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Original Article

Silencing CCAT2 inhibited proliferation and invasion of epithelial ovarian carcinoma cells by regulating Wnt signaling pathway

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Abstract: Long non-coding RNA CCAT2 (colon cancer-associated transcript 2) is dysregulated in varieties of human tumors. However, the role of CCAT2 in epithelial ovarian carcinoma (EOC) is not yet known clearly. The aim of this study is to investigate the effects of CCAT2 on proliferation and invasion of EOC cells and the potential mechanisms by which CCAT2 functions. In the present paper, we found that knockdown of CCAT2 impaired cell proliferation and invasion in vitro. Furthermore, we also studied the role of CCAT2 in the modulation of Wnt/β-catenin signaling pathway. Our results showed that knockdown of CCAT2 inhibited the expression of β-catenin and the activity of TCF/LEF (T-cell factor/lymphoid enhancer factor) acting as a key transcription factor of Wnt/β-catenin signaling pathway. In addition, we found that silencing CCAT2 down-regulated the expression of c-MYC and MMP-7. But, that was reversed by the treatment with LiCl (lithium chloride) which could activate canonical Wnt/β-catenin signaling pathway. Taken together, these results indicate that CCAT2 may promote ovarian cancer progression, at least partly, through Wnt/β-catenin signaling pathway. Thus, CCAT2 might represent a novel therapeutic target for ovarian cancer.

Keywords: CCAT2, Wnt signaling, MMP-7, c-MYC, epithelial ovarian carcinoma

Introduction

Ovarian carcinoma is one of the most common gynecological malignancies and comprises multiple histological subtypes. The most prevalent histological type is the epithelial ovarian carcinoma (EOC) that represents 85% of ovarian carcinoma [1]. As a result, this histological subtype is one of the most serious threats to female health all over the world. Cause of the absence of specific signs/symptoms and sensitively screening methods in the early stage, EOC is characterized by a frequent development of metastases in the pelvic and abdominal cavities at the time of first diagnosis [2]. Despite improvement in surgery and chemotherapy for EOC in the last decades, the 10-year survival rate of patients with advanced stage was still below 27% [3]. The prognosis of ovarian cancer is associated with FIGO stage, therefore it is urgently needed to understand the molecular mechanisms responsible for ovarian cancer progression and identify the reliable markers of EOC.

Long non-coding RNAs (lncRNAs), which are defined as RNA sequences longer than 200 bp with no protein-coding function, were previously disregarded as transcriptional noise [4, 5]. However, recent studies have demonstrated that lncRNAs play key roles in cellular biological processes and diseases [6]. LncRNAs modulate the expression of target genes at the epigenetic, transcriptional, and posttranscriptional levels [7]. Increasing evidence has shown that some lncRNAs can function as oncogenes or tumor suppressors that have significant effects on the progression of some types of human tumors such as lung, colorectal, breast, and liver cancers [8-10]. To date, only a small fraction of lncRNAs have been characterized, while the functions of most of them remain unclear.

As a novel lncRNA transcript, Colon cancer-associated transcript 2 (CCAT2) mapping to 8q24 was originally detected in colorectal cancer [11]. CCAT2 has also been reported to be overexpressed in breast cancer and promote

Table 1. PCR Primer and siRNA sequences

PCR Primer	Sequence
CCAT2	5'-CCAGGCAATAACTGTGCAACTC-3' (sense)
	5'-ACTTACGTAGGGCATGCCAAA-3' (antisense)
β-actin	5'-GTCAGGTCATCACTATCGGCAAT-3' (sense)
	5'-AGAGGTCTTTACGGATGTCAACGT-3' (antisense)
Target RNA	siRNA sequence
CCAT2	5'-AGGUGUAGCCAGAGUUAAUTT-3' (sense)
	5'-AUUAACUCUGGCUACACCUTT-3' (antisense)

tumor growth and metastasis [12]. Recently, Qiu et al. showed that CCAT2 was highly expressed in non-small cell lung cancer (NSCLC), especially in lung adenocarcinoma. Their study also demonstrated that silencing CCAT2 by siRNA could inhibit cell proliferation and invasion of NSCLC cell lines in vitro [13]. The abnormal expression of CCAT2 has gradually become known as a significant feature of some human solid carcinomas including gastric cancer [14]. However, the detailed functions and molecular mechanisms of CCAT2 in EOC are not fully understood and need to be further investigated.

In the present study, the effects of CCAT2 on EOC cells proliferation, colony formation and invasion were investigated in vitro. In addition, we further explored the potential mechanisms by which CCAT2 may exert its biological functions.

Materials and methods

Cell lines and cell culture

Two human EOC cell lines (SKOV3, A2780) were purchased from the Institute of Biochemistry and Cell Biology of Chinese Academy of Science (Shanghai, China). Cell lines were cultured in RPMI-1640 medium (Corning, Manassas, VA, USA) containing 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA, USA), penicillin (100 U/ml, Beyotime, Jiangsu, China) and streptomycin (100 mg/ml, Beyotime, Jiangsu, China). Cell lines maintained in a humidified 5% CO_2 incubator at 37°C.

Total RNA extraction and quantitative real-time PCR (qPCR)

Total RNA was isolated from cell lines using Trizol reagent (Invitrogen, Aukland, New Zealand). RNA was reversely transcribed into cDNAs using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Indianapolis, IN, USA) according to the manufacturer's protocols. QPCR reactions performed using a Stratagene Mx3000p QPCR System (Agilent Technologies, Santa Clara, CA, USA) and SYBR Green PCR Master Mix (Roche, Indianapolis, IN, USA). The expression levels of CCAT2 were detected by qPCR. The specific primer sequences for CCAT2 and β-actin were in **Table 1**. Relative expression of CCAT2 was calculated using the $2^{-\Delta\Delta Ct}$ method. Each qPCR amplification was performed in triplicate to verify the results.

Small interfering RNAs (siRNAs) and transfection

For the assays in vitro, SKOV3 and A2780 cells were cultured on 6-well plate and respectively transfected with either siRNAs targeting CCAT2 or negative controls (Genechem, Shanghai, China) using lentivirus as vector (hU6-MCS-Ubiquitin-EGFP-IRES-puromycin) according to the manufacturer's instructions. The siRNA sequences were shown in **Table 1**. The knockdown efficiency was determined by qPCR at 48 hours after transfection. Next, cells were harvested for other experiments.

Cell proliferation and colony formation assays

The Cell Counting Kit-8 Kit (CCK-8 Kit, Dojindo Laboratories, Japan) was used to determine relative cell growth according to the instructions of the manufacturer. The infected cells were seeded into 96-well plates (2000 cells/well) with 100 μL complete medium and incubated at 37°C and 5% CO_2 atmosphere, and 10 μL CCK-8 solution was added into each well after 24 hours, 48 hours, 72 hours and 96 hours of culture respectively. The absorbance was measured at 450 nm with a Microplate Reader (Bio-Rad, Hercules, California, USA). The transfected cells were seeded into 12-well plates (100 cells/well) and cultured for 10-14 dates with complete medium to form colonies. The colonies were fixed with methanol for 30 min and stained with 0.1% crystal violet for 10 min. The plates were photographed and the numbers of visible colonies were counted. Each experiment was repeated in triplicate independently.

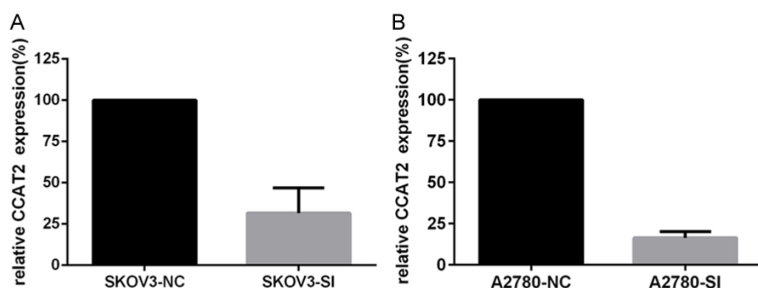

Figure 1. The relative expression of CCAT2. A, B: QPCR revealed that CCAT2 was efficiently knocked down by treatment with CCAT2-siRNA in SKOV3 and A2780 cells. The values of the controls were set as 1. The data represent the mean ± SD of three independent experiments (P<0.01, Student's t-test). NC, negative control; siRNAs, small interfering RNAs. qPCR, quantitative polymerase chain reaction.

Transwell invasion assay

Cell invasion assay was performed using Transwell chambers (8 μm pore size, Corning, Tewksbury, MA, USA). For this assay, 100 μL Matrigel (BD Biosciences, San Jose, CA, USA) was coated onto the upper chamber. A samble of 5 × 10^4 cells suspended in 100 μL FBS-free medium were seeded into the upper chamber, while the lower chambers were filled with 600 μL 20% FBS medium. Following incubation for 24 hours, the top layer of the insert was scrubbed with a sterile cotton swab to remove any remaining cells. The invading cells that had permeated the Matrigel and migrated to the bottom surface were fixed with methanol for 30 minutes, stained with 0.1% crystal violet for 10 minutes and imaged using digital microscopy (× 10 objective, Nikon, Japan). Cell numbers were calculated in five random fields for each chamber at least and then the average value was calculated.

Western blot assay

The harvested cells were disrupted on ice for 30 minutes in RIPA lysis buffer (Beyotime, Jiangsu, China) containing protease inhibitor (1 mM PMSF, phenylmethylsulfonyl fluoride). After centrifugation at 12000 g for 20 minutes, the supernatant fraction was harvested as the total cellular protein extract. β-actin was used as the loading control. The total cellular protein was separated by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gels) and then transferred to PVDF (polyvinylidene fluoride, Life Technologies, Carlsbad, CA, USA) membranes. After blocking with 5% fat-free milk for 1 hour, the membrane was incubated with primary antibodies (Abcam, Cambridge, UK) against c-MYC (1:200 dilution), MMP-7 (1:200 dilution) and β-actin (1:2000 dilution) overnight at 4°C and then blots were washed 3 times in 1 × TBS-T (Tris Buffered Saline with 0.5% Tween), followed by incubation with an appropriate secondary antibody for 1 hour. The protein bands were visualized using an enhanced chemiluminescence kit (Beyotime, Jiangsu, China). Grayscale scanning for western blots of three independent experiments was performed and the relative intensity of the bands was normalized to the controls.

TOP-FLASH luciferase assay

Cells were transfected with TOP-FLASH or FOP-FLASH reporter plasmid (300 ng) together with 30 ng of the Renilla luciferase reporter plasmid. Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA) was used to detect the luciferase activity at 48 hours after transfection. Renilla luciferase was used as the internal control, and TOP-FLASH or FOP-FLASH values were normalized to Renilla values. The TOP/FOP ratios were calculated and used as indicators of the level of Wnt signaling pathway.

Statistical analysis

All of the statistical analyses were performed with SPSS 18.0 software. Differences/correlations between two groups were assessed by the Student's t-test. *P* values < 0.05 were considered as statistical significance.

Results

Silencing CCAT2 inhibited proliferation of EOC cell lines in vitro

To investigate the biological functions of CCAT2 in EOC cells, specific siRNA was transfected into SKOV3 and A2780 cell lines. As was shown in **Figure 1**, qPCR assay was used to demonstrate that the expression of CCAT2 was significantly inhibited by siRNA in comparison with the negative control. The role of CCAT2 on the

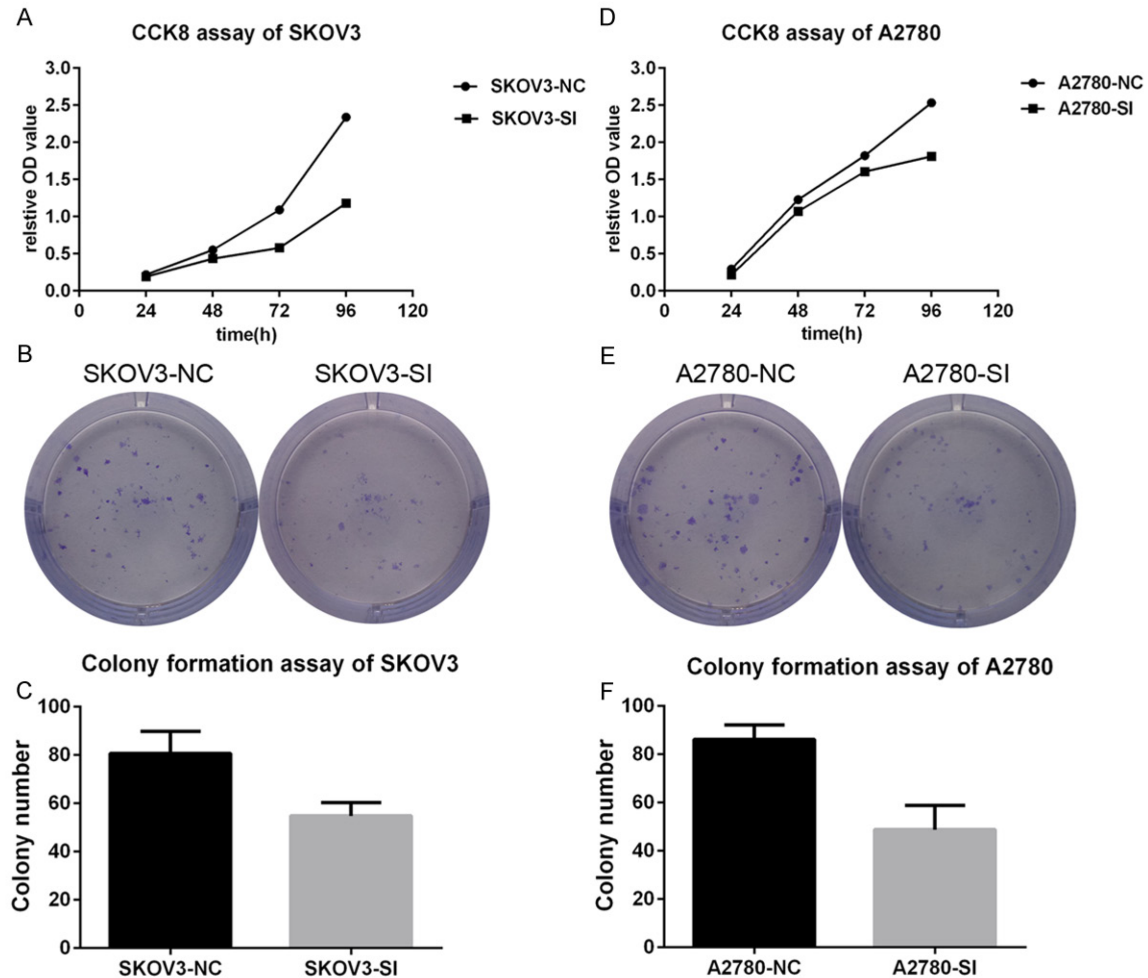

Figure 2. Silencing CCAT2 inhibited proliferation of EOC cells in vitro. A, D: Cell proliferation assay by CCK-8 kit. Knockdown of CCAT2 significantly inhibited cell proliferation, compared with NC (P<0.05, Student's t-test). C, F: Silencing CCAT2 also resulted in smaller and fewer colonies of EOC cells, compared with NC (P<0.05, Student's t-test). The data represent the means ± SD of three independent experiments. B, E: Representative images are presented. NC, negative control; SI, small interfering RNAs. CCK-8, cell counting kit-8.

proliferation of EOC cells was checked using CCK-8 assay and colony formation assay. As was shown in **Figure 2A** and **2D**, CCK8 assay revealed that cellular proliferation was inhibited by silencing CCAT2. In addition, knockdown of CCAT2 resulted in smaller and fewer colonies of EOC cells in vitro (**Figure 2C** and **2F**). These results indicate that CCAT2 may promote ovarian cancer cells proliferation in vitro.

Silencing CCAT2 inhibited invasion of EOC cell lines in vitro

Invasion ability is critical for the progression of cancers. To investigate whether CCAT2 plays a key role in facilitating cell invasion in EOC, transwell assay was performed. As was shown in **Figure 3**, knockdown of CCAT2 significantly impaired invasion of SKOV3 and A2780 cells. The results suggest that CCAT2 may facilitate invasion of ovarian cancer cells in vitro.

Silencing CCAT2 affected Wnt/β-catenin signaling pathway

To better reveal the underling mechanism by which CCAT2 functions in ovarian cancer cells, we examined whether knockdown of CCAT2 affected Wnt signaling pathway. As a key component of Wnt/β-catenin signaling pathway, the expression of β-catenin was detected at the protein level. The data showed that silencing CCAT2 decreased the expression of β-catenin both in the nucleus and cytoplasm (**Figure 4A**).

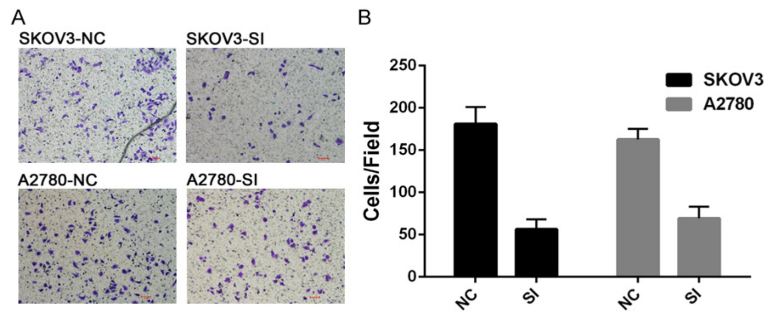

Figure 3. Silencing CCAT2 inhibited invasion of EOC cells in vitro. B: Cell invasion was evaluated using a Matrigel invasion chamber. Silencing CCAT2 decreased the invasive capacity of SKOV3 and A2780 cells. The data represent the mean ± SD of three independent experiments (P<0.01, Student's t-test). A: Representative images are presented. NC, negative control; SI, small interfering RNAs.

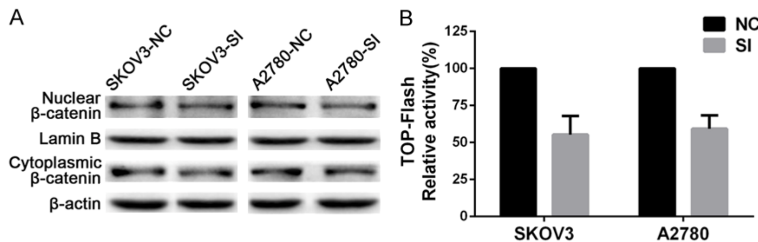

Figure 4. Knockdown of CCAT2 inhibited the activity of Wnt signaling. A: TOP-FLASH luciferase reporter assay showed that silencing CCAT2 reduced the activity of TCF in SKOV3 and A2780. B: Knockdown of CCAT2 decreased the expression of β-catenin both in the nucleus and cytoplasm at the protein level. The values of the controls were set as 1. The data represent the mean ± SD of three independent experiments (P<0.05, Student's t-test). NC, negative control; SI, small interfering RNAs.

The luciferase reporter system was also employed in SKOV3 and A2780 cells. The results showed that silencing CCAT2 significantly decreased the activity of TCF that acted as a important factor influencing the transcription of several target genes of Wnt signaling pathway (**Figure 4B**). The above results revealed that knockdown of CCAT2 inhibited the activity of Wnt/β-catenin signaling patheway.

To investigate the detailed mechanisms by which CCAT2 induced cell proliferation and invasion, several downstream target genes of Wnt signaling pathway were tested. We found that knockdown of CCAT2 reduced the expression of MMP-7 and c-MYC genes at the protein level (**Figure 5**). Moreover, ovarian cancer cells were treated with LiCl (lithium chloride, 20 mM for 24 h) to further activate canonical Wnt/β-catenin signaling pathway through the inhibition of GSK3B (glycogen synthesis kinase 3 beta). The results showed that treatment with LiCl reversed the effects of silencing CCAT2 on the expression of MMP-7 and c-MYC (**Figure 5**). These results strongly suggest that CCAT2 may elevate the activity of Wnt signaling pathway to exert the biological functions in EOC cells.

Discussion

Recently, accumulating evidence has demonstrated the aberrant expression of lncRNAs in various cacinomas, including ovarian cancer [15-17]. As a novel lncRNA, CCAT2 was initially reported for its overexpression in primary colorectal cancer and promotion of cancer proliferation, invasion, and metastasis [11]. In recent years, abnormal expression of CCAT2 has been reported in other cancer tissues [18-21]. In our previous study, the data revealed that the expression levels of CCAT2 in EOC tissues were clearly higher than those in noncancerous tissues. Furthermore, the elevated expression of CCAT2 was associated with advanced FIGO stages, indicating that overexpression of CCAT2 may facilitate a more malignant phenotype as well as metastasis in EOC.

Induced proliferation and invasion are some of the defining features of cancer cells, particularly EOC cells. The mechanisms behind these biological events and factors involved in these processes need to be further investigated for understanding of cancer progression. In the current study, we found that proliferation of SKOV3 and A2780 cells was inhibited by the treatment with siRNA targeting CCAT2. Moreover, silencing CCAT2 resulted in smaller and fewer colonies of EOC cells. We next investigated the effects of CCAT2 on ovarian cancer invasion. The results demonstrated that knockdown of CCAT2 obviously inhibited invasion of EOC cells in vitro. Collectively, these results

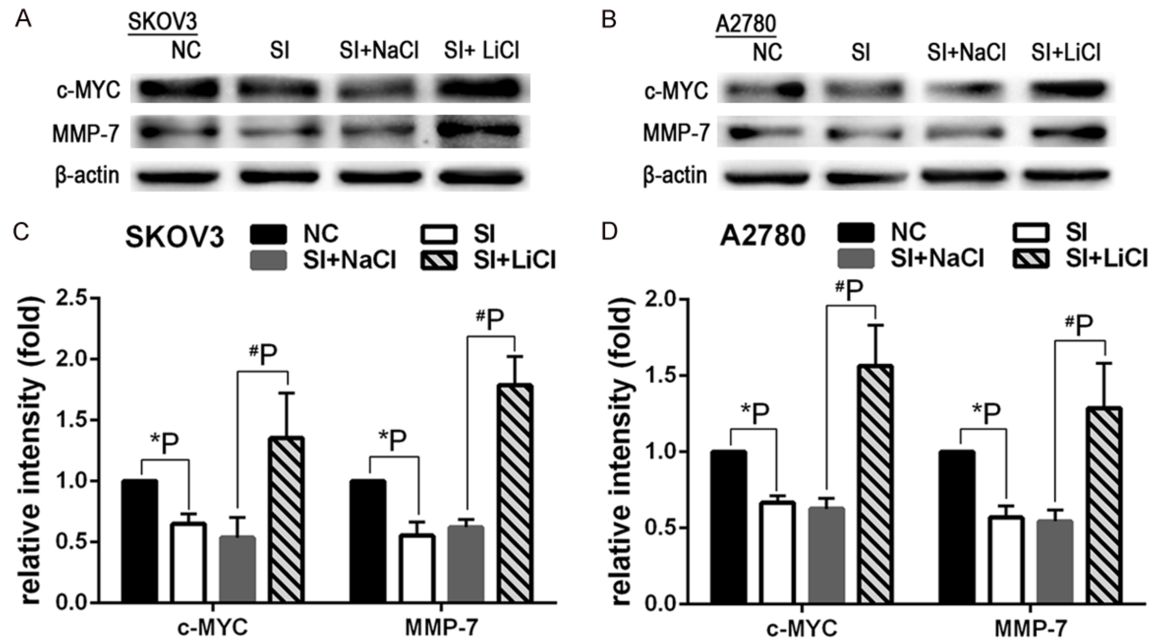

Figure 5. CCAT2 affected the expression of downstream target genes of Wnt signaling pathway in EOC cells. C, D: Silencing CCAT2 down-regulated the expression of c-MYC and MMP-7 at the protein level. Moreover, treatment with 20 mM LiCl for 24 hours (20 mM NaCl as control), an activator of Wnt signaling, reversed the effects of silencing CCAT2 on the expression of MMP-7 and c-MYC. The levels of β-actin were served as the internal controls. The data represent the mean ± SD of three independent experiments. The values of the controls were set as 1. *$P<0.05$ compared to NC group. #$P<0.05$ compared to the SI+NaCl group (Student's t-test). A, B: Representative images are presented. NC, negative control; SI, small interfering RNAs; LiCl, lithium chloride.

indicate that CCAT2 may promote ovarian cancer progression.

Dysregulated signal of the Wnt/β-catenin pathway has been shown to increase proliferation and malignancy of various human cancers, such as EOC [22]. A study showed that the abnormal expression of CCAT2 could influence Wnt signaling pathway in glioma cells [23]. Ling et al. found that CCAT2 might modulate the downstream genes of Wnt pathway by combining with TCF7L2 and increasing its transcriptional activity in colon cancer cells [11]. In this present study, we observed that silencing CCAT2 inhibited the activity of TCF/LEF acting as a key transcription factor of Wnt signaling pathway in ovarian cancer cells. Moreover, our investigation showed that knockdown of CCAT2 decreased the expression of β-catenin protein in the nucleus and cytoplasm. These results demonstrated that silencing CCAT2 inhibited the activity of Wnt/β-catenin signaling pathway in EOC cells, which was in line with the finding in breast cancer [24].

MMP-7 and c-MYC represent the downstream target genes of Wnt signaling pathway [25-27]. The matrix metalloproteinases (MMPs) such as MMP-2, MMP-7 and MMP-9 are capable of degrading extracellular matrix components, thereby contributing to the cellular invasion [28-30]. It has been established that c-MYC plays a key role in controlling cell proliferation [31]. In this study, we found that knockdown of CCAT2 decreased the expression of MMP-7 and c-MYC genes at the protein level. To further study the underlying mechanisms by which CCAT2 promotes ovarian cancer progression, EOC cells were treated with LiCl to activate canonical Wnt/β-catenin signaling pathway. The data revealed that LiCl treatment reversed the effect of silencing CCAT2 on the expression of MMP-7 and c-MYC. These results strongly suggest that CCAT2 may promote ovarian cancer progression, at least partly, through Wnt signaling pathway in EOC cells.

In summary, the present study highlights the importance of CCAT2 in promoting ovarian can-

cer progression and reveals the potential molecular mechanisms by which CCAT2 functions in ovarian cancer. Thus, CCAT2 might be a valuable target for the treatment of epithelial ovarian cancer.

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Disclosure of conflict of interest

None.

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References

[1] Jelovac D and Armstrong DK. Recent progress in the diagnosis and treatment of ovarian cancer. CA Cancer J Clin 2011; 61: 183-203.

[2] Bast RC Jr, Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. Nat Rev Cancer 2009; 9: 415-428.

[3] Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. Lancet 2014; 384: 1376-88.

[4] Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev 2009; 23: 1494-504.

[5] Nagano T, Fraser P. No-nonsense functions for long noncoding RNAs. Cell 2011; 145: 178-81.

[6] Tsai MC, Spitale RC, Chang HY. Long intergenic noncoding RNAs: new links in cancer progression. Cancer Res 2011; 71: 3-7.

[7] Maruyama R, Shipitsin M, Choudhury S, Wu Z, Protopopov A, Yao J, Lo PK, Bessarabova M, Ishkin A, Nikolsky Y, Liu XS, Sukumar S, Polyak K. Altered antisense-to-sense transcript ratios in breast cancer. Proc Natl Acad Sci U S A 2012; 109: 2820-4.

[8] Ren S, Peng Z, Mao JH, Yu Y, Yin C, Gao X, Cui Z, Zhang J, Yi K, Xu W, Chen C, Wang F, Guo X, Lu J, Yang J, Wei M, Tian Z, Guan Y, Tang L, Xu C, Wang L, Gao X, Tian W, Wang J, Yang H, Wang J, Sun Y. RNA-seq analysis of prostate cancer in the Chinese population identifies recurrent gene fusions, cancer-associated long noncoding RNAs and aberrant alternative splicings. Cell Res 2012; 22: 806-21.

[9] Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, Tanaka F, Shibata K, Suzuki A, Komune S, Miyano S, Mori M. Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res 2011; 71: 6320-6.

[10] Yang Z, Zhou L, Wu LM, Lai MC, Xie HY, Zhang F, Zheng SS. Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. Ann Surg Oncol 2011; 18: 1243-50.

[11] Ling H, Spizzo R, Atlasi Y, Nicoloso M, Shimizu M, Redis RS, Nishida N, Gafà R, Song J, Guo Z, Ivan C, Barbarotto E, De Vries I, Zhang X, Ferracin M, Churchman M, van Galen JF, Beverloo BH, Shariati M, Haderk F, Estecio MR, Garcia-Manero G, Patijn GA, Gotley DC, Bhardwaj V, Shureiqi I, Sen S, Multani AS, Welsh J, Yamamoto K, Taniguchi I, Song MA, Gallinger S, Casey G, Thibodeau SN, Le Marchand L, Tiirikainen M, Mani SA, Zhang W, Davuluri RV, Mimori K, Mori M, Sieuwerts AM, Martens JW, Tomlinson I, Negrini M, Berindan-Neagoe I, Foekens JA, Hamilton SR, Lanza G, Kopetz S, Fodde R, Calin GA. CCAT2, a novel noncoding RNA mapping to 8q24, underlies metastatic progression and chromosomal instability in colon cancer. Genome Res 2013; 23: 1446-61.

[12] Redis RS, Sieuwerts AM, Look MP, Tudoran O, Ivan C, Spizzo R, Zhang X, de Weerd V, Shimizu M, Ling H, Buiga R, Pop V, Irimie A, Fodde R, Bedrosian I, Martens JW, Foekens JA, Berindan-Neagoe I, Calin GA. CCAT2, a novel long non-coding RNA in breast cancer: expression study and clinical correlations. Oncotarget 2013; 4: 1748-62.

[13] Qiu M, Xu Y, Yang X, Wang J, Hu J, Xu L, Yin R. CCAT2 is a lung adenocarcinoma-specific long non-coding RNA and promotes invasion of non-small cell lung cancer. Tumour Biol 2014; 35: 5375-80.

[14] Wang CY, Hua L, Yao KH, Chen JT, Zhang JJ, Hu JH. Long non-coding RNA CCAT2 is up-regulated in gastric cancer and associated with poor prognosis. Int J Clin Exp Pathol 2015; 8: 779-85.

[15] Crea F, Clermont PL, Parolia A, Wang Y, Helgason CD. The non-coding transcriptome as a dynamic regulator of cancer metastasis. Cancer Metastasis Rev 2014; 33: 1-16.

[16] Richards EJ, Permuth-Wey J, Li Y, Chen YA, Coppola D, Reid BM, Lin HY, Teer JK, Berchuck A, Birrer MJ, Lawrenson K, Monteiro AN, Schildkraut JM, Goode EL, Gayther SA, Sellers TA, Cheng JQ. A functional variant in HOXA11-AS, a novel long non-coding RNA, inhibits the oncogenic phenotype of epithelial ovarian cancer. Oncotarget 2015; 6: 34745-57.

[17] Qiu JJ, Lin YY, Ye LC, Ding JX, Feng WW, Jin HY, Zhang Y, Li Q, Hua KQ. Overexpression of long non-coding RNA HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial ovarian cancer. Gynecol Oncol 2014; 134: 121-8.
[18] Li J, Zhuang C, Liu Y, Chen M, Zhou Q, Chen Z, He A, Zhao G, Guo Y, Wu H, Cai Z, Huang W. shRNA targeting long non-coding RNA CCAT2 controlled by tetracycline-inducible system inhibits progression of bladder cancer cells. Oncotarget 2016; 7: 28989-97.
[19] Wu L, Jin L, Zhang W, Zhang L. Roles of long non-coding RNA CCAT2 in cervical cancer cell growth and apoptosis. Med Sci Monit 2016; 22: 875-9.
[20] Zhang X, Xu Y, He C, Guo X, Zhang J, He C, Zhang L, Kong M, Chen B, Zhu C. Elevated expression of CCAT2 is associated with poor prognosis in esophageal squamous cell carcinoma. J Surg Oncol 2015; 111: 834-9.
[21] Li JJ, Liu Y, Li PF, Guo YW, Liu YN, Ren Y. Long noncoding RNA CCAT2 promotes proliferation and metastasis in non-small cell lung cancer through the Wnt pathway. Int J Clin Exp Pathol 2017; 10: 7983-7990.
[22] Ford CE, Jary E, Ma SS, Nixdorf S, Heinzelmann-Schwarz VA, Ward RL. The Wnt gatekeeper SFRP4 modulates EMT, cell migration and downstream Wnt signalling in serous ovarian cancer cells. PLoS One 2013; 8: e54362.
[23] Guo H, Hu G, Yang Q, Zhang P, Kuang W, Zhu X, Wu L. Knockdown of long non-coding RNA CCAT2 suppressed proliferation and migration of glioma cells. Oncotarget 2016; 7: 81806-81814.
[24] Cai Y, He J, Zhang D. Long noncoding RNA CCAT2 promotes breast tumor growth by regulating the Wnt signaling pathway. Onco Targets Ther 2015; 8: 2657-64.
[25] He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW. Identification of c-MYC as a target of the APC pathway. Science 1998; 281: 1509-12.
[26] Lemieux E, Cagnol S, Beaudry K, Carrier J, Rivard N. Oncogenic KRAS signalling promotes the Wnt/β-catenin pathway through LRP6 in colorectal cancer. Oncogene 2015; 34: 4914-27.
[27] Yoshioka S, King ML, Ran S, Okuda H, MacLean JA 2nd, McAsey ME, Sugino N, Brard L, Watabe K, Hayashi K. WNT7A regulates tumor growth and progression in ovarian cancer through the WNT/β-catenin pathway. Mol Cancer Res 2012; 10: 469-82.
[28] Yokoyama Y, Grünebach F, Schmidt SM, Heine A, Häntschel M, Stevanovic S, Rammensee HG, Brossart P. Matrilysin (MMP-7) is a novel broadly expressed tumor antigen recognized by antigen-specific T cells. Clin Cancer Res 2008; 14: 5503-11.
[29] Zhao H, Yang Z, Wang X, Zhang X, Wang M, Wang Y, Mei Q, Wang Z. Triptolide inhibits ovarian cancer cell invasion by repression of matrix metalloproteinase 7 and 19 and upregulation of E-cadherin. Exp Mol Med 2012; 44: 633-41.
[30] Zhan Y, Abi Saab WF, Modi N, Stewart AM, Liu J, Chadee DN. Mixed lineage kinase 3 is required for matrix metalloproteinase expression and invasion in ovarian cancer cells. Exp Cell Res 2012; 318: 1641-1648.
[31] Thomas LR, Foshage AM, Weissmiller AM, Tansey WP. The MYC-WDR5 nexus and cancer. Cancer Res 2015; 75: 4012-5.