

Original Article

Long non-coding RNA CCAT2 promotes gastric cancer proliferation and invasion by regulating the E-cadherin and LATS2

Yong-Jun Wang^{1*}, Jian-Zhen Liu^{2*}, Pei Lv³, Yi Dang⁴, Jiang-Yan Gao¹, Yong Wang¹

Departments of ¹Cardiovascular, ²Urology, ³Nephrology, Hebei Chest Hospital, Shijiazhuang, Hebei, China; ⁴Department of Cardiovascular, Hebei general Hospital, Shijiazhuang, Hebei, China. *Co-first authors.

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Abstract: Dysregulation of long non-coding RNAs (lncRNAs) play important roles in tumor development and progression. The long non-coding RNA CCAT2 has been identified to be up-regulated in gastric cancer (GC). However, the detailed molecular mechanism of CCAT2 involved in GC progression is still unknown. The aim of this study was to explore the expression and role of CCAT2 in GC progression. In the study, the expression levels of CCAT2 were significantly up-regulated in 108 cases GC tissues compared with adjacent non-tumor tissues by qRT-PCR analysis. Higher CCAT2 expression was correlated with tumor size, lymph node metastasis and Tumor Node Metastasis (TNM) stage in GC patients. Multivariate analysis showed that lymph node metastasis, TNM stage and the expression of CCAT2 were independent prognostic indicator for disease-free survival (DFS) and the over survival time (OS) for GC patients. Further function analysis demonstrated that knockdown of CCAT2 inhibited the cell migration and invasion, whereas, the overexpression of CCAT2 showed the opposite results in GC cells. Our results also demonstrated that CCAT2 promoted the GC cells epithelial-mesenchymal transition (EMT) by downregulated the E-cadherin expression and upregulated the ZEB2, Vimentin and N-cadherin expression. Moreover, RNA immunoprecipitation (RIP) and Chromatin immunoprecipitation (ChIP) revealed that CCAT2 interacted with EZH2 and regulated the E-cadherin and LATS2 expression. Thus, our results demonstrated that CCAT2 functioned as an oncogene in GC and was involved in gastric cancer progression. Targeting CCAT2 might be a potential therapeutic target for GC.

Keywords: Gastric cancer, CCAT2, epithelial-mesenchymal transition, E-cadherin, LATS2

Introduction

Gastric cancer (GC) is one of the most common digestive malignant tumors and the second leading cause of cancer deaths worldwide [1]. Over the past decades, although the incidence of gastric cancer has decreased due to improved living standards, a reduction in chronic H. pylori infection and remarkable advances in diagnosis and treatment, unfortunately, the 5-year survival rate for advanced-stage patients remains low [2, 3]. Therefore, it is essential to elucidate the underlying molecular mechanisms of initiation and metastasis and to develop novel therapeutic approaches for GC.

In the recent years, some studies have revealed that long non-coding RNAs, consisting of more than 200 nucleotides (nt) in length, are involved in molecular mechanism to regulate cell structure, function, and physiological development

[4, 5]. Long non-coding RNAs have been confirmed to play key roles in cancer development and progression [6]. For example, lncRNA H19 expression was higher in gastric cancer (GC) tissues and over-expression of H19 promoted the features of GC including proliferation, migration, invasion and metastasis [7]. lncRNA HOTAIR was also significantly up-regulated in cancerous tissues than that in corresponding normal mucosa, and higher expression of HOTAIR significantly correlated with peritoneal metastasis in GC patients [8]. Long non-coding RNA Linc00152 acted as an oncogene, which was involved in cell cycle arrest, apoptosis, epithelial to mesenchymal transition, cell migration and invasion in gastric cancer [9].

CCAT2 is a novel non-coding RNA located at 8q24. Ling *et al* found CCAT2 was highly over-expressed in microsatellite-stable colorectal cancer and promoted tumor growth, metasta-

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sis, and chromosomal instability [10]. Another study reported that CCAT2 was over-expressed in breast cancer tissues and up-regulated cell migration and down-regulated chemosensitivity to 5-FU in an rs6983267-independent manner [11]. Wang *et al* confirmed that up-regulation of lncRNA CCAT2 was correlated with gastric cancer progression and might be a potential molecular biomarker for predicting the prognosis in GC patients [12]. These investigation suggested that CCAT2 functioned as an oncogene in cancer development and progression, however, the detailed function of CCAT2 in gastric cancer was still unknown.

In present study, we showed that CCAT2 was significantly up-regulated in GC tissues and higher CCAT2 expression was correlated with poor survival outcome. Furthermore, knock-down of CCAT2 inhibited the cell migration, invasion and promoted the GC cells epithelial-mesenchymal transition (EMT) by downregulating the E-cadherin expression and upregulated the ZEB2, Vimentin and N-cadherin expression. Moreover, we also revealed that CCAT2 interacted with EZH2, LSD1, and H3k27me3, which regulated the E-cadherin and LATS2 expression. Therefore, these results indicated that CCAT2 functioned as an oncogene and was involved in gastric cancer progression.

Methods

Patient tissue samples

The gastric cancer tissue and adjacent normal tissue were obtained from 108 patients who had undergone surgical resection between March 2008 and February 2013 at the Hebei Chest Hospital and Hebei general Hospital. All of the patients were diagnosed by two experienced pathologists. The fresh tissue samples were obtained and then immediately frozen in liquid nitrogen, and stored at -80°C for further analysis. TNM staging classification was based on criteria of American Joint Committee on Cancer (AJCC, 6th edition). The written informed consent had been obtained from all the patients, and this study was approved by the Ethical Committee of Hebei Chest Hospital.

Cell lines and culture

The human gastric epithelial cell line GES-1 and the four gastric cancer cell lines (SGC7901, MKN45, BGC-823 and MKN-28) were purchased from Shanghai Institute of Cell Biology

(Shanghai, China). All of the cell lines were maintained routinely in RPMI Media 1640 (Gibco) supplemented with 100 U/mL penicillin, and 100 µg/mL streptomycin. All cells were maintained in a humidified incubator containing 5% carbon dioxide at 37°C.

Cell transfection

Two siRNA oligos specifically targeting CCAT2 were constructed by GenePharma Co. Ltd (Shanghai, China) and two silencing sequences were si-CCAT2-1, 5'-GUGCAACUCUGCAAUUUAUUU-3'; si-CCAT2-2, 5'-UUAAAUUGCAGAGUUGCACUU-3'. The siRNA against EZH2 was 5'-GACUCUGAAUGCAGUUGCUTT-3'. GC Cells were transfected with siRNAs oligos by using Lipofectamine 3000 reagent (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. The CCAT2 full length sequence was subcloned into the pcDNA3.1 vector (Invitrogen). Ectopic CCAT2 expression was achieved through pcDNA3.1-CCAT2 transfection using lipofectamine 3000 (Invitrogen) and the empty pcDNA3.1 vector was used as a control.

RNA extraction and qRT-PCR

Total RNA was extracted from tissues or cells by using the Trizol reagent (TAKALA, Dalian, China) according to the manufacturer's instructions. The quantitative real-time polymerase chain reaction (PCR) was performed by using SYBR-green PCR Master Mix in a Fast Real-time PCR 7500 System (Applied Biosystems). The gene-specific primers were as follows: CCAT2, forward: 5'-AGACAGTGCCAGCCACC-3', reverse: 5'-TGCCAAACCCTCCCTTA-3'; E-cadherin, forward: 5'-TCT TCCAGGAACCTCTGTGATG-3'; reverse: 5'-CAATGCCGCCATCGCTTACACC-3', LATS2, forward: 5'-ACCCCAAAGTTGGACCTTAT-3', 5'-reverse: CATTGCGGTTCACTTCTGC-3'. the GAPDH was used as an internal control, forward: 5'-AATGGACAAGTGGTCGTGGAC-3', and reverse: 5'-CCCTCCAGGGGATCTGTTT-3'. The reactions were carried out at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s. Fold change of mRNA was calculated by the equation $2^{-\Delta\Delta Ct}$.

Cell proliferation, migration and invasion assays

Cell proliferation was measured in MKN45 and BGC-823 cells using the Cell Counting Kit-8 assays (Dojindo) according to the manufacturer's instructions. 3000 cells per well was

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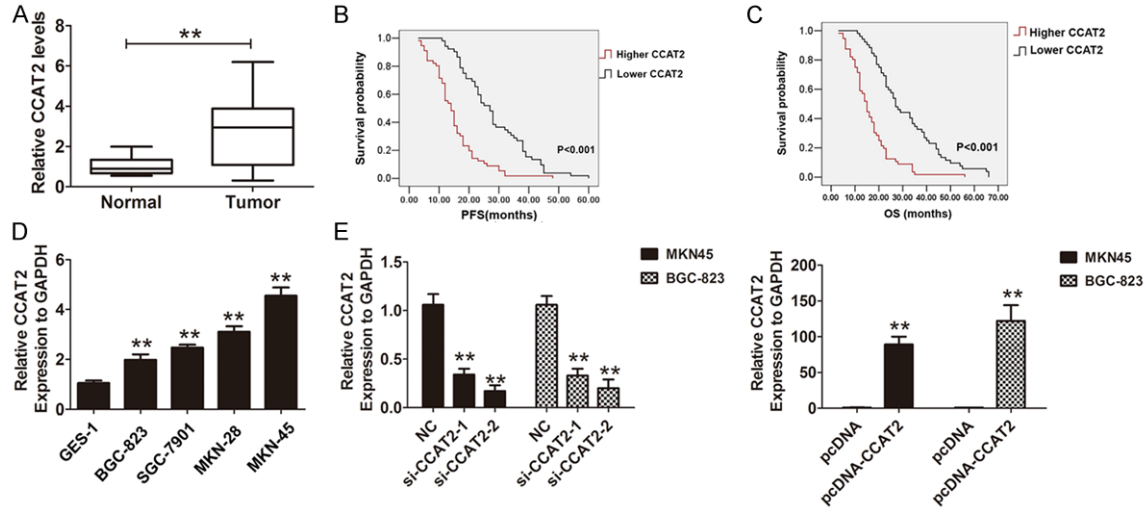


Figure 1. CCAT2 was up-regulated in GC tissues and cells. (A) The Relative expression of CCAT2 in 108 cases gastric tissues and adjacent normal tissues was analyzed by using qRT-PCR assays and normalized to GAPDH expression. Data are presented as mean \pm S.D. (B, C) The expression of CCAT2 was classified into two groups according to the median value expression level in GC tissues sample. Kaplan-Meier method and the log-rank test were used to analyze the progression-free survival curves and the overall survival curves of GC patients in higher and lower CCAT2 expression groups. (D) The Relative expression of CCAT2 in four gastric cells and human gastric epithelial cell line GES-1 was analyzed by using qRT-PCR assays and normalized to GAPDH expression. Data are presented as mean \pm S.D. (E) The Relative expression of CCAT2 was analyzed after silencing CCAT2 in MKN45 and BGC-823 cells and (E) was evaluated by transfecting pcDNA3.1-CCAT2 into MKN45 and BGC-823 cells, the normal control was GAPDH expression. The mean values and S.D were calculated from triplicates of a representative experiment. ** $P < 0.05$.

seeded in 96-well plate. The 100 μ l of cell suspension was placed in 96-well plate and pre-incubated for 12 h. Then, 10 μ l of CCK-8 solution was added to each well. The cell number was evaluated at 0, 24 h, 48 h, 72 h, 96 h and the absorbance was 450 nm using Epoch Microplate Spectrophotometer (Bio Tek). The cell migration and invasion assays were measured by transwell invasion assay. Briefly, GC cells in different groups cultivated with DMEM medium without fetal bovine serum were put on the upper chamber coated (the invasion assays using BD Matrigel) in the 24-well plates. DMEM medium with 10% fetal bovine serum was put to the lower chamber. After 48 h incubation, cotton swabs were used to wipe off the GC cells from the upper chamber. The number of cells migrating or invasion to the lower chamber was calculated by inverted microscopy after Crystal violet staining. And the number of and the mean of number of cells in each field represented the invasive ability of the cells.

Western blotting analysis

The cells were lysed using RIPA Lysis buffer (Beyotime, China) supplemented with a Protease and Phosphatase Inhibitor Cocktail (Sigma). Cell lysates were centrifuged and then

the protein concentration was calculated using the Pierce BCA protein assay kit (Beyotime, China). Proteins were separated by a 10% polyacrylamide gel and transferred to a PVDF membrane (Millipore, USA), then detected with anti-E-cadherin (1:1000, CST, USA), Vimentin (1:1000, CST, USA), N-cadherin (1:1000, CST, USA), ZEB2 (1:1000, CST, USA), LATS2 (1:1000, CST, USA) and The GAPDH (1:1000, CST, USA) was as the internal control. The protein bands were analyzed using Image J software.

RNA immunoprecipitation (RIP)

RNA immunoprecipitation (RIP) experiments were performed according to previous describe [13], and was used by a Magna RIPTM RNA-Binding Protein Immunoprecipitation Kit (Millipore, USA) according to the manufacturer's instructions. EZH2 (CST, USA), LSD1 (CST, USA) and H3 trimethyl Lys 27(CST, USA) antibodies was used for RIP assays.

Chromatin immunoprecipitation (ChIP) assay

ChIP assay was performed as described previously [14], and an EZ-CHIP KIT was used according to the manufacturer's instruction (Millipore, USA). EZH2, LSD1 and H3 trimethyl Lys

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Table 1. Correlation between CCAT2 expression levels and clinicopathologic parameters in 108 GC patients was analyzed

Clinicopathologic features	GC patients	CCAT2 expression level		χ^2 test p-value
		Lower	Higher	
Gender				0.749
Female	44	22	22	
Male	64	30	34	
Age (years)				0.106
≤ 60	62	34	28	
> 60	46	18	28	
Tumor size				0.036**
< 5 cm	68	38	30	
> 5 cm	40	24	26	
Histological grade				0.175
High, middle differentiation	55	30	25	
Low differentiation	53	22	31	
Lymph node metastasis				0.001**
No	50	35	15	
Yes	58	17	41	
Local invasion				0.076
T1, T2	59	33	26	
T3, T4	49	19	30	
TNM stage				0.001**
I-II	54	38	16	
III-IV	54	14	40	

**p-value < 0.05 was considered statistically significant.

27 antibody was used in the experiments. Quantification of immunoprecipitated DNA was detected by qRT-PCR with SYBR Green Mix (Takara). The ChIP data was calculated as a percentage relative to the input DNA.

Statistical analysis

The SPSS 18.0 was used for the statistical analysis. All of the values were analyzed as the mean \pm SD and performed at least three independent experiments. Data were evaluated by using two-tailed Student's t-test and one-way ANOVA. The $P < 0.05$ was considered to be statistically significant.

Results

CCAT2 is up-regulated in GC tissues and cells

QRT-PCR was used to analyze CCAT2 expression levels in 108 cases GC tissues and adjacent normal tissues. The results showed that

CCAT2 was markedly up-regulated in GC tissues, compared with the adjacent normal tissues (**Figure 1A**, $P < 0.05$). We further detected the association between CCAT2 expression and clinicopathological features in GC patients. The GC patients were divided into two groups according to the CCAT2 median expression levels (2.92) in the GC tissues: lower CCAT2 expression group ($n=52$) (the change of relative expression < median CCAT2 fold) and higher CCAT2 expression group ($n=56$) (the change of relative expression > median CCAT2 fold). The results indicated that CCAT2 expression levels were significantly correction with tumor size, lymph node metastasis and TNM stage ($P < 0.05$, **Table 1**), but did not correlate with age, gender, Histological grade and so on in GC patients ($P > 0.05$, **Table 1**). Moreover, Multivariate analysis showed that lymph node metastasis, TNM stage and the expression of CCAT2 were independent prognostic indicator for disease-free survival (DFS) and the over survival (OS) time for GC patients. Patients with higher CCAT2 expression had a significantly poor disease-free survival (DFS) and the over survival (OS) time than those with lower expression (**Figure 1B, 1C; Tables 2, 3**). These results indicated that CCAT2 could act as a oncogene in GC patients.

CCAT2 promotes cell proliferation, migration and invasion in vitro

We further investigated the role of CCAT2 in human GC cancer cells, the results suggested that CCAT2 was higher expression in four GC cells compared with human gastric epithelial cell line GES-1 (**Figure 1D**). Two si-CCAT2 oligos targeting CCAT2 were designed and transfected into MKN45 and BGC-823 cells, the results demonstrated that CCAT2 was effectively knocked down and especially was used by the si-CCAT2-2. Thus, the si-CCAT2-2 was used to perform the knockdown of CCAT2 in following experiments. The pcDNA3.1-CCAT2 was used to over-expression of CCAT2 in MKN 45 and BGC-823 cells (**Figure 1E**). Furthermore, we explored the effects of CCAT2 knock-

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Table 2. Univariate and multivariate analysis of disease-free survival in GC patients (n=108)

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.335	0.919-1.979	0.151			
Gender	0.839	0.553-1.272	0.408			
Tumor size	1.213	0.786-1.645	0.118			
Histological grade	1.129	0.712-1.446	0.457			
Lymph node metastasis	2.146	1.138-3.569	0.008**	1.778	1.210-2.612	0.003**
Local invasion	1.313	0.812-1.844	0.123			
TNM stage	3.604	2.353-5.552	0.001**	2.508	1.687-3.728	0.001**
CCAT2	2.526	1.700-3.755	0.001**	2.305	1.554-3.418	0.001**

**p-value < 0.05 was considered statistically significant.

Table 3. Univariate and multivariate analysis of overall survival in GC patients (n=108)

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.378	0.901-1.923	0.189			
Gender	0.805	0.521-1.282	0.469			
Tumor size	1.385	0.568-2.166	0.286			
Histological grade	1.169	0.668-1.421	0.385			
Lymph node metastasis	2.053	1.391-3.303	0.001**	1.913	1.301-2.813	0.001**
Local invasion	1.332	0.823-1.855	0.154			
TNM stage	3.545	2.221-5.345	0.001**	2.442	1.553-3.556	0.001**
CCAT2	2.346	1.537-3.556	0.001**	2.108	1.442-3.202	0.001**

**p-value < 0.05 was considered statistically significant.

down on cells proliferation abilities by CCK8 assays and the results showed that CCAT2 knockdown significantly inhibited the cells growth abilities in MKN45 and BGC-823 cells (**Figure 2A, 2B**). Moreover, we found that CCAT2 knockdown also significantly inhibited cell migration and invasion in MKN45 cells, and promoted the cell migration and invasion by introducing pcDNA3.1 into BGC-823 cells (**Figure 2C-F**). Therefore, our results demonstrated that CCAT2 could promote GC cell proliferation, migration and invasion in vitro.

CCAT2 promotes the epithelial-mesenchymal transition (EMT) in GC cells

To further study the role of CCAT2 in GC development, we investigated whether CCAT2 promoted the epithelial-mesenchymal transition (EMT) in MKN45 and BGC-823 cells. The results showed that knockdown of CCAT2 in MKN45 cells decreased the transcription factor ZEB2 and EMT mesenchymal markers Vimentin and N-cadherin expression, but up-regulating the epithelial marker E-cadherin

expression (**Figure 3A**). Over-expression of CCAT2 in BGC-823 cells markedly inhibited the protein expression of E-cadherin, but up-regulated that of Vimentin and N-cadherin and transcription factor ZEB2, which contributed to promote GC cells EMT (**Figure 3B**). Previous study demonstrated that ZEB2 was a vital EMT inducer through suppressing E-cadherin expression or inducing Vimentin expression in human cancer [15]. Taken together, the present data indicated that CCAT2 could directly promote ZEB2 expression and regulate EMT process in GC cells.

CCAT2 interacts with EZH2, H3K27me3 and LSD1 in GC cells

Recent evidences have reported that lncRNAs recruited polycomb-group proteins to specific loci and repressed gene expression, and some lncRNAs have been shown to physically associate with Polycomb Repressive Complex 2 (PRC2 complex) [16]. Furthermore, the RNA immunoprecipitation was performed and the results showed that the endogenous CCAT2 was

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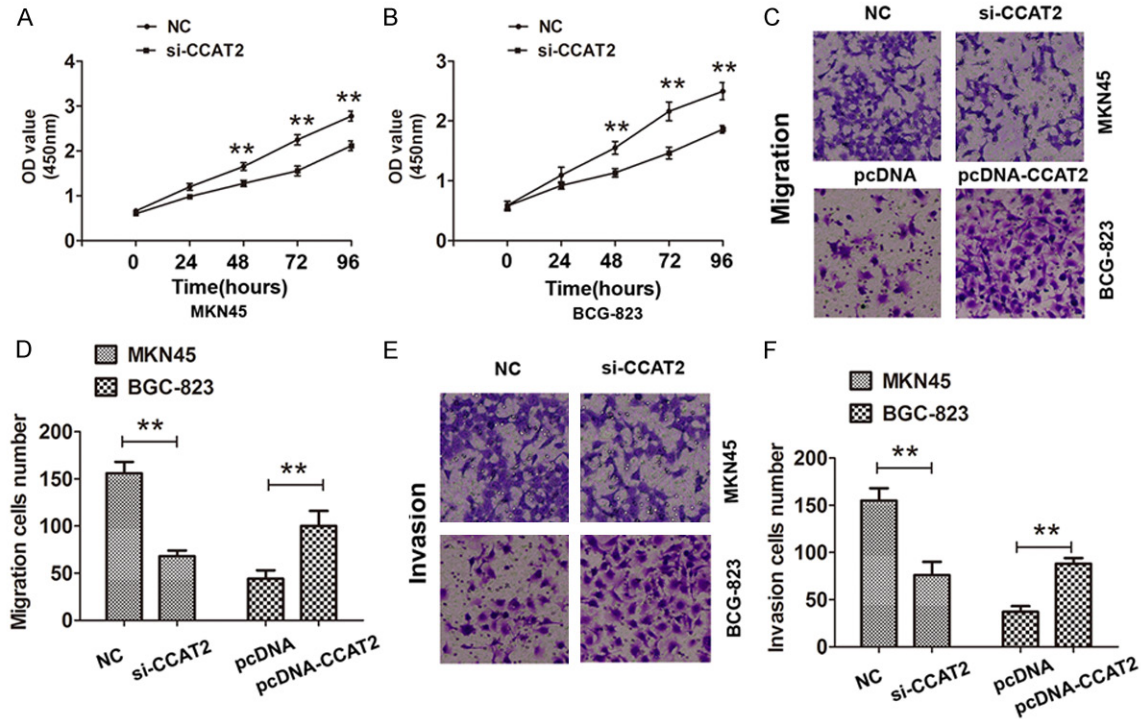


Figure 2. The effects of CCAT2 on GC cell proliferation migration, and invasion *in vitro*. A, B. CCK8 cell proliferation assays were used to determine the cell proliferation abilities in si-CCAT2-transfected MKN45 cells or pcDNA-CCAT2-transfected BCG-823 cells. C, D. Transwell migration assays were used to determine the cell migration abilities in si-CCAT2-transfected MKN45 cells or pcDNA-CCAT2-transfected BCG-823 cells. E, F. Transwell invasion assays were used to determine the cell invasion abilities in si-CCAT2-transfected MKN45 cells or pcDNA-CCAT2-transfected BCG-823 cells. The mean values and S.D were calculated from triplicates of a representative experiment. **P < 0.05.

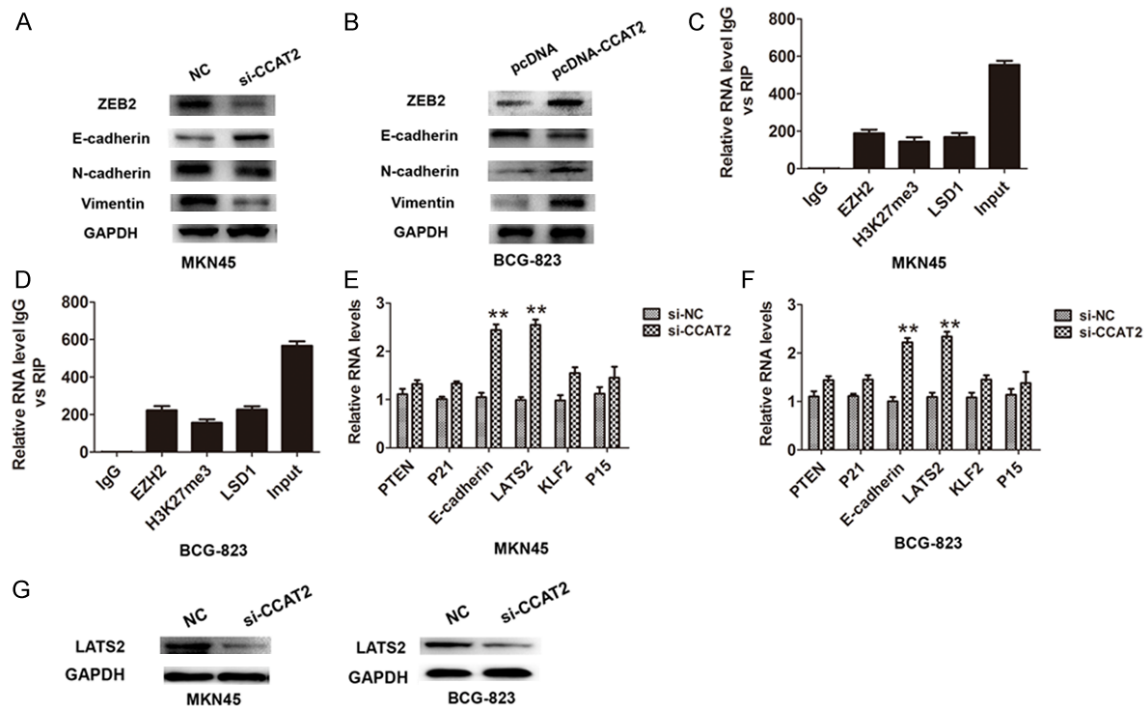


Figure 3. CCAT2 promoted GC cells epithelial-to-mesenchymal-transition and interacted with EZH2, H3k27me3 and LSD1. A, B. Western-blotting analysis was used to detect the protein expression of Transcription factor ZEB2 and

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EMT markers (E-cadherin, N-cadherin and Vimentin) in si-CCAT2-transfected MKN45 cells or pcDNA-CCAT2-transfected BGC-823 cells. C, D. RNA levels in immunoprecipitates with EZH2, LSD1, and H3K27me3 were determined by qRT-PCR analysis in MKN45 and BGC-823 cells. Expression levels of CCAT2 were presented as fold enrichment relative to IgG immunoprecipitate. E, F. The levels of PTEN, P21, E-cadherin, LAST2, KLF2 and P15 mRNA were detected by qRT-PCR after knockdown of CCAT2 in MKN45 and BGC-823 cells. The mean values and S.D were calculated from triplicates of a representative experiment. $**P < 0.05$. G. The protein expression of LAST2 were detected by western-blotting analysis after knockdown of CCAT2 in MKN45 and BGC-823 cells.

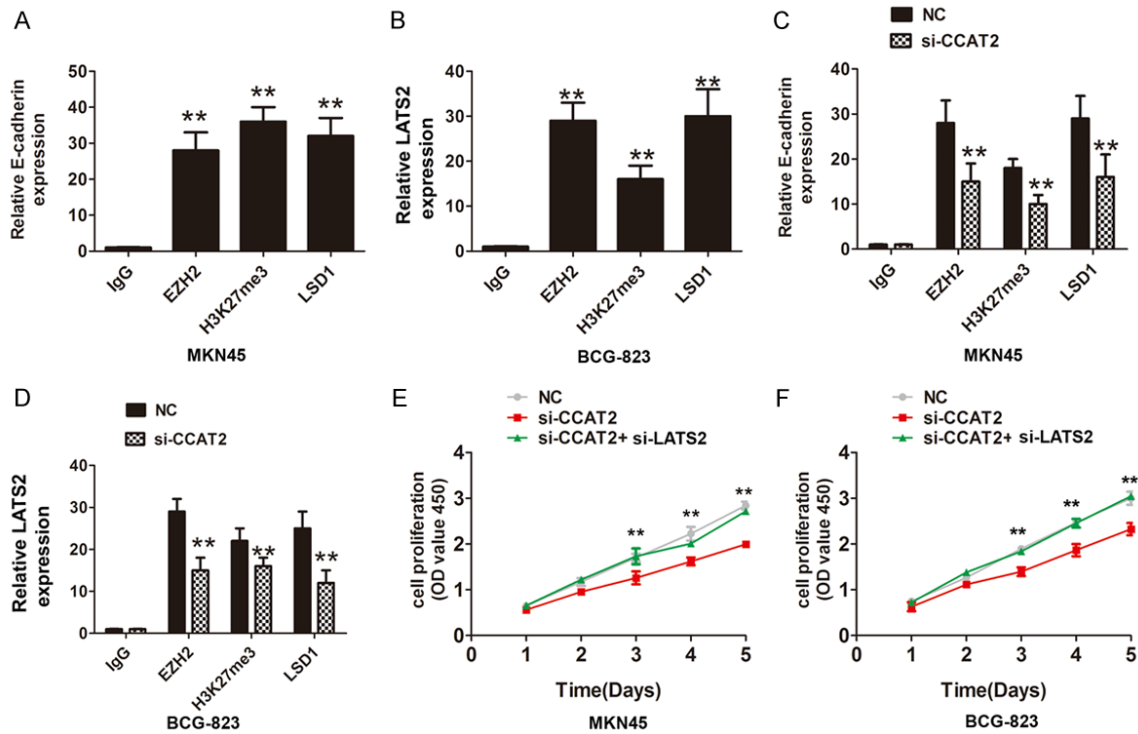


Figure 4. CCAT2 interacted with EZH2, H3k27me3 and LSD1 and repressed the LATS2 expression. A, B. Chromatin immunoprecipitation-qPCR was used to analyze EZH2 and LSD1 occupancy, H3K27me3 binding to E-cadherin and LATS2 promoter regions in MKN45 and BGC-823 cells, C, D. Chromatin immunoprecipitation-qPCR was used to analyze EZH2 and LSD1 occupancy, H3K27me3 binding to the LATS2 promoter regions after knockdown of CCAT2 in MKN45 and BGC-823 cells, the IgG was acted as a negative control. The mean values and S.D were calculated from triplicates of a representative experiment. $**P < 0.05$. E, F. CCK8 cell proliferation assays were performed to evaluate the cell growth when transfected si-NC, si-CCAT2 or si-CCAT2+si-LATS2 in MKN45 cells or BGC-823 cells.

enriched in the anti-EZH2, anti-H3K27me3 and anti-LSD1 immunoprecipitation (RIP) fraction relative to the input compared to the IgG fraction in MKN45 and BGC-823 cells (**Figure 3C, 3D**). Together, our results demonstrated a specific correlation between EZH2, H3K27me3 or LSD1 and CCAT2.

CCAT2 interacts with EZH2, H3k27me3, and LSD1 occupancy and epigenetically regulates the expression of E-cadherin and LATS2

Based on our findings that CCAT2 bound to EZH2, H3K27me3 and LSD1, we detected some targets of EZH2 and found that E-cadherin and LATS2 expression were significantly

up-regulated when CCAT2 was knockdown in MKN45 and BGC-823 cells (**Figure 3E, 3F**). The protein expression analysis confirmed that the expression of LATS2 were also up-regulated in both cells (**Figure 3G**). Furthermore, the E-cadherin and LATS2 mRNA expression levels were also increased after EZH2 silencing in MKN45 and BGC-823 cells (**Figure 4A, 4B**). Further experiments to evaluate whether CCAT2 functioned as a transcriptional repression through EZH2 and targeted the E-cadherin and LATS2 promoters, we performed the ChIP assays and demonstrated that knockdown of CCAT2 significantly decreased the binding of EZH2, H3K27me3 and LSD1 levels across the E-cadherin and LATS2 promoters

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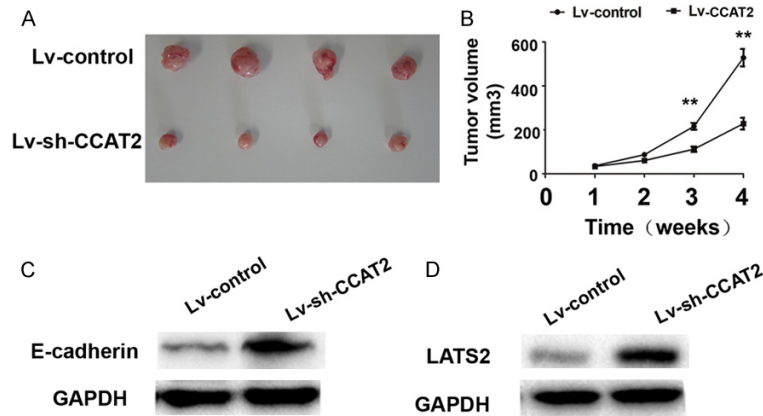


Figure 5. CCAT2 inhibited the tumor growth and up-regulated the expression of E-cadherin and LATS2 in vivo. (A) The stable CCAT2 knockdown MKN45 cells were used for the in vivo study. The nude mice carrying tumors from Lv-shRNA-CCAT2 group and Lv-control group were shown and (B) tumor growth curves were measured per week after the injection of MKN45 cells in Lv-shRNA-CCAT2 group and Lv-control group were shown. (C, D) Western-blotting analysis was performed to detect the expression of E-cadherin and LATS2 in tumor tissues in Lv-shRNA-CCAT2 knockdown and control group. The mean values and S.D were calculated from triplicates of a representative experiment. ** $P < 0.05$.

(Figure 4C, 4D). Moreover, functional experiments also demonstrated that knockdown of CCAT2 inhibited the cell proliferation and the effects were reversed by co-transfection of si-LATS2 and si-CCAT2 into MKN45 and BGC-823 cells. These results indicated that CCAT2 interacted with EZH2, H3k27me3, and LSD1 occupancy and epigenetically modulated the expression of E-cadherin and LATS2.

CCAT2 inhibits the tumor growth and up-regulated the expression of E-cadherin and LATS2 in vivo

To explore the effect of lncRNA CCAT2 on tumor growth in vivo, we constructed stable cell lines by using the lentivirus vector to knockdown CCAT2 in MKN45 cells, the results indicated that the tumor volume in CCAT2 knockdown group was significantly smaller than that in the control group and the tumor growth increased slower in CCAT2 knockdown group than that in the control group (Figure 5A, 5B). Furthermore, we also demonstrated that E-cadherin and LATS2 expression were up-regulated in CCAT2 knockdown group than that in the control group (Figure 5C, 5D). Thus, our results indicated that CCAT2 inhibited the tumor growth and up-regulated the expression of E-cadherin and LATS2 in vivo.

Discussion

LncRNAs play a critical role in cancer development and progression, previously some study reported that abnormally expressed cancer-related lncRNAs were identified involving in GC and affected the development and progression of GC [17, 18]. For example, H19 was up-regulated in GC and promoted cell proliferation by partly inactivating p53, decreasing its activity, and suppressing the expression of the p53 target protein Bax [19]. Xu *et al* reported that high expression level of HOTAIR was a predictor of poor over-all survival in GC patients and suppression of HOTAIR could reverse GC cells EMT process [20]. Other lnc-

RNA such as, SPRY4-IT1 [21], LEIGC [22], TUG1 [23], NEAT1 [24] and so on, were also involving in GC carcinogenesis. In our study, we proved that the expression levels of CCAT2 was up-regulated in GC tissues and higher CCAT2 expression had a significantly poor disease-free survival (DFS) and the over survival (OS) time than those with lower expression in GC patients. Over-expression of CCAT2 promoted the GC cell proliferation, migration and invasion, which indicated the oncogene role of CCAT2 in GC.

Further we found that over-expression of CCAT2 promoted the epithelial-mesenchymal transition (EMT) by up-regulating the ZEB2 expression and inhibiting the E-cadherin expression. Zheng *et al* demonstrated that up-regulation of long non-coding RNA CCAT2 indicated a poor prognosis for prostate cancer and promoted metastasis by affecting epithelial-mesenchymal transition [25], which was consistent with our findings. Moreover, our results revealed the potential Molecular Mechanisms that CCAT2 interacted with EZH2, H3k27me3 and LSD1 occupancy and epigenetically regulated the expression of E-cadherin and LATS2. Recent studies had identified many promoter-associated RNAs that altered gene transcription through interaction with protein complexes.

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Several studies have suggested that the Lnc-RNAs were related to interact with EZH2 and inhibited EZH2 related target genes. Kong *et al* reported that Long non-coding RNA PVT1 indicated a poor prognosis of gastric cancer and promoted cell proliferation through epigenetically regulating p15 and p16 [26]. Liu *et al* reported that HOTAIR epigenetically silenced miR34a by binding to PRC2 to promote the epithelial-to-mesenchymal transition in human gastric cancer [27]. Long non-coding RNA HOXA-AS2 also been indicated to promote gastric cancer proliferation by epigenetically silencing P21/PLK3/DDIT3 expression [28]. Our results demonstrated that CCAT2 also bound to EZH2 and LSD1 and promoted GC progression through epigenetically regulating E-cadherin and LAST2.

In conclusion, our results revealed that the lncRNA CCAT2 was over-expression in GC and higher CCAT2 predicted a poor survival outcome in GC patients. Further analysis suggested that the lncRNA CCAT2 recruited EZH2 to repress E-cadherin and LATS2, which promoted gastric cancer progression. To sum up, our study revealed CCAT2 served as an oncogene in GC and provided a therapeutic target for GC patients.

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Disclosure of conflict of interest

None.

Authors' contribution

Yong-Jun Wang and Pei Lv contributed in study concept and design, execution of experiments and writing of the manuscript. Jian-Zhen Liu, Yi Dang and Jiang-Yan Gao performed statistical analysis and interpretation of data. Yong Wang, Yong-Jun Wang and Pei Lv collected tissue samples and clinical data and performed the experiments.

Address correspondence to: Pei Lv, Department of Nephrology, Hebei Chest Hospital, No. 372, Sheng Li Street, Shijiazhuang 050041, Hebei, China. Tel: 86-311-86911243; Fax: 86-311-86911243; E-mail: longpei1252@sina.com

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