Long non-coding RNA-CCAT2 promotes the occurrence of non-small cell lung cancer by regulating the Wnt/β-catenin signaling pathway

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Abstract. The present study aimed to investigate the biological function of colon cancer-associated transcript 2 (CCAT2) in the occurrence and progression of non-small cell lung carcinoma (NSCLC) and its potential use in the early diagnosis and molecular-targeted therapy of NSCLC. The tumor tissues, para-carcinoma tissues and associated clinical data of 36 patients with NSCLC were collected in order to detect the expression of CCAT2 and assess the impact of factors including histopathological type, Tumor-Node-Metastasis stage and lymph node metastasis on CCAT2 expression. The lung cancer NCI-H1975 cell line was transfected with a small interfering RNA (siRNA) plasmid to determine the effect of si-CCAT2 on NSCLC proliferation, invasion and metastasis. The effect of si-CCAT2 on the expression of nuclear and cytoplasmic β-catenin protein in the lung cancer NCI-H1975 cell line was detected using western blot analysis. The expression levels of CCAT2 in the tumor tissues of patients with NSCLC were significantly higher than those in the normal para-carcinoma tissues (t=8.580, P<0.01). Subsequent to CCAT2 silencing, the proliferation and invasive abilities of NCI-H1975 cells were significantly decreased compared with control cells (P<0.05). In the si-CCAT2 group, the level of nuclear and cytoplasmic β-catenin proteins was decreased, and the activity of the Wnt signaling pathway was significantly inhibited compared with the control cells (P<0.01), and a synergistic effect was exerted with the Wnt signaling inhibitor FH535. CCAT2 may therefore promote the occurrence of NSCLC by regulating the Wnt/β-catenin signaling pathway.

Introduction

Lung cancer has the highest mortality rates of all cancer types, and non-small cell lung cancer (NSCLC) accounts for 85% all mortality cases globally (1). The metastasis of NSCLC cells is rapid, the disease is usually at an advanced stage by the time it is diagnosed and the effectiveness of current treatments is limited, resulting in a low 5-year survival rate and a poor prognosis (2). Therefore, in order to increase the survival rate of patients with NSCLC, in-depth studies on the pathogenesis of NSCLC focusing on inhibiting the malignant proliferation and metastasis of the tumor are required. Long non-coding RNAs (lncRNAs) are non-coding RNAs with a length of >200 nucleotides, and are a novel research area that has emerged in previous years (3). Studies have revealed that lncRNAs are involved in a number of biological regulation processes, such as cell differentiation, proliferation and immune response (3,4). A number of studies have revealed that lncRNAs are abnormally expressed in intestinal cancer (5), ovarian cancer (6), gastric cancer (7) and numerous other cancer types. The abnormal expression of lncRNA may be associated with the occurrence and metastasis of cancer (8). Evidence has demonstrated that certain lncRNAs are associated with the occurrence and progression of lung cancer (9). The lncRNA colon cancer-associated transcript 2 (CCAT2) is located on chromosome 8 (10), and is involved in the occurrence and progression of breast cancer, gastric cancer, colon cancer and lung cancer (9,10). LncRNA CCAT2 is a novel lncRNA, first identified to have abnormal expression in the tissues of colon cancer, and may promote the growth and metastasis of tumor tissues and induce chromosomal instability (11). The Wnt/β-catenin pathway is a classic Wnt signaling pathway and β-catenin protein is its main member. This pathway is able to activate the transcriptional activity of its target gene through the nuclear translocation of β-catenin (9-11). Previous studies have revealed that the abnormal activation

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of the Wnt/β-catenin signaling pathway served a role in the process of cell carcinogenesis, tumorigenesis and tumor invasion (12), and the abnormalities of the classic Wnt/β-catenin signaling pathway were associated with the occurrence of a variety of malignant tumor types (13,14). Previous studies have revealed that the lncRNA CCAT2 enhances Wnt/β-catenin pathway activity, affecting glioma (15), oral squamous cell carcinoma (16), breast cancer (17), renal cell carcinoma (18) and other tumor types. However, there are few studies on the effect of CCAT2 and the Wnt/β-catenin signaling pathway in NSCLC. The present study aims to study the association between CCAT2 and the Wnt/β-catenin pathway in NSCLC and investigate its association with clinical features, in order to provide insight for the early diagnosis and molecular targeted therapy of NSCLC.

Materials and methods

Patient grouping. Patients were divided into two groups for each characteristic, according to the median age (≥56 and <56 years), sex, smoking history, tumor size (>3 and ≤3 cm), Tumor-Node-Metastasis (TNM) staging (16) and the presence or absence of lymph node metastasis. Samples were divided into a high expression group and a low expression group according to the cut-off point of 2.58 times the CCAT2 expression level in normal tissues, with 2.58 included within the high expression group, including 15 cases in the high expression group and 21 cases in the low expression group.

Instruments and reagents. The lung cancer NCI-H1975 cell line was purchased from the American Type Culture Collection cell bank (Manassas, VA, USA). RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco; Thermo Fisher Scientific, Inc. (Waltham, MA, USA). The Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). The cell apoptosis kit was purchased from Beyotime Institute of Biotechnology (Haimen, China). The RNA extraction reagent TRIzol was obtained from Invitrogen; Thermo Fisher Scientific, Inc. The reverse transcription-quantitative polymerase chain reaction (RT-qPCR) kit was obtained from Takara Biotechnology Co., Ltd. (Dalian, China). The gel imaging system and ViiA7 type Real-Time PCR instrument were purchased from Applied Biosystems (Thermo Fisher Scientific, Inc.). The total protein extraction kit was purchased from BestBio (Shanghai, China). The Coomassie Brilliant Blue Protein assay kit was purchased from Shanghai Majorbio Pharmaceutical Technology Co., Ltd. (Shanghai, China). SDS-PAGE, PBS with 0.1% Tween-20 (PBST) solution, vertical electrophoresis apparatus and GIS-2020D gel image analysis system were purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany). β-actin antibody (cat no. MS123A1) was purchased from Abcam (Cambridge, MA, USA). CCAT2 small interfering RNA (siRNA) and negative control (NC) siRNA were purchased from Guangzhou RiboBio Co., Ltd. (Guangzhou, China). Lipofectamine® 2000 was purchased from Invitrogen; Thermo Fisher Scientific, Inc.

Patients. A total of 36 cases of cancerous tissue resection specimens were collected from patients with lung cancer who attended in the First Affiliated Hospital of Bengbu Medical College (Bengbu, China) between January 2013 and December 2015. Each specimen included tumor tissue and normal para-carcinoma tissue (2 cm from the edge of the tumor). None of the patients had received radiotherapy, chemotherapy or other treatments prior to surgery. The fresh tissue specimens *in vitro* were placed in a -80°C refrigerator for preservation subsequent to liquid nitrogen freezing at -4°C for 30 min. All lung cancer cases were pathologically confirmed with NSCLC. Relevant clinical data of the patients were collected, including 28 male and 8 female cases, with a mean age of 54.91±6.48 years (range, 43-65 years). Patients provided written informed consents prior to the study, which was ethically approved by the ethics committee of the First Affiliated Hospital of Bengbu Medical College.

Cell lines and cell culture. NCI-H1975 cells were cultured in RPMI-1640 medium containing, 100 U/ml penicillin, 10% FBS at 37°C, 20% O_2 and 5% CO_2.

si-CCAT2 transfection. The sequence of si-CCAT2 was 5'-GUGCAACUCUGCAAUUUAAUU-3' and the control sequence of NC siRNA was: 5'-AATGGACAACTGGTC GTGGAC-3'. NCI-H1975 cells were cultured at a density of 1,000 cells/well in a 96-well plate and transfected with 1.0 μl siRNA for 24 h using Lipofectamine 2000 according to the manufacturer's protocol; they were divided into a transfection group and an untransfected control group (control). Subsequent experiments were performed 24-72 h following transfection.

RT-qPCR. RNA of cancerous tissues, para-carcinoma tissues and NCI-H1975 cells was extracted using TRIzol reagent. The RNA purity and content were detected using a nucleic acid protein analyzer (DU640; Beckman Coulter, Inc., Brea, CA, USA). The RNA integrity was identified using 1% agarose gel electrophoresis. A total of 1 μg RNA was reverse transcribed to obtain cDNAs using the RT-qPCR kit (Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. Next, the RT-qPCR reaction system was prepared as follows: 5 μl 2X SYBR-Green Mixture, 0.5 μl cDNA, 0.5 μl Primer (10 μM) and 4 μl ddH2 (Takara Bio, Inc., Otsu, Japan). The thermocycling conditions were as follows: 95°C pre-denaturation for 10 min, 95°C denaturation for 15 sec and annealing extension for 60 sec at 60°C and extension for 15 sec at 72°C for a total of 40 PCR cycles performed on a ViiA7 type fluorescence qPCR apparatus. Three parallel samples were established for each experiment and β-actin was used as the reference gene. RT-qPCR was conducted as previously described (15). The primer sequence used were: CCAT2, forward, 5'-GTTGTT GGGAGCTACATTGTCTGC-3', and reverse, 5'-GTGTCG TGAACTCGGCAATTC-3'; and β-actin, forward, 5'-GAA CCCTAAGGCCAAC-3', and reverse, 5'-TGTCACGCACGA TTTCC-3'.

CCK-8 assay for cell proliferation. Cells (5x10⁶) in the transfection, NC and control groups following digestion and counting were inoculated into 96-well plates, and cultured at 37°C in a 5% O_2 and 5% CO_2 atmosphere. In the transfection group, experiments were performed 24, 48 and 72 h following transfection, 5 repeated wells were used at each time point

and a blank control group treated with PBS was established. Subsequently, the cells were washed 3 times with PBS and 100 μl CCK-8 mixture (CCK-8 reagent; Qiagen GmbH, Hilden, Germany) was added to each well, incubated at 37°C for 2 h, and then the absorbance value was measured a wavelength of 450 nm using a microplate reader. Cell proliferation was calculated according to the absorbance value.

Transwell assay. Cells were digested to adjust the concentration to 2x10^5/ml in serum-free Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich; Merck KGaA). A total of 200 ml cell suspension was transferred to the upper chamber (8 mm pore size) and placed in a 24-well plate, and 500 ml complete DMEM culture medium containing 10% FBS was added to the lower chamber. The cells were then incubated at 37°C for 24 h in an incubator, prior to the chamber being removed and the cells on the upper surface being removed using a sterile cotton swab. The cells were subsequently fixed with 5% methanol at 4°C overnight and stained at 37°C using crystal violet for 10 min. The number of membrane-penetrating cells under 5 high magnification fields was counted under a light microscope (x400), to evaluate the invasion ability of the transfection group.

Western blot analysis. Cytoplasmic proteins and nucleoproteins were separated and purified by radioimmunoprecipitation assay lysate (Thermo Fisher Scientific, Inc.), the proteins were quantified using the Coomassie Brilliant Blue Protein assay kit, and then boiling at 100°C for 5 min to prepare the 10% SDS-PAGE gel (Sigma-Aldrich; Merck KGaA). The protein samples (10 μl/lane) were loaded for electrophoresis, prior to being transferred to an electrophoresis tank for nitrocellulose membrane transfer by the addition of transfer buffer containing 25 ml Tris, 0.193 M glycine and 20% methanol (Sigma-Aldrich, Merck KGaA). The membranes were blocked with PBST containing 5% skimmed milk for 2 h at room temperature and washed three times using PBST, followed by incubation with primary antibodies (cat. no. A21422; dilution: 1:2,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) overnight at 4°C. Membranes were washed twice using PBST for 30 min, and the horseradish peroxidase (HRP)-labeled secondary antibody (cat. no. A66585; dilution, 1:10,000; Santa Cruz Biotechnology, Inc.) prepared using PBST containing 2.5% skimmed milk was added for incubation at 37°C for 60 min. Once membranes were washed 3 times with PBST, the proteins were evenly applied to the nitrocellulose membrane, exposed in the dark room, the films were developed for 5 min in the 3,3'-Diaminobenzidine developing solution (Sigma-Aldrich; Merck KGaA). Subsequent to washing with the developing solution, the membranes were placed in the PBST fixing solution (Sigma-Aldrich; Merck KGaA) at 37°C for 5 min, then dried following washing. The optical density of the β-catenin protein, β-actin protein and Histone3 protein bands in the si-CCAT2 group, β-catenin inhibitor FH535 (37°C for 30 min; Sigma-Aldrich, Merck KGaA) (15 μmol/l) group and si-CCAT2 + FH535 group was visualized by Supersignal West Femto HRP sensitive chemiluminescent substrate (Sigma-Aldrich, Merck KGaA) and analyzed using the GIS-2020D gel image analysis system (Ningbo Shuangjia Instrument Co., Ltd., Hangzhou, China), and the ratio of the
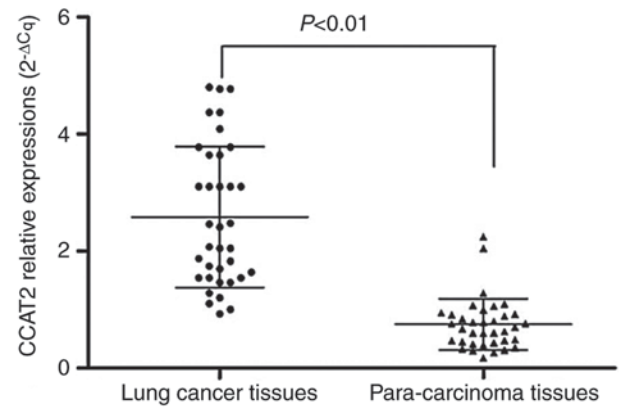

Figure 1. Relative mRNA expression levels of CCAT2 in lung cancer tissues and para-carcinoma tissues. P<0.01 with comparisons shown by lines. CCAT2, colon cancer-associated transcript 2.

optical density of β-catenin protein bands to that of β-actin bands or Histone3 bands was used as the expression intensity of β-catenin protein.

TOP/FOP luciferase assay. Lung cancer NCI-H1975 cells at the logarithmic phase were inoculated in a 96-well cell culture plate. Lung cancer NCI-H1975 cells (1x10^6) were transfected with si-CCAT2 using Lipofectamine 2000 according to the manufacturer's protocol. A total of 24 h later, cells were transfected with TOP/FOP luciferase plasmids at 37°C for 30 min, according to the manufacturer's protocols of Lipofectamine 2000 detection kit (Invitrogen; Thermo Fisher Scientific, Inc.). The experiment was repeated three times. The data are expressed as the ratio of TOP/FOP. At 48 h after transfection, the TOP/FOP luciferase assay was performed.

Statistical analysis. Data statistical analysis was performed using SPSS 19.0 statistical software (IBM Corp., Armonk, NY, USA). Statistical data with a normal distribution are expressed as the mean ± standard deviation. The comparison between CCAT2 expression in lung cancer tissues and adjacent tissues was performed using a paired Student's t-test. Analysis of the association between CCAT2 expression levels and the clinical characteristics of patients was performed by χ^2 test. Comparisons of measured data between two groups with two independent samples were performed using a paired Student's t-test. Comparisons of measured data between multiple groups were performed using one-way analysis of variance followed by Dunnett's test for comparisons between specific groups. P<0.05 was considered to indicate a statistically significant difference.

Results

mRNA expression levels of CCAT2 in lung cancer tissues and normal para-carcinoma tissues. The mRNA expression levels of CCAT2 in lung cancer tissues and normal para-carcinoma tissues in 36 patients with lung cancer were detected using RT-qPCR. Results revealed that the expression levels of CCAT2 in lung cancer tissues were significantly higher compared with those in the normal para-carcinoma tissues (t=8.580, P<0.01), as presented in Fig. 1.

Table I. Association between CCAT2 expression and patient clinicopathological features.

Clinicopathological feature	No. cases	Relative expression level of CCAT2		P-value
		Low	High	
Age, years				0.311
≥56	19	13	6	
<56	17	8	9	
Sex				0.236
Male	28	18	10	
Female	8	3	5	
Smoker				0.219
Yes	29	17	12	
No	7	2	5	
Tumor size, cm				0.039
≥3	22	7	15	
<3	14	10	4	
Tumor-Node-Mode stage				0.071
I and II	12	8	4	
III and IV	24	7	17	
Lymph node metastasis				0.006
Yes	20	4	16	
No	16	11	5	

CCAT2, colon cancer associated transcript 2.

Association between CCAT2 mRNA expression levels and the clinicopathological features of patients. The association between the CCAT2 expression level and clinicopathological features was investigated by Fisher's exact test. As presented in Table I, the expression level of CCAT2 in samples with a >3 cm tumor tissue size and lymph node metastasis was significantly increased compared with samples with a ≤3 cm tumor tissue size and no lymph node metastasis, respectively (P<0.05). The expression level of CCAT2 was not significantly associated with age, sex, smoking history or TNM staging (P>0.05).

Expression levels of CCAT2 in lung cancer NCI-H1975 cells following transfection with si-CCAT2. NCI-H1975 lung cancer cells were transfected with siRNA to inhibit the expression of CCAT2. RT-qPCR results revealed that following the transfection of si-CCAT2, the mRNA expression levels of CCAT2 in lung cancer NCI-H1975 cells were significantly inhibited (t=19.98, P<0.001), as presented in Fig. 2.

Effect on proliferation and apoptosis of lung cancer NCI-H1975 cells following CCAT2 silencing. A CCK-8 assay was performed to detect the effect on the proliferation of lung cancer NCI-H1975 cells subsequent to CCAT2 silencing, and the optical density values are presented in Fig. 3. At 24-72 h after transfection, the cell proliferation rate in the si-CCAT2 transfection group was significantly lower than that of the NC

siRNA group and the control group (P<0.05). There was no significant difference between the cell proliferation rate of the NC siRNA group and that of the control group (P>0.05).

A Transwell assay was performed to detect the effect on the invasion ability of lung cancer NCI-H1975 cells subsequent to CCAT2 silencing. The number of cells that passed through the chamber is presented in Fig. 4. Results revealed that the cell invasion ability in the si-CCAT2 group was significantly lower than that in the NC siRNA group (t=39.50, P<0.01) and the control group (t=29.86, P<0.01).

Effect of si-CCAT2 on Wnt pathway β-catenin proteins. FH535 was used as an inhibitor of Wnt pathway β-catenin proteins. The effect of CCAT2 silencing on the protein expression of β-catenin protein in the nucleus and cytoplasm of lung cancer NCI-H1975 cells and the effect of CCAT2 on the entry of β-catenin into the nucleus were detected using western blot analysis, and the results are presented in Fig. 5. The expression of β-catenin proteins in the cell nucleus and cytoplasm were decreased in the si-CCAT2 group compared with that in the NC siRNA group; in the FH535 group, the protein expression of β-catenin was decreased in the cell nucleus and maintained in the cell cytoplasm compared with the NC siRNA group; and in the si-CCAT2+FH535 group, the protein expression of β-catenin in the cell nucleus and cytoplasm was markedly decreased compared with that in the NC siRNA group.
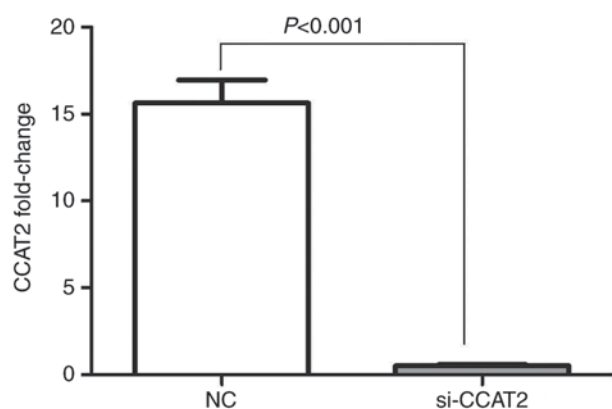
Figure 2. CCAT2 expression levels in lung cancer NCI-H1975 cells transfected with si-CCAT2 or NC siRNA detected by reverse transcription-quantitative polymerase chain reaction. P<0.01 with comparisons shown by lines. CCAT2, colon cancer-associated transcript 2; siRNA/si-, small interfering RNA; NC, negative control.
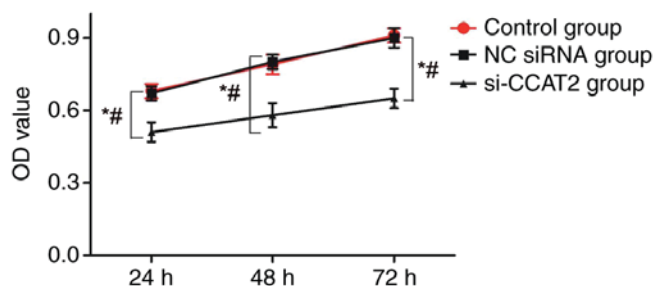

Figure 3. Effect on the proliferation of lung cancer NCI-H1975 cells following CCAT2 silencing. *P<0.05, si-CCAT2 group vs. control group; #P<0.05, si-CCAT2 group vs. NC siRNA group. CCAT2, colon cancer-associated transcript 2; si-/siRNA, small interfering RNA; NC, negative control; OD, optical density.
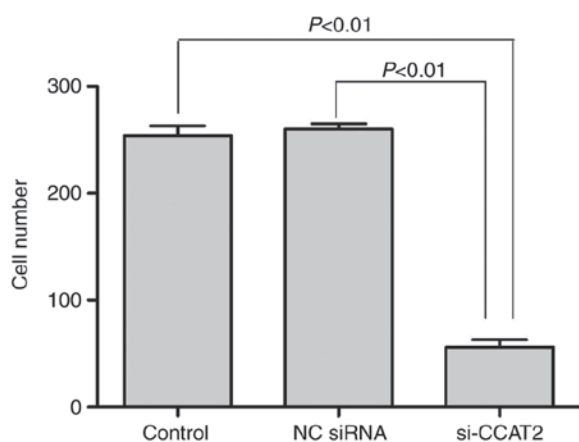

Figure 4. Effect on the invasion ability of lung cancer NCI-H1975 cells following CCAT2 silencing. P<0.01 with comparisons shown by lines. CCAT2, colon cancer-associated transcript 2; si-/siRNA, small interfering RNA; NC, negative control.

Detection of Wnt/-β-catenin signaling pathway activity using a TOP/FOP luciferase ratio assay. In order to further verify the effect of si-CCAT2 on the Wnt/-β-catenin signaling pathway, a TOP/FOP luciferase ratio assay was performed to detect the activity of the Wnt/-β-catenin signaling pathway. Results
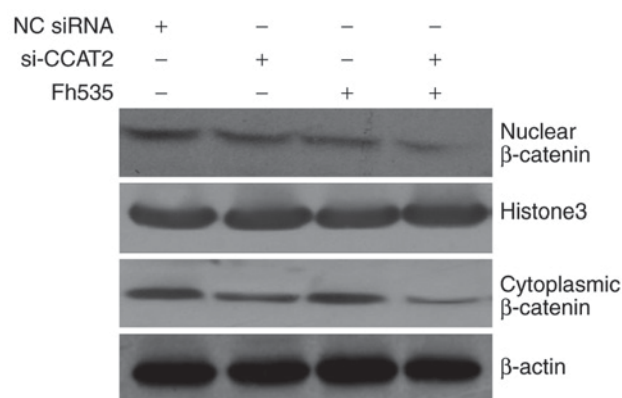

Figure 5. Effect of si-CCAT2 and FH535 on β-catenin proteins. CCAT2, colon cancer-associated transcript 2; si-/siRNA, small interfering RNA; NC, negative control.
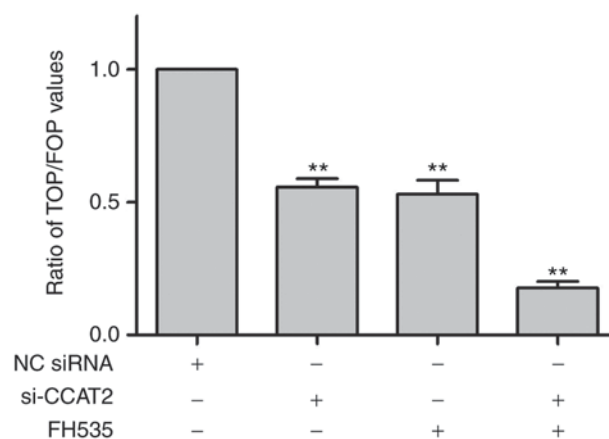

Figure 6. Detection of β-catenin activity following CCAT2 silencing and FH535 treatment, using a TOP/FOP luciferase ratio assay. **P<0.01 vs. NC siRNA. CCAT2, colon cancer-associated transcript 2; si-/siRNA, small interfering RNA; NC, negative control.

revealed that si-CCAT2 and FH535 significantly decreased the activity of the β-catenin signaling pathway compared with the NC siRNA group (t=12.49, P<0.01 and t=11.23, P<0.01, respectively). si-CCAT2 and FH535 combined exerted a synergistic, significant inhibitory effect on the activity of the β-catenin signaling pathway in lung cancer NCI-H1975 cells compared with the NC siRNA group (t=23.56, P<0.01), as presented in Fig. 6.

Discussion

A previous study concluded that lncRNAs are novel tumor biomarkers that may provide a novel approach to the early diagnosis and treatment of cancer (19). Qiu *et al* (20) reported that CCAT2 is an lncRNA specifically expressed in lung adenocarcinoma, and that it promotes tumor proliferation and invasion. CCAT2 combined with carcinoembryonic antigen may be used to predict the potential for lymph node metastasis in patients (20). In the present study, the expression level of CCAT2 in the lung cancer tissues was higher compared with that in the normal para-carcinoma tissues of 36 patients with lung cancer, suggesting that CCAT2 may be associated with

the occurrence of lung cancer. In addition, Fisher's exact test revealed that the high expression of CCAT2 was associated with lymph node metastasis, consistent with the results reported by Qiu et al (20). Wang et al (21) collected the tumor samples and clinical data of patients with gastric cancer and revealed that the patients with a high expression of CCAT2 were at an increased risk of lymph node metastasis and distant metastasis. In the present study, the high expression of CCAT2 was associated with lymph node metastasis and tumor size, suggesting that CCAT2 was highly expressed in lung cancer. A previous study demonstrated that CCAT2 may promote the invasion and proliferation of gastric cancer by regulating E-cadherin and large tumor suppressor kinase 2, and that CCAT2 serves the biological role of an oncogene (22). Zhao et al (23) believed that CCAT2 may promote the occurrence of NSCLC through the tumor suppressor gene cyclin-dependent kinase inhibitor 1. In the present study, CCK-8 and Transwell assays revealed that CCAT2 may promote the proliferation and metastasis of NSCLC, indicating that CCAT2 may promote the occurrence and progression of NSCLC, which was consistent with the results reported by Qiu et al (20) and Wang et al (21).

The Wnt signaling pathway is a complex and conservative signal transduction pathway, serving an important role in embryonic development, regulating cell growth and differentiation, as well as tumor occurrence, development and metastasis (24). The activity of the Wnt signaling pathway is regulated by a variety of proteins (25). The abnormal activation of Wnt signaling results in Wnt activating the disheveled protein, transducing intracellular signals and β-catenin is inhibited by the activated disheveled protein to form a complex with Axin-APC and Wnt signaling pathway regulator-glycogen synthase kinase-3β (26). Through combining with T-cell factor/lymphoid enhancer factor, the transcription of downstream target genes matrix metalloproteinase-7, MYC proto-oncogene, BHLH transcription factor (c-Myc) is stimulated to induce cell proliferation (26). The abnormal activation of the Wnt signaling pathway is associated with cellular malignant transformation and tumorigenesis (27). The core of the Wnt signaling pathway is the accumulation of β-catenin in the cell, which results in the transcription of specific target genes through its downstream pathway (15). CCAT2 is a downstream target site of the Wnt signaling pathway (9,10), which is involved in the occurrence and progression of cancer (15,28). Ling et al (10) reported that CCAT2 may upregulate Myc, microRNA (miR)-17-5p and miR-20a via transcription factor 7-like 2 in colon cancer tissues and that it may enhance the activity of the Wnt signaling pathway. In the present study, the expression of Wnt signaling pathway β-catenin protein in the nucleus and cytoplasm of NCI-H1975 cells and the activity of the Wnt/β-catenin signaling pathway was detected using a TOP/FOP luciferase assay following CCAT2 silencing. The results revealed that si-CCAT2 may reduce the expression of β-catenin in the cytoplasm and nucleus of NCI-H1975 cells and inhibit the Wnt signaling pathway, while FH535 may only inhibit the expression of β-catenin in the nucleus. The combination of the two may further reduce the expression of β-catenin in the nucleus of NCI-H1975 cells and may further inhibit the activity of the Wnt signaling pathway. This suggests that CCAT2 activates the Wnt signaling pathway by enhancing β-catenin stability and the entry of β-catenin protein into the nucleus.

In the present study, it was revealed that CCAT2 may be involved in the occurrence and progression of NSCLC through the Wnt/β-catenin signaling pathway. To investigate the potential use of CCAT2 in the early diagnosis and molecular targeted therapy of patients with NSCLC, further in vivo experiments are required.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CZ is responsible for study conception and design. CQ analyzed and interpreted the data. LZ was responsible for clinical sampling collection and manuscript draft. YC was in charge of cell experiment and manuscript revision.

Ethics approval and consent to participate

All specimens were collected with the patient's written informed consent and approved by the Ethics Committee of the First Affiliated Hospital of Bengbu Medical College.

Patient consent for publication

Witten informed consent was obtained from the patients for the publication of any associated data.

Competing interests

The authors declare that they have no competing interests.

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