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Original Article

Long noncoding RNA CCAT2 as an independent prognostic marker in various carcinomas: evidence based on four studies

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Abstract: Objective: Numerous studies have demonstrated that long noncoding RNAs (lncRNAs) are involved in various biological processes, including cancer progression and metastasis. CCAT2, a newly discovered lncRNA, has been reported that is aberrantly expressed in many types of cancers. However, the clinical application value of CCAT2 in cancers remains to be clarified. We aimed to explore comprehensively potential role of CCAT2 as a prognostic biomarker in malignancy. Methods: Systematic search was performed in Embase, Medline and Cochrane Library until June 2015. Pooled hazard ratios (HRs) with 95% confidence interval (95% CI) were calculated to summarize the effect. Results: A total of 576 patients from four studies were included in this meta-analysis. A significant association was found between high CCAT2 expression and poor overall survival (OS) and progression-free survival (PFS) in patients with cancers, with pooled hazard ratio (HR) of 1.99 (95% CI: 1.25-3.17, P=0.004) and 2.10 (95% CI: 1.36-3.22, P=0.0007), respectively. Conclusion: the findings from this present meta-analysis suggest that CCAT2 abundance may serve as a novel predictive factor for poor prognosis in different types of cancers.

Keywords: Long noncoding RNA, CCAT2, cancer, prognosis, meta-analysis

Introduction

Recently, genome-wide transcriptome studies have confirmed that there are a large number of long intergenic noncoding RNAs (lncRNAs) and growing evidence indicates many lncRNAs act as critical regulators of tumorigenesis and are believed to be involved in tumor development and progression [1]. LncRNAs are non-protein coding RNA molecules greater than 200 nucleotides in length and most lack protein coding capability [2]. Their ability to regulate essential pathways for tumor initiation and progression together with their tissue and stage specificity, promotes them as valuable biomarkers and therapeutic targets [3, 4]. Increasing evidence has demonstrated that lncRNAs can function as oncogenes and tumor suppressors [5-9]. To date, only a few lncRNAs have been characterized functionally, while the functions of most of lncRNAs remain unknown.

CCAT2 (Colon cancer-associated transcript 2) is located within the 8q24 gene desert region and includes a cancer-related single nucleotide polymorphism (SNP) rs6983276. It was firstly identified and found highly expressed in microsatellite-stable colorectal cancer [10]. The CCAT2 genomic locus similar to ultra-conserved regions (UCRs) is highly conserved and harbors the SNP rs6983267, which was shown to be associated with predisposition to colon, ovarian and prostate cancer [11-13]. CCAT2 is aberrantly expressed in a variety of human cancers. It has been suggested that CCAT2 expression may play a useful prognostic role in some tumors. Such as breast cancer, lung cancer, esophageal squamous cell carcinoma, gastric cancer and colon cancer [14-16]. In addition, overexpression of CCAT2 was discovered to be associated with survival of patients in multiple malignant tumors. It promotes tumor growth, metastasis, and chromosomal instability. But

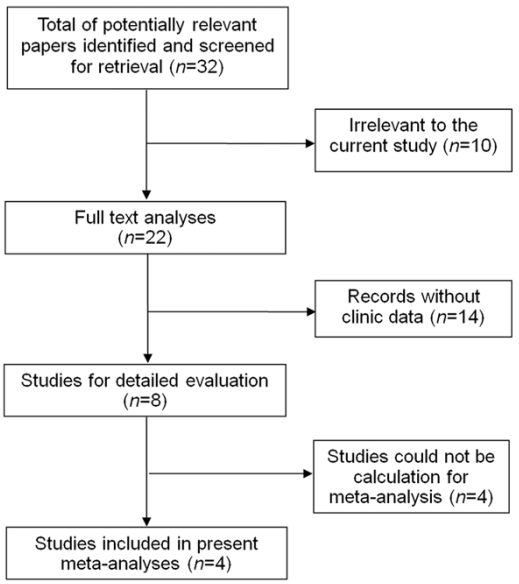

Figure 1. Flow diagram of the study selection process.

the limitation of those studies is the small sample size. According to this, we systematically reviewed the previous literature to investigate and clarify the prognostic value of CCAT2 expression in human cancers.

Materials and methods

Search strategy

The PubMed, EMBASE, and MEDLINE databases were searched, in addition to the Cochrane Central Register of Controlled Trials, to locate articles (published between January 1995 and June 2015), including articles referenced in the publications. The search strategy used both MeSH terms and free-text words to increase the sensitivity of the search. The following search terms were used: "long non-coding RNA", "lncRNA", "CCAT2", "cancer", "prognosis", "survival" and "clinical outcome". Internet search engines were also used to perform a manual search for abstracts from international meetings, which were then downloaded and studied. The reference lists of original articles and review articles were also hand searched to increase the search sensitivity. The published language was limited to English.

Inclusion and exclusion criteria

Two investigators independently assessed all the eligible studies and extracted the data. Studies met the inclusion criteria if they studied the patients with any type of carcinoma, measured the expression of CCAT2 in cancer tissues and investigated the association between CCAT2 expression levels and patients survival outcome. CCAT2 expression was determined in human tissue using quantitative PCR or microarray expression analysis. The sample size was sufficient to calculate the hazard ratios (HR) and 95% confidence interval (CI) for survival rates. Studies have no restrictions on the methods of obtaining the tissue specimens and detection of CCAT2 expression. If the same patient population was used in more than one study, only the complete study would be included. Studies of case reports, letters, and reviews without original data; non-English papers; animal or laboratory studies; and studies of non-dichotomous CCAT2 expression levels and absence of survival outcome were excluded. If any doubt of suitability remained after the abstract was examined, the full manuscript was obtained. If the data could not be extracted or calculated from the original article, the study was excluded.

Data extraction

To validate the accuracy of extraction data, two investigators extracted data independently and reached a consensus on all items. Data regarding the following for each included studies were extracted: first authors' surname, publication year, origin country, sample size, tumor types, CCAT2 assessment methods and the cut-off definition, HR of CCAT2 expression for overall survival (OS) and progression-free survival (PFS) as well as corresponding 95% confidential interval (CI) and *P* value. Multivariate Cox hazard regression analysis reported in the article was included in the present analysis. Disagreements were discussed by the authors and resolved by consensus.

Statistical analysis

Pooled hazard ratios (HRs) were extracted from the included studies. All these HRs and 95% confidence interval (CI) were calculated following Tierney's method and the log HR and standard error (SE) were used for aggregation of the survival results [17]. Univariate meta-regression was conducted to explore the potential heterogeneity in the analysis of the association between CCAT2 and survival. Furthermore, fac-

Table 1. Characteristics of studies included in the meta-analysis

Study	Year	Disease	Number	CCAT2 assay	Survival analysis	Multivariate analysis	Hazard ratios	Follow-up, months
Ling et al. [10]	2013	BC	129	qRT-PCR	PFS	Yes	Reported	NA
Redis RS et al. [14]	2013	BC	134	qRT-PCR	OS, MFS	Yes	Reported	NA
Wang et al. [15]	2015	GC	85	qRT-PCR	OS, PFS	Yes	Reported	60
Zhang et al. [16]	2015	ESCC	229	qRT-PCR	OS	Yes	Reported	66

ESCC esophageal squamous cell carcinoma, *BC* breast cancer, *GC* gastric cancer, *qRT-PCR* quantitative real-time PCR, *OS* overall survival, *PFS* progression-free survival, *NA* not available.

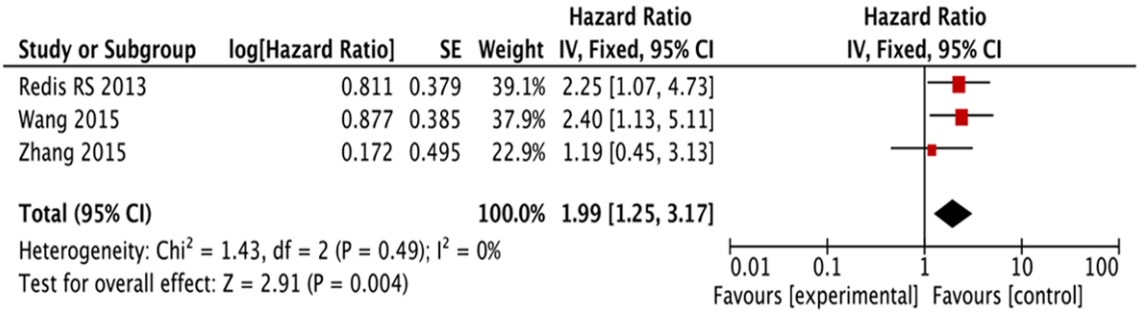

Figure 2. Estimated Hazard Ratio (HR) Summary for overall survival (OS) with CCAT2 expression.

tors identified as significant by univariate analysis were further analyzed with multivariate meta-regression if necessary. I^2 statistics was used to evaluate the between-study heterogeneity analysis in this meta-analysis [18]. The fixed effects model was adopted in the initial calculation of HR with corresponding 95% CIs. If there was a significant statistical heterogeneity among the studies, the random-effects model was applied for the analysis. The meta-analysis results were displayed as forest plots. Sensitivity analysis was performed to test the impact of individual study on the pooled data. Begg's funnel plots and Egger's linear regression test were performed to estimate potential publication bias. The statistical analysis was carried out using the Review Manager (RevMan) software version 5.0. All the *P* values were two-sided. Differences were considered statistically significant at $P < 0.05$.

Results

Eligible studies

Our initial search in electronic database retrieved a total of 32 references. After carefully screening the abstract and full-text of these references, 10 were excluded because they were not related to the current study. Upon further review, 14 were excluded because they were either laboratory studies or records without survival data. Then we evaluated 8 potential candidate studies in full text. Four papers were excluded because of insufficient data to estimate HR for further analysis. Finally, 4 [10, 14-16] studies were included in this meta-analysis. The selection steps were summarized in the flow diagram shown in **Figure 1**. Among these 4 studies, a total of 576 patients were included, with a maximum sample size of 229 and a minimum sample size of 85 patients. The accrual period of these studies ranged from 2013 to 2015. In the four included studies, 2 from China, 1 from the Netherlands and 1 from the United States. All of them were retrospective in design. The types of cancers in these studies included esophageal squamous cell carcinoma, gastric cancer and breast cancer. All of the studies used qRT-PCR to measure the expression level of CCAT2. In each study, the cut-off values of CCAT2 appeared to be different. No patient received chemotherapy or radiotherapy before surgery. The participants in all of the studies were divided into high CCAT2 expression group and low CCAT2 expression group. The main characteristics of included studies were summarized in **Table 1**.

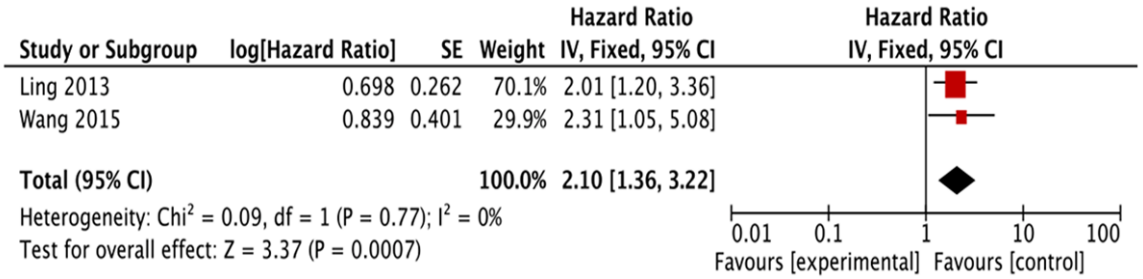

Figure 3. Estimated Hazard Ratio (HR) Summary for progression-free survival (PFS) with CCAT2 expression.

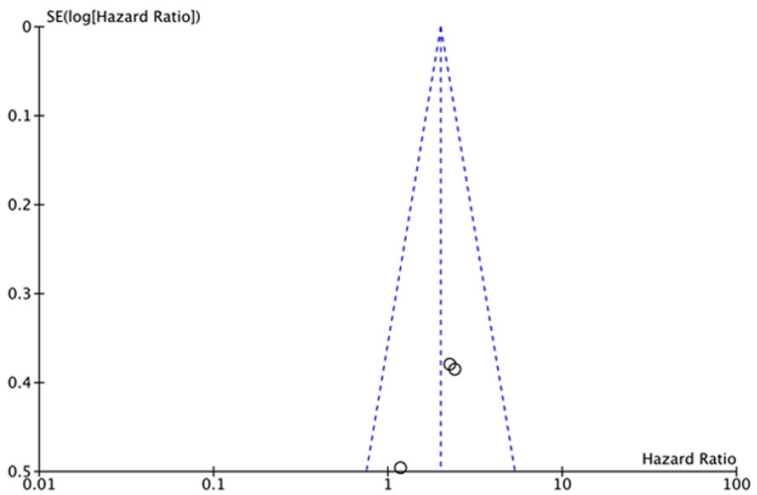

Figure 4. Funnel plot for the publication bias.

Meta-analysis

Three studies reported the overall survival (OS) of 347 patients according to CCAT2 expression levels. Heterogeneity analysis revealed that there was no obvious between-study heterogeneity among those three studies for CCAT2 expression (I^2=0%), so the fixed-effect model was used to calculate the pooled HR with corresponding 95% CI. The result indicated that high expression of CCAT2 might be associated with poor overall survival outcome in various carcinomas, with the pooled HR of 1.99 (95% CI: 1.25-3.17, *P*=0.004) (**Figure 2**). Two studies reported the progression-free survival (PFS) of 214 patients according to CCAT2 expression levels. Heterogeneity analysis revealed that there was no obvious between-study heterogeneity among those three studies for CCAT2 expression (I^2=0%), so the fixed-effect model was used to calculate the pooled HR with corresponding 95% CI. The result indicated that high expression of CCAT2 might be associated with poor progression-free survival outcome in various carcinomas, with the pooled HR of and 2.10 (95% CI: 1.36-3.22, *P*=0.0007) (**Figure 3**). Combining these results suggest that CCAT2 expression might be an independent prognostic factor for cancer patients.

Publication bias

Funnel plot and Egger's test were used to evaluate the publication bias of the literatures. The shapes of the funnel plot did not reveal any evidence of obvious asymmetry (**Figure 4**).

Discussion

Cancer is one of the most common causes of death worldwide, and has become a major public health challenge. Accompanied with the advance of research on carcinogenesis, more and more studies focus on novel strategies for early detection and prevention [19]. Emerging evidence has suggested and proposed long noncoding RNAs (lncRNAs) as promising biomarkers for early cancer detection and accurate prognosis as well as targets for more efficient treatment [20].

LncRNA is a kind of RNA with transcripts longer than 200nt. They were initially regarded as spurious transcriptional noise, without biological functions. LncRNAs regulate the expression levels of target genes at epigenetic, transcriptional, and post-transcriptional levels [21, 22]. There are no doubts that lncRNAs are important cancer players, thus the field of lncRNA research is very promising in the area of cancer research. CCAT2, a novel lncRNA transcript

maps to the highly conserved 8q24.21 region encompassing rs6983267 and is overexpressed in microsatellite-stable CRC samples. The CCAT2 genomic locus similar to UCRs is highly conserved and harbors the SNP rs6983267, which was shown to be associated with predisposition to colon, ovarian and prostate cancer [11, 12]. CCAT2 promotes metastasis and chromosomal instability in microsatellite stable (MSS) colon cancer through a mechanism involving transcription factors, oncogenes and microRNAs [10]. Recently, many studies have indicated that lncRNA CCAT2 plays a crucial role in progression and metastasis of diverse cancers. But the prognostic role of CCAT2 in different types of cancer is unclear. Therefore, we conduct this present meta-analysis to investigate the relationship between CCAT2 expression and the overall survival rate in patients with various cancers. Our results demonstrated that high expression of CCAT2 did predict poor survival in patients with a variety of carcinomas. Significant heterogeneity did not exist across studies.

To our limited knowledge, this is the first meta-analysis focus on the prognostic value of CCAT2 in cancers. However, it should be circumspect to make a verdict of the association with CCAT2 and human cancers, because there are still several limitations should be considered. First, the cut-off definition of CCAT2 appeared to be different in each study. Although most of them defined median as the cut-off of elevated CCAT2 expression, the accurate values could be various in the different study populations. It was difficult to set a standard cut-off value. Second, sample size of the study is limited. We only recruited 4 studies with 576 patients in this meta-analysis. It might weaken the reliability of our results. Third, most of the included studies reported positive results because those with negative results are generally less likely to be published. Therefore we strongly suggest conducting more larger-size and better design studies to confirm our results.

In conclusion, this meta-analysis provides evidence that the high expressed CCAT2 is significantly associated with poor survival in patients with various types of carcinoma. CCAT2 may be useful prognostic markers in several cancers. However, if we consider applying CCAT2 in prognosis of a single type of cancer, more studies and more subjects are needed, especially a single type of cancer.

Disclosure of conflict of interest

None.

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