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Long non-coding RNA CCAT1 regulates the biological behavior of osteosarcoma cells through the miR-454-3p/ZEB2 axis

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Abstract. – OBJECTIVE: To investigate the clinical value and mechanism of action of lncRNA CCAT1 in OS.

PATIENTS AND METHODS: Serum, tumor tissue and corresponding adjacent tissue of 84 cases of patients receiving treatment in our hospital from February 2014 to June 2015 were obtained. Healthy volunteers were recruited during the same period for serum collection. CCAT1 expression in serum and tissue samples, were detected, and the value of its expression in osteosarcoma (OS) diagnosis and prognosis w lyzed. Stable and transient over-express inhibition vectors were established and s- fected into OS cells. CCK-8, transwell, and cytometry were applied to determine the pr eration, invasion, and apoptosis of transfecte cells, and the Dual-Luciferase was uti lized to determine the correl T1 with miR-454-3p, miR-454-3p, a EB2 c-finger E-box-binding homeobo

RESULTS: In OS, C as and serum CCAT1 c d b a marker for OS diagnosis, an AU e of 0.930. High CCAT1 expr n predicte survival rate in patients ion of CCAT d sup- press the prol th l invasion of S cells, and increase apop rate. Over-expression of mi 54-3p and in n of ZEB2 could also ach the above effec al-Luciferase report dicated that CCAT1 uld target miR-454-3 nd mi 4-3p could target ZEB2. The res exper t pro d that CCAT1 could regu gressi hrough the miR-454-3p/ZEB

NCL S: T1 can be used as a di- ic and tic marker for OS, promote OS prolifer n and invasion, and inhibit ap osis through the miR-454-3p/ZEB2 axis, wh herapeutic target for OS.

Words:
osarcoma, CCAT1, MiR-454-3p, ZEB2, Biologi- ca n.

duction

coma (OS) i imary bone tumor t nds to occur in adolescents and children, cause of whi has not been elucidated[1]. Ac- ding to stati surgery and chemotherapy the standa eatment for OS in 2014. In lone, th year survival rate of patients with s was 70%, but the 5-year survival rate of patients with metastasis was only 15% to 30%[2]. Since the application of chemother- 1970s, there has been little change in treatment received by OS patients and no significant improvement in patient outcomes[3]. Therefore, the exploration of OS pathogenesis and potential therapeutic targets is extremely important to improve the diagnosis, treatment and prognosis of the disease.

Although lncRNA cannot directly encode proteins, it can participate in various biological processes such as cell growth, embryonic development and tumorigenesis by regulating chromatin, transcription and post-transcriptional gene expression[4,5]. CCAT1, as a member of lncRNA, is highly expressed in a variety of cancers and can be used as a potential tumor promoter[6]. MicroRNA (miR) is considered a key regulator of gene expression, and its disorder is related to the occurrence and progression of various cancers[7]. CCAT1 can act as a tumor promoter in a variety of tumors through miRNAs. Notably, in the study of Li et al[8], CCAT1 expression increased in gastric cancer, and knockdown CCAT1 could inhibit the growth of gastric cancer and promote the progression of gastric cancer by negatively regulating miR-219-1. Cui et al[9] also reported that up-regulation of CCAT1 in glioma can promote tumor formation and growth by sponging miR-181b. Besides, CCAT1 could promote OS migra-

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tion and proliferation by regulating the miR-148a/PIK3IP1 axis[10]. However, there are relatively few studies on the clinical value and possible molecular mechanisms of CCAT1 in OS. Thus, potential targeting sites of CCAT1 and miR-454-3p were found through online bioinformatics analysis, but the relationship between the two is still unknown.

In this research, the expression of CCAT1 in OS tissues, serum and cells was first observed. Secondly, the correlation of CCAT1 with pathological features and survival rate of patients was analyzed. Finally, the role and molecular mechanism of CCAT1 in OS were investigated through *in vitro* experiments.

Patients and Methods

Source of Serum and Tissue Samples

This investigation was approved by the Institute of Medical Ethics of our Institute and followed the Declaration of Helsinki. The subjects involved in this study were 84 cases of OS patients (patient group) undergoing surgery in our hospital from February 2014 to June 2015. ...sion criteria: patients were diagnosed as C... logically. Patients and their family had sig... the informed consent. Patients had complete cl... data. Patients did not treatment of chemot... apy or radiotherapy before. Exclusion criter... patients were complicated wi... malignant tumors. Patients had liver a... eart ...ase. Patients were infected befo... rollme... Patients whose expected survival... ss th... During the surgery, bl... d, c... es and corresponding adjacent ...mal tiss... the patients were collected. Th... ssues were ... in liquid nitrogen, and th... as centrifug... om the blood. Then, ... stored in the refrigerator at -80°C for future ... Meanwhile, healthy subjects (... mal group) were ... ited and their serum ... obtained. The patient group was followe... for 3 y..., and the overall survival rate wa... rded ... elepho... and outpatient review. Follow... conduc... very one month.

Proces...

C... ell line... OSP-9607, KHOS, U2OS, MG... and normal osteoblast cell lines (h... e bought from Xin Yu Biotech Ltd., Shanghai, China. Cells were placed in ...becco's Modified Eagle's Medium (DMEM) c... ing 10% fetal bovine serum (FBS, Gibco, ...nd Island, NY, USA), 100 UI/ml penicillin/100 μg/ml streptomycin (100X, Thermo Fisher Scientific, Waltham, MA, USA) ... placed in an incubator at 37°C ... % C... Cell transfection: CCAT1 inhibit... plasmid (si-CCAT1), miR-454-3p over-ex... on plasmid (miR-454-3p-mimics), zinc-finger ... binding homeobox-2 (ZEB2) inhibi... y plasm... d cor... responding negative con... (CCAT1-N... NC, ZEB2-NC) were ... blished ...sing pc... 3.1 as plasmid. The... ey we... ansfected by the aid of Lipofecta... 2... kit (In... gen, Carlsbad, CA, U...). S... s later ... transfected cells we... ansferr... edi... comprising 10% ph... te-buffered s... BS).

qRT-PC...

qRT-PCR was use... tect CCAT1 and miR-454... ression. The ... RNA was extracted f... he samples in the light of the manufacturer instructions of Trizol kit (Invitrogen, Carlsbad, CA, USA). ...ltraviolet spectrophotometer ... agarose gel ...ctrophoresis were used for d... ination ... rity, concentration and integrity ... ted RNA. 2 μg total RNA was taken and reverse-transcribed into cDNA using ...everse transcription kit (Invitrogen, Carlsbad, ...A). SYBR_Premix ExTaq II (TaKaRa, ...alian, China) was used for amplification, and qRT-PCR quantitative analysis was carried out on ABI 7500 PCR (Applied Biosystems, Foster City, CA, USA). Amplification system: 10 μL of SYBR Premix Ex Taq II (2X), 2 μL of cDNA, 0.8 μL of upstream primers, 0.8 μL of downstream primers, sterile purified water was supplemented to 20 μL. Amplification conditions: pre-denaturation at 95°C for 30 s, denaturation at 95 °C for 5 s, and annealing and extension at 60 °C for 30 s. A total of 40 cycles were performed. CCAT1 and miR-454-3p expression was calculated using $2^{-\Delta\Delta ct}$[11], which was normalized to GADPH and U6. Primer sequence was shown in Table I.

Western Blot

Western blot was applied to detect ZEB2 protein expression. Total protein was extracted by the aid of radio immunoprecipitation assay (RIPA, Thermo Fisher Scientific, Waltham, MA, USA), and protein concentration was measured with the help of bicinchoninic acid assay (BCA) kit (Thermo Fisher Scientific, Waltham, MA, USA). The protein concentration was adjusted to 4 μg/μL and separated by 12% polyacrylamide gel electrophoresis. After that, it was transferred to polyvinylidene difluoride membrane, soaked with

Table I. Primer sequences.

Groups	Forward	Reverse
CCAT1	5'-TTTATGCTTGAGCCTTGA-3'	5'-CTTGCCTGAAATAC[illegible]C-3'
GADPH	5'-CATGAGAAGTATGACAACAGCCT-3'	5'-AGTCCTTCCACGA[illegible]AAGT-3'
miR-454-3p	5'-ACCCTATCAATATTGTCTCTGC-3'	5'-GCGAGCACAGAAT[illegible]GAC-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCAC[illegible]'

phosphate-buffered saline and tween (PBST) for 5 min and washed, blocked with 5% skimmed milk powder for 2 h. Then, ZEB2 (1:1000), β-catenin (1:1000) (Abcam, Cambridge, MA, USA) primary antibody was added to seal overnight at 4°C. The primary antibody was removed by washing membrane, and horseradish peroxidase (HRP)-labeled goat anti-rabbit (Abcam, Cambridge, MA, USA) secondary antibody (1:2000) was added, incubated at 37°C for 1 h, then rinsed with phosphate-buffered saline (PBS) for 3 times, with 5 min each time. Excess liquid was absorbed from the membrane with filter paper. Enhanced chemiluminescence (ECL) was used to illuminate and develop in a dark room. The protein bands were scanned to calculate the relati[illegible] pression level of the target protein.

CCK-8 Proliferation Detection

Cell proliferation was determined by CC[illegible] (Beyotime Biotechnology Co., Ltd., Shangha[illegible] China). After 24 h of transfect[illegible]get cells were cultured in a 96-well [illegible] at a [illegible] density of 2.5×10^3 per well. 10 µl [illegible]CK-8 w[illegible]dropped into the wells at 24 h, 4[illegible]2 h, a[illegible] the plate was incubat[illegible] at [illegible]erature for 2 h. Multimode read[illegible] Thermo[illegible]r Scientific, Waltham, MA, U[illegible] was adopted [illegible]essment of the absorban[illegible] well at 490 [illegible]

Cell Invasion Detecti[illegible]

Target[illegible]s after 24 h [illegible]sfection were collect[illegible]nd adjusted to 5*10[illegible], inoculated to a 6-we[illegible]ate. It[illegible] rinsed twice with PBS and inc[illegible]d in [illegible]upper [illegible]partment. The upper compa[illegible]as ad[illegible]with 200 µL DMEM [illegible]trient [illegible]n, a[illegible]he lower compartment [illegible]dded [illegible] mL DMEM (containing 20%[illegible]S), cult[illegible] at 37°C for 48 h. Cells that faile[illegible] penetrate the upper compartment were re[illegible]for 3 times with PBS, fixed with [illegible]ormaldenyde for 10 min, and rinsed with [illegible]le distilled water for 3 times. After drying, c[illegible]re stained with 0.5% crystal violet and its inva[illegible] was observed with microscope.

Cell Apoptosis D[illegible]tion

Target cells afte[illegible]h o[illegible]nsfecti[illegible]were collected and di[illegible]ted [illegible]5% try[illegible]. After digestion, the[illegible]re rinse[illegible]e w[illegible]PBS and added with [illegible]µL binding [illegible]They were configure[illegible]10^6 /mL susp[illegible]sion. Annexin-FIT[illegible]0 µL[illegible] propidium iodide (PI, 5 µL) were successiver[illegible]d, incubating at room tem[illegible]e in the dark [illegible]min. Determination o[illegible] apoptosis was conducted with the help [illegible]low cytomet[illegible] (Thermo Fisher Scientific, [illegible]ltham, MA, [illegible]) and the apoptosis rate was [illegible]ulated.

Du[illegible]e Reporter

The lncRNA and miR target genes were pre-[illegible]icted using starBase 3.0 and Targetscan 7.2. [illegible] ZEB2 was cloned into the pmirGLO [illegible]ual-[illegible]ciferase target expression vector using Lipofectamine™ 2000 kit. CCAT1-3'UTR wild-type (Wt), CCAT1-3'UTR mutant (Mut), ZEB2-3'UTR Wt and ZEB2-3'UTR Wt were transferred [illegible]o the downstream of the Luciferase reporter gene for sequencing and identification of the constructed plasmids. The correctly sequenced plasmids were co-transfected with miR-424-3p-mimics or miR-NC into target cells, respectively. Dual-Luciferase reporter assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was utilized when determining Luciferase activity.

Statistical Analysis

Statistical analysis of the data collected in this study was conducted using SPSS 20.0 (IBM, Armonk, NY, USA) software package, and GraphPad 7 software package was used to draw the required images. Comparison of enumeration data between the two groups adopted chi-square test. Measurement data comparison between the two groups adopted independent sample *t*-test. One-way analysis of variance was utilized for comparison among groups, represented with F. LSD-*t* test was used for afterwards pairwise comparison. Comparison of expression among multiple time points was analyzed using repeated mea-

sures, denoted by F. Bonferroni was used for back testing. ROC was used to draw the diagnostic curve of serum CCAT1 for OS. The overall survival of patients was plotted using K-M survival curve, and Log-rank test was used for analysis. When $p<0.05$, there were statistical differences.

Results

Clinical Value of CCAT1 in OS

According to qRT-PCR, CCATI expression was up-regulated in OS tissue, serum and cells. ROC curve exhibited that serum could be used as a marker for OS diagnosis, with an AUC of 0.930, a sensitivity of 88.10%, and a specificity of 85.71%. Patients were divided into low-expression group and high-expression group according to the median value of CCAT1 expression, and the correlation of CCAT1 with patients' clinicopathological data and survival rate was analyzed. The results revealed that high CCAT1 expression was associated with patients' high TNM staging (stage III+IV), lymph node metastasis, low differentiation and poor survival rate (Table I and Figure 1).

CCAT1 can Promote OS Developmen

This study had revealed that CCAT1 was regulated in OS and was related to high TN staging, lymph node metasta ntiation, and survival of patients, suggesting that CCAT1 plays an important role in OS de Therefore, we inhibited CCAT1 i SP-9 (CCAT1 expression in this c howed the greatest difference). After th CK-8, transwell and flow cytometry were to observe the proliferation, inv on and is of SOSP-9607 after CCAT ibition. Th revealed that after SO 607 w inhibit CCAT1, CCAT1 ex sion w own-regulated, the proliferati in on abil were inhibited, and a tos s incr d. This indicated that AT1 had ear effect on OS (Figure

MiR-45 p ca Targeting Regulated by CCAT1

A g to the s paragraph of the R s section, it could be known that CCAT1 d inhibit the ogression of OS, but the spec mechanism not clear. To further explore mechanism CAT1 in OS, targeted bind- betwe CAT1 and miR-454-3p were foun to online software starBase 3.0. The relationship between them was proved Dual-Luciferase activity, and it was found fection of miR-454-3p-mimics could nhib e activity of CCAT1-3'UTR Wt Luciferase in SOSP-9607 without affecting CCAT1-3'UTR Mut Luciferase activity. Furthermore, we found through qRT-PCR that miR-454-3p

Table II. CCAT1 and clinicop gical dat

Factors		CCAT1 expression		χ^2	*p*-value
		High expression (n = 42)	Low expression (n = 42)		
Gender				0.223	0.640
	Mal	28 (66.67)	30 (71.43)		
	Female	14 (33.33)	12 (28.57)		
Age (ye				0.192	0.661
	< 18 years old (n=46)	24 (57.14)	22 (52.38)		
	≥ 18 years old (n=38)	18 (42.86)	20 (47.62)		
Tu				0.441	0.664
	≥ (n=49)	23 (58.97)	26 (71.79)		
	m (n=35)	19 (41.03)	16 (28.21)		
	tagin			5.845	0.016
	rade I+II (n=47)	18 (42.86)	29 (69.05)		
	Grade III+VI (n=37)	23 (57.14)	14 (30.95)		
Lyn	de metastasis			4.773	0.029
	Metastasis (n=40)	25 (59.52)	15 (35.71)		
	Without metastasis (n=44)	17 (40.48)	27 (64.29)		
	ferentiation			4.762	0.029
	Low differentiation (n=42)	26 (61.90)	16 (38.10)		
	Middle+high differentiation (n=42)	16 (38.10)	26 (61.90)		

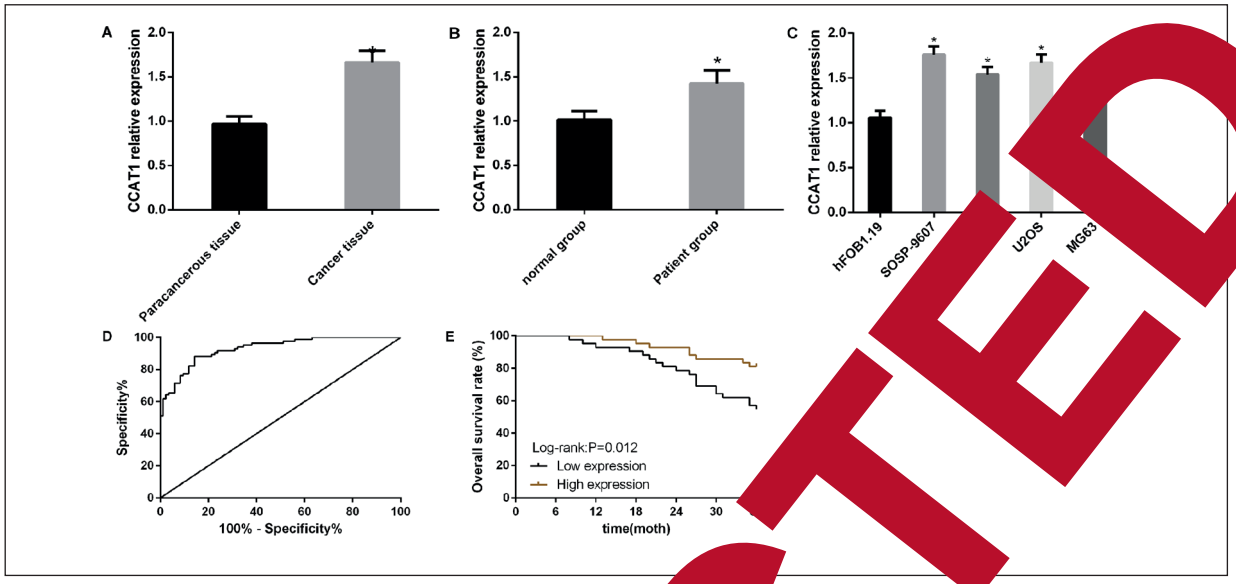
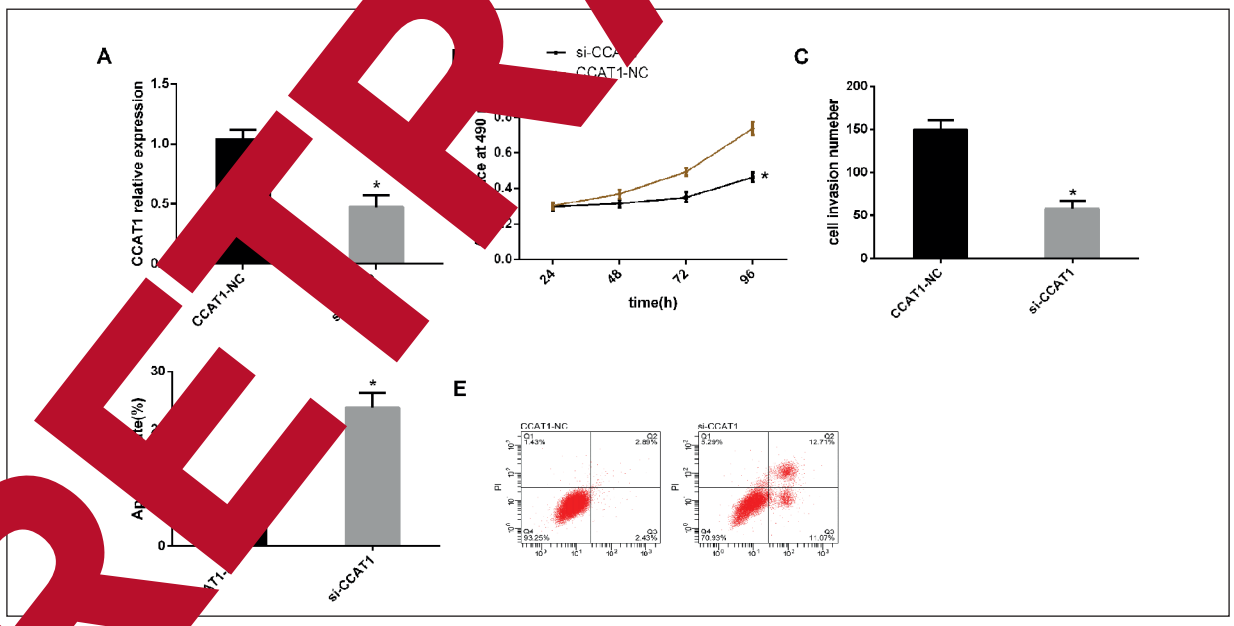


Figure 1. **A**, CCAT1 expression in the cancer tissues of OS pati was higher than that in the adjacent normal tissues. **B**, CCAT1 expression in serum of the patient group was higher tha t of the norma p. **C**, CCAT1 expression increased in OS cells. **D**, ROC curve of serum CCAT1 in diagnosing OS, t C value was 0.9 he sensitivity was 88.10%, and the specificity was 85.71%. **E**, Patients with high expression had a lowe r overall sur rate than those with low expression (Log-rank: p=0.012). Note: * denotes p<0.05.

was increased when si-CCAT1 was tra

CAT1 can Promote OS Development by MiR-454-3p

with SOSP-9607. This suggested that mi 4-3p could be targeting regulated by CCAT1 . MiR-454-3p was proved to be the downstream target gene of CCAT1 in the third paragraph of the ure 3).



ure 2. Effects of CCAT1 on cell biological function of OS. **A**, CCAT1 expression was down-regulated when SOSP-9607 nsfected with si-CCAT1. **B**, The proliferation ability was decreased when SOSP-9607 was transfected with si-CCAT-1. **C**, asion ability was decreased when SOSP-9607 was transfected with si-CCAT1. **D**, The apoptosis rate was increased when P-9607 was transfected with si-CCAT1. **E**, Flow cytometry image. Note: * denotes p<0.05.

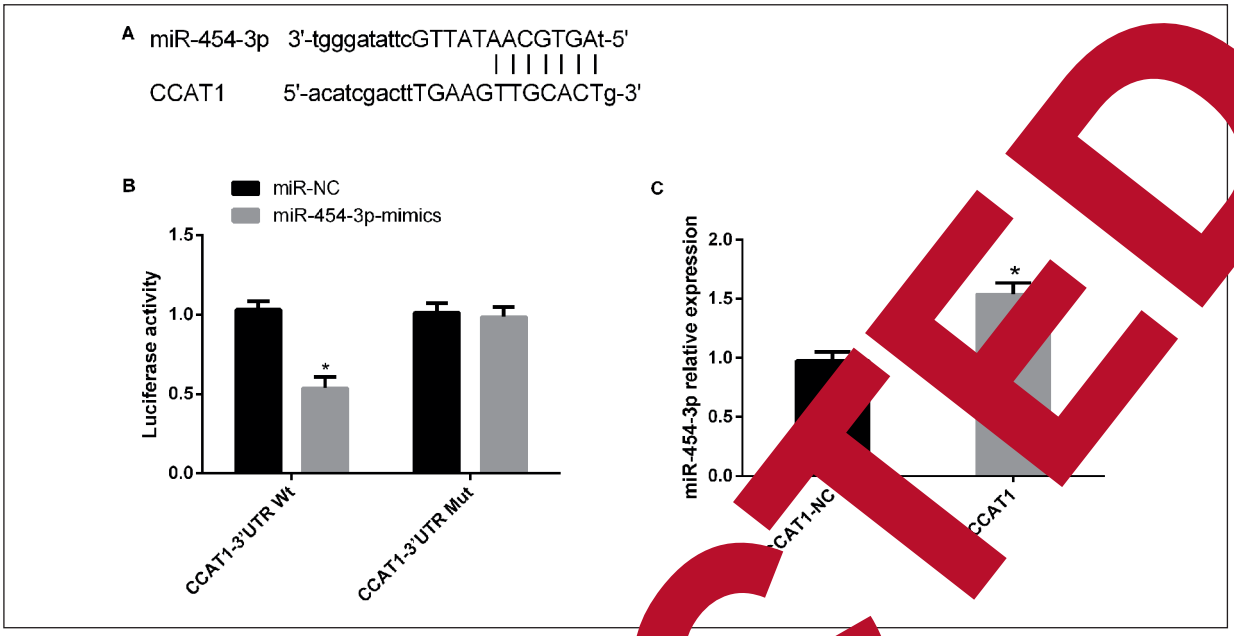


Figure 3. Search of downstream target genes of CCAT1. **A**, Targ binding sites ex between CCAT1 and miR-454-3p. **B**, Transfection of miR-454-3p-mimics could inhibit Luciferase ac f CCAT1-3' Wt in SOSP-9607 without affecting Luciferase activity of SIRT1-3' UTR Mut. **C**, miR-454-3p was inc fter SO 07 was transfected with si-CCAT1. Note: * denotes $p<0.05$.

Results section, while whether CCAT1 cou o-mote the development of OS through miR-4 remained unknown. To determine whether CC could inhibit OS progression through miR-454-3 we over-expressed miR-454-3p 9607 and observed the effects of such t ent oliferation, invasion and apoptosi OSP-9 The results showed that after tr tion with miR-454-3p-mim expression was increased, the p eration vasion ability of SOSP-9607 nhibited, an poptosis rate was incre sequently, C T1 and miR-454-3p i SP- ere over-expressed at the same ti e. By observ biological functions of t lls after treatme as found that there w o diff rence in the proliferation and apopt after t ection with sh-CCAT1+miR-454 imic miR-N Compared with transfection 54-3p ics, however, the proliferation vasi bility increased, and the is rat d. It suggested that at least part CCAT1 d play a carcinogenic role in OS ugh miR-454-3p (Figure 4).

-454-3p can Inhibit OS Progression ough ZEB2

e above results, the CCAT1/miR-454-3p axis been found to produce a marked effect on OS progression. To further improve the echa sm of this axis, Targetscan 7.2 online software was applied for miR-454-3p target gene prediction, and binding sites between ZEB2 and miR-454-3p were found. The activity of Dual-Luciferase was detected, and it was found that transfection of miR-454-3p-mimics could inhibit the activity of ZEB2-3' UTR Wt without affecting the Luciferase activity of ZEB-3' UTR Mut. Through qRT- PCR, ZEB2 was found to be down-regulated after SOSP-9607 was transfected with ZEB2. Subsequently, we investigated the role of ZEB2 in OS, and inhibited ZEB2 in SOSP-9607. The results showed that after transfection of SOSP-9607 with si-ZEB2, ZEB2 was down-regulated, proliferation and invasion of SOSP-9607 were inhibited, and the apoptosis rate was increased. This indicated that miR-454-3p could play a role through ZEB2. Subsequently, SOSP-9607 was treated with ZEB2 and miR-454-3p inhibition simultaneously. After observing the biological functions of the cells after treatment, it was found that there was no difference in the proliferation and apoptosis after transfection with sh-CCAT1+miR-454-3p-mimics or miR-NC. Compared with transfection of si-ZEB2, however, the proliferation and invasion ability increased, and the apoptosis

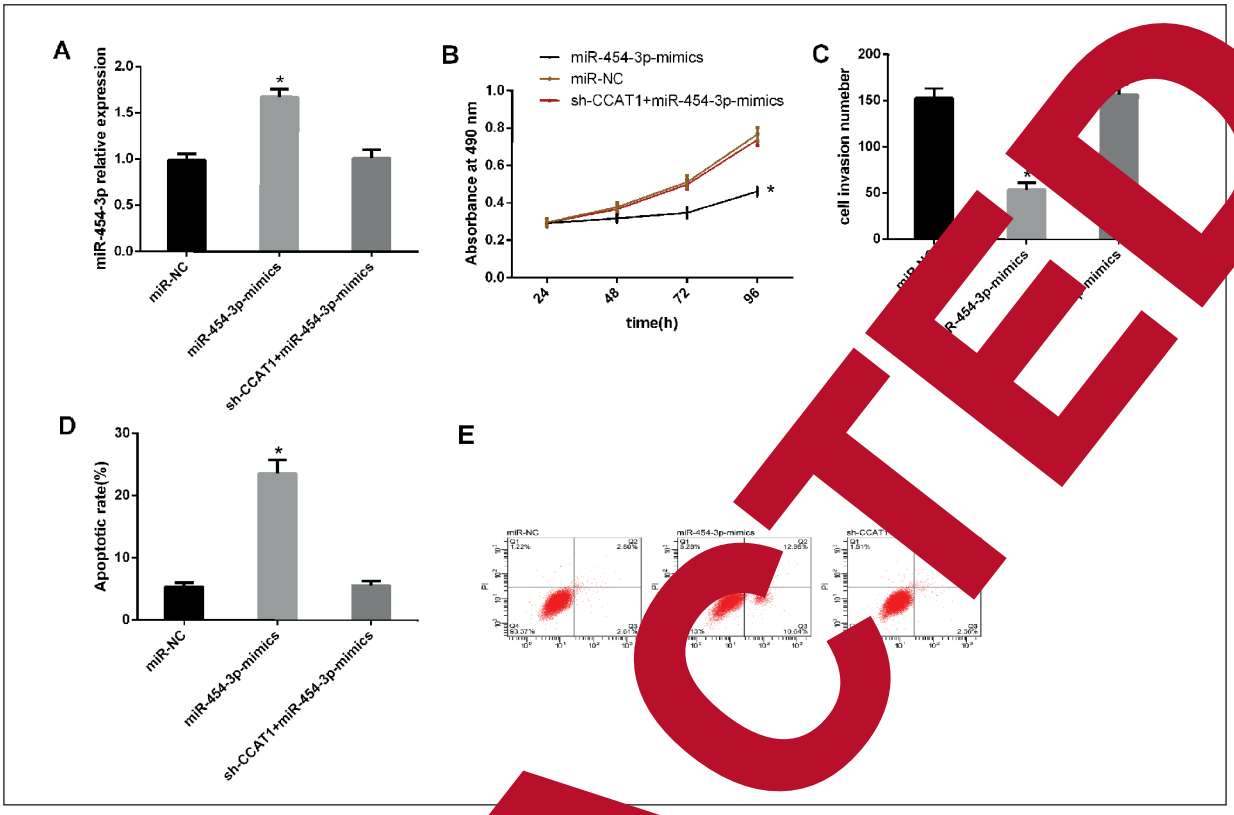


Figure 4. CCAT1 can promote OS development th... After SOSP-9607 was transfected with miR-454-3p-mimics, miR-454-3p increased. After SOSP-9607 w... AT1+miR-454-3p-mimics, miR-454-3p showed no significant change. **B**, After SOSP-9607 was transfe... -454-3p-mimics, the proliferation ability decreased. After SOSP-9607 was transfected with sh-CCAT1+miR-454-... s, the proliferation ability showed no significant change. **C**, After SOSP-9607 was transfected with miR-454-3p-mimi... invasion ability decreased. After SOSP-9607 was transfected with sh-CCAT1+miR-454-3p-mimics... n ability s... no significant change. **D**, After SOSP-9607 was transfected with miR-454-3p-mimics, the apo... ab... creased. A... OSP-9607 was transfected with sh-CCAT1+miR-454-3p-mimics, the apoptosis showed no... ficant c... e. **E**, Flow c... ometry image. Note: * denotes $p<0.05$.

rate decreased, su... ing tha... ast part of miR-454-3p coul... a carcinoge... le in OS through ZEB2...

Discussi...

Ac... ing to... study, CCAT1 was upregulat... OS ti... s, ser... and cells, and serum CCA... e used... marker for OS diagnosis, wi... AUC ... e of 0.930. High CCAT1 ... ion ... d to high TNM staging (sta... I+IV), ly... node metastasis, low differenti... and poor survival rate. In addition, cell ex... ealed that CCAT1 could promote ... cell proliferation and invasion and inhibit ... tosis through miR-454-3p/ZEB2 axis.

...ent years, with the further understanding of li... NA, its dysregulation has been considered as one of the important factors in the development of multiple tumors including OS[12-14]. CCAT1 is a member of lncRNA, which is generally highly expressed in tumors and associated with poor prognosis. In cholangiocarcinoma, CCAT1 is up-regulated and predicts a poor prognosis[15]. In oral squamous cell carcinoma, CCAT1 is also up-regulated and associated with poor therapeutic outcomes[16]. CCAT1 is elevated in ovarian cancer and is related to poor prognosis of patients. In addition, CCAT1 can promote cancer cell migration, invasion and epithelial mesenchymal transformation (EMT)[17]. However, the mechanism of CCAT1 in OS is still largely unknown. Here, we measured the expression of CCAT1 in OS and explored its clinical value. CCAT1 increased in OS tissues, serum and cells. By drawing the ROC curve, it was found that serum CCAT1 could be used as the marker for OS diagnosis, with an

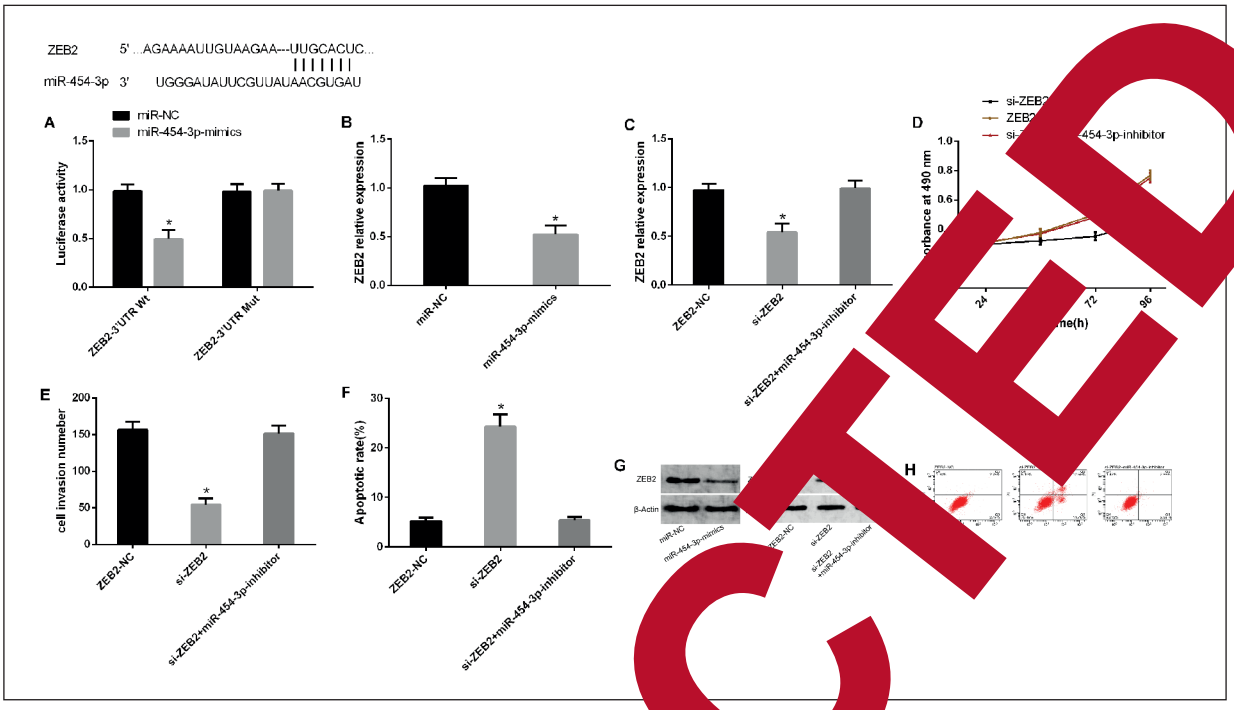


Figure 5. MiR-454-3p can inhibit OS progression through ZEB2. **A**, ites existed between miR-454-3p and ZEB2, and transfection of miR-454-3p-mimics could inhibit Luciferase EB2-3'UTR Wt in SOSP-9607 without affecting Luciferase activity of SIRT1-3' UTR Mu was inhibited after transfection of SOSP-9607 with miR-454-3p-mimics. **C**, After transfection of SOSP-9607 decreased. After transfection of SOSP-9607 with si-ZEB2+miR-454-3p-inhibitor, ZEB2 showed no sig nt ch transfection of SOSP-9607 with si-ZEB2, the proliferation ability was decreased. After transfecti SOSP -ZEB2+miR-454-3p-inhibitor, the proliferation ability showed no significant change. **E**, After transfe P-9607 with si-ZEB2, the invasion ability was decreased. After transfection of SOSP-9607 with si-ZEB2+miR-45 ibitor, the invasion ability showed no significant change. **F**, After transfection of SOSP-9607 with 2, the apop ate was increased. After transfection of SOSP-9607 with si-ZEB2+miR-454-3p-inhibitor, the ap owed no s ant change. **G**, WB image. H, Flow cytometry image. Note: * denotes $p<0.05$.

AUC value of 0.930, nsi 3.10%, and specificity of 85.7 Moreo gh CCAT1 expression was d to high staging (stage III+IV), de metastas ow differentiation poo ival rate, indicating that CCAT could be us a marker for OS diagnosis prognosis, and be involved in the pat nesis of the disease. hen, *in vitro* experi s reve that knocking down CCAT1 co pres cell p iferation and invasion, and p optosis ich was similar to previous stu sug g that CCAT1 plays a genic s.

W the purp of further understanding the fun of CCAT1 in promoting OS progressi starBase 3.0 software was used alyze the presence of targeted binding sites een CCAT1 and miR-454-3p, and Dual-Lu- activity test showed that miR-454-3p coul regulated by CCAT1 in a targeted manner. miR-454-3p has been previously reported to be a tumor suppressor, which can block the progression of glioblastoma[18], gastric cancer[19] and cervical cancer[20]. MiR-454-3p also acts as a tumor suppressor in OS. According to Niu et al[21], miR-454-3p is reduced in OS and inhibits the growth and invasion of cancer cells by regulating c-Met. Through cell experiments, it could be seen that miR-454-3p over-expression could inhibit the proliferation and invasion of OS cells and promote apoptosis, suggesting that miR-454-3p could suppress OS progression.

CCAT1 contributes to the progression of a variety of tumors through miR. In laryngeal squamous cell carcinoma, CCAT1 can promote cancer through miR-218/ZFX axis[22]. In intrahepatic cholangiocarcinoma, CCAT1 can promote the migration, invasion and EMT of cancer cells via inhibiting miR-152[23]. Subsequently, this study found that the proliferation and apoptosis

after transfection with sh-CCAT1+miR-454-3p-mimics showed no statistical difference when compared with those transfected with miR-NC, while the proliferation and invasion capacity increased and the apoptosis rate decreased compared with those transfected with miR-454-3p-mimics. These results demonstrated that at least part of CCAT1 could play a carcinogenic role in OS through miR-454-3p.

To further improve the mechanism of CCAT1/miR-454-3p in OS, Targetscan 7.2 online software was utilized miR-454-3p target gene prediction, and it was found that there were binding sites between ZEB2 and miR-454-3p. After Dual-Luciferase activity detection, it was observed that ZEB2 could be targeted by miR-454-3p. As a member of the ZEB family, ZEB2 is considered to be a transcription factor involved in proliferation, apoptosis, invasion and other cellular functions[24]. It is thought to be a carcinogenic gene in tumor[25]. ZEB2 is up-regulated in OS and promotes the development of the disease; in addition, it can be regulated by miR-187 to play a role in cancer inhibition[26]. In this paper, ZEB2 in OS cells was inhibited, and it was found that this treatment could suppress the proliferation and invasion of OS cells and increase the apoptosis rate. Subsequently, both ZEB2 and miR-454-3p in OS cells were inhibited at the same time. By observing the biological functions of the cells, it was found that there was no difference in proliferation and apoptosis after transfection with si-ZEB2+miR-454-3p-inhibitor or with si-NC. Compared with transfection with si-ZEB2, the proliferation and invasion ability increased, and the apoptosis decreased. Combined with the above results, it could be known that CCAT1 could promote OS cell proliferation and invasion and inhibit apoptosis through the miR-454-3p/ZEB2 axis.

We showed that CCAT1 could be used as a diagnostic and prognostic marker for OS, and found for the first time that CCAT1 could promote OS cell proliferation and invasion and inhibit apoptosis through miR-454-3p/ZEB2 axis. It is well known that the resistance of tumor cells to chemotherapeutic drugs will cause chemotherapy to fail. Further, CCAT1 affects the chemical sensitivity of paclitaxel in nasopharyngeal carcinoma through the miR-181a/CPEB2 axis[27]. Therefore, it indicates that CCAT1 may exert a certain influence on chemotherapy resistance of OS cells through miR-454-3p/ZEB2 axis, which can be explored in future studies. There are also some shortcomings in this study. For example, the relationship between CCAT1 and other targets has not been explored. Moreover, tumor formation in nude mice experiments have not been conducted to prove the effect of CCAT1 on tumors *in vivo*. We hope that more research can be included in future researches to supplement and improve our results.

Conclusions

To sum up, CCAT1 can be used as a diagnostic and prognostic marker for OS and can promote OS cell proliferation and invasion and inhibit apoptosis through the miR-454-3p/ZEB2 axis, which can be a therapeutic target for OS. The results indicates that CCAT1 is an important regulator in the development of OS and a potential target for OS therapy.

Conflict of Interest

The authors declare that they have no conflict of interests.

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