Original Article
The association of long non-coding RNA TCF7 with tumor features, prognosis, cell proliferation and stemness in gastric cancer

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Abstract: This study aimed to evaluate the correlation of long non-coding RNA transcription factor 7 (lnc-TCF7) with the clinicopathological features and survivals in gastric cancer (GC) patients as well as the effect of its knockdown on GC cell proliferation and stemness. Lnc-TCF7 expression in tumor tissues from 236 GC patients and adjacent tissues from 120 GC patients were detected by RT-qPCR. Patients' clinicopathological properties were reviewed. Meanwhile, disease free survival (DFS) and overall survival (OS) were calculated. *In vitro*, the effect of lnc-TCF7 knockdown on GC cell proliferation and stemness was measured by lnc-TCF7 shRNA lentivirus transfection into AGS cells. Lnc-TCF7 expression was increased in tumor tissues compared with adjacent tissues, and its high expression was correlated with advanced pathological grade, larger tumor size, higher T stage and TNM stage in GC patients. Besides, lnc-TCF7 high expression predicted worse DFS and OS in GC patients. *in vitro*, lnc-TCF7 was upregulated in GC cell lines (HGC-27, AGS and BGC-823) compared with human normal gastric mucosal cell line (GSE-1), and lnc-TCF7 knockdown suppressed cell proliferation, CD44 and CD133 expressions, CD44$^+$CD133$^+$ cell proportion, drug resistance to 5-fluorouracil and sphere formation efficiency in AGS cells. Lnc-TCF7 is upregulated and associates with advanced tumor features as well as poor prognosis, and its knockdown suppresses cancer cell proliferation and stemness in GC.

Keywords: lnc-TCF7, gastric cancer, prognosis, proliferation, stemness

Introduction

Gastric cancer (GC), as one of the most frequently diagnosed cancers, is responsible for over 1 million new cases and approximately 0.783 million deaths worldwide in 2018 according to the Globally Cancer Statistics [1, 2]. Meanwhile, it is the fifth most incident and the third most common cause of cancer-related death [3]. Multimodality treatments, consisting of chemotherapy, radiotherapy, surgery and molecular targeted agents, play important roles in the treatment of GC, and increase disease free and overall survival in GC patients. However, the metastasis rate and 5-year recurrence rate of GC are still high, contributing to the high mortality as well as poor prognosis in GC patients [4-7]. Thus, it is important to investigate more novel prognostic biomarkers and potential therapeutic targets, which may assist

with improving the clinical outcomes in GC patients.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNA molecules longer than 200 nucleotides, and some of which function as oncogenes or tumor-suppressors in cancers, attracting the attention of numerous researchers [8]. As for GC, some abnormally expressed lncRNAs (such as: lnc-PVT1, lnc-ROR and lnc-MALAT1) have been reported to regulate the proliferation and stemness of cancer cell, thus subsequently contributing to GC development and progression [8-12]. Recently, some papers reveal that lncRNA transcription factor 7 (lnc-TCF7) serves as an oncogene in tumorigenesis of some common solid tumors including colorectal cancer, glioma, liver cancer, and promotes the self-renewal of human liver cancer stem cells through activation of Wnt signaling

pathway, however, the function of lnc-TCF7 in GC still remains unknown [13-16]. Given that lnc-TCF7 acts as an oncogene during the tumorigenesis in several cancers, we speculated that lnc-TCF7 might also be involved in the pathogenesis of GC. Thus, this study aimed to investigate the association of lnc-TCF7 expression with clinicopathological features and survival in GC patients as well as the effect of lnc-TCF7 knockdown on GC cell proliferation and stemness.

Methods

Patients

Between 2014/1/1 and 2016/12/31, 236 GC patients underwent gastrectomy in Daqing Oilfield General Hospital were retrospectively reviewed in the current study. These patients were screened according to the following inclusion criteria: (1) diagnosed as primary GC confirmed by the endoscopic, imaging and histopathological examinations; (2) underwent gastrectomy with snap-frozen tumor tissues reserved and accessible; (3) clinical data were complete; (4) with at least 2-year follow-up records. Patients who received neoadjuvant therapy or accompanied with other malignancies were excluded from the analysis. This study was approved by the Ethics Committee of Daqing Oilfield General Hospital. Written informed consents or oral agreements with recording were collected from patients or their guardians.

Specimen collection and detection

All tumor tissues were obtained from the gastrectomy. Immediately after surgery, the tissues were removed, snap frozen in liquid nitrogen, and were stored at -80°C. After approval by the Hospital, GC tumor specimens were collected from the sample storage room. Besides, 120 adjacent tissues (defined by the pathologist) of GC were also collected from the sample storage room, which were used as control in the present study. After sample acquisition, total RNAs were extracted using RNeasy Protect Mini Kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, German), and then lnc-TCF7 relative expressions in the tumor and adjacent tissues were determined by the real-time quantitative polymerase chain reaction (RT-qPCR) analysis.

Data collection

Patients' clinical data were reviewed, and following information were collected: (1) demographic information: age and gender; (2) history of familial cancer, smoking, and drinking; (3) clinical characteristics: H.pylori infection, tumor location, pathological grade, tumor size, T stage, N stage, M stage and TNM stage (according to the 7th edition of the American Joint Committee on Cancer (AJCC) cancer staging manual); (3) treatments: adjuvant chemotherapy and adjuvant radiotherapy. Moreover, follow-up records of patients were also reviewed. The last follow-up date was 2018/12/31, and the median follow-up duration was 35.0 months (range: 3.0-59.0 months). Disease-free survival (DFS) was calculated from the date of gastrectomy to the date of relapse or death of any cause. Overall survival (OS) was calculated from the date of gastrectomy to the date of death of any cause.

Cells culture

Human GC cell lines (HGC-27, AGS, BGC-823 and NCI-N87) and human normal gastric mucosal cells GSE-1 were purchased from Chinese Academy of Sciences Affiliated Cell Resource Center of Shanghai Institute of Life Sciences (Shanghai, China) or American Type Culture Collection (ATCC) (Manassas, USA). HGC-27, BGC-823, NCI-N87 and GSE-1 cells were cultured in 90% RPMI 1640 medium (Sigma-Aldrich, USA) and 10% fetal bovine serum (FBS) (Gibco, USA); AGS cells were cultured in 90% F12K medium (Sigma-Aldrich, USA) and 10% FBS (Gibco, USA). All the cell lines were incubated in a humidified incubator under 95% air and 5% CO_2 condition at 37°C.

Transfection

Control shRNA and lnc-TCF7 shRNA lentivirus were constructed by Shanghai GenePharma Company (Shanghai, China) and then transfected into AGS cells to obtain stably transfected AGS cells and were divided into NC (-) group and Lnc-TCF7 (-) group. Then lnc-TCF7 expression was detected by RT-qPCR in NC (-) group and Lnc-TCF7 (-) group.

Detection of GC cell proliferation

Cells of NC (-) group and Lnc-TCF7 (-) group were cultured for 72 h, and cell proliferation
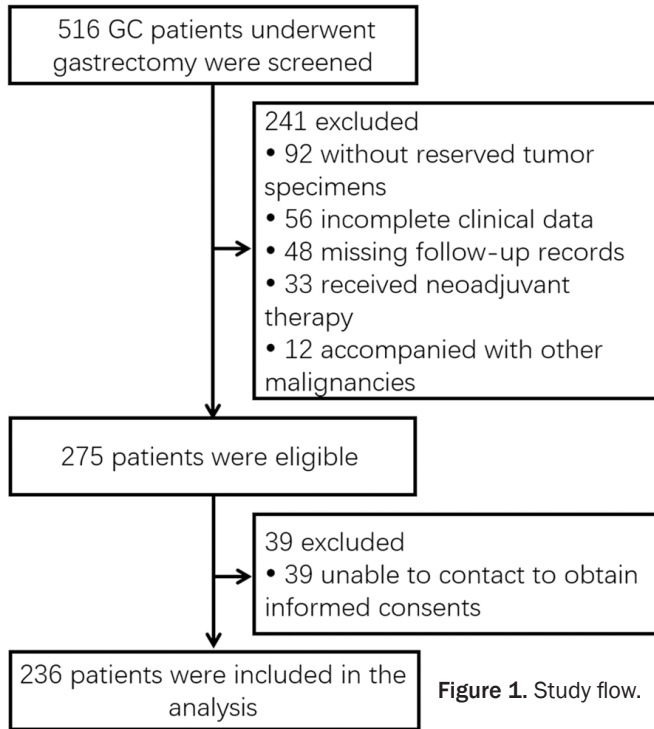
Figure 1. Study flow.

was detected at 0 h, 24 h, 48 h and 72 h using Cell Counting Kit-8 (Sigma, USA) according to the instructions of manufacturers.

Detection of GC cell stemness

In order to detect the GC cell stemness, CSCs markers (CD44, CD133), CD44⁺CD133⁺ cell proportion, drug resistance to 5-fluorouracil (5-FU) and sphere formation efficiency was measured. In brief, in both NC (-) and Lnc-TCF7 (-) cells: (1) CD44 and CD133 expressions were detected by RT-qPCR; (2) CD44⁺ CD133⁺ cell proportion was detected by flow cytometry using BD FACS Calibur (BD, USA) with CD44 rat mAb (APC Conjugate) (CST, UST) and CD133 mouse mAb (Alexa Fluor® 488 Conjugate) (CST, UST); (3) 200 ng/ml 5-FU was added to treat the cells, and then cell proliferation was detected at 72 h using Cell Counting Kit-8 (Sigma, USA) according to the instructions of manufacturers, and relative cell proliferation of Lnc-TCF7 (-) group was calculated according to the proliferation of NC (-) group; (4) Sphere formation efficiency was detected using sphere formation assay and observed under microscope (Olympus, Japan).

RT-qPCR

Total RNA was extracted from tissues or cells using TRIzol™ reagent (In-vitrogen, USA) and then reversely transcribed to cDNA using PrimeScript™ RT reagent Kit (Takara, Dalian, Liaoning, China). Following that, RT-qPCR was performed using SYBR® Premix DimerEraser™ (Takara, Dalian, Liaoning, China) to quantify lnc-TCF7, CD44, CD133 relative expressions. And the result was calculated using $2^{-\Delta\Delta Ct}$ method with GAPDH as an internal reference. The primers used in RT-qPCR were listed in Supplementary Table 1.

Statistical analysis

Data were displayed as mean ± standard deviation (SD), median and inter-quartile range (IQR) or count (percentage). Comparison among groups was determined by one-way ANOVA followed by multiple comparisons test. Comparison between two groups was determined by the t test, Wilcoxon rank sum test or Chi-square test. Receiver-operating characteristic (ROC) curve and the area under the ROC curve (AUC) were used to assess the feasibility of using lnc-TCF7 to distinguish tumor tissues from adjacent tissues. Survival curves were constructed with the Kaplan-Meier method and compared by the log-rank test. Univariate and multi-variate Cox's proportional hazard regressions analyses were performed to screen for risk factors of survival. All tests were two sided. A *P* value <0.05 was considered significant. All statistics were performed using SPSS 20.0 for windows (IBM Corporation, Armonk, NY, USA), and all figures were plotted using GraphPad Prism 6.01 (GraphPad Software Inc., San Diego, CA, USA).

Results

Study flow

516 GC patients underwent gastrectomy were initially screened, and 241 of them were excluded (including 92 without reserved tumor specimens, 56 with incomplete clinical data, 48 without follow-up records, 33 receiving neoadjuvant therapy and 12 accompanied with other

Table 1. Patients' characteristics

Parameters	GC patients (N=236)
Age (years), mean ± SD	60.1 ± 10.9
Gender (male/female), No.	136/100
History of familial cancer, No. (%)	33 (14.0)
History of smoke, No. (%)	107 (45.3)
History of drink, No. (%)	94 (39.8)
H.pylori Infection, No. (%)	
Positive	87 (36.9)
Negative	149 (63.1)
Tumor location, No. (%)	
Cardia	59 (25.0)
Gastric body	27 (11.4)
Gastric antrum	150 (63.6)
Pathological grade, No. (%)	
G1	41 (17.3)
G2	167 (70.8)
G3	28 (11.9)
Tumor size (cm), median (IQR)	3.0 (2.5-4.0)
T stage, No. (%)	
T1	5 (2.1)
T2	23 (9.7)
T3	204 (86.4)
T4	4 (1.7)
N stage, No. (%)	
N0	76 (32.2)
N1	58 (24.6)
N2	91 (38.5)
N3	11 (4.7)
M stage, No. (%)	
M0	236 (100.0)
M1	0 (0.0)
TNM stage, No. (%)	
I	28 (11.9)
II	101 (42.8)
III	107 (45.3)
Treatments, No. (%)	
Adjuvant chemotherapy	155 (65.7)
Adjuvant radiotherapy	30 (12.7)

GC: gastric cancer; SD: standard deviation; IQR: inter-quartile range.

malignancies) (**Figure 1**). 275 patients were further reviewed for eligibility, while 39 of them were excluded as they were unable to be contacted to obtain informed consents. The remaining 236 patients were included in the analysis.

Baseline characteristics

A total of 236 GC patients with mean age of 60.1 ± 10.9 were enrolled in the study (**Table 1**). The number of male and female were 136 and 100 respectively. There were 87 (36.9%) patients with H.pylori infection positive and 149 (63.1%) patients with H.pylori infection negative. The number of patients with tumor location at cardia, gastric body and gastric antrum were 59 (25.0%), 27 (11.4%) and 150 (63.6%) respectively. With respect to pathological grade, there were 41 (17.3%) patients in G1, 167 (70.8%) patients in G2 and 28 (11.9%) in G3. As for TNM stage, the number of patients with TNM stage I, II and III were 28 (11.9%), 101 (42.8%) and 107 (45.3%). Other detailed baseline information of GC patients was shown in **Table 1**.

Comparison of lnc-TCF7 expression between tumor tissues and adjacent tissues in GC

The median of lnc-TCF7 relative expression in tumor tissues (N=236) was 2.481 (1.223-3.999), which was elevated compared with adjacent tissues (N=120) (1.000 (0.509-1.596)) (P<0.001) (**Figure 2A**). And ROC curve revealed that lnc-TCF7 was of good value in distinguishing tumor tissues from adjacent tissues with AUC of 0.807 (95% CI: 0.763-0.851) (**Figure 2B**).

Correlation of lnc-TCF7 level with clinicopathological features in GC patients

All GC patients were divided into lnc-TCF7 high group and lnc-TCF7 low group according to the median value of baseline tumor lnc-TCF7 expression for correlation analysis. Lnc-TCF7 high expression was associated with increased pathological grade (P=0.006) (**Figure 3A**), larger tumor size (P=0.046) (**Figure 3B**), higher T stage (P=0.014) (**Figure 3C**), and elevated TNM stage (P=0.011) (**Figure 3E**), while lnc-TCF7 low expression was numerically correlated with elevated N stage but without statistical significance (P=0.067) (**Figure 3D**) in GC patients. These data suggested that lnc-TCF7 was correlated with advanced tumor features in GC patients.

Correlation of lnc-TCF7 level with DFS and OS

All GC patients were divided into lnc-TCF7 high group and lnc-TCF7 low group according to the
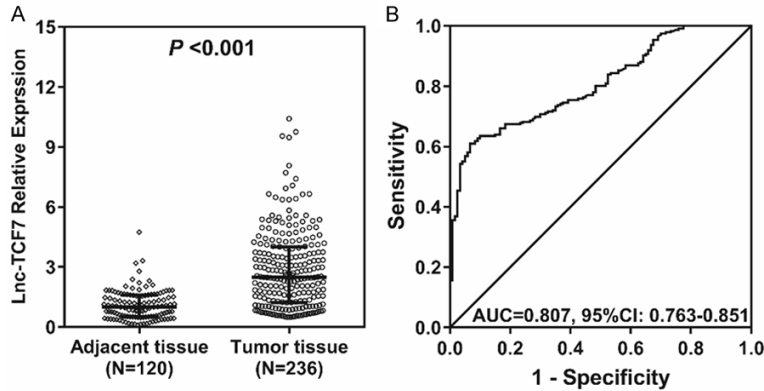
Figure 2. Lnc-TCF7 expression in GC tumor tissue and adjacent tissue. The lnc-TCF7 relative expression was elevated in tumor tissues compared with adjacent tissues in GC patients (A). Besides, ROC curve analysis revealed that lnc-TCF7 was of good value in distinguishing tumor tissues from adjacent tissues (B). P<0.05 was considered as significant. lnc-TCF7, long noncoding RNA transcription factor 7; GC, gastric cancer; ROC, receiver operating characteristic.

median value of baseline tumor lnc-TCF7 expression for survival analysis. DFS in lnc-TCF7 high group was shorter compared with that in lnc-TCF7 low group (P=0.001) (**Figure 4A**), and OS in lnc-TCF7 high group was also decreased compared with that in lnc-TCF7 low group (P<0.001) (**Figure 4B**). These data indicated that high expression of lnc-TCF7 was correlated with poor survivals in GC patients.

Factors affecting DFS by Cox's proportional hazards regression model analyses

Univariate Cox's proportional hazards regression model presented that lnc-TCF7 high expression (HR=2.176, P<0.001), higher pathological grade (HR=3.069, P<0.001), tumor size >3 cm (HR=1.728, P=0.002), increased T stage (HR=1.796, P=0.010), N stage (HR=1.525, P<0.001) and TNM stage (HR=1.877, P<0.001) were associated with decreased DFS in GC patients (**Table 2**). And multivariate Cox's proportional hazard regression was further conducted, which revealed that lnc-TCF7 high expression (HR=1.416, P=0.068) was not an independent risk factor for DFS, while higher pathological grade (HR=3.066, P<0.001) was an independent predictive factor for worse DFS in GC patients. Combining the aforementioned analysis, we speculated that lnc-TCF7 high expression might contribute to worse DFS via the interaction of other tumor features such as pathological grade and TNM stages.

Factors affecting OS by Cox's proportional hazards regression model analyses

Univariate Cox's proportional hazards regression exhibited that lnc-TCF7 high expression (HR=2.176, P<0.001), higher pathological grade (HR=3.385, P<0.001), tumor size >3 cm (HR=1.738, P=0.008), higher T stage (HR=1.788, P=0.036), elevated N stage (HR=1.521, P<0.001) and advanced TNM stage (HR=1.895, P<0.001) were correlated with decreased OS in GC patients (**Table 3**). And multivariate Cox's proportional hazard regression further indicated that lnc-TCF7 high expression (HR=1.685, P=0.021) and advanced pathological grade (HR=3.323, P<0.001) were both independent predictive factors for worse OS in GC patients.

Lnc-TCF7 relative expression in GC cells and the effect of its knockdown on cell proliferation and stemness

To further investigate the potential of lnc-TCF7 as a target in GC treatment, *in vitro* experiments were conducted by lnc-TCF7 knockdown. lnc-TCF7 relative expression was upregulated in HGC-27 cells (P<0.05), AGS cells (P<0.01), BGC-823 cells (P<0.05) but similar in NCI-N87 cells (P>0.05) compared with that in human normal gastric mucosal cells GSE-1 (**Figure 5A**). The highest expression of lnc-TCF7 was exhibited in AGS cells, which were chosen to be used in the following experiments. Lnc-TCF7 expression was downregulated in Lnc-TCF7 (-) group compared with that in NC (-) group (P<0.01), which indicated the successful transfection (**Figure 5B**). Cell proliferation was reduced in Lnc-TCF7 (-) group compared with that in NC (-) group at 48 h (P<0.05) and 72 h (P<0.05) after transfection (**Figure 5C**). As to GC cell stemness, both CD44 (P<0.01) and CD133 (P<0.05) mRNA expressions were reduced in Lnc-TCF7 (-) group compared with those in NC (-) group (**Figure 5D**, **5E**). CD44+CD133+ cell proportion was decreased in Lnc-TCF7 (-) group compared with that in NC (-) group (P<0.01) (**Figure 5F**). The drug resistance of GC cells was investigated by detecting cell proliferation after
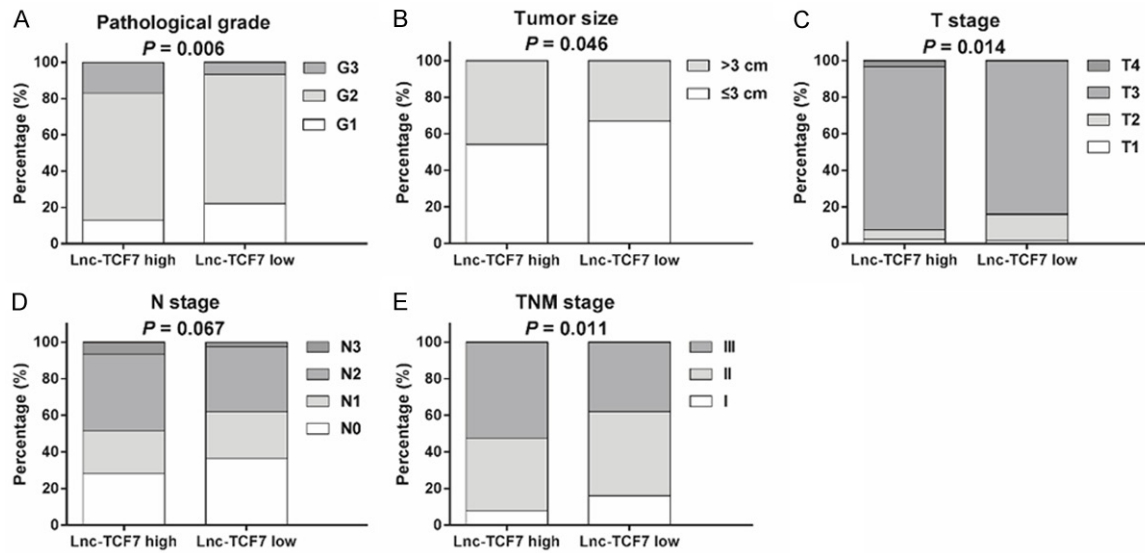
Figure 3. Association of lnc-TCF7 relative expression with clinicopathological features in GC patients. Patients with lnc-TCF7 high expression presented higher pathological grade (A), larger tumor size (B), elevated T stage (C), and advanced TNM stage (E), while numerical elevated N stage (D) (without statistical significance) compared with patients with lnc-TCF7 low expression. P<0.05 was considered as significant. lnc-TCF7, long noncoding RNA transcription factor 7; GC, gastric cancer.
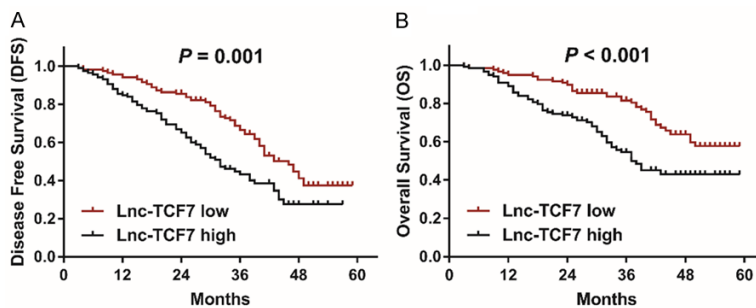

Figure 4. Comparison of DFS and OS between lnc-TCF7 high expression patients and lnc-TCF7 low expression patients. DFS was decreased in lnc-TCF7 high expression patients compared with lnc-TCF7 low expression patients (A). OS was also reduced in lnc-TCF7 high expression patients compared with lnc-TCF7 low expression patients (B). P<0.05 was considered as significant. DFS, disease free survival; OS, overall survival; lnc-TCF7, long noncoding RNA transcription factor 7.

adding 5-FU, which revealed that relative cell proliferation was lower in Lnc-TCF7 (-) & 5-FU group compared with that in NC (-) & 5-FU group (P<0.05) (**Figure 5G**). Regarding sphere formation efficiency, number of spheres/1000 cells was decreased in Lnc-TCF7 (-) group compared with NC (-) group (P<0.01) (**Figure 5H**, **5I**). These data disclosed that knockdown of lnc-TCF7 suppressed cell proliferation as well as stemness in GC.

Discussion

In the present study, we observed that (1) lnc-TCF7 expression was increased in GC tumor tissues compared with adjacent tissues, and its high expression was correlated with advanced pathological grade, larger tumor size, higher T stage and TNM stage in GC patients; (2) Lnc-TCF7 high expression was a predictive factor for poor survivals in GC patients; (3) *In vitro* experiments disclosed that lnc-TCF7 knockdown suppressed GC cell proliferation and stemness.

As a common reported oncogenetic lncRNA, lnc-TCF7 has been disclosed to be dysregulated in several cancers [13-15]. For example, a study indicates that lnc-TCF7 expression is upregulated in cancer tissues compared with adjacent normal tissues and correlates with larger tumor size, higher differentiation degree and elevated TNM stage in colorectal cancer; another study reveals that lnc-TCF7 level is elevated in glioma tissues compared with normal brain tissues [14, 17]. In addition, lnc-TCF7 is also indicated to be highly expressed in hepatocellular carcinoma tumor tissues in compassion with peri-tumor tissues [15]. These previous studies indicated that lnc-TCF7 is involved in the susceptibility of several cancers and cor-

Table 2. Analysis of factors affecting DFS

Parameters	Univariate Cox's proportional hazard regression				Multivariate Cox's proportional hazard regression			
	P value	HR	95% CI		P value	HR	95% CI	
			Lower	Higher			Lower	Higher
Lnc-TCF7 high	<0.001	2.176	1.429	3.312	0.068	1.416	0.975	2.057
Age >60 years	0.605	1.113	0.741	1.674	0.464	1.158	0.782	1.713
Gender (male)	0.242	1.287	0.843	1.962	0.803	0.949	0.629	1.432
History of familial cancer	0.244	0.696	0.379	1.280	0.386	0.796	0.476	1.332
History of smoke	0.286	0.825	0.580	1.174	0.293	0.804	0.535	1.208
History of drink	0.293	0.823	0.573	1.183	0.293	0.798	0.524	1.215
H.pylori Infection (positive)	0.554	1.114	0.779	1.593	0.727	0.932	0.629	1.382
Tumor location (antrum)	0.251	1.236	0.860	1.777	0.090	1.404	0.949	2.078
Higher pathological grade	<0.001	3.069	2.174	4.332	<0.001	3.066	1.981	4.744
Tumor size >3 cm	0.002	1.728	1.218	2.451	0.109	1.428	0.924	2.205
Higher T stage	0.010	1.796	1.148	2.810	0.380	1.457	0.629	3.376
Higher N stage	<0.001	1.525	1.263	1.841	0.390	0.814	0.509	1.301
Higher TNM stage	<0.001	1.877	1.418	2.486	0.070	2.101	0.941	4.692
Adjuvant chemotherapy	0.647	1.092	0.749	1.593	0.188	0.747	0.483	1.153
Adjuvant radiotherapy	0.988	0.996	0.590	1.683	0.290	0.731	0.410	1.305

Univariate and multivariate Cox's proportional hazard regressions were performed to analyze factors affecting DFS. P value <0.05 was considered significant. Pathological grade was scored as G1=1, G2=2, G3=3; T stage was scored as T1=1, T2=2, T3=3, T4=4; N stage was scored as N0=0, N1=1, N2=2, N3=3; TNM stage was scored as I=1, II=2, III=3. The statistical analysis was carried out based on these definitions. DFS: disease free survival; HR: hazard ratio; CI: confidence interval.

**Table 3.** Analysis of factors affecting OS

Parameters	Univariate Cox's proportional hazard regression				Multivariate Cox's proportional hazard regression			
	P value	HR	95% CI		P value	HR	95% CI	
			Lower	Higher			Lower	Higher
Lnc-TCF7 high	<0.001	2.176	1.429	3.312	0.021	1.685	1.084	2.619
Age >60 years	0.605	1.113	0.741	1.674	0.124	1.438	0.906	2.283
Gender (male)	0.242	1.287	0.843	1.962	0.881	0.964	0.601	1.547
History of familial cancer	0.244	0.696	0.379	1.280	0.379	0.750	0.395	1.423
History of smoke	0.583	0.892	0.592	1.343	0.401	0.815	0.505	1.314
History of drink	0.221	0.765	0.499	1.174	0.328	0.784	0.482	1.276
H.pylori Infection (positive)	0.379	1.205	0.795	1.826	0.816	1.056	0.668	1.668
Tumor location (antrum)	0.140	1.386	0.899	2.138	0.138	1.423	0.893	2.268
Higher pathological grade	<0.001	3.385	2.268	5.051	<0.001	3.323	1.980	5.577
Tumor size >3 cm	0.008	1.738	1.157	2.611	0.218	1.374	0.829	2.278
Higher T stage	0.036	1.788	1.038	3.081	0.301	1.677	0.630	4.461
Higher N stage	<0.001	1.521	1.217	1.900	0.428	0.803	0.466	1.383
Higher TNM stage	<0.001	1.895	1.355	2.651	0.169	1.907	0.760	4.788
Adjuvant chemotherapy	0.838	0.956	0.621	1.472	0.184	0.713	0.433	1.174
Adjuvant radiotherapy	0.938	1.024	0.558	1.879	0.604	0.839	0.432	1.629

Univariate and multivariate Cox's proportional hazard regressions were performed to analyze factors affecting OS. P value <0.05 was considered significant. Pathological grade was scored as G1=1, G2=2, G3=3; T stage was scored as T1=1, T2=2, T3=3, T4=4; N stage was scored as N0=0, N1=1, N2=2, N3=3; TNM stage was scored as I=1, II=2, III=3. The statistical analysis was carried out based on these definitions. OS: overall survival; HR: hazard ratio; CI: confidence interval.

relates with advanced tumor features. However, the role of lnc-TCF7 in GC is still unknow. In the present study, we found that lnc-TCF7 relative expression in GC tumor tissues was increased
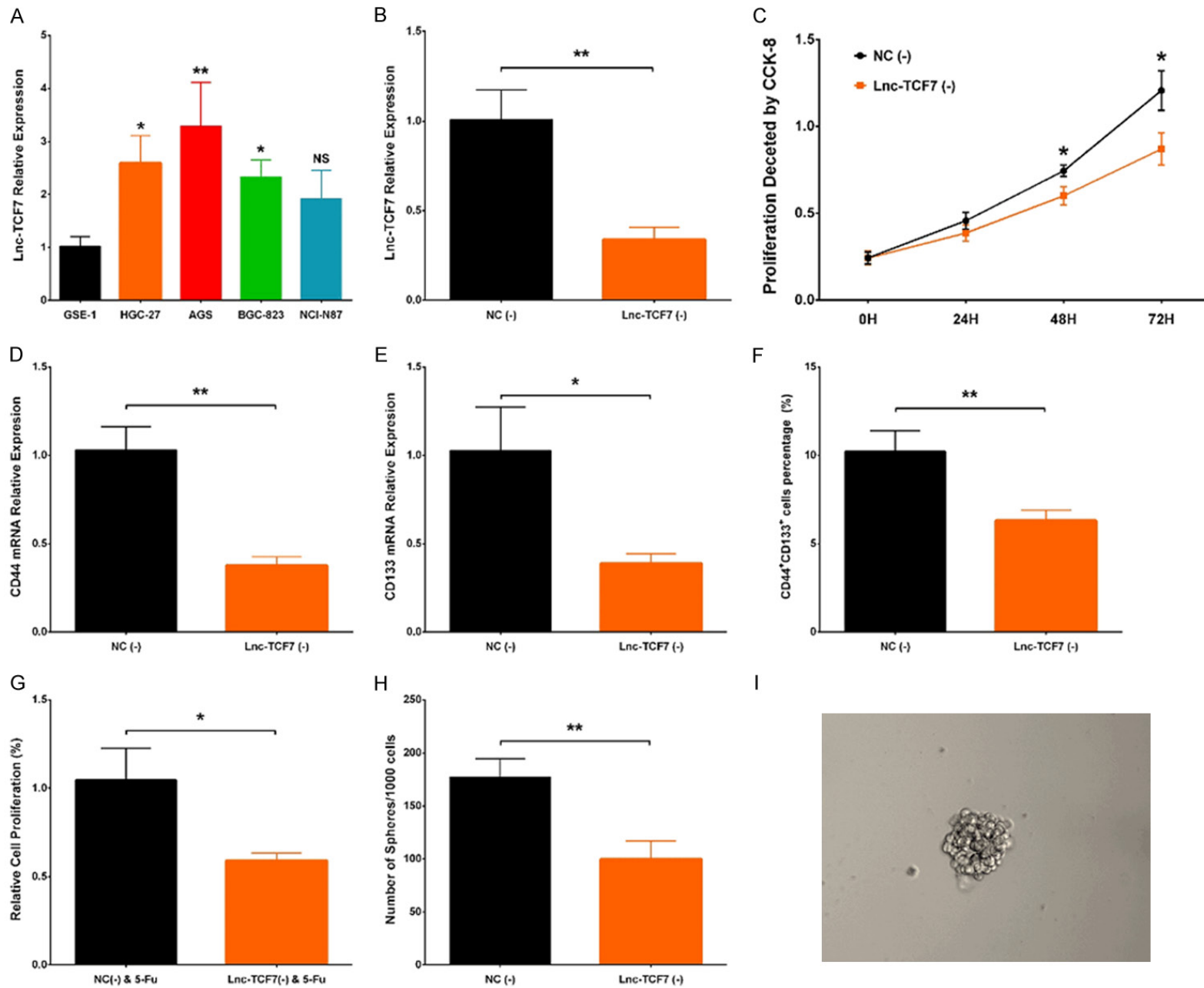
Figure 5. Effect of lnc-TCF7 knockdown on cell proliferation and stemness in AGS cells. Lnc-TCF7 expression was increased in HGC-27, AGS, BGC-823 cells but similar in NCI-N87 cells compared with human normal gastric mucosal cells GSE-1 (A). Lnc-TCF7 expression was decreased in Lnc-TCF7 (-) group compared with NC (-) group (B), indicating good transfection efficiency. Proliferation was decreased in Lnc-TCF7(-) group compared with NC (-) group at 48 h and 72 h (C). Lnc-TCF7 (-) group presented reduced expression of CD44 (D) and CD133 (E) compared with NC (-) group. Lnc-TCF7 (-) exhibited decreased CD44$^+$CD133$^+$ cell percentage compared with NC (-) group (F). Lnc-TCF7 (-) & 5-Fu group presented lower relative cell proliferation compared with NC (-) & 5-Fu group (G). Lnc-TCF7 (-) exhibited reduced number of spheres/1000 cells compared with NC (-) group (H), and examples of spheres were presented in (I). P<0.05 was considered as significant. NS, non-significant, *P<0.05, **P<0.01. Lnc-TCF7, long noncoding RNAs transcription factor 7; 5-Fu, 5-fluorouracil.

compared with adjacent tissues and it was of good value in differentiating tumor tissues from adjacent tissues in GC patients by ROC curve analysis. We further analyzed the correlation of lnc-TCF7 expression with clinicopathological features in GC patients, and disclosed that lnc-TCF7 high expression was associated with advanced pathological grade, larger tumor size, higher T stage and TNM stage in GC patients. And these might be explained by the following reasons: (1) Lnc-TCF7 acted as an oncogene that promoted cell proliferation, migration and invasion in cancers, which might lead to its elevated level in GC tumor tissues and advanced GC tumor features. (2) Lnc-TCF7 enhanced cancer stemness which resulted in increased pathological grade of GC. (3) Lnc-TCF7 positively regulated several oncogene pathways such as Wnt signaling to induce cancer progression, which might give rise to increased tumor stages of GC.

Currently, lnc-TCF7 has been exhibited to serve as a potential prognostic biomarker in several cancers [14, 18]. For example, lnc-TCF7 is associated with higher susceptibility and predicts unfavorable prognosis in glioma [14]. Another study reports that lnc-TCF7 high expression is associated with worse 3-year survival in colorectal cancer patients [18]. Based on the two studies and the aforementioned data that lnc-TCF7 high expression was correlated with advanced clinicopathological features in GC, we hypothesized that lnc-TCF7 might be of prognostic value in GC patients as well. This present study further observed that lnc-TCF7 high expression predicted worse survivals in GC patients. The possible explanations included that: (1) Lnc-TCF7 correlated with advanced disease stages and higher pathological grade, which directly resulted in worse survivals in GC patients. (2) Lnc-TCF7 might increase chemoresistance and aggressiveness of GC cell by enhancing cancer cell stemness,

leading to elevated drug resistance, therefore patients with lnc-TCF7 high expression would response ineffectively to drugs and have poor survival [15, 19, 20].

Existing *in vitro* experiments indicate that lnc-TCF7 actively participates in the tumorigenesis by promoting cell proliferation as well as stemness in some cancers [14, 16-18]. For instance, overexpression of lnc-TCF7 promotes the cell proliferation in glioma and enhances the stemness of glioma cells via upregulating the expression of cell stemness markers such as epithelial cell adhesion molecule (EpCAM) [20]. In addition, lnc-TCF7 is reported to trigger TCF7 expression via binding to the T-cell factor 7 (TCF7) gene, further activating Wnt signaling pathway, which is reported to play an important role in tumorigenesis in GC as well, and contributing to the increased stemness in hepatocellular carcinoma as well as colorectal cancer [10, 15, 18]. According to the previous studies, we speculated that lnc-TCF7 might regulate the cell proliferation and stemness in GC as well. In the present study, we discovered that lnc-TCF7 knockdown suppressed cell proliferation, CD44 and CD133 expressions, CD44$^+$CD133$^+$ cell proportion, drug resistance to 5-FU, as well as sphere formation efficiency in GC cells, indicating that lnc-TCF7 knockdown reduced proliferation and stemness of GC cells. These data further validated the oncogene role of lnc-TCF7 in GC especially its potential to be a treatment target for GC.

In conclusion, lnc-TCF7 is upregulated and associates with advanced tumor features as well as poor prognosis, and its knockdown suppresses cancer cell proliferation and stemness in GC.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Primers applied in RT-qPCR

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
lnc-TCF7	ACACAGTTGGCTGCGGATTA	CAGATTCAGGAGTAGAACACAGG
CD44	ACATCCTCACATCCAACACCTC	CCTCCTGAAGTGCTGCTCCT
CD133	GCTGCTTGTGGAATAGACAGAATG	GAAGGACTCGTTGCTGGTGAAT
GAPDH	TGACCACAGTCCATGCCATCAC	GCCTGCTTCACCACCTTCTTGA