Overexpression of lncRNA TCLlnc1 in gastric cancer predicts postoperative distant recurrence and poor survival

Kan Hu, Yugui Zhang, Jun Rong, Wensheng Deng and Benping Xiao

TCLlnc1 was characterized as a lncRNA with oncogenic roles in T cell lymphoma, whereas its role in other diseases is unknown. We then explored the involvement of TCLlnc1 in gastric cancer. Paired gastric cancer and nontumor tissues from 66 gastric cancer patients were used to extract total RNA samples, which were used to perform RT-qPCRs to determine the expression of TCLlnc1. Plasma samples from these 66 gastric cancer patients and 66 healthy controls were also used to detect circulating TCLlnc1. Correlations of TCLlnc1 in both plasma and tissue samples with patients' clinical data were analyzed by chi-square *t*-test. The diagnostic value of TCLlnc1 for early-stage gastric cancer was analyzed with the receiver operating characteristic curve. A 5-year follow-up study was performed to explore the prognostic value of TCLlnc1 for the survival of gastric cancer patients. TCLlnc1 expression in tissue was increased in gastric cancer. Plasma TCLlnc1 was also increased in gastric cancer. Plasma TCLlnc1 was closely correlated with TCLlnc1 in gastric cancer tissues, but not TCLlnc1 in nontumor tissues. TCLlnc1 in plasma was only correlated with tumor distant metastasis, but not other clinical data. TCLlnc1 in plasma showed promising diagnostic value for stage I and II gastric cancer. Increased accumulation of TCLlnc1 was closely correlated with distant recurrence and poor survival during a 5-year follow-up. Therefore, TCLlnc1 is overexpressed in gastric cancer predicts postoperative distant recurrence and poor survival. *Anti-Cancer Drugs* 33: 999–1003 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Gastric cancer, a frequently diagnosed malignancy in clinical practice, has been a leading cause of death worldwide [1]. Each year, more than 1 million gastric cancer patients are diagnosed and about 80 0000 of these patients are estimated to die of gastric cancer in near future [2]. Besides salty and smoked foods and poor dietary structure, gastric cancer is closely related to the infections of Helicobacter pylori [3], which is common in China [4]. As a consequence, China accounts for about 50% of gastric cancer cases and gastric cancer -related deaths [5]. The survival of gastric cancer is mainly related to clinical stages [6,7]. No less than 70% of localized gastric cancer patients can survive 5 years after total or subtotal gastrectomy followed by chemotherapy [6,7]. However, most gastric cancer patients at an early stage are asymptomatic [8]. Therefore, early diagnosis is critical.

Previous studies have developed different types of biomarkers for the early detection of gastric cancer [9–11]. These biomarkers may include c carbohydrate antigens, arcinoembryonic antigen, α-fetoprotein, pepsinogen and methylated DNA markers [9–11]. However, these biomarkers are usually limited by the low specificity of tissue expression [12]. This is to say currently available biomarkers are not sufficient to diagnose cancer, and other tests, such as radiology tests and biopsy are still needed [12]. In both pathological and physiological processes, lncRNAs affect protein synthesis rather than directly code proteins to play critical roles [13,14]. In effect, recent studies have reported the potential application of lncRNAs as biomarkers for cancers [15,16]. However, the clinical values of most lncRNAs for gastric cancer remain unknown. TCLlnc1 was characterized as a lncRNA with oncogenic roles in T cell lymphoma, whereas its role in other diseases is unknown. We then explored the involvement of TCLlnc1 in gastric cancer [17].

Methods

Study population

The study population of the present study included a total of 62 gastric cancer patients (22 females and 40 males, 53.4+/7.8 years) and 62 healthy controls (22 females and 40 males, 53.8+/7.6 years), who were admitted to Jiangxi PingXiang People's Hospital from March 2014 to May 2016 (ethics committee of this hospital approved this study). All gastric cancer patients were diagnosed with imaging techniques and endoscopy. Other malignancies,

chronic infections and metabolic diseases were excluded from these patients. Healthy controls passed systemic physiological examinations at the aforementioned hospital. No history of malignancies was observed in healthy controls. Patient and control groups showed a similar distribution of age and sex. All participants signed informed consent. The patient's clinical data were presented in Table 1.

Collection of clinical samples

Fasting blood in EDTA tubes was used for the isolation of plasma samples by centrifuging the samples for 10 min at 1200 g. All gastric cancer patients were subjected to the resection of the primary tumors, which were dissected by histopathological experts to separate nontumor tissues from the gastric cancer tumor tissues. A tank filled with liquid nitrogen was used to store all tissue and plasma samples before use.

Follow-up

Through out-patient visits and telephone, all patients were followed up for 5 years to monitor their survival and tumor recurrence. Patients were checked every month. This study excluded deaths unrelated to gastric cancer ($n=2$). All patients completed the follow-up.

RNA isolation and purification

TRIzol (Invitrogen, Carlsbad, CA, USA) was used to prepare total RNA samples. Chloroform purification was performed to reduce protein and DNA contamination. RNA samples were precipitated using isopropanol and washed with 70% ethanol to remove the salt. RNA samples were dissolved in nuclear-free water, followed by the addition of DNase I (Invitrogen) and incubation at 37 °C for 1 h to totally eliminate genomic DNA contamination. RNA concentration was determined using Bioanalyzer, and RNA concentration was adjusted to about 2000 ng/μl. RNA integrity was also determined using Bioanalyzer. All RNA samples were with a RIN value higher than 9.0.

Table 1 Chi-square analysis of the associations between plasma TCLlnc1 and patients' clinical data

		TCLlnc1			
Clinical parameter	Cases ($n=62$)	High expression ($n=31$)	Low expression ($n=31$)	χ^2-test	*P* value
Age					
<50 years	27	12	15	0.59	0.44
≥50 years	35	19	16		
Sex					
Male	40	22	18	1.13	0.29
Female	22	9	13		
Tumor size					
<5 cm	29	13	16	0.58	0.45
≥5 cm	33	18	15		
Location					
Cardia + body	39	20	19	0.07	0.79
Pylorus	23	11	12		
Histologic differentiation					
Well + moderate	34	14	20	2.34	0.13
Poor + undifferentiated	28	17	11		
TNM stage					
I + II	28	11	17	2.34	0.13
III + IV	34	20	14		
Lymphatic metastasis					
No	20	7	13	2.66	0.10
Yes	42	24	18		
Distant metastasis					
No	50	21	29	6.61	0.01
Yes	12	10	2		

Real time quantitative polymerase chain reaction

PrimeScript RT reagent Kit (Takara Bio, China) was used to prepare cDNA samples with about 2000 ng total RNA as a template. After that, template RNA was removed through RNase H digestion. To determine the expression of TCLlnc1, qPCRs were performed on ABI 7500HT real-time PCR system (Applied Biosystems, Forster City, CA, USA) with 18S rRNA as an internal control. All qPCR mixtures were prepared using SYBR Premix Ex Taq (Takara Bio). The method of $2^{-\Delta\Delta C}$q was used to normalize Ct values.

Statistical analysis

The preparation of images and the analysis of data were all performed using Graphpad Prism 6 software. Differences between groups were explored by performing Student's *t*-test. The role of plasma TCLlnc1 in predicting stage I/II ($n=28$) or III/IV ($n=34$) gastric cancer patients was explored with a receiver operating characteristic curve, in which true positive and negative cases were gastric cancer patients and controls, respectively. Patients were divided into high and low plasma TCLlnc1 level groups ($n=31$) groups. A chi-square analysis of the correlations between patients' clinical data and TCLlnc1 in plasma was conducted. Survival and distant metastasis-free curve were plotted. $P<0.05$ was statistically significant.

Results

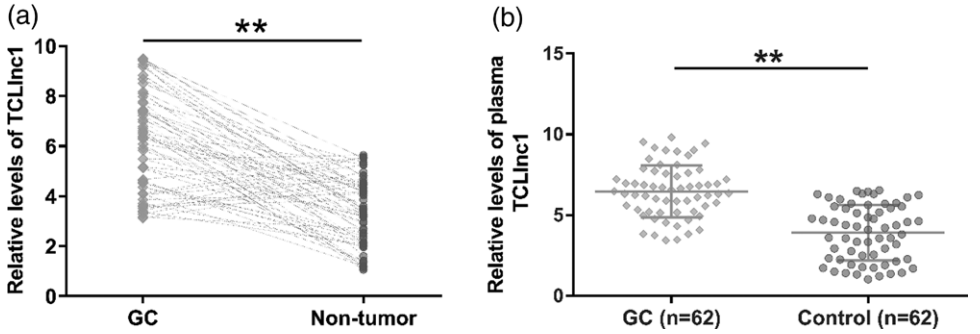
Expression pattern of TCLlnc1in gastric cancer and controls

RNA was isolated from tissue samples donated by 62 gastric cancer patients, followed by RT-qPCRs to determine the expression of TCLlnc1. TCLlnc1 accumulation was increased in gastric cancer tissues (Fig. 1a, $P<0.01$). Plasma samples donated by the 62 gastric cancer patients and 62 healthy controls were also subjected to RNA isolation and RT-qPCRs. Our data revealed that plasma TCLlnc1 was also increased in gastric cancer patients (Fig. 1b, $P<0.01$). Our data suggested the potential involvement of TCLlnc1in gastric cancer.

Correlations between TCLlnc1 in plasma and tissue samples

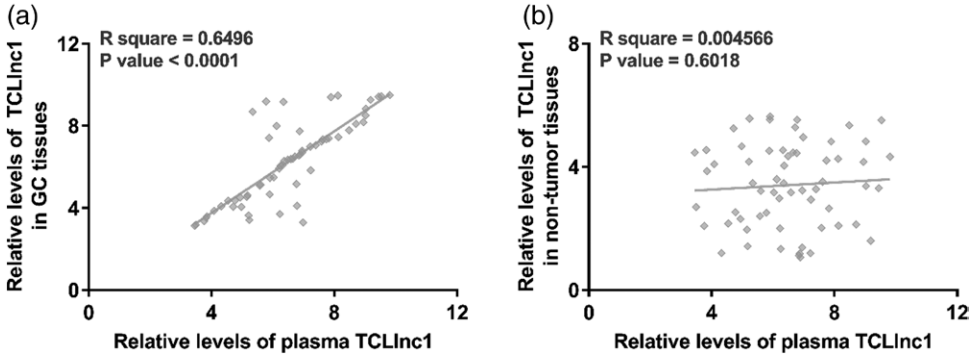
Plasma TCLlnc1 was closely correlated with TCLlnc1 in gastric cancer tissues (Fig. 2a), but not TCLlnc1 in nontumor tissues (Fig. 2b). Therefore, the increased level of TCLlnc1 in plasma of gastric cancer patients is likely a

Fig. 1



Expression pattern of TCLlnc1in gastric cancer (GC) and controls. RNA was isolated from tissue samples donated by 62 GC patients (a) and plasma samples donated by the 62 GC patients and 62 healthy controls (b), followed by RT-qPCRs to determine the expression of TCLlnc1. **,$P<0.01$.

Fig. 2



Correlations between TCLlnc1 in plasma and tissue samples. Pearson's correlation coefficient was applied to explore the correlations of plasma TCLlnc1 with TCLlnc1 in gastric cancer (GC) (a) or non-tumor (b) tissue samples.

consequence of the overexpression of TCLlnc1 in gastric cancer tissues of gastric cancer patients.

Chi-square analysis of the associations between plasma TCLlnc1 and patients' clinical data

Clinical data of the 62 gastric cancer patients are listed in Table 1. Patients were divided into high and low plasma TCLlnc1 level groups ($n=31$) groups. A chi-square test was applied to analyze the correlations between patients' clinical data and TCLlnc1 in plasma. TCLlnc1 in plasma was only correlated with tumor distant metastasis, but not other clinical data (Table 1). Therefore, TCLlnc1 is likely involved in the distant metastasis and recurrence of gastric cancer.

Analysis of the diagnostic value of plasma TCLlnc1 for different stages of gastric cancer

The role of plasma TCLlnc1 in predicting stage I/II ($n=28$) or III/IV ($n=34$) gastric cancer patients was explored with a ROC curve, in which true positive and negative cases were gastric cancer patients and controls, respectively. TCLlnc1 in plasma showed promising diagnostic value for both stage I/II (Fig. 3a) and stage III/IV gastric cancer (Fig. 3b) patients.

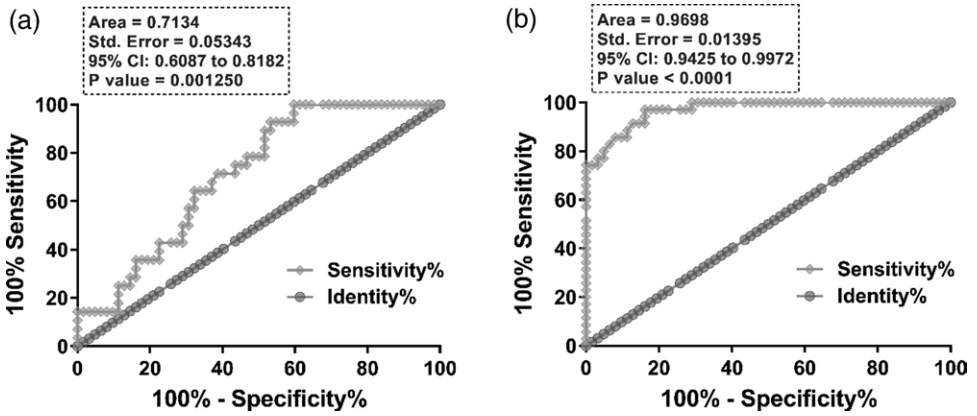
Analysis of the predictive value of plasma TCLlnc1 for survival and distant recurrence

Patients were divided into high and low plasma TCLlnc1 level groups ($n=31$). Distant metastasis-free and overall survival curves were plotted. High expression levels of TCLlnc1 were closely correlated with distant recurrence (Fig. 4a) and poor survival (Fig. 4b) during a 5-year follow-up.

Discussion

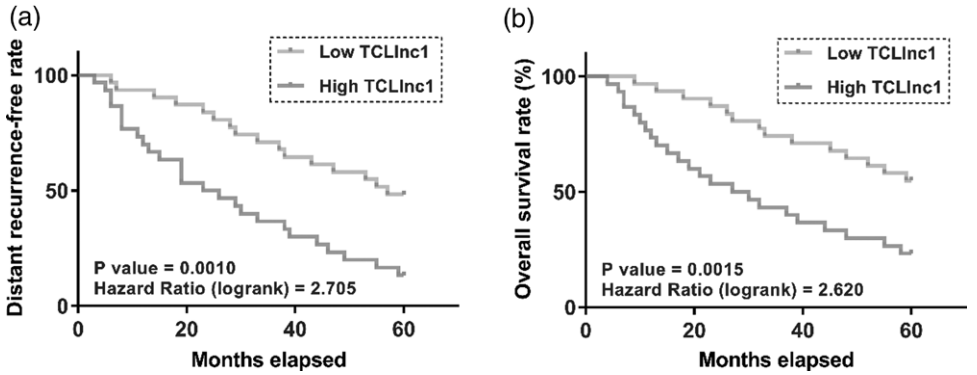
The present study analyzed the expression pattern of TCLlnc1 in gastric cancer and explored its values in the diagnosis and prognosis of gastric cancer. We showed that the increased TCLlnc1 in plasma is correlated with distant metastasis of gastric cancer and may serve as a potential biomarker to diagnose gastric cancer and predict the survival of gastric cancer patients.

Fig. 3



Analysis of the diagnostic value of plasma TCLlnc1 for different stages of gastric cancer (GC). The role of plasma TCLlnc1 in predicting stage I/II (n=28) or III/IV (n=34) GC patients was explored with ROC curve, in which true positive and negative cases were GC patients and controls, respectively.

Fig. 4



Analysis of the predictive value of plasma TCLlnc1 for survival and distant recurrence. Patients were divided into high and low plasma TCLlnc1 level groups (n=31) groups. Distant metastasis-free (a) and overall survival (b) curve were plotted for both groups.

In a recent study, Zhao *et al.* [17] functionally characterized a novel lncRNA named TCLlnc1 in T cell lymphoma. In T cell lymphoma, TCLlnc1 is significantly overexpressed and it plays a role as the modular scaffold of YBX1 and HNRNPD complexes to promote cancer progression [17]. However, the expression pattern of TCLlnc1 in other cancers is unknown. In the present study, we observed increased levels of TCLlnc1 in both gastric cancer tissues and gastric cancer plasma compared to controls. Interestingly, increased plasma levels of TCLlnc1 were only closely correlated with patients' distant tumor metastasis, but not other clinical parameters. Therefore, TCLlnc1 may participate in gastric cancer mainly by promoting tumor metastasis. Future studies are needed to further confirm the role of TCLlnc1 in cell movement and tumor metastasis of gastric cancer.

Compared to biomarkers in cancer tissues, plasma markers are less invasive and can be accepted by most cancer patients [18]. In the present, we showed that plasma TCLlnc1 was closely correlated with TCLlnc1 in gastric cancer tissues, but not TCLlnc1 in nontumor tissues. Therefore, TCLlnc1 in plasma should be mainly from gastric cancer tissues, and measuring the plasma levels of TCLlnc1 main fully reflect TCLlnc1 expression in gastric cancer tissues. We showed that increased plasma TCLlnc1 could effectively separate early-stage gastric cancer patients from healthy controls. Therefore, plasma TCLlnc1 may serve as a potential biomarker for the early detection of gastric cancer. Moreover, TCLlnc1 was found to be closely correlated with distant tumor recurrence and poor survival. Therefore, measuring the plasma levels of TCLlnc1 before treatment may assist the development of personalized treatment approaches to prolong the survival of patients.

In conclusion, TCLlnc1 may participate in gastric cancer distant metastasis. Increased plasma levels of TCLlnc1 may serve as a potential early diagnostic and prognostic biomarker for gastric cancer.

Acknowledgements

This study passed the review board of the Ethics Committee of the Jiangxi PingXiang People's Hospital. Informed consent was obtained from all individual participants included in the study.

K.H. designed the study, performed experiments, wrote the article and revised the article, Y.Z., J.R., W.D. and B.X. collected patient specimens and related information and contributed to data analysis.

Data are available upon reasonable request from corresponding author.

Conflicts of interest

There are no conflicts of interest.

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