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High long non-coding LIFR-AS1 expression correlates with poor survival in gastric carcinoma

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Abstract. – OBJECTIVE: Abundant evidence has demonstrated that long non-coding RNAs (lncRNAs) play key roles in the development of human neoplasms. A novel cancer-related lncRNA, leukemia inhibitory factor receptor antisense RNA 1 (LIFR-AS1), has been reported to be under-expressed in breast cancer and associated with poor prognosis, but its significance in gastric cancer (GC) remains to be determined. Therefore, we assessed the prognostic and diagnostic value of LIFR-AS1 in GC.

PATIENTS AND METHODS: Quantitative RT-PCR assay was used to detect the expression levels of LIFR-AS1 in GC tissues and adjacent normal tissues. The correlation between LIFR-AS1 expression and clinicopathological features was analyzed by Pearson's χ^2-test. The disease-free survival and overall survival rates of GC patients were calculated by the Kaplan-Meier method. Cox regression analysis was used to assess factors related to survival.

RESULTS: In this study, levels of LIFR-AS1 were significantly higher in GC tumor samples relative to adjacent normal tissue samples. A ROC analysis suggested LIFR-AS1 expression could be reliably used to differentiate between normal and GC tumor tissue. In addition, elevated LIFR-AS1 expression was positively correlated with more advanced and aggressive GC features, such as larger tumor size, lymphatic metastasis, and more advanced TNM stage. Survival analyses revealed that elevated LIFR-AS1 expression was correlated with worse overall survival and disease-free survival. Multivariate analysis further confirmed the relevance of LIFR-AS1 as an independent predictor of GC patient outcomes.

CONCLUSIONS: In summary, these results indicate that the lncRNA LIFR-AS1 is a promising prognostic indicator in GC patients.

Key Words:
Lnc-LIFR-AS1, Gastric cancer, Prognosis, Biomarker.

Abbreviations

GC = gastric cancer; LIFR-AS1 = leukemia inhibitory factor receptor antisense RNA 1; HR = hazard ratio; SPSS = statistical package for the social sciences; OS = overall survival; DFS = disease-free survival; GEPIA = gene expression profiling interactive analysis.

Introduction

Gastric cancer (GC) is one of the deadliest forms of cancer in the world[1-3], and it is highly prevalent in China where it is the second most common and third deadliest cancer subtype[4,5]. While there have been many complex and comprehensive analyses of GC conducted to date, the mechanisms governing the onset and progression of this disease remain incompletely understood, thus limiting patient access to efficacious treatment options. As such it is important to identify new biomarkers of diagnostic or prognostic utility in an effort to improve patient survival rates.

Long noncoding RNAs (lncRNAs) are RNA molecules that do not encode protein despite their active transcription[6]. There is clear evidence[7] that some lncRNAs are able to act through a variety of mechanisms in order to influence diverse processes including transcription, mRNA stability, and epigenetic regulatory pathways. In addition, several lncRNAs are dysregulated in tumors, with those being overexpressed often helping to initiate or drive tumor development[8,9]. Leukemia inhibitory factor receptor antisense RNA 1 (LIFR-AS1), a novel cancer-related lncRNA, is transcribed from the LIFR gene located on human chromosome 5p13.1 in an antisense manner[10]. LIFR-AS1 is under-expressed in breast cancer and associated with poor survival of breast cancer patients[11,12]. However, the exact role of LIFR-AS1 in breast cancer and its potential molecular mechanisms remain largely unknown. Whether LIFR-AS1 exhibits any clinical relevance in patients with GC, however, is unclear. As such, this study sought to produce novel evidence examining the

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potential for LIFR-AS1 to be a diagnostic and/or prognostic biomarker in GC patients.

Patients and Methods

Patients and Clinical Specimens

A total of 265 pairs of GC tumor tissue and adjacent healthy tissue were collected by Shaoxing People's Hospital. These patients (148 males, 117 female) were 31-79 years old (median: 57.3 years) and had been diagnosed with GC based upon both clinical and histopathological assessments. Samples used in this study were from patients who had not undergone preoperative chemotherapy or radiotherapy. Samples were snap-frozen using liquid nitrogen prior to -80°C storage. Patient follow-up information and survival were determined based upon medical records and or direct contact with the patients or their families. Patient demographic and clinical characteristics are compiled in Table I. The Ethics Committee of the Shaoxing People's Hospital approved this investigation.

Quantitative RT-PCR

RNA was extracted from samples using the miRNeasy Mini-kit (Qiagen, Xuhui, Shanghai, China), after which Reverse Transcriptase (Transgene, Xuhui, Shanghai, China) was used to prepare cDNA from all samples. A 7300-sequence detection system (Applied Biosystems, Foster City, CA, USA) was used to conduct qRT-PCR reactions with SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) using 35 cycles of 12 s at 95°C and 1 min at 60°C. For normalization, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control gene. The comparative cycle threshold (CT) approach was used for de-

Table II. Association between LIFR-AS1 expression and clinicopathological features of human GC.

Clinical features	Total	LIFR-AS1 expression		*p*-value
		High (N= 135)	Low (N = 130)	
Age (years)				0.069
< 60	78	33	45	
≥ 60	187	102	85	
Gender				0.519
Male	148	78	70	
Female	117	57	60	
Tumor size (cm)				0.024
< 5	106	45	61	
≥ 5	159	90	69	
Differentiation grade				0.543
Well	89	43	46	
Moderate + Poor	176	92	84	
TNM stage				0.003
I + II	166	78	88	
III	99	57	42	
Depth of invasion				0.055
T1 + T2	124	77	89	
T3 + T4	141	58	41	
Lymph node metastasis				0.030
No	166	76	90	
Yes	99	59	40	
Distant metastasis				0.151
No	204	99	105	
Yes	61	36	25	
CEA level, μg/l				0.202
< 5	126	59	67	
≥ 5	139	76	63	
CA19-9, kU/L				0.439
< 40	145	77	68	
≥ 40	120	58	62	

CEA; Carcinoembryonic antigen, CA19-9 carbohydrate antigen 19-9; Pearson chi-square test was used for comparison between subgroups.

termining relative LIFR-AS1 expression levels, with primers used shown as below. Lnc-LIFR-AS1 forward: 5'-AAGTTTCAGGCTCCTGACAGC-3'; Lnc-HANR-AS1 reverse: 5'-TTCGCCCACGTTCTTCTCGC-3', GAPDH forward: 5'-AGAAGGCTGGGGCTCATTTG-3'; GAPDH reverse: 5'-AGGGGCCATCCACAGTCTTC-3'.

Statistical Analysis

SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical testing. Student's *t*-tests were used to compare data between groups, while the association between LIFR-AS1 expression and clinicopathological findings was assessed *via* chi-squared test. A receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic relevance of LIFR-AS1 expression in GC patients, while Kaplan-Meier and multivariate analyses were used to gauge the prognostic value of LIFR-AS1 expression. $p < 0.05$ was the significance threshold.

Results

GC Tumors Exhibit Elevated LIFR-AS1 Expression

To first assess how the lncRNA LIFR-AS1 might be linked to GC progression, we explored its expression levels in tumor samples from those patients suffering from GC. This analysis revealed that tumor samples exhibited significantly higher LIFR-AS1 expression relative to normal control samples ($p<0.001$) (Figure 1A), with 198/256 (77.3%) patients exhibited significantly higher LIFR-AS1 expression than compared normal control samples (Figure 1B). Together these findings suggested the possibility that the lncRNA LIFR-AS1 is expressed at high levels in GC and may play a role in disease progression.

The Diagnostic Value of LIFR-AS1 Expression in GC Patients

As LIFR-AS1 expression was apparently dysregulated in GC patient samples, we next explored its potential utility as a diagnostic biomarker of GC. A ROC curve analysis suggests that LIFR-AS1 expression levels allowed for reliable differentiation between normal and GC tumor tissues (AUC: 0.922; 95% confidence interval: 0.897-0.946) (Figure 2). The sensitivity and specificity of LIFR-AS1 in this analysis were 0.755 and 0.996, respectively. The lncRNA LIFR-AS1 may thus be a useful diagnostic biomarker for GC.

Elevated LIFR-AS1 Expression Correlated With GC Patient Clinical Characteristics

To further assess the clinical relevance of LIFR-AS1 expression in patients with GC, we divided the 265 patient samples according to their levels of LIFR-AS1 expression (LIFR-AS1-high or LIFR-AS1-low; n=83 and 82, respectively) based on the median LIFR-AS1 expression level in GC tumor tissue samples. Chi-squared tests were then used to compare clinical characteristics

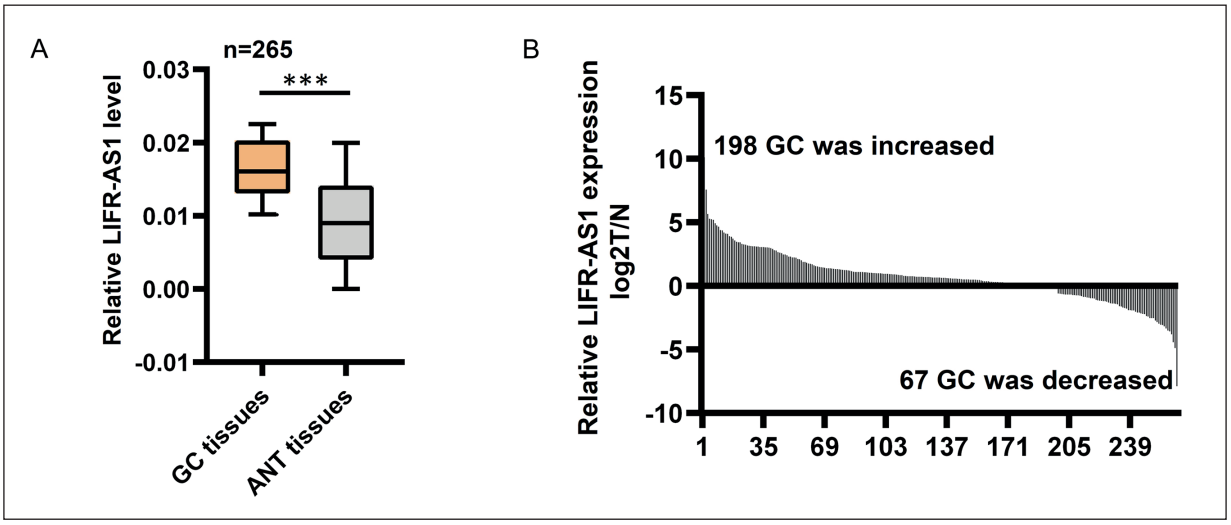

Figure 1. GC tumors exhibit decreased LIFR-AS1 expression. **A, B,** qRT-PCR was used to assess LIFR-AS1 expression in 265 paired normal and GC tumor tissues. ***$p<0.001$.

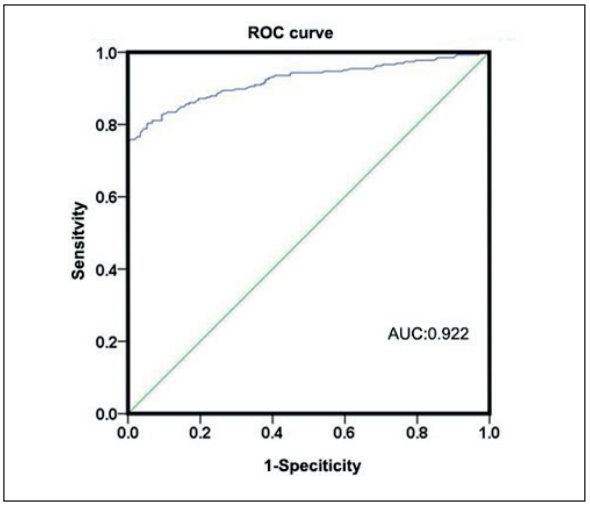

Figure 2. ROC curve analysis of the diagnostic value of the lncRNA LIFR-AS1 in GC.

between groups, revealing that higher LIFR-AS1 levels were associated with tumor size (p=0.024), lymphatic metastasis (p=0.030), and advanced TNM stage (p=0.003) (Table I). In contrast, there was no relationship between LIFR-AS1 expression and other clinical characteristics (p>0.05).

LIFR-AS1 Offers Prognostic Utility in GC Patients

Finally, from the Gene Expression Profiling Interactive Analysis (GEPIA) data indicated that high LIFR-AS1 level had poor overall survival (OS) (p=0.0002, Figure 3A) as well as disease-free survival (DFS) (p=0.0073, Figure 3B). Meanwhile, from our cohort we assessed the prognostic relevance of LIFR-AS1 in GC *via* a Kaplan-Meier approach, revealing a significant association between elevated LIFR-AS1 expression and reduced overall survival (OS) (p=0.003, Figure 3C), as well as disease-free survival (DFS)

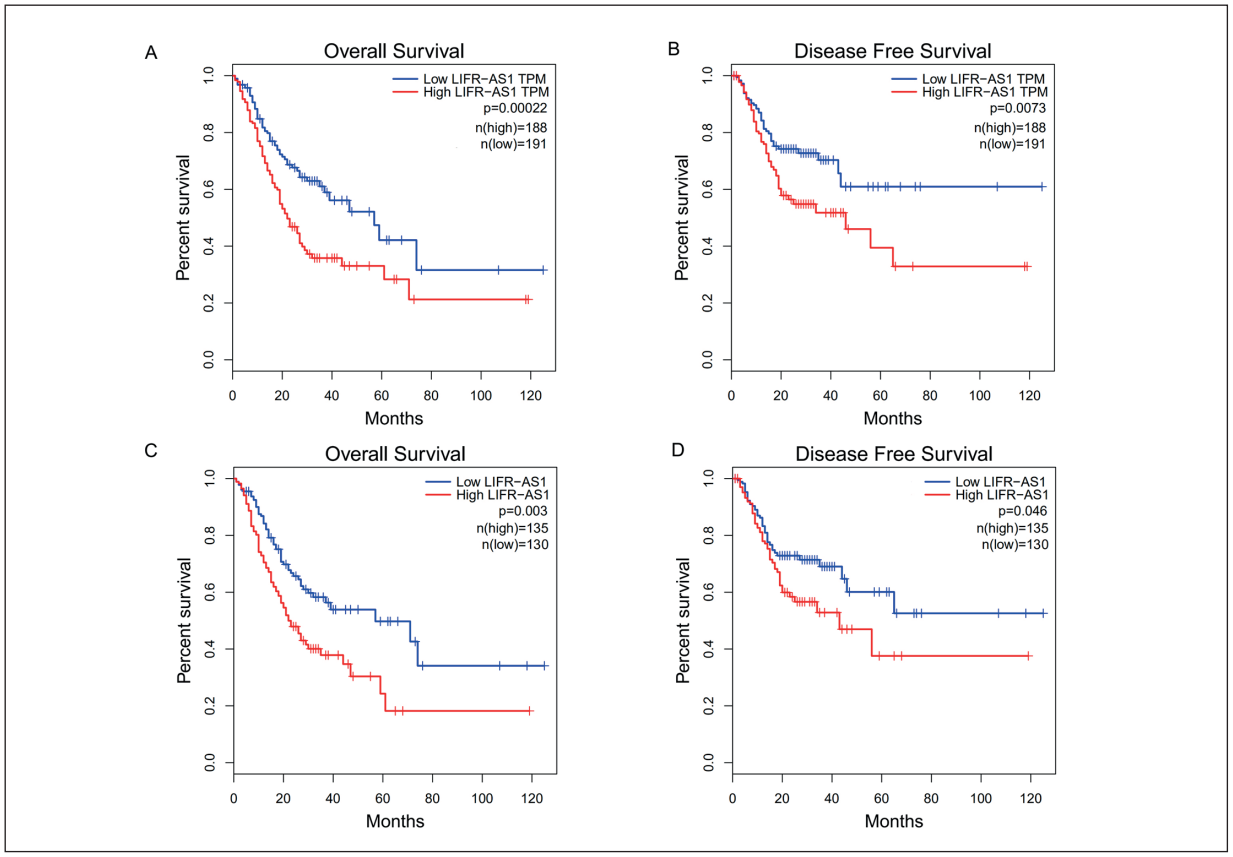

Figure 3. GC patient survival is associated with LIFR-AS1 expression levels. **A**, GEPIA data indicated GC patients with high LIFR-AS1 expression exhibited significantly reduced overall survival relative to LIFR-AS1-high patients (p=0.002). **B**, GEPIA data indicated GC patients with high LIFR-AS1 expression exhibited significantly reduced disease-free survival relative to LIFR-AS1-high patients (p=0.007). **C**, GC patients with high LIFR-AS1 expression exhibited significantly reduced overall survival relative to LIFR-AS1-high patients (p=0.003). **D**, GC patients with high LIFR-AS1 expression exhibited significantly reduced disease-free survival relative to LIFR-AS1-high patients (p=0.046).

(p=0.046, Figure 3D), meaning that higher levels of this lncRNA are correlated with a worse prognosis. A multivariate analysis was additionally conducted to identify factors predictive of OS and DFS (Table II), revealing that elevated LIFR-AS1 expression independently predicted reduced OS (HR=1.501, 95% CI: 1.356-2.108, p=0.033) and DFS (HR=2.314, 95% CI: 1.713-3.956, p=0.012) in GC patients.

Discussion

GC is the fifth leading form of cancer globally, making it a key public health issue[1-3]. As there are no effective means of screening the general population for GC and it is often asymptomatic until the disease is advanced, many patients with GC are first diagnosed when the disease is already in its advanced stages. For these patients, surgery and chemotherapy are of limited value, and as a result the prognosis for these GC patients is poor. Several studies[13,14] have highlighted the potential of lncRNAs to serve as tumor diagnostic biomarkers, given that these RNA molecules are often dysregulated in tumors in a manner functionally linked to tumor progression. As high throughput sequencing technologies have become increasingly prevalent, it has become far easier to readily detect expression patterns of many lncRNAs at the same time, making them ideal targets worth of study as putative diagnostic and prognostic biomarkers[15,16].

Many reports have sought to characterize patterns of lncRNA expression and their functional relevance in GC[17]. Zhang et al[18] found HCG11 to be expressed at high levels in GC in a manner correlated with reduced GC patient survival and enhanced tumor growth owing to its ability to activate Wnt signaling pathway by miR-1276/CTNNB1axis. Wang et al[19] found OIP5-AS1 to be expressed at high levels in GC and to correspond with a worse patient prognosis. Chen et al[20] found the lncRNA PCAT18 to similarly be overexpressed in GC patients in a manner correlating with poorer survival, with *in vitro* analyses

Table II. Univariate and multivariate Cox regression analyses of overall survival and disease-free survival in GC patients.

Variables	Univariate analyses			Multivariate analyses		
	Hazard ratio	95% CI	*p*-value	Hazard ratio	95% CI	*p*-value
Overall survival						
Age (years) ≥ 60/< 60	1.322	0.765-3.882	0.213	–	–	–
Gender Male/female	0.845	0.574-1.551	0.589	–	–	–
Tumor size (cm) ≥ 5/< 5	2.578	1.226-3.244	0.022	2.162	1.792-3.174	0.044
Differentiation grade Poor + moderate/well	1.668	1.112-3.526	0.017	2.611	1.145-3.152	0.034
TNM stage III/I + II	2.589	1.559-4.210	0.029	1.226	0.856-1.684	0.276
Depth of invasion pT3-4/pT1-2	1.878	0.451-2.998	0.465	–	–	–
Lymph node metastasis Yes/no	1.965	1.712-2.926	0.018	1.709	1.267-3.264	0.021
Distant metastasis Yes/no	2.633	1.506-3.526	0.012	1.305	0.574-2.856	0.255
CEA level, μg/l ≥ 5/< 5	0.921	0.772-2.520	0.398	–	–	–
CA19-9, kU/L ≥ 40/< 40	1.725	0.795-2.515	0.098	–	–	–
LIFR-AS1 expression High/Low	2.829	1.824-3.119	0.011	1.501	1.356-2.108	0.033
Disease-free survival						
Age (years) ≥ 60/< 60	1.365	0.556-2.558	0.584	–	–	–
Gender Male/female	0.769	0.369-1.478	0.862	–	–	–
Tumor size (cm) ≥ 5/< 5	1.754	1.165-2.634	0.024	1.043	0.992-1.374	0.461
Differentiation grade Poor + moderate/well	2.877	1.654-4.766	0.017	2.025	1.145-3.152	0.021
TNM stage III/I + II	2.564	1.564-3.478	0.033	1.543	0.559-2.784	0.369
Depth of invasion pT3-4/pT1-2	2.589	1.365-3.687	0.024	1.921	1.767-3.264	0.032
Lymph node metastasis Yes/no	2.456	1.875-3.147	0.020	2.429	1.767-3.265	0.025
Distant metastasis Yes/no	2.059	1.643-4.563	0.029	2.725	1.665-3.656	0.048
CEA level, μg/l ≥ 5/< 5	0.869	0.791-1.458	0.312	–	–	–
CA19-9, kU/L ≥ 40/< 40	0.799	0.392-1.364	0.568	–	–	–
LIFR-AS1 expression High/Low	2.597	1.486-5.155	0.005	2.314	1.713-3.956	0.012

CEA; Carcinoembryonic antigen, CA19-9 carbohydrate antigen 19-9; HR, hazard ratio; 95% CI, 95% confidence interval.

demonstrating the ability of this lncRNA to regulate PTEN/PI3K/AKT signaling pathway, so as to control tumor cell invasion. Leukemia inhibitory factor receptor antisense RNA 1 (LIFR-AS1), a novel cancer-related lncRNA, is transcribed from the LIFR gene located on human chromosome 5p13.1 in an antisense manner[21]. LIFR-AS1 is under-expressed in breast cancer and associated with poor survival of breast cancer patients[11,12]. Whether LIFR-AS1 is similarly relevant to the prognosis of GC patients has not been assessed previously.

In this report we observed a significant up-regulation of LIFR-AS1 in GC tumors relative to adjacent normal controls, with ROC curve analyses confirming that LIFR-AS1 may be an effective marker well-suited to differentiating between normal and tumor tissue. We further provided novel insight into the clinical relevance of LIFR-AS1, determining that it was significantly associated with tumor size, lymphatic metastasis, and more advanced TNM stage, indicating that LIFR-AS1 may be positively associated with GC progression in patients. Importantly, when we assessed patient survival as a function of LIFR-AS1 expression we found that individuals with higher LIFR-AS1 expression suffered poorer clinical outcomes, with shorter average OS and DFS. We then employed a multivariate analysis to demonstrate that LIFR-AS1 was an independent predictor of OS and DFS in GC patients, confirming its potential relevance. However, it is necessary to note that we employed an arbitrary LIFR-AS1 expression level cut-off value in this study, and future research should seek to identify an optimal clinically relevant cut-off value. In addition, our study population was relatively small, and as such additional research will be needed to confirm that LIFR-AS1 is relevant as a biomarker in GC patients.

Conclusions

Shortly, these results indicate that LIFR-AS1 has potential as a novel biomarker useful for diagnosing GC and/or for predicting patient prognosis, with higher levels of this lncRNA being correlated with poorer patient prognosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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