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Original Research

CCAT1 expressed in malignant and pre-malignant human gastric tissues

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Abstract: CCAT1 has been reported to implicate in various human malignancies. However, its expression in the oncogenesis of gastric cancer (GC) remains unknown. Quantitative reverse transcription (RT)-qPCR assay was performed to detect the expression of CCAT1 in 68cases of GC tissues,paired tumor-proximal gastric mucosa and normal gastric mucosa, 19 cases of gastric intraepithelial neoplasia (GIN), and the sera of these 68 GC patients and 22 healthy volunteers. And the relationship between CCAT1 and clinicopathologic features of GC patients were analyzed. Inthe present study, we found that CCAT1 expression was significantly abnormally deregulated in GC tissues (185.43 ± 21.37), GIN tissues (121.30 ± 43.61), tumor-proximal mucosa (8.9 ± 1.21), and normal mucosa (1.5 ± 0.55). CCAT1 wasalso overexpressed in the sera of GC patients (47.40 ± 6.60) compared with healthy controls (0.62 ± 0.06; $p < 0.001$). And high CCAT1 expression in GC tissues was associated with high tumor burden, including larger tumor size, lymphatic metastasis and advanced TNM stage (all $p < 0.05$). These results reveal that CCAT1 is a detectable biomarkerforgastric cancertumorigenesis and may be utilized as adiagnosticand prognosticindicator.

Key words: CCAT1; Gastric cancer; Expression; Tissue, sera.

Introduction

Gastric cancer (GC) is one the most common malignancies worldwide with a very high morbidity and mortality in China (1). The prognosis of GC is poor and most patients are generally diagnosed at a rather advanced stage(2). The five-year survival rate of GC is less than 30%, and patients succumb to frequent metastasis is more than 10% despite recent advances in surgical techniques and treatment regimens (1, 3). Although previous studies identified many aberrantly expressed molecules in GC, novel markers are still urgently needed for early diagnosis and prognostication (4).

Homo sapiens colon cancer associated transcript 1 (CCAT1), also known as CARLo-5 (onco-lncRNA-40), as along non-coding RNA located at chromosome 8q24.21, was originally found in 2014 and interactes with MYC enhancer by the CCAT1 promoter, thus implicated in tumor development (5). CCAT1 plays vital roles in various biological processes, such as cell-cycle, cell proliferation, migration and invasion (5, 6).

In recent years, CCAT1 has received more and more attention in the role of tumor development and progression in many types of human malignancies (7). CCAT1 has been considered as a diagnostic marker and correlates with poor prognosis in various types of cancer, including colorectal cancer (8), gastric cancer (6), hepatocellular carcinoma (9), and gallbladder cancer (10).

This study is designed to evaluate the level of CCAT1 expression in the process of GC development. Our results showed that CCAT1 was altered drastically during GC tumorigenesis and the increase of CCAT1 is also exists in the sera of patients with GC compared with healthy controls.

Materials and Methods

Patients and samples collection

The study was approved by our Institutional Review Board, and informed consent was obtained from all patients and controls. In total, 68 patients (aged 38~79 years; mean, 61.0 years) diagnosed with primary GC who underwent gastrectomyat the first Affiliated Hospital of Soochow University between April 2015 and April 2016 were selected. Patients who received preoperative chemo-radiotherapy were excluded from the study. To check the tumor cell concentration, we get frozen sections from all resected fresh tissues, followed byhistologic analyses, and only samples with tumor cell concentration > 60% can be included, and then immediately frozen in liquid nitrogen and stored at -80°C. All specimens underwent routine histopathologic analysis. Paired tumor-proximal mucosa was isolated 2~5cm away from the tumor border, and normal GC mucosa was isolated from 5~20cm away from the tumor border. The tumor grades were defined in accordance with the criteria outlined by the World Health Organization (WHO) Classification of Tumors of the Digestive System, 2010 edition (2). Tumors were staged according to the criteria enumerated in the AJCC Cancer Staging Manual, seventh edition (11). We also included the fresh tissues from 19 cases of patients with gastric intraepithelial neoplasia (GIN). All GC and GIN patients' clinicopathological features are shown in Table 1. Addi-

tional set of sera from aforementioned 68 hospitalized GC patients and 22 healthy volunteers were employed in the study.

RNA isolation

Total RNA of tissue sample was extracted from frozen tissues using TRIzol® reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Serum RNA isolation was performed as published earlier (12) . In brief, total RNA was extracted from 300 µl of sera using a Blood Total RNA Isolation Kit (RP4001, BioTeke, Beijing, China) and eluted in 50µL of pre-heated (95°C) Elution Solution according to the manufacturer's recommendation. RNA quantity and purity was determined using the NanoDrop 1000 spectrophotometer (ThermoScientific, Wilmington, DE; USA). The RNA specimens were stored at –80°C.

Quantitative reverse transcription (RT)-qPCR

cDNA was synthesized by Prime Script RT Master Mix kit (Takara, Japan). The expression of CCAT1 was quantified by Quantifast SYBR Green RT-qPCR kit (Qiagen, Dusseldorf, Germany). PCR was then performed at 94°C for 2 min, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. GAPDH was used as a control gene. The primer sequences used were as follows: for CCAT1, forward, 5'- TCACTGACAACATCGACTTTGAAG-3'; and reverse, 5'- GGAGAAAACGCTTAGCCATACAG-3'; for GAPDH, forward, 5'-GTCAACGGATTTGGTCTGTATT-3'and reverse, 5'--AGTCTTCTGGGTGGCAGTGAT-3'. Every independent experiment was performed three times. Results were analyzed as relative quantity (RQ) expression of CCAT1.

Statistical analysis

Statistical analysis was performed using IBM-SPSS® statistical package Version 22.0 (SPSS Inc. Chicago, IL). The significance of differences between groups was analyzed using Student's t-test, Chi-squared test, or Fisher's exact test, as appropriate. A $p<0.05$ was considered statistically significant.

Results

Expression levels of CCAT1 in GC tissues

In order to explore the level of CCAT1 in gastric carcinogenesis, we quantified CCAT1 expression in gastric tissues using RT-qPCR assay. GC tissues (mean ± SD:185.43 ± 21.37) showed more than 100 - fold expression of CCAT1 levels over normal mucosa (1.5 ± 0.55; $p< 0.001$; Figure 1). Meanwhile, we also detected the level of CCAT1 in GIN and tumor-proximal mucosa tissues. GIN tissues (121.30 ± 43.61) showed more than 80-fold upregulation of CCAT1 levels compared to normal mucosa ($p < 0.001$). In addition, the CCAT1 level in tumor-proximal mucosa tissues (6.8 ± 1.21), albeit to a lesser extent, showed a mean overexpression of 4.5-fold over normal mucosa ($p = 0.048$; Figure 1). And our results showed that CCAT1 expression were also increased dramatically in GIN tissues compared with tumor-proximal mucosa tissues (Figure 1; $p < 0.001$). Specifically,CCAT1 expression was dramatically high in normal mucosa obtained 2–5 cm proximal to nine tumors compared with the mean value of the tumor-proximal group, and only twosamples in normal mucosa 5-20 cm proximal to the tumor showed significant overexpression compared with the mean value of the normal group (Figure 1).

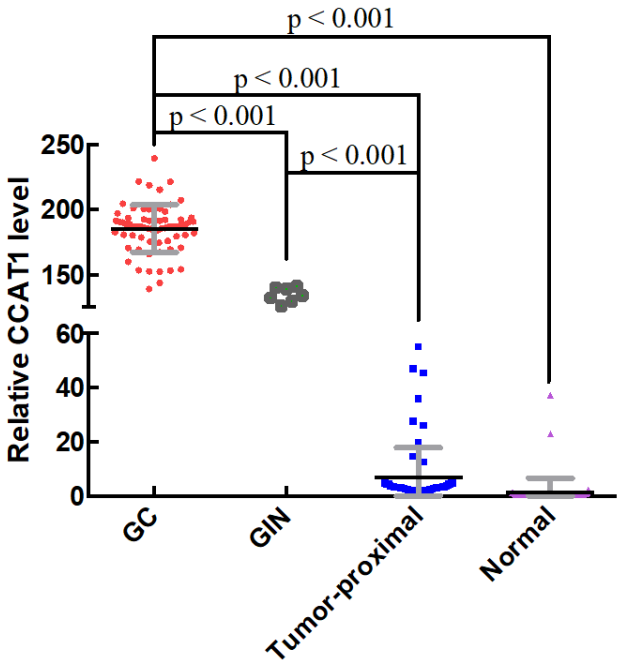

Figure 1. Expression of CCAT1 in gastric tissues. CCAT1 expression was detected in GC tissues (n = 68), paired tumor-proximal gastric mucosa (n = 68), normal gastric mucosa (n = 68) and GIN (n = 19) by RT-qPCR. Normalized by the level of GAPDH.

Expression levels of CCAT1 in GC sera

We also detected CCAT1 expression in sera from the aforementioned 68 GC patients and 22 healthy volunteers by RT-qPCR. The results showed that CCAT1 expression in sera of GC patients (47.40 ± 6.60) were about 76-fold compared to that obtained from healthy volunteers (0.62 ± 0.06; Figure2).

CCAT1 positively correlates with the GC progression

We further determined the relationship between CCAT1 expression and GC patients' clinicopathologi-

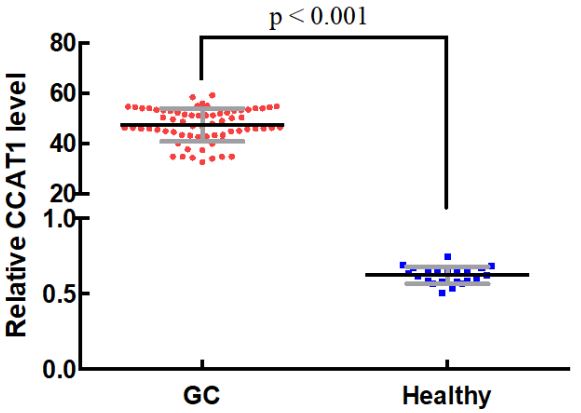

Figure 2. Expression of CCAT1 in the sera of GC patients (n =68) and healthy volunteers (n = 22) detected by RT-qPCR. Normalized by the level of GAPDH.

Table 1. Correlations between CCAT1 expression levels and clinical parameters.

Variables	GC patients (n = 68)	GC patients		GIN patients (n = 19)	P value
		Low expression	High expression		
Age (years)					0.237
<60	18	7	11	16	
≥60	50	25	35	14	
Gender					0.205
male	40	23	17	20	
female	28	13	15	10	
Tumor size					0.032*
<5cm	39	27	12		
≥5cm	27	11	16		
Histologic grade					0.178
Well and moderate	23	14	9		
Poor and others	45	22	23		
Depth of tumor					0.152
T1, T2	19	10	9		
T3, T4	42	23	19		
Lymphatic metastasis					0.031*
Absent	25	16	9		
Present	43	12	31		
Distant metastasis					0.059
Absent	48	21	27		
Present	20	9	11		
TNM stage[a]					0.027*
I and II	22	14	8		
III and IV	46	11	35		

[a] Tumor stage was obtained according to the TNM criteria.

* $p < 0.05$.

cal features (Table 1).The expression levels of CCAT1 in tumor tissues were categorized as low or high in relation to the mean expression level of CCAT1 in GC tissues (185.43 ± 21.37). The high CCAT1 group (n = 41) showed larger tumor size (p = 0.032), more lymphatic metastasis (p = 0.031) and advanced TNM stage (p = 0.027) compared with the low CCAT1 expression group (n =27). However, there was no significant correlation between CCAT1 expression and other clinicopathologic features, such as age, gender, histologic grade or distance metastasis, (all $p > 0.05$; Table 1).

Discussion

LncRNAs are a family of long non-coding RNA molecules, which reveal complex functions by involves inmultiple perspectives (genomic, transcriptional and posttranscriptional regulation) in almost all physiological processes including differentiation, proliferation, apoptosis and invasion (11). In addition, the lncRNAs are important regulators in the carcinogenesis and progression of various malignancies (13). CCAT1 has been previously reported to be overexpressed in various types of cancer tissues and may execute pivotal function in human neoplasms (14, 15).

In the present study, we sought to determine the expression level of CCAT1inGC and normal tissues/ sera samples. We confirmed that CCAT1 was upregulated in gastric cancer. Our results were consistent with that of previous reports (14). Particularly, our result showed that normal mucosa, paired tumor-proximal GC mucosa, GIN and GC tissues exhibit rising CCAT1expression, and CCAT1 is overexpressed in sera of GC patients compared with healthy controls. These results suggest that CCAT1 is involves in the occurrence and development of GC.Differential expression profile may reveal the mechanism of functional CCAT1 in GC. Based on the RT-qPCR results and in light of recent reports(6, 16, 17), we proposed that CCAT1 potentially be a biomarker and therapeutic target for gastric cancer. Indeed, CCAT1 was reported associated with the clinical outcome of colonic adenoma-carcinoma (8), hepatocellular carcinoma (18), and breast cancer (19).

In addition, we found that the expression level of CCAT1 was significantly associated with important clinicopathologic parameters in GC including tumor size, lymphatic metastasis and TNM stage, showing the pivotal role of CCAT1 in GC. Mechanically, CCAT1 has been reported to function as a Myc-activated oncogene in GC that regulate GC migration by forming a CCAT1/ miR-490/hnRNPA1 axis (17). It was found that CCAT1 facilitates cell growth and migration by serving as a scaffold for SPRY4 in the nucleus, and as a decoy for miR-7 in cytoplasm(20). As a Myc enhanced regulator, CCAT1promotes cancer cell malignant phenotype in multiple human tumors(5, 6, 18, 21).So, except for two

aspects of the mechanism of CCAT1 in the prognosis of cancer, more studies need to be directed to the complex networks between CCAT1, SPRY4, MYC, miRNA, and other genes.

There are some limitations in the present study. Depend on the existing study, the reliability of GAPDH as a control gene in tissue and sera, whether the sample size and the population include is representative, the sera levels of gastric adenocarcinoma to other conditions that affect the stomach (such as patients with h. pylori gastritis and patients with atrophic gastritis, etc.), all these aspects need to be focus on to make more depth analyses. Although until now there is no indirectly data show the relationship between CCAT1 expression and radiotherapy in cancer, nowadays some studies show that long non-coding RNA can modulates radiosensitivity of cancer cells by involving in multiple mechanisms(22, 23). It has been found that chemoradiotherapy is an effective treatment for localized gastric cancer without distant metastases, and intraoperative radiotherapy have a favorable effect for cancer in stage II~IIIto show loco-regional control(24, 25). These suggest that radiotherapy and lncRNA, to some extent, may link with each other. Therefore, in this present study, we exclude patients receiving preoperative chemo-radiotherapy. Further studies are required to determine the specific relationship between radiotherapy and CCAT1 in GC.

In conclusion, we demonstrated that CCAT1 is increasingly expressed during human GC tumorigenesis and development. Enhanced expression of CCAT1was associated with tumor size, invasion to the lymph nodes and advanced tumor stage. Our results indicate that CCAT1 is a potential biomarker for GC. Screening for CCAT1 expression could potentially improve early diagnosis, prognostication and therapy.

Author's contribution

Haixin Qian had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, planned and supervised the project, performed data analysis and wrote the manuscript. Xiding Liand Yongping Zhouperformed the clinical part of the work, did the data analysis and interpretation, performed experimental work and statistical analysis. Haixin Qian revised the manuscript. All authors have read and approved the final manuscript.

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