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Long noncoding RNA SNHG16 acts as an oncogene in Wilms' tumor through sponging miR-200a-3p

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Abstract. – OBJECTIVE: Recently, the role of long noncoding RNA (lncRNAs) in tumor progression has attracted much attention. The aim of this study was to investigate the role of lncRNA SNHG16 in the development of Wilms' tumor, and to explore the underlying mechanism.

PATIENTS AND METHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was used to detect SNHG16 expression in Wilms' tumor patients' tissues. Function assays, including wound healing assay, and transwell assay, were conducted to detect the cha biological behaviors in Wilms' tumor cel gain or loss of SNHG16. Besides, the luc se reporter gene assay was performed to ex the underlying mechanism.

RESULTS: The expression level of SNHG16 w significantly up-regulated in Wil or tissues when compared with adjace Cell migration and invasion abilit were s ficantly repressed *via* down-regul of SN How-ever, opposite results we ained ulation of SNHG16 *in tro*. down-regulation of SNHG16, th press miR-200a-3p increased signific . However, pression of miR-200a-3p remarkably ed *via* up-regulation *in vitro*. Fur rmore, SNHG16 acte a co g endogenous RNA *via* spongi miR-200a-3p ms' tumor.

CONC ONS: SNHG16 ed the metastasis o ms' tumor *via* spo ging miR-200a-3p. O ndings ght provide a new prospect for iagno and therapy of Wilms' tumor.

Key Wo
ng n ng SNHG16, Wilms' tumor, a-3p.

Introduction

Wilms' tumor is the most frequent pediatric ncer, which affects one in 10,000 children annu y. Currently, the overall survival rate of Wilms' or is than 90%[1]. When embryonic nephrogenic ce l to undergo terminal diff ion, Wilms' occurs. In recent y advances have been made in combination apy to improv the prognosis of most patients. wever, almos % of patients with Wilms or may dev metastasis and recurrence, c uting to prognosis[2,3]. Therefore, there is a d to understand the underlying mechanism of Wilms' tumor progression and to find out potential therapeutic strategies.

sub-type of noncoding RNA (ncRNA), cRN s participate in a variety of cellular processes and pathways in cancer development. LncRNA ZNF667-AS1 promotes the progression of cervical cancer and is correlated with poor prognosis[4]. LncRNA MEG8 enhances epigenetic induction of epithelial-mesenchymal transition (EMT) in pancreatic carcinoma cells[5]. By modulating OIP5 expression, lncRNA OIP5-AS1 promotes cell proliferation and inhibits cell apoptosis in bladder cancer[6]. The expression level of lncRNA-CCHE1 is positively associated with the malignancy of colorectal carcinoma by regulating the ERK/COX-2 pathway[7]. Activated by ZEB1, lncRNA HCCL5 accelerates cell viability, cell migration, EMT, and the malignancy of hepatocellular carcinoma[8]. LncRNA SNHG16, as a newly discovered lncRNA, has been reported as a vital regulator in tumor development. However, the clinical role and biological mechanisms of SNHG16 in the metastasis of Wilms' tumor have not been fully elucidated.

Our study found that SNHG16 expression was remarkably up-regulated in Wilms' tumor tissues. Meanwhile, SNHG16 promoted the migration and invasion of Wilms' tumor cells *in vitro*. Furthermore, we explored the underlying mechanism of SNHG16 function in Wilms' tumor.

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Patients and Methods

Tissue Specimens

50 Wilms' tumor patients who received surgery at The First Hospital of Jilin University were enrolled in this study. Tissue samples were collected from these patients for subsequent experiments. All collected tissues were maintained at –80°C. Written informed consent was obtained from each subject before the operation. This study was approved by the Ethics Committee of The First Hospital of Jilin University.

Cell Lines

Cells were collected and digested from fresh Wilms' tumor tissues. All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Hyclone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Hyclone, South Logan, UT, USA) and penicillin. Besides, the cells were maintained in an incubator with 5% CO_2 at 37°C.

Cell Transfection

After synthesis, short hairpin RNA (shRNA) targeting SNHG16 (sh-SNHG16) or scra[illegible] oligonucleotides (NC), lentivirus target[illegible] HG16 (SNHG16) or empty vector (EV) [illegible]re cloned into pGLVH1/GFP+Puro vector ([illegible] Pharma, Shanghai, China). Subsequently, Wil[illegible] tumor cells were transfected with sh-SNHG16 [illegible] NC, and SNHG16 lentivirus [illegible]) or EV, respectively. Real Time-qua[illegible]tive [illegible]merase Chain Reaction (RT-qPC[illegible]as use[illegible] detect the transfection efficien[illegible]

RNA Extraction [illegible] RT-qP[illegible]

Total RNA in[illegible]es and cell[illegible]xtracted by using TRIzo[illegible] (Thermo Fishe[illegible]ientific; Waltham, MA[illegible]A). [illegible]uently, total RNA was reverse-tran[illegible]ribed into co[illegible]entary deoxyribose nucleic a[illegible](cDNAs) throug[illegible]rse Transcription Ki[illegible]KaRa Biotechnology [illegible]o., Ltd., Dalian, Chin[illegible]lyceral[illegible]de 3-phosphate dehydrogenase (G[illegible]) wa[illegible]d as an[illegible]ternal reference. Primers us[illegible]-qPCR[illegible]e as follows: SNHG16 f[illegible]ward [illegible]GCCT[illegible]GGAAGTCTCTTGCC-3′, [illegible]ACAAGTTATCACACAG-CA[illegible]glycerald[illegible]de 3-phosphate dehydrogenase (GA[illegible]) primers forward 5′-GGGAGCCAAAAG-G[illegible]reverse 5′- GAGTCCTTCCACGA-[illegible]CAA-3′. The thermal cycle was as follows: 30 [illegible]t 95°C, 5 sec for 40 cycles at 95°C, and 35 sec a[illegible]. The relative expression level of target genes was [illegible]ulated by the $2^{-\Delta\Delta Ct}$ method.

Wound Healing Assay

Cells were first seeded into 6-well[illegible] cultured overnight. After scratched[illegible]a pip[illegible] tip, the cells were cultured in seru[illegible]ee DMEM. Relate distance was viewed un[illegible]light micro-scope (Olympus, Tokyo, Japan)[illegible]h. Each assay was independently re[illegible]ted for[illegible]imes.

Transwell Assay

A total of 5 ×10^4 t[illegible]ected [illegible]in serum-free DMEM were replant[illegible]e u[illegible]chamb[illegible]Corning, Corning, NY[illegible]SA)[illegible]ell plat[illegible]e-coated with Matris[illegible]atrix di[illegible](BD[illegible]sciences, San Jose, CA[illegible]A). Meanwhil[illegible]er chamber was added[illegible]EM and FBS.[illegible]h later, after wiped b[illegible]cotton[illegible] the top surface of chambers was immersed w[illegible] paraformaldehyde for 10 [illegible] stained with[illegible]crystal violet for 30 m[illegible]ree fields were randomly selected for each [illegible]ple. Finally, th[illegible]number of invading cells was [illegible]nted under a [illegible] DMI4000B microscope (Lei-[illegible]icrosystems[illegible]delberg, Germany).

Luc[illegible]orter Gene Assay

DIANA LncBASE Predicted v.2 was used to [illegible]rch for miRNAs that contained complementa-[illegible]ith SNHG16. The 3′-untranslated region [illegible]UTR of SNHG16 was cloned into pGL3 vector (Promega, Madison, WI, USA) as wild-type (WT) 3′-UTR. Site-direction mutagenesis of the miR-200a-3p binding site in SNHG16 3′-UTR was conducted through quick-change site-directed mutagenesis kit (Stratagene, Cedar Creek, TX, USA), as mutant (MUT) 3′-UTR. Subsequently, they were transfected into Wilms' tumor cells. Luciferase activity was finally determined by the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA).

Statistical Analysis

GraphPad Prism 5.0 (La Jolla, CA, USA) was used for all statistical analysis. Experimental data were expressed as mean ± standard deviation (SD). The Student's *t*-test was utilized to compare the difference between the two groups. $p<0.05$ was considered statistically significant.

Results

SNHG16 Expression Level in Wilms' Tumor Tissues

To determine the biological function of SNHG16 in the tumorigenesis of Wilms' tumor, we first detected SNHG16 expression in 50 paired

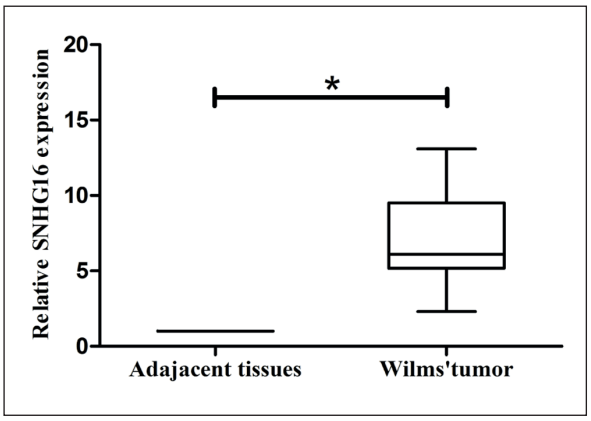

Figure 1. Expression levels of SNHG16 in Wilms' tumor tissues and cell lines. SNHG16 expression was significantly up-regulated in Wilms' tumor tissues compared with adjacent tissues. Data were presented as mean ± standard error of the mean. *$p<0.05$.

Wilms' tumor tissues and adjacent tissues by RT-qPCR. The results showed that SNHG16 was significantly up-regulated in Wilms' tumor tissues when compared with adjacent tissues (Figure 1).

Knockdown of SNHG16 Inhibited Migration and Invasion Abilities of Wilms' Tumor Cells

The cells were first collected and diges from Wilms' tumor tissues. Subsequently, t cells were transfected with s or NC, respectively. Transfection ef ncy verified by RT-qPCR (Figure 2A) ound he assay demonstrated that knock f SN cantly reduced the m ate of Wilms tumor cells (Figur). Besi e transwell assay demonstrat at SNHG16 regulation remarkably the invasion Wilms' tumor cells (F e 2

Overex sion of SNHG promoted Migra and Invasion Abilities of W s' Tu Cells

ells e first llected and digested from umor es. Subsequently, the lls wer fecte h SNHG16 lentivirus or pecifi arly, transfection efficiency w detected T-qPCR (Figure 3A). Wound heal assay illustrated that overexpression of SN cantly increased the migrated nce of Wilms' tumor cells (Figure 3B). Besides, the results of transwell assay indicated that o ression of SNHG16 promoted the invasive abili f Wilms' tumor cells (Figure 3C).

The Association Between MiR-200a-3p and SNHG16 in Wilms' Tumor T and Cells

DIANA LncBASE Predicted was used to search for miRNAs that conta omplementary base with SNHG16. As sh Figure 4A, miR-200a-3p was sele d from miR NAs that interacted with HG16. As s Figure 4B, RT-qPCR ts de nstrated the expression of mi 0a-3p significantly higher in sh-SNH ou an tha NC group. However e e n of mi 0a-3p was significa lower H G entivirus group than group (Figu To further identify th on between mi -200a-3p and SNHG1 cifera rter gene assay was conducted. As shown in e 4D, co-transfection of 6-WT and 0a-3p remarkably d sed luciferase activity. However, no signifi changes in l iferase activity were observed r co-transfec of SNHG16-MUT and miR-3p. Furthe re, correlation analysis was c ted in W s' tumor tissues. The results foun 0a-3p expression was negatively correlated with SNHG16 expression in Wilms' mor tissues (Figure 4E).

Discussion

In recent years, increasing researches have revealed that noncoding RNAs function as important regulators in Wilms' tumor. This may help to understand the molecular mechanism in the development of Wilms' tumor. So, lncRNA SOX-2OT promotes the development of Wilms' tumor by regulating miR-363/FOXP4 axis. LINC00473 functions as an oncogene in Wilms' tumor[9] by antagonizing miR-195. MiR-21 regulates PTEN, which also inhibits the proliferation and metastasis of Wilms' tumor cells[10]. MiR-613 represses cell proliferation and migration in Wilms' tumor by targeting FRS2. All these findings may provide a potential target for the treatment of Wilms' tumor[11].

Small nucleolar RNA host gene 16 (SNHG16), one of the noncoding RNAs, functions as an oncogene in multiple malignant tumors. SNHG16 promotes the progression of cervical cancer through the miR-216-5p/ZEB1 signal pathway[12]. SNHG16 enhances cell proliferation and migration in gastric cancer[13]. SNHG16 promotes the growth and migration, whereas represses the apoptosis of colorectal cancer cells through the

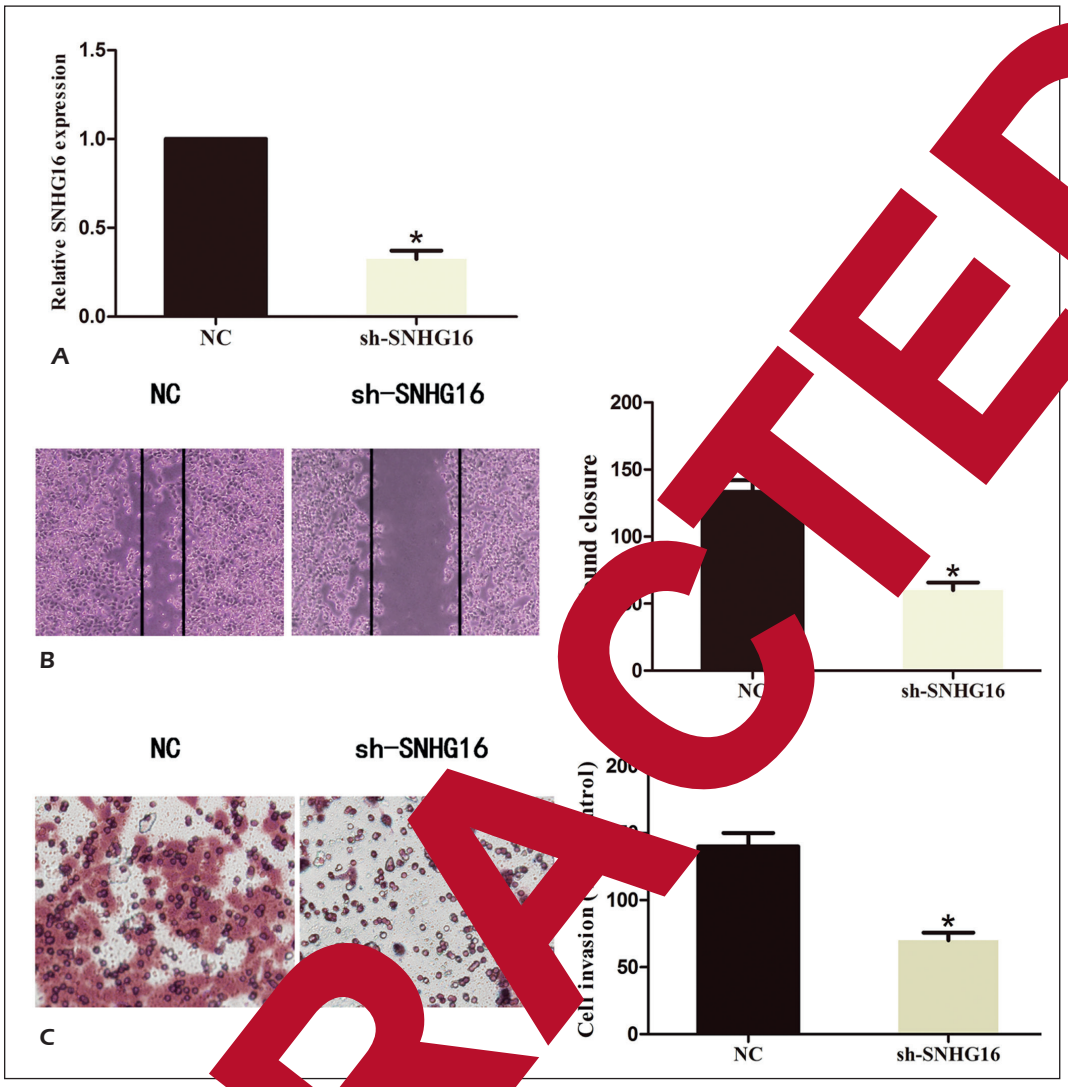

Figure 2. Knockdown o HG Wilms' tumor cell migration and invasion. **A**, SNHG16 expression in Wilms' tumor cells transfected control s NC) or SNHG16 shRNA (sh-SNHG16) was detected by RT-qPCR. **B**, Wound healing assay showed nockdown of significantly decreased migrated distance of Wilms' tumor cells (magnification: 40×). **C**, Tra y showed that th nber of invaded cells was significantly reduced via knockdown of SNHG16 (magnification: 4 $p<$

Wnt pa y[14]. SNHG16 accele tes cell viability in bl canc targeting p21. Meanwhile, it is ated poor p nosis of bladder cancer pa oweve exact role in Wilms' t or re unk so far. In the present ve th vestigated the role of SNHG Wilms or.

C results revealed that SNHG16 was highly ex lms' tumor tissues. After SN- was knocked down, the migration and sion of Wilms' tumor cells were significantly i d. After SNHG16 over-expression, the mig n and invasion of Wilms' tumor cells were significantly promoted. All the above results indicated that SNHG16 acted as an oncogene in Wilms' tumor and promoted its metastasis.

Recently, increasing studies have uncovered the mechanism that lncRNAs function as competing endogenous RNAs for microRNAs, thereby participating in tumor progression. Consistently, lncRNA TP73AS1 significantly promotes cell apoptosis and depresses cell proliferation in colorectal cancer by functioning as a competing endogenous RNA for miR-103 to modulate PTEN expression[16]. By sponging miR-27b-3p, lncRNA KCNQ1OT1 facilitates the proliferation

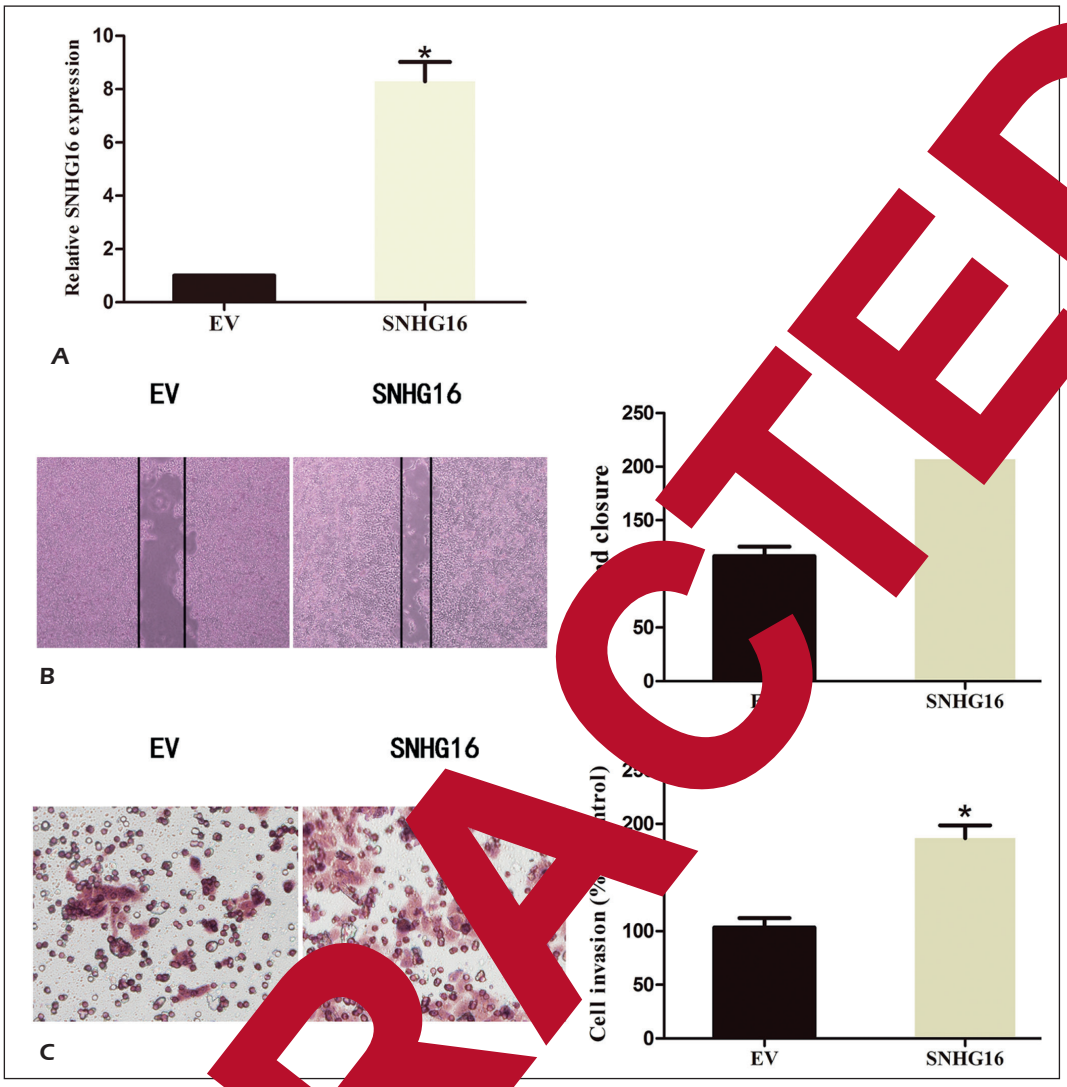

Figure 3. Overexpressio NH ted Wilms' tumor cell migration and invasion. **A**, SNHG16 expression in Wilms' tumor cells transfected empty ve) or SNHG16 lentivirus (SNHG16) was detected by RT-qPCR. GAPDH was used as an internal c **B**, Wound hea ay showed that overexpression of SNHG16 significantly increased the migrated distance of l or cells (magni on: 40×). **C**, Transwell assay showed that the number of invaded cells was significantly elev ia ression of SNHG16 (magnification: 40×). *p<0.05.

and i ion of -small cell lung cancer cells by ulat HSP90 A1[17].

To veal h NHG16 functioned in Wilms' t DIA LncBASE Predicted v.2 ed to e potential microRNAs of SN 6. As a , miR-200a-3p was screened out further experiments. MiR-200 family (in-ch a, -200b, -200c, -141, and -429) been reported to inhibit cell proliferation, astasis, and EMT in various malignant tu-i iR-200a-3p inhibits cell proliferation and pro s cell apoptosis of renal cell carcinoma through regulating SPAG9[18]. MiR-200a-3p, regulated by lncRNA HULC, inhibits the metastasis and reverses EMT of hepatocellular carcinoma[19]. Our results showed that SNHG16 knockdown significantly up-regulated miR-200a-3p expression. However, an opposite trend was observed after SNHG16 overexpression. Further experiments revealed that SNHG16 acted as a sponge for miR-200a-3p in Wilms' tumor. In addition, miR-200a-3p expression was negatively correlated with SNHG16 expression in Wilms' tumor tissues.

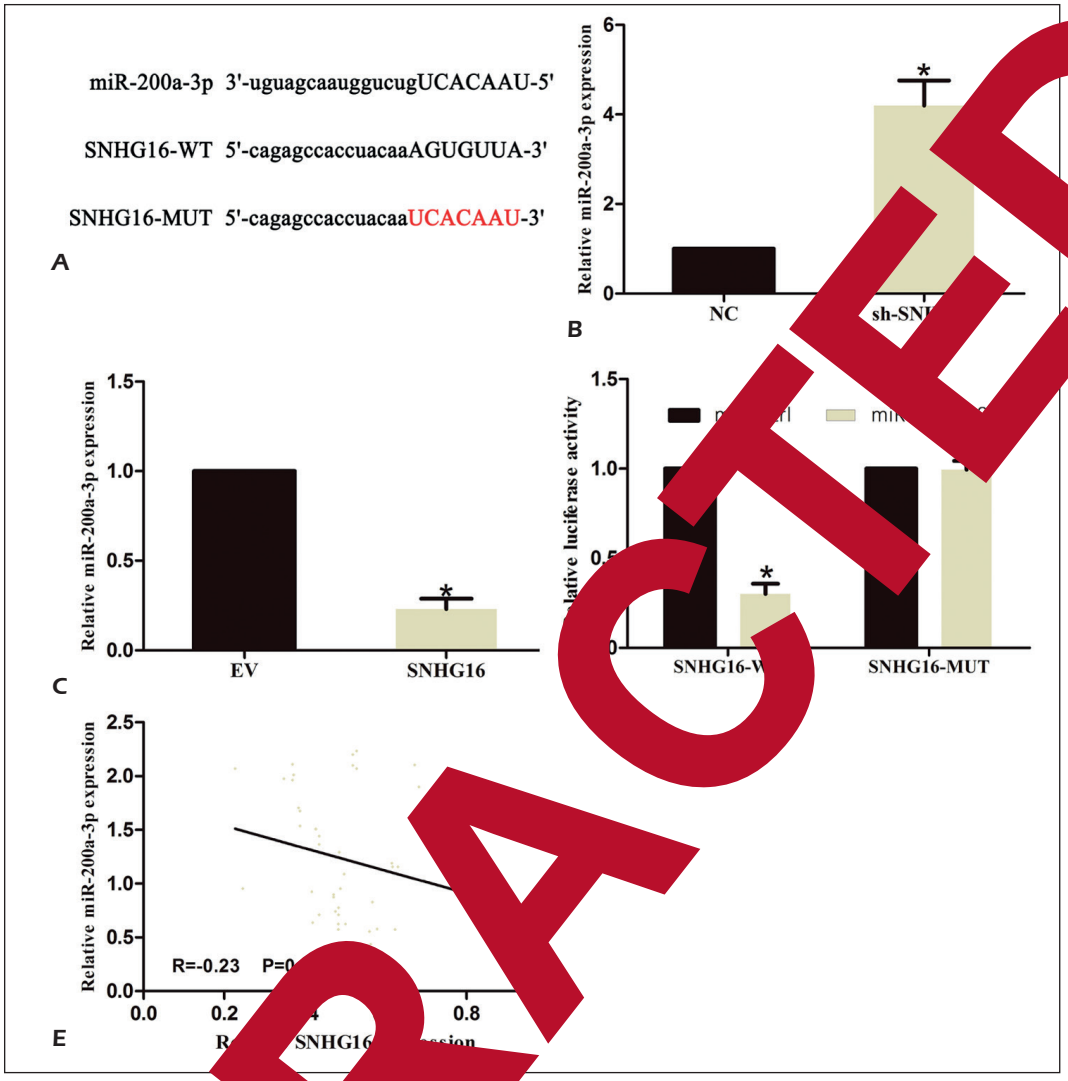

Figure 4. The associatio[n] wee[n] [a]nd miR-200a-3p in Wilms' tumor tissues and cells. **A**, The binding sites of miR-200a-3p on SNHG16. **B**, [M]200a-3p [e]xpression increased significantly in sh-SNHG16 group when compared with NC group. **C**, MiR-200a-3p expr[e]ssion decreased ob[v]iously in SNHG16 lentivirus (SNHG16) group when compared with EV group. **D**, Co-transfection of [w]t-200a-3p and SNHG[1]6 strongly decreased luciferase activity. However, no significant changes in luciferase activity [w]ere observed after co-transfection of miR-200a-3p and SNHG16-MUT. E, MiR-200a-3p expression was negatively correlated with S[N]HG16 expression in Wilms' tumor tissues. The results represented the average of three independent experiments. Data were presented as mean ± standard error of the mean. *$p<0.05$.

Conclusions

We found that SNHG16 was remarkably up-regulated in Wilms' tumor, which could enhance its metastasis by sponging miR-200a-3p. All the findings suggested that SNHG16 might contribute to therapy for Wilms' tumor as a prospective

Conflict of Interests

The authors declared that they have no conflict of interests.

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