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# Overexpression of IncRNA LINC01793 acts as a potential predictor for progression and poor prognosis of gastric cancer

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**Summary.** Objective. Accumulating evidence has shown that differentially expressed long non-coding RNA (lncRNA) is closely related to the development of gastric cancer. This study aims to explore the role of potential lncRNAs in the development of gastric cancer.

Methods. TCGA database of gastric cancer were analyzed by bioinformatics. LINC01793 was overexpressed in gastric cancer. Furthermore, LINC01793 level in 21 pairs of gastric cancer and paracancerous tissues were detected by qRT-PCR (quantitative real-time polymerase chain reaction). Prognostic analysis was performed to investigate the predictive effect of LINC01793 on the prognosis of patients with gastric cancer. GSEA analysis was performed to investigate the possible biological processes of LINC01793 involved in the development of gastric cancer.

Results. LINC01793 was overexpressed in gastric cancer tissues compared to paracancerous tissues. Moreover, LINC01793 overexpression in gastric cancer was closely related to patients with poor prognosis. GSEA analysis found that LINC01793 was closely related to apoptosis, cell cycle, invasion and metastasis of gastric cancer cells.

Conclusions. Our study showed that overexpression of LINC01793 indicates the poor prognosis of gastric cancer patients. Therefore, LINC01793 may serve as a target for the diagnosis and treatment of gastric cancer. Key words: LncRNA, LINC01793, Gastric cancer, Poor prognosis, Biomarker

#### Introduction

Gastric cancer is a common malignancy in the world, the poor prognosis of which seriously threatens human health (Patru et al., 2013). Data from the International Agency for Research on Cancer suggested that there were about 95.1 million new cases of gastric cancer and about 723,000 cases of gastric cancer deaths in 2012 (Ferlay et al., 2015; Torre et al., 2016; Yuan et al., 2016). However, the potential mechanism of development and progression of gastric cancer remains unclear (Lee et al., 2014; Yang et al., 2015). Therefore, further exploration of the intrinsic molecular mechanism of gastric cancer is of great significance, so as to find a new target for diagnosis and treatment of gastric cancer. With the rapid development of bioinformatics and next generation sequencing technologies, it has been found that only about 2% of the human genomes could encode proteins. The majority of genes did not or barely have the RNA-coding function, including miRNA, piRNA, cirRNA and lncRNA (Lee et al., 2014; Yang et al., 2015). Long non-coding RNA (lncRNA) has been well studied in the field of non-coding RNA in recent years (Li et al., 2014; Schmitt and Chang, 2016). Functionally, IncRNA is capable of regulating biological processes at transcriptional or post-transcriptional level (Nie et al., 2012; Jacob et al., 2017; Huang et al., 2018). In recent years, a great number of studies have reported that lncRNA is widely involved tumorigenesis, which serves

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as an oncogene or tumor suppressor gene (Huarte, 2015; Sun, 2018). For example, Sun et al. found that overexpressed lncRNA HOXA-AS in gastric cancer tissues is related to poor prognosis of gastric cancer patients (Sun et al., 2016); Li et al. found that lncRNA FOXP4-AS1 served as a tumor suppressor in colorectal cancer and FOXP4-AS1 knockdown can inhibit cell proliferation and promote apoptosis (Sun et al., 2016). Therefore, in order to further investigate the mechanism of gastric cancer development and discover new biomarkers, differentially expressed lncRNAs in gastric cancer and their biological functions need to be explored.

LncRNA LINC01793 is a non-coding RNA with 2020 bp in length located at locus 2p16.1 (https://www.ncbi.nlm.nih.gov/nuccore/NR\_110219.1). To our best knowledge, no research on LINC01793 has been reported yet. We analyzed the gastric cancer data through TCGA to screen out the related lncRNA. Biological function of the lncRNA in gastric cancer was then verified.

#### Materials and methods

#### Data acquisition and collection

Genome-wide expression profiles of gastric cancer and normal gastric mucosa in the TCGA database were downloaded. Prognostic survival of these patients were also downloaded using the GDC Data Transfer Tool. Differentially expressed lcnRNA was analyzed by edgerR package, and prognosis-related genes were analyzed by survival analysis.

Table 1. Summary of the cohorts clinical characteristics.

Characteristics	Number of patients(%)
Total cases	21 (100%)
Gender Male Female	13 (61.9%) 9 (38.1%)
Age(years) <60 ≥60	5 (23.8%) 16 (76.2%)
Tumor size <5cm	7 (33.3%)
≥5cm	14 (66.7%)
Differentiation Well/ Moderate Poor	6 (28.6%) 15 (71.4%)
TNM stage I/II III/IV	5 (23.8%) 16 (76.2%)
Lymph-node metastasis Negative Positive	6 (28.6%) 15 (71.4%)

#### Specimen collection and processing

Gastric cancer tissues and paracancerous tissues  $(1 \times 1 \times 1 \text{ cm})$  were surgically resected. Tissues were washed repeatedly with PBS until no blood remained, placed in liquid nitrogen, and transferred to -80°C refrigerator for further study. Our study was approved by the Research Ethics Committee of Zhejiang University (Hangzhou, Zhejiang, PR China), and written informed consent was obtained from all patients. The clinical characteristics of the gastric cancer patients are summarized in Table1.

QRT-PCR The total RNA was extracted from gastric cancer tissues and paracancerous tissues by RNA extraction kit. After transcribing into cDNA, the target gene was amplified by polymerase chain reaction (PCR) to detect the different mRNA expressions of LINC01379 in gastric cancer and paracancerous tissues. Sequences used in this study were as follows: GAPDH: forward primer: 5'-GACTCATGACCACAGTCCATGC-3', reverse prime: 5'-AGAGGCAGGGATGATGTTCTG-3'. The final expression was expressed as 2 - $\Delta$ Ct.

#### Gene set enrichment analysis (GESA)

GSEA 2.2.1 software was used for analysis. The gastric cancer expression profiles were divided into high and low expression groups based on the median expression of LINC01379. GESA was performed according to the default weighted enrichment statistics and the random combination number was set as 1000.

#### Statistical analysis

All statistical analyses in our experiment were performed using the SPSS version 20.0 software system (IBM, Armonk, NY, USA). Data are shown as mean  $\pm$ standard error of mean (SEM). The differences between groups were analyzed by the Student's t-test, Wilcoxon test, or  $\chi^2$  test. The Kaplan-Meier method was performed for patients' overall survival analysis. All experiments were run in triplicate. P<0.05 was considered statistically significant (\*P<0.05; \*\*P<0.01; \*\*\*P<0.001).

#### Results

## LncRNA LINC01793 was overexpressed in gastric cancer

A total of 60115 genes were detected by TCGA database, including 14,464 lncRNAs. EdgerR package was utilized to analyze the differentially expressed genes in TCGA database. We found that LINC01793 expression was significantly increased in gastric cancer (Fig. 1A,B). In addition, LINC01793 is a long non-coding RNA without any functional report in GC yet. To validate the expression results from TCGA database, we detected the level of LINC01793 in 21 paired GC tissues

and adjacent normal tissues by qRT-PCR. As shown in Fig. 1C, LINC01793 expression was significantly upregulated in GC tissues.

### LncRNA LINC01793 can predict the prognosis of gastric cancer patients

In order to verify whether LINC01793 can predict prognosis of gastric cancer patients. We analyzed the prognostic information of gastric cancer in the TCGA database. LINC01793 expression was negatively related to the prognosis of gastric cancer patients. The higher the expression, the worse the prognosis (Fig. 2). Subsequently, gastric cancer patients were divided into 10 groups according to their age, sex, grading, staging and lymph node metastasis. The relationship between LINC01793 expression and overall survival (OS) of gastric cancer patients in each group was analyzed. Among them, LINC01793 was a risk factor for OS of gastric cancer patients who were younger than 60 years old (Fig. 2B), older than 60 years old (Fig. 2A), male patients (Fig. 2D), female patients (Fig. 2C), pathological grading with G1+G2 (Fig. 2E), pathological grading with G3 (Fig. 2F), lymph node metastasis (Fig. 2H), and clinical stage with stage 3+4 (Fig. 2G). In summary, overexpression of LINC01793 may indicate poor prognosis of gastric cancer patients.

#### LncRNA LINC01793 regulated cell cycle, apoptosis, invasion, metastasis and other biological processes of gastric cancer cells

According to the median expression of LINC01793, the patients' genomic expression profiles were divided into high expression group and low expression group. GESA analysis showed that LINC01793 mainly regulated cell cycle, apoptosis, cell adhesion junction, MAPK signaling pathway, JAK-STAT signaling pathway and ERBB pathway (Fig. 2A-I). In summary, LINC01793 may be involved in the development of



Fig. 1. LncRNA LINC01793 was overexpressed in gastric cancer tissues. A. The heatmap of dysregulated lncRNAs in gastric cancer in TCGA. B. LINC01793 was overexpressed in gastric cancer. C. LINC01793 was overexpressed in gastric cancer tissue compared to paracancerous tissues by qRT-PCR.



LINC01793 predicted poor prognosis of gastric cancer patients. A. Gastric cancer patients older than 60 years with high expression level of LINC01793 had a shorter OS than those with lower level. B. Gastric cancer patients younger than 60 years with high expression level of LINC01793 had a shorter OS than those with lower level. C. Female patients with high expression level of LINC01793 had a shorter OS than those with lower level. D. Male patients with high expression level of LINC01793 had a shorter OS than those with lower level. E. In gastric cancer patients with pathological stage of G1 + G2, patients with high expression of LINC01793 had a shorter OS than those with lower level. F. In gastric cancer patients with histological stage G3, patients with high expression of LINC01793 had a shorter OS than those with lower level. G. In gastric cancer patients with stage 3 and 4, patients with high expression of LINC01793 had a shorter OS than those with lower level. H. In gastric cancer patients with lymph node metastasis, patients with high expression of LINC01793 had a shorter OS than those

gastric cancer and serve as an oncogene.

#### Discussion

Gastric cancer is a common malignancy with a poor

prognosis in the world, which seriously threatens human health (Hamashima, 2014). In China, the incidence of gastric cancer is relatively high (Yang, 2006; Sugano, 2015). The mortality and mobility of gastric cancer in China accounted for about 50% of the world (Yang,



Fig. 3. Possible biological process and pathway that LINC01379 was involved in. A. LINC01379 was associated with cell apoptosis. B. LINC01379 was associated with cell cycle. C. LINC01379 was associated with focal adhesion. D. LINC01379 was associated with the regulation of actin cytoskeleton. E. LINC01379 was involved in adherent junction. G, H and I. LINC01379 could regulate MAPK, JAK-STAT and ERBB pathway.

2006; Sugano, 2015). Prevention and treatment of gastric cancer have been well reconized (de Martel et al., 2013; Plummer et al., 2015). Therefore, it is urgent to further study the molecular mechanism of the occurrence and development of gastric cancer, so as to search for new molecular targets for the diagnosis and treatment of gastric cancer.

Long non-coding RNA (lncRNA) is a kind of DNA transcription product that does not encode protein. LncRNA plays an important role in cell differentiation and regulation of gene expressions and other activities (Li et al., 2013; Fatica and Bozzoni, 2014). In recent studies, relative results have shown that lncRNA is involved in chromatin modification, transcriptional activation, transcriptional interference, nuclear transport and other important regulatory processes, which is closely related to many tumor and non-tumor diseases (Marchese and Huarte, 2014; Gonzalez et al., 2015). The current studies showed that differentially expressed lncRNA is associated with development, invasion, metastasis and prognosis of gastric cancer, which may be used as a diagnostic tumor marker and therapeutic target (Sun et al., 2016).

So far, biological functions of lncRNA LINC01793 have not been reported yet. In the present study, we first found that LINC01793 was overexpressed in gastric cancer tissues by analyzing the TCGA data of gastric cancer. Furthermore, we confirmed that LINC01793 was overexpressed in gastric cancer tissues compared with that of paracancerous tissues. GESA analysis indicated that LINC01793 may serve as an oncogene in the development of gastric cancer. Therefore, the biological function and molecular mechanism of LINC01793 in gastric cancer need to be further studied.

A large amount of evidence has suggested that the differential expressed lncRNA in tumor tissues may be a new molecular marker for survival prognosis of cancer patients (Hu et al., 2014, 2015). To explore the predictive value of LINC01793 in clinical outcomes of gastric cancer patients, prognostic analysis of lncRNA LINC01793 was performed. TCGA data showed that OS was shorter in gastric cancer patients with higher expression level of lncRNA LINC01793 than those with lower level. The higher the LINC01793 expression, the worse the gastric cancer prognosis. In addition, GSEA analysis found that the main functions of LINC01793 were enriched in cell cycle, apoptosis, invasion, metastasis and cancer-related signaling pathways. In summary, overexpression of LINC01793 was a risk factor for poor prognosis of gastric cancer patients. LINC01793 may affect cell proliferation, invasion and metastasis-related malignant phenotype of gastric cancer. This study first demonstrated that lncRNA LINC01793 is overexpressed in gastric cancer. Overexpression of LINC01793 in gastric cancer may predict poor prognosis. Further bioinformatics analysis revealed that LINC01793 may be involved in the development of gastric cancer. However, we did not verify the cytology of these mechanisms in this experiment which requires further in-depth studies.

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