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LncRNA SNHG8 participates in the development of endometrial carcinoma through regulating c-MET expression by miR-152

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Abstract. – OBJECTIVE: To investigate the possible function and mechanism of lncRNA SNHG8 in the pathogenesis of endometrial carcinoma.

PATIENTS AND METHODS: We utilized qRT-PCR to detect the expression of SNHG8 in 60 cases of endometrial carcinoma and 25 cases of normal endometrium; after that, the endometrial carcinoma cell lines were screened. SNHG8 was transfected into endometrial carcinoma cells by Lipofectamine and the proliferative activity of cells was detected by cell counting kit-8 (CCK-8) assay. Bioinformatics methods were used to detect the target microRNA. miR-152 is predicted to bind to SNHG8 and target genes of c-MET. Luciferase reporter assay was performed to detect the relative luciferase activity between miR-152 and c-MET, SNHG8. The interactions between SNHG8, miR-152, and c-MET were further verified by transfection of miR-152 mimics, miR-152 mimics + OE-SNHG8, SNHG8 siRNA, and SNHG8 siRNA + miR-152 inhibitor.

RESULTS: SNHG8 expression in endometrial carcinoma tissue was significantly higher than that in normal endometrium. After transfection with SNHG8 siRNA, the cell viability of AN3CA cells decreased, whereas the activity of Ishikawa was increased after transfection with SNHG8 overexpression plasmid. Bioinformatics predictions and dual luciferase reporter assay illustrated that SNHG8 was bound to miR-152 and miR-152 targeted on c-MET. In addition, miR-152 mimics inhibited the expression of c-MET, and the inhibitory effect was reversed after SNHG8 overexpression. Silencing SNHG8 reduced c-MET expression, and c-MET expression was reversed after addition of miR-152 inhibitor.

CONCLUSIONS: SNHG8 is highly expressed in endometrial carcinoma, and SNHG8 targets c-MET through miR-152 to regulate the proliferation of endometrial cancer cells.

Key Words:
Endometrial carcinoma, SNHG8, MiR-152, c-MET, Proliferation.

Introduction

Endometrial cancer (EC) is a common epithelial malignancy in the female reproductive system, and its incidence ranks the fourth among all the female cancers, only lower than those in colorectal cancer, lung cancer and breast cancer[1]. According to its relationship with the estrogen, EC is classified into estrogen-dependent type and non-estrogen-dependent type. Type I EC presents good cell differentiation, positive expressions of estrogen and progesterone receptor and good prognosis. Type II EC presents a high degree of malignancy with poor prognosis, in which estrogen and hormone receptors are not or weakly expressed. Although the prognosis of EC is better than ovarian cancer and cervical cancer, its 5-year survival rate is not satisfactory, with only 83% in stage I-II, 73% in stage III and 62% in stage IV. More studies have shown that the occurrence and development of EC are closely linked to epigenetic changes[2,3].

In the 3 billion base pairs that make up the human genome, only less than 2% of the nucleic acid sequences encode proteins, and over 98% are non-protein-coding sequences. LncRNAs are non-coding RNAs with over 200 nucleotides in length[4,5]. Earlier researches have shown that lncRNAs have no effect, but recent studies have found that some lncRNAs are dysregulated in some cancerous tissues. They exhibit a high

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degree of tissue specificity and are significantly associated with the proliferation, invasion of cancer cells, and prognosis of cancer patients, which are a new target for cancer diagnosis and treatment[6,7]. Moreover, lncRNAs act as competitive endogenous RNAs (ceRNAs) to bind miRNAs and regulate the expression of target mRNAs. This mechanism is becoming new hotspot in epigenetic research[8,9].

Small nucleolar RNA host gene 8 (SNHG8) has a total length of 1062 nt and is defined as lncRNA. It has been reported that the expression of SNHG8 in EBV-associated gastric cancer is significantly increased and may be involved in its development[10], but the current expression of SNHG8 in endometrial cancer has not been reported.

Patients and Methods

Patients' Information

Tumor tissues from 60 EC patients who underwent surgical resection from June 2008 to December 2011 were selected as the disease group. Neither hormone treatment nor chemoradiation was performed before surgery. Patients were followed-up for 5 years. Then, the clinical data was collected for further analysis. Normal endometrial tissues of 25 patients with hysteromyoma undergoing total hysterectomy in the same period were selected as the control group. This study was approved by the Hospital Ethics Committee; the patients signed the informed consent form.

Cell Culture and Transfection

Human endometrial cancer cells (Ishikawa, Japan, HEC1-A, HEC1-B, AN3CA, RL95-2) were cultured in Dulbecco's modified eagle medium (DMEM), high glucose containing 10% fetal bovine serum (FBS) at 37°C with 5% CO_2. Cells with good viability in logarithmic phase were selected and transfected according to Lipofectamine 2000 instructions. SNHG8 overexpression plasmid and siRNA, miR-152 NC, miR-152 mimics, miR-152 inhibitors, c-MET siRNA (Genepharma, Shanghai, China) were transfected and the medium was changed 6 h after transfection. The interference sequences were: miR-152 mimics (Forward: 5'-UCAGUGCAUGACAGAACUUGG-3', Reverse: 5'-AAGUUCUGUCAUGCACUGAUU-3'), miR-152 inhibitor (Forward: 5'- CCAAGUUCUGUCAUGCACUGA-3', Reverse: 5'-CTCATTTGGATAGGCTTGTAA-3'), negative control (5'-TTACAAGCCTATCCAAATGAG-3').

RNA Extraction and qRT-PCR

Total RNA was extracted by TRIzol, chloroform and isopropanol for both cell samples and tissue samples. The extracted RNA was stored at -80°C until the concentration was determined by a microtiter plate reader. cDNA was reverse transcribed by the loop method and 1 μL of cDNA was used for the detection of miR-152. SYBR Green method was used for polymerase chain reaction (PCR) detection. PCR amplification conditions were: 5 min pre-denaturation at 94°C followed by 40 cycles of (30 s at 94°C, 30 s at 55°C, and 90 s at 72°C). The primer sequences were: internal reference U6 primer (Forward: 5'-CTCGCTTCGGCAGCAGCACATATA-3 ', downstream was a universal primer for the kit), SNHG8 (Forward: 5'AAGTTTACAAGCATGCGCGG-3'; Reverse: 5'-TCAAACTGACGGTTCTCGGG-3'), miR-152 (Forward: 5'-GTGCAGGGTCCGAGGT-3'; Reverse: 5'-TGACAGAACTTGGGTCGT-3'), c-MET (Forward: 5'-AGCAATGGGGAGTGTAAAGAGG-3', Reverse: 5'-CCCAGTCTTGTACTCAGCAAC-3').

Cell Counting Kit-8 (CCK-8) Assay

Transfected cells after 24 h were digested and collected. These cells were inoculated into 96-well plates at 2×10^3 per well. 6 replicate wells were set in each group. 6 h after cells were adherent to the plate, CCK-8 method was used to detect cell viability. 2 h before the assay, 10 μL of CCK-8 were added to each well and incubated at 37°C for 2 h. The absorbance of each well at 450 nm was recorded by a microplate reader.

Bioinformatics Prediction

MiR-152 was obtained from predicting miRNAs that bound to SNHG8 by bioinformatics (starbase), followed by prediction of miR-152 target genes using TargetScan, mirBase and mirDB to obtain c-MET. Screening strategies: tumor-related genes were selected, high scores of candidate genes bound to miRNA were finally chosen.

Construction of Luciferase Reporter Gene Vector and its Activity Assay

The sequences of SNHG8 and c-MET were downloaded from the NCBI website to construct the wild-type sequence SNHG8 WT 3'UTR and the mutant sequence SNHG8 MUT 3'UTR, as

well as the wild type sequence c-MET WT 3'UTR and the mutant sequence c-MET MUT 3'UTR. AN3CA cells were then seeded in 96-well plates and co-transfected with 50 pmol/L miR-152 mimics or negative control and the constructed 80 ng of c-MET or SNHG8 WT 3'UTR or SNHG8 MUT 3'UTR into AN3CA cells. After transfection for 48 h, cells were lysed using dual luciferase reporter assay system to detect the fluorescence intensity.

Western Blot

The transfected cells were seeded in 6-well plates containing 2.5 mL of cell culture medium and cultured for 72 h. After that, they were collected. For protein extraction, cell lysate was added on ice and centrifuged,; next, the supernatant was harvested. The protein concentration was calculated according to bicinchoninic acid (BCA) protein kit instructions. Proteins were boiled at 100°C after adding sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) protein loading buffer. Proteins were blotted onto polyvinylidene difluoride (PVDF) membranes and blocked in 5% skimmed milk; c-MET primary antibody was used for incubation, and after washing, the second antibody was utilized for incubation. At last, immunoblots were visualized.

Statistical Analysis

We used Statistical Product and Service Solutions (SPSS) 19.0 statistical software (IBM, Armonk, NY, USA) for analysis; measurement data were expressed by mean ± standard deviation, comparison of data between groups were obtained by using *t*-test. $p < 0.05$ indicated the difference was statistically significant.

Results

LncRNA SNHG8 Promotes the Development of Endometrial Carcinoma

We detected the lncRNA SNHG8 expression in 60 cases of EC and 25 cases of normal endometrium by Real-time fluorescence quantitative PCR. Higher expression level of SNHG8 in EC was observed than that in normal endometrial tissues ($p < 0.001$) (Figure 1A). Median expression of SNHG8 in EC was served as the boundary; the disease group was further assigned into high expression group and low expression group. Survival time of EC patients with high expression of SNHG8 was shorter than that in lower expression group ($p = 0.0332$) (Figure 1B). The SNHG8 expression in positive lymph node metastasis was significantly higher than that with negative lymph node metastasis ($p < 0.001$) (Figure 1C). The expression level of SNHG8 in EC patients with FIGO stage III was significantly higher than those in stage I and II ($p < 0.001$) (Figure 1D).

Effect of lncRNA SNHG8 on Cell Phenotype

First, we detected the expression level of SNHG8 in EC cells (Ishikawa, HEC1-A, HEC1-B, AN3CA, RL95-2) by qRT-PCR. We found that the expression level of SNHG8 was the lowest in Ishikawa and the highest in AN3CA. Therefore, Ishikawa and AN3CA were selected for subsequent cell experiments. Subsequently, SNHG8 overexpression plasmid was transfected in Ishikawa and the expression level of SNHG8 was detected. The results indicated that SNHG8 expression was significantly elevated after SNHG8 overexpression plasmid transfection in Ishikawa cells (Figure 2C). CCK-8 assay revealed that the viability of Ishikawa cells was significantly increased after transfection with SNHG8 overexpression plasmid (Figure 2D). Interference efficiency of SNHG8 siRNA1 and SNHG8 siRNA2 was evaluated by Real-time PCR after SNHG8 transfected in AN3CA. We found that both siRNAs significantly down-regulated mRNA level of SNHG8, of which, inhibitory effect of siRNA1 was more significant (Figure 2E). So, we selected siRNA1 for subsequent functional verification. After transfection with SNHG8 siRNA1 for 96 h, CCK8 assay demonstrated that viability of AN3CA cells were significantly reduced (Figure 2F).

SNHG8 Regulates c-MET via miR-152

We proposed that it is possible for SNHG8 to bind to and promote the degradation of some miRNAs and then to prevent the degradation of its target gene as ceRNA. In order to obtain miRNAs that are bound to SNHG8, we predicted a very large number of miRNAs by starbase, and miR-152 was chosen because of its function and score. In order to further verify whether miR-152 can bind to SNHG8, we first detected the expression level of miR-152 in EC (Figure 2B), which was remarkably decreased in EC. In contrast, miR-152 expression was subsequently significantly elevated by transfection of SNHG8 siRNA into AN3CA cells (Figure 3A). By constructing the SNHG8 WT 3'UTR and the mutant

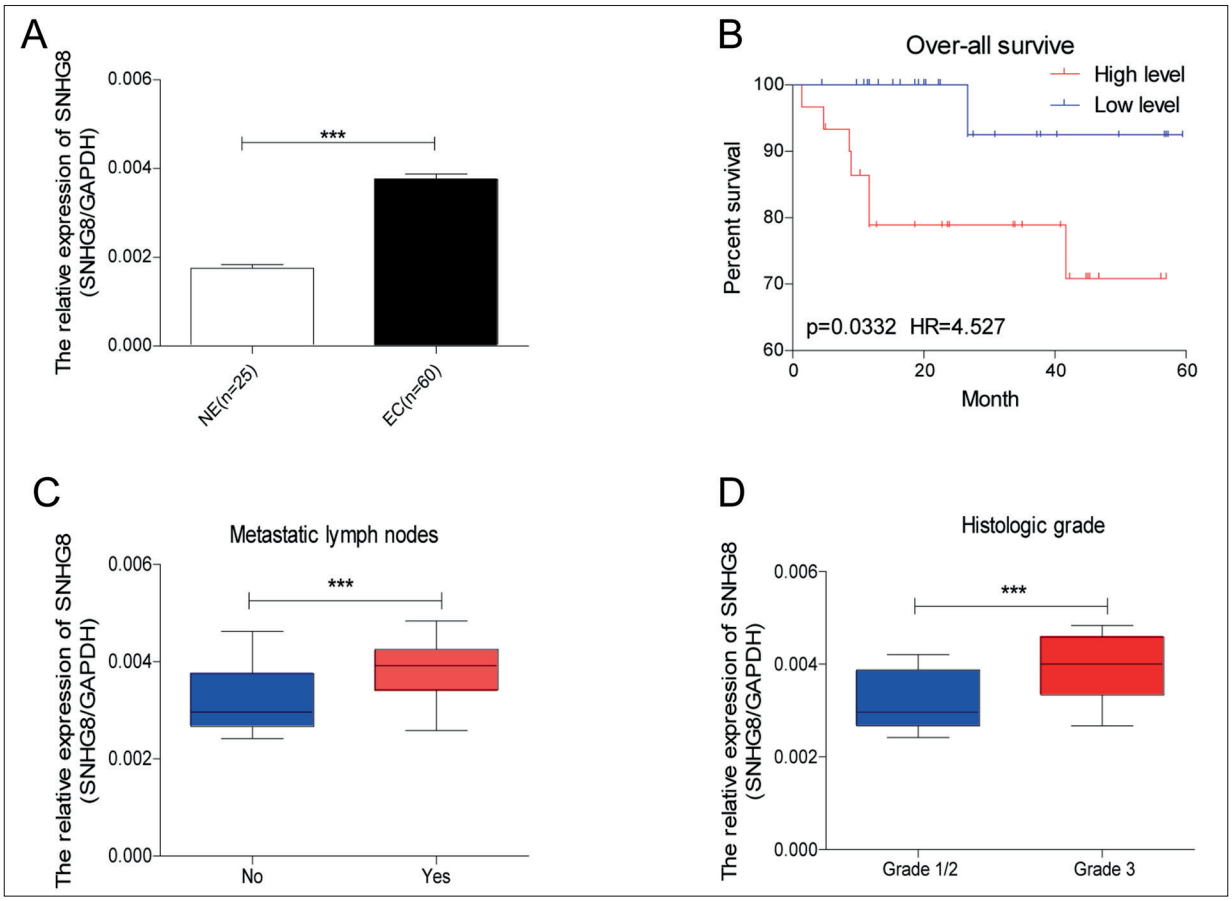

Figure 1. LncRNA SNHG88 plays a cancer-promoting role in EC. ***A,*** The expression of SNHG8 in 60 EC patients was significantly higher than the expression of 25 normal endometrium. ***B,*** EC patients were divided into SNHG8 high and low expression group. The overall survival rate of SNHG8 high group was significantly lower than that of SNHG8 low expression group. ***C,*** SNHG8 expression was positively correlated with lymph node metastasis. ***D,*** SNHG8 expression was positively correlated with histological grade.

sequence SNHG8 MUT 3'UTR (Figure 3B), the SNHG8 WT 3'UTR luciferase was decreased after transfection of miR-152 in AN3CA cells. No significant difference in luciferase activity before and after SNHG8 MUT 3'UTR transfection was observed, suggesting that SNHG8 is bound to miR-152 to quench fluorescence (Figure 3C).

Next, bioinformatics was utilized to predict target genes of miR-152 and functional analysis was performed; thereby, c-MET was obtained. We next verified whether miR-152 can bind to c-MET, and after AN3CA cells were transfected with SNHG8 siRNA, the expression level of c-MET was remarkably reduced (Figure 3D). Further validation pointed out that the luciferase activity of c-MET WT 3' UTR was decreased after AN3CA cells were transfected with miR-152, while there was no significance in luciferase activity before and after transfecting c-MET MUT 3'UTR (Figure 3E). As a result, c-MET is the target gene of miR-152.

Interactions Between SNHG8, miR-152 and c-MET

For further verifying the effect of SNHG8 on regulating the expression level of c-MET as ceRNA, we examined the expression level of c-MET in EC and analyzed the association between the expressions of SNHG8 and c-MET in EC. We found a positive link between SNHG8 and c-MET (Figure 4A). To determine whether SNHG8 regulates c-MET expression by binding to miR-152, we determined protein and mRNA levels of c-MET after altering the expression of SNHG8 and miR-152. The miR-152 mimics inhibited c-MET expression, and the inhibitory effect was reversed after overexpressing SNHG8

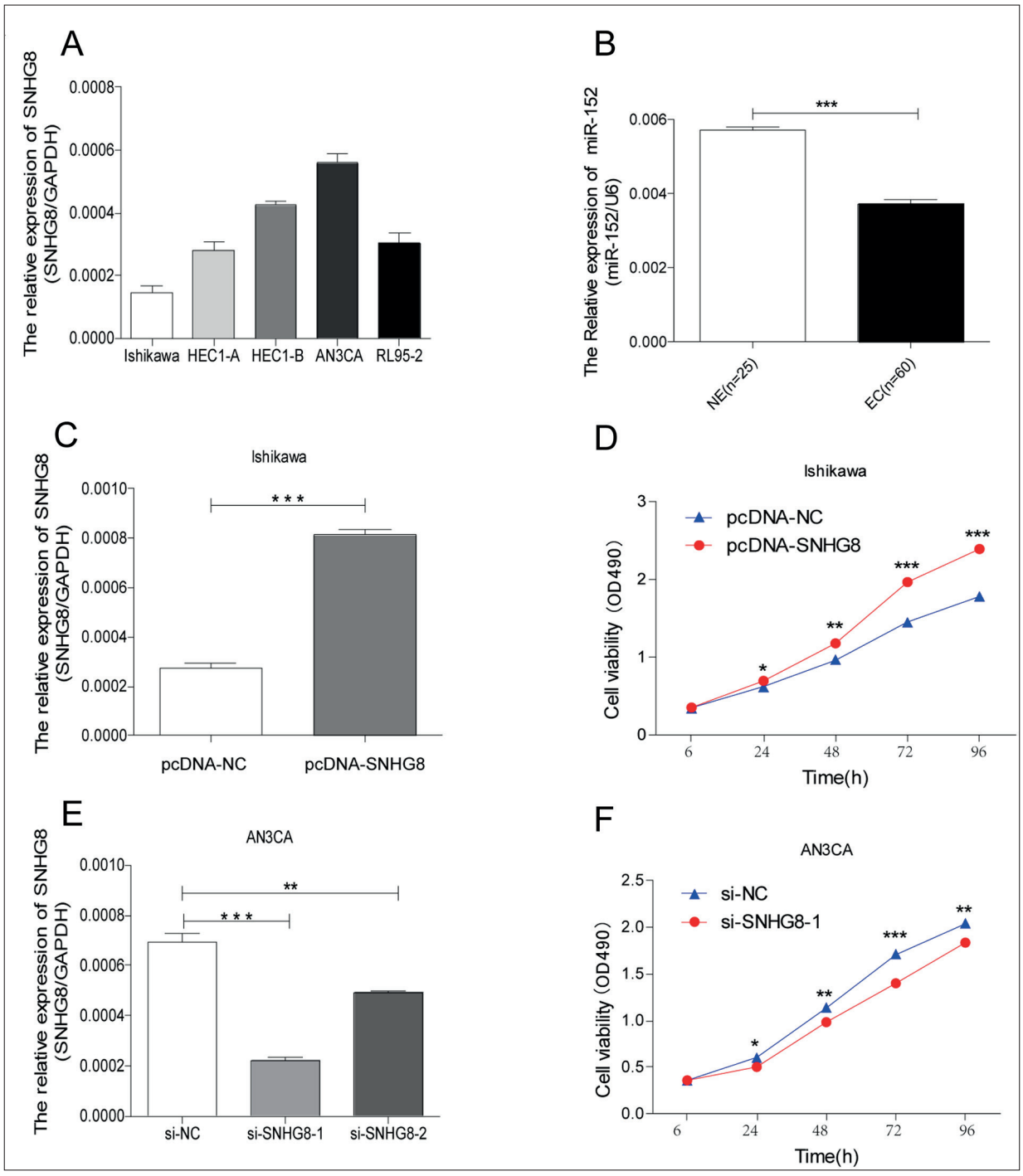

Figure 2. Effect of LncRNA SNHG8 on cell phenotype. ***A,*** SNHG8 expression in EC cells (Ishikawa, HEC1-A, HEC1-B, AN3CA, RL95-2). ***B,*** MiR-152 was lowly expressed in EC tissues. ***C,*** The interference efficiency of pcDNA-SNHG8 in Ishikawa cells. ***D,*** CCK-8 assay showed that overexpression of SNHG8 promoted cell viability of Ishikawa cells. E, Interference efficiency of si-SNHG8-1 was the best in AN3CA cells. ***F,*** Viability of AN3CA cells weakened after knockdown of SNHG8.

(Figures 4B-D). Silencing of SNHG8 reduced c-MET expression, and c-MET expression was reversed after addition of miR-152 inhibitor (Figures 4C and 4E). These results indicated that SNHG8 regulates c-MET expression by binding to miR-152.

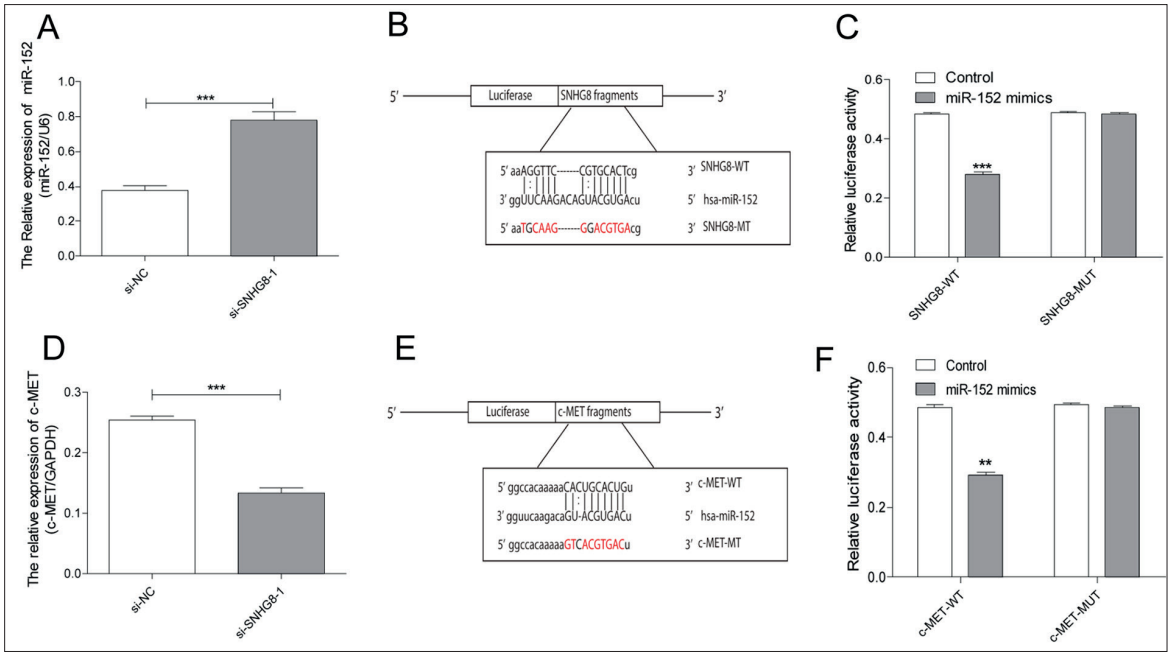

Figure 3. SNHG8 regulated c-MET via miR-152. ***A,*** MiR-152 expression in AN3CA cells increased after knockdown of SNHG8. ***B,*** Predicted binding sites for miR-152 and 3'-UTR regions of SNHG8. ***C,*** Luciferase reporter gene indeed showed that miR-152 binds to SNHG8. ***D,*** Knockdown of SNHG8 reduced c-MET expression in AN3CA cells. ***E,*** Predicted binding sites for miR-152 with the 3'-UTR region of c-MET. ***F,*** Luciferase reporter gene assay showed that miR-152 was bound to c-MET.

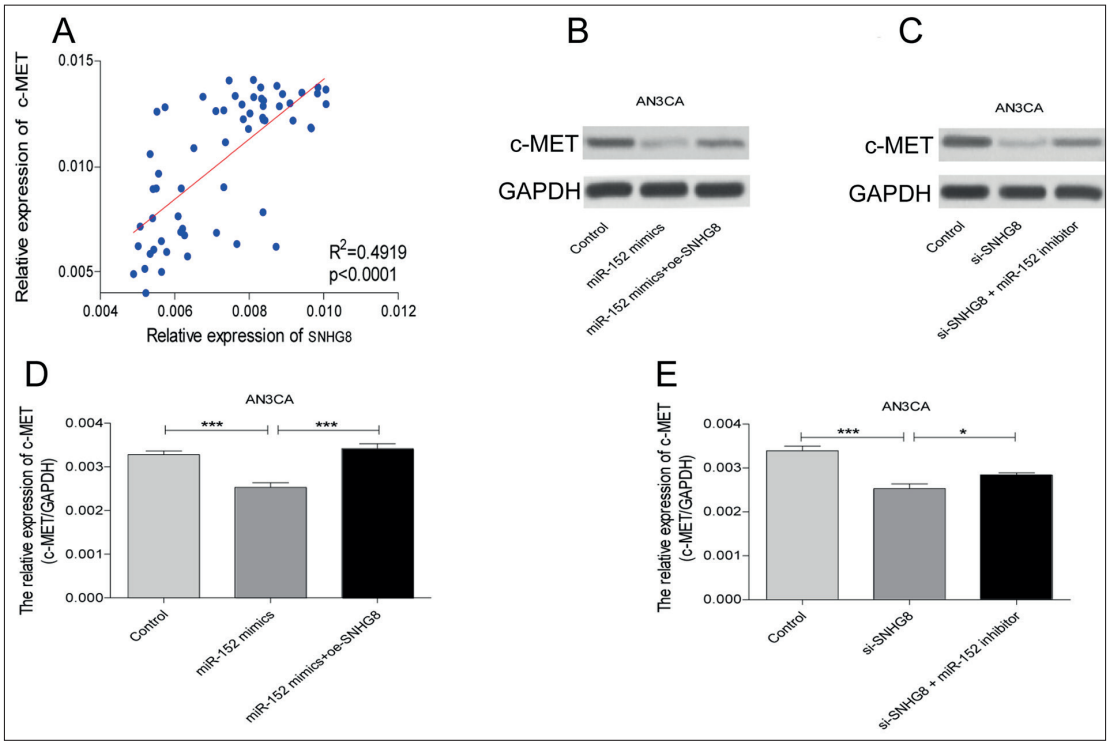

Figure 4. Interaction between c-MET, SNHG8 and miR-152. ***A,*** Correlation analysis of SNHG8 and c-MET expression. ***B,*** Protein expression of c-MET in AN3CA cells was decreased after transfection of miR-152 mimics, which was reversed by oe-SNHG8. ***C,*** Protein expression of c-MET in AN3CA cells was decreased after knockdown of SNHG8, which can be reversed by miR-152 inhibitor. ***D,*** The mRNA expression of c-MET in AN3CA cells was decreased after transfection of miR-152 mimics, which was reversed by oe-SNHG8. ***E,*** The mRNA expression of c-MET in AN3CA cells was decreased after knockdown of SNHG8, which can be reversed by miR-152 inhibitor.

Discussion

Endometrial carcinoma is one of the three most common malignant tumors in the female reproductive tract and is the most common gynecological tumor in developed countries. More than 90% of EC occurs in women over 50 years old. 69% of EC patients with stage I and II according to the FIGO staging are surgically treated. In addition to surgery, 28% of EC patients require radiotherapy and/or chemotherapy, and they generally have a better prognosis with a 5-year survival rate of 95%. However, EC patients in stage III and IV require radiotherapy and/or chemotherapy. Patients with advanced disease usually have a poor prognosis and a high recurrence rate. The estimated 5-year survival rate is 47-69% and 15-17%, respectively. Surgical removal of the uterus leads to loss of fertility, ovariectomy causes menopausal symptoms in premenopausal women, lymphadenectomy causes lower extremity edema, radiation therapy induces gastrointestinal reactions, chemotherapy and other complications of chemotherapy serious influences life quality. They also severely limit the progress of diagnosis and treatment of endometrial cancer. It is of great importance to explore the pathogenesis as well as to find new diagnostic and therapeutic targets for the prognosis of EC. In recent years, many other etiologies of EC have been reported. Mutations and loss of PTEN gene, type I EC, is related to microsatellite instability (MI) led by mismatch, mutation of K-ras oncogene and abnormally high expression of estrogen receptor subtype ERα. Type II EC is mainly characterized by increased mutation levels of mTOR or P53 in serous carcinoma[11-14]. However, the molecular biology of the occurrence and development of endometrial cancer remains unclear. LncRNA was originally considered as "transcriptional noise". As the research progresses, lncRNAs regulate the expression of genes at the epigenetic, transcriptional and post-transcriptional levels. LncRNAs are dysregulated in various tumor tissues and are involved in the occurrence and development of the tumor tissue. Therefore, lncRNAs have been new targets for cancer diagnosis and treatment[15]. Researches have found that lncRNA are greatly involved in the tumorigenesis of EC. For example, HOTAIR is one of the most studied lncRNAs; it is upregulated in EC and is associated with poor prognosis. Currently, research on lncRNA in EC is still small, and its role in EC is still worth exploring[16,17].

LncRNA SNHG8 is a type of small nucleolar RNA (snoRNA). Most of the snoRNAs have been considered to have no function of coding proteins except for guiding RNA in post-translational ribosomal RNA modification. Several studies[18,19] illustrated that this type of RNA is closely related to the development of tumors. For example, SHNG5 is downregulated in cancer tissues, such as colon cancer and gastric cancer, and it also exhibits tumor suppressor activity. We utilized RT-PCR to detect the mRNA expression of LncRNA SNHG8 in EC tissues and normal endometrium tissues. The results demonstrated that compared with normal endometrium, the expression of SNHG8 in EC was significantly higher, which was consistent with the previous report[10] as the expression level of SNHG8 in gastric cancer tissue was higher than adjacent tissues. This indicated that SNHG8 may play an essential role in EC. In this investigation, it was difficult to obtain the matched normal endometrial tissues from the EC patients as the control group due to the morphology and location characteristics of EC tissue, which may cause some bias.

Kaplan-Meier analysis was performed on the SNHG8 expression and the survival of EC patients. Results illustrated that the overall survival of EC patients with overexpressed SNHG8 was shorter, high expression of SNHG8 in EC patients had a poor prognosis. SNHG8 is expected to become a prognostic marker of EC.

Several studies[20-23] have shown that miR-152 is down-regulated in many solid tumor tissues. In this experiment, we also confirmed that miR-152 was overexpressed in EC tissues compared with normal tissues, which is consistent with previous researches. Some research findings[24] on miR-152 in other tumors are obtained. In gliomas, miR-152 inhibits invasion and angiogenesis of tumor cells by directly regulating the expressions of NRP-2 and MMP-3. Studies[25] have shown that upregulation of miR-152 expression in EC significantly inhibits tumor growth.

Bioinformatics predicts that c-MET is a potential target gene for miR-152. In comparison with the control group, the mRNA and protein expressions of c-MET were significantly decreased after transfecting miR-152 mimics. We determined c-MET as the direct target gene of miR-152 by dual luciferase reporter gene. Previously, overexpression of c-MET gene is observed in many tumor tissues, including gastric cancer, liver cancer, breast cancer, etc.[26-28], so that the ability of tumor cells is prior to normal cells, which is consistent with our investigation.

We explored the possible function of SNHG8 in the pathogenesis of EC and found that SNHG8 was abnormally expressed in EC. In *in vitro* experiments, low expression of SNHG8 inhibited cell viability. Bioinformatics predicted that miR-152 was bound to SNHG8 and c-MET was bound to miR-152. A series of experiments demonstrated that SNHG8 exerted sponge function of miR-152 to positively regulate its target gene c-MET, thus promoting cell activity. The above results provide a new possibility to explain the pathogenesis of EC.

Conclusions

We showed that SNHG8 was highly expressed in EC, and SNHG8 targets c-MET to modulate the proliferative activity of EC cells through miR-152.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Matias-Guiu X, Catasus L, Bussaglia E, Lagarda H, Garcia A, Pons C, Munoz J, Arguelles R, Machin P, Prat J. Molecular pathology of endometrial hyperplasia and carcinoma. Hum Pathol 2001; 32: 569-577.
2) Hakim SA, Raboh NM. Immunohistochemical expression of glypican 3 in endometrial carcinoma and correlation with prognostic parameters. Int J Clin Exp Pathol 2015; 8: 13225-13232.
3) Goff BA. Uterine papillary serous carcinoma: what have we learned over the past quarter century? Gynecol Oncol 2005; 98: 341-343.
4) Zhang CG, Yin DD, Sun SY, Han L. The use of lncRNA analysis for stratification management of prognostic risk in patients with NSCLC. Eur Rev Med Pharmacol Sci 2017; 21: 115-119.
5) Maher CA, Kumar-Sinha C, Cao X, Kalyana-Sundaram S, Han B, Jing X, Sam L, Barrette T, Palanisamy N, Chinnaiyan AM. Transcriptome sequencing to detect gene fusions in cancer. Nature 2009; 458: 97-101.
6) Guerra E, Trerotola M, Dell' AR, Bonasera V, Palombo B, El-Sewedy T, Ciccimarra T, Crescenzi C, Lorenzini F, Rossi C, Vacca G, Lattanzio R, Piantelli M, Alberti S. A bicistronic CYCLIN D1-TROP2 mRNA chimera demonstrates a novel oncogenic mechanism in human cancer. Cancer Res 2008; 68: 8113-8121.
7) Yang X, Duan B, Zhou X. Long non-coding RNA FOXD2-AS1 functions as a tumor promoter in colorectal cancer by regulating ECT and notch signaling pathway. Eur Rev Med Pharmacol Sci 2017; 21: 3586-3591.
8) Sun C, Li S, Zhang F, Xi Y, Wang L, Bi Y, Li D. Long non-coding RNA NEAT1 promotes non-small cell lung cancer progression through regulation of miR-377-3p-E2F3 pathway. Oncotarget 2016; 7: 51784-51814.
9) Yu S, Zhao Y, Lai F, Chu M, Hao Y, Feng Y, Zhang H, Liu J, Cheng M, Li L, Shen W, Min L. LncRNA as ceRNAs may be involved in lactation process. Oncotarget 2017; 8: 98014-98028.
10) Huang T, Ji Y, Hu D, Chen B, Zhang H, Li C, Chen G, Luo X, Zheng XW, Lin X. SNHG8 is identified as a key regulator of epstein-barr virus(EBV)-associated gastric cancer by an integrative analysis of lncRNA and mRNA expression. Oncotarget 2016; 7: 80990-81002.
11) Colombo N, Creutzberg C, Amant F, Bosse T, Gonzalez-Martin A, Ledermann J, Marth C, Nout R, Querleu D, Mirza MR, Sessa C. ESMO-ESGO-ESTRO consensus conference on endometrial cancer: diagnosis, treatment and follow-up. Int J Gynecol Cancer 2016; 26: 2-30.
12) Burke WM, Orr J, Leitao M, Salom E, Gehrig P, Olawaiye AB, Brewer M, Boruta D, Villella J, Herzog T, Abu Shahin F. Endometrial cancer: a review and current management strategies: part I. Gynecol Oncol 2014; 134: 385-392.
13) Burke WM, Orr J, Leitao M, Salom E, Gehrig P, Olawaiye AB, Brewer M, Boruta D, Herzog TJ, Shahin FA; Society of Gynecologic Oncology Clinical Practice Committee. Endometrial cancer: a review and current management strategies: part II. Gynecol Oncol 2014; 134: 393-402.
14) Bonaventura A, O'Connell RL, Mapagu C, Beale PJ, McNally OM, Mileshkin LR, Grant PT, Hadley AM, Goh J, Sjoquist KM, Martyn J, DeFazio A, Scurry J, Friedlander ML. Paragon (ANZGOG-0903): phase 2 study of anastrozole in women with estrogen or progesterone receptor-positive platinum-resistant or -refractory recurrent ovarian cancer. Int J Gynecol Cancer 2017; 27: 900-906.
15) Chandra GS, Nandan TY. Potential of long non-coding RNAs in cancer patients: from biomarkers to therapeutic targets. Int J Cancer 2017; 140: 1955-1967.
16) Dhamija S, Diederichs S. From junk to master regulators of invasion: LncRNA functions in migration, ECT and metastasis. Int J Cancer 2016; 139: 269-280.
17) Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464: 1071-1076.
18) Zhao L, Guo H, Zhou B, Feng J, Li Y, Han T, Liu L, Li L, Zhang S, Liu Y, Shi J, Zheng D. Long non-coding RNA SNHG5 suppresses gastric cancer progression by trapping MTA2 in the cytosol. Oncogene 2016; 35: 5770-5780.

19) Siprashvili Z, Webster DE, Johnston D, Shenoy RM, Ungewickell AJ, Bhaduri A, Flockhart R, Zarnegar BJ, Che Y, Meschi F, Puglisi JD, Khavari PA. The noncoding RNAs SNORD50A and SNORD50B bind K-Ras and are recurrently deleted in human cancer. Nat Genet 2016; 48: 53-58.

20) Wen YY, Liu WT, Sun HR, Ge X, Shi ZM, Wang M, Li W, Zhang JY, Liu LZ, Jiang BH. IGF-1-mediated PKM2/beta-catenin/miR-152 regulatory circuit in breast cancer. Sci Rep 2017; 7: 15897.

21) Schaefer A, Jung M, Mollenkopf HJ, Wagner I, Stephan C, Jentzmik F, Miller K, Lein M, Kristiansen G, Jung K. Diagnostic and prognostic implications of microRNA profiling in prostate carcinoma. Int J Cancer 2010; 126: 1166-1176.

22) Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, Morgan DL, Postier RG, Brackett DJ, Schmittgen TD. Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer 2007; 120: 1046-1054.

23) Mercatelli N, Coppola V, Bonci D, Miele F, Costantini A, Guadagnoli M, Bonanno E, Muto G, Frajese GV, De Maria R, Spagnoli LG, Farace MG, Ciafre SA. The inhibition of the highly expressed miR-221 and miR-222 impairs the growth of prostate carcinoma xenografts in mice. PLoS One 2008; 3: e4029.

24) Shi XB, Xue L, Yang J, Ma AH, Zhao J, Xu M, Tepper CG, Evans CP, Kung HJ, DeVere WR. An androgen-regulated miRNA suppresses Bak1 expression and induces androgen-independent growth of prostate cancer cells. Proc Natl Acad Sci U S A 2007; 104: 19983-19988.

25) Navarro A, Marrades RM, Vinolas N, Quera A, Agusti C, Huerta A, Ramirez J, Torres A, Monzo M. MicroRNAs expressed during lung cancer development are expressed in human pseudoglandular lung embryogenesis. Oncology-Basel 2009; 76: 162-169.

26) Peng Z, Zhu Y, Wang Q, Gao J, Li Y, Li Y, Ge S, Shen L. Prognostic significance of MET amplification and expression in gastric cancer: a systematic review with meta-analysis. PLoS One 2014; 9: e84502.

27) Shirahata A, Fan W, Sakuraba K, Yokomizo K, Goto T, Mizukami H, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y, Hibi K. MACC 1 as a marker for vascular invasive hepatocellular carcinoma. Anticancer Res 2011; 31: 777-780.

28) Baccelli I, Schneeweiss A, Riethdorf S, Stenzinger A, Schillert A, Vogel V, Klein C, Saini M, Bauerle T, Wallwiener M, Holland-Letz T, Hofner T, Sprick M, Scharpff M, Marme F, Sinn HP, Pantel K, Weichert W, Trumpp A. Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. Nat Biotechnol 2013; 31: 539-544.