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Increased expression of long non-coding RNA CCAT2 predicts poorer prognosis in patients with hepatocellular carcinoma

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Abstract

Background: Long non-coding RNA colon cancer-associated transcript 2 (CCAT2) is a 1752-bp lncRNA transcribed from m8q24 genomic region. A lot of investigations have confirmed the involvement of CCAT2 in the tumorigenesis of many cancer types. Previous studies found that over-expression of CCAT2 significantly promoted cell migration and proliferation, and inhibited apoptosis of HCC cells. In the present investigation, the clinical value and prognostic significance of CCAT2 were investigated.

Methods: The 122 pairs of HCC tissues and adjacent normal liver tissues were acquired between September 2013 and February 2018. The expression levels of CCAT2 in HCC tissues and their corresponding adjacent normal liver tissues were examined by RT-qPCR analysis. Survival was calculated using the Kaplan-Meier method and analyzed using the log-rank test. Independent prognostic indicators were determined in the multivariate analysis using Cox's proportional hazard model.

Results: CCAT2 expression levels were significantly increased in HCC tissues compared to that in their normal counterparts ($P < .001$). CCAT2 expression was significantly correlated with vascular invasion ($P = .001$), histopathologic grading ($P = .001$), distant metastasis ($P = .002$) and TNM stage ($P = .018$). A Kaplan–Meier survival curve showed that the overall survival rate of HCC patients in high CCAT2 expression group markedly decreased as compared with that of low CCAT2 expression group ($P = .016$). In addition, COX multivariate analysis showed that high expression of CCAT2 was an independent risk factor for predicting shorter overall survival time in HCC (HR = 2.126, 95%CI:1.273–8.775, $P = .021$).

Conclusions: Taken together, this research revealed that lncRNA CCAT2 may serve as a potential biomarker for predicting overall survival time in HCC.

Abbreviations: CCAT2 = colon cancer-associated transcript 2, CRC = colorectal cancer, ESCC = esophageal squamous cell carcinoma, HCC = hepatocellular carcinoma, lncRNAs = long noncoding RNAs, NSCLC= non-small cell lung cancer, RCC = renal cell carcinoma.

Keywords: HCC, hepatocellular carcinoma, long non-coding RNA CCAT2, prognosis

1. Introduction

Hepatocellular carcinoma (HCC) ranks as the fourth most common malignance and the third leading cause of cancer-associated mortality worldwide.[1] The underlying pathological mechanisms of HCC may be very complicated, though most HCC cases may be attributed to chronic infection with hepatitis B or C virus.[2,3] Even after surgical resection, the 5-year survival rate of HCC patients remains poor, owing to high rates of metastasis and recurrence.[4] To date, there are few reliable markers available to accurately predict prognosis of HCC. Therefore, there is a strong need to identify novel biomarkers that better conduct the option of treatments and predict overall survival of HCC patients.

Long noncoding RNAs (lncRNAs) are a class of noncoding RNA, which is longer than 200 nucleotides in length.[5,6] Accumulating evidence has demonstrated that lncRNAs play important roles in various physiological and pathological processes.[7] lncRNAs have been proved to play important roles as oncogenes or tumor suppressor genes in different cancer types.[8–10] Current researches have found that lncRNA abnormal expression profile has close relationships with HCC developments.

Long non-coding RNA colon cancer-associated transcript 2 (CCAT2) is a 1752-bp lncRNA transcribed from m8q24 genomic region. A lot of investigations have confirmed the involvement of CCAT2 in the tumorigenesis of many cancer types, including pancreatic ductal adenocarcinoma, endometrial cancer, renal cell carcinoma(RCC), breast cancer, gastric cancer, non-small cell lung cancer(NSCLC), glioma, ovarian cancer, esophageal squamous cell carcinoma(ESCC), and colorectal cancer (CRC).[11–22] Previously, Liu et al found that CCAT2 promoted HCC cells proliferation and metastasis through up-regulation of NDRG1.[23] Zhou et al found that CCAT2 was upregulated in

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human HCC tissues and human HCC cell lines. Furthermore, the over-expression of CCAT2 using a synthesized vector significantly promoted cell migration and proliferation, and inhibited apoptosis of HCC cells in vitro, indicating that CCAT2 might function as an oncogene in hepatocellular carcinoma.[24] In the present study, the relative expression levels of CCAT2 in HCC tissues and adjacent non-cancerous were measured. Furthermore, the clinical value and prognostic significance were also investigated.

2. Methods and materials

2.1. Patient and tissue samples

122 pairs of HCC tissues and adjacent normal liver tissues were acquired at the Department of hepatobiliary and pancreatic surgery, Hubei Provincial Cancer Hospital between September 2013 and February 2018. Tissue samples were immediately frozen in liquid nitrogen at the time of hepatectomy. The diagnosis was confirmed by histopathological examination, and complete clinical and laboratory data were available prior to surgery and during follow-up. Vascular invasion includes microvascular invasion and portal vein tumor thrombus. The diagnostic criterion of microvascular invasion was the presence of a tumor cell nest in the portal vein, hepatic artery, hepatic vein, bile duct or lymph duct in the tumor surrounding the liver tissue under microscopic examination. portal vein tumor thrombus was diagnosed when there were low-attenuation intraluminal masses that expanded the portal vein, or filling defects in the portal vein system, as presented in CT or MRI imaging. To control the potential confounding factors, patients who received treatments as chemotherapy or radiotherapy were excluded. The distribution of clinicopathologic data in the study is given in Table 1. Informed consent for tissue analysis was obtained before surgery. The study was approved by the Institutional Ethics Committee of Hubei Provincial Cancer Hospital.

2.2. RNA extraction and quantitative RT-PCR

Total RNA was isolated from patient tissues using TRIzol Reagent (Invitrogen, Carlsbad, CA) as per the manufacturer's instructions. qRT-PCR analyses were further performed using SYBR Premix Ex Taq (Takara Bio, Japan) in the ABI Prism 7500 Fast Real-Time PCR system (Applied Biosystems, USA). By using 2^{-Bios} method, the relative expression levels of the target genes were normalized to that of endogenous control GAPDH. The premier sequences were as follows:

CCAT2 (forward): 5′-CCACATCGCTCAGACACCAT-3′; (reverse):5′-ACCAGGCGCCCAATACG-3′; GAPDH (forward): 5′-GTCAACGGATTTGGTCTGTATT-3′, (reverse): 5′-AGTCTTCTGGGTGGCAGTGAT-3′.

2.3. Statistical analysis

Continuous data was analyzed by the Student *t* test to assess differences between the two groups. Categorical data was analyzed by the Chi-square test. Survival was calculated using the Kaplan–Meier method and analyzed using the log-rank test. Independent prognostic indicators were determined in the multivariate analysis using Cox's proportional hazard model. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) 19.0 (SPSS, Chicago, IL) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA). $P < .05$ was considered statistically significant.

Table 1

Association of lncRNA CCAT2 expression with clinicopathological features of HCC patients.

Variables	Cases (n)	LncRNA CCAT2 expression		*P* value
		High (n = 63)	Low (n = 59)	
Gender				
Male	79	43	36	.451
Female	43	20	23	
Age (years)				
< 65	45	22	23	.709
⩾65	77	41	36	
HBsAg status				
Positive	81	46	35	.127
Negative	41	17	24	
AFP (ng/ml)				
<200	48	21	27	.195
⩾200	74	42	32	
Tumor size (cm)				
<5	54	26	28	.585
⩾5	68	37	31	
Tumor number				
Multiple	43	24	19	.571
Single	79	39	40	
Liver cirrhosis				
Yes	77	42	35	.455
No	45	21	24	
Vascular invasion				
Yes	34	26	8	.001
No	88	37	51	
Histopathologic grading				
Well + moderately	85	35	50	.001
Poorly	37	28	9	
Distant metastasis				
Yes	16	14	2	.002
No	106	49	57	
TNM stage				
I/II	56	22	34	.018
III/IV	66	41	25	

CCAT2 = colon cancer-associated transcript 2, HCC = hepatocellular carcinoma.

3. Results

3.1. The expression level of lncRNA CCAT2 in HCC tissues

To understand the role of lncRNA CCAT2 in HCC, the expression levels of CCAT2 in HCC tissues and their corresponding adjacent normal liver tissues were examined by RT-qPCR analysis. We observed that CCAT2 expression levels were significantly increased in HCC tissues compared to that in their normal counterparts ($P < .001$, shown in Fig. 1). To examine the potential clinical significance of CCAT2 in HCC, a total of 122 HCC patients were divided into a high-expression group (n = 63) and a low expression group (n = 59) according to the median value of CCAT2 expression.

3.2. The relationships between clinicopathologic features and CCAT2 expression levels in HCC

To investigate the clinical significance of CCAT2 expression in HCC, we analyzed the relationship between clinicopathologic features and CCAT2 expression levels in HCC cases. We found that CCAT2 expression was significantly correlated with vascular invasion ($P = .001$), histopathologic grading ($P = .001$), distant metastasis ($P = .002$) and TNM stage ($P = .018$).

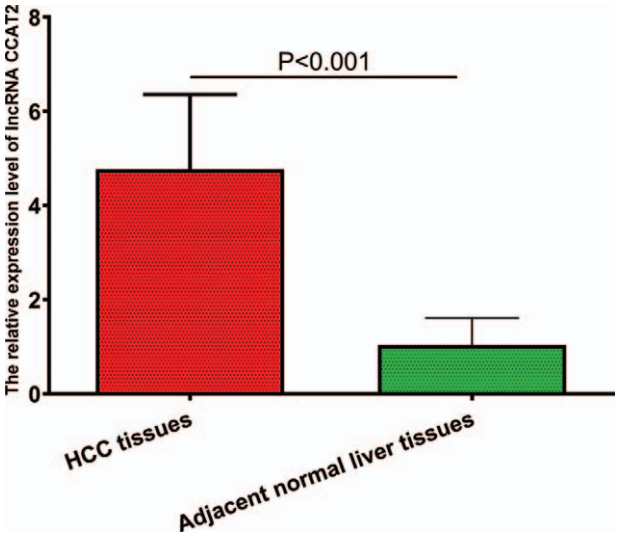

Figure 1. The qRT-PCR analysis revealed that colon cancer-associated transcript 2 expression levels were significantly increased in hepatocellular carcinoma tissues compared to that in their normal counterparts ($P < .001$).

However, there was no significant correlation between CCAT2 expression and age, gender, HBsAg status, serum AFP level, tumor size, or tumor number(all $P > .05$, shown in Table 1).

3.3. The prognostic value of CCAT2 in HCC patients

To explore the utility of CCAT2 as a promising molecular marker to predict the prognosis of HCC patients, we compared the overall survival times between 122 HCC patients who expressed high or low expression levels of CCAT2 based on extensive clinical follow-up data. A Kaplan–Meier survival curve showed that the overall survival rate of HCC patients in high CCAT2 expression group markedly decreased as compared with that of low CCAT2 expression group ($P = .016$, shown in Fig. 2). In addition, COX multivariate analysis showed that high expression

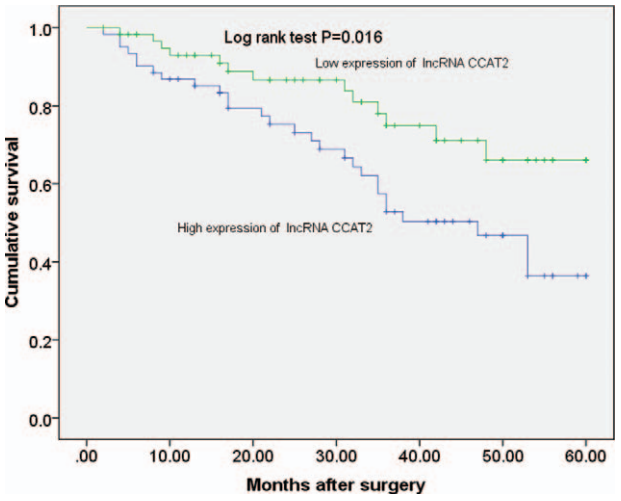

Figure 2. The Kaplan–Meier survival curve showed that the overall survival rate of hepatocellular carcinoma patients in high CCAT2 expression group markedly decreased as compared with that of low CCAT2 expression group ($P = .016$). CCAT2 = colon cancer-associated transcript 2.

Table 2

Multivariate analysis of overall survival in patients with HCC.

Variable	Hazard ratio	95% CI	*P* value
Gender	1.627	0.362–3.942	.873
Age	1.829	0.736–4.556	.375
HBsAg status	2.038	0.835–4.887	.279
AFP level	2.638	0.937–7.827	.182
Tumor size	2.835	0.892–7.465	.099
Tumor number	1.627	0.834–6.273	.103
Liver cirrhosis	2.835	0.933–4.836	.123
Vascular invasion	2.916	1.283–9.027	.017
Histopathologic grading	3.537	1.926–11.883	.008
Distant metastasis	3.728	2.019–16.728	.001
TNM stage	3.192	2.036–14.283	.003
LncRNA CCAT2 level	2.126	1.273–8.775	.021

CCAT2 = colon cancer-associated transcript 2, HCC = hepatocellular carcinoma.

of CCAT2 was an independent risk factor for predicting shorter overall survival time in HCC (HR = 2.126, 95%CI:1.273–8.775, $P = .021$, shown in Table 2).

4. Discussion

Aggressiveness, invasiveness, and frequent postoperative recurrence are the most significant characteristics of HCC.[25] Despite advances in early diagnosis and improved treatments for HCC, the early diagnosis rate and long-term survival rate remains poor.[26,27] The frequent recurrence and high metastasis rates of HCC hinder positive outcomes for patients. Because multiple genetic alterations are responsible for the progression of HCC, exploration of the molecular mechanism of HCC is important for disease diagnosis, treatment, and prediction of outcomes.

LncRNAs have been presumed to play a significant role in various cellular processes, including immune surveillance, cell-cycle regulation, stem cell pluripotency, and tumorigenesis.[28–31] Emerging studies reveal that lncRNAs participate in almost all processes of HCC development, including tumorigenesis, tumor proliferation and metastasis.[32,33] Therefore, exploring the cancerigenic or carcinostatic mechanism of lncRNAs in HCC is of great significance for comprehending the etiology and optimizing treatment.

CCAT2, a novel lncRNA, has been reported as an oncogene in several cancers. For example, Ruan et al demonstrated that lncRNA CCAT2 expression was significantly upregulated in osteosarcoma tissues compared to adjacent normal bone tissues. Higher lncRNA CCAT2 expression was positively associated with larger tumor size, advanced tumor stage and poor overall survival rate of patients. In vitro, knockdown of lncRNA CCAT2 suppressed cell proliferation and colony formation ability. They also found that knockdown of lncRNA CCAT2 inhibited GSK3β/β-catenin signaling by reducing p-GSK3β and β-catenin expression, but increasing GSK3β expression.[34] Zhao et al found that the expression levels of CCAT2 in the tumor tissues of patients with NSCLC were significantly higher than those in the normal para-carcinoma tissues ($t = 8.580$, $P < .01$). CCAT2 promoted the occurrence of NSCLC by regulating the Wnt/β-catenin signaling pathway.[35] Zhang et al found that CCAT2 was upregulated in ESCC tissues, especially in cases with lymph node metastasis (LNM), advanced TNM stages, and MYC amplification. Furthermore, the level of CCAT2 was positively correlated with TNM stages, LNM, and the number of positive

lymph nodes, indicating CCAT2 might be a prognostic biomarker and therapeutic target for ESCC.[22] Huang et al found that the expression levels of the lncRNA CCAT2 in ovarian cancer tissues and cell lines were significantly higher compared with values obtained for adjacent non-tumor tissues and normal ovarian epithelial cells. Interestingly, higher CCAT2 expression levels were associated with a shorter overall survival ($P = .006$) and disease-free survival ($P = .001$) in ovarian cancer patients. In addition, CCAT2 expression was positively correlated with FIGO stage ($P = .002$), tumor grade ($P = .006$) and distant metastasis ($P < .001$). Moreover, CCAT2 knockdown in ovarian cancer cells markedly suppressed cell proliferation, migration, and invasion.[20] Guo et al found that expression of CCAT2 was up-regulated in glioma tissues and significantly correlated with the advanced tumor stage (III/IV). Functional assays in vitro and in vivo demonstrated that knockdown of CCAT2 could inhibit proliferation, cell cycle progression and migration of glioma cells. Further analysis indicated the effect of CCAT2 knockdown on glioma cell phenotype through inhibiting Wnt/β-catenin signal pathway activity, providing evidence that CCAT2 may function as a potential biomarker for glioma.[18] Wu et al showed that lncRNA CCAT2 was upregulated in gastric cancer tissues ($P = .000$), and positively correlated with TNM stage ($P = .029$), lymphatic invasion ($P = .042$) and nervous invasion ($P = .024$) in gastric cancer patients. Furthermore, they also found that high expression of lncRNA CCAT2 was an unfavorable prognostic factor in gastric cancer patients. Silencing of lncRNA CCAT2 inhibits gastric cancer cell proliferation and invasion.[15]

Previous investigations demonstrated that CCAT2 might function as an oncogene in HCC. Liu et al found that CCAT2 promoted HCC cells proliferation and metastasis through up-regulation of NDRG1.[23] Zhou et al found that CCAT2 was upregulated in HCC tissues and human HCC cell lines. Furthermore, the over-expression of CCAT2 using a synthesized vector significantly promoted cell migration and proliferation, and inhibited apoptosis of HCC cells in vitro.[24] In the present investigation, to understand the role of lncRNA CCAT2 in HCC, the expression levels of CCAT2 in HCC tissues and their corresponding adjacent normal liver tissues were examined by RT-qPCR analysis. We observed that CCAT2 expression levels were significantly increased in HCC tissues compared to that in their normal counterparts. To investigate the clinical significance of CCAT2 expression in HCC, we analyzed the relationship between clinicopathologic features and CCAT2 expression levels in HCC cases. We found that CCAT2 expression was significantly correlated with vascular invasion, histopathologic grading, distant metastasis and TNM stage, suggesting that CCAT2 expression was associated with malignant behaviors of human HCC, such as cancer invasion and metastasis. Previously, Mokdad et al found that vascular invasion was a prognostic factor of human HCC.[36] Tan et al found that histopathologic grading was a significant predictor of microvascular invasion in HCC patients.[37] Furthermore, distant metastasis and high TNM stage are significantly associated with poor prognosis of human HCC.[2,3] Therefore, we speculated that CCAT2 might be a prognostic factor for human HCC. To explore the utility of CCAT2 as a promising molecular marker to predict the prognosis of HCC patients, we compared the overall survival times between 122 HCC patients who expressed high or low expression levels of CCAT2 based on extensive clinical follow-up data. A Kaplan–Meier survival curve showed that the overall survival rate of HCC patients in high CCAT2 expression group markedly decreased as compared with that of low CCAT2 expression group. In addition, COX multivariate analysis showed that high expression of CCAT2 was an independent risk factor for predicting shorter overall survival time in HCC. The present study has several limitations. Firstly, we have not investigated the targets of CCAT2 in prostate cancer cells. More in-depth study is needed in the future to clarify the role of CCAT2 in prostate cancer. Secondly, all the patients included in this study were Han ethnic groups in China, and the prognostic value of CCAT2 in patients of other races should be investigated in the future.

Taken together, this research revealed that lncRNA CCAT2 was upregulated in HCC tissues, and higher level of CCAT2 was found in advanced HCC. Furthermore, lncRNA CCAT2 may serve as a potential biomarker for predicting overall survival time in HCC.

Author contributions

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Writing – review & editing: Changbo Fu, Dongde Wu.

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