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LncRNA SNHG16 functions as an oncogene by sponging miR-200a-3p in pancreatic cancer

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Abstract. – OBJECTIVE: Recently, the role of long noncoding RNAs (lncRNAs) is vital in tumor progression. Our study aims to identify the role of SNHG16 in the metastasis of pancreatic carcinoma.

PATIENTS AND METHODS: Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) was used to measure SNHG16 expression in 56 pancreatic carcinoma patients' tissues. Function assays, including wound healing assay, and transwell assay, were conducted to detect the effect of SNHG16 on the metastasis of pancreatic carcinoma. Besides, the luciferase assay was performed to explore the underlying mechanism.

RESULTS: The expression level of SNHG16 was upregulated in pancreatic carcinoma samples compared with adjacent tissues. Moreover, cell migration and cell invasion were repressed *via* the knockdown of SNHG16, while cell migration and cell invasion were promoted *via* the overexpression of SNHG16. Moreover, the expression of miR-200a-3p was upregulated *via* knockdown of SNHG16 while the expression of miR-200a-3p wa downregulated *via* the upregulation of SNHG in vitro. Furthermore, it was discovered that S HG16 acted as a competing endogenous RNA *vi* sponging miR-200a-3p in pancreatic ca oma.

CONCLUSIONS: Our study sugge SNHG16 acts as an oncogene in pa eati arcinoma and promotes cell metasta *vi* ing miR-200a-3p, which migh e a the peutic strategy in pancrea carcino

Key Words:

Long noncoding R SNHG1 ncreatic carcinoma, MiR-200a-3p

Intro ion

Panc cane is the most lethal malignancy glo which may be the 2nd most common cause o ncer-related deaths by 2030[1]. It was estimated that pancreatic cancer was the 11th most frequent cancer worldwide, accounting for 458,918 new cases and 4.5% of all cancer-related deaths in 2018. In patients undergoing successful surgical resection, the 5-year survival rate is approximately 27%[2]. However, the median survival time of patients with metastasis is six to eleven months. Therefore, it's urgent to ha deep understanding of the underlying m of the progression of pancreatic ca and fi ut a potential therapeutic strategy.

Long non-coding R s (lnc As) a diverse group of trans ts wh ch a ger than 200 nucleotides in th thout the potential of coding prote . L As a differentially regulated in ariety gical behaviors, including pression cancers. In fact, the dow egulat lncRNA ZNF667-AS1 repres he progres f cervical cancer, which is related to the prognosis of cervical cancer. ncRNA T 73AS1 dramatically promotes cell toptosis d depresses cell proliferation in colore cer by functioning as a competing dogenous RNA for miR-103 to modulate the n of PTEN[4]. By sponging miR-27b-3p, cRNA KCNQ1OT1 facilitates cell proliferation and cell invasion in the progression of non-small cell lung cancer by upregulating HSP90AA1[5]. Recently, the role of lncRNAs in pancreatic carcinoma causes more attention of many researchers. LncRNA MEG8 enhances epigenetic induction of the epithelial-mesenchymal transition in pancreatic carcinoma cells[6]. LncRNA SNHG16, as a newly discovered lncRNA in malignant tumors, is reported to be a vital regulator in the tumor development. However, the clinical role and biological mechanisms of SNHG16 in the metastasis of pancreatic carcinoma remain unexplored.

We found that SNHG16 expression level was remarkably higher in pancreatic carcinoma tissues and SNHG16 promoted migration and invasion of pancreatic carcinoma cell *in vitro*. We also explored the underlying mechanism of how SNHG16 functioned in pancreatic carcinoma.

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Patients and Methods

Tissue Specimens

56 pancreatic carcinoma patients received surgery at Huaihe Hospital, Henan University, and their tissue samples were used for our further investigations, which were stored immediately at –80°C. The Ethics Committee of Huaihe Hospital, Henan University approved this study protocol, and all participants provided written informed consents.

Cell Lines

Human pancreatic carcinoma cell lines (BXPC3, CFPAC-1, Panc-1, and Capan-2) were purchased from the Institute of Biochemistry and Cell Biology (Shanghai, China). One immortalized pancreatic ductal epithelial cell line (H6C7) was also obtained from the Ontario Cancer Institute (Toronto University, Toronto, Canada). Those cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA) and penicillin. Besides, the incubator for cell culture contained 5% CO_2 at 37°C.

Cell Transfection

Specific short-hairpin RNA (shRNA; Biose Inc., San Diego, CA, USA) and lentivirus agains SNHG16 were synthesized. Negative trol shRNA and scramble vector were al sized. SNHG16 shRNA (sh-SNHG16) egative control (control) were used for tra ction cells. SNHG16 lentivirus (SN amble vector (NC) were used for t nsfe apan-2 cells. Real-Time quantita Polyme ain Reaction (RT-qPCR) wa d to detect th s-fection efficiency.

RNA Extraction and RT-qP

The total RNA s separated usi RIzol reagent (Invitroge arlsbad, CA, USA). Then, the total RNA was ersely scribed to complementary deoxyri acids DNAs) through reverse Trans it (T Ra Biotechnology Co., Ltd., Da The primers used for RT ere as . SNHG16 forward 5′-GT GGAAGTC CTTGCC-3′, reverse 5′-A CA TATCACACAGCAC-3′; gl deh te dehydrogenase (GAPDH) forward 5′-GGGAGCCAAAAGGGTCAT verse 5′-GAGTCCTTCCACGATACCAA-3′. rmal cycle was 30 sec at 95°C, 5 sec for 40 cycl at 95°C, and 35 sec at 60°C.

Wound Healing Assay

1.0×10^4 cells were seed a 6 te. Three parallel lines were e on the of each well. After growin bout 90% c u-ence, cells were scratched ipette nd cultured in a medium Cells hed under a light micros e after 0 a Each assay was indepen y re ted in tr cate.

Transwell As

5×10^4 treated tra rmed to top chamber of 8 µm insert (Corning, Corni NY, USA) a with 200 µL serum-free These inserts were previously coated Matrigel (BD Biosciences, Frankli Lakes, The bottom chamber co d DMEM c ing FBS. These cells w cultured for 48 h. Then, the top surface of mbers was wiped by a cotton swab and im- sed by precoo methanol for 20 min. Crystolet was us or staining the inserts.

Luc porter Gene Assay

DIANA LncBASE Predicted v.2 was used to find miRNAs that contained complementary base 16. 3′-untranslated region (3′-UTR) of was cloned into the pGL3 vector (Promega, Madison, WI, USA) as wild-type (WT) 3′-UTR. te-direction mutagenesis of the miR-200a-3p nding site in SNHG16 3′-UTR as mutant (MUT) UTR was conducted through quick-change te-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). Then, they were used for transfection of pancreatic cancer cells. The luciferase assay was conducted on the Dual Luciferase reporter assay system (Promega, Madison, WI, USA).

Statistical Analysis

All statistical analyses were performed by Statistical Product and Service Solutions (SPSS) 19.0 (SPSS, Chicago, IL, USA). Independent-sample *t*-test was selected when appropriate. Moreover, $p<0.05$ was considered as a statistically significant difference.

Results

SNHG16 Expression Level in Pancreatic Carcinoma Tissues

To determine the biological function of SNHG16 in the tumorigenesis of pancreatic carcinoma, we detected SNHG16 expression levels in 56 paired pancreatic carcinoma specimens by RT-qPCR. Re-

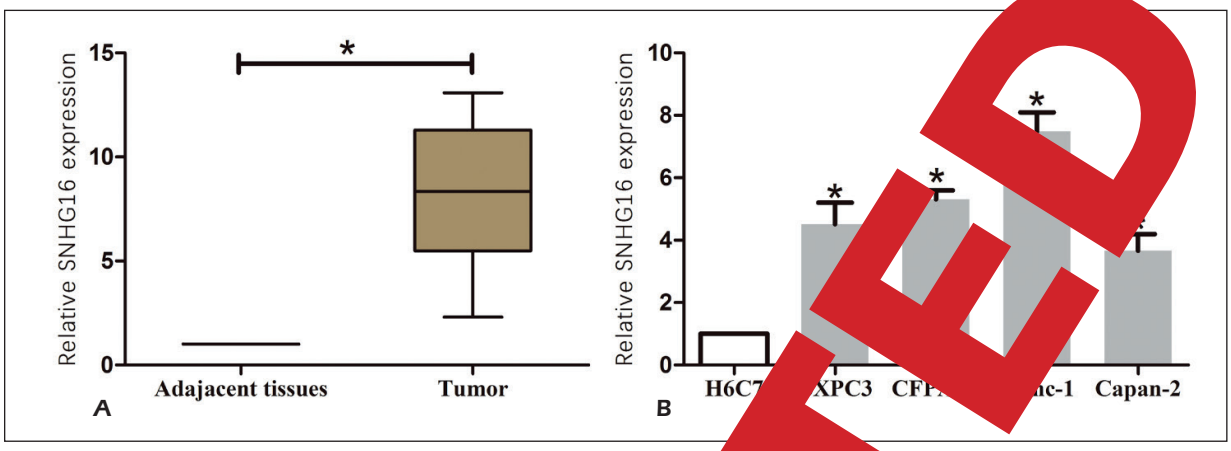

Figure 1. Expression levels of SNHG16 in pancreatic carcinoma tissue[s] and cell [l]ines. SNHG16 expression was significantly increased in the pancreatic carcinoma tissues compared with adjacent tissues. **B**, [E]xpression levels of SNHG16 relative to GAPDH were determined in the human pancreatic carcinoma cell[l]ines and H6C7 by RT-qPCR. Data are presented as the mean ± standard error of the mean. *p<0.05.

sults showed that SNHG16 was upregulated in tumor tissue samples compared with adjacent tissues (Figure 1A). Then, SNHG16 expression was detected in pancreatic carcinoma cells by RT-qPCR. SNHG16 expression level of pancreatic carcinoma cells was higher than H6C7 (Figure 1B).

Knockdown of SNHG16 Inhibited Migrated and Invaded Ability in Pancreatic Carcinoma Cells

According to the expression [l]evel in cells, we chose Panc-1 cell line for [s]iRNA transfection. The transfection efficiency was detected by RT-qPCR (Figure 2A). The wound healing assay demonstrated that knockdown of SNHG16 reduced the migrated distance of Panc-1 cells (Figure 2B). Besides, the results of transwell assay demonstrated that the knockdown of SNHG16 reduced the invasive ability of Panc-1 cells (Figure 2C).

Overexpression of SNHG16 Inhibited Migrated Ability in Pancreatic Carcinoma Cells

According to the expression level in cells, we chose Capan-2 cells for lentivirus transfection. The transfection efficiency was detected by RT-qPCR (Figure 3A). The results of wound healing assay demonstrated that overexpression of SNHG16 increased the migrated distance of Capan-2 cells (Figure 3B). Besides, results of transwell assay demonstrated that overexpression of SNHG16 increased the invaded ability of Capan-2 cells (Figure 3C).

Association Between MiR-200a-3p and SNHG16 in Pancreatic Carcinoma Tissues and Cells

DIANA LncBASE Predicted v.2 was used to predict the miRNAs that contained complementary with SNHG16. MiR-200a-3p was selected from these miRNAs, which were interacted with SNHG16 (Figure 4A). The RT-qPCR assay showed that the expression of miR-200a-3p was higher in sh-SNHG16 group than that in control group, and the expression of miR-200a-3p was lower in SNHG16 lentivirus group than that in NC group and (Figure 4B and 4C). Furthermore, the luciferase assay revealed that co-transfection of SNHG16-WT and miR-200a-3p largely decreased the luciferase activity, while co-transfection of SNHG16-MUT and miR-200a-3p had no effect on the luciferase activity either (Figure 4D). In addition, the correlation analysis demonstrated that miR-200a-3p expression level negatively correlated to SNHG16 expression in pancreatic carcinoma tissues (Figure 4E).

Discussion

In recent years, increasing researches have revealed that lncRNAs function as important regulators of pancreatic cancer, which may help to understand the molecular processes in the development of pancreatic cancer. Consistently, regulated by ALKBH5, lncRNA KCNK15-AS1 inhibits cell migration and cell motility in pancreatic cancer[7]. Downregulation of lncRNA HOST2 represses cell proliferation and promotes cell apoptosis in pan-

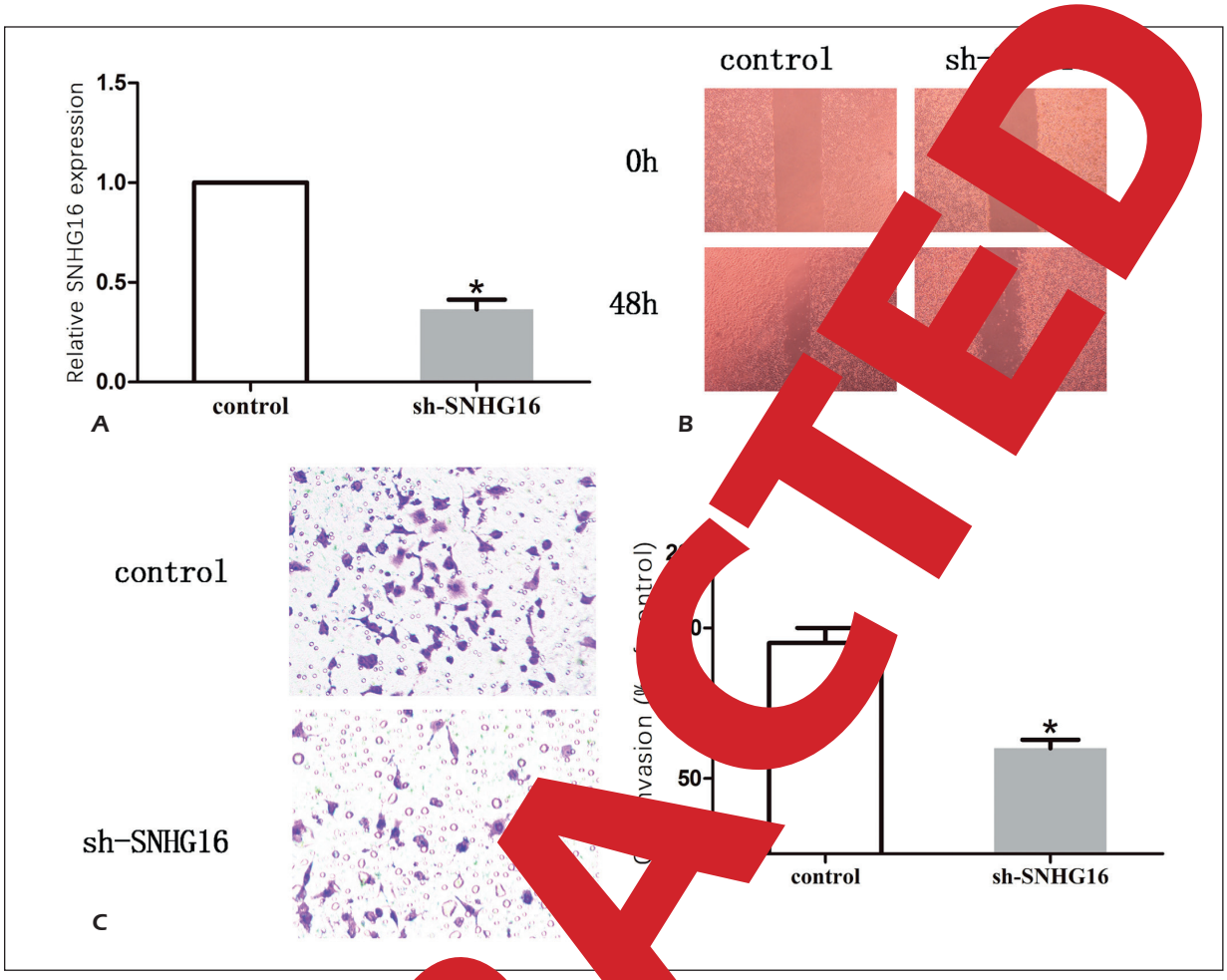

Figure 2. Knockdown of SNHG16 in[hibits] [pancr]eatic carcin[oma] cell migration and invasion. **A**, SNHG16 expression in Panc-1 cells transfected with control sh[RNA] (con[trol]) or SNHG16 shRNA (sh-SNHG16) was detected by RT-qPCR. **B**, Wound healing assay showed that knockdo[wn] SNHG[16] decreased cell migrated distance of Panc-1 cells (magnification: 40×). **C**, Transwell assay show[ed that] [the] number of in[vad]ed cells was significantly decreased via knockdown of SNHG16 in Panc-1 cells (magnification: 40×). *p

creatic cancer, which m[ay] offer a potent[ial] ther-apeutic target for pa[nc]ancer[8]. LncRNA H19 promotes cell [pro]liferation and cell migration in pancreatic cancer, which [is] regulated by miR-194[9]. By tar[get]ing miR-221/SO[CS3], lncRNA GAS5 suppress[es] cell proliferation, cell metastasis, and gemcitabi[ne] resista[nce] in pancreatic cancer[10].

Small nu[cleolar] RN[A] host ge[ne] 16 (SNHG16) is one of the none[ncoding] As wh[ich] has been reported to function as an o[nco]gene to promote tumor progressio[n] in multiple can[cers]. SNHG16 enhances cell viabil[ity] and migration, reduces cell apoptosis in c[olo]rectal [cancer] by regulating the Wnt pathway a[nd] me[diates] overexpression of SNHG16 co[ntributes] to cell proliferation and cell migration in gastri[c cancer][12]. By silencing p21 epigenetically, SNHG16 ac[celerates] tumor proliferation in bladder cancer, which is associated with poor prognosis of the patients[13]. SNHG16 promotes the tumorigenesis of cervical cancer through miR-216-5p/ZEB1 signal pathway[14].

We revealed that SNHG16 was highly-expressed in both pancreatic carcinoma samples and cells. After SNHG16 was knocked down, pancreatic carcinoma metastasis was found to be inhibited. Meanwhile, after SNHG16 was overexpressed, pancreatic carcinoma metastasis was promoted. Above results indicated that SNHG16 promoted metastasis of pancreatic carcinoma and might act as an oncogene.

DIANA LncBASE Predicted v.2 was used to predict the potential target miRNAs containing SNHG16 reaction sites, among which miR-200a-3p was used for our following investigations. Increasing evidence has suggested that the miR-200 family (miR-200a, -200b, -200c, -141, and -429)

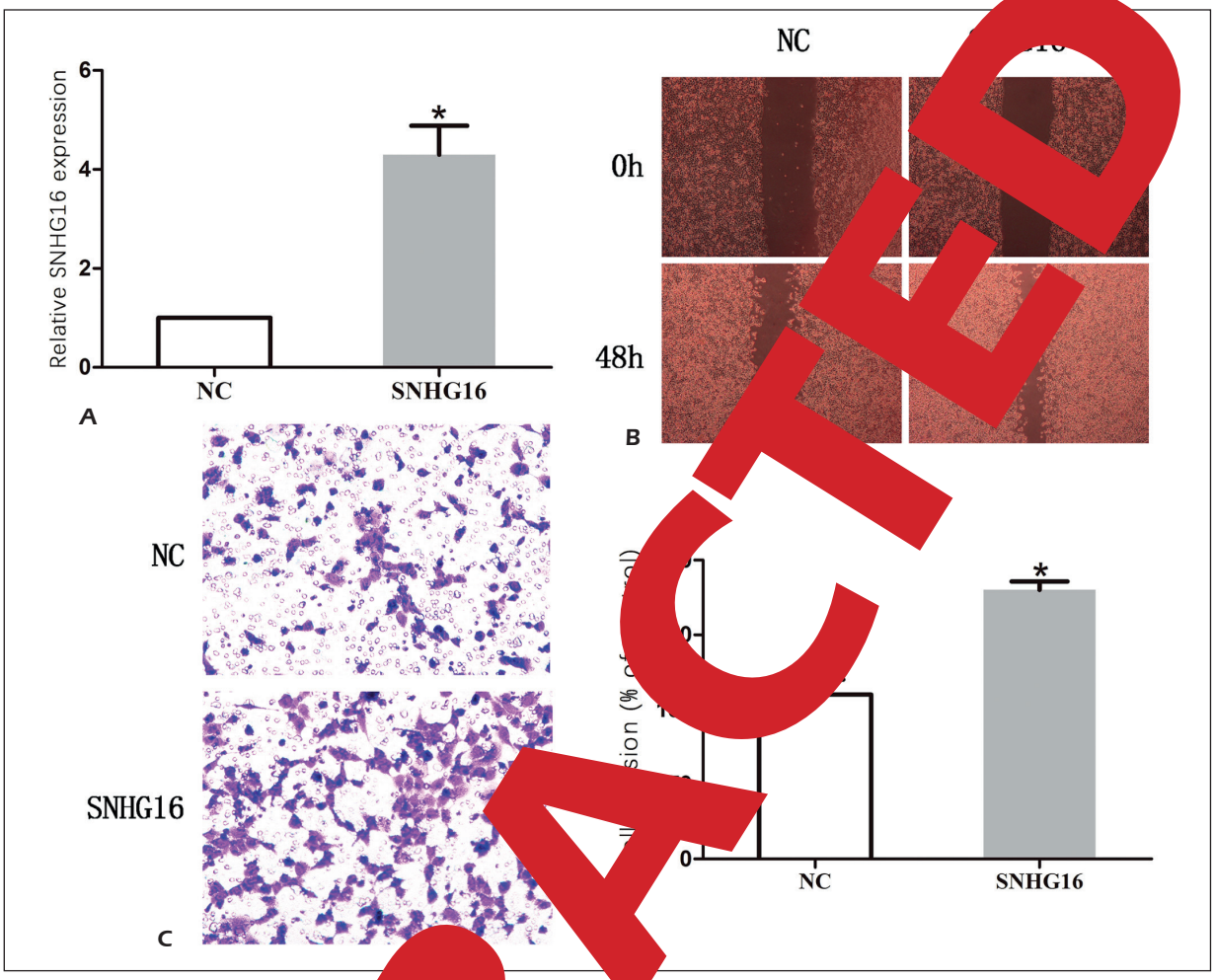

Figure 3. Overexpression of SNHG[illegible]romot[illegible]carcinoma cell migration and invasion. **A**, SNHG14 expression in Capan-2 cells transfected with s[illegible]e vect[illegible]G14 lentivirus (SNHG14) was detected by RT-qPCR. GAPDH was used as an internal control. **B**, [illegible] assay s[illegible]ved that overexpression of SNHG16 significantly increased cell migrated distance of Capan-2 c[illegible]s (ma[illegible]. 40×). **C**, Transwell assay showed that number of invaded cells was significantly increased via overexpre[illegible] of SNH[illegible]an-2 cells (magnification: 40×). *p<0.05.

modulates cell pro[illegible]atio[illegible]asis, epithelial-mesenchymal transition ([illegible] several cancers. LncRN[illegible]HULC promote[illegible]umorigenesis, metastas[illegible]nd EMT of hepatocellular carcinoma cell [illegible]ne m[illegible]00a-3p/ZEB1 signaling pathway[15]. [illegible]00[illegible] inhib[illegible]ell proliferation and induces [illegible]osis i[illegible]nal cell carcinoma *via* targeting [illegible]ne reports[17,18] have identi[illegible]miR-20[illegible]uppresses pancreatic carc[illegible]gression. In the present study, the mi[illegible]00a-3[illegible] could be upregulated h[illegible]SNHG16, and miR-200a-3p [illegible] could be downregulated by overexpressi[illegible]HG16. Moreover, miR-200a-3p expression [illegible]creatic carcinoma tissues was negatively related to SNHG16. Further works revealed that SNHG16 acted as a sponge for miR-200a-3p in pancreatic carcinoma.

Conclusions

We showed that SNHG16 was remarkably upregulated and could enhance metastasis of pancreatic carcinoma by sponging miR-200a-3p. The results suggest that SNHG16 may contribute to therapy for pancreatic carcinoma as a prospective therapeutic target.

Conflict of Interests

The Authors declare that they have no conflict of interests.

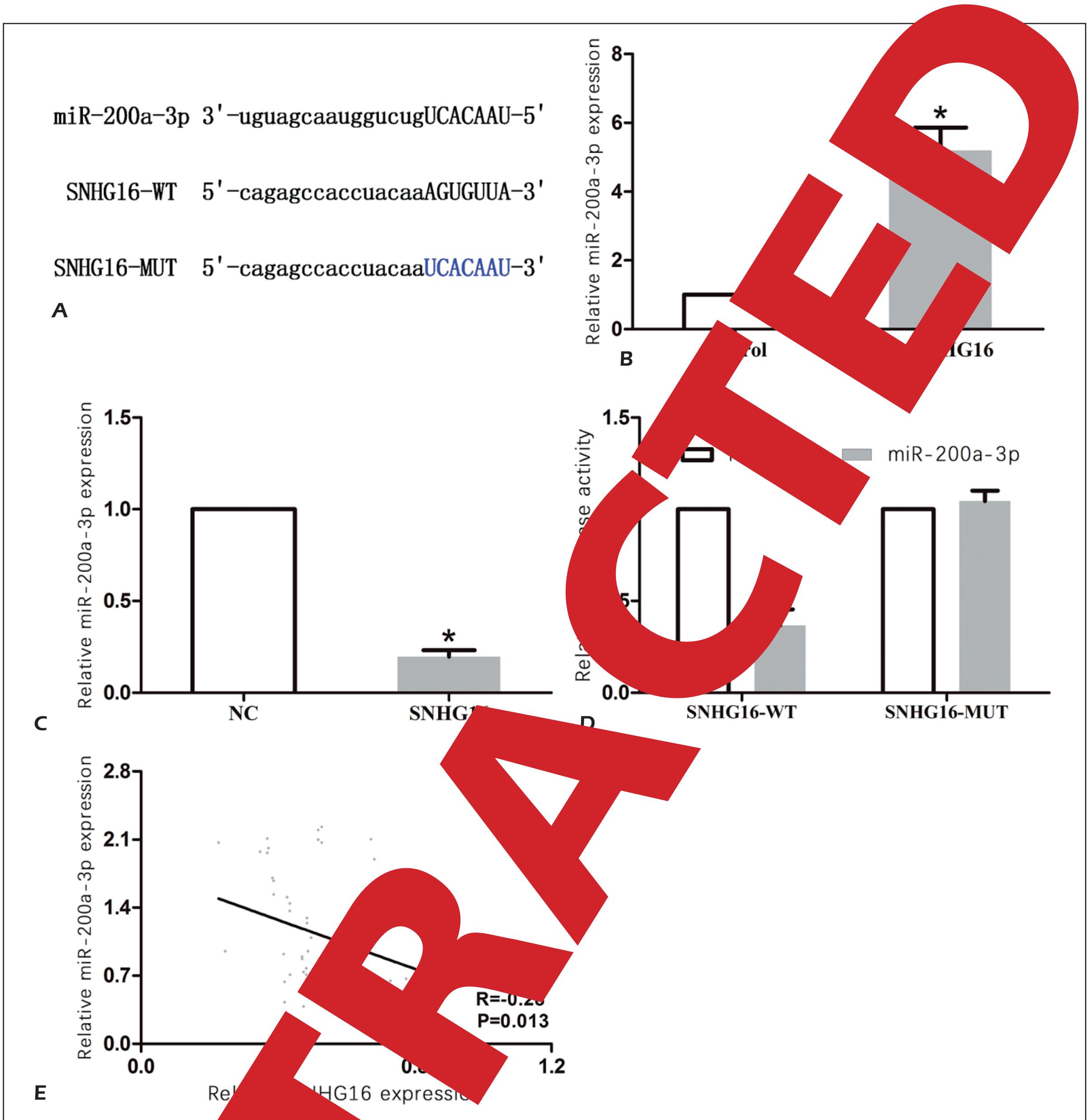

Figure 4. The association between SNHG16 and miR-200a-3p in pancreatic carcinoma tissues and cells. **A**, Binding sites of miR-200a-3p on SNHG16. **B**, MiR-200a-3p expression was increased in sh-SNHG16 group compared with control group. **C**, MiR-200a-3p expression was decreased in SNHG16 group compared with NC group. **D**, Co-transfection of miR-200a-3p and SNHG16-WT strongly decreased the luciferase activity, while co-transfection of miR-200a-3p and SNHG16-MUT did not change the luciferase activity. **E**, Negative correlation between the expression level of miR-200a-3p and SNHG16 in pancreatic carcinoma tissues. The results represent the average of three independent experiments. Data are presented as the mean ± standard error of the mean. *p<0.05.

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