

LncRNA CASC15 promotes migration and invasion in prostate cancer *via* targeting miR-200a-3p

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Abstract. – **OBJECTIVE:** Prostate cancer (PC) is one of the most ordinary malignant cancers. Recent researches have proved that long noncoding RNAs (lncRNAs) act as an important role in cancers. Our study aims to explore the function of lncRNA CASC15 in the tumor metastasis of PC.

PATIENTS AND METHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was utilized to detect CASC15 expression in 50 PC patients. Besides, the wound healing assay and transwell assay were performed to identify the biological behavior changes of PC cells after CASC15 was silenced in PC cells. In addition, the potential mechanism was also explored using the luciferase assay.

RESULTS: CASC15 expression level was significantly higher in PC tissues and cell lines. Results of wound healing assay and transwell assay revealed that cell migrated ability and invaded ability were suppressed via silence of CASC15 in PC cells. Furthermore, the expression of miR-200a-3p was upregulated via silence of CASC15 in PC cells. Luciferase assay showed that miR-200a-3p was a direct target of CASC15 in PC. In addition, miR-200a-3p expression was negatively correlated with CASC15 expression in PC tissues. Rescue experiments also revealed that the inhibition of migration and invasion by silence of CASC15 could be reversed through knockdown of miR-200a-3p.

CONCLUSIONS: CASC15, a novel lncRNA, could enhance cell migration and invasion of PC cells by sponging miR-200a-3p, which might be applied as a novel therapeutic target for PC treatment.

Key Words:

Long noncoding RNA, CASC15, PC, MiR-200a-3p.

Introduction

Prostate cancer (PC) is the most common male cancer which ranks the third highest cause of

cancer-related deaths in the developed countries¹. The rate of newly diagnosed PC patients has increased over the past decades due to an aging population, increased awareness, and the use of prostate-specific antigen (PSA) measurement for screening and diagnosis. There are approximately 233,000 new cases diagnosed with PC annually, accounting for 27% of all cancer cases². Due to the lack of specific and sensitive methods for early PC screening, most patients were diagnosed at advanced stages. In the past decades, numerous studies have been carried out to explore the molecular and biological mechanisms of the progression of PC. However, few advances have been made to improve the poor prognosis of advanced PC patients. Therefore, it is urgent to find out the underlying mechanism and figure out a new treatment strategy.

Long non-coding RNAs (lncRNAs) are a class of transcripts longer than 200 nucleotides without the ability of coding proteins. However, recent studies have revealed that lncRNAs are associated with a variety of biological behaviors, including the complicated networks of gene-regulation in tumorigenesis. For example, lncRNA HOTAIR facilitates cell invasion and metastasis in oral squamous cell carcinoma by indirectly recruiting EZH2 and inhibiting E-cadherin³. LncRNA CASC2 represses cell metastasis and epithelial-mesenchymal transition of lung cancer by downregulation of SOX4⁴. LncRNA CAMTA1 contributes to cell proliferation and cell mobility in breast cancer by targeting miR-20b⁵. Knockdown of lncRNA DGCR8 suppresses cell proliferation, cell migration, and cell invasion in ovarian cancer⁶.

Recently, lncRNA CASC15, a novel lncRNA, has caught much attention for its fundamental role in tumorigenesis. However, few studies have

uncovered the function of CASC15 in PC. Our study aims to identify whether CASC15 participates in the metastasis of PC and uncover the potential mechanism.

Patients and Methods

Tissue Samples

Totally, 50 PC patients who underwent surgical resection at The First Affiliated Hospital of Nanchang University were enrolled for tumor tissues. Written informed consents were gathered from all the patients. No radiotherapy or chemotherapy for any patients before surgery. Tissue samples were immediately snap-frozen in liquid nitrogen and stored immediately at -80°C . This study was approved by the Ethics Committee of The First Affiliated Hospital of Nanchang University. Signed written informed consents were obtained from all participants before the study.

Cell Culture

The PC cell lines (LNCaP, DU145 and 22Rv1) and normal human prostate epithelial cells (P69) were bought from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) in a humidified incubator at 37°C with 5% CO_2 .

Cell Transfection

Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was utilized to efficiently transfect PC cells with negative control of siRNA oligonucleotides against CASC15 (sh-CASC15) respectively, which were purchased by GenePharma (Shanghai, China). Cells were collected for following experiments after incubation for 24 h. MiR-200a-3p inhibitor was also purchased from GenePharma (Shanghai, China).

RNA Extraction and Real-time Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR)

According to the manufacturer's instructions, total RNAs were extracted and cells were separated by using RNeasy spin column (Invitrogen, Carlsbad, CA, USA) and using a Reverse Transcription Kit (TaKaRa, China), RNA was reverse-transcribed to cDNA for RT-qPCR. Primers was as follows: CASC15 primers forward: 5'-CACAC-

GCATGGAAAACCCAG-3', reverse: 5'-GAG-GACCTGAGCTGTAAGCC-3'. GAPDH primers forward: 5'-CACCCAC-3' and reverse: 5'-CC-3' and reverse: 5'-CCCTGTT-3'-TAG-3'. Following are the amplification conditions: 30 sec at 95°C , 5 sec for 40 cycles, 5 sec at 60°C .

Wound Healing Assay

1.0×10^4 cells were seeded into 6-well plate with cells in each well. Five parallel lines were made on the back of each well. After growing to about a confluence of 90%, cells were scratched with a sterile tip and cultured in a medium. Wound closure was viewed at specific time points. Each assay was independently repeated for three times.

Tube Formation Assay

24 h after transfection, 4×10^5 cells in 100 μl serum-free DMEM were transformed to top of 96-well culture inserts (Corning, Corning, USA) coated with or without $50 \mu\text{g}$ Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). 20% FBS-DMEM was added to the lower chamber of the culture inserts. 24 h later, these culture inserts were treated by methanol for 30 min and stained by hematoxylin for 20 min. An inverted microscope ($\times 40$) was utilized for counting invaded cells in three random fields.

Dual-Luciferase Reporter Assay

For the luciferase assay, the 3'-Untranslated Region (3'-UTR) of CASC15 was cloned into the pGL3 vector (Promega, Madison, WI, USA), which was identified as wild-type (WT) 3'-UTR. Quick-change site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) was used for site-directed mutagenesis of the miR-200a-3p binding site in CASC15 3'-UTR, which was named as mutant (MUT) 3'-UTR. Cells were transfected with WT-3'-UTR or MUT-3'-UTR and miR-control or miR-200a-3p for 48 h. Then the luciferase assay was conducted on the dual luciferase reporter assay system (Promega, Madison, WI, USA).

Statistical Analysis

All statistical analyses were carried out using GraphPad Prism 5.0 (La Jolla, CA, USA). The differences between two groups were compared by student *t*-test. The statistically significance was defined as $p < 0.05$.

Results

CASC15 Expression Level in PC Tissues and Cells

To determine the biological function of CASC15 in the development of PC, CASC15 expression was monitored by RT-qPCR in 50 PC patients' tissues. CASC15 was remarkably higher expressed in PC tissue samples compared with adjacent tissues (Figure 1A). CASC15 expression was also detected via RT-qPCR in three PC cell lines and CASC15 expression level was the highest in DU145 cells (Figure 1B). Therefore, DU145 cell line was chosen for the silence of CASC15 and transfection efficiency was detected by RT-qPCR (Figure 1C).

Cell Migration and Invasion Were Repressed Via Silence of CASC15 in PC Cells

To further investigate whether CASC15 is correlated to the metastasis of PC, we researched the function of CASC15 *in vitro*. Wound healing

assay revealed that the wound significantly reduced via silence of CASC15 in DU145 cells (Figure 2A). Besides, results of the cell assay showed that number of migrated cells was reduced after silence of CASC15 in DU145 cells (Figure 2B). Furthermore, the invasion assay also showed that number of invaded cells was reduced after silence of CASC15 in DU145 cells (Figure 2C).

The Interaction between miR-200a-3p and CASC15 in PC

DIANA-microTcPRED v.2 was used to find the miRNAs that contained complementary base pairs with CASC15. miR-200a-3p, as a tumor suppressor, was chosen from these miRNAs that interacted with CASC15 (Figure 3A). RT-qPCR analysis showed that the expression of miR-200a-3p was higher in sh-CASC15 group than that in negative control (NC) group (Figure 3B). Furthermore, the luciferase assay revealed that co-transfection of CASC15-WT and miR-200a-3p largely decreased luciferase activity, while co-trans-

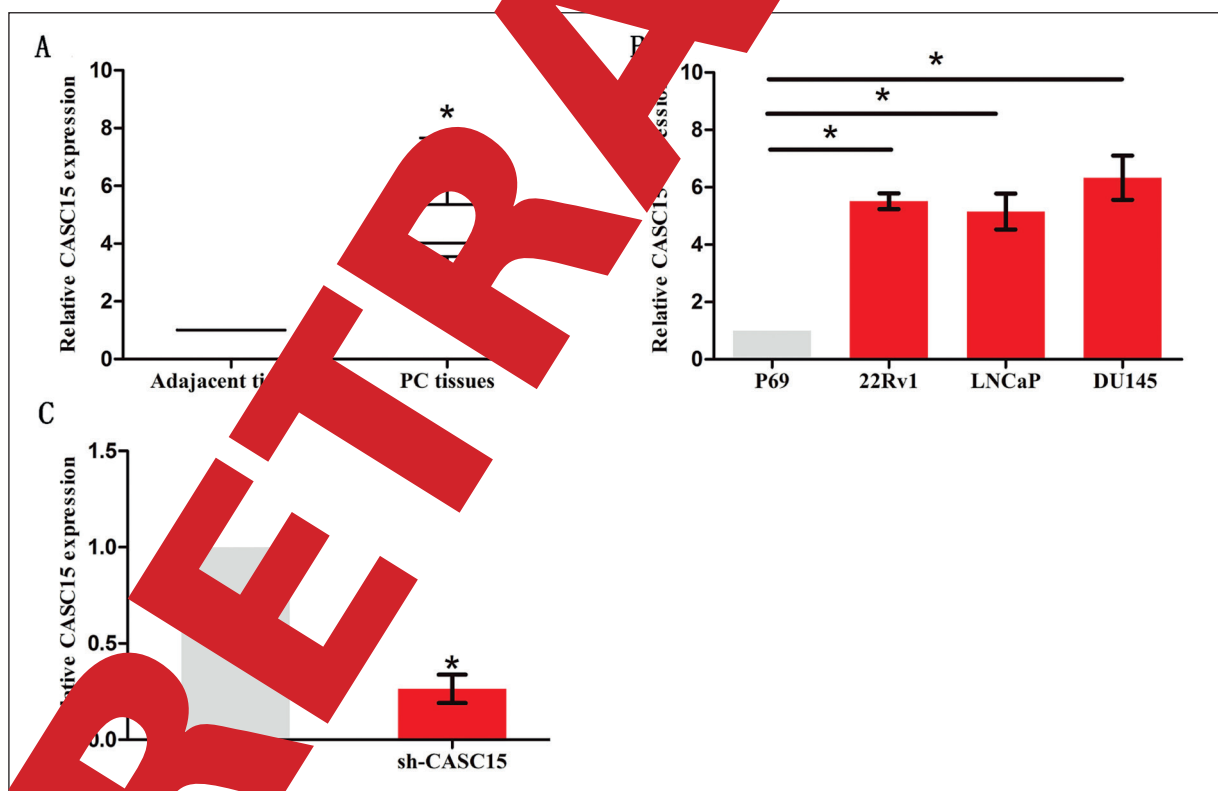


Figure 1. Expression levels of CASC15 in PC tissues and cell lines. **A**, CASC15 expression was significantly increased in the PC tissues compared with adjacent tissues. **B**, Expression levels of CASC15 were determined in the human PC cell lines and P69 by RT-qPCR. **C**, CASC15 expression in PC cells transfected with sh-CASC15 or negative control (NC) was detected by RT-qPCR. GAPDH was used as an internal control. Data are presented as the mean \pm standard error of the mean. * $p < 0.05$.

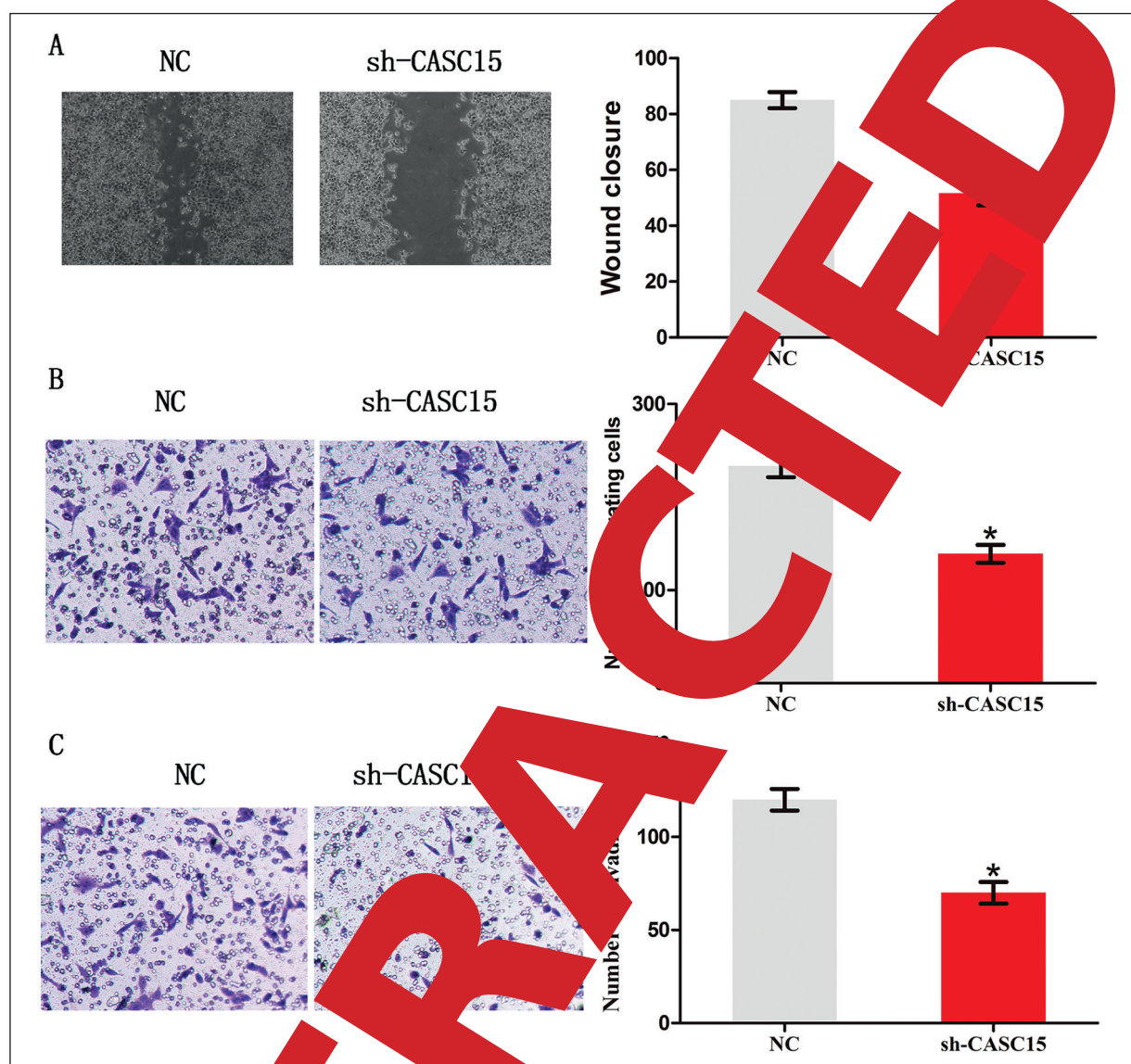


Figure 2. Functional assays showed that silenced CASC15 inhibited PC cell migration and invasion. **A**, Wound healing assay showed that wound closure was significantly decreased via silence of CASC15 in PC cells (magnification: 40 \times). **B**, Transwell assay showed that number of migrating cells was significantly decreased via knockdown of CASC15 in PC cells (magnification: 40 \times). **C**, Transwell assay showed that number of invaded cells was significantly decreased via knockdown of CASC15 in PC cells (magnification: 40 \times). * p <0.05, as compared with the control cells.

fection of CASC15 and miR-200a-3p had no effect on the cell viability either (Figure 3C). We further found a negative correlation between miR-200a-3p and CASC15 expression level in PC (Figure 3D).

The Association Between CASC15 and MiR-200a-3p in PC Migration and Invasion

To further verify the association between CASC15 and miR-200a-3p in the metastasis

of PC, we conducted rescue experiments in PC cells. Wound healing assay revealed that knockdown of miR-200a-3p could reverse the inhibition of cell migration by silence of CASC15 in DU145 cells (Figure 4A). Besides, results of transwell assay showed that knockdown of miR-200a-3p could reverse the inhibition of cell migration and invasion by silence of CASC15 in DU145 cells (Figure 4B and 4C).

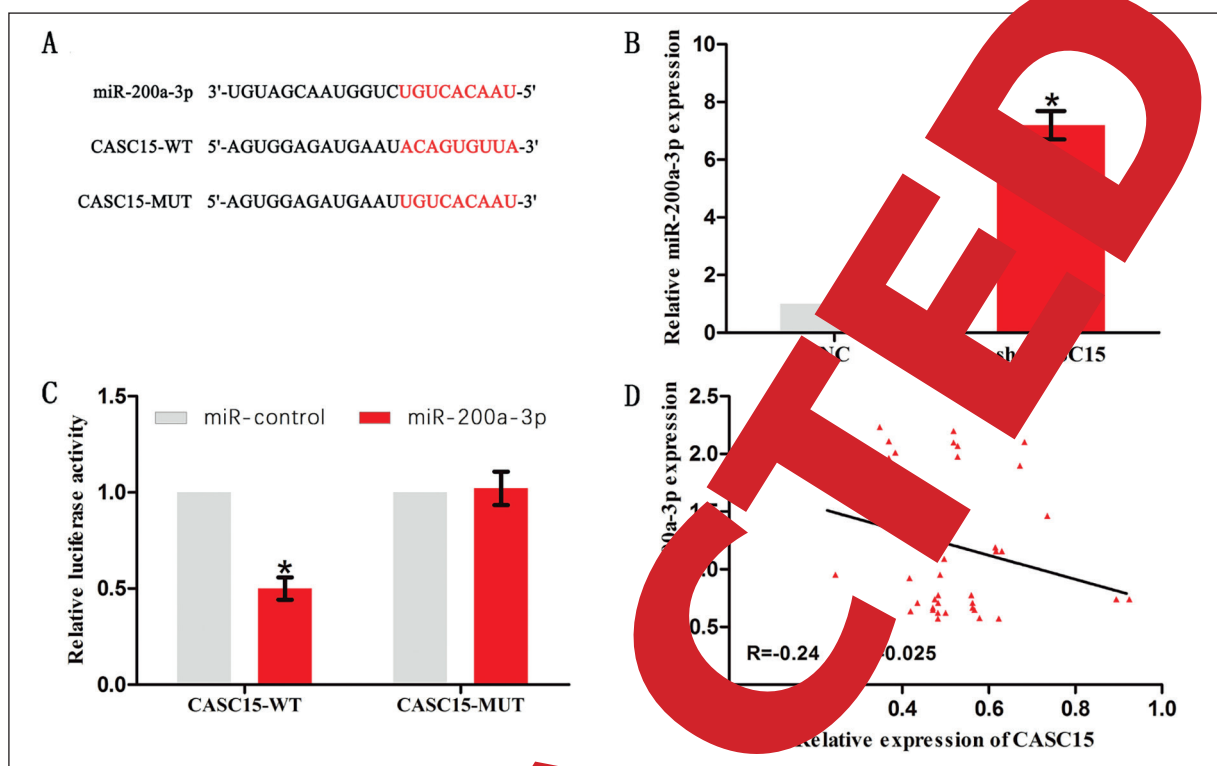


Figure 3. The interaction between miR-200a-3p and CASC15. **A**, The binding sites of miR-200a-3p on CASC15. **B**, The miR-200a-3p expression was increased in prostate cancer tissues compared with control vector group. **C**, The miR-200a-3p expression was decreased in CASC15 lentivirus (CASC15-sh) group compared with empty vector group. **D**, Co-transfection of miR-200a-3p and CASC15-WT strongly decreased the luciferase activity, while co-transfection of miR-control and CASC15-WT did not change the luciferase activity. **E**, The linear correlation between the expression level of miR-200a-3p and CASC15 in PC tissues. The results represent the average of three independent experiments. The data are presented as the mean \pm standard error of the mean. * $p < 0.05$.

Discussion

Recent evidence has indicated that lncRNA serves as a crucial regulatory role in the development of PC, including cell proliferation, apoptosis, and migration. For example, lncRNA ZEB1-AS1 acts as an oncogene in PC by activating ZEB1 and regulating the downstream molecules of TGF- β . LncRNA DANCR enhances the invasion of PC through inhibiting the expression of TIMP-1, which may provide a potential intervention for preventing PC metastasis⁸. In addition, lncRNA H19 contributes to the development of aggressive PC by antagonizing the function of the SWI/SNF complex⁹.

Cancer stem cell candidate 15 (CASC15), located on chromosome 6p22.3, is initially identified also as an active lncRNA. Overexpression of CASC15 is reported to promote cell proliferation in gastric cancer¹⁰. CASC15 enhances cell metastasis and development in hepato-

cellular carcinoma which is correlated with the poor prognosis¹¹. By regulating the expression of SOX4, CASC15 is found to participate in RUNX1-rearranged acute leukemia¹². In addition, overexpression of CASC15 is reported to promote metastatic progression and phenotype switching of melanoma¹³. Our study showed that CASC15 was higher expressed in PC tissues and cell lines. Besides, cell migration and invasion of PC cells were repressed via knockdown of CASC15. Above results suggested that CASC15 promoted aggressiveness of PC *in vitro*.

The interaction between lncRNAs and microRNAs has been widely explored recently. For example, by sponging miR-27b-3p, lncRNA KCNQ1OT1 facilitates cell proliferation and cell invasion in the progression of non-small cell lung cancer through upregulating HSP90AA1¹⁴. Through targeting miR-221/SOCS3, lncRNA GAS5 suppresses cell proliferation, cell metastasis and gemcitabine resistance in pancreatic can-

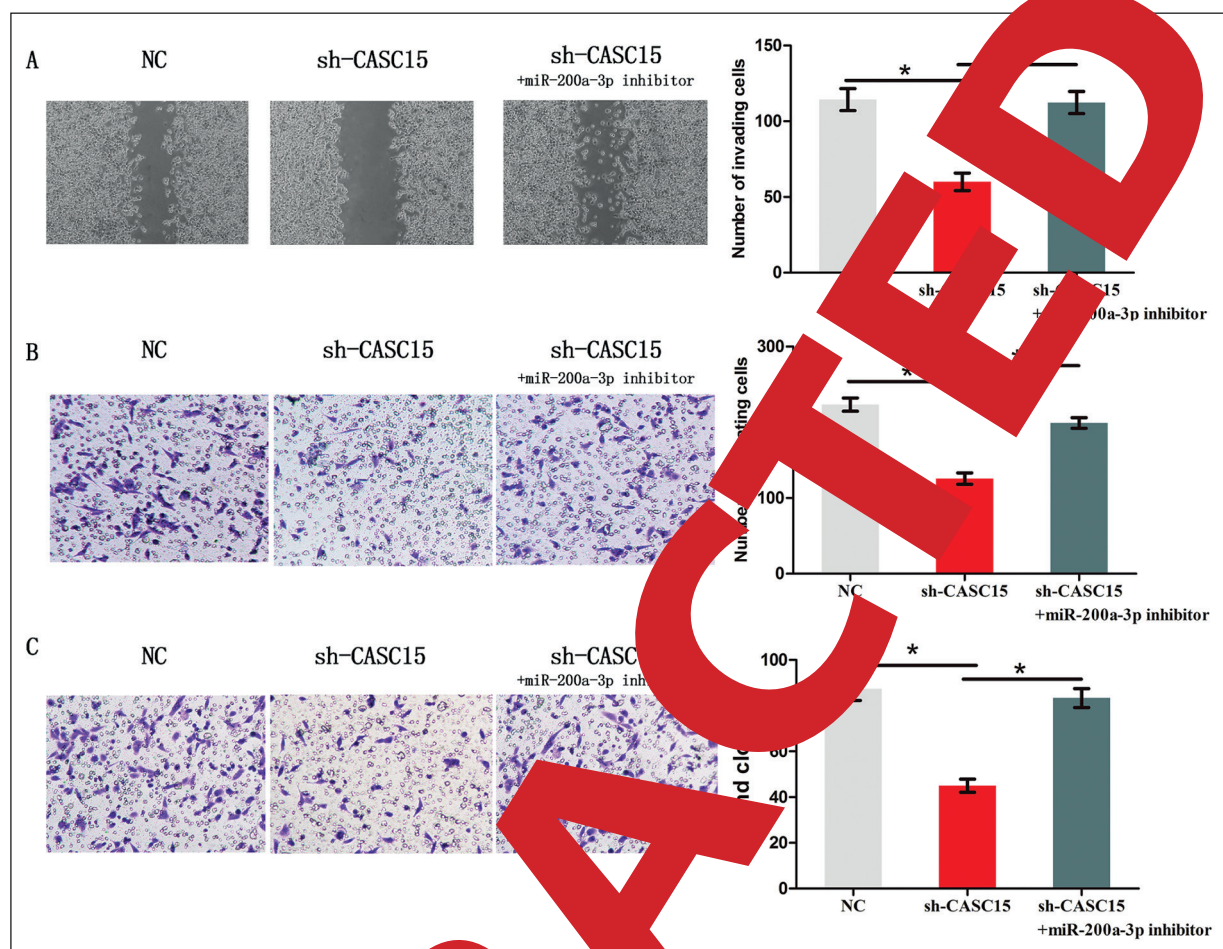


Figure 4. Rescue experiments show that knockdown of miR-200a-3p could reverse the effect of silenced CASC15 on PC migration and invasion. **A**, Wound healing assay showed that wound closure was significantly decreased via silence of CASC15 in PC cells, which could be reversed by knockdown of miR-200a-3p (magnification: 40×). **B**, The transwell assay showed that number of migrated cells was significantly decreased via knockdown of CASC15 in PC cells, which could be reversed by knockdown of miR-200a-3p (magnification: 40×). **C**, The transwell assay showed that number of invaded cells was significantly decreased via knockdown of CASC15 in PC cells, which could be reversed by knockdown of miR-200a-3p (magnification: 40×). * $p < 0.05$ compared with control cells.

cer¹⁵. LncRNA LINC00052 depends on migration and invasion of hepatocellular carcinoma cells through upregulation of EPB41L3, which is modulated by miR-52-5p.

Bioinformatics analysis shows that miR-200a-3p was a potential target mRNA of CASC15. MiR-200 family is involved in cell proliferation, metastasis, epithelial-mesenchymal transition (EMT) in several cancers. MiR-200a-3p has been reported to promote cell proliferation and induces EMT in pancreatic cancer cell carcinoma by targeting SP1. In addition, lncRNA HULC promotes tumorigenesis, metastasis and EMT of hepatocellular carcinoma cell through miR-200a-3p/ZEB1 signaling pathway¹⁸.

Therefore, we detected miR-200a-3p expression and CASC15 expression in PC tissues. Results showed that miR-200a-3p expression in PC tissues was negatively related to CASC15 expression. We further found that miR-200a-3p expression could be suppressed by knockdown of CASC15 in PC cells. Besides, luciferase assay indicated that miR-200a-3p could directly bind to CASC15. Rescue experiments showed that the inhibition of PC cell migration and invasion, which was triggered by silence of CASC15, was reversed through knockdown of miR-200a-3p. All the results above suggested that CASC15 might promote metastasis of PC through sponging miR-200a-3p.

Conclusions

LncRNA CASC15 could enhance PC cell metastasis through sponging miR-200a-3p *in vitro*, indicating that CASC15 might act as a candidate target for therapy of PC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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