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Overexpression of long non-coding RNA colon cancer-associated transcript 2 is associated with advanced tumor progression and poor prognosis in patients with colorectal cancer

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Abstract. The aim of the present study was to explore the clinicopathological and prognostic significance of long non-coding RNA (lncRNA) colon cancer-associated transcript 2 (CCAT2) expression in human colorectal cancer (CRC). Expression levels of lncRNA CCAT2 in CRC, adjacent non-tumor and healthy colon mucosa tissues were detected by quantitative polymerase chain reaction. The disease-free survival and overall survival rates were evaluated using the Kaplan-Meier method, and multivariate analysis was performed using Cox proportional hazard analysis. The expression level of lncRNA CCAT2 in CRC tissues was increased significantly compared with adjacent normal tissues or non-cancerous tissues. CCAT2 expression was observed to be progressively increased between tumor-node-metastasis (TNM) stages I and IV. A high level of CCAT2 expression was revealed to be associated with poor cell differentiation, deeper tumor infiltration, lymph node metastasis, distance metastasis, vascular invasion and advanced TNM stage. Compared with patients with low levels of CCAT2 expression, patients with high levels of CCAT2 expression had shorter disease-free survival and overall survival times. Multivariate analyses indicated that high CCAT2 expression was an independent poor prognostic factor. Therefore, increased lncRNA CCAT2 expression maybe a potential diagnostic biomarker for CRC, and an independent predictor of prognosis in patients with CRC.

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Introduction

Colorectal cancer (CRC) is the third most common type of human malignancy worldwide and the fourth leading cause of cancer-associated mortality (1-3). Despite the significant achievements that have been made in the treatment of early CRC, the long-term survival rate for advanced CRC remains low. The survival rate of CRC may benefit from 5-fluorouracil (5-FU) and oxaliplatin-based adjuvant chemotherapy, which has been accepted as a standard therapy (4,5). However, 20-40% of patients with advanced-stage CRC relapse following primary curative surgery (6,7). This is primarily attributed to the following reasons: Lack of diagnostic markers for early detection, weak prognostic value of histological indicators, limited efficiency of current treatment for advanced diseases and lack of genetic markers utilized for targeted therapy (2). Therefore, the identification of novel genetic markers for the improvement of diagnostic and prognostic techniques is required.

With the development of whole genome sequencing technology, it has been revealed that <2% of the mammalian genome is in protein-encoded regions and the remainder of the genome contains non-coding RNAs (ncRNAs) (8). Those ncRNAs >200 nucleotides in length are termed long non-coding RNAs (lncRNAs) (9). In recent years, lncRNAs have been regarded as a diagnostic biomarkers and prognostic factors (10).

Previous studies have identified a series of lncRNAs with aberrant expression in cancer (11,12). Upregulated lncRNA HOTAIR relative expression in primary tumors and in the blood of patients with CRC is associated with unfavorable prognosis (13). lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been shown to be upregulated in clinical CRC tissue samples (14). High-level MALAT1 expression is an independent prognostic risk factor for patients with CRC (14). One recent study has revealed that lncRNA colon cancer-associated transcript 2 (CCAT2) encompasses the rs6983267 single nucleotide polymorphism (15). Furthermore, lncRNA CCAT2 is highly overexpressed in CRC cell lines and promotes tumor growth, metastasis and chromosomal instability (15). In addition, it was revealed that CCAT2 promotes MYC expression (15).

To the best of our knowledge, the diagnostic and prognostic role of lncRNA CCAT2 expression in CRC remains unclear. In the present study, the levels of CCAT2 expression in clinical CRC tissues were tested. Subsequently, the association of CCAT2 with disease-free survival and overall survival of patients with CRC was analyzed.

Materials and methods

Cohort and tissue samples. According to The Code of Ethics of the World Medical Association, Declaration of Helsinki, the present study was approved by the Ethics Committee of Peking University (Beijing, China). All patients involved provided full consent for the study. All the fresh specimens were obtained from resected colorectal tissues of 218 patients, who were selected from patients diagnosed with CRC between January 2005 and December 2007 at the Department of General Surgery, Peking University First Hospital. A total of 218 patients (113 were male and 105 were female) were included in the study. The mean age of patients was 64 years (range 23-89 years). A total of 36 noncancerous healthy colon mucosa tissues served as controls. All specimens were snap-frozen in liquid nitrogen and kept at -80˚C immediately following surgical resection. All patients with CRC stage II-IV received adjuvant chemotherapy based on 5-FU. Patients with the following criteria were subsequently excluded: Received treatment prior to surgery, including neoadjuvant chemotherapy or neoadjuvant radiotherapy; had a diagnosis of additional malignant disease; and harvested insufficient specimens for RNA isolation. Clinicopathological information and follow-up data of all patients were entered into a database that was updated with respect to survival status every 3 months. All the specimens were histologically diagnosed by the Department of Pathology according to the criteria of the World Health Organization classification and the tumor-node-metastasis (TNM) stage set out by American Joint Committee on Cancer (16).

Total RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA from cancer tissues or adjacent normal tissues or non-cancerous tissues was isolated using TRIzol® according to the manufacturer's procedure (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and stored at -80˚C. RNA concentration and purity was measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc.). The OD260/280 ratios for all samples were between 1.8 and 2.0. RNA integrity was determined using Agilent 2100 Bioanalyzer, with RNA 6000 Nano Assay Chip (Agilent Technologies Inc., Santa Clara, CA, USA). The RNA integrity of all samples ranged between 6.0 and 10.0. cDNA was synthesized from 500 ng total RNA using the Promega A3500 Reverse Transcription System kit (Promega Corporation, Madison, WI, USA) with random hexamer primers in a final volume of 40 μl, according to the manufacturer's protocol. The cDNA was stored at -20˚C. RT-qPCR was performed using the SYBR Premix Ex TagTM II (Takara Biotechnology Co., Ltd., Dalian, China) and ABI 7300 System (Applied Biosystems; Thermo Fisher Scientific Inc.). The primers were as follows: CCAT2 forward, 5'-CCC TGGTCAAATTGCTTAACCT-3' and reverse, 5'-TTATTC GTCCCTCTGTTTTATGGAT-3'; and GAPDH forward, 5'-CCACATCGCTCAGACACCAT-3' and reverse, 5'-ACC AGGCGCCCAATACG-3'. The PCRs were performed in a total volume of 30 μl, containing 30 ng of cDNA for each sample. The cycling program was set for initial hold at 95˚C for 10 min, followed by 40 cycles of denaturation at 95˚C for 15 sec, annealing and extension at 50˚C for 30 sec and 72˚C for 15 sec. The relative expression of CCAT2 was analyzed using the $2^{-\Delta\Delta Cq}$ method (17). All fluorescent data were converted into relative quantitation (RQ; relative expression obtained by $2^{-\Delta\Delta Cq}$ method) measurements. CCAT2 levels were normalized to GAPDH. Sequencing of randomly selected qPCR products was utilized to ensure the quality of qPCR; sequencing services were provided by BBI Life Sciences Co. (Shanghai, China).

Measurement of disease-free survival and overall survival. Disease-free survival time was defined as the time elapsed between surgery and the first occurrence of any of the following events: Relapse of CRC, distant metastasis of CRC or mortality from any cause without documentation of a cancer-associated event. The diagnosis of relapse and distant metastasis was based on imaging methods, including computed tomography, magnetic resonance imaging or position emission tomography, and if possible, biopsy or cytological analysis. Overall survival time was defined as the time elapsed between surgery and mortality of patients with CRC.

Statistical analysis. Statistical analysis was performed using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation. The levels of CCAT2 expression from different tissues were analyzed by one-way analysis of variance followed by Bonferroni multiple comparisons. Associations between CCAT2 expression and categorical variables were analyzed by Pearson's χ^2 test or Fisher's exact test, as appropriate. Survival curves were estimated using the Kaplan-Meier method, and differences in survival distributions were evaluated by the log-rank test. Cox's proportional hazards modeling of factors potentially associated with survival was performed in order to identify factors that may have a significant independent effect on survival. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of patients. The characteristics of patients are shown in Table I. In total, there were 113 male (51.83%) and 105 female (48.17%) patients. There were 136 tumors (62.39%) located in the colon and 82 tumors (37.61%) located in the rectum. The tumor size of 70 patients (32.11%) with CRC was <3 cm and that of 148 patients (67.89%) was ≤3 cm. Poorly- and moderately-differentiated tumors were the most common histological type (71.10%), followed by well-differentiated (28.90%) tumors. According to the International TNM Classification, 32 (14.68%), 56 (25.69%), 89 (40.83%) and 41 (18.81%) of 218 patients with CRC were classified as TNM stages I, II, III and IV, respectively (Table I).

lncRNA CCAT2 is significantly upregulated in CRC tissues. The levels of lncRNA CCAT2 expression were detected in all

Table I. Statistical results of CCAT2 expression.

Parameters	No. (%)	CCAT2 expression, n (%)		P-value
		Low	High	
Total	218 (100.00)	89 (100.00)	129 (100.00)	
Sex				0.7839
Male	113 (51.83)	45 (39.82)	68 (60.18)	
Female	105 (48.17)	44 (41.9)	61 (58.1)	
Age, years				0.1592
<60	87 (39.91)	41 (47.13)	46 (52.87)	
≥60	131 (60.09)	48 (36.64)	83 (63.36)	
Location				0.7762
Colon	136 (62.39)	57 (41.94)	79 (58.06)	
Rectum	82 (37.61)	32 (39.02)	50 (60.98)	
Smoking status				0.8873
Never smoked	135 (61.92)	56 (41.48)	79 (58.51)	
Smoker	83 (38.08)	33 (39.76)	50 (60.24)	
BMI, kg/m^2				0.5150
<25	112 (51.38)	44 (39.29)	58 (60.71)	
≥25	106 (48.62)	45 (42.45)	71 (57.54)	
Diameter, cm				0.1186
<3	70 (32.11)	36 (51.43)	39 (48.57)	
≥3	148 (67.89)	53 (35.81)	90 (64.19)	
Differentiation				0.0006
High	63 (28.90)	37 (58.73)	26 (41.27)	
Poor/moderate	155 (71.10)	53 (34.19)	103 (65.81)	
TNM stage				<0.0001
I/II	88 (40.37)	65 (73.86)	23 (26.14)	
III/IV	130 (59.63)	24 (18.46)	106 (81.54)	
Tumor infiltration				<0.0001
T1/T2	36 (16.51)	28 (77.78)	8 (22.22)	
T3/T4	182 (83.49)	61 (33.52)	121 (66.48)	
Lymph node metastasis				<0.0001
N0	76 (34.86)	59 (77.63)	17 (32.37)	
N1-N3	142 (66.14)	30 (21.23)	112 (78.77)	
Distant metastasis				<0.0001
M0	167 (76.61)	80 (47.90)	87 (52.10)	
M1	51 (23.39)	9 (17.65)	42 (82.35)	
Vascular invasion				<0.0001
Absent	112 (51.38)	61 (54.46)	51 (45.54)	
Present	108 (49.54)	28 (25.93)	78 (74.07)	

CCAT2, colon cancer-associated transcript 2; BMI, body mass index; TNM, tumor-node-metastasis.

human tissues by qPCR. Following normalization to GAPDH expression, the levels of lncRNA CCAT2 expression in CRC tissues were significantly increased compared with those of adjacent non-tumor tissues or noncancerous mucosa tissues (P<0.001; Fig. 1A). The RQ of lncRNA CCAT2 in CRC samples was 3.91±2.37, whereas the relative CCAT2 expression detected in matched adjacent normal tissues was 1.00±0.47, and that of noncancerous colon mucosa tissues was 0.86±0.44. The CCAT2 expression levels of cancer tissues from patients with stage III and IV CRC were significantly higher than those from patients with stage I and II CRC (P<0.001; Fig. 1B).

Association between lncRNA CCAT2 expression and clinicopathological characteristics of CRCs. On the basis of these aforementioned data, the mean expression level of lncRNA CCAT2 in CRC tissues (3.91) was used as a cutoff point to

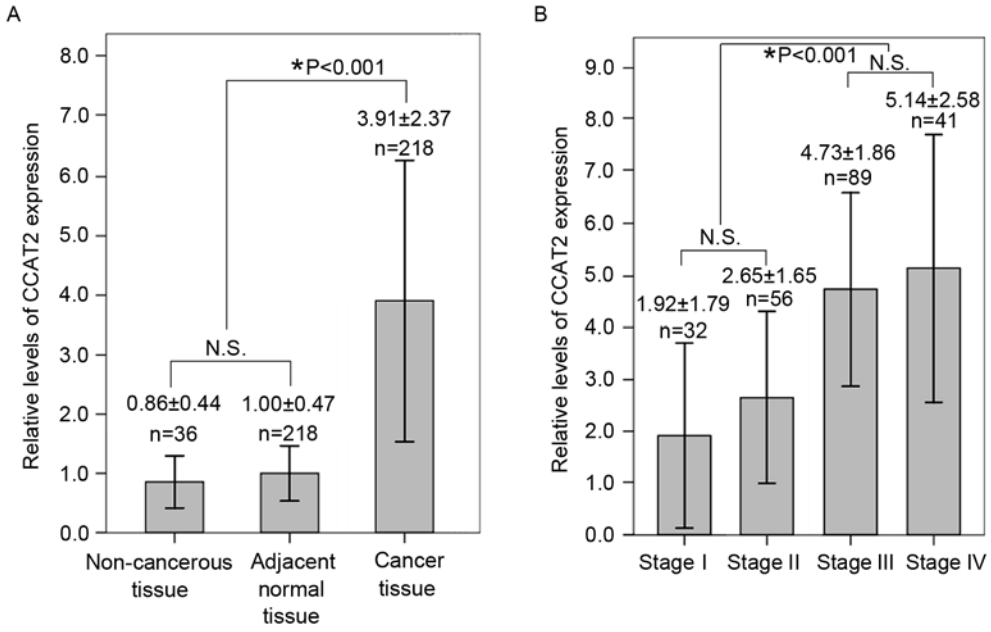


Figure 1. The level of lncRNA CCAT2 expression was upregulated in CRC tissues. (A) The level of lncRNA CCAT2 expression in all human tissues was detected by quantitative polymerase chain reaction. lncRNA CCAT2 expression levels were normalized to GAPDH. (B) The levels of lncRNA CCAT2 expression in CRC tissues were stratified by TNM stages of primary cancers. Results are presented as the mean ± standard deviation. *P<0.001. CCAT2, colon cancer-associated transcript 2; lncRNA, long non-coding RNA; CRC, colorectal cancer; N.S., not significant.

classify all 218 cancerous tissues into two groups: Low expression (n=98) and high expression (n=120). The associations between CCAT2 expression levels and different clinicopathological factors are shown in Table I. No statistically significant associations were observed between CCAT2 expression and sex, age at diagnosis, tumor location, smoking status, body mass index (BMI) status and tumor size. Statistically significant associations were observed between high CCAT2 expression and low degree of tumor cell differentiation (P=0.0006), advanced TNM staging and deeper tumor infiltration, high incidence of lymph node metastasis, distant metastasis and vascular invasion (all P<0.0001).

Association between lncRNA CCAT2 expression and disease-free survival of patients with CRC. Kaplan-Meier analysis revealed that patients with low lncRNA CCAT2 expression had increased disease-free survival times compared with those with high lncRNA CCAT2 expression (P<0.001; Fig. 2A). In addition, patients with poor differentiation, tumor diameter >3 cm, vascular invasion, deeper tumor infiltration, lymph node metastasis, distant metastasis or advanced TNM stage had shorter disease-free survival times and an increased risk of relapse compared with those without. However, other clinical parameters had no prognostic value on disease-free survival of patients with CRC. Unadjusted risk ratios (RR) are shown in Table II.

Kaplan-Meier analysis was also performed with stratification by TNM stage. Since TNM stage IV tumors were defined as tumors with distant metastasis, and itself was a marker of high risk, patients were classified into groups of TNM I, II and III tumors, respectively. Results demonstrated that the levels of lncRNA CCAT2 expression were associated with disease-free survival in three groups of patients with TNM I, II and III CRC. Disease-free survival was significantly shorter in patients with high CCAT2 expression vs. low CCAT2 expression (Fig. 2B-D). In addition, both colon cancer and rectal cancer patients with low CCAT2 expression had more favorable disease-free survival compared with those with high CCAT2 expression (Fig. 2E and F).

To verify the independent prognostic value of CCAT2 expression, the Cox proportional hazards model adjusted for the presence of other clinical parameters was utilized to control for other prognostic factors. As a result, the level of CCAT2 expression was shown to be an independent prognostic factor, subsequent to controlling for all other clinical parameters. Adjusted RR was 1.00 (as a reference) in low CCAT2 expression patients, while the adjusted RR of patients with CRC with a high level of CCAT2 expression was 1.561 (P=0.030; Table II). Thus, CCAT2 maybe an independent prognostic factor of disease-free survival for patients with CRC. In addition, vascular invasion, lymph node metastasis, distant metastasis and TNM stages were also revealed to be independent prognostic factors for disease-free survival of patients with CRC (Table II).

Association between lncRNA CCAT2 expression and overall survival of patients with CRC. A statistically significant association between high level of CCAT2 expression and poor overall survival times was observed in patients with CRC (P<0.001; Fig. 3A). Kaplan-Meier analysis revealed that patients with CRC with high CCAT2 expression had shorter overall survival times compared with patients with a low level of CCAT2 expression (Fig. 3A; P<0.001). Similar to results of disease-free survival, compared with other patients, patients with poor differentiation, deeper tumor infiltration, increased incidence of lymph node metastasis, distant metastasis, vascular invasion and advanced TNM stage experienced shorter overall survival times compared with those without. However, other clinical factors had no prognostic value on

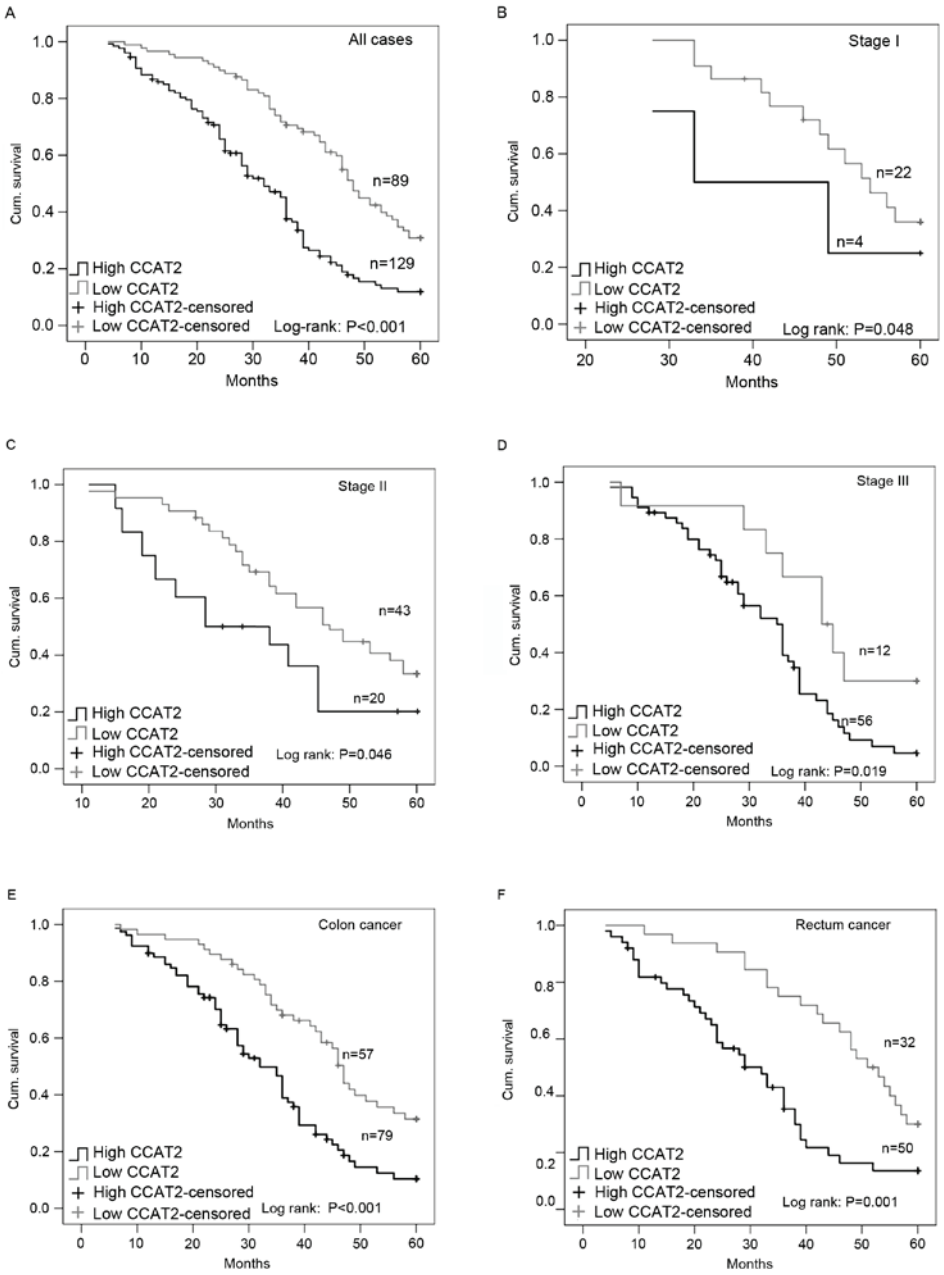


Figure 2. Association between lncRNA CCAT2 expression and disease-free survival: (A) All cases; (B) TNM stage I tumors; (C) TNM stage II tumors; (D) TNM stage III tumors; (E) colon cancers; and (F) rectum cancers. TNM, tumor-node-metastasis; CCAT2, colon cancer-associated transcript 2.

overall survival of patients with CRC. Unadjusted RR values are shown in Table III.

With regard to TNM stage, significant association with overall survival was observed in patients with high CCAT2 expression vs. those with low CCAT2 expression with TNM I, II and III tumors (P<0.05; Fig. 3B-D). In all three groups, patients with high expression of CCAT2 had shorter overall survival times than patients with low expression of CCAT2 (Fig. 3B-D). With regard to tumor location, patients with colon cancer and patients with rectal cancer with high CCAT2 expression had shorter overall survival times compared with those with high CCAT2 expression (Fig. 3E and F). Multivariate analysis revealed that CCAT2 expression may be a prognostic factor for overall survival of patients with CRC, independent of other clinical parameters. The adjusted RR of CRC patients with high level CCAT2 expression was 1.584 (P=0.044; Table III). In addition, TNM stages, lymph node metastasis and distant metastasis were also shown to be independent prognostic factors, subsequent to controlling for all other clinical parameters (Table III).

Discussion

The incidence of CRC has been increasing in the last few decades, particularly in China; CRC has >340,000 newly diagnosed cases and leads to >80,000 mortalities each year in China, and ranks as the fifth cause of cancer-associated mortalities among all malignant diseases (18).

Current clinical TNM staging systems often fail to discriminate the biological features of a number of tumors (19).

Table II. Disease-free risk ratio.

	Univariate		Multivariate	
Parameters	Unadjusted RR (95% CI)	P-value	Adjusted RR (95% CI)	P-value
CCAT2	2.340 (1.677-3.264)	<0.001	1.561 (1.045-2.331)	0.030
Sex	1.162 (0.849-1.592)	0.348	1.111 (0.798-1.548)	0.533
Age, years	1.159 (0.840-1.598)	0.369	0.994 (0.696-1.419)	0.972
Location	0.901 (0.655-1.238)	0.518	1.013 (0.721-1.424)	0.939
Smoking status	0.913 (0.689-1.427)	0.891	0.987 (0.616-1.299)	0.808
BMI status, kg/m^2	1.038 (0.758-1.422)	0.815	1.003 (0.713-1.412)	0.984
Tumor diameter, cm	1.462 (1.036-2.064)	0.031	1.380 (0.918-2.073)	0.121
Differentiation	1.445 (1.022-2.042)	0.037	1.119 (0.725-1.727)	0.613
TNM stage	1.482 (1.065-2.064)	0.020	0.616 (0.375-1.011)	0.045
Tumor infiltration	1.707 (1.111-2.625)	0.015	0.954 (0.524-1.739)	0.878
Lymph node metastasis	2.351 (1.658-3.332)	<0.001	1.767 (1.128-2.769)	0.013
Distant metastasis	2.578 (1.792-3.710)	<0.001	2.376 (1.557-3.625)	<0.001
Vascular invasion	1.766 (1.286-2.426)	<0.001	1.573 (1.125-2.200)	0.008

RR, risk ratio; CI, confidence interval; TNM, tumor-node-metastasis; CCAT2, colon cancer-associated transcript 2; BMI, body mass index.

Table III. Overall risk ratio.

	Univariate		Multivariate	
Parameters	Unadjusted RR (95% CI)	P-value	Adjusted RR (95% CI)	P-value
CCAT2 expression	2.387 (1.663-3.424)	<0.001	1.584 (1.013-2.477)	0.044
Sex	0.920 (0.660-1.281)	0.620	0.886 (0.627-1.252)	0.493
Age, years	1.207 (1.106-1.481)	0.085	1.196 (0.821-1.744)	0.351
Location	0.877 (0.620-1.239)	0.456	0.872 (0.612-1.241)	0.446
Smoking status	0.891 (0.658-1.347)	0.869	0.831 (0.601-1.278)	0.727
BMI, kg/m^2	1.194 (0.858-1.661)	0.293	1.071 (0.747-1.535)	0.708
Tumor diameter, cm	0.991 (0.701-1.402)	0.960	0.872 (0.612-1.241)	0.253
Differentiation	1.727 (1.173-2.542)	0.006	1.468 (0.927-2.327)	0.102
TNM stage	1.501 (1.265-1.948)	0.048	0.413 (0.249-0.684)	0.001
Tumor infiltration	2.819 (1.905-4.174)	<0.001	1.691 (0.860-3.327)	0.128
Lymph node metastasis	2.819 (1.905-4.174)	<0.001	2.136 (1.314-3.472)	0.002
Distant metastasis	2.533 (1.761-3.645)	<0.001	2.032 (1.328-3.108)	0.001
Vascular invasion	1.605 (1.151-2.239)	0.005	1.334 (0.942-1.889)	0.104

RR, risk ratio; CI, confidence interval; BMI, body mass index; TNM, tumor-node-metastasis; CCAT2, colon cancer-associated transcript 2.

Patients with the same stage of disease even exhibited a marked discrepancy in prognosis and survival (19). Genetic alterations involved in cancer recurrence and outcome may serve as biomarkers for early detection of metastasis and as a measure for therapeutic intervention (1). In previous years, a number of molecules have been used for the prediction of the prognosis of patients with CRC, but their roles in determining the individual risk level of the patient are limited (20). Therefore, identification of new prognostic markers remains important for the prevention and treatment of CRC (21).

More than 50% of patients have a diagnosis of stage II or III tumors (1,22). Following curative surgery, patients with stage III CRC experience 50-60% chance of developing recurrence (23). 5-FU-based adjuvant chemotherapy has been accepted as a standard therapy for patients with stage III CRC, and it has been demonstrated that the overall survival rate of these patients benefit from it (7,24). Considering that the role of neoadjuvant chemotherapy for CRC is also controversial, and neoadjuvant therapy may affect the level of CCAT2 expression, patients who received neoadjuvant therapy were excluded.

Early studies proposed that lncRNAs may be simply transcriptional noise (25). However, recent studies have revealed that numerous lncRNAs perform important roles

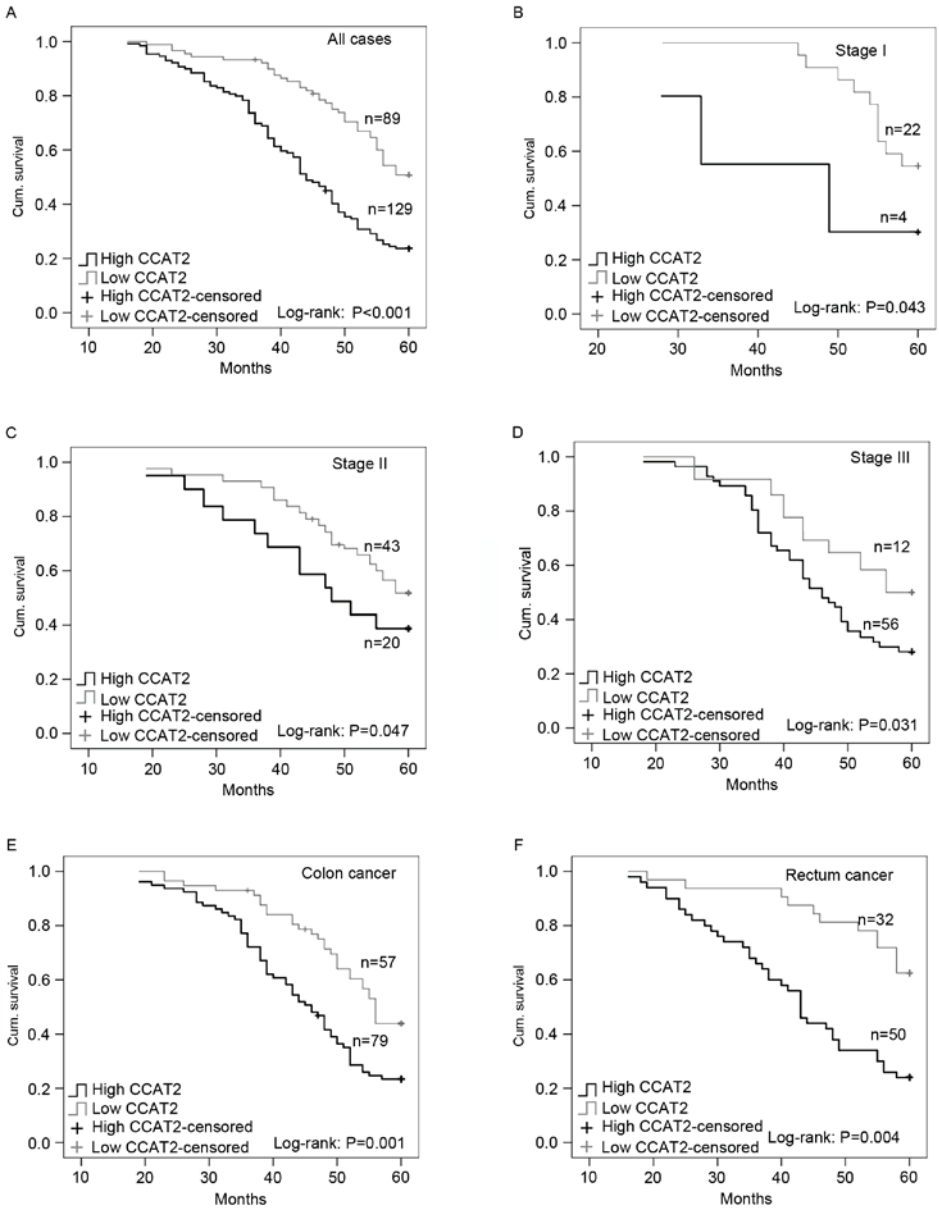


Figure 3. Association between lncRNA CCAT2 expression and overall survival: (A) All cases; (B) TNM stage I tumors; (C) TNM stage II tumors; (D) TNM stage III tumors; (E) colon cancers; and (F) rectum cancers. CCAT2, colon cancer-associated transcript 2; TNM, tumor-node-metastasis.

in the regulation of gene transcription, cell differentiation, genetic and epigenetic and other cellular activities (10,25,26). lncRNAs are considered to be new tumor biomarkers for cancer diagnosis and prognosis (8). The latest studies have demonstrated that the lncRNA CCAT2 promotes the MYC and Wnt signaling pathways in CRC in a positive feedback loop model (15). Furthermore, it is considered that MYC induces 5-FU-based chemoresistance (27). Regarding these findings, CCAT2 overexpression may induce chemoresistance.

The present study focused on the association of lncRNA CCAT2 expression with clinicopathological features and prognosis of patients with CRC. Results of the present study uncovered that lncRNA CCAT2 expression in CRC tissues was significantly increased compared with that in adjacent non-tumor tissues or in noncancerous healthy colon tissues. The qPCR results revealed that CCAT2 expression progressively increased between TNM stage I and IV. These results supported the hypothesis that CCAT2 facilitates carcinogenesis and is associated with CRC progression.

In addition, statistical analysis demonstrated that high CCAT2 expression was associated with poor cell differentiation, deeper tumor infiltration, vascular invasion, lymph node metastasis and distance metastasis, which indicated that CCAT2 upregulation performs an important role in CRC progression. However, CCAT2 expression was not associated with age, sex, BMI status, smoking status or tumor size.

The primary goal of the present study was to determine whether the CCAT2 expression level in primary CRC could predict disease relapse. In the present cohort, it was demonstrated that high levels of CCAT2 expression were associated with unfavorable disease-free survival and overall survival. The prognostic value of CCAT2 expression for disease-free survival and overall survival was statistically significant in univariate and multivariate analyses.

Kaplan-Meier analysis revealed that the patients with CRC with a high level of CCAT2 expression had shorter disease-free survival and overall survival times compared with patients with CRC with a low level of CCAT2 expression. Multivariate analysis revealed that patients with a high level of CCAT2 expression had an increased risk of relapse and mortality. Although survival patterns of colon cancer and rectum cancer were found to be different, sub-analysis stratified by primary cancer location showed that these findings for overall CRC could also be applied to colon cancer and rectal cancer separately. The Kaplan-Meier analysis stratified by TNM stage demonstrated that CCAT2 expression was associated with disease-free and overall survival in patients with TNM stage I-III tumors.

Thus far, there is no definitive approach to predict which patients will develop recurrent disease. Prolongation of disease-free survival means prevention or delay of recurrence or metastasis. In this regard, results of the present study demonstrated that measurements of CCAT2 expression may be helpful to identify patients who were at high risk of early recurrence or metastasis. Therefore, this may contribute to a tailored treatment regime for individual patients, thus preventing patients from receiving insufficient or excessive adjuvant treatment.

In summary, the present study demonstrated that lncRNA CCAT2 expression may be an independent biomarker for CRC diagnosis. Furthermore, CCAT2 may be a prognostic marker to evaluate recurrence, early metastasis and prognosis of CRC, and it also may be a potential therapeutic target in molecular therapy.

Acknowledgements

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References

1. Brenner H, Kloor M and Pox CP: Colorectal cancer. Lancet 383: 1490-1502, 2014.
2. Fedirko V, Romieu I, Aleksandrova K, Pischon T, Trichopoulos D, Peeters PH, Romaguera-Bosch D, Bueno-de-Mesquita HB, Dahm CC, Overvad K, *et al*: Pre-diagnostic anthropometry and survival after colorectal cancer diagnosis in Western European populations. Int J Cancer 135: 1949-1960, 2014.
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
4. Gustavsson B, Carlsson G, Machover D, Petrelli N, Roth A, Schmoll HJ, Tveit KM and Gibson F: A review of the evolution of systemic chemotherapy in the management of colorectal cancer. Clin Colorectal Cancer 14: 1-10, 2015.
5. Teufel A, Gerken M, Hartl J, Itzel T, Fichtner-Feigl S, Stroszczynski C, Schlitt HJ, Hofstädter F and Klinkhammer-Schalke M: Benefit of adjuvant chemotherapy in patients with T4 UICC II colon cancer. BMC Cancer 15: 419, 2015.
6. Barton MK: Oxaliplatin in the adjuvant treatment of colon cancer. CA Cancer J Clin 62: 3-4, 2012.
7. Wolpin BM, Meyerhardt JA, Mamon HJ and Mayer RJ: Adjuvant treatment of colorectal cancer. CA Cancer J Clin 57: 168-185, 2007.
8. Angrand PO, Vennin C, Le Bourhis X and Adriaenssens E: The role of long non-coding RNAs in genome formatting and expression. Front Genet 6: 165, 2015.
9. Esteller M: Non-coding RNAs in human disease. Nat Rev Genet 12: 861-874, 2011.
10. Xie X, Tang B, Xiao YF, Xie R, Li BS, Dong H, Zhou JY and Yang SM: Long non-coding RNAs in colorectal cancer. Oncotarget 7: 5226-5239, 2016.
11. Deng Q, He B, Gao T, Pan Y, Sun H, Xu Y, Li R, Ying H, Wang F, Liu X, *et al*: Up-regulation of 91H promotes tumor metastasis and predicts poor prognosis for patients with colorectal cancer. PLoS One 9: e103022, 2014.
12. Yu H, Xu Q, Liu F, Ye X, Wang J and Meng X: Identification and validation of long noncoding RNA biomarkers in human non-small-cell lung carcinomas. J Thorac Oncol 10: 645-654, 2015.
13. Svoboda M, Slyskova J, Schneiderova M, Makovicky P, Bielik L, Levy M, Lipska L, Hemmelova B, Kala Z, Protivankova M, *et al*: HOTAIR long non-coding RNA is a negative prognostic factor not only in primary tumors, but also in the blood of colorectal cancer patients. Carcinogenesis 35: 1510-1515, 2014.
14. Zheng HT, Shi DB, Wang YW, Li XX, Xu Y, Tripathi P, Gu WL, Cai GX and Cai SJ: High expression of lncRNA MALAT1 suggests a biomarker of poor prognosis in colorectal cancer. Int J Clin Exp Pathol 7: 3174-3181, 2014.
15. Ling H, Spizzo R, Atlasi Y, Nicoloso M, Shimizu M, Redis RS, Nishida N, Gafà R, Song J, Guo Z, *et al*: CCAT2, a novel noncoding RNA mapping to 8q24, underlies metastatic progression and chromosomal instability in colon cancer. Genome Res 23: 1446-1461, 2013.
16. Edge SB and Compton CC: The American Joint Committee on Cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 17: 1471-1474, 2010.
17. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
18. Zheng R, Zeng H, Zhang S, Chen T and Chen W: National estimates of cancer prevalence in China, 2011. Cancer Lett 13: 00620-00625, 2015.
19. Brenner H, Kloor M and Pox CP: Colorectal cancer. Lancet 383: 1490-1502, 2014.
20. Li W, Qiu T, Ling Y, Guo L, Li L and Ying J: Molecular pathological epidemiology of colorectal cancer in Chinese patients with KRAS and BRAF mutations. Oncotarget 6: 39607-39613, 2015.
21. Carethers JM: Biomarker-directed Targeted therapy in colorectal cancer. J Dig Cancer Rep 3: 5-10, 2015.
22. Tsikitis VL, Larson DW, Huebner M, Lohse CM and Thompson PA: Predictors of recurrence free survival for patients with stage II and III colon cancer. BMC cancer 14: 336, 2014.
23. Rodriguez-Moranta F, Saló J, Arcusa A, Boadas J, Piñol V, Bessa X, Batiste-Alentorn E, Lacy AM, Delgado S, Maurel J, *et al*: Postoperative surveillance in patients with colorectal cancer who have undergone curative resection: A prospective, multicenter, randomized, controlled trial. J Clin Oncol 24: 386-393, 2006.
24. Schmoll HJ, Tabernero J, Maroun J, de Braud F, Price T, Van Cutsem E, Hill M, Hoersch S, Rittweger K and Haller DG: Capecitabine plus oxaliplatin compared with fluorouracil/folinic acid as adjuvant therapy for stage III colon cancer: Final results of the NO16968 randomized controlled phase III trial. J Clin Oncol 33: 3733-3740, 2015.
25. Schmitt AM and Chang HY: Long Noncoding RNAs in Cancer Pathways. Cancer Cell 29: 452-463, 2016.
26. Meseure D, Drak Alsibai K, Nicolas A, Bieche I and Morillon A: Long noncoding RNAs as new Architects in cancer epigenetics, prognostic biomarkers, and potential therapeutic targets. Biomed Res Int 2015: 320214, 2015.
27. Kugimiya N, Nishimoto A, Hosoyama T, Ueno K, Enoki T, Li TS and Hamano K: The c-MYC-ABCB5 axis plays a pivotal role in 5-fluorouracil resistance in human colon cancer cells. J Cell Mol Med 19: 1569-1581, 2015.