Original Paper

Low Expression of LncRNA Cancer Susceptibility Candidate 2 and its Clinical Significance in Cancer Tissues

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Key Words

Cancer susceptibility candidate 2 • Long noncoding RNA • Overall survival • Tumor stage

Abstract

Background/Aims: Long noncoding RNA (lncRNA) cancer susceptibility candidate 2 (CASC2) is downregulated in various cancers and involved in both tumorigenesis and progression. The aim of this study was to assess the prognostic value of lncRNA CASC2 in cancer patients. **Methods:** We searched the Web of Science, PubMed, EMBASE, China National Knowledge Infrastructure (CNKI), and the Wanfang database to identify studies evaluating the prognostic value of lncRNA CASC2 in cancer patients. Pooled hazard ratios (HRs) or odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using fixed-effects/random-effects models. **Results:** A total of eight studies were included. The combined results showed that lncRNA CASC2 was significantly associated with decreased overall survival (OS) (HR = 0.37, 95% CI, 0.27–0.46, $P < 0.001$). Subgroup analyses further indicated that low expression of lncRNA CASC2 predicted decreased OS in cancer patients. Additionally, low CASC2 expression levels in cancer tissues appeared to be correlated with advanced clinical staging (OR = 3.32, 95% CI, 2.29–4.80, $P < 0.001$). **Conclusions:** Low CASC2 expression appears to be predictive of poor OS and advanced tumor stage in multiple cancers. CASC2 expression may serve as unfavorable prognostic factor for clinical outcomes in cancer patients.

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Introduction

Cancers are among the major most serious diseases causing morbidity and mortality worldwide. In 2012, there were some 14.1 million new cancer cases and 8.2 million cancer deaths [1]. Although there have recently been advances in the diagnosis and treatment of cancer [2–4], the prognosis for most cancer patients has remained poor, especially in terms of their long-term survival, which has not dramatically improved. Thus, there is an urgent

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need for the identification of new potential biomarkers that might improve the prediction of prognosis.

Long noncoding RNAs (lncRNAs) are a group of ncRNAs greater than 200 nucleotides in length that lack an obvious open reading frame; for decades, they were perceived as mere transcriptions of “noise” [5, 6]. However, now interest in these RNAs has grown, and they are thought to have indispensable roles in diverse biological processes [7-10]. More and more lncRNAs have been identified and found to function as oncogenes or suppressive factors in cancers [11-12]. Furthermore, some of them may come to serve as promising prognostic biomarkers and therapeutic targets in various types of malignancies [13-15].

The lncRNA cancer susceptibility candidate 2 (CASC2), located at chromosome 10q26, has been identified as a novel lncRNA that was first found to be downregulated in endometrial cancer [16]. Now increasing numbers of studies have shown that CASC2 is also significantly downregulated in other cancers, such as hepatocellular carcinoma, colorectal cancer, and bladder cancer [17-19], and it could act as a tumor suppressor with implications for diagnosis and therapy [20]. Numerous researchers have reported that CASC2 is implicated in tumor initiation and progression and has shown a potential for predicting cancer prognosis [21-23]. However, up to now there has been no specific meta-analysis to systematically elucidate the prognostic value of CASC2 in cancers. We therefore conducted the present meta-analysis to assess the potential value of CASC2 as a prognostic biomarker.

Materials and Methods

Publication search

A comprehensive search was conducted in a number of electronic databases, including the Web of Science, PubMed, EMBASE database, China National Knowledge Infrastructure (CNKI), and Wanfang database. The latest search was updated on September 30, 2017. The following key words were used for the initial search: “CASC2,” “cancer susceptibility 2,” and “C10orf5.” Manual searches were also performed through the reference lists of the retrieved articles. There is no restriction on the language of publication.

Inclusion and exclusion criteria

Inclusion criteria: (1) the expression of lncRNA CASC2 was detected in cancerous tissues; (2) the relationship between CASC2 expression and prognosis were investigated; (3) sufficient data were provided for calculating the hazard ratio (HR) with 95% CI for survival rates; and (4) patients were divided into high- and low-expression groups.

Exclusion criteria: (1) reviews, conference abstracts, or non-original articles; (2) duplicate publications; (3) studies focused only on the molecular mechanisms or functions of CASC2.

Data extraction

The following data were extracted from every study by two investigators acting independently: first author, publication year, country, tumor type, sample size, expression pattern, clinical staging, criterion of low expression, detection method, follow-up time, outcome measures, and type of analysis.

To extract the OS data, if a study provided only Kaplan-Meier curves, the HRs and 95% CIs were retrieved using the Engauge Digitizer version 4.1. If a study reported the data in univariate and multivariate analysis, the latter was applied directly.

For quality assessment, the Newcastle-Ottawa quality assessment scale (NOS) was used to assess the quality of eligible studies with scores ranging from 0 to 9 points. A high- quality study was identified as with a score of ≥ 6 .

Statistical analysis

Stata statistical software version 12.0 was used to analyze the relationship between lncRNA CASC2, OS, and clinical staging.

Heterogeneity among studies was identified via I^2 statistics and the chi-square Q test. $I^2 \geq 50\%$ and/or $P_Q < 0.05$ was deemed to indicate significant heterogeneity, in which case a random-effects model was

adopted. Otherwise, the fixed-effects model was used. Potential publication bias was assessed with the funnel plot and Begg's test. Sensitivity analysis was used to evaluate the stability of the results. A difference with $P < 0.05$ was regarded to be statistically significant.

Results

Study characteristics

Initially, after duplications were excluded, 26 articles were obtained. After 15 of these had been thoroughly evaluated, 7 were excluded because they did not meet the inclusion criteria. Finally, 8 articles [21-28] were included in our meta-analysis. The detailed process of study, search, and selection is presented in Fig. 1.

Our studies comprised 580 patients; they were enrolled with mean sample size of 72.5, with a wide range from 47 to 110. Seven different kinds of cancers were included: cervical cancer (CC), hepatocellular carcinoma (HCC), thyroid cancer (TC), pancreatic cancer (PC), glioma, gastric cancer (GC) and non-small-cell lung cancer (NSCLC). These cancers ranged from an early stage to advanced clinical stages (I, II, III, and IV). Of the 8 studies, 3 included a follow-up of greater than or equal to 60 months [23, 27, 28]; the remaining 5 studies covered less than 5 years [21, 22, 24-26]. The expression of lncRNA CASC2 in all eligible studies was measured by quantitative real-

Fig. 1. The steps for screening eligible articles.

Table 1. Main characteristics of all studies included in the meta-analysis. CC: cervical cancer; HCC: hepatocellular carcinoma; TC: thyroid cancer; C: pancreatic cancer; GC: gastric cancer; NSCLC: non-small cell lung cancer; LE: low expression; OM: Outcome measures; UVM: univariate analysis; MVM: multivariate analysis

First author, Year	Cancer type	Sample size	LE (N, %)	Follow-up	Criterion of LE	OM	Analysis	NOS score
Feng Y, 2017 [21]	CC	60	30, 50.0%	< 5 years (1-40 months)	below the median	OS	UVM	7
Liao Y, 2017 [22]	Glioma	57	28, 49.1%	< 5 years (1-40 months)	below the median	OS	MVM	6
Wang R, 2017 [23]	Glioma	47	25, 53.2%	≥ 5 years (max. 60 months)	less than the median	OS	MVM	6
Wang Y, 2017 [24]	HCC	75	37, 49.3%	< 5 years (max. 36 months)	below the median	OS	UVM	7
Xiong X, 2017 [25]	TC	86	16, 18.6%	< 5 years (max. 32 months)	below the mean	OS	MVM	7
Yu Y, 2017 [26]	PC	110	54, 49.1%	< 5 years (max. 48 months)	below the median	OS	MVM	9
Zhou J, 2017 [27]	GC	69	35, 50.7%	≥ 5 years (above 60 months)	expression ratio ≤ median	OS	UVM	8
He X, 2016 [28]	NSCLC	76	38, 50.0%	≥ 5 years (max. 60 months)	below the mean	OS	MVM	8

time polymerase chain reaction (qRT-PCR) and normalized relative to GAPDH or RNU6B using the $2^{-\Delta\Delta Ct}$ method. LncRNA CASC2 expression was found to be down-regulated in 18.6% to 50.7% cancerous tissues. All 8 articles were written in English, all were from China, and all the patients were Chinese. The publication period ranged from 2016 to 2017. All studies investigated the relationship between lncRNA CASC2 and cancer prognosis. The main characteristics of all the included studies are summarized in Table 1.

Results of the meta-analysis LncRNA CASC2 and OS

All included studies comprising 580 cases reported the relationship between lncRNA CASC2 and OS in cancer patients. As seen in Fig. 2A, the pooled results showed that low expression of lncRNA CASC2 was significantly more marked with poorer OS (HR=0.37, 95% CI, 0.27–0.46, $P < 0.001$) with no significant heterogeneity ($I^2 = 0.0\%$; $P = 0.879$). These observations suggest that lncRNA CASC2 may serve as a valuable biomarker for prognosis in human cancers.

Furthermore, subgroup meta-analyses stratified by cancer type, follow-up time, and analysis type were conducted to assess the prognostic value of lncRNA CASC2. The results revealed that lncRNA CASC2 could act as prognostic indicator of OS for patients with gastrointestinal (GI) cancer (HR=0.40, 95% CI, 0.26–0.53, $P < 0.001$) (Fig. 2B). After stratification by follow-up time, we found that lncRNA CASC2 could act as a prognostic factor in groups with follow-up time < 5 years (HR=0.41, 95% CI, 0.28–0.55, $P < 0.001$) or groups with follow-up time ≥ 5 years (HR=0.32, 95% CI, 0.19–0.46, $P < 0.001$) (Fig. 2C). In addition, the pooled HRs were significantly and consistently less than 1 in subgroup meta-analyses based on the analysis type, indicating that lncRNA CASC2 was an independent prognostic marker for OS (HR= 0.35, 95% CI, 0.23–0.47, $P < 0.001$) (Fig. 2D).

Fig. 2. Meta-analysis of the pooled HRs of OS of patients with low lncRNA CASC2 expression. (A) overall, (B) stratified by cancer type, (C) stratified by follow-up time, (D) stratified by type of analysis.

Fig. 3. Association between lncRNA CASC2 and clinical stage.

Fig. 4. Funnel plots for publication bias test for OS (A) and clinical stages (B).

LncRNA CASC2 and clinical stage

Additionally, we explored the association between lncRNA CASC2 expression and clinical stage. Our 8 studies comprising 580 patients reported on this the relationship. As seen in Fig. 3, the pooled results showed that a low expression level of CASC2 significantly correlated with advanced clinical stage (OR=3.32, 95% CI, 2.29–4.80, $P < 0.001$). We therefore concluded that lncRNA CASC2 might be involved in oncogenesis and tumor progression.

Publication bias

As presented in Fig. 4A and B, no significant publication bias was found among studies. The test results also demonstrated that the answers were negative (OS: $P_{\text{Begg's test}} = 0.174$; clinical staging: $P_{\text{Begg's test}} = 0.108$).

Sensitivity analysis

As illustrated in Fig. 5A and B, the results of sensitivity analysis for OS and clinical staging were negative.

Discussion

lncRNA CASC2 was first discovered in 2004 by Baladinu et al [16]. Subsequent studies detected the expression of CASC2 in various types of cancer and found it to be downregulated in cancer tissues as compared with para-cancer or normal samples. The correlations between CASC2 expression and the clinicopathologic features of various human tumors or patient prognosis were also investigated and reported. As a result, it was found that CASC2 appeared to be acting as a cancer suppressor [23–26].

CASC2 is an indispensable cancer-related lncRNA, upregulation of CASC2 may inhibit the proliferation, migration, and invasion of cancer cells and promote early apoptosis [20]. A large number of studies have demonstrated that lncRNA CASC2 plays a vital role in the development and progression of cancer. For example, in esophageal carcinoma, CASC2 was shown to be an oncogene suppressor through inhibition of the NF- κ B pathway [29] or that it acted as a ceRNA of miR-18a-5p to exert its biological effects by modulating the expression of PTEN [30]. In hepatocellular carcinoma, lncRNA CASC2 may regulate cell biological behavior through the MAPK signaling pathway [17]. Additionally, it suppressed the epithelial-mesenchymal transition of hepatocellular carcinoma cells through the CASC2/miR-367/FBXW7 axis [24]. CASC2 was also reported to act as a tumor suppressor lncRNA by regulating several signaling pathways, such as the Wnt/ β -catenin signaling pathway [23] or the MAPK signaling pathway [31] or to be a direct target gene of miR-21, suppressing malignancy [32, 33]. Although many studies have explored the interaction partners and molecular functions of CASC2 in multiple cancers, the mechanisms underlying CASC2 and cancer progression remain elusive.

For all we know, ours is the first meta-analysis revealing that lncRNA CASC2 expression is significantly related to patient survival and clinical staging in human cancers. Patients with low expression of CASC2 exhibited decreased OS, and the subgroup analyses for OS also demonstrated the prognostic value of CASC2 in cancers. Furthermore, the pooled results showed that low expression of CASC2 was significantly associated with advanced clinical

Fig. 5. Sensitivity analysis of the relationship between CASC2 and OS (A) or clinical stage (B).

staging. Taken together, these data indicate an important role of CASC2 in the development and progression of cancer, suggesting the potential clinical value of CASC2 as a novel prognostic marker in various cancers.

Nevertheless, it should be noted that our study has several limitations. First, only 8 studies comprising 580 cases were enrolled; moreover, the number of articles and the sample size were relatively small. In addition, all of the studies included in the meta-analysis were conducted in China; there were no researchers from other countries. This might limit the application of our conclusions. In addition, we failed to assess the correlations between CASC2 expression and some other clinicopathologic features, such as lymph node metastasis, since we had no relevant data in this regard. Finally, other factors, such as treatment, that could affect survival time were not factored into our analyses.

The translational value of CASC2 as a promising tumor biomarker deserves further attention. Our findings will strongly contribute to the development of CASC2 as a prognostic marker applicable to the practice of clinical medicine. Of course, before CASC2 can be utilized in clinical prognostication, much work remains to be done. A most important point is that the study of CASC2 should be established and standardized, including sampling, treatment, the use of stable and commercially available reagents, and that procedures cutoff values be unified. Based on the established determination method, a well-designed large-scale multicenter case-control clinical trial should be performed. The results could serve to support the practical application of CASC2 in the clinical prognostication of cancer.

In aggregate, we preliminarily concluded that CASC2 could be applied for the improvement of prognosis estimation and that it constitutes a promising biomarker for human cancers. Prospective multicenter studies designed with larger sample sizes and other populations will be necessary to verify and strengthen the prognostic value of CASC2 in cancer patients.

Disclosure Statement

The authors declare no conflicts of interest.

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