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Original Article

Identification of long noncoding RNA TC0101441 as a novel biomarker for diagnosis and prognosis of gastric cancer

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Abstract: The present work aimed to explore the prognostic values of lncRNA TC0101441 (TC0101441) in patients with gastric cancer (GC). The expression of TC0101441 in a total of 159 GC specimens and matched normal specimens was detected by quantitative RT-PCR. ROC assays were conducted to determine the diagnostic value of TC0101441 expression in GC patients. The association of TC0101441 expression with clinical characteristics of 159 patients was analyzed using chi-square test. Kaplan-Meier methods were employed to determine the prognostic value of TC0101441 expression in the survival rate of GC patients. Multivariate Cox regression assays were used to identify whether TC0101441 could be a prognostic biomarker for GC patients. We found that TC0101441 expression was significantly increased in GC specimens compared to that in the normal specimens ($P < 0.01$). High TC0101441 expression was correlated with lymphatic metastasis ($P = 0.027$) and TNM stage ($P = 0.015$). TC0101441 could distinguish GC specimens from adjacent normal gastric specimens with an area under the receiver operating characteristic curve (AUC) of 0.8082. Survival data revealed that patients with high TC0101441 expression had worse overall survival ($P = 0.0009$) and disease-free survival ($P < 0.0001$) rates than those with low TC0101441 expression. Multivariate assays showed that TC0101441 expression was an independent biomarker for GC patients. The present study suggested that TC0101441 expression was increased in GC and may be aprognostic and diagnostic biomarker for GC.

Keywords: lncRNA TC0101441, prognosis, diagnosis, gastric cancer

Introduction

Gastric cancer (GC), a gastrointestinal neoplasm originating from the epithelium, is one of the most frequent causes of death from cancer in both sexes around the world, and it isstrongly linked to a prolonged inflammatory response [1, 2]. More than 960,000 new GC cases and 710,000 deaths were estimatd to occur in 2018 [3]. Despite advances in diagnostic methods, surgery and other comprehensive treatments, the clinical outcome of GC remains unsatisfactory [4, 5]. Many patients are first diagnosed at an advanced stage and exhibit a positive distant metastasis, resulting in frequent treatment failure [6]. Hence, it is urgently needed to improve management by identifying specific and sensitive biomarkers in GC patients.

Long noncoding RNAs (lncRNAs) are a class of RNAs with a length longer than 200 nucleotides (nt) [7]. With the extensive application of high-throughput sequencing, many lncRNAs have been found to be expressed in a specimen-specific manner and in several conditions connected with diseases [8]. It has been confirmed that lncRNAs exhibit critical effects on the modulation of gene splicing, neurologic biochemistry, chromatin organization, and gene transcription [9, 10]. In recent years, studies have frequently reported that lncRNAs can be involved in many pathophysiologic processes of various tumors, including tumor growth, metastasis, pyroptosis, and apoptosis [11, 12]. In addition, several lncRNAs have been confirmed to possess clinical potential, as novel prognostic and diagnostic biomarkers for various tumors, such as

Table 1. Sequences of primers for RT-PCR used in this study

Name	Sequence (5'→3')
TC0101441: Forward	AAGGCAGGTGAGAACGAGT
TC0101441: Reverse	CTCGACTTAGGGAGCTGCAC
GAPDH: Forward	GGAGCGAGATCCCTCCAAAAT
GAPDH: Reverse	GGCT GTTGTCATACTTCTCATGG

lncRNA CASC9, lncRNA MEG3, and lncRNA BCYRN1 [13-15].

LncRNA TC0101441 (TC0101441), also known as estrogen receptor responsive lncRNA 1, is a newly-identified metastasis-related lncRNA in epithelial ovarian cancer [16, 17]. In addition, TC0101441 was functionally reported to promote endometriosis migration/invasion [18]. However, its expression and function in other tumors remain unclear. In this study, we aim to explore its expression pattern and clinical significance in GC.

Materials and methods

Patients and tissue samples

159 paired GC tissues and para-carcinoma specimens were collected from the Heping Hospital affiliated with Changzhi Medical College during March 2012 to May 2016. The inclusion criteria for the patient cohorts included: (1) having definite pathologic diagnosis of GC; (2) surgical resection, defined as the complete resection of all tumor nodules with the cut margin being free of cancer by histologic examination; and (3) having complete clinical and survival data. An exclusion criterion was those patients who received anticancer treatment before surgical operation. The diagnosis of all 159 cases was histopathologically certified by two experienced pathologists and staged based on the TNM staging of the 8th edition of AJCC guidelines. A tumor that does not resemble the original tissue is termed poorly differentiated. A tumor that resembles the original tissue is termed well-differentiated. All GC patients received neither chemotherapy nor radiotherapy prior to surgery. Prior to RT-PCR assays, all collected samples were frozen in liquid nitrogen and stored at -80°C. Regular follow-up was carried out by field visits or telephone interviews every three months until September 2019 or the point of death. All patients signed a written informed and the present study protocol received the approval from the Ethics Committee of our hospital.

Quantitative RT-PCR (qRT-PCR)

Total RNAs were extracted from tumor and normal specimens by Trizol (Invitrogen, Hangzhou, Zhejiang, China) according to protocols, and were reverse-transcribed into cDNA using Takara-PrimeScriptTM# RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, Niaoning, China). RT-PCR assays were performed by SYBR green qPCR SuperMix (Bofang Technology, Pudong, Shanghai, China) in the ABI prism 7800 detection system (Biosystems, Haidian, Beijing, China). The expression of GAPDH served as an endogenous control. The relative expressions of RNAs were calculated by the use of the comparative C_T methods. All reactions were carried out at least three times. The primer sequences used are listed in **Table 1**.

Statistical analysis

All statistical analyses were performed using SPSS 20.0 statistical software (SPSS, Inc., Chicago, IL, USA). The correlation between TC0101441 expressions and clinicopathologic data in GC specimens was examined by the chi-square test. Receiver operating characteristic (ROC) curves were applied to explore the diagnostic values of TC0101441 expression. Survival curves were calculated by Kaplan-Meier method and compared with log-rank tests. Overall survival (OS) was defined as the time from randomization to death from any cause. Disease-free survival (DFS) was defined as the time from randomization to recurrence of tumor or death.

Multivariate analyses were used to estimate prognostic factors for survival. A two-sided *p* value of < 0.05 was considered significant.

Results

Increased expression of TC0101441 in GC patients

To explore whether TC0101441 was a dysregulated lncRNA in GC, we performed RT-PCR to examine its expression. As presented in **Figure 1**, TC0101441 was significantly upregulated in GC specimens compared to matched normal samples ($P < 0.01$).

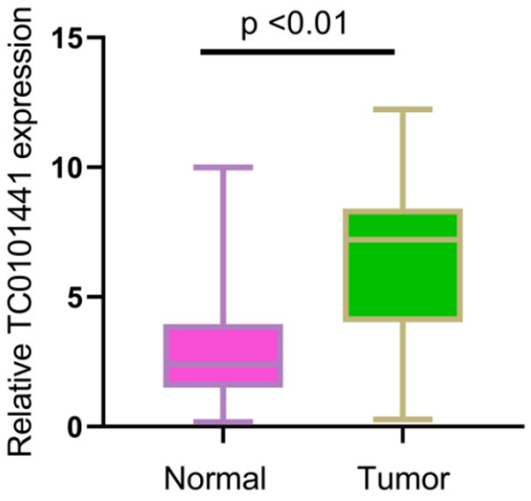

Figure 1. Real-time PCR results revealed that TC0101441 was distinctly upregulated in GC tissues.

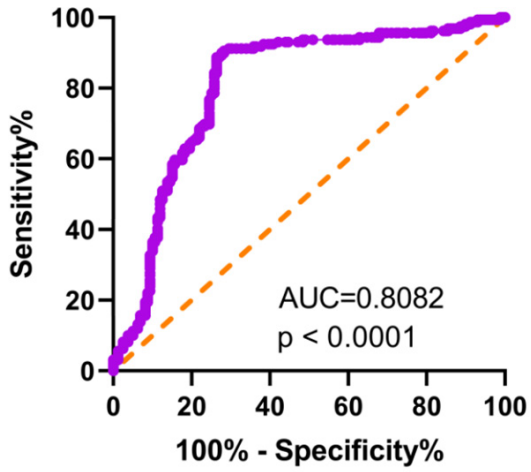

Figure 2. ROC curve of TC0101441 expression to distinguish GC specimens from normal gastric tissues.

Upregulation of TC0101441 as a diagnostic marker for GC

To study whether TC0101441 has diagnostic significance in GC patients, we performed ROC curves analysis and further calculated the AUC values. As presented in **Figure 2**, overexpression of TC0101441 was robust in distinguishing GC specimens from normal gastric tissues, with an AUC value of 0.8082 (95% CI, 0.7574 to 0.8590). Using a Youden's index of 8.45 as the cutoff value for relative TC0101441 levels, the sensitivity and specificity of TC0101441 as a biomarker for the diagnosis of glioma were 81.7% and 92.3%, respectively (**Figure 2**).

Table 2. Correlation of clinicopathologic features of GC with TC0101441 expression level

Characteristic	All cases	TC0101441 expression		*p* value
		High	Low	
Age				0.304
≥ 60	76	35	41	
< 60	83	45	38	
Gender				0.476
Male	95	50	45	
Female	64	30	34	
Differentiation				0.227
Well-moderate	89	41	48	
Poor	70	39	31	
Lauren type				0.383
Intestinal	79	37	42	
Diffuse and mixed	80	43	37	
Tumor size				0.160
≥ 5 cm	61	35	26	
< 5 cm	98	45	53	
TNM stage				0.015
I/II	102	44	58	
III/IV	57	36	21	
Lymphatic metastasis				0.027
Negative	112	50	62	
Positive	47	30	17	

Association of TC0101441 expression with clinical features of GC patients

For the exploration of the clinical relevance of TC0101441 expression in GC patients, we divided all 159 cases into a high expression group (n = 80) and a low expression group (n = 79) according to the median expression level of TC0101441 (7.35) in GC specimens. Based on the results from the chi-square test, our group observed that high TC0101441 expression was correlated with lymphatic metastasis (P = 0.027) and TNM stage (P = 0.015) (**Table 2**). However, there were no significant associations between TC0101441 expression and other clinical features.

TC0101441 up-regulation is associated with shorter survival time

To explore the clinical potential of TC0101441 levels in GC, we performed five-year follow-up. Eight cases were lost to follow up and the median follow up time was 32 months. By Kaplan-

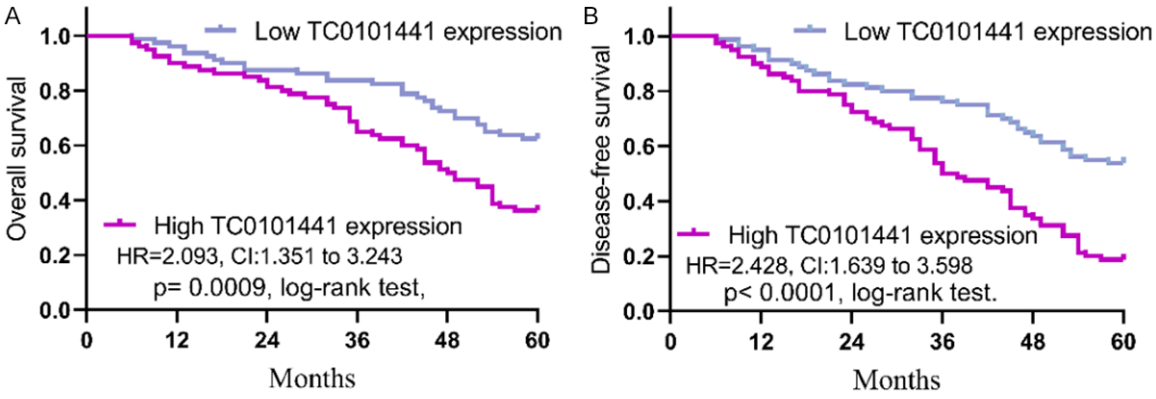

Figure 3. Kaplan-Meier survival curves for GC patients according to the expression of TC0101441. Overall survival rate (A) and disease-free survival rate (B) in patients with high TC0101441 expression were distinctly higher than in those with low TC0101441 expression.

Table 3. Multivariate analysis of overall survival and disease-free survival in GC patients

Variable	Overall survival			Disease-free survival		
	Risk ratio	95% CI	P	Risk ratio	95% CI	P
Age	0.872	0.422-1.452	0.325	0.933	0.562-1.773	0.231
Gender	1.125	0.562-1.778	0.267	1.322	0.672-1.994	0.189
Differentiation	1.354	0.732-2.158	0.158	1.291	0.882-2.019	0.211
Lauren type	0.986	0.572-1.662	0.238	1.217	0.687-1.884	0.169
Tumor size	1.375	0.721-2.328	0.185	1.441	0.983-2.321	0.113
TNM stage	2.895	1.321-4.882	0.012	3.017	1.472-5.218	0.005
Lymphatic metastasis	3.083	1.285-4.527	0.009	3.275	1.385-5.762	0.001
TC0101441 expression	2.798	1.389-4.563	0.015	2.986	1.427-4.896	0.008

Meier method, patients with high TC0101441 expression had significantly worse overall survival (OS) (38.0% vs. 63.8%; P = 0.0009, **Figure 3A**) and disease-free survival (DFS) (22.4% vs 56.8%; *P* < 0.0001, **Figure 3B**) rates than those with low TC0101441 expression. Moreover, multivariate assays revealed that TC0101441 expression was an independent prognostic marker for both OS (RR = 2.798, *P* = 0.015) and DFS (RR = 2.986, *P* = 0.008) of GC patients (**Table 3**).

Discussion

To date, barium meal and gastroscopy have been extensively used for early diagnosis of gasttic carcinoma (GC) patients [19]. In regard of serum biomarkers, the detection of serum CEA, CA19-9, and CA72-4 is used as the most frequent method for GC [20]. However, the insufficiency of specificity limits the clinical application of the above biomarkers used as a routine screening tool for GC. In recent years, with the development of targeted therapy, the accurate prognosis of GC patients has been needed for the guidance of therapeutic scheduling [21]. However, sensitive biomarkers are limited in clinical practice. In recent years, lncRNA-based assays were used as a novel diagnostic tool for GC and attracted growing attention because most protein-based assays have not enough accuracy [22].

LncRNAs were initially considered to be "transcriptional noise", but studies indicated that they may be involved in tumor progression [10]. For instance, lncRNA SNHG3 is highly expressed in GC and predicts a short survival time. Functionally, overexpression of lncRNA SNHG3 was confirmed to promote the proliferation and invasion of GC cells by modulating neighboring MED18 gene methylation [23]. LncRNA CASC2, an overexpressed GC-related lncRNA induced by E2F6, was shown to suppress the metastasis of GC cells [24]. LncRNA NEAT1 was found to be overexpressed in GC, and its knockdown

could suppress the growth of GC cells by modulation of the miRNA-497-5p/PIK3R1 axis [25]. Recently, Qiu et al reported that TC0101441 expression was distinctly upregulated in epithelial ovarian cancer and associated with poor prognosis [16]. In their functional study, the forced expression of TC0101441 was found to promote the metastasis of epithelial ovarian cancer cells through decreasing KiSS1. However, the possible function of TC0101441 in other tumors had not been investigated.

In this study, we first provided robust evidence that TC0101441 expression was increased in GC tissues compared to normal gastric tissues. Then, we analyzed the diagnostic value of TC0101441 in GC patients, finding that high TC0101441 expression could effectively identify GC tissues from normal specimens based on ROC analysis. Moreover, clinical assays revealed that higher TC0101441 expression was associated with advanced clinical stage and lymphatic metastasis. Because the above two factors were considered to be independent prognostic biomarkers for GC patients, we wondered whether TC0101441 could affect the five-year survival of GC patients. Kaplan-Meier assays demonstrated that patients with higher TC0101441 expression had shorter OS and DFS time than those with low TC0101441 expression. More importantly, multivariate Cox analysis proved that TC0101441 was a prognostic indicator for GC patients. Recently, lncRNAs have been shown to serve as oncogenes or tumor suppressors in tumor progression by acting as competing endogenous (ce) RNA to regulate tumor-related genes through sponging miRNAs [26, 27]. Thus, we suggest overexpression of TC0101441 may promote clinical progression of GC patients by acting as ceRNA.

There are some limitations in our study. First, the sample size is relatively small, and large clinical trials are needed. Secondly, we just explored the clinical significance of TC0101441 expression in GC patients. Further exploration of the biologic function of TC0101441 in GC cells using in vitro and in vivo is important to confirm whether it serves as a tumor promotor.

Our study provided the first evidence that TC0101441 overexpression may be an independent unfavorable prognostic and diagnostic factor for GC patients.

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Disclosure of conflict of interest

None.

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