 2020; 24: 3572-3578

LncRNA SNHG16 promotes migration and invasion through suppression of CDKN1A in clear cell renal cell carcinoma

S.-B. LIU[1], H.-F. WANG[2], Q.-P. XIE[1], G. LI[1], L.-B. ZHOU[3], B. HU[1]

[1]Department of Urological Surgery, Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute, Shenyang, China
[2]Department of Urological Surgery, The Second Affiliated Hospital of Kunming Medical University, Yunnan Institute of Urology, Kunming, China
[3]Department of Urological Surgery, Ningbo Medical Center Lihuili Hospital, Ningbo, China

RETRACTED

Abstract. – OBJECTIVE: Researchers have discovered the important role of long noncoding RNAs (lncRNAs) in tumorigenesis. In this study, lncRNA SNHG16 was studied to identify whether it influences metastasis of clear cell renal cell carcinoma (ccRCC).

PATIENTS AND METHODS: SNHG16 expression was detected by quantitative real-time polymerase chain reaction (qRT-PCR) in both ccRCC cells and tissue samples. The association between prognosis of ccRCC patients and the expression of SNHG16 was analyzed through Kaplan-Meier method. Moreover, functional assays including wound healing assay and transwell assay were conducted. Bioinformatics analysis, qRT-PCR and Western blot assay were used to explore the underlying mechanism. Furthermore, animal experiment[illegible] conducted to explore the function of S[illegible] *in vivo*.

RESULTS: SNHG16 expression level was [illegible]er in ccRCC samples when com[illegible] with in adjacent ones. Moreover, [illegible]mi[illegible]tion a[illegible] cell invasion capacities we[illegible]ibit[illegible] SNHG16 was silenced *in vit*[illegible] In[illegible]n, [illegible]R-NA and protein expres[illegible] of C[illegible]1A was up-regulated after silen[illegible]NHG16. [illegible]hermore, the expression lev[illegible] of [illegible]1A was negatively related to the e[illegible]ession [illegible]NHG16 in ccRCC tissues. The e[illegible]eriments *in v*[illegible] showed that silence of SN[illegible]6 re[illegible]rkably repressed the metastasis of [illegible]

CON[illegible]SIO[illegible] The[illegible]esults suggest that silen[illegible]G16 [illegible]nhibit cell migration and in[illegible]ion [illegible] upreg[illegible]ing CDKN1A in ccRCC.

Key [illegible]:
Long [illegible]coding RNA, SNHG16, Clear cell renal cell carcinoma, [illegible]DKN1A.

Introducti[illegible]

Renal cell carcinoma [illegible]C) acco[illegible]s for the most common kidne[illegible]arc[illegible] Ma[illegible] are more likely to be dia[illegible]sed of R[illegible]n the ratio of 1.6:1.0. Cle[illegible]ll R[illegible] (ccR[illegible]), originating from the e[illegible]the[illegible] the [illegible]oximal tubule of the neph[illegible] is the [illegible]ype of RCC with a preval[illegible] 70–80%[illegible]lthough most ccRCC pati[illegible] ca[illegible]ured by surgical excision, 30% [illegible]wly diag[illegible] ccRCC cases presents with a [illegible]edian survival [illegible]me of 13 months due to local [illegible] distant metastasis[4]. Therefore, it is urgent to [illegible]lize the [illegible]derlying mechanism and find a new t[illegible]trategy.

Long noncoding RNAs (lncRNAs) are one of [illegible] newly discovered subgroups of noncoding [illegible]As (ncRNAs) which are longer than 200 nucleotides. Although lncRNAs cannot be translated into proteins, lncRNAs play a crucial role in a variety of biological processes, including proliferation, migration and metastasis in cancers. Notably, through negatively modulating miR-330-3p, LINC00052 serves as a tumor inhibitor in pancreatic cancer[5]. By competitively bind to miR-144, lncRNA LINC00483 modulated epithelial-mesenchymal transition and radiosensitivity in lung adenocarcinoma *via* interacting with HOXA10[6]. Moreover, lncRNA ZNFX1 is significantly increased in hepatocellular carcinoma and gastric cancer which contributes to the development and progression of hepatocellular cancer[7].

LncRNA SNHG16 functions as a novel oncogene in tumorigenesis. However, the function of SNHG16 in ccRCC remains unknown so far.

Corresponding Author: Bin Hu, Ph.D; e-mail: hubin@cancerhosp-ln-cmu.com

Our study revealed that the expression of SNHG16 was remarkably higher in ccRCC tissues. Moreover, silence of SNHG16 suppressed the migration and invasion of ccRCC cell *in vitro* and *in vivo*. Furthermore, the underlying mechanism how SNHG16 functioned in ccRCC development was also studied.

Patients and Methods

Cell Lines and Clinical Samples

48 ccRCC patients were enrolled for human tissues who received surgery at Liaoning Cancer Hospital & Institute. No radiotherapy or chemotherapy was performed before the surgery. Samples got from the surgery were saved immediately at –80°C. The Research Ethics Committee of Liaoning Cancer Hospital & Institute approved this study. Written informed consent was offered by the patients.

Cell Culture

Human renal cancer cells (Caki-1, ACHN, 786-O) and human kidney epithelial cells ([illegible] were got from American Type Culture [illegible]tion (ATCC). Culture medium consisted of [illegible] fetal bovine serum (FBS; Invitrogen Life Te[illegible]nologies), Dulbecco's Modified Eagle's Mediu[illegible] (DMEM) as well as penicillin. [illegible], cells were cultured in an incubator con[illegible]% CO_2 at 37°C.

Cell Transfection

Lentivirus expressing [illegible]hort[illegible] RNA (shRNA; BiosettiaInc., [illegible] Diego, [illegible]A) targeting SNHG16 wa[illegible]ed into the p[illegible]ti-EF1a-EGFP-F2A-Pur[illegible]iosettiaInc., San Diego, CA, USA). T[illegible] SN[illegible]NA (sh-SNHG16) and the negative control [illegible]re packaged and used fo[illegible]nsfection in cc[illegible] cells. 48 h later, quanti[illegible]e real-time polymerase chain reaction (qRT[illegible]R) w[illegible]sed to detect SNHG16 expression [illegible] th[illegible] cells.

RNA [illegible]ion an[illegible]-PCR

T[illegible]ent (Invitrogen, Carlsbad, CA, US[illegible] was [illegible]parate the total RNA. T[illegible]re[illegible]iption Kit (TaKaRa Biotec[illegible]o., Ltd., Dalian, China), the total RNA wa[illegible]-transcribed to complementary deoxyribose[illegible]c acids (cDNAs). The primer sequences are as follows: SNHG16 forward: 5′-GTGCCTCAGGAAGTCTC[illegible] and reverse: 5′-ATCCAAACAA[illegible]ATCA[illegible]G-CAC-3′; glyceraldheyde 3-[illegible]sphate dehy[illegible]e-nase (GAPDH), forward: [illegible]AAAATCA[illegible]T-GGGGCAATGCTGG-3′ a[illegible]se: 5′-[illegible]AT-GGCATGGACTGTG[illegible]TCAT[illegible]rmal cycle was as follows[illegible] sec at 95°C[illegible] for 40 cycles at 95°C, 35[illegible] at 60[illegible]

Wound Hea[illegible]

After tran[illegible]ferre[illegible]wel[illegible]ates, Caki-1 cells were c[illegible]red in D[illegible]ium overnight. After scra[illegible] with a plast[illegible], cells were cultured in[illegible]e DMEM. Each assay was repeated[illegible]ripl[illegible]ependently. Wound closure was viewed at 24 [illegible]

T[illegible]swell Assay

[illegible]or detecting cell invaded ability, 5 ×10^4 Ca[illegible] cells in 20[illegible]L serum-free DMEM were [illegible]formed to [illegible]chamber of an 8 µm pore size i[illegible], Billerica, MA, USA) coated with [illegible]atrigel (BD Biosciences, Franklin Lakes, NJ, USA). DMEM and FBS was added [illegible] bottom chamber. 48 h later, the top sur[illegible]mbers was immersed for 10 min with [illegible]g methanol after wiped by cotton swab. [illegible]llowing were stain in crystal violet for 30 min. The data for invaded membrane was counted in [illegible]ree fields.

Bioinformatics Analysis

In this research, Starbase v2.0 (http://starbase.sysu.edu.cn/starbase2/rbpLncRNA.php) was used to predict the target proteins of SNHG16.

Western Blot Analysis

Protein was extracted from cells by Reagent radioimmunoprecipitation assay (RIPA) (Beyotime, Shanghai, China). Bicinchoninic acid (BCA) protein assay kit was used for quantifying protein concentrations (TaKaRa Biotechnology Co., Ltd., Dalian, China). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was utilized to separate the target proteins. After replaced to the polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), they were incubated with antibodies. Rabbit anti-GAPDH, rabbit anti-CDKN1A and goat anti-rabbit secondary antibody were provided by Cell Signaling Technology (CST, Danvers, MA, USA). Image J software (NIH, Bethesda, MD, USA) was applied for assessment of protein expression.

RETRACTED

RETRACTED

Tumor Metastasis Assay

Transfected cells were injected into tail vein of NOD/SCID mice (6 weeks old). The mice were sacrificed, and the lung were extracted after 4 weeks. Then the number of metastatic nodules in the lung was counted. The research was approved by the Animal Ethics Committee of Liaoning Cancer Hospital & Institute.

Statistical Analysis

Statistical analysis was conducted by Statistical Product and Service Solutions (SPSS) 20.0 (SPSS, Chicago, IL, USA). Data were presented as mean ± SD (standard deviation). Student *t*-test and Kaplan-Meier method were performed to the data. It was considered of statistically significance, when $p<0.05$.

Results

SNHG16 Expression Level in ccRCC Tissues

Firstly, SNHG16 expression was detect[illegible] qRT-PCR in 48 patients' tissues. As a[illegible]nt, SNHG16 was significantly upregulated [illegible]mor tissue samples (Figure 1A). Those pati[illegible] were divided into high SNHG16 expression gro[illegible] and low SNHG16 expression group [illegible]ording to the median expression. Kaplan[illegible]nalysis showed that patients in high [illegible]HG[illegible] expression group had a poorer di[illegible]e-free[illegible] when compared with the low[illegible]16 [illegible]ressio[illegible] group (Figure 1B).

Silence of SNHG16 Inhibite[illegible]ration and Invasion of Caki-1 c[illegible]

SNHG16 expression le[illegible]f ccRCC c[illegible]was higher than that of HK[illegible]igure 2A). [illegible]i-1 ccRCC cell line was sel[illegible] silence [illegible]SNHG16. Then SNHG1[illegible] expr[illegible]ected by qRT-PCR (Figu[illegible]B). More[illegible]ults of wound healing as[illegible] show[illegible] that sile[illegible]e of SNHG16 significan[illegible]epres[illegible] the ability of migration in ccRCC[illegible]2C). [illegible] outcome of transwell as[illegible]y a[illegible]d th[illegible]umber of invaded cells[illegible]s rema[illegible]ased after SNHG16 wa[illegible]enced in ccR[illegible]ls (Figure 2D).

The [illegible]ra[illegible]etween CDKN1A and SNH[illegible]6 in cc[illegible]

[illegible]e results of b[illegible]ormative analysis, CD[illegible]A was predicted as one of the potential target [illegible]teins of SNHG16. Besides, qRT-PCR results [illegible]wed that ex[illegible]sion level of CDKN1A in [illegible]C cells w[illegible]gnificantly higher in SNHG16 [illegible] (sh-[illegible]G16) group when compared with the[illegible] level in negative control (NC) group (Figure 3A). The result of Western blot assay showed that after SNHG16 was silenced, [illegible] could be upregulated at protein level [illegible]3B). Furthermore, correlation analysis [illegible]monstrated that CDKN1A expression level negatively correlated to SNHG16 expression in [illegible]cRCC tissues (Figure 3C).

Silence of SNHG16 Inhibited Tumor Metastasis In Vivo

The ability of SNHG16 in tumor metastasis was detected *in vivo*. The number of metastatic

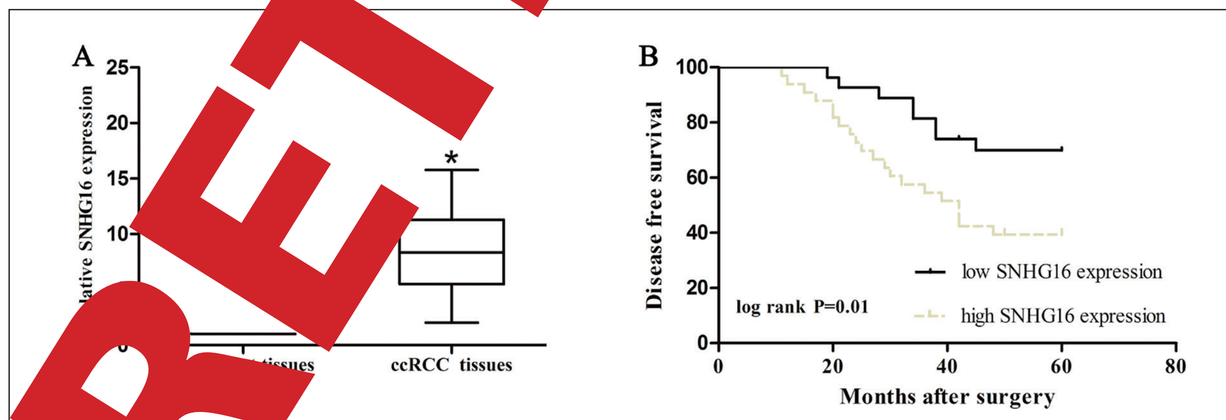


Figure [illegible]on level of SNHG16 was increased in ccRCC patients. **A,** SNHG16 expression was significantly increased in the ccRC[illegible]ompared with adjacent tissues. **B,** High level of SNHG16 was associated with poorer disease-free survival of ccRC[illegible]ients. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

RETRACTED

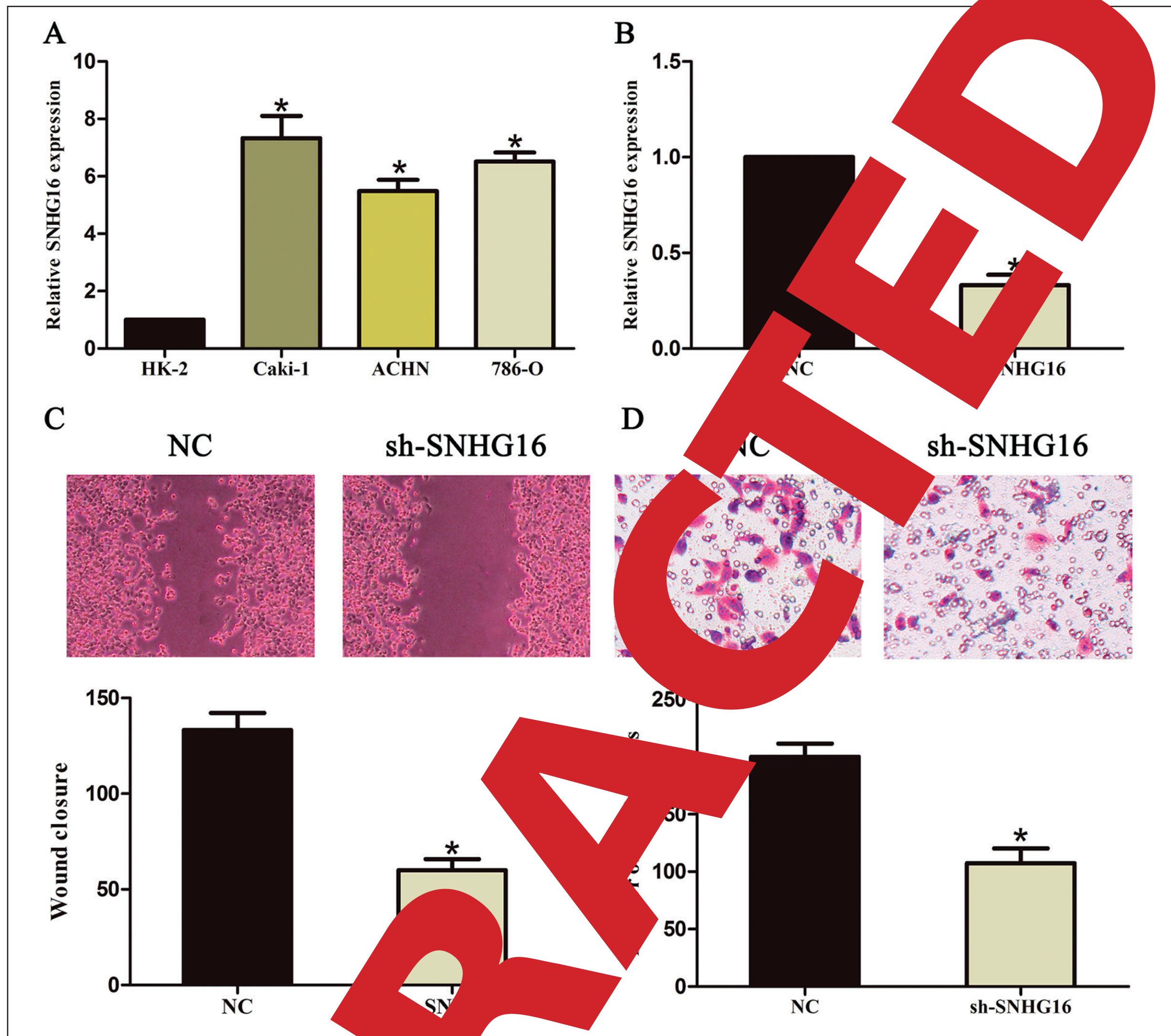


Figure 2. Silence of SNHG16 [illegible]bited Caki-[illegible]ll migration and invasion. **A,** Expression levels of SNHG16 relative to GAPDH were determined [illegible] human ccRCC c[illegible]es and human kidney epithelial cell (HK-2) by qRT-PCR. **B,** SNHG16 expression in ccRCC cells t[illegible]ith SNHG16 shRNA (sh-SNHG16) and the negative control (NC) was detected by qRT-PCR. GAPDH was used a[illegible]ol. **C,** Wound healing assay showed that silence of SNHG16 significantly decreased cell migration in ccRCC [illegible] (magn[illegible]×). **D,** Transwell assay showed that number of invaded cells was decreased *via* silence of SNHG16 in [illegible]RCC cells (mag[illegible] 40×). The results represent the average of three independent experiments (mean ± standard er[illegible] the mean). *$p<0.0$[illegible] compared with the control cells.

nodules in the [illegible]m the [illegible]-SNHG16 group was significantly r[illegible]ared to NC group (Figur[illegible] Moreover[illegible] expression level of SNH[illegible]DKN1A in extracted tumor tissues wa[illegible]tecte[illegible]R. The results showed th[illegible]G[illegible]regulated in sh-SNHG16 grou[illegible]red with NC group (Figure 4B). CDKN1[illegible]regulated in sh-SNHG16 group compared w[illegible]C group (Figure 4C). Above results suggested that silence of SNHG16 could inhibit tumor metastasis *via* upregulating CDKN1A *in vivo*.

Discussion

LncRNAs take part in a variety of important biological processes, which include the growth and metastasis of cancer. Moreover, lncRNAs may offer potential therapeutic targets in the

RETRACTED

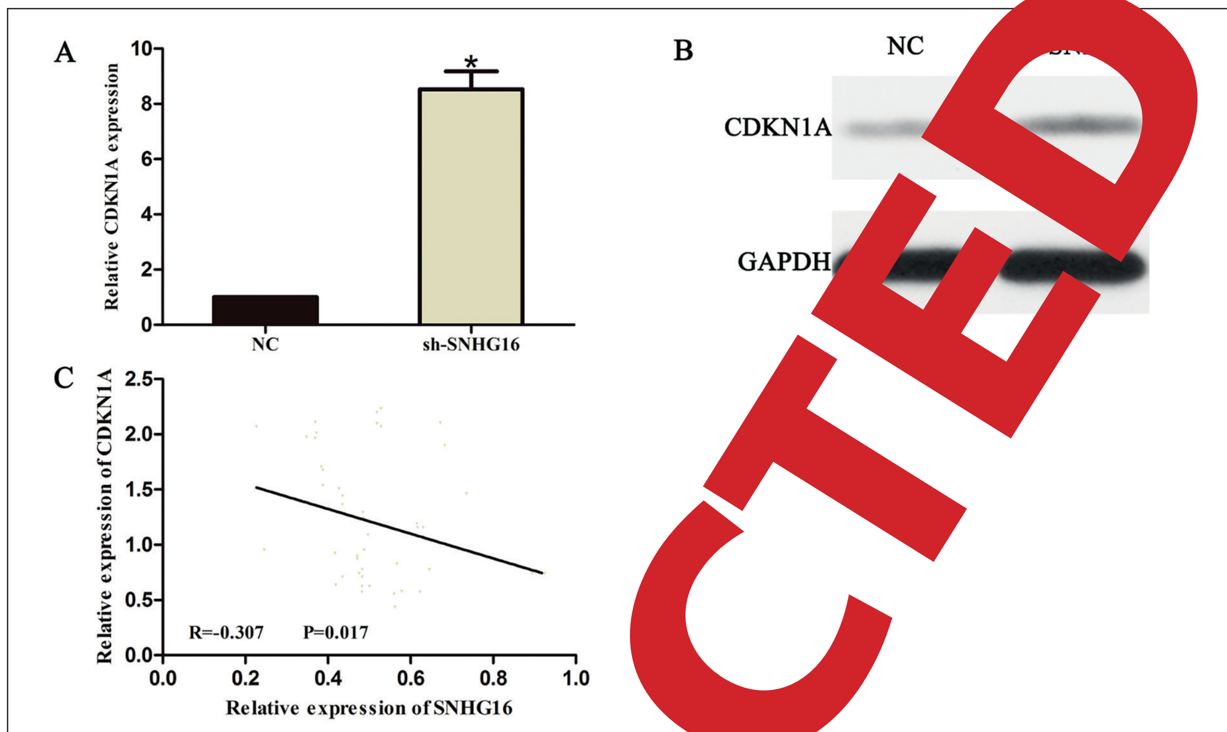
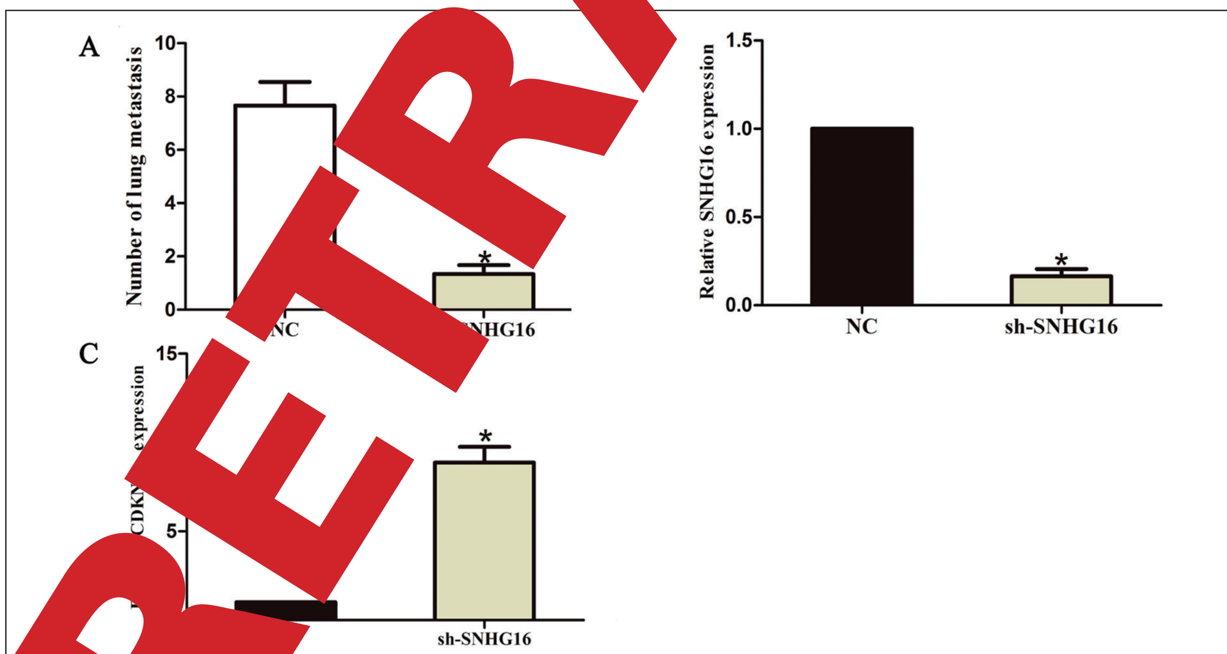


Figure 3. Interaction between SNHG16 and CDKN1[…] RT-qPCR results showed that CDKN1A expression was increased in SNHG16 shRNA (sh-SNHG16) compared with t[…] (NC). **B,** Western blot assay revealed that CDKN1A protein expression was increased in SNHG16 shR[…] with the negative control (NC). **C,** The linear correlation between the expression level of CDKN1A[…] tissues. The results represent the average of three independent experiments Data are presented as the me[…] error of the mean. *$p<0.05$.



Figu[…]kdown of SNHG16 inhibited tumor metastasis *in vivo*. **A,** The number of metastatic nodules in the lung from the s[…] group was significantly reduced compared to negative control (NC) group. **B,** The relative expression of SNHG16 in […]tic nodules were examined by RT-qPCR. **C,** The relative expression of CDKN1A in metastatic nodules were examined by […]-qPCR. Data are presented as the mean ± SD of three independent experiments. *$p<0.05$.

RETRACTED

future for most cancers. A lot of lncRNAs participate in the development of ccRCC. Consistently, lncRNA DHRS4-AS1 inhibits the progression of ccRCC and promotes the apoptosis of ccRCC cells[8]. By downregulating the expression of miR-196a, lncRNA TUG1 promotes cell proliferation and cell migration in ccRCC[9]. LncRNA DHRS4-AS1 functions as a tumor inhibitor and may be a potential prognostic biomarker in ccRCC[8]. LncRNA OTUD6B-AS1 suppresses cell proliferation in RCC and is associated with poor prognosis through Wnt/β-catenin signaling pathway[10].

Small nucleolar RNA host gene 16 (SNHG16) is one of noncoding RNAs which has been reported to function as an oncogene and promote tumor progression in multiple cancers. Upregulation of SNHG16 contributes to cell proliferation and cell migration in gastric cancer[11]. SNHG16 enhances cell viability and cell migration, reduces cell apoptosis in colorectal cancer through regulating the Wnt pathway and lipid metabolism[12]. SNHG16 promotes the tumorigenesis of cervical cancer through miR-216-5p/ZEB1 signal pathway[13]. By silencing p21 epigenetically, SNHG16 accelerates tumor proliferation in bladder cancer which [illegible] sociated with poor prognosis of the patients [illegible].

Our study revealed that SNHG16 was [illegible]er-expressed in both ccRCC carcinoma samp[illegible] and cells. The high expression of SNHG16 wa[illegible] associated with the poor prognosi[illegible] ccRCC. After SNHG16 was knocked do[illegible]igrated ability of ccRCC was found [illegible] be [illegible]. Meanwhile, after SNHG16 w[illegible] nock[illegible] the invaded ability of ccRC[illegible] to be inhibited. Above results i[illegible]icate[illegible]HG16 promoted metastasis of [illegible]C and m[illegible]s an oncogene.

To further explore [illegible]ing mechanism of SNHG16 in ccR[illegible] bio[illegible]ive analysis was utilized to predict the pot[illegible]get proteins of SNHG16[illegible]ong which Cyc[illegible]ependent kinase inhibit[illegible] espe[illegible]lly CDKN1A ($p21^{Cip1}$) was a canoni[illegible]olyc[illegible] target gene and tumor suppressor. [illegible]ar[illegible], by [illegible]ressing CDKN1A, a posit[illegible]ck l[illegible] EZH2 controls the proliferation o[illegible]enters B cell and enable[illegible]le prog[illegible]. CDKN1A gene is signi[illegible]related wi[illegible] prognosis of patients wi[illegible]astric[illegible]oma resection[16]. CDKN[illegible]ld[illegible] response of cutaneous tum[illegible]iotherapy by manipulating langerhans cel[illegible] and promoting Treg cell generation[17]. In the[illegible]nt study, we firstly discovered the interaction between CDKN1A and SNHG16. The results showed that the e[illegible]el of CDKN1A could be upregula[illegible]fter k[illegible]wn of SNHG16. Furthermore[illegible]KN1A exp[illegible]on in ccRCC tissues was n[illegible]ly related t[illegible]N-HG16 expression. Experi[illegible] *vivo* [illegible]ved that the lower-expres[illegible]d SNH[illegible] the less lung metastasi[illegible]dules and t[illegible]er-expressed CDKN1A [illegible]mpar[illegible] with the negative control group. [illegible]he r[illegible]s abo[illegible] suggested that silence of [illegible]ght in[illegible] metastasis of ccRCC thr[illegible]gh u[illegible]ing [illegible]KN1A.

Conclusions

Ab[illegible]ve data id[illegible]hat SNHG16 was rema[illegible]y upregulated [illegible]cRCC tissues and was a[illegible]iated with ccRCC patients' poor survival. [illegible]des, silence [illegible]f SNHG16 could inhibit cell [illegible]ration and i[illegible]ion in ccRCC through up[illegible]ting CDK[illegible]A *in vitro* and *in vivo*. These fi[illegible]hat SNHG16 may contribute to thera[illegible]CC as a candidate target.

Conflict of Interest

[illegible] Authors declare that they have no conflict of interests.

Acknowledgements

This work was partly supported by the Natural Science Foundation of Liaoning (grant no. 20180550399); the Nation Cancer Center Tumor Research Special Subject (grant no. NCC2017A13); the Natural Science Foundation of Ningbo (grant no. 2017A610186); and the Medicine and Health Project of Zhejiang Province (grant no. 2019KY603).

References

1) Morris MR, Latif F. The epigenetic landscape of renal cancer. Nat Rev Nephrol 2017; 13: 47-60.
2) Turajlic S, Larkin J, Swanton C. SnapShot: renal cell carcinoma. Cell 2015; 163: 1556.
3) Cai Q, Zhao A, Ren LG, Chen J, Liao KS, Wang ZS, Zhang W. MiR-425 involves in the development and progression of renal cell carcinoma by inhibiting E2F6. Eur Rev Med Pharmacol Sci 2018; 22: 6300-6307.
4) Gupta K, Miller JD, Li JZ, Russell MW, Charbonneau C. Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. Cancer Treat Rev 2008; 34: 193-205.

5) Xiong X, Shi Q, Yang X, Wang W, Tao J. LINC00052 functions as a tumor suppressor through negatively modulating miR-330-3p in pancreatic cancer. J Cell Physiol 2019: doi.org/10.1002/jcp.28209 (Epub ahead of print).
6) Yang QS, Li B, Xu G, Yang SQ, Wang P, Tang HH, Liu YY. Long noncoding RNA LINC00483/microRNA-144 regulates radiosensitivity and epithelial-mesenchymal transition in lung adenocarcinoma by interacting with HOXA10. J Cell Physiol 2019; 234: 11805-11821.
7) He A, He S, Li X, Zhou L. ZFAS1: A novel vital oncogenic lncRNA in multiple human cancers. Cell Prolif 2019; 52: e12513.
8) Wang C, Wang G, Zhang Z, Wang Z, Ren M, Wang X, Li H, Yu Y, Liu J, Cai L, Li Y, Zhang D, Zhang C. The downregulated long noncoding RNA DHRS4-AS1 is protumoral and associated with the prognosis of clear cell renal cell carcinoma. Onco Targets Ther 2018; 11: 5631-5646.
9) Yang Y, Sun DM, Yu JF, Zhang M, Yi C, Yang R, Dan BH, Li AJ. Long noncoding RNA TUG1 promotes renal cell carcinoma cell proliferation, migration and invasion by downregulating microRNA196a. Mol Med Rep 2018; 18: 5791-5798.
10) Wang G, Zhang ZJ, Jian WG, Liu PH, Xue W, Wang TD, Meng YY, Yuan C, Li HM, Yu YP, Liu ZX, Wu Q, Zhang DM, Zhang C. Novel long noncoding [illegible] OTUD6B-AS1 indicates poor prognosis [illegible]hibits clear cell renal cell carcinoma pro[illegible]on via the Wnt/beta-catenin signaling pathwa[illegible] Cancer 2019; 18: 15.
11) Lian D, Amin B, Du D, Yan W[illegible] expression of the long non-codi[illegible] contributes to gastric cancer [illegible]gression a[illegible]astasis. Cancer Biomark [illegible], 21: 151-160.
12) Christensen LL, True K[illegible] MP, Niels[illegible]MM, Damas ND, Damgaard C[illegible] Der[illegible]is E, Bramsen JB, Pederse[illegible]S, Lund [illegible]olt K, Madsen MR, Laur[illegible], McGuire S[illegible] TF, Andersen CL. SNHG[illegible]s regulated by the [illegible] pathway in colorectal c[illegible]er and [illegible]cts genes involved in lipid metabo[illegible] Mol C[illegible] 2016; [illegible]1266-1282.
13) Zhu H, Zeng [illegible] Ye W. S[illegible]G16/miR-216-5p/ZEB1 [illegible]gnal p[illegible]nt[illegible]es to the tumorigenes[illegible] cervical [illegible]s. Arch Biochem Bioph[illegible]018; 637: 1-8.
14) Ca[illegible] Yue D. LncRNA-SNHG16 predicts p[illegible]d promotes tumor proliferation th[illegible]gh epig[illegible] silencing p21 in bladder [illegible]ncer. Cancer [illegible]er 2018; 25: 10-17.
15) [illegible]eguelin W, Rivas MA, Calvo FM, Teater M, Purwada A, Redmond D, Shen H, Challman MF, Elemento O, Singh A, Melni[illegible] AM. EZH2 enables germinal centre formation [illegible]ugh epigenetic silencing of CDKN1A and a[illegible]-E2F1 feedback loop. Nat Commun 201[illegible]77.
16) [illegible] Latif F. The epigenetic landscape of renal cancer. Nat Rev Nephrol 2017; 13: 47-60.
17) Price JG, Idoyaga J, Salmon H, Hogstad B, Bigarel[illegible] Ghaffari S, Leboeuf M, Merad M. CDKN1A [illegible]ates Langerhans cell survival and promotes Treg cell generation upon exposure to ionizing irradiation. Nat Immunol 2015; 16: 1060-1068.

RETRACTED