European Review for Medical and Pharmacological Sciences 2017; 21: 1242-1247

LncRNA CCAT1 as the unfavorable prognostic biomarker for cholangiocarcinoma

X.-M. JIANG[1], Z.-L. LI[1], J.-L. LI[1], W.-Y. ZHENG[1], X.-H. LI[1], Y.-F. CUI[1], D.-J. SUN[2]

[1]Department of Hepatopancreatobiliary Surgery, Second Affiliated Hospital of Harbin Medical University, Harbin, China
[2]Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin Medical University, Harbin, China

Xing-Ming Jiang and *Zheng-Long Li* contributed equally to this work

Abstract. – OBJECTIVE: To investigate the expression of long noncoding RNA CCAT1 in cholangiocarcinoma (CCA) and to assess the CCAT1 expression as a prognostic biomarker for CCA.

PATIENTS AND METHODS: The CCAT1 expression in tumor tissues and paired adjacent normal tissues from 91 CCA patients was detected by quantitative real-time PCR. The association of the CCAT1 expression with clinicopathological features of CCA patients and the prognostic value of the CCAT1 expression for overall survival was also evaluated by Kaplan-Meier, Cox regression model and ROC analysis.

RESULTS: The CCAT1 expression was significantly upregulated in CCA tumor tissues compared with adjacent normal tissues. The CCAT1 expression was obviously associated with histological differentiation, lymph node invasion, and TNM stage. The overall survival of CCA patients with high CCAT1 expression was worse. Furthermore, the CCAT1 expression could be considered as an independent prognostic factor in predicting the overall survival for CCA patients.

CONCLUSIONS: Our study showed that lncRNA CCAT1, which was upregulated and associated with aggressive malignant behavior, may serve as a novel prognostic biomarker and potential therapeutic target for cholangiocarcinoma.

Key Words:
LncRNA CCAT1, Cholangiocarcinoma, Prognosis.

Introduction

Cholangiocarcinoma (CCA), originating from the cholangiocytes malignant transformation, is the second most common fatal primary hepatobiliary cancer and characterized by poor prognosis and growing incidence worldwide[1]. Without typical symptoms or effective preventive measures, the majority of CCA patients were usually diagnosed at an advanced stage with distant metastasis. Because of resistance to traditional chemotherapy or radiotherapy, the efficacy of cholangiocarcinoma treatment is limited, and the radical surgical resection is still the only possible curative intervention for this lethal disease[2]. However, the 5-year survival rate of CCA patients was less than 30%[3,4]. Therefore, the discovery of novel biological markers and therapeutic targets will be beneficial for the future treatment of cholangiocarcinoma including early diagnosis and improved prognosis.

Long non-coding RNAs (lncRNA), whose transcripts are longer than 200 nucleotides, are a class of non-coding RNA without an open reading frame (ORF) or a protein-coding function[5]. In the early decades, lncRNAs were considered as the transcriptional noise during the genes expression[6]. In recent years, many studies have demonstrated that lncRNAs are involved in multiple pathophysiological processes of the human body by regulating downstream genes expression in epigenetic, transcriptional and post-transcriptional levels[7-9]. Furthermore, there is increasing evidence that the dysregulation of lncRNAs is closely associated with the occurrence and progression of various carcinomas including liver cancer, gastric cancer and colon cancer through modulating proliferation, dedifferentiation, anti-apoptosis and metastasis[10-12]. Meanwhile, detecting the expression of lncRNAs, which have been recognized as oncogenes or tumor suppressors, could be used for diagnosis and prognosis

Corresponding Author: Cui Yunfu, MD; e-mail: yfcui777@hotmail.com
Sun Dianjun, MD; e-mail: hrbmusdj@hotmail.com

prediction of cancers[13-14]. However, the emerging functional roles of lncRNAs in cholangiocarcinoma still remains unclearly.

In the present study, we investigated the expression of lncRNA CCAT1 (colon cancer associated transcript 1) in cholangiocarcinoma and evaluated the association between CCTA1 expression and clinicopathological characteristics of CCA patients. Our results showed that lncRNA CCAT1, which was significantly upregulated in tumor tissues, could represent a promising prognostic biomarker and potential therapeutic target for cholangiocarcinoma.

Patients and Methods

Patients and Tissues Samples

A total of 91 specimens including tumor tissues and paired adjacent normal tissues were obtained intraoperatively from CCA patients who underwent surgical resection at Second Affiliated Hospital of Harbin Medical University from August 2011 to October 2013. The patients were all diagnosed with cholangiocarcinoma and confirmed pathologically. None of the recruited patients, who received the same medical care and follow-up, received chemotherapy or radiotherapy preoperatively or postoperatively. The tissues samples were immediately frozen in liquid N2 and stored at -80°C until use after the surgical resection. The research protocols of this study were approved by the Ethical Committee of Second Affiliated Hospital of Harbin Medical University and written informed consent was obtained from each recruited patient for this study.

RNA Isolation and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from tissues by using TRIzol reagent according to the manufacturer's protocol (Invitrogen Co, Carlsbad, CA, USA). RNA was reverse transcribed into cDNA using the Prime-Script™ one step RT-PCR kit (Takara, Dalian, Liaoning, China). Quantitative real-time PCR was conducted using SYBR Green Master Mix (Biosystems, Foster City, CA, USA) to detect the CCAT1 expression on the ABI 7500 system (Biosystems, Foster City, CA, USA). GAPDH was used as an endogenous control, and the relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method. The primers for CCAT1 and GAPDH were as follows: CCAT1 forward 5'-TCACTGACAACATCGACTTTGAAG-3', CCAT1 reverse 5'-GGAGAAAACGCTTAGCCATACAG-3' and GAPDH forward 5'-CCCATCACCATCTTCCAGGAG-3', GAPDH reverse 5'-GTTGTCATGGATGACCTTGGC-3'.

Statistical Analysis

The SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was applied for the statistical analysis. Data was expressed as mean ± SD. The statistical differences between groups were analyzed by Student's *t*-test or chi-square test. Survival curves were estimated with Kaplan-Meier method, and the significance was determined by log-rank test. The univariate and multivariate analysis were performed using the Cox proportional-hazards regression model. The association of CCAT1 expression with overall survival and prognostic value of CCAT1 expression was evaluated by Pearson correlation and receiver operating characteristic (ROC) curve analysis. The $p<0.05$ was considered to be statistically significant.

Results

LncRNA CCTA1 Expression is Upregulated in Cholangiocarcinoma

We detected the expression of CCAT1 in 91 pairs of CCA tumor tissues and matched adjacent normal tissues by qRT-PCR. The results data showed that the expression level of CCTA1 in CCA tumor tissues was significantly increased than that in adjacent normal tissues (Figure 1). This suggested that CCAT1 might act as an oncogene in cholangiocarcinoma.

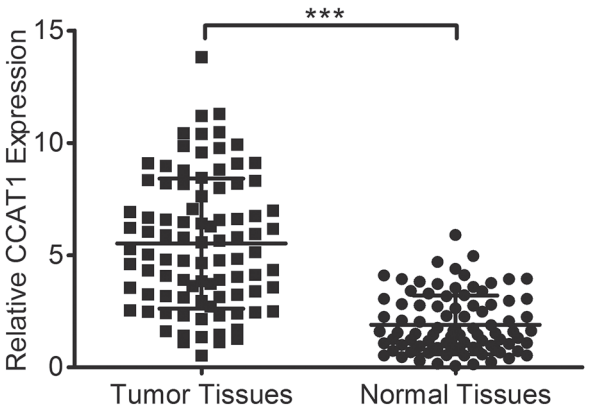

Figure 1. The expression of lncRNA CCAT1 was detected by qRT-PCR in 91 paired CCA tumor tissues and adjacent normal tissues. ***$p < 0.0005$.

Correlation Between CCAT1 Expression and Clinicopathological Features of CCA Patients

To explore the oncogenic function of lncRNA CCAT1, the relationship between the clinicopathological characteristics of CCA patients and CCAT1 expression was analyzed. According to the median value of CCAT1 expression level, the all 91 patients were divided into low CCAT1 expression group (n=44) and high CCAT1 expression group (n=47). As shown in Table I, the CCAT1 expression was significantly associated with histological differentiation, TNM stage, and lymph node invasion. However, there were no obvious correlations between CCAT1 expression and other clinicopathological features. The relative CCAT1 expression of CCA patients with different clinicopathological features including histological differentiation, TNM stage, and lymph node invasion was shown in Figure 2.

Prognostic Value of CCAT1 Expression For CCA Patients

To assess the prognostic value of the CCAT1 expression for CCA, survival curves were analyzed by Kaplan-Meier method and compared by log-rank test. The results data indicated that the low CCAT1 expression group patients had longer overall survival ($p < 0.0001$, Figure 3) and the correlation analysis between the CCAT1 expression and overall survival of the CCA patients was shown in Figure 4. We also performed univariate and multivariate analysis to evaluate the prognostic value of the CCAT1 expression and other clinicopathological features for the overall survival of the CCA patients. The univariate analysis showed that the lymph node invasion, histological differentiation, TNM stage and CCAT1 expression were significantly correlated with the overall survival, and the CCAT1 expression was confirmed as an independent prognostic factor

Table I. Correlation between CCTA1 expression and clinicopathological characteristics of CCA patients.

Variables	Cases (n = 91)	CCAT1 expression		p-value
		Low	High	
Gender				0.875
Male	53	26	27	
Female	38	18	20	
Age (years)				0.413
< 60	35	15	20	
≥ 60	56	29	27	
T umor location				0.930
Intrahepatic	21	11	10	
Perihilar	38	17	21	
Distal	32	16	16	
Differentiation				0.01
Well+Moderate	58	34	24	
Poor	33	10	23	
Histological type				0.636
Adenocarcinoma	80	39	41	
Mucinous adenocarcinoma	6	2	4	
Papillary carcinoma	5	3	2	
TNM stage				0.005
I+II	38	25	13	
III+IV	53	19	34	
Lymph node invasion				0.01
Positive	52	19	33	
Negative	39	25	14	
HBV infection				0.909
Positive	44	21	23	
Negative	47	23	24	
Serum CEA				0.851
> 5 ng/ml	34	16	18	
≤ 5 ng/ml	57	28	29	
Serum CA199				0.490
> 37 u/ml	63	32	31	
≤ 37 u/ml	28	12	16	

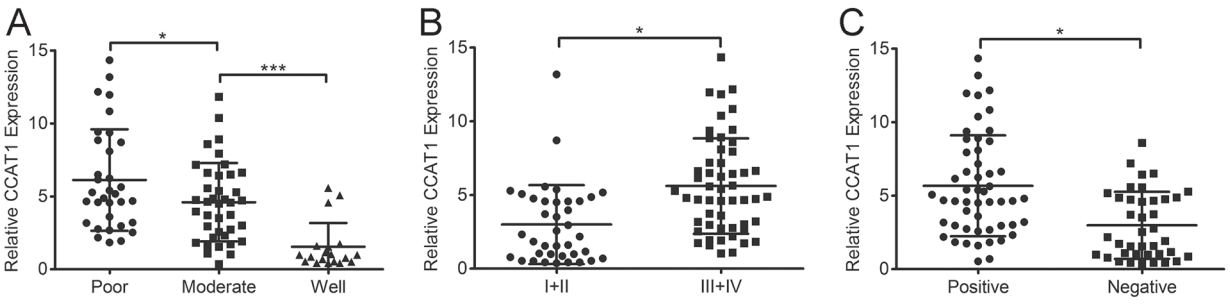

Figure 2. The relative expression level of CCAT1 in CCA patients with various histologic differentiation ***(A)***, different TNM stage ***(B)***, with or without lymph node invasion. *$p < 0.05$, ***$p < 0.0005$.

for CCA patients by multivariate analysis (Table II). Also, Figure 5 showed the sensitivity and specificity of CCAT1 expression as a prognostic factor in predicting the overall survival by ROC analysis (sensitivity: 81.8%, specificity: 74.5%).

Discussion

Cholangiocarcinoma is the second most common primary hepatobiliary malignancy with less than 30% 5-year survival[15]. Lack of effective preventive measures and obvious symptoms, the CCA patients were usually diagnosed at the advanced clinical stage with local and/or distant metastasis[16]. Therefore, it is of great importance to identify the efficient biomarkers for diagnosis, prognostic prediction, and therapeutic strategies.

LncRNAs, which are more than 200 nucleotides in length, are a group of RNA molecules without ORF or protein-coding capacity[5]. It has been confirmed that lncRNAs are involved in diverse pathophysiological processes of the human body through regulating genes expression by means of different mechanisms[17]. Mounting evidence suggest that dysregulated lncRNAs, acting as oncogenes or tumor suppressors, have critical roles in the regulation of development and progression of various cancers[18]. These aberrantly expressed lncRNAs could be tracked in malignant biological behaviors including proliferation, cell cycle, anti-apoptosis, and metastasis, which indicate the biomarkers and therapeutic targets potential of lncRNAs[19,20]. However, the dysregulation and function of lncRNAs in cholangiocarcinoma are still poorly explored.

The lncRNA CCAT1 (colon cancer associated transcript 1), with 2795nt in length, is a discovered lncRNA and located on chromosome 8q24.1. The expression of CCAT1 was found upregulated in colorectal carcinoma at first, and the upregulation of CCAT1 promoted the carcinogenesis of colon cancer[21]. Moreover, CCTA1 has been found to be consistently upregulated in many cancers and enhanced the tumor occurrence and progression including proliferation, dedifferenti-

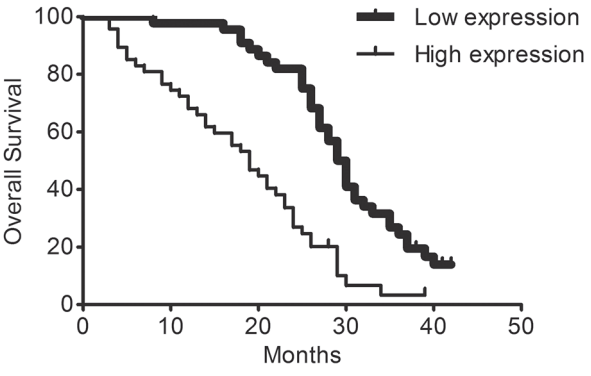

Figure 3. Kaplan-Meier curves for overall survival of CCA patients with high and low CCTA1 expression ($p < 0.0005$).

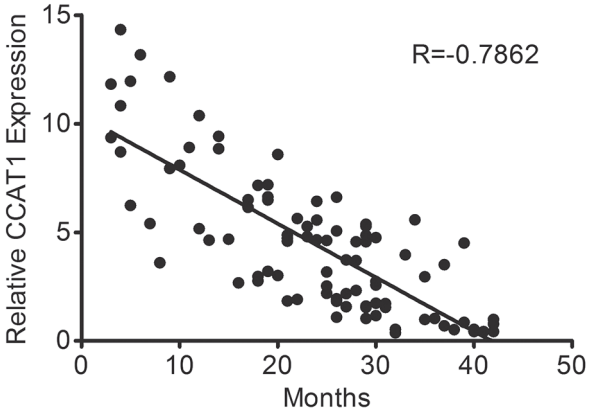

Figure 4. The correlation analysis between relative CCAT1 expression level and overall survival of CCA patients.

Table II. Univariate and multivariate analysis for overall survival by Cox regression model.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p*-value
Gender						
Male *vs*. Female	0.970	0.618-1.493	0.857			
Age (years)						
≥ 60 *vs*. < 60	1.273	0.814-1.991	0.290			
HB V infection						
Positive *vs*. Negative	1.203	0.776-1.864	0.408			
Lymph node invasion						
Positive *vs*. Negative	2.546	1.600-4.051	0.005	1.925	1.193-3.107	0.007
Differentiation						
Poor *vs*. (Moderate+Well)	5.450	3.106-9.565	0.008	2.750	1.524-4.960	0.001
TNM stage						
(I+II) *vs*. (III+IV)	5.596	3.260-9.605	0.009	4.331	2.428-7.725	0.003
CCAT1 expression						
Low *vs*. High	3.139	1.968-5.008	0.005	2.250	1.395-3.630	0.001

ation, and chemoresistance[22-24]. And these studies indicated that there was a close relationship between aberrant expression of CCAT1 and human cancers. However, the expression and functional roles of lncRNA CCTA1 in cholangiocarcinoma are still not clear.

In the present study, we investigated the expression and prognostic biomarker potential of lncRNA CCAT1 in cholangiocarcinoma for the first time. The results showed that the expression of CCAT1 in CCA tumor tissues was significantly higher than that in paired adjacent normal tissues and the upregulated expression of CCAT1 was associated with poor histological differentiation, lymph node invasion, and advanced TNM stage. The overall survival of patients with low CCAT1 expression was longer than that with high CCAT1 expression. Furthermore, we applied multivariate and ROC analysis to confirm that lncRNA CCAT1 expression could be considered as an independent prognostic factor for CCA patients with acceptable sensitivity and specificity.

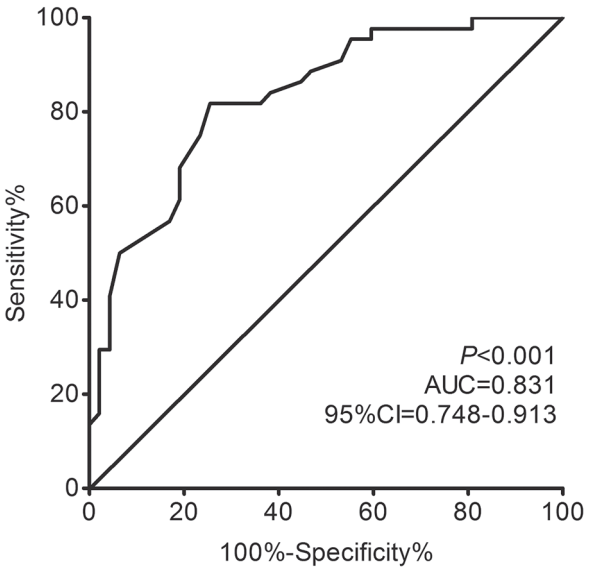

Figure 5. Receiver operating characteristic curve analysis of CCAT1 expression in predicting the overall survival of CCA patients.

Conclusions

The expression of lncRNA CCTA1 was significantly upregulated in CCA and the high expression of CCAT1 was closely associated with poor histological differentiation, lymph node invasion, advanced TNM stage and worse overall survival. Our findings revealed that lncRNA CCAT1 could serve as the independent prognostic biomarker and potential therapeutic target for cholangiocarcinoma, and further studies will be needed to focus on the underlying regulatory mechanisms of CCAT1 in cancers.

Acknowledgements

This study was funded by National Natural Science Foundation of China (Grant No. 81602088), Health and Family Planning Commission Research Project of Heilongjiang Province (Grant No. 2016-049), Heilongjiang Postdoctoral Science Foundation (Grant No. LBH-Z16096) and Innovative Science Foundation of Harbin Medical University (Grant No. 2016LCZX09).

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Groen PC, Gores GJ, Larusso NF, Gunderson LL, Nagorney DM. Biliary tract cancers. N Engl J Med 1999; 341: 1368-1378.
2) Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. Lancet 2005; 366: 1303-1314.
3) Malhi H, Gores GJ. Cholangiocarcinoma: modern advances in understanding a deadly old disease. J Hepatol 2006; 45: 856-867.
4) Gatto M, Alvaro D. Cholangiocarcinoma: risk factors and clinical presentation. Eur Rev Med Pharmacol Sci 2010; 14: 363-367.
5) Lau E. Non-coding RNA: Zooming in on lncRNA functions. Nat Rev Genet 2014; 15: 574-575.
6) Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 2016; 17: 47-62.
7) Engreitz JM, Pandya-Jones A, Mcdonel P, Shishkin A, Sirokman K, Surka C, Kadri S, Xing J, Goren A, Lander ES, Plath K, Guttman M. The Xist lncRNA exploits three-dimensional genome architecture to spread across the X chromosome. Science 2013; 341: 1237973.
8) Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell 2013; 152: 1298-1307.
9) Wierzbicki AT. The role of long non-coding RNA in transcriptional gene silencing. Curr Opin Plant Biol 2012; 15: 517-522.
10) Wang X, Sun W, Shen WF, Xia MY, Chen C, Xiang DM, Ning BF, Cui XL, Li HY, Li XF, Ding J, Wang HY. Long non-coding RNA DILC regulates liver cancer stem cells via IL-6/STAT3 axis. J Hepatol 2016; 64: 1283-1294.
11) Zou JH, Li CY, Bao J, Zheng GQ. High expression of long noncoding RNA Sox2ot is associated with the aggressive progression and poor outcome of gastric cancer. Eur Rev Med Pharmacol Sci 2016; 20: 4482-4486.
12) Di-Cecilia S, Zhang F, Sancho A, Li S, Aguiló F, Sun Y, Rengasamy M, Zhang W, Del-Vecchio L, Salvatore F, Walsh MJ. RBM5-AS1 is critical for self-renewal of colon cancer stem-like cells. Cancer Res 2016; 76: 5615-5627.
13) Yang L, Wei H, Xiao HJ. Long non-coding RNA Loc554202 expression as a prognostic factor in patients with colorectal cancer. Eur Rev Med Pharmacol Sci 2016; 20: 4243-4247.
14) Huang B, Song JH, Cheng Y, Abraham JM, Ibrahim S, Sun Z, Ke X, Meltzer SJ. Long non-coding antisense RNA KRT7-AS is activated in gastric cancers and supports cancer cell progression by increasing KRT7 expression. Oncogene 2016; 35: 4927-4936.
15) Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. Semin Liver Dis 2004; 24: 115-125.
16) Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, Hama N, Hosoda F, Urushidate T, Ohashi S, Hiraoka N. Genomic spectra of biliary tract cancer. Nat Genet 2015; 47: 1003-1010.
17) Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet 2014; 15: 7-21.
18) Huarte M. The emerging role of lncRNAs in cancer. Nat Med 2015; 21: 1253-1261.
19) Evans JR, Feng FY, Chinnaiyan AM. The bright side of dark matter: lncRNAs in cancer. J Clin Invest 2016; 126: 2775-2782.
20) Yang JP, Yang XJ, Xiao L, Wang Y. Long noncoding RNA PVT1 as a novel serum biomarker for detection of cervical cancer. Eur Rev Med Pharmacol Sci 2016; 20: 3980-3986.
21) Nissan A, Stojadinovic A, Mitrani-Rosenbaum S, Halle D, Grinbaum R, Roistacher M, Bochem A, Dayanc BE, Ritter G. Colon cancer associated transcript-1: a novel RNA expressed in malignant and pre-malignant human tissues. Int J Cancer 2012; 130: 1598-1606.
22) Zhu HQ, Zhou X, Chang H, Li HG, Liu FF, Ma CQ, Lu J. CCAT1 promotes hepatocellular carcinoma cell proliferation and invasion. Int J Clin Exp Pathol 2015; 8: 5427-5434.
23) Chen LX, Wang W, Cao LX, Li ZJ, Wang X. Long Non-Coding RNA CCAT1 acts as a competing endogenous RNA to regulate cell growth and differentiation in acute myeloid leukemia. Mol Cells 2016; 39: 330-336.
24) Chen J, Zhang K, Song HZ, Wang R, Chu XY, Chen LB. Long noncoding RNA CCAT1 acts as an oncogene and promotes chemoresistance in docetaxel-resistant lung adenocarcinoma cells. Oncotarget 2016; 7: 62474-62489.