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Clinical prognostic value of A FOXM1 related long non-coding RNA expression in gastric cancer

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Abstract. – OBJECTIVE: The aim of this study was to explore FOXM1-related LncRNA 1(FRLnc1) expression level in gastric cancer (GC) and demonstrate its association with the prognosis.

PATIENTS AND METHODS: A total of 173 GC patients from Affiliated Hospital of Jining Medical University were enrolled in the study. GC tissue samples were quantified for FRLnc1 expression level using quantitative PCR (qPCR) method. The relevance between FRLnc1 expression and clinicopathological features was determined by x2-test. The association between FRLnc1 expression and overall survival was estimated by the Kaplan-Meier method. Multivariate and univariate analysis were performed to explore whether FRLnc1 was an independent prognostic factor for GC patients.

RESULTS: We found that FRLnc1 expression was higher in GC tissues than corresponding adjacent tissues ($p < 0.01$). Increased FRLnc1 expression was associated with depth of tumor ($p = 0.004$), differentiation degree ($p = 0.032$), distant metastasis ($p = 0.007$), TNM stage ($p = 0.006$) and lymph node metastasis ($p = 0.012$). More importantly, Kaplan-Meier survival analysis demonstrated that overall patient survival for those with low FRLnc1 expression was significantly longer than those patients with high FRLnc1 expression ($p < 0.001$). Multivariate analysis suggested that FRLnc1 expression was an independent prognostic marker for survival in patients with GC.

CONCLUSIONS: The data presented in this work firstly suggested that FRLnc1 may be a prognostic predictor in GC.

Key Words:
FRLnc1, Gastric cancer, Prognosis.

Introduction

Gastric cancer is one of the most common gastrointestinal tumors, which still has significant impact on cancer related death worldwide, accounting for 8.8% of the total deaths from cancer[1-3]. In 2015, more than 248,000 GC-related deaths were found in China, accounting for nearly half of all the GC deaths worldwide[4]. Even with advanced systematic therapy, the 5-year overall survival of patients with advanced GC is < 20%[5]. The outcome of advanced GC still remains poor due to the recurrence, invasion and metastasis. Therefore, it is imperative to explore the biomarkers help in the early diagnosis and prognosis prediction for GC.

Long noncoding RNAs (lncRNAs) are a group of transcribed RNA consisting of more than 200 nucleotides and cannot be translated into a protein[6]. More and more studies have indicated that lncRNAs participate in various biological processes, such as cell proliferation, differentiation, apoptosis, and cell cycle progression[7,8]. Indeed, it has been confirmed that lncRNAs was involved in the regulation of gene expression at different levels, including transcriptional processing, microRNA sponging, and other processes[9,10]. Furthermore, increased evidence have suggested that abnormal expression of lncRNAs is correlated with various human diseases like coronary artery diseases and tumor[11,12]. Recently, some lncRNAs have been well studied. For instance, lncRNA URHC[13] was high expressed in hepatocellular carcinoma than in normal hepatocellular tissues; it decreased URHC expression and inhibited the proliferation of human hepatocellular carcinoma via ZAK through the ERK/MAPK signaling pathway. Qi et al[14] found that lncRNA-ATB was increased in renal cell carcinoma, and lncRNA-ATB expression level in renal cell carcinoma is a powerful prognostic marker for patients with renal cell carcinoma. Moreover, lncRNA AGAP2-AS1[15], lncRNA GAPLINC[16] and lncRNA Sox2ot[17] were also reported to play an important role in progression of GC. However, the functional involvement of lncRNAs in gastric carcinogenesis has not yet been fully elucidated.

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FRLnc1 (FOXM1-related LncRNA 1) was a newly identified lncRNA. To our best knowledge, only Cai et al[18] reported that FRLnc1 expression was significantly upregulated in GC tissues. However, the clinical significance of FRLnc1 in GC remains unknown. Thus, we investigated the feasibility of FRLnc1 as a novel prognostic biomarker for GC.

Patients and Methods

Patients

This study was approved by the Ethics Committee of Affiliated Hospital of Jining Medical University, and all patients provided the informed consent. A total of 178 patients with histologically confirmed EOC were included into the cohort study. None of the patients had received preoperative therapy. Resected tissue samples were immediately cut and snap-frozen in liquid nitrogen until RNA extraction. Overall survival (OS) was calculated from the day of surgery to the day of death. Follow-up was done through hospital medical record and telephone interview. Clinical data of GC patients were collected from the medical records and shown in Table I.

RNA Extraction and Quantitative Real-time PCR (qRT-PCR)

The total RNA was isolated from tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was generated using the PrimeScript™ RT Reagent Kit (TaKaRa, Otsu, Shiga, Japan). The level of FRLnc1 was detected by qRT-PCR, which was carried out using SYBR Premix Ex Taq II (Perfect Real-time; TaKaRa, Otsu, Shiga, Japan). GAPDH was used as an internal control. The primer sequences were as follows: FRLnc1 forward, 5' ATGCGTGATTGCAGTCTCTG-3' and reverse, 5'- TCTTGCAATATTTCCTGTGA-3'; GADPH forward, 5'-GTCAACGGATTTGGTCTGTATT-3' and reverse, AGTCTTCTGGGTGGCAGTGAT-3'. The relative expression levels of FRLnc1 were calculated using the $2^{-\Delta Ct}$ method.

Table I. Correlations between FRLnc1 expression and clinicopathological features in GC patients.

		FRLnc1 expression		
Parameters	n	Low	High	p
Age (years)				0.565
<60	73	34	39	
≥60	100	51	49	
Gender				0.252
Male	88	47	41	
Female	85	38	47	
Tumor size(cm)				0.176
≥5	51	21	30	
<5	122	64	58	
Vascular invasion				0.096
Absent	119	64	55	
Present	54	21	33	
Depth of tumor				0.004
T1 and T2	116	66	50	
T3 and T4	57	19	38	
Differentiation degree				0.032
Well/Moderately	119	65	54	
Poorly	54	20	34	
Distant metastasis				0.007
Yes	60	21	39	
No	113	64	49	
TNM stage				0.006
I+II	124	69	55	
III+IV	49	16	33	
Lymph node metastasis				0.012
Present	52	18	34	
Absent	121	67	54	

Statistical Analysis

SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All experimental data were presented as means ± standard deviation (S.D.). The difference in the expression of FRLnc1 between GC and the normal tissues was analyzed with Student's *t*-test. Associations between clinicopathological parameters and FRLnc1 expression were evaluated using χ^2-tests. Kaplan-Meier method was used for survival analysis, and differences in survival were determined using the log-rank test. Cox regression analysis was used for multivariate analysis of independent prognostic factors for OS. Statistical significance was determined at the level of $p < 0.05$.

Results

FRLnc1 Level Was Upregulated in GC

We first compared the expression levels of FRLnc1 in 173 GC tissue samples to those in the adjacent normal tissues using RT-PCR. As shown in Figure 1, we found that FRLnc1 expression was higher in GC tissues than corresponding adjacent tissues ($p < 0.01$). This result suggested that FRLnc1 probably played a crucial role in GC.

Clinicopathological Significance of FRLnc1 Expression in Patients with GC

For better understanding of the clinical significance of FRLnc1 expression in GC, the GC patients were classified into two groups (high and low) according to the median of FRLnc1 expression level. Table II showed the relationships between FRLnc1 expression and the clinical pathological characteristics of GC. Increased FRLnc1 expression was associated with depth of tumor ($p = 0.004$), differentiation degree ($p = 0.032$), distant metastasis ($p = 0.007$), TNM stage ($p = 0.006$) and lymph node metastasis ($p = 0.012$). However, no significant correlation was found between the expression of FRLnc1 and age, gender, tumor size, and vascular invasion (all $p > 0.05$).

Association of FRLnc1 Overexpression with Poor Prognosis of GC Patients

Kaplan-Meier survival curves of association between overall survival and FRLnc1 levels are shown in Figure 2. Overall patient survival for those with low FRLnc1 expression was significantly longer than those patients with high FRLnc1 expression ($p < 0.001$). Then, univariate analysis of prognostic parameters for overall survival was performed. The results showed that depth of tumor, differentiation degree, distant metastasis, TNM stage, lymph node metastasis and FRLnc1 was a prognostic factor in GC patients (Table II). Those factors, which correlated with overall survival in the univariate analysis, were further demonstrated by multivariate analysis. The results confirmed that increased FRLnc1 expression ($p = 0.003$) was an independent predictive factor of unfavorable prognosis GC patients (Table II).

Discussion

In China, GC is the primary public health problem, which arouses more and more attention

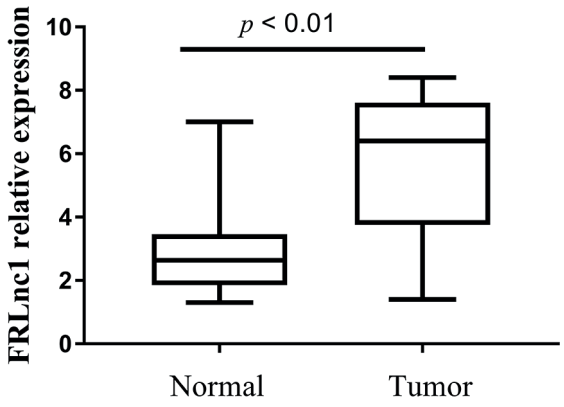

Figure 1. The expression of FRLnc1 in 173 GC tissues and adjacent non-tumor tissues were examined by qRT-PCR. FRLnc1 was significantly up-regulated in GC tissues compared to non-tumor tissues ($p < 0.01$).

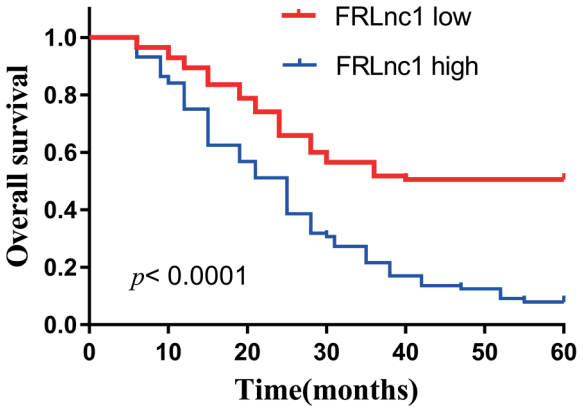

Figure 2. Kaplan-Meier postoperative survival curve for patterns of patients with GC and FRLnc1 expression. Patients with higher FRLnc1 expression level had significantly poorer OS ($p < 0.0001$).

Table II. Univariate and multivariate analysis for overall survival in patients with GC.

		Univariate analysis		Multivariate analysis	
Risk factors	**Category**	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Age	<60/≥60	1.341 (0.683-2.231)	0.492	-	-
Gender	Male/Female	1.673 (0.821-2.191	0.277	-	-
Tumor size	≥5/<5	2.563 (0.672-2.933)	0.113	-	-
Vascular invasion	Present/ Absent	2.731 (0.823-3.387)	0.081	-	-
Depth of tumor	T3 and T4/ T1 and T2	3.891 (1.532-6.482)	0.007	3.431 (1.239-5.783)	0.009
Differentiation degree	Poorly/ Well/Moderately	3.263 (1.482-5.433)	0.011	2.893 (1.219-4.372)	0.017
Distant metastasis	Yes/No	4.231 (1.783-7.983)	0.003	3.532 (1.321-6.119)	0.006
TNM stage	III+IV/ I+II	3.672 (1.549-5.732)	0.009	3.013 (1.139-4.328)	0.015
Lymph node metastasis	Present/ Absent	3.213 (1.239-5.328)	0.018	2.783 (1.023-4.213)	0.024
FRLnc1 expression	High/Low	3.982 (1.623-8.921)	0.001	3.129 (1.271-7.893)	0.003

nowadays. Exploring new biological molecular markers is of considerable importance to improve the prognosis of GC patients[19]. Growing evidence indicated that dysregulated lncNRAs were reported to serve as critical regulators in progression and development of GC and could be used for predicting the outcome of this disease[20,21]. In this investigation we focused on FRLnc1.

It has been reported that aberrant expression of lncRNAs performed an important function in various tumors, including GC. For instance, Ma et al[22] reported that the expression levels of lncRNA XIST was significantly up-regulated in GC and over-expression of lncRNA XIST promoted cell growth and invasion through regulating miR-497/ MACC1 axis in GC. Bi et al[23] found that lncRNA PCAT-1 could promote proliferation and metastasis in GC cells through regulating CDKN1A, suggesting that PCAT-1 plays an oncogenic role in GC. Another study by Liu et al[24] found that up-regulation of lncRNA CARLo-5 was associated with poor prognosis of GC patients. Moreover, they confirmed lncRNA CARLo-5 as an independent marker for predicting the clinical outcome of GC patients. Cai et al[18] identified a novel lncRNA, designated as FRLnc1. They found that FRLnc1 expression was significantly upregulated in GC tissues and up-regulation of FRLnc1 could promote GC cells migration by regulating the levels of TGFb1 and Twist. However, to our knowledge, no studies have reported the clinical significance of FRLnc1 in GC patients.

In the present work, we observed that increased FRLnc1 expression was evident in GC tissues compared with normal gastric tissues. These results were in line with previous data. Then, we also found that increased FRLnc1 expression was associated with depth of tumor, differentiation degree, distant metastasis, TNM stage and lymph node metastasis. Kaplan-Meier analysis indicated that patients with high FRLnc1 expression had a poor overall survival. The multivariate analysis results indicated that FRLnc1 might be as an independent prognostic factor for GC patients. All the results suggested that decreased expression of FRLnc1 was been fitting to predict prognosis of GC patients.

Conclusions

Evidence from this research demonstrated that FRLnc1 was an independent prognostic indicator in GC. Future investigation should be done to elucidate the molecular mechanisms underlying the role of FRLnc1 in progression of GC. On the other hand, our results must be verified by large-scale prospective studies with standardized methodology.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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