Serum CCAT2 as a Biomarker for Adjuvant Diagnosis and Prognostic Prediction of Cervical Cancer

Xiaoli Cao
   Nantong Tumor Hospital

Juan Yao
   Nantong Tumor Hospital

Meiqun Jia
   Nantong Tumor Hospital

Xianjuan Shen
   Nantong University Affiliated Hospital: Affiliated Hospital of Nantong University

Jinye Zhang
   Nantong Tumor Hospital

shaoqing ju (✉ jsq814@hotmail.com)
   Nantong University Affiliated Hospital: Affiliated Hospital of Nantong University

Research

Keywords: cervical carcinoma, IncRNA CCAT2, Real-time quantitative PCR

DOI: https://doi.org/10.21203/rs.3.rs-117958/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Growing evidence indicates that IncRNA colon cancer-associated transcript 2 (CCAT2) is associated with cancers. However, the clinical value of CCAT2 in cervical cancer (CC) remains unclear. In this study, serum CCAT2 level was detected by real-time quantitative PCR (RT-qPCR). Carbohydrate antigen 125 (CA125) and squamous-cell carcinoma antigen (SCC) were detected by electrochemiluminescence. A receiver operating characteristic (ROC) curve was utilized to estimate the diagnostic efficiency of CCAT2. Kaplan-Meier survival analysis and univariable and multivariable analyses were performed to assess the prognostic value of CCAT2. The relative expression level of CCAT2 in primary CC patients was significantly higher than that in cervical intraepithelial neoplasias (CIN) patients and healthy controls (both \( P < 0.001 \)). CCAT2 relative expression was positively correlated with tumor FIGO stage, SCC-Ag and LNM (all \( P < 0.05 \)). CCAT2 expression in recurrent/metastatic CC was significantly higher compared with primary CC (\( P < 0.0001 \)) or operated CC (\( P < 0.0001 \)) and during follow-up, CCAT2 expression was increased before surgery and decreased significantly after surgery (\( P < 0.0001 \)). Furthermore, the overall survival rate of CC patients with high CCAT2 expression group markedly decreased as compared with that of low CCAT2 expression group (\( P = 0.026 \)). Univariate analyses indicated that CCAT2 was a poor prognostic factor associated with overall survival (OS). Our study indicates that CCAT2 may be valuable in complementary diagnosis and monitoring of progression and prognosis of CC patients. Combined detection of CCAT2, CA125 and SCC can greatly improve the diagnostic efficiency of primary CC.

Background

Cervical cancer (CC) ranks the second in female malignant tumors, and the second cause of cancer-related death in cancer patients aged 20–39 years. In addition, the incidence of CC tends to increase gradually each year [13]. Early surgical intervention, radiotherapy and chemotherapy can offer a high survival rate in CC patients. However, many CC patients have already been in the middle or late state and lost the chance of surgery at the time of diagnosis. Radiochemotherapy for such patients is often ineffective and their survival and prognosis are usually poor. With technical advances in recent years, tumor markers have gradually been used for early screening of malignant tumors due to simplicity, microinvasiveness and quickness, and play a significant role in early diagnosis, therapeutic assessment and prognostic prediction of cancer patients.

Carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125) and squamous cell carcinoma antigen (SCC) are three main tumor markers for early screening, therapeutic monitoring and prognostic assessment of CC at present. But as they are not sensitive and specific as expected, more reliable, sensitive and specific serum markers are required.

LncRNA colon cancer associated transcript 2 (CCAT2) is highly expressed in colon cancer and can promote tumor growth, metastasis and chromosomal instability. Ling et al [10] found the mutual physical interaction between CCAT2 and TCF7L2 can enhance the activity of the WNT signaling pathway. Sun et al [14] reported that low expression of miR-424 in normal human astrocytes (NHA) was accompanied with
a high expression of CCAT2 and vascular endothelial growth factor-A (VEGFA). Xu et al.\(^{[18]}\) discovered that CCAT2 promoted the development and progression of triple-negative breast cancer (TNBC) by up-regulating the expression of OCT4-PG1 and activating Notch signaling. Roxana et al.\(^{[12]}\) first analyzed the expression of CCAT2 in normal breast tissue and breast cancer tissue by RT-qPCR and observed that breast cancer patients with high CCAT2 expression could not benefit from cyclophosphamide (CTX) + 5-fluorouracil (5-FU) + methotrexate (CMF) adjuvant chemotherapy, and these patients often had a shorter survival duration. CCAT2 expression in liver cancer tissue was significantly higher than that in normal control. High CCAT2 expression is an independent risk factor of predicting shorter survival of hepatocellular carcinoma (HCC) patients.\(^{[5]}\).

We hypothesize that there is a correlation between CCAT2 and CC. In the present study, we explored CCAT2 expression in CC and evaluated the value as a serum biomarker for clinical adjuvant diagnosis and prognostic prediction of CC.

**Results**

**Serum relative expression of CCAT2 in primary patients, CIN patients and healthy controls**

The median value of serum CCAT2 relative expression in the 100 healthy controls was 0.727 (0.544, 0.990), which was not correlated with age \( (P > 0.05) \), and 0.795 (0.591, 0.979) in the 80 CIN patients. There was no significant difference in serum CCAT2 relative expression between CIN patients and healthy controls \( (P > 0.05) \). The median value of serum CCAT2 relative expression in 180 primary CC patients was 1.616 (1.068, 2.219), which was significantly higher than that in CIN patients and healthy controls (both \( P < 0.001 \)) (Fig. 1).

**Correlation between serum CCAT2 relative expression and clinicopathological features of primary CC patients**

Correlation analysis of serum CCAT2 relative expression with age, menopause, tumor size, FIGO stage, pathological type, SCC-Ag and the presence or absence of lymph node metastasis (LNM) in 180 primary CC patients showed that serum CCAT2 relative expression was positively correlated with tumor FIGO stage, SCC-Ag and LNM (all \( P < 0.05 \)) (Table 1).
| Clinicopathologic features | n= | CCAT2 relative expression | P value |
|---------------------------|----|---------------------------|---------|
| [median (upper and lower quartile)] | | | |
| Total (n) | 180 | | |
| Age (yr) | | | 0.222 |
| ≤ 50 | 79 | 1.434(1.050,2.343) | |
| >50 | 101 | 1.679(1.079,2.131) | |
| Menopause | | | 0.460 |
| yes | 94 | 1.586(1.043,2.282) | |
| no | 86 | 1.675(1.110,2.219) | |
| Tumor size | | | 0.101 |
| ≤ 4 cm | 90 | 1.357(1.021,2.129) | |
| >4 cm | 90 | 1.749(1.181,2.343) | |
| FIGO stage | | | 0.038* |
| IA1-Ib1 | 63 | 1.283(1.007,2.056) | |
| Ib2-Ila2 | 112 | 1.722(1.189,2.298) | |
| ≥IIb | 5 | 3.411(1.052,3.822) | |
| Pathological type | | | 0.263 |
| aquamous Ca | 152 | 1.675(1.110,2.131) | |
| adenal Ca | 22 | 1.503(1.021,3.541) | |
| adenosqua. Ca | 6 | 1.153(0.924,1.357) | |
| SCC-Ag(ng/ml) | | | 0.001* |
| negative | 98 | 1.352(1.014,1.866) | |
| positive | 82 | 1.886(1.266,2.453) | |
| LNM | | | 0.046* |
| negative | 150 | 1.517(1.047,2.129) | |

Note: *P<0.05; FIGO: International Federation of Gynecology and Obstetrics; CC: cervical cancer; CCAT2: colon cancer-associated transcript 2; LNM: lymph node metastasis
Clinicopathologic features | n= | CCAT2 relative expression | P value
---|---|---|---
[median (upper and lower quartile)] | | | |
positive | 30 | 1.904 (1.257, 2.596) | |

Note: *P < 0.05; FIGO: International Federation of Gynecology and Obstetrics; CC: cervical cancer; CCAT2: colon cancer-associated transcript 2; LNM: lymph node metastasis

Application of CCAT2, CA125 and SCC detection to diagnosis of primary CC

Pairwise comparison demonstrated that the serum concentration of CA125 in 180 primary CC patients was 16.14 (10.55, 23.08) U/ml vs. 11.50 (7.53, 16.50) U/ml in 100 healthy controls, showing a significant difference between the two groups (P < 0.05). The serum concentration of SCC and CCAT2 in primary CC patients was 2.10 (1.20, 6.60) ng/ml vs. 0.90 (0.60, 1.30) ng/ml in healthy controls and 1.616 (1.068, 2.219) vs. 0.727 (0.544, 0.990) respectively, showing significant differences between the two groups (P < 0.001) (Table 2).

Table 2
The median and cutoff values of CCAT2, CA125 and SCC in primary CC patients and healthy controls

| Parameters | Primary CC | Healthy controls | Cutoff | AUC   | p     |
|------------|------------|------------------|--------|-------|-------|
| CCAT2      | 1.616 (1.068, 2.219) | 0.727 (0.544, 0.990) | 1.102  | 0.897 | < 0.001 |
| CA125 (U/ml) | 16.14 (10.55, 23.08) | 11.50 (7.53, 16.50) | 9.69   | 0.678 | < 0.05 |
| SCC (ng/ml) | 2.10 (1.20, 6.60) | 0.90 (0.60, 1.30) | 1.55   | 0.815 | < 0.001 |

Serum CCAT2 relative expression was not significantly correlated with the CA125 concentration in 180 primary CC patients (P = 0.926, r² < 0.0001) but significantly correlated with the SCC concentration (P < 0.0001, r² = 0.120) (Fig. 2, 3).

Evaluation of the diagnostic value of serum CCAT2, CA125 or SCC for primary CC

When the cutoff value of CCAT2 for diagnosing CC was 1.102 (73.33% sensitivity and 87.00% specificity), the area under the curve for CCAT2 was 0.897 (95%CI: 0.862–0.933). By that analogy, the cutoff value used for CA125 was 9.69 U/ml (81.67% sensitivity and 46.00% specificity). The area under the curve for CA125 was 0.678 (95%CI: 0.614–0.743). The cutoff value used for SCC was 1.55 ng/ml (69.44%
sensitivity and 90.00% specificity). The area under the curve for SCC was 0.815 (95%CI: 0.767–0.863) (Fig. 4).

Next, a combined analysis of CCAT2, CA125 and SCC was performed using a tandem model of the three markers. The sensitivity, specificity, accuracy, positive prediction and negative prediction of this panel in differentiating the healthy controls were 94.44%, 96.00%, 95.00%, 97.70% and 90.57% respectively, which greatly improved the diagnostic efficiency in diagnosing CC (Table 3).

| Molecular marker | Sensitivity | Specificity | Accuracy | Positive prediction | Negative prediction |
|------------------|-------------|-------------|----------|---------------------|---------------------|
|                  | (%)         | (%)         | (%)      | (%)                 | (%)                 |
| CCAT2            | 73.33       | 87.00       | 78.21    | 91.03               | 64.44               |
|                  | (132/180)   | (87/100)    | (219/280)| (132/145)           | (87/135)            |
| CA125            | 81.67       | 46.00       | 68.93    | 73.13               | 58.23               |
|                  | (147/180)   | (46/100)    | (193/280)| (147/201)           | (46/79)             |
| SCC              | 69.44       | 90.00       | 76.79    | 92.59               | 62.07               |
|                  | (125/180)   | (90/100)    | (215/280)| (125/135)           | (90/145)            |
| CCAT2 + CA125    | 91.67       | 89.00       | 90.71    | 93.75               | 85.58               |
|                  | (165/180)   | (89/100)    | (254/280)| (165/176)           | (89/104)            |
| CCAT2 + SCC      | 82.22       | 96.00       | 87.14    | 97.37               | 75.00               |
|                  | (148/180)   | (96/100)    | (244/280)| (148/152)           | (96/128)            |
| Three combination| 94.44       | 96.00       | 95.00    | 97.70               | 90.57               |
|                  | (170/180)   | (96/100)    | (266/280)| (170/174)           | (96/106)            |

**CCAT2 relative expression in postoperative CC patients and recurrent/metastatic CC patients**

The median value of serum CCAT2 relative expression in 165 postoperative CC patients was 0.790 (0.607, 1.165), showing a significant difference from that in primary CC patients ($P<0.0001$). The median value of serum CCAT2 relative expression in 44 recurrent and metastatic CC patients was 1.752 (1.003, 2.787), showing a significant difference from that in primary CC patients and postoperative CC patients ($P=0.0471$, $P<0.0001$) (Fig. 5).
CCAT2 dynamic in postoperative CC patients

Pre- and postoperative CCAT2 was measured in 30 of the 180 primary CC who received surgery and were followed up. CCAT2 was determined before surgery (day 0) in these patients, ranging from 0.732 to 4.5 (median 1.651). At 5–10 days after surgical intervention, a significant decreasing was observed in these subjects. CCAT2 ranged from 0.325 to 2.694 (median 0.786). At 30–60 days after surgery, CCAT2 ranged from 0.444 to 1.779 (median 0.823). CCAT2 ranged from 0.442 to 1.079 (median 0.682) during 90–120 days post-operation, and between 0.298 and 1.421 (median 0.585) during 150–180 days post-operation (Fig. 6). There was a general trend that CCAT2 was significantly higher before surgery, and decreased progressively in the follow-up period after surgery.

Prognostic value of CCAT2 in CC patients

We compared the overall survival times between 154 CC patients who expressed high or low expression levels of CCAT2 and SCC based on extensive clinical follow-up data. A Kaplan-Meier survival curve showed that the overall survival rate of CC patients in high CCAT2 expression group markedly decreased as compared with that of low CCAT2 expression group ($P = 0.026$) (Fig. 7). In addition, univariate analysis and multivariate analyses were performed by a Cox proportional hazards regression model to further assess the prognostic value of CCAT2. In the univariate analysis, FIGO stage ($P = 0.021$), lymph node metastasis ($P = 0.001$) and CCAT2 ($P = 0.032$) were associated with OS. In the multivariate analysis, lymph node metastasis (HR = 0.553, 95%CI: 1.684–14.690, $P = 0.004$) were independent factors associated with OS, but the influence of CCAT2 (HR = 0.654, 95%CI:0.737–9.575, $P = 0.135$) on OS was lost (Table 4). Thus, our results may indicate that CCAT2 was not an independent prognostic factor for CC.
| Variables               | Univariate analysis |          |          |          | Multivariate analysis |          |          |
|------------------------|---------------------|----------|----------|----------|-----------------------|----------|----------|
|                        | HR                  | 95% CI of HR | P value  | HR                  | 95% CI of HR | P value  |
| Age                    | 0.142               | 0.450–3.251 | 0.706    |          |                      |          |          |
| Tumor size             | 0.265               | 0.472–3.620 | 0.607    |          |                      |          |          |
| FIGO stage             | 5.310               | 1.215–11.113 | 0.021*   | 0.671               | 0.773–10.727 | 0.115   |
| pathological types     | 0.549               | 0.183–2.151 | 0.459    | 0.654               | 0.737–10.774 | 0.135   |
| Lymph node metastasis  | 15.260              | 2.672–19.329 | 0.001*   | 0.553               | 1.684–14.690 | 0.004*  |
| CCAT2                  | 4.606               | 1.114–10.774 | 0.032*   | 0.654               | 0.737–9.575  | 0.135   |
| SCC                    | 0.042               | 0.407–3.025 | 0.838    | 0.654               | 0.737–9.575  | 0.13      |

*P< 0.05

**Discussion**

Tremendous progress has been made in the research of lncRNAs. Scientists have discovered that lncRNAs are functional transcripts that play key roles in gene regulation and therefore are regarded as new regulators of gene expression. In addition, they are closely related to tumor development and progression [15]. Kim et al [9] found that there was antisense HOXA11 (HOXA11-AS) expression in CC patients, indicating that HOXA11-AS expression in CC patients was higher than that in the control group. In vitro experiments showed that HOXA11-AS overexpression promoted cell proliferation, migration and invasion, while HOXA11-AS knockout inhibited these biological properties. Jin et al [8] found that the expression level of TCONS_00026907 in the CC tissue was significantly higher than that in the para-carcinoma tissue, and that patients in the high-expression group had a lower survival rate, while silencing TCONS_00026907 expression and overexpressing miR-143-5p inhibited CC cell proliferation, migration and invasion, and promoted cell apoptosis. Hu et al [7] reported significantly high expression of TUG1 in the CC tissue and this high expression was correlated with tumor size, FIGO stage and LNM. Chen et al [3] found that CCHE1 high expression in the CC tissue was correlated with FIGO stage, tumor size, LNM and human papillomavirus. Kaplan-Meier survival curve showed that patients with low CCHE1 expression had better OS and recurrence-free survival (RFS).

CCAT2 is found to play an oncogene role in multiple cancers. Zhao et al [19] reported that the nucleus and cytoplasmic β-catenin protein level in CCAT2 group was reduced and the Wnt signaling pathway was inhibited significantly (P < 0.01), which had a synergistic effect with the Wnt signal inhibitor FH535. CCAT2 promoted the occurrence of non small-cell lung cancer (NSCLC) by regulating the Wnt/β-catenin signaling pathway. Wang et al [17] reported that CCAT2 promoted epithelial-mesenchymal transition
(EMT) of gastric cancer cells through downregulating the expression of E-cadherin and upregulating Zinc finger E-box binding homeobox 2 (ZEB2), Vimentin and N-cadherin. However, the clinical value of CCAT2 in cancers remains unclear.

The result of RT-qPCR in the present study showed that the median value of serum CCAT2 relative expression was 1.616 in 180 primary CC patients, 0.795 in 80 CIN patients, and 0.727 in 100 health controls. Mann-Whitney U test showed that the serum CCAT2 relative expression level in primary CC patients was significantly higher than that in CIN patients and healthy controls ($P < 0.001$). Chen et al$^2$ used RT-qPCR detected the expression level of CCAT2 in SCC tissues of 123 patients and found that CCAT2 expression was upregulated in the SCC tissues as compared with the corresponding para-cancer tissues. The CCK8 result by Wu et al$^{16}$ showed that CCAT2 knockout inhibited the proliferation of HeLa, CaSki and SiHa cells and promoted the proliferation and survival of CC cells. All these findings provide theoretical clues to support the possibility of CCAT2 as an adjuvant diagnostic marker for CC. In addition, we found that CCAT2 relative expression was positively correlated with tumor FIGO stage, SCC-Ag and LNM (all $P < 0.05$), suggesting that CCAT2 expression level may prove to be an adjuvant marker for monitoring the degree of malignancy and disease progression.

CCAT2 relative expression was correlated with the SCC content in primary CC patients ($P < 0.0001, r^2 = 0.120$) and not with the CA125 content ($P = 0.926, r^2 < 0.0001$). The AUC value of CCAT2 in differential diagnosis between primary CC patients and healthy controls was 0.897 (95%CI:0.862–0.933) and the cutoff value was 1.102; under this critical value, the sensitivity, specificity, accuracy, positive prediction and negative prediction were 73.33%, 87.00%, 78.21%, 91.03% and 64.44% respectively, indicating that CCAT2 has a relatively high sensitivity and specificity. More importantly, combination detection of CCAT2, CA125 and SCC could greatly improve the diagnostic efficiency of CC.

The median value of serum CCAT2 relative expression in 165 postoperative CC patients was 0.790, showing a significant decreasing as compared with that in the preoperative CC patients. The median of CCAT2 relative expression in the 44 recurrent and metastatic CC patients was 1.752, showing significant increasing from that in the primary CC patients and postoperative CC patients, suggesting that serum CCAT2 has certain significance in assessing the prognosis of CC patients.

Finally, we assessed the postoperative dynamic change of CCAT2 in 30 of the 180 primary CC patients through 6-month follow-up observation. The results showed that there was a significantly decreasing tendency in CCAT2 expression during D5-10 post-operation, which may be because the serum content of CCAT2 was decreased after resection of the tumor. Serum CCAT2 showed a slowly decreasing tendency during D30-180 post-operation, except in one patient whose CCAT2 showed an increasing tendency. Imaging and clinical analysis of the case suggested the possibility of cancer recurrence in this patient. Therefore, detection of dynamic change in serum CCAT2 could be used to dynamically monitoring the postoperative prognosis of surgical treatment in CC patients, though larger-sample studies are required to verify our conclusion. In the prognostic aspect, Kaplan-Meier survival curve showed that the overall survival rate of CC patients in high CCAT2 expression group markedly decreased as compared with that
of low CCAT2 expression group. Univariable analysis showed that FIGO stage, Lymph node metastasis and CCAT2 were all significantly associated with OS.

**Conclusions**

CCAT2 was also found to be highly expressed in bladder cancer, pancreatic ductal adenocarcinoma and ovarian cancer tissues [1][6][11]. Above all, serum CCAT2 relative expression is to some extent associated with the development and progression of CC, suggesting that CCAT2 may prove to be an important biomarker. Analysis and assessment of the CCAT2 value in treatment and prognostic prediction of CC showed that CCAT2 detection in combination with CA125 and SCC could improve the diagnostic efficiency of CC. Our study also lays a foundation for further clarification of the action mechanism of CCAT2 in CC. Nevertheless, the above results have some limitations. First, all samples were from the same hospital and further prospective multicenter studies will be needed. In addition, our assessment was based on the relative expression of CCAT2 in serum, and its expression in primary CC tumor tissues and cells remains to be further studies for the sake of providing more solid evidence for early diagnosis and prognostic prediction of CC.

**Methods**

**Sample collection**

Serum samples were collected from 180 primary CC patients aged 28-76 years with a median of 52 (45.0, 58.0) years, 165 postoperative CC patients aged 28-76 years with a median of 51 (46.0, 58.0) years, 44 inoperable recurrent and metastatic CC patients aged 31-70 years with a median of 54 (49.0, 60.0) years, 80 CIN patients aged 23-74 years with a median of 47 (40.0, 51.5) years, and 100 healthy individuals aged 24-69 years with a median of 48 (40.0, 56.0) years as control. In addition, serum samples were collected consecutively from 30 of the 180 primary CC patients who underwent operation during the follow-up periods of 1-120 days. The primary CC patients and CIN patients were selected from the patients who were clinic pathologically confirmed as having CC and received treatment in the department of gynecology of Nantong Tumor Hospital between January 2015 and December 2018. The healthy controls were selected from individuals who underwent physical examination in the PE Center of the same hospital during the same period. Tumor staging and typing were according to the American Joint Committee on Cancer (AJCC) guidelines [4].

This research protocol was approved by the ethics committee of the said hospital (LW2020002), and all samples were anonymous. Informed consent was obtained from all patients and controls. Serum samples (5ml each) were centrifuged at 1000r/min for 10 min to collect the sera, which were restored at 80°C for use.

**RNA extraction and cDNA synthesis**
Serum total RNA was extracted using the serum RNA extraction kit (Beijing Patek Biotechnology Co., Ltd., Beijing, China). RNA optical density (OD) was measured by ultraviolet spectrophotometry to calculate the $OD_{260}/OD_{280}$ ratio of the RNA samples, knowing that the ratio between 1.8 and 2.0 indicates good purity of the RNA extracted. The extracted RNA was reverse transcribed into cDNA by using RevertAid First Strand cDNA Synthesis Kit (Thermo, USA). The reaction system is as follows: 10 μlRNA, 4μl 5× reaction buffer, 2μl (10nM) deoxynucleoside triphosphate (dNTP), 1μl oligonucleotide as the primer (dT), 1μl (200 U /μl) reverse transcriptase ribonuclease inhibitor, and addition of enzyme-free H$_2$O to 20 μl. They were mixed thoroughly, centrifuged and reverse transcribed at 42°C for 60 min and 70°C for 5min.

**RT-qPCR assay**

RT-qPCR assay was performed with Light Cycler 480 RT-QPCR using the following reaction system: 10μl SYBR Green I mix, 3μl cDNA,1μl up-stream primer, 1μl down-stream primer, and 5 μl enzyme-free H$_2$O to a total volume of 20μl under the reaction condition of 95 °C for 5 min, one cycle; 95 °C, 15 s; 61 °C, 30s; 72°C, 30s, totaling 45 cycles. Two wells were set for each sample and the mean value was used for analysis. IncRNA relative expression (RQ)=$2^{\Delta\Delta CT}$ was used to indicate the relative expression of CCAT2, and $\Delta\Delta$ cycle threshold (Ct)=study group (CT$_{CCAT2}$-CT$_{GAPDH}$)−control group (CT$_{CCAT2}$-CT$_{GAPDH}$) was used as the mean value. The primer sequences are as follows: CCAT2 up-stream primer: 5′-CCCTGGTCAAATTGCTAAACCT-3′, down-stream primer: 5′-TTATTCGTCCCTCTGTTTTATGGAT-3′; GAPDH up-stream primer: 5′-TGATGACATCAAGAAGGTGGTGAAG-3′, down-stream primer: 5′-TCCTTGGAGGCCCAGTGGGCCAT-3′.

**CA125 and SCC detection**

CA125 and SCC were detected by electrochemiluminescence using the E601 electrochemiluminescence instrument (Roche, Germany) and Maglumi automatic chemiluminescence instrument (New industry Biomedical Engineering Co., Ltd., Shenzhen, China) respectively.

**Follow-up**

We collected information on 5-year survivors from 154 of the 180 primary CC patients. Follow-up with all CC patients occurred by telephone once every 3 months in the first 2 years and every 6 months after that.

**Statistical analysis**

Statistical analysis and ROC curve mapping were performed by SPSS20.0. Pair-wise comparison of CCAT2 relative expression between primary CC patients, CIN patients and healthy controls was performed by Mann-Whitney U test. The correlation between CCAT2 relative expression and clinicopathological
features was analyzed by $\chi^2$ test. OS was analyzed by Kaplan-Meier analysis. Univariate and multivariate analyses were evaluated with Cox proportional hazards models. Mapping was performed by GraphPad Prism 5. Statistically significant difference was set as $P<0.05$.

**Declarations**

**Ethics approval and consent to participate**

The study was approved by the Ethics Committee of Tumor Hospital affiliated to Jiangsu Nantong University.

**Consent for publication**

Not applicable.

**Availability of data and materials**

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

This project was supported by the National Natural Science Foundation of China (No. 82072363; No.81871720); the mandatory project of Nantong Municipal Health and Family Planning Commission (WKZL2018051).

**Authors' contributions**

XLC and JYZ researched the literature and conceived the study. XLC,JY, MQJ,XJS, JYZ and SQJ were involved in protocol development, gaining ethical approval, patient recruitment and data analysis. XLC wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

**Acknowledgements**
We would like to thank Tumor Hospital affiliated to Jiangsu Nantong University and the affiliated hospital of Jiangsu Nantong University.

Authors' information

1Laboratory Medicine of Tumor Hospital affiliated to Jiangsu Nantong University, #48 West Qingnian Road, Nantong 226019, Jiangsu Province, China. 2Gynecology of Tumor Hospital affiliated to Jiangsu Nantong University, #48 West Qingnian Road, Nantong 226019, Jiangsu Province, China. 3Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, #20 Xisi Road, Nantong 226001, Jiangsu Province, China.

References

1. Cai Y, Li XM, Shen P, Zhang D. CCAT2 is an oncogenic long non-coding RNA in pancreatic ductal adenocarcinoma. Biol Res. 2018;51(1):1.
2. Chen X, Liu LF, Zhu WP. Up-regulation of long non-coding RNA CCAT2 correlates with tumor metastasis and poor prognosis in cervical squamous cell cancer patients. Int J Clin Exp Pathol. 2015;8(10):13261–6.
3. Chen Y, Wang CX, Sun XX, Wang C, Liu TF, Wang DJ. Long non-coding RNA CCHE1 overexpression predicts a poor prognosis for cervical cancer. Eur Rev Med Pharmacol Sci. 2017;21(3):479–83.
4. Edge SB, Compton CC. AJCC cancer staging manual The American Joint Committee on Cancer: the 7th Edition of the AJCC Cancer Staging Manual and the Future of TNM. Annals of Surgical Oncology. 2010;17(6):1471–1474.
5. Fu CB, Xu X, Lu WJ, Nie L, Yin T, Wu DD. Increased expression of long non-coding RNA CCAT2 predicts poorer prognosis in patients with hepatocellular carcinoma. Medicine (Baltimore). 2019;98(42):17412.
6. Huang SY, Qing C, Huang ZK, Zhu YF. The long non-coding RNA CCAT2 is up-regulated in ovarian cancer and associated with poor prognosis. Diagn Pathol. 2016;11(1):49.
7. Hu YY, Sun XW, Mao CC, Guo GQ, Ye SS, Xu JF, et al. Upregulation of long noncoding RNA TUG1 promotes cervical cancer cell proliferation and migration. Cancer Med. 2017;6(2):471–82.
8. Jin XJ, Chen XG, Hu Y, Ying FR, Zou R, Lin F, et al. LncRNA-TCONS_00026907 is involved in the progression and prognosis of cervical cancer through inhibiting miR-143-5p. Cancer Med. 2017;6(6):1409–1423.
9. Kim HJ, Eoh KJ, Kim LK, Nam EJ, Yoon SO, Kim KH, et al. The long noncoding RNA HOXA11 antisense induces tumor progression and stemness maintenance in cervical cancer. Oncotarget. 2016;7(50):83001–16.
10. Ling H, Spizzo R, Atlasi Y, Nicoloso M, Shimizu M, Redis RS, et al. CCAT2, a novel noncoding RNA mapping to 8q24, underlies metastatic progression and chromosomal instability in colon cancer.
11. Li JF, Zhuang CL, Liu YC, Chen MW, Zhou Q, Chen ZC, et al. shRNA targeting long non-coding RNA CCAT2 controlled by tetracycline-inducible system inhibits progression of bladder cancer cells. Oncotarget. 2016;7(20):28989–97.

12. Roxana SR, Anieta MS, Maxime PL, Oana T, Cristina I, Riccardo S, et al. CCAT2, a novel long non-coding RNA in breast cancer: expression study and clinical correlations. Oncotarget. 2013;4:1748–62.

13. Siegel R, Ma JM, Zou ZH, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64(1):9–29.

14. Sun SL, Shu YG, Tao MY. LncRNA CCAT2 promotes angiogenesis in glioma through activation of VEGFA signalling by sponging miR-424. Mol Cell Biochem. 2020;468(1–2):69–82.

15. Wang KC, Chang HY. Molecular mechanisms of long non-coding RNAs. Mol Cell. 2011;43(6):904–14.

16. Wu L, Jin LX, Zhang WM, Zhang LF. Roles of Long Non-Coding RNA CCAT2 in Cervical Cancer Cell Growth and Apoptosis. Med Sci Monit. 2016;22:875–9.

17. Wang YJ, Liu JZ, Lv P, Dang Y, Gao JY, Wang Y. Long non-coding RNA CCAT2 promotes gastric cancer proliferation and invasion by regulating the E-cadherin and LATS2. Am J Cancer Res. 2016;6(11):2651–60.

18. Xu Z, Liu C, Zhao Q, Lü JH, Ding X, Luo A, et al. Long non-coding RNA CCAT2 promotes oncogenesis in triple-negative breast cancer by regulating stemness of cancer cells. Pharmacol Res. 2020;152:104628.

19. Zhao CL, Qiao CC, Zong LG, Chen YQ. Long noncoding RNA CCAT2 promotes the occurrence of nonsmall cell lung cancer by regulating the Wnt/βcatenin signaling pathway. Oncology Letters. 2018;16:4600–4606.

Figures
Figure 1

A scatter diagram of serum CCAT2 in healthy controls, CIN patients and primary CC patients. CCAT2 was detected by RT-qPCR. Pair-wise comparison of the relative expression of CCAT2 between primary CC patients, CIN patients and healthy controls was performed by Mann-Whitney U test, and $P<0.05$ was considered statistically significant. NS: no significance.
Figure 2

A scatter diagram of CCAT2 and CA125 correlation in primary CC patients, $P=0.926$, $r^2<0.0001$.

Figure 3

A scatter diagram of CCAT2 and SCC correlation in primary CC patients, $P<0.0001$, $r^2=0.120$. 
Figure 4

RCO differentiation between primary CC patients and healthy controls. The AUC value of CCAT2, CA125 and SCC was 0.897 (95%CI:0.862-0.933), 0.678 (95%CI:0.614-0.743) and 0.815 (95%CI:0.767-0.863), respectively.
Figure 5

A scatter diagram of CCAT2 relative expression in primary CC patients, postoperative CC patients, and recurrent/metastatic CC patients. Man-Whiney U test, $P<0.05$; the horizontal line indicates the median line of each group.
Figure 6
A straight line diagram of serum CCAT2 relative expression in the 30 follow-up primary CC patients.

![Figure 6](image)

Figure 7
Kaplan-Meier curve of CCAT2 for the overall survival of 154 patients with cervical cancer (CC).

![Figure 7](image)