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Elevated long non-coding RNA LINC00958 was associated with metastasis and unfavorable prognosis in gastric cancer

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Abstract. – OBJECTIVE: To investigate the clinicopathological significance of long non-coding LINC00958 and its expression in gastric cancer (GC).

PATIENTS AND METHODS: A total of 200 patients with GC whose sample tissues were enrolled. Total RNA was isolated using the TRIzol method followed by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) detection of LINC00958 on mRNA level. The correlation was analyzed using Chi-square test, between the LINC00958 expression versus clinicopathological variables, including age, gender, clinical stage, tumor size, TNM classification and overall prognosis. The Kaplan-Meier survival curve was used to assess the prognostic value of LINC00958 expression, after which univariate and multivariate analysis was carried out with the COX proportional hazard analysis.

RESULTS: LINC00958 was shown to be dramatically elevated in GC relative to normal controls. Elevated LINC00958 significantly correlated with lymph nodes metastases, distance metastasis, peritoneal dissemination, and peritumoral tissues infiltration ($p<0.05$). The Kaplan-Meier survival analysis showed that up-regulated LINC00958 was markedly associated with inferior overall survival ($p<0.001$). In addition, multivariate analysis highlighted that LINC00958 expression was an independent prognostic factor in GC.

CONCLUSIONS: Our observation revealed that elevated LINC00958 was significantly associated with metastasis and was an independent prognostic factor in GC, indicating that LINC00958 can serve as a novel prognostic predictor in GC.

Key Words:
Long non-coding LINC00958, Gastric cancer, Metastasis, Prognosis.

Introduction

Gastric cancer (GC) is the fourth most common cancer and the third most frequent cause of cancer-related death worldwide[1]. In China, the incidence remains high, resulting in an estimated 221,478 deaths[2], almost half of the world's GC deaths, despite a worldwide decline in the incidence of GC[3]. Due to the lack of typical symptoms at the early stage, most GC patients are diagnosed at an advanced stage or metastasis may occur at the time of diagnosis. Although great advances have been made in diagnosis and therapeutic strategies in the past decades, the prognosis is still disma[l4]. Hence, there is a pressing need to look for and identify key potential biomarkers related to metastasis and prognosis of GC to improve its prognosis. Long non-coding RNAs (lncRNAs) are a class of RNA polymerase II transcripts which are greater than 200 nucleotides in size. Increasing evidence showed that lncRNAs are involved in the pathogenesis and are pointed out to be an important regulator of almost every cellular process; the expression of lncRNAs seems to be strictly regulated in physiological condition as well as in several human disorders, including cancer, as reviewed[5]. In recent years, a potential role for this biomarker-serving lncRNAs has been proposed[6,7] and has come

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to be appreciated by more and more studies[6,8-10] investigating the diagnostic and prognostic value of lncRNAs in GC. LINC00958, originally reported by Seitz et al[11] in bladder cancer, was revealed to be metastasis-associated and plays an oncogenic role. Following-up studies of LINC00958 was subsequently extended to glioma[12] and endometrial cancer[13], suggesting that LINC00958 closely correlated with metastases of cancers. It has been, however, little described in GC in the case of clinicopathological significance and its expression. To understand the expression, as well as its clinicopathological correlation, we undertook this work detecting and analyzing the clinicopathological meaning of LINC00958 expression in GC.

Patients and Methods

GC Patients and Tissue Specimens

A total of 200 cases of GC tissues and its paired normal controls were collected from the Department of Surgery, Zhangqiu District Maternal and Child Health Care Hospital, from January 2008 to January 2018. No patient was given any radiotherapy or chemotherapy before undergoing a gastrectomy. All cases were pathologically diagnosed by two experienced gastroenterological pathologists blindly and staged according to the TNM staging of the American Joint Committee on Cancer (AJCC 7th). All tissues were immediately frozen in liquid nitrogen following resection and stored at -80°C until use. The study was approved by the Committee for Ethical Review of Research involving Human Subjects of Zhangqiu District Maternal and Child Health Care Hospital, and informed consent was obtained from each participant involved.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from fresh frozen tissues using TRIzol (Invitrogen, Carlsbad, CA, USA) following the instruction. Total RNA (0.5 μg) were reversely transcribed into cDNA with Maxima^{TM} H Minus cDNA Synthesis Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). The quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed on a 7500 fast real-time PCR platform (Applied Biosystems, Foster City, CA, USA) with Power SYBR-Green Master Mix (Applied Biosystems, Foster City, CA, USA) to detect the expression of LINC00958. Data were normalized to the expression of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as an internal loading control. The primers of LINC00958 (NR_038904.1) were Forward 5'-GGATGGGCTCCAGCCTGGCAC-3'; Reverse 5'-GAGAAGGCAAAAAAGCCTTTT-3'; GAPDH (NM_002046.6) Forward 5'-CTCCTCCTGTTCGACAGTCAGC-3'; Reverse 5'-CCCAATACGACCAAATCCGTT-3'. The PCR program was denatured at 95°C for 5 sec; annealing was performed at 55°C for 30 sec and elongation at 65°C for 20 sec for 40 cycles. Each sample was performed in triplicates and fold changes were calculated using the $2^{-\Delta\Delta CT}$ method. The tissue cohort was stratified into two subcohorts in light of the expression of LINC00958 (a fold-change ≥ 0.5 stands for high expression of LINC00958, whereas a fold-change < 0.5 represents low expression), high expression group (n=146) and low expression group (n=54).

Statistical Analysis

All statistical analyses were analyzed with SPSS 18.0 (SPSS, Chicago, IL, USA) or GraphPad Prism 7.0 version (GraphPad Prism, La Jolla, CA, USA). The data of qRT-PCR are expressed as mean ± standard deviation (SD). The paired Student's *t*-test was employed to compare the means of the two groups. The correlation between expression of LINC00958 and clinicopathological variables was calculated using Chi-square test or Fisher's exact test when the number was less than 5 in Crosstable analysis. The Kaplan-Meier survival curve was plotted and the Log-Rank test was used to draw the survival curve and compare the differences between the survival curves, respectively. *p*-values less than 0.05 were taken as statistically significant.

Results

LINC00958 Was Elevated in RCC Tissues

To investigate the expression of LINC00958 on mRNA level, qRT-PCR was performed in GC tissues and its matched normal controls, totaling 200. It was exhibited that LINC00958 was dramatically up-regulated in GC relative to its paired normal controls (Figure 1), which is suggestive of its oncogenic characteristic of LINC00958 in GC.

Elevated LINC00958 Markedly Correlated With Metastasis in GC

Next, to understand the clinicopathological meaning of LINC00958 expression in GC, detailed statistical analysis was conducted with the clinicopathological variables available, including gender,

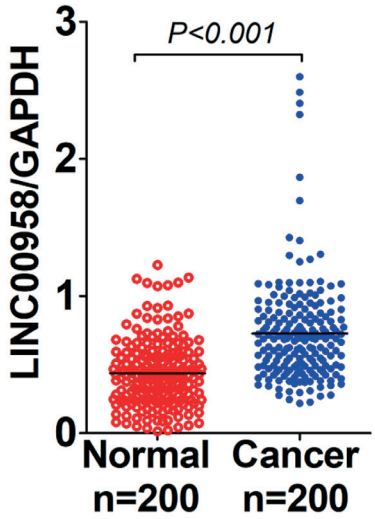

Figure 1. Detection of LINC00958 expression using qRT-PCR in GC and matched normal control tissues, total of 200 cases each. Normal, matched normal control tissues. Cancer, gastric cancer. Paired Student's *t*-test was utilized to analyze the significant difference. $p<0.001$ in comparison with the control group.

age, tumor size, T classification, N classification, M classification, Clinical stage and peritoneal dissemination. Statistical analysis revealed that elevated LINC00958 pronouncedly correlated with lymph nodes metastases ($p=0.004$), distant metastases ($p=0.001$), peritumoral tissue infiltration ($p=0.003$), and peritoneal dissemination ($p=0.013$). No significant correlation was observed between elevated LINC00958 *versus* gender, age, tumor size, differentiation, T classification, and clinical stage (Table I), suggesting that LINC00958 was associated with metastasis in GC.

Elevated LINC00958 Was an Independent Prognostic Factor in GC

Having seen that elevated LINC00958 correlated with metastasis, we wondered whether there would be a linkage between LINC00958 expression and overall prognoses in GC. The Kaplan-Meier survival curve was plotted on the basis of the survival data. The analysis of the Kaplan-

Table I. Clinicopathological significance of LINC00958 expression in GC.

Clinical variables	No	LINC00958 Expression		χ^2	*p*-value
		High (No., %)	Low (No., %)		
Gender					
Female	83	57 (68.7%)	26 (31.3%)	1.347	0.261
Male	117	89 (76.1%)	28 (23.9%)		
Age (years)					
< 60	76	52 (68.4%)	24 (31.6%)	1.304	0.257
≥ 60	124	94 (75.8%)	30 (24.2%)		
Tumor size					
< 5 cm	64	49 (76.6%)	15 (23.4%)	0.606	0.497
≥ 5 cm	136	97 (71.3%)	39 (28.7%)		
Differentiation					
Well	36	27 (75%)	9 (25%)	0.991	0.609
Moderate	39	26 (72.2%)	13 (27.8%)		
Poor	125	93 (74.4%)	32 (25.6%)		
T classification					
T1-2	67	46 (68.7%)	21 (31.3%)	0.964	0.399
T3-4	133	100 (75.2%)	33 (24.8%)		
N classification					
N0	51	29 (56.9%)	22 (43.1%)	9.045	**0.004**
N1-3	149	117 (78.5%)	32 (21.5%)		
Clinical stage					
I-II	61	43 (70.5%)	18 (29.5%)	0.280	0.607
III-IV	139	103 (74.1%)	36 (25.9%)		
M classification					
No	113	72 (63.7%)	41 (36.3%)	11.358	**0.001**
Yes	87	74 (85.1%)	13 (14.9%)		
Peritumoral tissue infiltration					
No	131	87 (66.4%)	24 (33.6%)	9.679	**0.003**
Yes	69	39 (56.5%)	30 (43.5%)		
Peritoneal dissemination					
No	145	113 (77.9%)	32 (22.1%)	6.505	**0.013**
Yes	55	33 (60.0%)	22 (40.0%)		

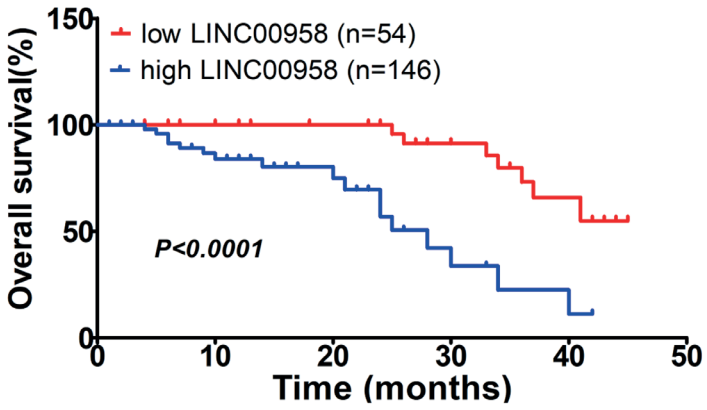

Figure 2. Kaplan-Meier survival analysis of LINC00958 expression. Of 200 cases of GC, the patients with high LINC00958 expression were 146 cases, while the patients with low LINC00958 were 54. Log-Rank test was taken to analyze the significant difference.

Meier survival curve displayed that there was an exceedingly significant difference of overall survival between high expression of LINC00958 and low expression of LINC00958 (p<0.001, Figure 2), indicating that LINC00958 expression was linked with overall prognosis in GC. Considering that LINC00958 markedly correlated with lymph nodes metastases, distant metastases, peritumoral tissue infiltration and peritoneal dissemination (Table I), we next carried out the multivariate COX repression analysis which is more stringent than univariate analysis. The multivariate analysis highlighted that, among all the significant parameters analyzed using univariate analysis, LINC00958 expression was still significant in addition to distant metastases and peritoneal dissemination (Table II), demonstrating that LINC00958 expression was an independent prognostic factor in GC.

Discussion

In the present work, LINC00958 was displayed to be dramatically elevated in GC tissues compared with normal controls and elevated LINC00958 was markedly associated with poor overall survival. Elevated LINC00958 also remarkably correlated with lymph nodes metastases, distant metastasis, peritoneal dissemination

Table II. Univariate and multivariate Cox regression analysis of LINC00958 for overall survival in GC.

Clinicopathological variables	Univariate analysis		*p*-value	Multivariate analysis		*p*-value
	HR	95% CI		HR	95% CI	
Overall survival						
Gender (Male *vs.* Female)	0.612	0.457-1.184	0.262			
Age (≥60y *vs.* <60y)	0.641	0.514-1.384	0.318			
(Down vs. Upper/Middle)						
Tumor size (≥5 cm *vs.* <5 cm)	1.413	0.881-2.046	0.203			
Differentiation (well/moderate *vs.* poor)	1.409	0.760-2.787	0.176			
T classification (T1-T2 *vs.* T3-T4)	1.126	1.512-4.322	0.278			
N classification (N0 *vs.* N1-N3)	2.015	1.254-3.616	**0.005**			
Clinical stage (I-II *vs.* III-IV)	0.641	0.514-1.384	0.318			
M classification (Yes *vs.* No)	3.052	1.706-4.459	**0.001**	1.855	1.024-3.360	**0.042**
Peritoneal dissemination (Yes *vs.* No)	2.381	1.532-3.379	**0.001**	1.792	1.025-2.973	**0.031**
Peritumoral infiltration (Yes *vs.* No)	2.218	1.010-5.835	**0.045**			
LINC00958 expression (High *vs.* Low)	0.345	0.217-0.547	**0.001**	0.46	0.287-0.736	**0.001**

HR, Hazard ratio; CI, confidence interval.

and peritumoral tissue infiltration after detailed statistical analysis. Subsequent multivariate COX regression analysis underscored that up-regulated LINC00958 was an independent prognostic factor in GC. LINC00958 was first identified as a meaningful biomarker that can predict metastasis and overall prognoses in GC. In our previous work, to search for the potential lncRNAs that were associated with metastasis of GC, lncRNA microarray was performed using 10 paired cases of GC with metastasis and its matched normal controls. As a result, some significant differential lncRNAs were screened out and emerged (data not shown). Among these differential lncRNAs that were linked to metastasis of GC, LINC00958 was eventually picked out as lncRNA of interest in consideration that it has been little described ever before in GC. Considering its scant evidence, we therefore focused on LINC00958 trying to explore the clinicopathological significance of its expression in GC. The first report regarding LINC00958 came from bladder cancer[11,14], followed by extension to endometrial cancer[13] and glioma[12]. In bladder cancer by Seitz et al[11], *in vitro* functional analysis using cell lines showed that silencing of LINC00958 can drastically suppress the invasion and migration of bladder cancer cells, suggesting its oncogenic trait especially in terms of promotion of motility. Despite this, the study was short of any data concerning the expression of LINC00958 on tissue level, let alone its clinicopathological meaning. In another recent study on bladder cancer carried out by He et al[14], the authors not only mechanistically demonstrated the metastasis-associated characteristic using animal model and *in vitro* cell lines, but they also provided evidence on tissue level exhibiting that LINC00958 was markedly up-regulated in lymph node metastatic bladder cancer and correlated with lymph node metastasis, strongly indicating metastasis-associated trait of LINC00958 in bladder cancer. Consistently, the observation that elevated LINC00958 in cancer tissues compared with normal control can also be made in endometrial cancer[13] and glioma[12]. Nevertheless, the expression of LINC00958 remains poorly known in GC. Suggested by these earlier investigations as discussed above, we postulated that LINC00958 would be elevated in GC, likely independent of the tissue-specific expression. Our results showed that LINC00958 was elevated in GC and elevated LINC00958 was strikingly linked with metastasis and overall prognosis in GC, which was fully in concordance with what has been earlier reported in glioma[12] but seems to be partially in line with what described in endometrial cancer[13] where the authors failed to analyze the prognostic value of LINC00958. Using multivariate COX regression analysis, we further revealed that LINC00958 was an independent prognostic factor in GC in addition to distant metastasis and peritoneal dissemination in our setting. Although LINC00958 was first described in our setting in GC tissues, there were several limitations that should be acknowledged. First, the sample size of our study may need to be warranted further using another independent cohort with larger sample size. Second, the relevant working mechanistic of LINC00958 is lacking, performance of which will go beyond our original intention. Additionally, it should be noted that the correlation we described here could be varying with different cut-off values adopted in stratifying the high and low expression of LINC00958 using the qRT-PCR technique. Another different method that will complement to qRT-PCR, therefore, may be required.

Conclusions

LINC00958 was first showed to be strikingly up-regulated in GC and elevated LINC00958 pronouncedly correlated with metastasis and unfavorable overall survival, which can serve as an independent prognostic factor in GC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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