Long noncoding RNA colon cancer associated transcript-1 promotes the proliferation, migration and invasion of cervical cancer

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Abstract. Previous studies have revealed significant roles for long noncoding RNA (lncRNA) in the tumorigenesis, metastasis and invasion of various tumors, including cervical cancer. The present study aimed to investigate the potential roles of lncRNA colon cancer associated transcript 1 (CCAT1) in the metastasis and invasion of cervical cancer, and to reveal the potential underlying mechanism. The mRNA expression of lncRNA CCAT1 in cervical cancer tissue was measured using the reverse transcription-quantitative polymerase chain reaction, and the association between lncRNA CCAT1 and the metastasis of cervical cancer was analyzed. The effects of lncRNA CCAT1 expression on HeLa cell viability, and migration and invasion were also analyzed by MTT and Transwell assays. The results demonstrated that lncRNA CCAT1 was highly expressed in the cervical cancer tissue compared with the adjacent normal tissue. High expression of lncRNA CCAT1 was positively associated with tumor size, and there was correlation between high lncRNA CCAT1 expression and a poor survival rate of cervical cancer. The cell viability, and migratory and invasive abilities were suppressed by silencing CCAT1. The results of the present study indicate that lncRNA CCAT1 was highly expressed in cervical cancer, and may serve important roles in promoting the progression and metastasis of cervical cancer.

Introduction

Cervical cancer remains one of the most common female malignancies worldwide (1), and is characterized by migration and invasion (2), resulting in a poor 5-year survival rate (3). Therefore, the development of novel methods for the improvement of cervical cancer treatment and prognosis is vital for improving the diagnosis and treatment of cervical cancer.

Long noncoding RNAs (IncRNAs; >200 nucleotides) have been demonstrated to exhibit crucial roles in tumorigenesis, migration and invasion in recent years (4,5). For example, the upregulated IncRNA HOX transcript antisense RNA (HOTAIR) promotes the progression of hepatocellular cancer (6), and IncRNA H19 promotes the tumorigenesis and metastasis of gastric cancer (7). Generally, the underlying mechanism of IncRNA gene expression regulation may be at the transcriptional or post-transcriptional level (8). To date, several IncRNAs, including focally amplified lncRNA in epithelial cancer (FAL1), HOTAIR, metastasis-associated lung adenocarcinoma transcript-1 and cervical carcinoma expressed proliferating cell nuclear antigen regulatory IncRNA, have been reported to serve pivotal roles in the progression or metastasis of cervical cancer (9-12). IncRNA colon cancer associated transcript 1 (CCAT1) is highly expressed in colon cancer tissue, and has been reported to be useful in the detection of colon cancer and tumor-associated tissues (13). The roles of IncRNA CCAT1 in the biology of tumors has only been reported in a number of tumor types (14,15). The proliferation and invasion of colon cancer cells was promoted by the c-myc-mediated activation of IncRNA CCAT1 (16). Ma et al (17) demonstrated that IncRNA CCAT1 promotes the development of gallbladder cancer by negatively modulating the expression of miR-218-5p. These previous studies indicate that IncRNA CCAT1 may function as an oncogene in tumors. However, few studies have reported the association between IncRNA CCAT1 expression, and the progression and development of cervical cancer.

The present study investigated the expression of IncRNA CCAT1 in cervical cancer tissue, and analyzed the association between IncRNA CCAT1 expression and HeLa cell viability, metastasis and invasion. The present study aimed to investigate the potential roles of IncRNA CCAT1 in the metastasis of cervical cancer and to identify the potential underlying mechanism.
Materials and methods

Patients and samples. A total of 30 patients aged 23–62 years who were diagnosed with cervical cancer at Hebei Provincial People's Hospital (Shijiazhuang, China) between June 2014 and December 2015 were enrolled in the present study. Informed consent was obtained from all patients and all procedures were approved by the Protection of Human Ethics Committee of the Hebei Provincial People's Hospital. The cervical cancer diagnosis was pathologically confirmed, and cancer tissues and adjacent normal tissue were obtained from clinically ongoing surgical specimens with informed consent. Tissues size was measured and all of the samples were snap-frozen with liquid nitrogen and stored at -80°C until RNA extraction.

Cell culture and transfection. HeLa human cervical cancer cells (European Collection of Authenticated Cell Cultures; Public Health England, Salisbury, UK) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.) at 37°C in an atmosphere containing 5% CO₂. The full-length of CCAT1 encoding sequences were amplified by using the PfuUltra II fusion HS DNA Polymerase (Agilent Technologies, Inc., Santa Clara, CA, USA). The thermocycling conditions were: 95°C 45 sec, 60°C 15 sec, 95°C 30 sec, 30 cycles. Then CCAT1 sequences were cloned into pcDNA3.1 vector (Invitrogen; Thermo Fisher Scientific, Inc.) as pcDNA3.1-CCAT1. The experimental procedures were performed as previously described (18). Primers used for CCAT1 amplification were 5'-CTGCTAGCAACATCGACTTTAGTGTT-3' (forward) and 5'-CCCAAGCCTTAGACTTAATATACCTTATTTA-3' (reverse). The small interfering (si)RNA for CCAT1 was designed and synthesized by RiboBio (Guangzhou RiboBio Biotechnology, Co., Ltd., Guangzhou, China), and the sequence was as follows: CCAT1 siRNA (si-CCAT1: 5'-CAU UAA CCU GCU AUC CUC A-3' (reverse). The small interfering (si)RNA for CCAT1 was designed and synthesized by RiboBio (Guangzhou RiboBio Biotechnology, Co., Ltd., Guangzhou, China), and the sequence was as follows: CCAT1 siRNA (si-CCAT1: 5'-CAU UAA CCU GCU AUC CUC A-3' (reverse). The small interfering (si)RNA for CCAT1 was designed and synthesized by RiboBio (Guangzhou RiboBio Biotechnology, Co., Ltd., Guangzhou, China), and the sequence was as follows: CCAT1 siRNA (si-CCAT1: 5'-CAU UAA CCU GCU AUC CUC A-3' (reverse).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA from cervical cancer tissue was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), followed by treatment with RQ1 RNase-free DNase I (Promega Corporation, Madison, WI, USA). Purified RNA mixed with nuclease-free water was used for cDNA synthesis using the reverse transcriptase PrimerScript 1st Strand cDNA Synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.). Expression was detected in an Eppendorf Mastercycler using the SYBR ExScript RT-PCR kit (Takara Biotechnology, Co., Ltd., Dalian, China) according to the manufacturer’s protocol. The reaction procedure was as follows: 95°C 45 sec, 95°C 15 sec, 60°C 15 sec, 45 cycles. All the tests were repeated 3 times, and the relative quantitative analysis by 2-ΔΔCt method for the determination of LncRNA CCAT1 changes (20). Primers used were: LncRNA CCA T1, 5'-TATGCTGCTTGAAGTGAATGTT-3' (forward) and 5'-CTT GCCCTGAATCTTTG-3' (reverse); internal GAPDH primer: 5'-TGTTGCCATCAATGACCCCTT-3' (forward) and 5'-CTCCACGGCTACTCCAGCG-3' (reverse). GAPDH was selected as the internal control.

Statistical analysis. All experiments were repeated three times independently. Data are presented as the mean ± standard deviation. Statistical analysis was performed using SPSS 19.0 software (IBM SPSS, Armonk, NY, USA). Significant differences were calculated using one-way analysis of variance followed by Dunnett's test. The correlation analysis was performed by using log rank to compare survival curves. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of lncRNA CCAT1 in cervical cancer tissue. The present study analyzed the relative mRNA expression of lncRNA CCAT1 in cervical cancer tissue by RT-qPCR analysis. The results demonstrated that lncRNA CCAT1 exhibited increased expression in the cervical cancer tissue compared with the adjacent tissue (P<0.05; Fig. 1A). Consequently, the association between relative mRNA expression of lncRNA CCAT1 in tumor tissue and tumor size was also analyzed, and the results demonstrated that as the expression of lncRNA CCAT1 increased the tumor size increased (Fig. 1B).
addition, the correlation between survival and lncRNA CCAT1 expression was also investigated, and the results demonstrated that high lncRNA CCAT1 expression was associated with a decreased survival rate compared with low CCAT1 expression (Fig. 1C). The results may indicate that the expression of lncRNA CCAT1 is positively associated with the tumor size, and associated with a poor survival rate in patients with cervical cancer.

Effects of lncRNA CCAT1 expression on HeLa cell viability in vitro. lncRNA CCAT1 was overexpressed and silenced in HeLa cells (Fig. 2). Expression of lncRNA CCAT1 was significantly increased following pcDNA3.1-CCAT1 transfection compared with the control, while it was significantly reduced by transfection with si-CCAT1 transfection compared with the control (P<0.05; Fig 2A). Subsequently, the viability of HeLa cells was analyzed, and results demonstrated that cell viability was increased by lncRNA CCAT1 overexpression, while it was reduced by silencing CCAT1 expression compared with control cells (Fig. 2B), indicating a positive association between lncRNA CCAT1 expression and HeLa cell viability. In addition, the colony assay also revealed that lncRNA
CCAT1 overexpression increased the number of HeLa cell colonies compared with the control (Fig. 2C and D).

*IncRNA CCAT1 overexpression promotes migration and invasion in HeLa cells.* The influence of IncRNA CCAT1 expression on HeLa cell migration (Fig. 3A) and invasion (Fig. 3B) was investigated. The number of migratory or invasive cells was significantly increased by CCAT1 overexpression compared with the control group (P<0.05), while the number of migratory or invasive cells was decreased by silencing CCAT1, indicating that IncRNA CCAT1 expression was positively associated with cell migration and invasion in HeLa cells.

**Discussion**

Increasing evidence has demonstrated that IncRNAs serve pivotal roles in the biology of various tumors, including cervical cancer (21). Previous studies have demonstrated that IncRNA CCAT1 is abundantly expressed in several types of cancer, including colon and gastric cancer (14,15). The present study analyzed the expression of IncRNA CCAT1 in cervical cancer tissue, and investigated the potential roles of IncRNA CCAT1 in the development and metastasis of cervical cancer. The results of the present study revealed that IncRNA CCAT1 was highly expressed in cervical cancer tissue and that its expression was positively associated with tumor size, indicating that the abnormal expression of IncRNA CCAT1 may be associated with cervical cancer.

In addition, the present study investigated the influence of IncRNA CCAT1 expression on the proliferation of HeLa cells and the results demonstrated that silencing IncRNA CCAT1 reduced the viability of HeLa cells compared with control HeLa cells. The roles of IncRNA CCAT1 in cervical cancer have not, to the best of our knowledge, previously been completely investigated. However, previous studies have demonstrated that colon cancer cell proliferation is promoted by the overexpression of IncRNA CCAT1 (22,23). Similarly, Wang *et al* (24) demonstrated that upregulation of IncRNA CCAT1 promoted cell proliferation in esophageal squamous carcinoma and Liang *et al* (25) reported that IncRNA CCAT1 promoted the progression of hepatocellular carcinoma by a complex mechanism. Based on the results of the current study, we hypothesize that IncRNA CCAT1 upregulation may enhance cell proliferation in cervical cancer.

The present study also examined the effects of abnormal IncRNA CCAT1 expression on the migration and invasion of HeLa cells. Cell migration and invasion are involved in the metastasis of tumors (26). IncRNA CCAT1 was previously demonstrated to have crucial roles in the proliferation and invasion in various types of tumor, including colon cancer and hepatocellular cancer (22,27). Additionally, previous studies have revealed that several IncRNAs serve roles in the invasion and migration of cervical cancer cells, including FAL1, MALAT1 and LET IncRNAs (10,28,29). The results of the present study revealed that HeLa cell migration and invasion was suppressed by silencing IncRNA CCAT1, indicating that abnormal expression of IncRNA CCAT1 may be associated with cervical cancer migration and invasion.

In conclusion, the results of the present study revealed that IncRNA CCAT1 was upregulated in cervical cancer tissues, and that the abnormal expression of IncRNA CCAT1 may increase the progression and metastasis of cervical cancer by affecting cell proliferation, migration and invasion. The present study may provide a theoretical basis for the potential role of IncRNA CCAT1 as an oncogene in cervical cancer. Further studies are required to investigate the signaling pathways underlying the effects of abnormal IncRNA CCAT1 expression on cervical cancer in depth.
References


