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Long non-coding RNA CCHE1 overexpression predicts a poor prognosis for cervical cancer

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Abstract. – OBJECTIVE: Our study is intended to explore the correlation of long non-coding RNA CCHE1 (CCHE1) and clinicopathological factors of cervical cancer patients, and evaluate the impact of CCHE1 on the prognosis of cervical cancer.

PATIENTS AND METHODS: The CCHE1 expression in cervical cancer tissues was examined by quantitative Real-time PCR (qRT-PCR) and its correlation with clinicopathological features was also analyzed. A Kaplan-Meier survival curve was generated following a log-rank test. Multivariate Cox regression analyses were finally used to determine the independent factors for overall survival (OS) and recurrence-free survival (RFS) times.

RESULTS: Our results showed that higher CCHE1 expression was found in cervical cancer tissues compared with the match normal cervical tissues. Then, we found that high levels of CCHE1 expression correlated with FIGO stage (p= 0.014), tumor size (p = 0.007), lymph node metastasis (p = 0.022) and HPV (p = 0.001). Results of Kaplan-Meier survival curve revealed that patients with low CCHE1 expression had better OS (p = 0.019) and RFS (p = 0.006) than patients with high CCHE1 expression. Finally, overexpression of CCHE1 was supposed to be an independent poor prognostic factor for predicting the 5-year RFS and OS of cervical cancer patients through multivariate analysis.

CONCLUSIONS: Increased CCHE1 was associated with poor survival in cervical cancer patients, suggesting that CCHE1 was a potential prognostic biomarker for cervical cancer.

Key Words:
Long non-coding RNA, CCHE1, Cervical cancer, Prognosis.

Introduction

Cervical cancer is one of the most common malignant gynecological disorders, especially in developed countries. It is the major cause of death from gynecological cancers in developing countries like India[1-3]. Despite the progress in early diagnosis and multimodal therapeutic modalities, the recurrence rate is high, and surgery affects long-term recovery and survival in cervical cancer patients after curative resection[4,5]. Therefore, to assess prognosis and establish reasonable treatment of cervical cancer patients, it is necessary to reveal the mechanism of cancer progression and to search an ideal tumor molecular marker. However, up to date, no satisfying biological marker can be used in cervical cancer routinely.

It is well known that long noncoding RNA (lncRNA) is transcribed RNA molecules more than 200 nucleotides and lack protein-coding potential[6,7]. Previous studies[8-10] have revealed that lncRNAs are involved in many biological processes, and some lncRNAs have been found playing vital roles in the progression of various types of cancers. Some lncRNAs have been studied deeply. For instance, lncRNA MALAT1 was reported to function as a tumor promoter in many tumor including cervical cancer[11], non-small cell lung cancer[12], and clear cell renal cell carcinoma[13]. lncRNA HMlincRNA717 was observed to play an anti-oncogene in several tumors including pancreatic cancer[14], lung cancer[15], and gastric cancer[16]. However, to our knowledge, the clinical significance and biological function of CCHE1 in cervical cancer remain largely unclear.

CCHE1 was a newly identified lncRNA. To our best knowledge, up to date, very little papers reported the effect of CCHE1 in tumors. In the present study, we firstly explored the possibility that CCHE1 could be useful as a novel prognostic biomarker for cervical cancer.

Patients and Methods

Patients and Clinical Samples

Patients with cervical cancer admitted to the oncology from July 2008 to July 2011 were drawn from the hospital tumor registry. All the patients did not receive prior anticancer treatment. All spe-

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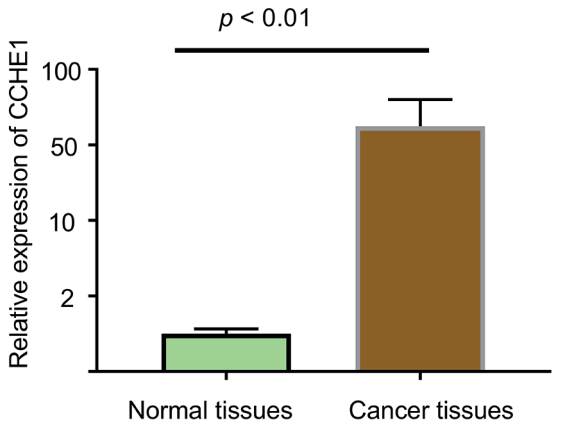

Figure 1. CCHE1 up-regulated in cervical cancer patients. RT-PCR of 141 paired cervical cancer and match normal tissues.

cimens were handled and made anonymous based on the ethical and legal standards. The tissue samples were immediately snap-frozen in liquid nitrogen and stored at -80°C until RNA extraction. Clinicopathological characteristics of cervical cancer patients were detailed in Table I. Written informed consent was obtained from all of the patients. This study was approved by the Research Ethics Committee of Linyi People's Hospital.

RNA Isolation, Reverse Transcription and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated from frozen specimens using Trizol (Invitrogen, Carlsbad, CA, USA). For qPCR, RNA reverse transcribed to complementary DNA (cDNA) from 1 μg of total RNA was reverse transcribed in a final volume of 10 μL using a Reverse Transcription Kit (Takara, Dalian, Niaoning, China). Real-time PCR was performed using the Applied Biosystems 7500 Sequence Detection system. The expression of lncRNAs was defined based on the threshold cycle (Ct). The relative expression of miR-130b was shown as fold difference relative to U6. Three experimental replicates were performed. The primer sequences used were as follows: CCHE1: 5′-AAGGTCCCAGGATACTCGC-3′ (forward) and 5′-GTGTCGTGGACTGGCAAAAT-3′ (reverse);

U6: 5′-ACAGUAGUCUGCACAUUGGUUA-3′ (forward) and

5′-ACGCAAATTCGTGAA GCG TT-3′ (reverse).

Statistical Analysis

The GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA, USA) was applied for chi-square test and Two-tailed Student's *t*-test. The overall survival and recurrence-free survival of patients with different expression of CCHE1 were estimated by the Kaplan-Meier analysis. The multivariate analyses were evaluated with Cox proportional hazards models. $p < 0.05$ was considered statistically significant.

Table I. Association between CCHE1 expression and clinicopathological parameters in 141 patients with cervical cancer.

		CCHE1 expression		
Clinicopathological features	No. of cases	High (no., %)	Low (no., %)	*p*-value
Age				0.449
<50	51	30	21	
≥50	90	47	43	
Tumor size (cm)				0.007
<4.0	101	48	53	
≥4.0	40	29	11	
Histological grades				0.075
Well/Moderately differentiated	102	51	51	
Poorly differentiated	39	26	13	
FIGO stage				0.014
Ib-IIa	84	39	45	
IIb-IIIa	57	38	19	
Lymph node metastasis				0.022
No	80	37	43	
Yes	61	40	21	
HPV				0.001
(−)	81	33	48	
(+)	60	44	16	

Results

CCHE1 Expression is Increased in Human Cervical Cancer

To determine whether CCHE1 expression was changed in human cervical cancer, the expression levels of CCHE1 in 141 pairs of cervical cancer tissues and their matched non-tumor tissues were examined by quantitative reverse transcription polymerase chain reaction (qRTPCR). As shown in Figure 1, the results showed that CCHE1 was frequently upregulated in cervical cancer specimens compared with adjacent noncancerous tissues ($p < 0.001$).

Relationship Between CCHE1 Expression and Clinicopathologic Characteristics

To explore the potential role of CCHE1 in the progression of tumors, we manually divided the cervical cancer patients into two groups accor-

Table II. Multivariate analysis of the correlation of RFS and OS with clinicopathological factors in cervical cancer patients.

Clinicopathological factors	5-year RFS			5-year OS		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	2.961	0.663-3.354	0.247	2.176	0.615-3.238	0.281
Tumor size (cm)	1.432	0.818-1.993	0.246	1.217	0.616-2.347	0.196
Histological grades	2.471	0.836-3.224	0.127	2.933	0.652-3.994	0.166
FIGO stage	1.893	0.895-2.431	0.018	2.644	1.146-3.147	0.008
Lymph node metastasis	2.574	1.931-3.226	0.014	2.865	2.136-4.127	0.006
HPV	2.245	0.855-2.994	0.147	2.883	1.034-3.552	0.189
CCHE1 expression	3.341	1.447-4.465	0.011	3.664	1.674-4.714	0.008

ding to the expression of CCHE1. Next, we evaluate the associations between CCHE1 expression level and clinical characteristics. As shown in Table I, The results showed that high levels of CCHE1 expression correlated with FIGO stage ($p = 0.014$), tumor size ($p = 0.007$), lymph node metastasis ($p = 0.022$) and HPV ($p = 0.001$). However, there was no correlation of CCHE1 expression with other clinical features, such as age and histological grades ($p > 0.05$).

Association of CCHE1 Expression with Patients' Survival Time

Additionally, we collected information on 5-year survivors from 141 patients. The relationship between CCHE1 expression and OS and RFS in 141 cervical cancer patients was analyzed by Kaplan-Meier using the log-rank test. The data indicated that patients with low CCHE1 expression had better OS ($p = 0.019$, Figure 2) and RFS ($p = 0.006$, Figure 3) than patients with high CCHE1 expression. These data demonstrated that increased CCHE1 was correlated with poor survival of cervical cancer patients. We next evaluated the expression of CCHE1 and other clinicopathologic parameters on the prognosis of cervical cancer with multivariate analysis. For the cervical cancer patients, FIGO stage (HR = 2.644, 95% CI, 1.146-3.147; $p = 0.008$), lymph node metastasis (HR = 2.865, 95% CI, 2.136-4.127; $p = 0.006$), and elevated CCHE1 level (HR = 3.664, 95% CI, 1.674-4.714; $p < 0.008$) were independent factors associated with OS (Table II). The similar finding was also observed in RFS. Thus, our results suggested that CCHE1 expression could be used as a powerful independent prognostic factor in cervical cancer patients.

Discussion

Finding new molecular targets for its diagnosis, prognosis, and treatment is very important to improve the clinical strategies and outcomes of cervical cancer. More and more evidence showed that dysregulation in lncRNAs involved in tumor development in different cancer, and some lncRNAs have been reported to serve as valuable

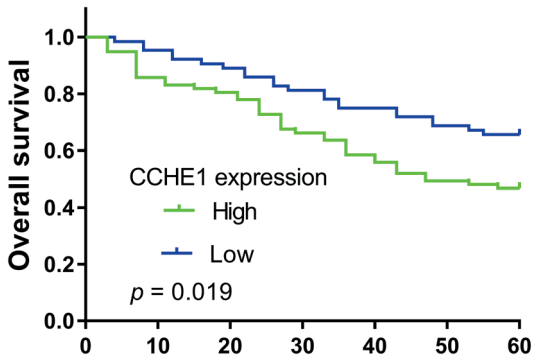

Figure 2. Overall survival curves according to status of CCHE1 expression, $p = 0.019$.

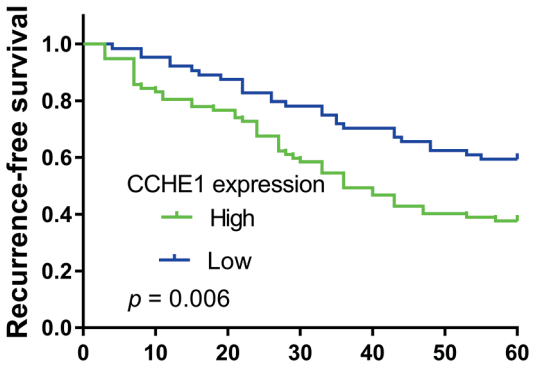

Figure 3. Recurrence-free survival curves according to status of CCHE1 expression, $p = 0.006$.

prognostic markers including cervical cancer[17,18]. In the present study, our attention focused on CCHE1.

CCHE1 encodes a long noncoding RNA and maps to chromosome 10[19]. To our best knowledge, only two papers reported the effect of CCHE1 on the progression of tumors. Peng et al[20] reported that the higher expression of CCHE1 was observed in hepatocellular carcinoma tissues and significantly correlated with tumor number, tumor size, and TNM stage. They further performed cells experiments and found that CCHE1 knockdown significantly promoted growth arrest and cell apoptosis in the activation of the ERK/MAPK pathway. Their results indicated that CCHE1 played an oncogene in hepatocellular carcinoma. Yang et al[21] showed that CCHE1 was significantly upregulated in cervical cancer tissues compared with normal tissues. By gain-of-function and loss-of-function experiments, they revealed that CCHE1 overexpression promotes the proliferation of cervical cancer cell. On the contrary, the depletion of CCHE1 inhibits the proliferation of cervical cancer cells. At the same time, they used Kaplan-Meier analyses and found that significantly shorter overall survival was observed in patients with higher expression of the CCHE1. However, they don't perform the univariate and multivariate analysis to confirm the prognosis effect of CCHE1 in cervical cancer.

In the present work, consistent with the previous study[21], we also observed that CCHE1 was significantly overexpressed in cervical cancer. Moreover, high CCHE1 expression was found to be closely correlated with advanced FIGO stage, tumor size, lymph node metastasis and positive HPV. Furthermore, Kaplan-Meier curves showed patients with low CCHE1 expression had better OS and RFS than patients with high CCHE1 expression. Finally, by multivariate analysis, we confirmed that CCHE1 can serve as a significant and independent predictor of OS and RFS.

Conclusions

Our findings provided the first evidence that CCHE1 was an independent prognostic marker in cervical cancer.

Conflict of interest

The authors declare no conflicts of interest.

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