hospital and Fifth Hospital of Wuhan. Tissue samples were immediately frozen in liquid nitrogen and kept at -80°C. The histopathological diagnosis of all samples was respectively diagnosed by two pathologists. The clinical staging was based on the 7th edition of the AJCC Cancer Staging Manual. The system treatments were performed according to NCCN guideline.

Cell lines and cell cultures

Two gastric cancer cell lines (SGC-7901 and AGS) and a normal gastric epithelial cell line (GES-1) were obtained from Chinese Type Culture Collection, Chinese Academy of Sciences, and were cultured in DMEM (Gibco) supplemented with 10% Fetal Bovine Serum (Hyclone) at 37°C in a humidified CO_2 (5%) atmosphere.

Real-time PCR

Total RNA was extracted from tissues and cell lines using RNAiso Plus (Takara) according to the manufacturer's instructions. 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 Sox2ot were 5'-GCTCGTGGCTTAGGA-GATTG-3' and 5'-CTGGCAAAGCATGAGGAACT-3', respectively. Forward and reverse primers sequences for GAPDH mRNA were 5'-ATGGG-GAAGGTGAAGGTCG-3' and 5'-GGGGTCATTGAT-GGCAACAATA-3', respectively. The reactions were performed using SYBR Premix Ex TaqTM II (Takara) on a LightCycler (Roche). Relative expression was calculated via the comparative cycle threshold method and was normalized to the expression of GAPDH. Relative quantification was calculated by using the $2^{-\Delta\Delta Ct}$ method. All reactions were run in triplicate.

Cell transfection

Sox2ot siRNA (si-Sox2ot) and non-targeting siRNA (si-NC) were purchased from Gene Pharma and used at 20 mM Opti-MEM transfection media (Invitrogen) and Lipofectamine 3000 reagent (Invitrogen) were used to transfect the cells once they reached 60% confluency. Knockdown was assessed by Real-time PCR after 48 hours of transfection.

Cell proliferation analysis

Cell proliferation was analyzed using MTT assay. Briefly, 1×10^3 cells were seeded into a 96-well plate with quadruplicate repeat for each condition. The cells were incubated for 1, 2, 3, and 4 days. Twenty microliters of MTT (5 mg/ml) (Sigma) was added to each well and incubated for 4 h. At the end of incubation, the supernatants were removed and 150 µl of DMSO (Sigma) was added to each well. The absorbance value (OD) of each well was measured at 490 nm. Experiments were performed three times.

Colony formation assay

Briefly, Cells (0.5×10^3) were plated into six well plates and cultured for ten days. Colonies were then fixed for 5 min with 10% formaldehyde and stained with 1.0% crystal violet for 30 s. The number of colonies containing ≥50 cells was counted under a microscope. Experiments were performed three times.

Cell migration and invasion assays

Briefly, 1×10^5 cells were seeded on a fibronectin-coated polycarbonate membrane insert in a
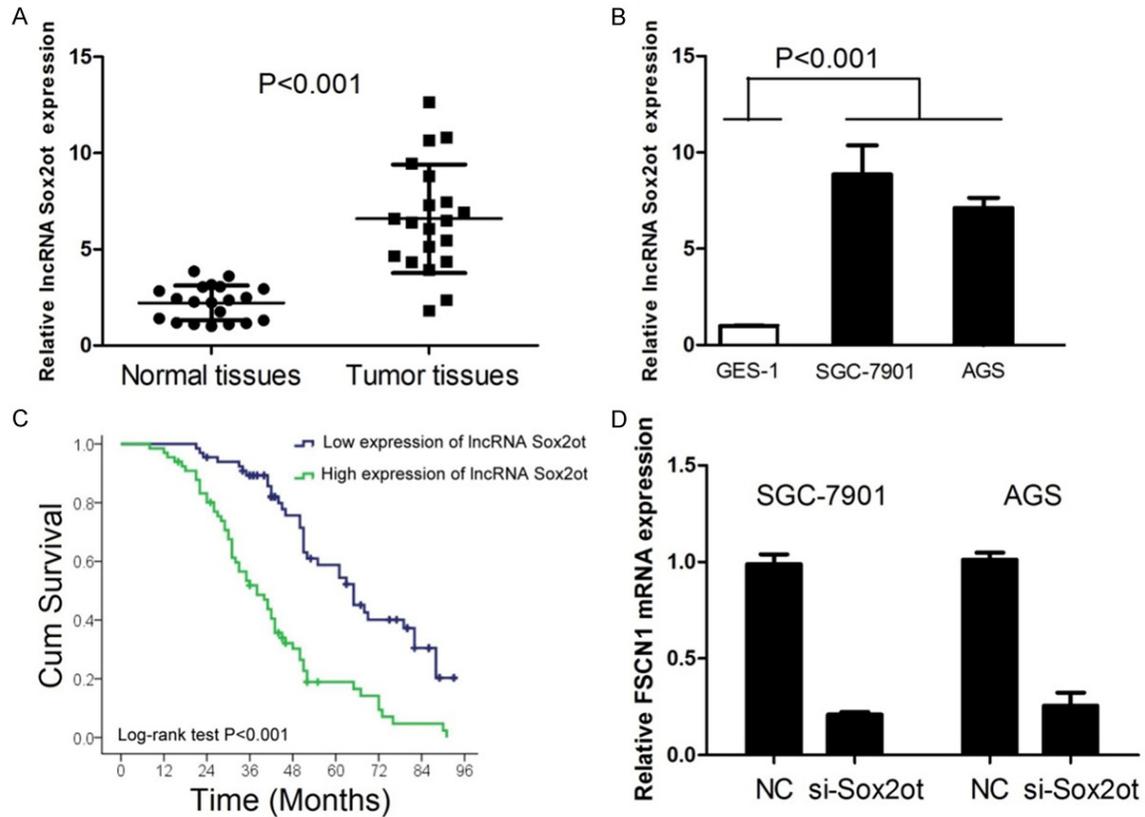
Figure 1. Sox2ot expression is elevated in gastric cancer tissues and cell lines, and associates with overall survival in gastric cancer patients. A: Expression of Sox2ot is increased in gastric cancer tissues compared with paired normal gastric tissues (*P*<0.001). B: Sox2ot is overexpressed in gastric cancer cell lines compared with normal gastric epithelial cell line (*P*<0.001). C: High expression of Sox2ot predicts a poor prognosis in gastric cancer patients (*P*<0.001). D: Sox2 expression is suppressed by small interfering RNA in SGC-7901 and AGS cells.

transwell apparatus (Corning). After the cells were incubated for 12 h, Giemsa-stained cells adhering to the lower surface were counted under a microscope in five predetermined fields (100×). For the cell invasion assay, the procedure was similar to the cell migration assay, except that the transwell membranes were pre-coated with 24 mg/ml Matrigel (Corning). Experiments were performed three times.

Statistical analysis

All data were analyzed for statistical significance using SPSS 17.0 software. The chi-square test was applied to the examination of correlation between Sox2ot expression and clinicopathological characteristics. Survival curves were plotted using the Kaplan-Meier method and the log-rank test. Cox regression was used for univariate analysis. The signifi-

cance of survival variables (P<0.05) in univariate analysis were included into the final multi-variable Cox proportional hazards model. Two-tailed Student's t test was used for comparisons of two independent groups. One-way ANOVA was used to determine the differences between groups or all in vitro analyses. The data are shown as the mean ± SD from at least three independent experiments. A *P*-value of less than 0.05 was considered statistically significant.

Results

The expression of Sox2ot was overexpressed in gastric cancer tissues and cell lines

In order to assess the role of Sox2ot in gastric cancer, we performed real-time PCR to measure the expression of Sox2ot in twenty paired

Table 1. Correlations between lncRNA Sox2ot expression and clinicopathological characteristics in gastric cancer

Characteristics	n	High expression (%)	Low expression (%)	P
Gender				
Female	47	26 (55.3)	21 (44.7)	0.363
Male	85	40 (47.1)	45 (52.9)	
Age(y)				
<50	54	26 (48.1)	28 (51.9)	0.723
≥50	78	40 (51.3)	38 (48.7)	
Histological type				
Differentiated	78	36 (46.2)	42 (58.3)	0.288
Undifferentiated	54	30 (55.6)	24 (44.4)	
Clinical stage				
I-II	61	21 (34.4)	40 (65.6)	0.001
III-IV	71	45 (63.4)	26 (36.6)	
Tumor depth				
T1-T2	68	27 (39.7)	41 (60.3)	0.015
T3-T4	64	39 (60.9)	25 (39.1)	
Lymph node metastasis				
N0-N1	68	25 (36.8)	43 (63.2)	0.002
N2-N3	64	41 (64.1)	23 (35.9)	
Distant metastasis				
M0	121	56 (46.3)	65 (53.7)	0.005
M1	11	10 (90.9)	1 (9.1)	

tumor depth (T1-T2 vs. T3-T4; $P=0.015$), lymph node metastasis (N0-N1 vs. N2-N3; $P=0.002$), and distant metastasis (M0 vs. M1; $P=0.005$). However, there was no significant correlation between Sox2ot expression and gender ($P=0.363$), age ($P=0.723$), and histological type ($P=0.288$).

Sox2ot overexpression was a poor independent prognostic factor in gastric cancer patients

To explore the prognostic value of Sox2ot expression in gastric cancer, we measured the association between the levels of Sox2ot expression and patients' survival using Kaplan-Meier analysis with the log-rank test. In 132 gastric cancer patients with prognosis information, we found that the level of Sox2ot expression was significantly associated with the overall survival of gastric cancer patients, as patients with lower levels of Sox2ot expression had better survival than those with higher levels of Sox2ot expression ($P<0.001$, **Figure 1C**). Furthermore, we also found that overexpression of Sox2ot showed poor prognosis in gastric cancer patients ($P<0.001$, **Table 2**), regardless of clinical stage, tumor depth, lymph node metastasis, and distant metastasis. Multivariate analysis showed that high expression of Sox2ot was a poor independent prognostic factor for patients with gastric cancer ($P=0.003$, **Table 2**).

Knockdown of Sox2ot expression suppressed gastric cancer cells growth

The efficiency of si-Sox2ot was confirmed by RT-PCR in gastric cancer cells (**Figure 1D**). Subsequently, we examined the effect of decreased Sox2ot expression on gastric cancer cells growth in vitro. The growth curves determined by MTT assay showed that si-Sox2ot gastric cancer cells had an decreased growth rate to si-NC cells over a four day period (**Figure 2A**, $P<0.05$). The results of MTT assay were

gastric cancer tissues and adjacent normal tissues, and two gastric cancer cell lines (SGC-7901 and AGS) and a normal gastric epithelial cell line (GES-1). Compared with adjacent normal tissues, gastric cancer tissues showed higher expression levels of Sox2ot ($P<0.001$, **Figure 1A**). Moreover, Sox2ot was significantly overexpressed in gastric cancer cell lines compared with normal gastric epithelial cell line ($P<0.001$, **Figure 1B**).

Correlation between Sox2ot and clinicopathological features in gastric cancer patients

We next analyzed the correlation between the expression of Sox2ot and clinicopathological characteristics of gastric cancer. Gastric cancer tissue samples were classified into the low expression group (n=66) and the high expression group (n=66) according to the median expression level of all gastric cancer samples. This classification was based on published study [16]. As summarized in **Table 1**, The levels of Sox2ot expression was associated significantly with clinical stage (I-II vs. III-IV; $P=0.001$),

Table 2. Univariate and multivariate Cox regression analyses of overall survival in gastric cancer

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Gender (Female vs. Male)	0.768	0.613-1.434	0.938			
Age (<50 vs. ≥50)	1.131	0.749-1.708	0.558			
Histological grade (Differentiated vs. Undifferentiated)	0.960	0.631-1.463	0.850			
Clinical stage (I-II vs. III-IV)	4.044	2.469-6.626	<0.001	2.640	0.783-8.899	0.117
Tumor depth (T1-T2 vs. T3-T4)	2.050	1.352-3.107	0.001	1.576	1.008-2.465	0.046
Lymph node metastasis (N0-N1 vs. N2-N3)	3.829	2.368-6.191	<0.001	1.030	0.309-3.438	0.962
Distant metastasis (M0 vs. M1)	10.827	5.137-22.815	<0.001	5.384	2.412-12.020	<0.001
lncRNA Sox2ot (Low vs. High)	3.119	2.033-4.787	<0.001	2.052	1.278-3.298	0.003

HR, hazard ratio; 95% CI, 95% confidence interval.
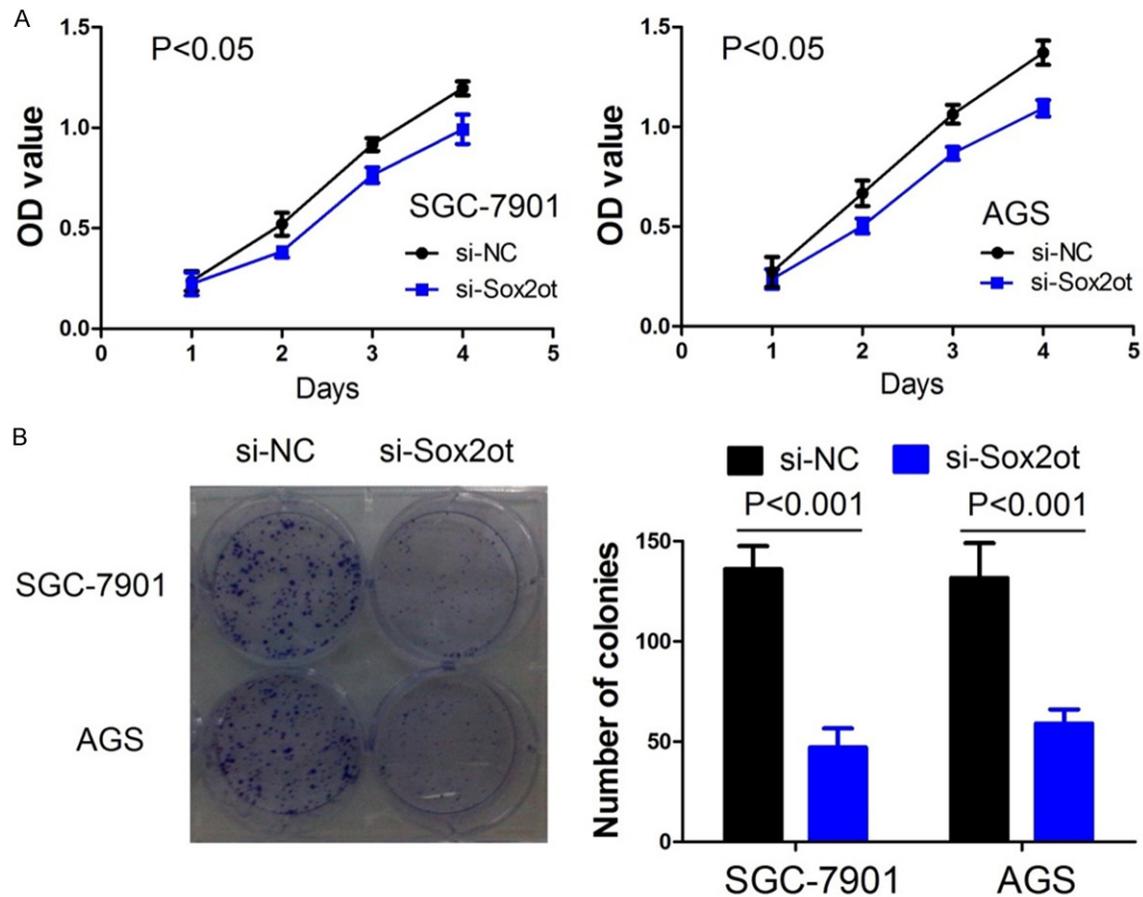
Figure 2. Knock-down of Sox2ot expression suppressed cell proliferation and plate clone formation. A: In vitro viabilities of SGC-7901 and AGS cells were decreased in Sox2ot-suppressed cells by MTT assay (both *P*<0.05). B: In vitro proliferative ability of SGC-7901 and AGS cells were decreased in Sox2ot-suppressed cells by colony formation assay (both *P*<0.001).

also consistent with clonogenicity tests as si-Sox2ot cells formed a decreased number of colonies compared to si-NC cells over a four-teen days period (**Figure 2B**, *P*<0.001). This suggested that knockdown of Sox2ot dramatically suppress gastric cancer cells growth.
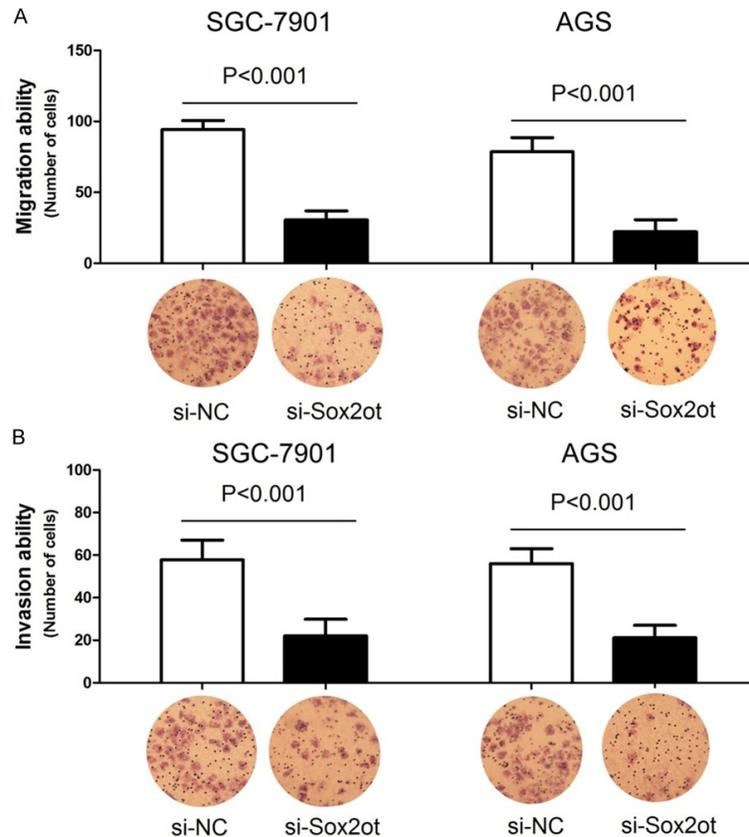
Figure 3. Down-regulation of Sox2ot inhibited gastric cancer cells migration and invasion. A: Down-regulated Sox2ot expression dramatically decreased the ability of SGC-7901 and AGS cells migration in vitro (both *P*<0.001). B: Suppressed Sox2ot expression inhibited invasiveness of SGC-7901 and AGS cells (both *P*<0.001).

Knockdown of Sox2ot inhibits gastric cancer cells migration and invasion

To examine the effect of Sox2ot on cell migration, after 24 hours of transfection, the number of migrated cells in both si-Sox2ot gastric cancer cell groups was significantly less than that in the si-NC gastric cancer cells (for both *P*<0.001, **Figure 3A**). Using a boyden chamber coated with matrigel, we determined changes in cell invasiveness after 24 hours of transfection. Compared with the si-NC gastric cancer cells, si-Sox2ot gastric cancer cells showed significantly decreased invasiveness (for both *P*<0.001, **Figure 3B**).

Discussion

Despite of declining mortality rate of gastric cancer, gastric cancer remains the fifth most frequently occurring malignant tumor and the third leading cause of cancer-related death [23, 24]. Many patients are diagnosed at an advanced stage with lymphatic or distant metastasis because of no early specific symptoms. The value of traditional biomarkers is limited for gastric cancer patients in diagnosing and predicting prognosis [25, 26]. Novel screening strategies for gastric cancer will be of great significance to reduce the mortality and elevate the quality of life of patients.

Long noncoding RNAs (lncRNAs) are broadly defined as RNA longer than 200 nucleotides lacking extended open reading frames [27]. Recent studies have revealed that lncRNAs are often aberrantly expressed in gastric cancer. LINC00152 has been suggested to overexpress in gastric cancer tissues, cell lines, blood, and gastric juice [28-30]. Moreover, MALAT1 is upregulated in gastric cancer tissues and cell lines compared with normal gastric tissues and normal gastric epithelial cell lines [31, 32]. Long non-coding RNA Sox2 overlapping transcript (Sox2ot) acts as an enhancer for SOX2 transcription and localizes on human chromosome 3q26.33. The level of Sox2ot in gastric cancer is still unknown. Our study first found the expressions of Sox2ot were obviously increased in gastric cancer tissues and cell lines compared with paired adjacent normal gastric tissues and normal gastric epithelial cell line. Similarly, Shi X.M. et al showed the expression of Sox2ot was significantly higher in hepatocellular carcinoma tissues compared with adjacent non-tumor tissues [33]. In esophageal squamous cell carcinoma, Shahryari A. et al suggested that Sox2ot was upregulated in tumor samples in comparison to their apparently normal marginal tissues [34].

Recently, the clinical significance of Sox2ot has been reported in several types of human can-

cers such as hepatocellular carcinoma [33], lung cancer [35], breast cancer [36, 37]. In hepatocellular carcinoma, overexpression of Sox2ot was associated with vein invasion, histological grade, and TNM stage [33]. Hou Z.B et al reported that the expression level of Sox2ot is markedly higher in lung squamous cell carcinomas than that in lung adenocarcinomas [35]. In breast cancer, Askarian-Amiri M.E. et al indicated that Sox2ot was overexpressed in estrogen receptor positive breast cancer cell lines, in comparison with the estrogen receptor negative breast cancer cell lines [36]. However, Iranpour M. et al showed no association was found between gene expressions and individual clinical data including PR status and Estrogen receptor status [37]. The discrepancy between Askarian-Amiri M.E. et al's data and Iranpour M. et al's data would be most likely due to the different samples between both studies and limited samples in Iranpour M. et al's study. In gastric cancer, we presented the evidence that Sox2ot expression was positively associated significantly with clinical stage, tumor depth, lymph node metastasis, and distant metastasis. In addition, we further explored the biological function of Sox2ot in gastric cancer cell lines, and found that knocking down Sox2ot expression obviously suppressed gastric cancer cells proliferation, migration, and invasion abilities. Similarly, Hou Z.B et al's report in lung cancer indicated that knocking down Sox2ot inhibited cell proliferation by inducing G2/M arrest, and reduced protein levels of Cyclin B1 and Cdc2 [35]. Meanwhile, the metastasis ability of hepatocellular carcinoma cells was markedly suppressed by knocking down Sox2ot expression [33].

In recent studies, high level of Sox2ot expression has been shown to be an independent poor prognostic factor in lung cancer and hepatocellular carcinoma. In lung cancer, high Sox2ot expression predicted poor survival for lung cancer patients, and the Risk Ratio (HR) and 95% confidence interval (95% CI) was 2.808 (1.131-6.967) [35]. According to multivariate Cox regression analysis, Sox2ot overexpression was an independent unfavorable prognostic factor for hepatocellular carcinoma patient's overall survival and the HR (95% CI) was 2.644 (1.473-5.845) [33]. In our study, we first reported that patients with lower levels of Sox2ot expression had better survival than those with higher levels of Sox2ot expression.

Based on multivariate Cox analysis, high expression of Sox2ot was a poor independent prognostic factor for patients with gastric cancer, and the HR (95% CI) was 2.052 (1.278-3.298).

In conclusion, Sox2ot was overexpressed in gastric cancer tissues and cell lines, and correlated with malignant status and poor prognosis of gastric cancer patients. Knockdown of Sox2ot expression suppressed gastric cancer cell proliferation, migration and invasion in vitro. Sox2ot could act as a novel biomarker and a potential therapeutic target for gastric cancer patients.

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Disclosure of conflict of interest

None.

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References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.

[3] Chen W, Zheng R, Zhang S, Zhao P, Li G, Wu L and He J. The incidences and mortalities of major cancers in China, 2009. Chin J Cancer 2013; 32: 106-112.

[4] Pasechnikov V, Chukov S, Fedorov E, Kikuste I and Leja M. Gastric cancer: prevention, screening and early diagnosis. World J Gastroenterol 2014; 20: 13842-13862.

[5] Yoon H and Kim N. Diagnosis and management of high risk group for gastric cancer. Gut Liver 2015; 9: 5-17.

[6] Sun M, Nie FQ, Wang ZX and De W. Involvement of lncRNA dysregulation in gastric cancer. Histol Histopathol 2016; 31: 33-39.

[7] Zhao J, Liu Y, Huang G, Cui P, Zhang W and Zhang Y. Long non-coding RNAs in gastric cancer: versatile mechanisms and potential for clinical translation. Am J Cancer Res 2015; 5: 907-927.

[8]   Yang F, Bi J, Xue X, Zheng L, Zhi K, Hua J and Fang G. Up-regulated long non-coding RNA H19 contributes to proliferation of gastric cancer cells. FEBS J 2012; 279: 3159-3165.

[9]   Zhuang M, Gao W, Xu J, Wang P and Shu Y. The long non-coding RNA H19-derived miR-675 modulates human gastric cancer cell proliferation by targeting tumor suppressor RUNX1. Biochem Biophys Res Commun 2014; 448: 315-322.

[10]   Zhang EB, Han L, Yin DD, Kong R, De W and Chen J. c-Myc-induced, long, noncoding H19 affects cell proliferation and predicts a poor prognosis in patients with gastric cancer. Med Oncol 2014; 31: 914.

[11]   Li H, Yu B, Li J, Su L, Yan M, Zhu Z and Liu B. Overexpression of lncRNA H19 enhances carcinogenesis and metastasis of gastric cancer. Oncotarget 2014; 5: 2318-2329.

[12]   Sun M, Xia R, Jin F, Xu T, Liu Z, De W and Liu X. Downregulated long noncoding RNA MEG3 is associated with poor prognosis and promotes cell proliferation in gastric cancer. Tumour Biol 2014; 35: 1065-1073.

[13]   Peng W, Si S, Zhang Q, Li C, Zhao F, Wang F, Yu J and Ma R. Long non-coding RNA MEG3 functions as a competing endogenous RNA to regulate gastric cancer progression. J Exp Clin Cancer Res 2015; 34: 79.

[14]   Kong R, Zhang EB, Yin DD, You LH, Xu TP, Chen WM, Xia R, Wan L, Sun M, Wang ZX, De W and Zhang ZH. Long noncoding RNA PVT1 indicates a poor prognosis of gastric cancer and promotes cell proliferation through epigenetically regulating p15 and p16. Mol Cancer 2015; 14: 82.

[15]   Ding J, Li D, Gong M, Wang J, Huang X, Wu T and Wang C. Expression and clinical significance of the long non-coding RNA PVT1 in human gastric cancer. Onco Targets Ther 2014; 7: 1625-1630.

[16]   Li L, Zhang L, Zhang Y and Zhou F. Increased expression of LncRNA BANCR is associated with clinical progression and poor prognosis in gastric cancer. Biomed Pharmacother 2015; 72: 109-112.

[17]   Liu Z, Shao Y, Tan L, Shi H, Chen S and Guo J. Clinical significance of the low expression of FER1L4 in gastric cancer patients. Tumour Biol 2014; 35: 9613-9617.

[18]   Sarkaria I, P Oc, Talbot SG, Reddy PG, Ngai I, Maghami E, Patel KN, Lee B, Yonekawa Y, Dudas M, Kaufman A, Ryan R, Ghossein R, Rao PH, Stoffel A, Ramanathan Y and Singh B. Squamous cell carcinoma related oncogene/DCUN1D1 is highly conserved and activated by amplification in squamous cell carcinomas. Cancer Res 2006; 66: 9437-9444.

[19]   Hussenet T, Dali S, Exinger J, Monga B, Jost B, Dembele D, Martinet N, Thibault C, Huelsken J, Brambilla E and du Manoir S. SOX2 is an oncogene activated by recurrent 3q26.3 amplifications in human lung squamous cell carcinomas. PLoS One 2010; 5: e8960.

[20]   Fantes J, Ragge NK, Lynch SA, McGill NI, Collin JR, Howard-Peebles PN, Hayward C, Vivian AJ, Williamson K, van Heyningen V and FitzPatrick DR. Mutations in SOX2 cause anophthalmia. Nat Genet 2003; 33: 461-463.

[21]   Boumahdi S, Driessens G, Lapouge G, Rorive S, Nassar D, Le Mercier M, Delatte B, Caauwe A, Lenglez S, Nkusi E, Brohee S, Salmon I, Dubois C, del Marmol V, Fuks F, Beck B and Blanpain C. SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. Nature 2014; 511: 246-250.

[22]   Shahryari A, Jazi MS, Samaei NM and Mowla SJ. Long non-coding RNA SOX2OT: expression signature, splicing patterns, and emerging roles in pluripotency and tumorigenesis. Front Genet 2015; 6: 196.

[23]   Bertuccio P, Chatenoud L, Levi F, Praud D, Ferlay J, Negri E, Malvezzi M and La Vecchia C. Recent patterns in gastric cancer: a global overview. Int J Cancer 2009; 125: 666-673.

[24]   Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-386.

[25]   Tian SB, Yu JC, Kang WM, Ma ZQ, Ye X, Cao ZJ and Yan C. Combined detection of CEA, CA 19-9, CA 242 and CA 50 in the diagnosis and prognosis of resectable gastric cancer. Asian Pac J Cancer Prev 2014; 15: 6295-6300.

[26]   Zhou YC, Zhao HJ and Shen LZ. Preoperative serum CEA and CA19-9 in gastric cancer--a single tertiary hospital study of 1,075 cases. Asian Pac J Cancer Prev 2015; 16: 2685-2691.

[27]   Ponting CP, Oliver PL and Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.

[28]   Zhao J, Liu Y, Zhang W, Zhou Z, Wu J, Cui P, Zhang Y and Huang G. Long non-coding RNA Linc00152 is involved in cell cycle arrest, apoptosis, epithelial to mesenchymal transition, cell migration and invasion in gastric cancer. Cell Cycle 2015; 14: 3112-3123.

[29]   Pang Q, Ge J, Shao Y, Sun W, Song H, Xia T, Xiao B and Guo J. Increased expression of long intergenic non-coding RNA LINC00152 in gastric cancer and its clinical significance. Tumour Biol 2014; 35: 5441-5447.

[30]   Li Q, Shao Y, Zhang X, Zheng T, Miao M, Qin L, Wang B, Ye G, Xiao B and Guo J. Plasma long noncoding RNA protected by exosomes as a potential stable biomarker for gastric cancer. Tumour Biol 2015; 36: 2007-2012.

[31] Okugawa Y, Toiyama Y, Hur K, Toden S, Saigusa S, Tanaka K, Inoue Y, Mohri Y, Kusunoki M, Boland CR and Goel A. Metastasis-associated long non-coding RNA drives gastric cancer development and promotes peritoneal metastasis. Carcinogenesis 2014; 35: 2731-2739.

[32] Wang J, Su L, Chen X, Li P, Cai Q, Yu B, Liu B, Wu W and Zhu Z. MALAT1 promotes cell proliferation in gastric cancer by recruiting SF2/ASF. Biomed Pharmacother 2014; 68: 557-564.

[33] Shi XM and Teng F. Up-regulation of long non-coding RNA Sox2ot promotes hepatocellular carcinoma cell metastasis and correlates with poor prognosis. Int J Clin Exp Pathol 2015; 8: 4008-4014.

[34] Shahryari A, Rafiee MR, Fouani Y, Oliae NA, Samaei NM, Shafiee M, Semnani S, Vasei M and Mowla SJ. Two novel splice variants of SOX2OT, SOX2OT-S1, and SOX2OT-S2 are coupregulated with SOX2 and OCT4 in esophageal squamous cell carcinoma. Stem Cells 2014; 32: 126-134.

[35] Hou Z, Zhao W, Zhou J, Shen L, Zhan P, Xu C, Chang C, Bi H, Zou J, Yao X, Huang R, Yu L and Yan J. A long noncoding RNA Sox2ot regulates lung cancer cell proliferation and is a prognostic indicator of poor survival. Int J Biochem Cell Biol 2014; 53: 380-388.

[36] Askarian-Amiri ME, Seyfoddin V, Smart CE, Wang J, Kim JE, Hansji H, Baguley BC, Finlay GJ and Leung EY. Emerging role of long non-coding RNA SOX2OT in SOX2 regulation in breast cancer. PLoS One 2014; 9: e102140.

[37] Iranpour M, Soudyab M, Geranpayeh L, Mirfakhraie R, Azargashb E, Movafagh A and Ghafouri-Fard S. Expression analysis of four long noncoding RNAs in breast cancer. Tumour Biol 2015; 37: 2933-40.