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REVIEW

lncRNAs as potential molecular biomarkers in the clinicopathology and prognosis of cholangiocarcinoma: a systematic review and meta-analysis

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Background: Cholangiocarcinoma (CCA) is the second most common fatal primary hepatobiliary malignant carcinoma, characterized by early invasion and extremely poor outcomes. It is therefore necessary to identify a novel biomarker to better diagnose CAA and predict its prognosis. Recently, emerging evidence has revealed that some lncRNAs play an important role in the tumorigenesis and progression of CAA. In order to support this search for novel diagnostic and prognostic biomarkers for CAA, we conducted a meta-analysis to analyze the published association between lncRNA expression and its clinical value in CAA.

Methods: Eligible studies were pooled and analyzed according to our inclusion and exclusion criteria after a comprehensive literature search. Stata 14.0 software was used to analyze the data from relevant studies and to construct a forest plot. Different effect sizes were selected for the meta-analysis.

Results: In total, 24 publications were included in this meta-analysis. After review of their full-text, 16 articles studied the association between lncRNAs and clinicopathological characteristics, 2 discussing diagnosis and 16 discussing prognosis. Our results showed that overexpression of CCAT1 was significantly correlated with tumor stage (I + II vs III + IV) (OR, 4.99; 95% CI 2.77–8.99; $P<0.001$) and lymph node metastasis in CCA (OR, 4.75; 95% CI 2.65–8.52; $P<0.001$). Furthermore, elevated CCAT lncRNA family expression predicted a shorter overall survival (HR, 2.09; 95% CI 1.17–3.00; $P<0.001$), especially CCAT2. Upregulation of CCAT2 was also obviously associated with tumor stage in CCA (OR, 5.29; 95% CI 2.64–10.58; $P=0.001$).

Conclusion: This is the first meta-analysis to assess the relationship between expression of lncRNAs and the clinical values of patients with CCA. lncRNAs can function as potential molecular biomarkers of the clinicopathology and prognosis of CCA.

Keywords: lncRNA, cholangiocarcinoma, clinicopathological characteristics, diagnosis, prognosis

Introduction

Cholangiocarcinoma (CCA) originates in the epithelium of hepatic biliary trees and is the second most common fatal primary hepatobiliary malignant carcinoma.[1] According to recent epidemiological data, the incidence and mortality of CCA in the world has been increasing rapidly over the past decades.[2,3] However, due to the lack of a specific clinical presentation and effective diagnostic systems for CCA, most of CCA patients are diagnosed at advanced stages.[2,4] Additionally, due to tumor resistance to

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traditional chemotherapy and radiotherapy, surgery is currently the most effective treatment for CCA.[3] As a result, the prognosis of patients with CCA is extremely poor, with a high rate of recurrence, and a 5-year survival rate of only 5%.[5,6] For these reasons, identifying new therapeutic targets and novel biomarkers associated with CCA diagnosis and prognosis is very important to improve outcomes for those with this disease.

lncRNAs are RNA molecules transcribed without functional open reading frames and are >200 nucleotides in length.[7] They function in various biological processes mostly by binding with miRNAs as sponges or interacting with proteins, including those active in cell proliferation, migration, invasion, and apoptosis.[8] Recently, numerous studies indicated that aberrant expression of lncRNAs was involved in tumorigenesis and cancer progression, including CCA.[7–10] Emerging evidence also demonstrated that some lncRNAs are associated with the diagnosis and prognosis of CAA.[8,11,12]

However, due to limitations related to small sample sizes and various experimental protocols, a single study on these topics may be inaccurate and are therefore insufficiently powered to inform solid conclusions. Thus, the aim of the present study was to systematically analyze all studies of CAA to assess the potential clinical value of lncRNAs in CAA. Here, we identify the relationship between lncRNAs expression and three different clinical outcomes (clinicopathological characteristics, diagnosis, and prognosis).

Methods

Search strategy

Two of the authors (KD and JQ) independently searched several databases, including PubMed, Embase, the Cochrane Library, China National Knowledge Internet (CNKI), Wanfang and Weipu database, for studies on lncRNAs and CCA. The literature was searched up to September 13, 2018. The search terms were as follows: Search ((((((((((((((((((((((((((Non-coding RNA, Long[Title/Abstract]) OR lncRNA[Title/Abstract]) OR Long ncRNA[Title/Abstract]) OR ncRNA, Long[Title/Abstract]) OR RNA, Long Non-Translated[Title/Abstract]) OR Long Non-Translated RNA[Title/Abstract]) OR Non-Translated RNA, Long[Title/Abstract]) OR RNA, Long Non Translated[Title/Abstract]) OR Long Non-Coding RNA[Title/Abstract]) OR Long Non Coding RNA[Title/Abstract]) OR Non-Coding RNA, Long[Title/Abstract]) OR RNA, Long Non-Coding[Title/Abstract]) OR Long Non-Protein-Coding RNA[Title/Abstract]) OR Long Non Protein Coding RNA[Title/Abstract]) OR Non-Protein-Coding RNA, Long[Title/Abstract]) OR RNA, Long Non-Protein-Coding[Title/Abstract]) OR Long Noncoding RNA[Title/Abstract]) OR RNA, Long Untranslated[Title/Abstract]) OR Long Untranslated RNA[Title/Abstract]) OR Untranslated RNA, Long[Title/Abstract]) OR Long ncRNAs[Title/Abstract]) OR ncRNAs, Long[Title/Abstract]) OR Long Intergenic Non-Protein Coding RNA[Title/Abstract]) OR Long Intergenic Non Protein Coding RNA[Title/Abstract]) OR LincRNAs[Title/Abstract]) OR LINC RNA[Title/Abstract])) OR "RNA, Long Noncoding"[Mesh])) AND ((((((((((((((Cholangiocarcinomas[Title/Abstract]) OR Cholangiocellular Carcinoma[Title/Abstract]) OR Carcinoma, Cholangiocellular[Title/Abstract]) OR Carcinomas, Cholangiocellular[Title/Abstract]) OR Cholangiocellular Carcinomas[Title/Abstract]) OR Extrahepatic Cholangiocarcinoma[Title/Abstract]) OR Cholangiocarcinoma, Extrahepatic[Title/Abstract]) OR Cholangiocarcinomas, Extrahepatic[Title/Abstract]) OR Extrahepatic Cholangiocarcinomas[Title/Abstract]) OR Intrahepatic Cholangiocarcinoma[Title/Abstract]) OR Cholangiocarcinoma, Intrahepatic[Title/Abstract]) OR Cholangiocarcinomas, Intrahepatic[Title/Abstract]) OR Intrahepatic Cholangiocarcinomas[Title/Abstract])) OR "Cholangiocarcinoma"[Mesh]).

Inclusion and exclusion criteria

The inclusion criteria were as follows: 1) patients diagnosed with CCA by histopathology; 2) the expression level of lncRNAs divided into high and low, and the correlation between lncRNAs expression and clinicopathological features were detailed; 3) the relationship between lncRNA expression and survival outcome, hazard risk (HR), 95% CI, or *P*-value, and Kaplan–Meier curves were outlined; 4) the expression of lncRNAs was detected in the tissue or serum, and sufficient data on sensitivity, specificity, and sample size were presented.

The exclusion criteria were as follows: 1) non-human studies; 2) letters, case reports, commentaries, conference abstracts, or review articles; 3) articles unrelated to lncRNA and CCA; 4) insufficient data for extraction; 5) HRs calculated using multiple lncRNAs; 6) studies focused on genetic polymorphisms or modification of lncRNAs.

Data extraction and quality assessment

Two independent authors (KD and JQ) extracted the information from the included literature using a predefined template based on the reporting checklists of PRISMA:[13] 1) the first author's last name and publication year; 2) the type of lncRNA, study population, region, sample number, follow-up time (months), and detection methods; 3) clinicopathological

features: age, gender, tumor size, histological grade, tumor stage, lymph node metastasis, distant metastasis, carbohydrate antigen 199, alpha-fetoprotein (AFP), and hepatitis B virus (HBV) infection; 4) HRs, 95 % CI, and *P*-value for survival analysis. If HRs were directly available, we collected these from the original studies, otherwise these data were indirectly extracted from Kaplan–Meier curves according to the method of Tierney et al[14] or we asked the authors for these data; and 5) diagnostic data were included: sensitivity, specificity, and area under curve (AUC).

We assessed the quality of all the included diagnostic studies according to the Quality Assessment of Diagnostic Accuracy Studies-2[15] criteria and used the Newcastle-Ottawa Scale[16] to assess the quality of the selected prognostic studies (scores >5 was regarded as high quality).

Statistical analysis

Heterogeneity among articles was assessed with Higgin's I^2 statistic. I^2>50% indicated statistically significant heterogeneity. A fixed-effects or random-effects model was applied to evaluate the relationship between lncRNAs expression and survival outcomes. A fixed-effects model was used when heterogeneity among studies was not obvious. Otherwise, a random-effects model was used.[17,18] A different effect size was selected for each meta-analysis: 1) that of clinicopathological features analyzed OR and associated 95% CI. 2) In the diagnostic meta-analysis, sensitivity, specificity, and AUC were used. 3) The prognostic meta-analysis employed HR and associated 95% CI for each study to estimate the survival outcomes associated with the expression of lncRNA. HR >1 was regarded as the worse survival for the group with elevated lncRNA expression.[18] Stata 14.0 software (StataCorp LP, College Station, TX, USA) was used to analyze study data and construct the forest plot. $P<0.05$ was considered to be statistically significant.

Results

Study identification and characteristics

As shown in the search flowchart (Figure 1), 88 articles in total were retrieved from PubMed, Embase, Cochrane, and three Chinese databases (China Knowledge Resource Integrated, Wanfang, and Weipu databases). Further, 20 articles

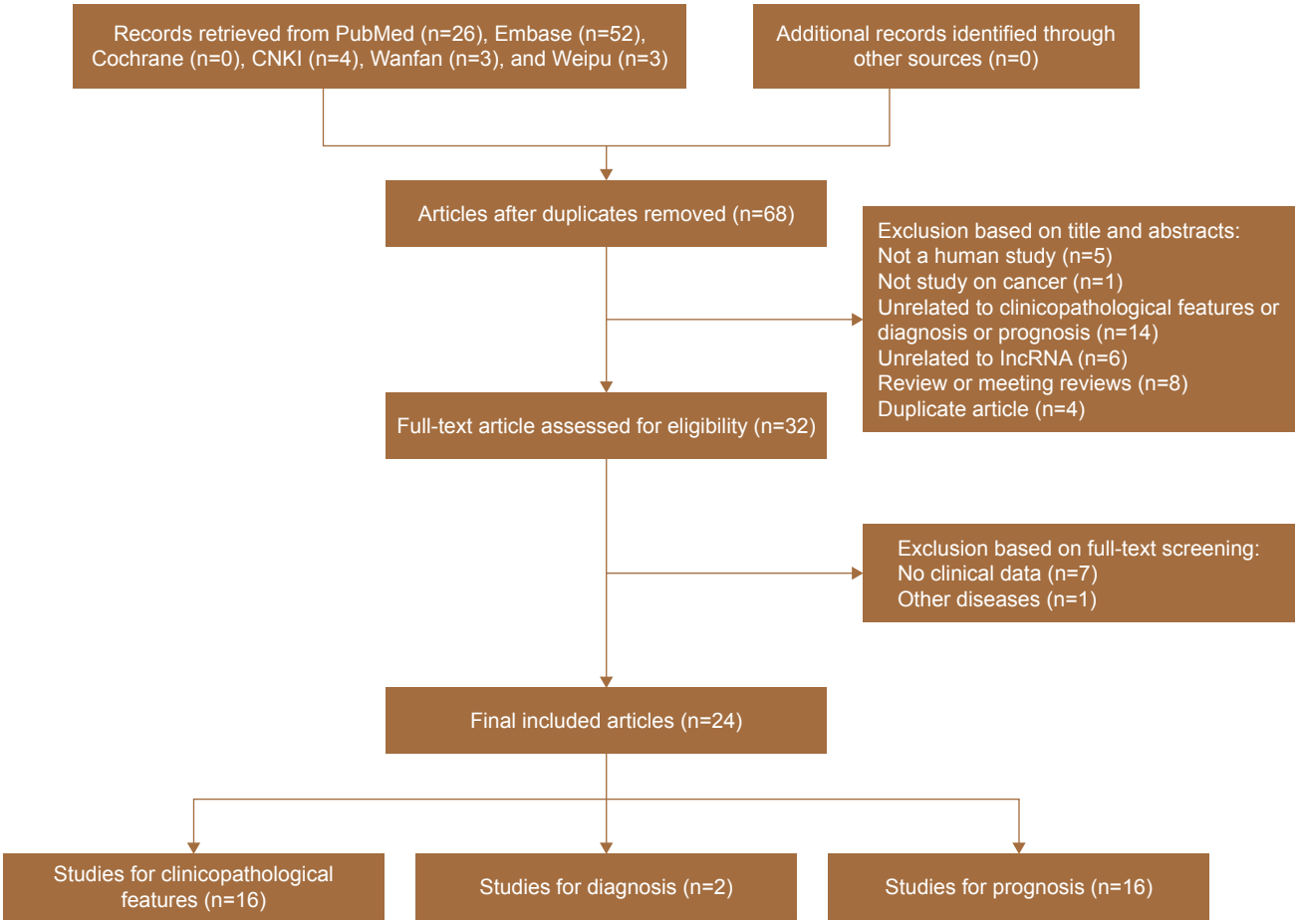

Figure 1 The study selection process.

Table 1 The association between lncRNAs and clinicopathological features

Study	Region	lncRNA	Simple size (n)	Detection method	Expression	Age	Gender (*P*-value)
Wang et al, 2016[20]	China	H19	72	RT-qPCR	Up	0.954	0.538
Ma et al, 2015[22]	China	CPS1-IT1	31	RT-qPCR	Up	0.862	0.693
Lv et al, 2017[19]	China	EMP1-008	72	RT-qPCR	Down	0.89	0.412
		ATF3-008			Down	0.388	0.431
		RCOR3-013			Up	0.917	0.359
		TMEM63A-005			Up	0.941	0.826
Xu et al, 2017[8]	China	CCAT1	91	RT-qPCR	Up	0.413	0.875
Zhang et al, 2017[24]	China	CCAT1	120	RT-qPCR	Up	0.938	0.407
Xu et al, 2017[25]	China	PANDAR	67	RT-qPCR	Up	0.307	0.457
Lu et al, 2017[11]	China	AFAP1-AS1	56	RT-qPCR	Up	0.768	1.000
Zhang et al, 2017[24]	China	Linc01296	57	RT-qPCR	Up	0.553	0.789
Tan et al, 2017[12]	China	MALAT1	62	RT-qPCR	Up	0.602	0.799
Xu et al, 2017[25]	China	UCA1	68	RT-qPCR	Up	0.621	0.807
Li et al, 2017[11]	China	Sox2ot	58	RT-qPCR	Up	0.301	0.571
Xu et al, 2017[25]	China	H19	56	RT-qPCR	Up	0.282	0.596
Xia et al, 2018[27]	China	CRNDE	118	RT-qPCR	Up	0.276	0.306
Bai et al, 2018[28]	China	CCAT2	106	RT-qPCR	Up	0.204	0.620
Xu et al, 2018[29]	China	CCAT2	60	RT-qPCR	Up	0.435	0.796
Xu et al, 2018[29]	China	HOTAIR	70	RT-qPCR	Up	0.624	0.544

Abbreviations: RT-qPCR, real-time quantitative PCR; CA199, carbohydrate antigen 199; AFP, alpha-fetoprotein; HBV, hepatitis B virus; NA, not available.

were excluded as duplicates. After reviewing the remaining titles and abstracts carefully, 36 studies were removed, including 5 non-human studies; 1 study not on CCA; 14 studies unrelated to clinicopathological features, diagnosis, or prognosis; 6 studies without lncRNAs; 8 reviews or meeting reviews; and 4 more duplicate articles. Finally, 24 studies were deemed eligible for meta-analysis upon further review of the full-text article, including 16 discussing on clinicopathological characteristics, 2 on diagnosis, and 16 on prognosis.

Clinicopathological characteristics

Herein, 14 lncRNAs were described in 13 included studies on clinicopathological characteristics. As shown in the Table 1, except EMP1-008[19] and ATF3-008,[19] almost all lncRNAs were upregulated in CCA, including H19,[20,21] CPS1-IT1,[22] RCOR3-013,[19] TMEM63A-005,[19] CCAT1,[23,24] PANDAR,[8] AFAP1-AS1,[11] Linc01296,[6] MALAT1,[12] UCA1,[25] Sox2ot,[26] CRNDE,[27] CCAT2,[28,29] and HOTAIR.[30] All studies indicated that lncRNAs were not significantly associated with patient age, gender, and HBV infection status. Few studies demonstrated that the expression levels of lncRNAs were associated with AFP, except H19.[20] Only one study indicated that CPS1-IT1 expression was significantly related to carcinoembryonic antigen (CEA),[22] and seven studies reported that six lncRNAs were significantly correlated with tumor size, including H19,[20,21] AFAP1-AS1,[11] Linc01296,[6] MALAT1,[12] CRNDE,[25] and HOTAIR.[30]

Only four studies found that lncRNAs were significantly associated with histological grade of CCA, while 13 studies claimed that lncRNAs were significantly correlated with tumor grade of CCA. Interestingly, Table 1 shows that AFAP1-AS1 and CCAT2 expressions were significantly related to vascular invasion.[11,28] Additionally, ten studies demonstrated that lncRNAs were significantly associated with lymph node metastasis, while three studies reported that lncRNAs were significantly correlated with distant metastasis. Table 1 also revealed that H19, CCAT1, and CCAT2 were all detected in two articles. Therefore, we combined these two studies with a total of six groups by constructing two-by-two tables. However, H19 could not be further analyzed due to insufficient data. Based on our meta-analysis of these articles describing CCAT1, the relationship between upregulation of CCAT1 and tumor stage (I + II vs III + IV) was indeed significant (OR, 4.99; 95% CI 2.77–8.99; $P<0.001$). Furthermore, overexpression CCAT1 was significantly correlated with the lymph node metastasis in CCA (OR, 4.75; 95% CI 2.65–8.52; $P<0.001$) (Figure 2).

Tumor size (cm)	Histological grade (I–IV)	Tumor stage	Vascular invasion	Lymph node metastasis	Distant metastasis	CA199	AFP	HBV
0.001 (5)	0.091	0.062	0.144	0.005	0.092	0.924	0.031	NA
0.677 (NA)	NA	NA	0.642	0.045	NA	0.044	NA	NA
0.314 (5)	NA	NA	NA	NA	0.012	0.819	0.603	0.914
0.304 (5)	NA	NA	NA	NA	0.001	0.321	0.482	0.484
0.379 (5)	NA	NA	NA	NA	0.03	0.923	0.319	0.926
0.271 (5)	NA	NA	NA	NA	0.955	0.141	0.094	0.807
NA	0.636	0.005	NA	0.01	NA	0.490	NA	0.909
NA	0.612	<0.01	NA	<0.01	NA	NA	NA	NA
NA	0.014	0.034	0.794	0.004	NA	0.221	NA	0.788
0.031 (5)	0.003	0.013	0.011	0.540	0.177	NA	NA	1.000
0.003 (3)	NA	0.024	NA	0.031	NA	NA	NA	NA
0.042 (3)	NA	0.037	NA	0.037	NA	NA	NA	NA
NA	0.307	0.004	NA	0.027	NA	NA	NA	NA
NA	0.849	0.007	0.203	0.031	NA	0.067	NA	0.813
0.029 (3)	0.783	0.015	0.245	0.105	NA	0.173	NA	0.418
0.001 (5)	0.046	0.050	0.742	NA	0.114	0.172	0.374	NA
0.326 (5)	0.040	<0.001	<0.001	NA	NA	0.327	NA	0.919
0.228 (3)	0.414	0.010	NA	0.019	NA	NA	NA	NA
0.028 (3)	0.609	0.021	0.468	0.805	NA	0.609	NA	0.789

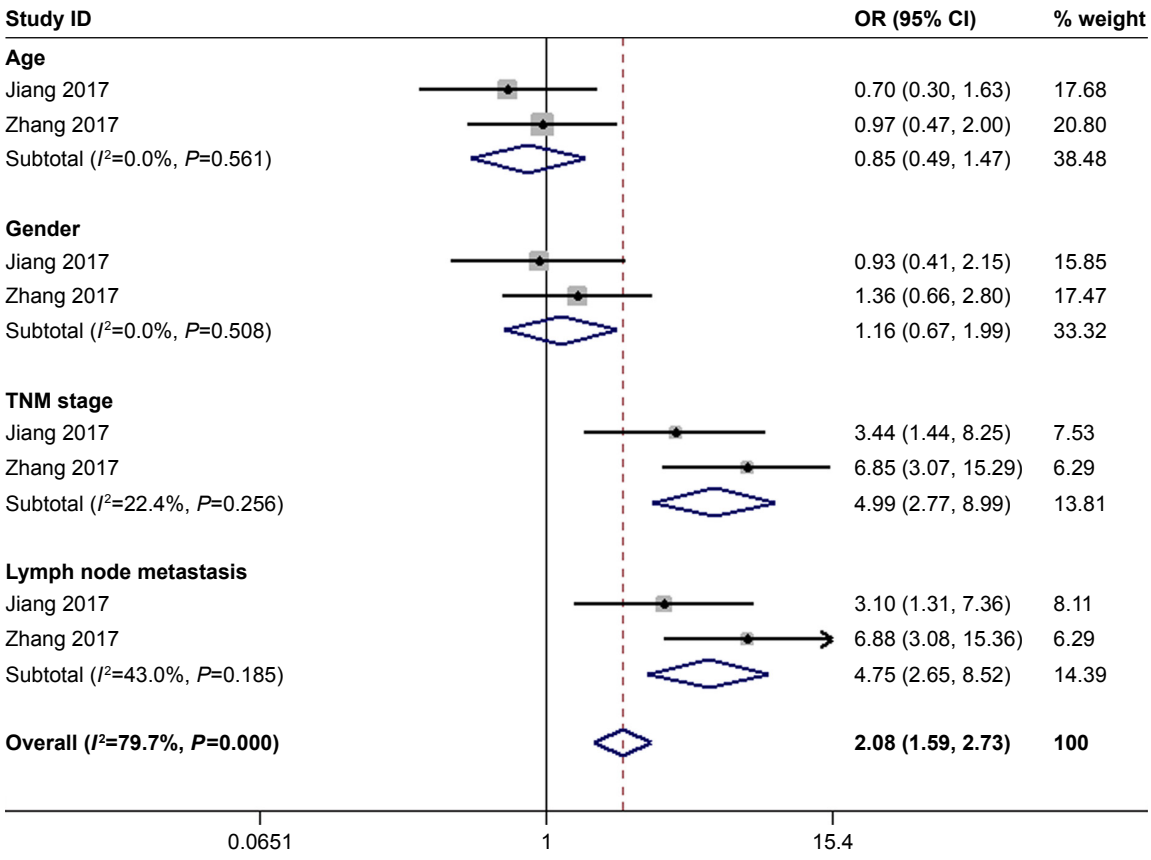

Figure 2 Forest plots of studies evaluating the odds ratio of upregulated CCAT1 expression and the clinicopathology of cholangiocarcinoma patients.

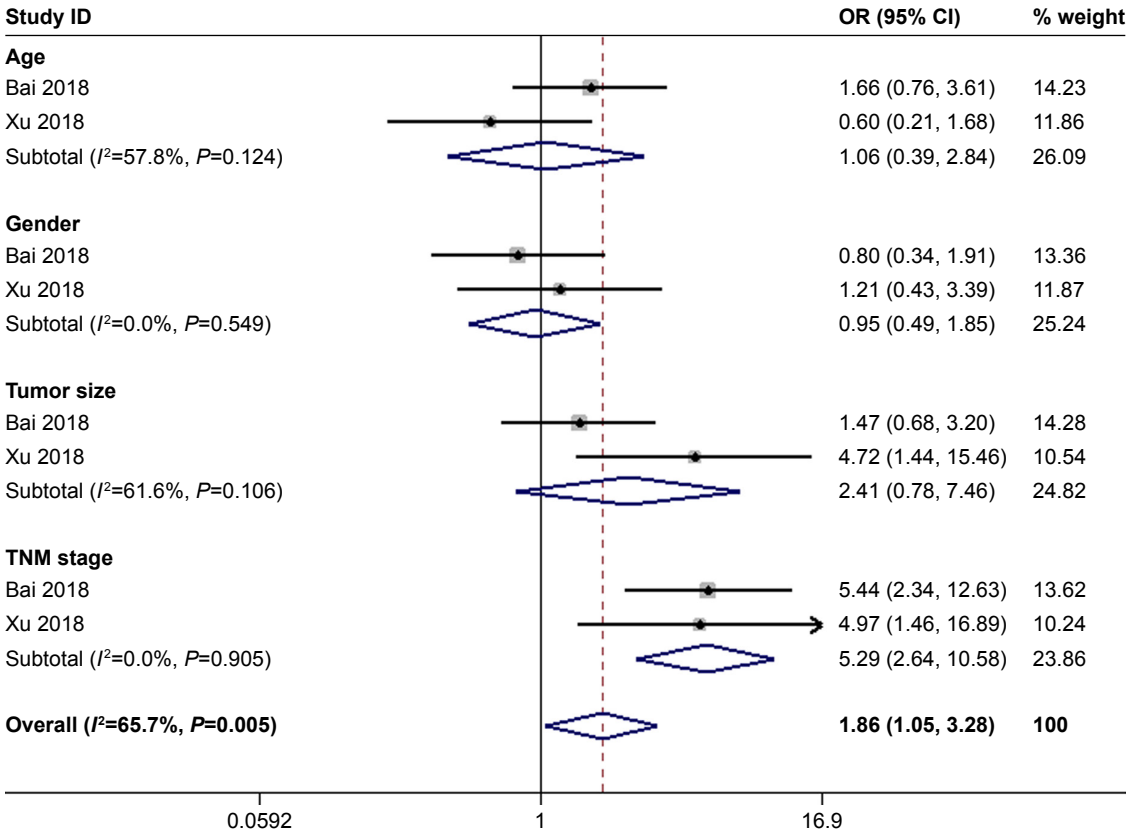

Figure 3 Forest plots of studies evaluating the odds ratio of upregulated CCAT2 expression and the clinicopathology of cholangiocarcinoma patients.
Note: Weights are from random-effects analysis.

Similarly, the result about CCAT2 also indicated that upregulation of CCAT2 was obviously associated with tumor stage in CCA (OR, 5.29; 95% CI 2.64–10.58; P=0.001) (Figure 3). However, dysfunction of CCAT2 was not related with age, gender, and tumor size (P>0.05).

Diagnosis

Only two studies about three lncRNAs were included in this topical analysis. All these lncRNAs were upregulated in different detected samples using real-time quantitative PCR (RT-qPCR). Jiang et al indicated that CCAT1 acted as a potential biomarker for the diagnosis of CCA with relative high sensitivity (81.80%) and specificity (74.50%).[23] Furthermore, Ge et al detected ENST00000517758.1 and ENST00000588480.1 in bile samples and found they were both potential biomarkers for the diagnosis of CCA.[31] The main characteristics of these two studies are presented in Table 2.

Prognosis

Briefly, 16 different lncRNAs were described in the 16 included studies on prognosis. The characteristics of these eligible studies are presented in Table 3. The lncRNA expression of all samples was detected from tissues by RT-qPCR. This analysis included 585 patients with high expression and 522 patients with low expression in total. Interestingly, all of these lncRNAs were overexpressed in CCA, with elevated expression levels associated with poor prognosis, including CPS1-IT1,[22] CCAT1,[23] TUG1,[32] PANDAR,[8] AFAP1-AS1,[11] LINC01296,[6] MALAT1,[12] UCA1,[25] Sox2ot,[26]

Table 2 Summary of lncRNAs used as diagnostic biomarkers of cholangiocarcinoma

Study	Region	lncRNA	Expression	SE (%)	SP (%)	AUC	Sample size		Detected sample	QUADAS
							Case	Control		
Xu et al, 2017[8]	China	CCTA1	Up	81.80	74.50	0.831	91	91	Tissue	7
Ge et al, 2017[31]	China	ENST00000517758.1	Up	–	–	0.613	35	56	Bile sample	5
		ENST00000588480.1	Up	62.90	73.20	0.680				

Abbreviations: SE, sensitivity; SP, specificity; AUC, area under curve; QUADAS, Quality Assessment of Diagnostic Accuracy Studies.

Table 3 Summary of lncRNAs used as prognostic biomarkers of cholangiocarcinoma

Study	Region	lncRNA	Expression	Detected sample	Test method	Cutoff	Sample size		Survival analysis	HR availability	Follow-up month	NOS
							High	Low				
Ma et al, 2015[22]	China	CPS1-IT1	Up	Tissue	RT-qPCR	FC>4	22	9	DFS	Indirectly	40	5
Tan et al, 2017[12]	China	MALAT1	Up	Tissue	RT-qPCR	Median	31	31	OS	Indirectly	30	6
Xu et al, 2017[8]	China	CCAT1	Up	Tissue	RT-qPCR	Median	47	44	OS	Indirectly	50	5
Zeng et al, 2017[32]	China	TUG1	Up	Tissue	RT-qPCR	NA	51	51	OS	Directly	100	6
									DFS			
Xu et al, 2017[8]	China	PANDAR	Up	Tissue	RT-qPCR	NA	40	27	OS	Directly	60	5
									DFS			
Lu et al, 2017[11]	China	AFAP1-AS1	Up	Tissue	RT-qPCR	Median	28	28	OS	Indirectly	80	5
Zhang et al, 2017[24]	China	LINC01296	Up	Tissue	RT-qPCR	Mean	35	22	OS	Indirectly	60	5
Xu et al, 2017[8]	China	UCA1	Up	Tissue	RT-qPCR	NA	38	30	OS	Directly	60	7
Li et al, 2015[36]	China	Sox2ot	Up	Tissue	RT-qPCR	Median	30	28	OS	Directly	60	6
Xia et al, 2017[27]	China	CRNDE	Up	Tissue	RT-qPCR	Mean	51	67	OS	Directly	60	6
Xu et al, 2018[24]	China	H19	Up	Tissue	RT-qPCR	NA	31	25	OS	Indirectly	60	5
Ge et al, 2017[31]	China	ENST00000588480.1	Up	Tissue	RT-qPCR	Median	18	17	OS	Indirectly	70	6
		ENST00000517758.1										
Xu et al, 2018[29]	China	SPRY4-IT1	Up	Tissue	RT-qPCR	Mean	41	29	PFS	Indirectly	60	5
									OS			
Bai et al, 2018[28]	China	CCAT2	Up	Tissue	RT-qPCR	Score =4.4	45	61	OS	Directly	72	7
									PFS			
Xu et al, 2018[29]	China	CCAT2	Up	Tissue	RT-qPCR	Mean	34	26	OS	Directly	60	7
Qin et al, 2018[30]	China	HOTAIR	Up	Tissue	RT-qPCR	NA	43	27	OS	Directly	60	6

Abbreviations: DFS, disease-free survival; OS, overall survival; PFS, progression-free survival; RT-qPCR, real-time quantitative polymerase chain reaction; NOS; Newcastle-Ottawa Scale; FC, fold-change; NA, not available.

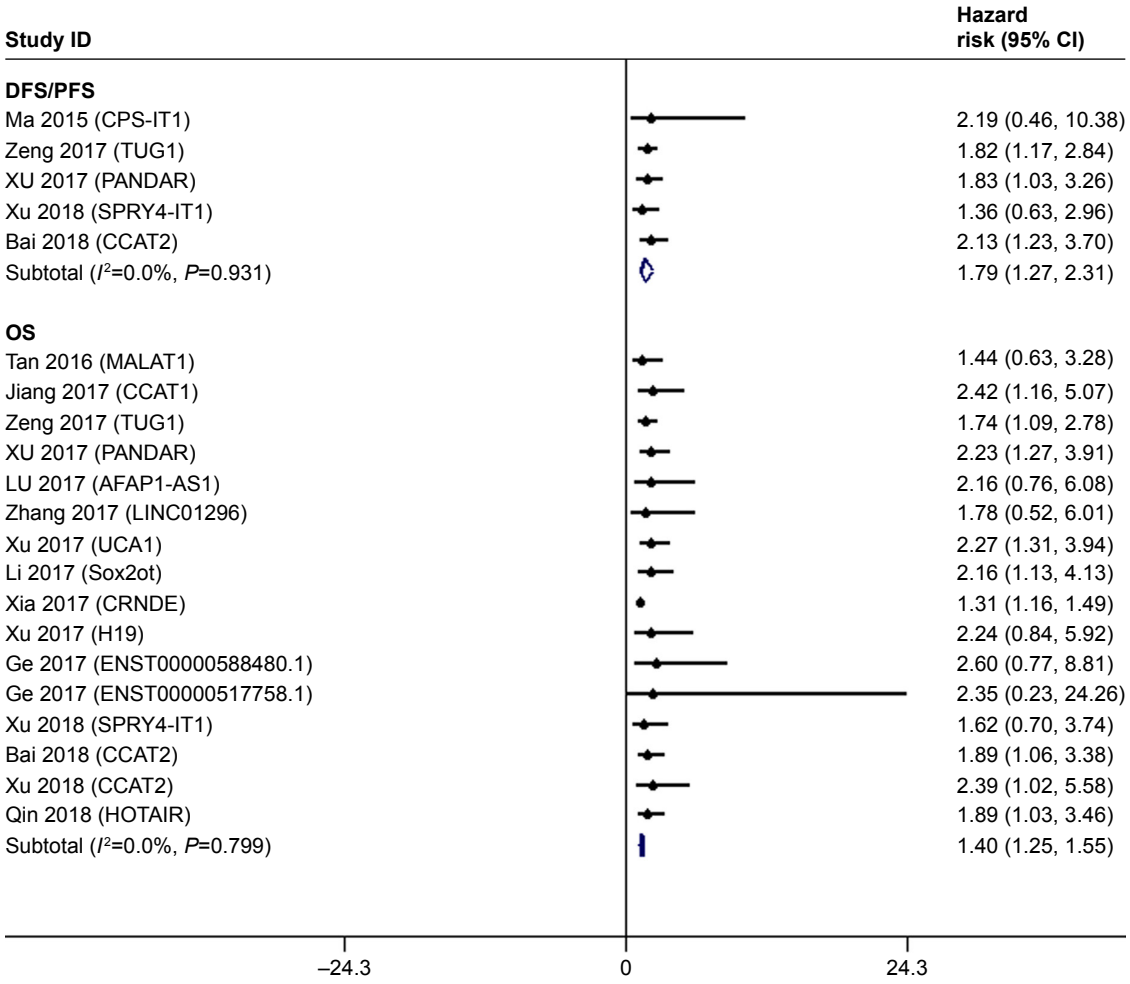

Figure 4 Display of the hazard risk (HR) of lncRNAs and overall survival (OS) in cholangiocarcinoma patients.
Abbreviations: DFS, disease-free survival; PFS, progression-free survival.

CRNDE,[27] H19,[21] SPRY4-IT1,[33] ENST00000588480.1,[31] ENST00000517758.1,[31] CCAT2,[28,34] and HOTAIR[30] (Figure 4). Among them, Sox2ot[26] had the highest HR of 2.936, while CRNDE[27] displayed the lowest HR with 1.309.

Among these 16 lncRNAs, the CCAT family was investigated in more than one study. Thus, we further analyzed the relationship between the expression of CCAT gene family and overall survival (OS; Figure 5). A fixed-effects model was used due to the lack of significant heterogeneity in the CCAT family (I^2=0.0%, P=0.867). As a result, high CCAT family expression predicted short OS (HR, 2.09; 95% CI 1.17–3.00; P<0.001). By further subgroup analysis, overexpression of CCAT2 was obviously associated with poor OS (HR, 2.00; 95% CI 0.96–3.03; P<0.001). Publication bias could not be assessed because of the small size of our study.

Discussion

CCA is still a deadly threat to human health due to its early invasion and metastatic characteristics and poor prognosis. According to relevant reports, the global incidence of CCA has clearly increased during the past decades, especially in Asia.[3,35] However, current therapeutics for CAA are unsatisfactory and so novel biomarkers to diagnose CAA and predict its prognosis are urgently needed. Recently, there has been emerging evidence showing that some lncRNAs play an important role in the tumorigenesis and progression of CAA. In order to codify some of these novel biomarkers for CCA, we conducted this systematic review and meta-analysis. As a result, this meta-analysis is the first to systematically analyze the association between lncRNA expression and their clinical value in CCA.

In terms of association with clinicopathological features, H19,[20,21] CPS1-IT1,[22] RCOR3-013,[19] TMEM63A-005,[19] CCAT1,[23,24] PANDAR,[8] AFAP1-AS1,[11] Linc01296,[6] MALAT1,[12] UCA1,[25] Sox2ot,[26] CRNDE,[27] CCAT2,[28,34] and HOTAIR[30] were overexpressed in CCA, while EMP1-008[19] and ATF3-008[19] were downregulated. All lncRNAs were not significantly associated with patient age, gender, and HBV infection.

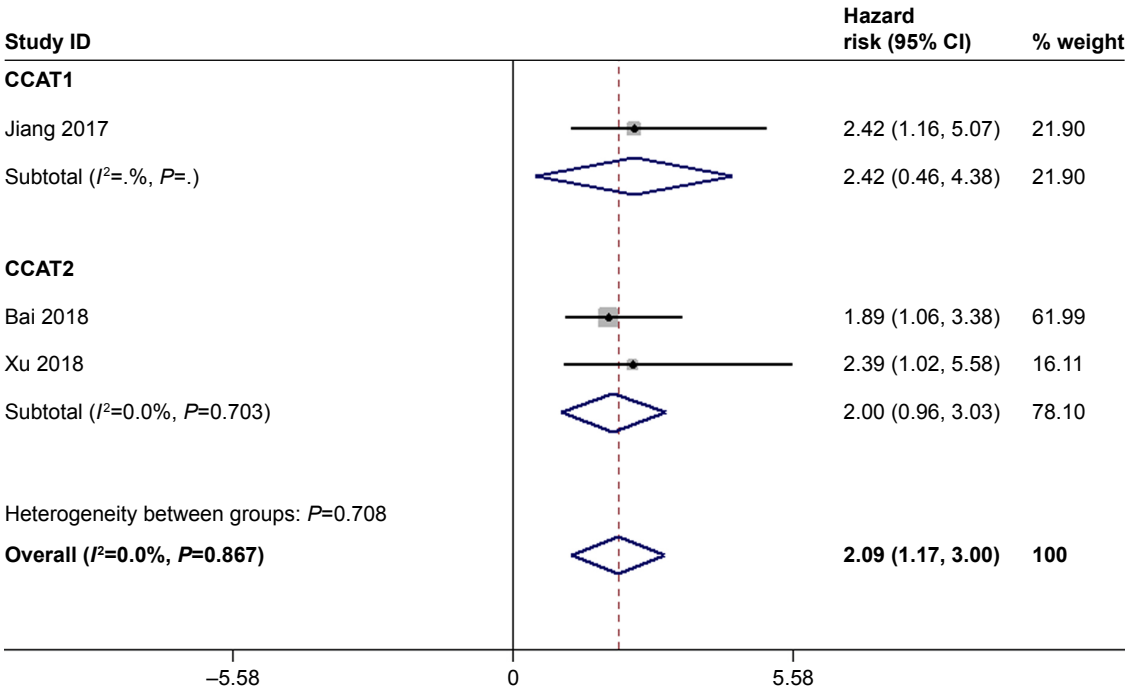

Figure 5 Forest plots of studies evaluating the hazard ratios (HRs) of upregulated CCAT family expression and the overall survival (OS) of cholangiocarcinoma patients.

It is well-known that AFP and CEA play an important role in the diagnosis of CCA.[36] However, studies did not indicate that dysregulation of lncRNAs was significantly related to AFP and CEA. Only two studies indicated that H19 expression was obviously associated with AFP,[20] and CPS1-IT1 expression was significantly related to CEA.[22] The reason for association of H19 with AFP is that the *Afp* and *H19* genes are regulated by *Afr1*, which was first identified in 1977 using persistent AFP serum levels.[37,38] There is no direct evidence for the relationship between CPS1-IT1 and CEA.

Similarly, we found that six lncRNAs were significantly correlated with tumor size, including H19,[20,21] AFAP1-AS1,[11] Linc01296,[6] MALAT1,[12] CRNDE,[25] CCAT2,[28,34] and HOTAIR.[30] However, the correlation remained uncertain due to the different evaluation criteria for tumor size. For example, in the study about association between H19 expression and tumor size, Wang et al[20] selected 5 cm as the criterion for tumor size, while Xu et al[21] used 3 cm as the threshold of tumor size. The reason why some lncRNAs were significantly correlated with tumor size is still uncertain. Moreover, most lncRNAs were significantly correlated with tumor grade of CCA, which meant these lncRNAs were correlated with the progression of CCA.

Ten studies demonstrated that lncRNAs were significantly associated with lymph node metastasis. Among them, we conducted a meta-analysis to further analyze the relationship between CCAT1 expression and clinical features. Our results indicated that CCAT1 expression was significantly correlated with tumor stage and lymph node metastasis in CCA. The above-mentioned results were also reported in other tumors, such as breast cancer[39] and esophageal squamous cell carcinoma.[40] However, Arunkumar et al found that CCAT1 was not obviously associated with tumor stage and the lymph node metastasis in oral squamous cell carcinomas ($P>0.05$).[41] Similarly, another meta-analysis was also performed to analyze the relationship between CCAT2 expression and clinical features. As a result, CCAT2 indicated that upregulation of CCAT2 was obviously associated with tumor stage in CCA.

In our analysis of the prognostic value of lncRNAs, all included studies demonstrated that the overexpression of lncRNAs was associated with a poor prognosis. Among these lncRNAs, the CCAT family was investigated in more than one study. After analysis, the results showed that high expression of CCAT family members predicted shorter OS, especially CCAT2. Our result is consistent with the previous findings, while more research is needed to verify this conclusion due to the small sample size of this study.[42,43]

This study was not without limitations. Firstly, the number of studies included was small. Most lncRNAs appeared only once within the incorporated studies and few lncRNAs appeared in more than two different studies, which influenced heterogeneity. Secondly, due to the lack of survival data, we extracted HR and 95% CI values from a Kaplan–Meier

curve according to Tierney et al methodology, which might also cause potential heterogeneity. Thirdly, because all the included studies were from China, these results might not be applicable to other ethnicities, such as Caucasians. Next, different cutoff values and follow-up end points were used among the included studies, leading to potential heterogeneity. Finally, studies with positive results were more likely to be published, which may result in an exaggeration of the clinical values of lncRNAs in CAA. In spite of these limitations, our study effectively confirmed the important role of various lncRNA expressions in CAA and encouraged researchers to explore the underlying mechanisms in the future.

So far, there are no reports about the application of lncRNA as a biomarker in clinical practice. However, some lncRNAs have been shown to be more sensitive and specific than existing markers.[44,45] So we believe that some lncRNAs will be identified for use in the clinic as biomarkers in the future.

Conclusion

Taken together, our results show that some specific lncRNAs are significantly associated with clinical value in CAA patients. Among them, upregulation of CCAT1 is significantly associated with tumor stage (I + II vs III + IV) and lymph node metastasis for CCA. Overexpression of CCAT2 is obviously related with tumor stage. Additionally, high expression of CCAT family members predicted shorter OS, especially with regards to CCAT2. However, further large-scale and high-quality studies should be included to confirm our findings and to verify the clinical value of lncRNAs in CCA.

Data sharing statement

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

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Author contributions

Kangfu Dai and Jing Quan are co-first authors. KD and JQ designed this study and were involved in data collection, data analysis, and manuscript writing. FY, XP, and XJ contributed to data collection and data analysis. XS and SZ contributed to data analysis. QR was involved in the language editing of the manuscript. All authors contributed to data analysis, drafting and revising the article, gave final approval to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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