Long non-coding RNA SNHG3 promotes prostate cancer progression by sponging microRNA-1827

MING HU[1,2*], MINGLIANG REN[2,3*], ZHENHUA ZHAO[1,2*], XUEJIANG CUI[1,2], MING SHI[1,2], YUNJIE YANG[1,2] and HAIYAN GUO[2,4]

[1]Department of Urology, The Sixth Affiliated Hospital, South China University of Technology (People's Hospital of Nanhai District); [2]The Affiliated Nanhai Hospital, Southern Medical University (People's Hospital of Nanhai District); Departments of [3]Spine Surgery and [4]Respiratory Medicine, The Sixth Affiliated Hospital, South China University of Technology (People's Hospital of Nanhai District), Foshan, Guangdong 528200, P.R. China

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Abstract. Long non-coding RNAs (lncRNAs) are important biological factors that contribute to the initiation and progression of different types of cancer, including gastric, bladder and colorectal cancer. Small nucleolar RNA host gene 3 (SNHG3) has been implicated in prostate cancer (PCa) progression. However, the expression pattern and function of SNHG3 in PCa remain unclear, impeding the development of novel treatment strategies for this cancer. The present study aimed to investigate a combination of molecular and biochemical approaches to determine the role of SNHG3 in patients at different stages of disease, and elucidate the pathway by which SNHG3 affects PCa progression. A Cell Counting Kit-8 assay was used to assess cell proliferation. Transwell assays were used to analyze cell migration and invasion. Reverse transcription-quantitative PCR and western blotting were used to evaluate the expression levels of RNAs and proteins, respectively. The results demonstrated that SNHG3 expression was upregulated in PCa tissues downloaded from The Cancer Genome Atlas database, which was associated with poor prognosis. Furthermore, cell proliferation, migration and invasion were significantly inhibited following SNHG3 knockdown *in vitro*, the effects of which were reversed following overexpression of SNHG3 in PCa cells. Bioinformatic analysis revealed that microRNA (miRNA/miR)-1827 was a downstream target of SNHG3. The direct interaction between SNHG3 and miR-1827 was validated via the dual-luciferase reporter and RNA immunoprecipitation assays. Pearson's correlation analysis demonstrated that SNHG3 expression was negatively correlated with miR-1827 expression at different stages of PCa. Furthermore, rescue assays indicated that cotransfection with small interfering-SNHG3 and miR-1827 inhibitor reversed the effects of SNHG3 knockdown on cell proliferation, migration and invasion. In addition, SNHG3 knockdown *in vivo* suppressed tumor growth. Notably, lncRNA SNHG3 promoted PCa progression through miR-1827 via the Wnt/AKT/mTOR pathway. Taken together, the results of the present study suggest that SNHG3 promotes PCa progression by sponging miR-1827, indicating that SNHG3 may be a promising diagnostic and therapeutic target of PCa.

Introduction

Prostate cancer (PCa) is one of the most frequently diagnosed malignancies and remains the second leading cause of cancer-associated mortality in men worldwide (1). Despite recent advancements in early diagnosis and the therapeutic treatment of PCa, the 5-year survival rate of this malignancy remains <30% (2). Thus, it is important to determine its underlying molecular mechanisms to identify novel diagnostic markers and develop targeted therapies to improve the prognosis of patients with PCa.

Long non-coding RNAs (lncRNAs) are a class of RNA molecules >200 nucleotides in length, without protein-encoding function (3). lncRNAs are dysregulated in PCa and can modulate multiple physiological and pathological processes, including cell proliferation, differentiation, migration, invasion and drug-resistance (4-7). For example, lncAMPC promotes metastasis and immunosuppression of PCa via the lncAMPC/LIF/LIFR axis (8). Furthermore, lncRNA FOXP4-AS1 can promote tumorigenesis and progression of PCa by regulating the microRNA (miRNA/miR)-3184-5p/FOXP4 axis (9). lncRNA625 is upregulated in PCa cells and modulates PCa progression via the miR-432/Wnt/β-catenin signaling pathway (10).

Recently, a newly identified lncRNA, small nucleolar RNA host gene 3 (SNHG3), was reported to be dysregulated in different types of cancer (11-14). However, the functions of SNHG3 are inconsistent. SNHG3 has been reported to act as

Correspondence to: Dr Haiyan Guo, Department of Respiratory Medicine, The Sixth Affiliated Hospital, Southern China University of Technology (People's Hospital of Nanhai District), 40 Foping Road, Nanhai, Foshan, Guangdong 528200, P.R. China
E-mail: haiyanguo_phd@163.com

*Contributed equally

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an oncogene in colorectal cancer and glioma (13,15); however, it acts as a tumor suppressor in papillary thyroid carcinoma (12). This may be due to different techniques or indicates the versatility of SNHG3. Although SNHG3 has been reported to accelerate PCa progression via the miR-577/SMURF1 axis (14), its molecular mechanism in PCa remains unclear.

The present study aimed to investigate the expression and function of SNHG3 in PCa cells, and determine the potential molecular mechanisms of SNHG3 in regulating the phenotypes of PCa cells.

Materials and methods

Human clinical samples. A total of 30 paired PCa tissues and adjacent normal tissues (5 cm away from tumor tissues) were collected from 30 patients during radical prostatectomy at the Affiliated Nanhai Hospital, Southern Medical University between January 2016 and May 2019. All samples were stored at -80˚C until subsequent experimentation. The mean age of patients with PCa 62.3 years (age range, 48-78 years) The present study was approved by the Medical Ethics Committee of Affiliated Nanhai Hospital, Southern Medical University (Foshan, China, IRB no. 2020-027-1 and no. IRB-NY-2020-018) and written informed consent was provided by all patients prior to the study start.

Bioinformatics analysis. The Cancer Genomic Atlas (TCGA, https://www.cancer.gov) prostate adenocarcinoma cohort, comprising 492 patients, was used for the expression and survival analyses. Briefly, 492 patients with PCa, with mRNA expression data from TCGA, were enrolled in the present study. Among these, 52 patients had SNHG3 expression data in both PCa tumor tissues and normal tissues. The DIANA (http://diana.imis.athena-innovation.gr/DianaTools/) and StarBase (http://starbase.sysu.edu.cn) databases were used to predict the target genes.

Cell culture. The human prostatic epithelial cell line (RWPE-1) and PCa cell lines (PC-3, DU145, VCaP and LNCaP) were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotics (all purchased from Gibco; Thermo Fisher Scientific, Inc.), at 37˚C with 5% CO_2.

Cell transfection. Cells ($2x10^5$) were seeded into 6-well plates at a density of 30-50%. Transient transfection with 20 nM small interfering si-SNHG3, miR-1827 mimics (3'-UAA GUUAGAUGACGGAGU-5'), miR-1827 inhibitor (3'-ACU CCGUCAUCUAACUUA-5'), miR-mimics-NC (3'-AACAUG AUGUGUUUUCAUGAC-5') or miR-inhibitor-NC (3'-UUU GCACUGUGCAAGC-5') was performed when cell reached 50-60% confluence using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature for 24 h, according to the manufacturer's instructions. For overexpression, lncSNHG3 was cloned into mammalian expression vector pcDNA3.1 (Invitrogen; Thermo Fisher Scientific, Inc.) to construct overexpressing plasmid pcDNA3.1- lncSNHG3. After transfection for 48 h, the transfected cells were used for subsequent experiments. si-SNHG3 (#1 and #2), SNHG3 overexpression plasmid, miR-1827 mimics, miR-1827 inhibitor and their respective negative controls (si-NC for si-SNHG3, empty vector of pcDNA3.1 for SNHG3 overexpression plasmid, miR-mimics-NC for miR-1827 mimics and miR-inhibitor-NC for miR-1827 inhibitor) were purchased from Guangzhou RiboBio Co., Ltd.

Reverse transcription-quantitative (RT-q)PCR. Total RNA was extracted from tissues or PCa cells using the TRIzol® RNA Purification kit (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse transcribed into cDNA using PrimeScript RT Master Mix (Takara Biotechnology Co., Ltd.) at 37˚C for 15 min. qPCR was subsequently performed using SYBR Premix Ex Taq (Takara Bio, Inc.) in a Roche LightCycler ®480II PCR instrument (Roche Diagnostics). The following primer sequences were used for qPCR: SNHG3 forward, 5'-GGAAATAAAGCTGGGCCTCG-3' and reverse, 5'-AAC AGAGCGACTCCATCTCC-3'; miR-1827 forward, 5'-GGT GAGGCAGTAGATTGAATCTC-3' and reverse, 5'-CTCAAC TGGTGTCGTGGAGTC-3'; GAPDH forward, 5'-AATGGG CAGCCGTTAGGAAA-3' and reverse, 5'-GCGCCCAAT ACGACCAAATC-3'; and U6 forward, 5'-TGCGGGTGCTCG CTTCGGCAGC-3' and reverse, 5'-CCAGTGCAGGGTCCG AGGT-3'. The thermocycling conditions for qPCR were as follows: Initial denaturation 95˚C for 30 sec, 40 of cycles of denaturation 95˚C for 5 sec, annealing and extension 60˚C for 30 sec and final extension 4˚C for 5 min. Relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method and U6 and GAPDH were used as the internal reference controls (16).

Cell viability assay. The Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Inc.) assay was performed to assess cell viability, according to the manufacturer's instructions. Transfected cells were seeded into 96-well plates ($2x10^3$) and treated with 10 μl CCK-8 reagent at 0, 24, 48 and 72 h. After 1.5 h, the absorbance values were measured at a wavelength of 450 nm, using a microplate reader (Thermo Fisher Scientific, Inc.).

Wound healing assay. The wound healing assay was performed to assess cell migration. Transfected cells were seeded into 6-well plates ($2x10^5$). After 24 h, once the cells reached >90% confluence, the monolayers were scratched using sterile pipette tips. Cells were cultured in serum-free medium (DMEM, Gibco; Thermo Fisher Scientific, Inc.) at 37˚C for 12 h. The wound healing process was observed under an inverted light microscope (Olympus Corporation, x20 magnification), and migration was evaluated by the percentage of wound width that were healed.

Invasion assay. The Transwell assay was performed to assess cell invasion, using BioCoat Matrigel Invasion Chambers (Corning, Inc.), according to the manufacturer's instructions. Briefly, transfected cells ($2x10^5$) in 0.2 ml serum-free DMEM were plated in the upper chambers of Transwell plates coated with Matrigel (Corning, Inc.) at 37˚C for 30 min, while DMEM supplemented with 20% FBS was plated in the lower chambers. Following incubation for 24 h at 37˚C, invasive cells in the lower chambers were fixed with 4% PFA and stained with 0.1% crystal violet for 5 min at room temperature. Stained

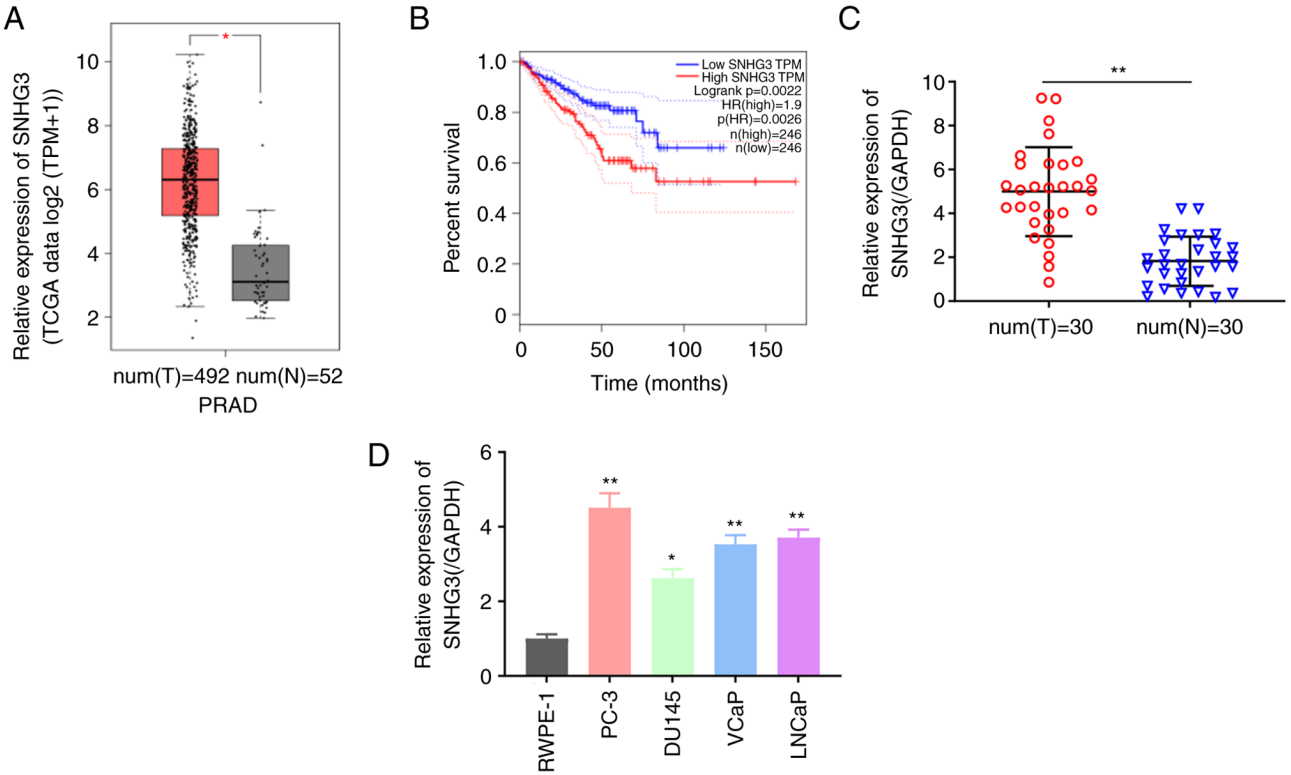

Figure 1. lncRNA SNHG3 expression in PCa tumor tissues and its clinical significance. (A) The expression of lncRNA SNHG3 in PCa tumor tissues (n=492) compared with normal tissues (n=52) in TCGA dataset. (B) Kaplan-Meier survival analysis of patients with high (n=246) and low (n=246) SNHG3 expression levels in TCGA dataset. (C) RT-qPCR analysis was performed to detect SNHG3 expression in PCa tumor tissues (n=30) compared with adjacent normal tissues (n=30). (D) RT-qPCR analysis was performed to detect SNHG3 expression in PCa cell lines (PC-3, DU145, VCaP and LNCaP) and the human prostatic epithelial cell line, RWPE-1. Data are presented as the mean ± standard error of the mean (n≥3). *P<0.05, **P<0.01 vs. RWPE-1 cells. lncRNA, long non-coding RNA; SNHG3, small nucleolar RNA host gene 3; PCa, prostate cancer; TCGA, The Cancer Genome Atlas; RT-qPCR, reverse transcription-quantitative PCR; T, tumor; N, normal.

cells were observed under an IX71 inverted light microscope (Olympus Corporation, x20 magnification).

Nuclear/cytoplasmic fractionation assay. The PARIS kit (Thermo Fisher Scientific, Inc.) was used for nuclear/cytoplasmic fractionation, according to the manufacturer's instructions. The expression levels of SNHG3 from the cell nuclear and cytoplasmic fractions were detected via RT-qPCR analysis. U6 and GAPDH served as the internal controls, respectively.

Dual-luciferase reporter assay. Wild-type (WT) or mutant (MUT) SNHG3 sequences were cloned and inserted into the pGL3-control vector (Promega Corporation) to construct SNHG3-WT and SNHG3-MUT. PC-3 cells were seeded into 24-well plates and co-transfected with SNHG3-WT or SNHG3-MUT along with miR-1827 mimics and miR-1827 inhibitor, using Lipofectamine® 3000 reagent for 48 h at 37˚C. Following incubation for 48 h, luciferase activities were detected using the luciferase reporter assay system (Promega Corporation), according to the manufacturer's instructions. *Renilla* luciferase activity (Promega Corporation) was used for normalization.

RNA immunoprecipitation (RIP) assay. The RIP assay was performed using the EZ-Magna RIP RNA-Binding Protein Immunoprecipitation kit (MilliporeSigma), according to the manufacturer's instructions. Cells ($5x10^6$) which transfected with siRNAs were collected by centrifugation at 800 x g for 5 min at 4˚C and then lysed with complete lysis buffer (cat. no. KT102-01; GZSCBio Co. Ltd.) containing RNase (Thermo Fisher Scientific, Inc.) and protease inhibitor cocktail (Roche Diagnostics). Subsequently, cell extracts (100 μl) were co-immunoprecipitated with protein G Sepharose beads (40 μl, included in the kit, according to the manufacturer's instructions) pre-coated with Argonaute2 (Ago2) antibody (dilution, 1:150, cat. no. P10502500, Otwo Biotech Inc.) or IgG (dilution, 1:150, Sigma-Aldrich; Merck KGaA). Following incubation for 2 h at 4˚C. The beads were centrifuged at 1,500 x g for 30 sec. The co-precipitated SNHG3 was isolated from the beads using the TRIzol® RNA Purification kit (Invitrogen; Thermo Fisher Scientific, Inc.) and assessed via RT-qPCR analysis.

Western blotting. Total proteins of the cells were isolated using RIPA buffer (cat. no. P0013B; Beyotime Institute of Biotechnology). After that, a BCA protein assay kit (Beyotime Institute of Biotechnology) was used to measure the protein concentration. Then, 40 μg proteins were loaded on the 10% sodium dodecyl sulfate-polyacrylamide gel and separated by electrophoresis. The proteins were transferred to polyvinylidene difluoride (PVDF) membranes and blocked for 2 h using 5% skimmed non-fat milk (Yili) at room temperature. Next, the corresponding primary antibodies, cleaved PARP (CST; cat. no. 5625), N-cadherin (CST; cat. no. 13116), MMP9 (CST;

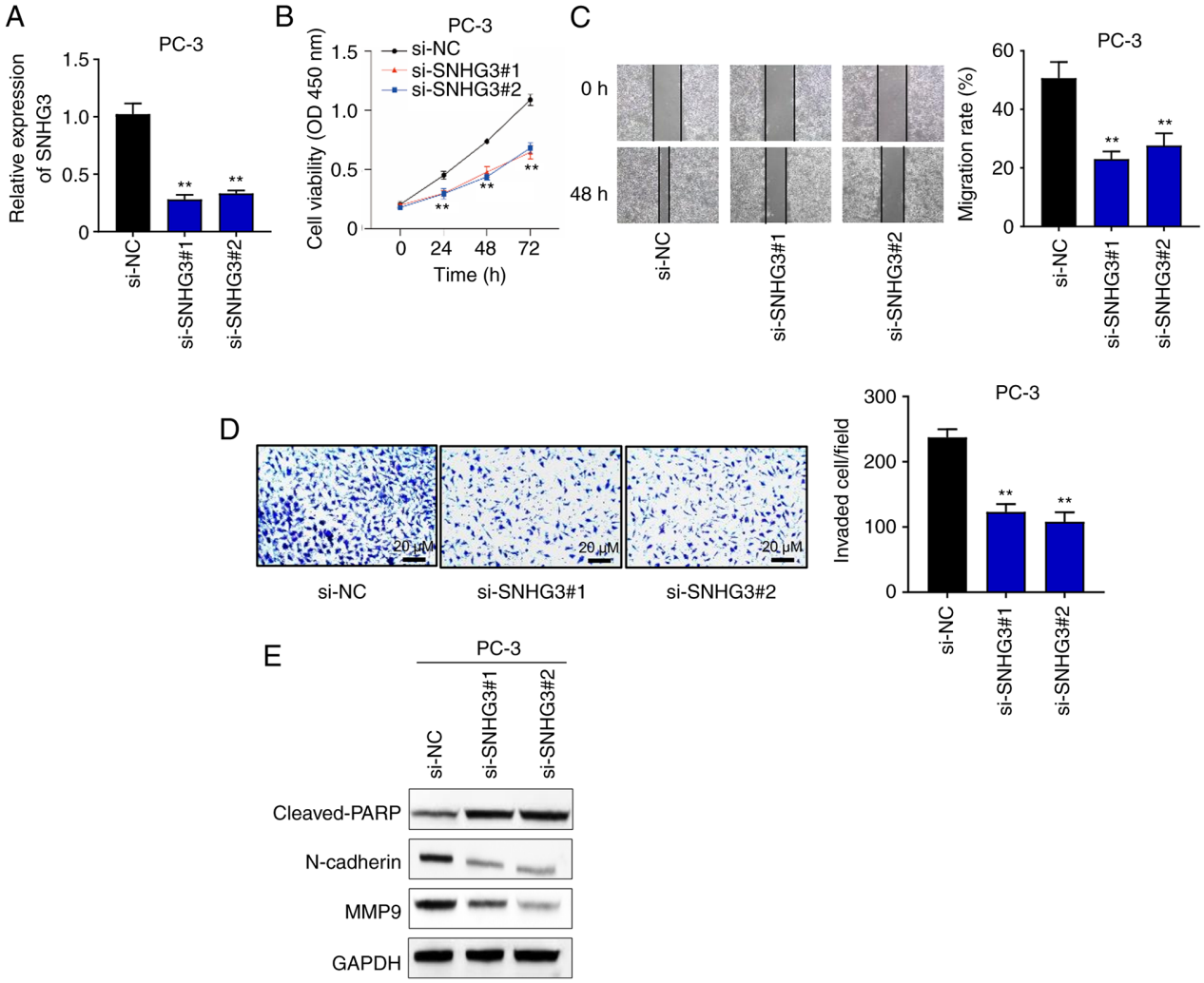

Figure 2. Effects of SNHG3 knockdown on the proliferation, migration and invasion of PC-3 cells. (A) SNHG3 expression was knocked down using siRNAs, and reverse transcription-quantitative PCR analysis was performed to detect SNHG3 expression. (B) The Cell Counting Kit-8 assay was performed to assess the proliferation of PC-3 cells following SNHG3 knockdown. (C) The wound healing assay was performed to assess cell migration following SNHG3 knockdown. (D) The Transwell assay was performed to assess cell invasion following SNHG3 knockdown. (E) Western blot analysis was performed to detect the protein expression levels of cleaved-PARP, N-cadherin and MMP9 following transfecting SNHG3 knockdown in PC-3 cells. Data are presented as the mean ± standard error of the mean (n≥3). **P<0.01 vs. control. SNHG3, small nucleolar RNA host gene 3; si, small interfering; MMP, matrix metalloproteinase; NC, negative control; OD, optical density.

cat. no. 13667), β-catenin (CST; cat. no. 8480), Akt (CST; cat. no. 4691), phosphorylated (p-)Akt (CST; cat. no. 4060) mTOR (CST; cat. no. 2983), p-mTOR (CST; cat. no. 5536) and GAPDH (CST; cat. no. 5174) were used to incubate the membranes at a dilution ratio of 1:1,000 with PBST containing 5% BSA overnight at 4˚C. After incubation, the membranes were treated with the secondary antibodies (anti-rabbit, CST; cat. no. 3900; dilution ratio of 1:5,000 with PBST containing 5% non-fat milk and 0.05% Tween) for 1 h at room temperature. Finally, the protein bands were developed with enhanced chemiluminescence (cat. no. 407207; EMD Millipore; Merck KGaA) on the Tanon 4600 imaging system (Tanon Science and Technology Co., Ltd.).

Tumor xenograft model in nude mice. A total of 12 male BALB/c nude mice (6-weeks-old; 16-20 g; n=6 for each group) were obtained from the Chinese Academy of Sciences. All mice were housed in cages with no more than 5 mice/cage. The room temperature was maintained at 20-25% and the humidity was at 40-55%, with a 12 h light/dark cycle. All mice were fed commercially available mouse food and sterilized water.

PC-3 cells transfected with si-SNHG3#1 or si-NC were subcutaneously injected into the right flanks of mice. Tumor volume was measured every week using the following formula: V=(tumor length x tumor width^2)/2. After 5 weeks, the mice were euthanized via cervical dislocation, and the absence of fluctuation in the chest cavity, breathing and heartbeat confirmed mortality. Subsequently, RNA was isolated from the tumor to detect the expression levels of SNHG3 and miR-1827. All animal experiments were reviewed and approved by the Animal Welfare Committee of Affiliated Nanhai Hospital, Southern Medical University (Foshan, China; IRB no. 2020-027-1).

Statistical analysis. Statistical analysis was performed using GraphPad Prism 7.0 software (GraphPad Software, Inc.) and SPSS 22.0 software (IBM Corp.). All experiments were performed in triplicate and data are presented as the mean ±

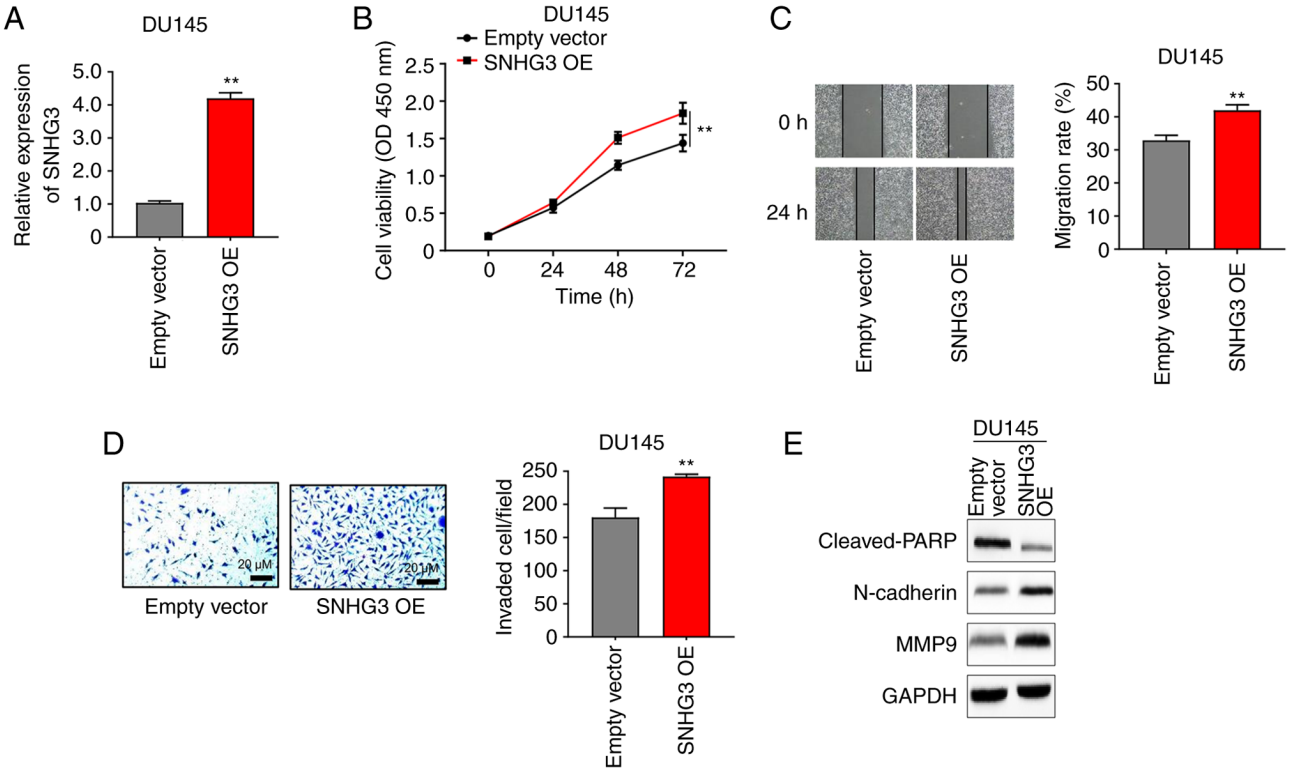

Figure 3. Effects of SNHG3 overexpression on the proliferation, migration and invasion of PC-3 cells. (A) Reverse transcription-quantitative PCR analysis was performed to detect SNHG3 expression following transfection with SNHG3 OE. (B) The Cell Counting Kit-8 assay was performed to assess cell proliferation following transfection with SNHG3 OE. (C) The wound healing assay was performed to assess cell migration following transfection with SNHG3 OE. (D) The Transwell assay was performed to assess cell invasion following transfection with SNHG3 OE in DU145 cells. (E) Western blot analysis was performed to detect the protein expression levels of cleaved-PARP, N-cadherin and MMP9 in DU145 cells transfected with SNHG3 OE. Data are presented as the mean ± standard error of the mean (n≥3). **P<0.01 vs. control. SNHG3, small nucleolar RNA host gene 3; OE, overexpression plasmid; MMP, matrix metalloproteinase; OD, optical density.

standard error of the mean. A paired Student's t-test was used to compare differences between tumor and adjacent normal tissues, and an unpaired Student's t-test was used to compare differences between the experimental and control groups. One-way ANOVA followed by Bonferroni's post hoc test were used to compare differences between multiple groups. Survival analysis was performed using the Kaplan-Meier method and log-rank test. Correlations were analyzed by Pearson's correlation test. P<0.05 was considered to indicate a statistically significant difference.

Results

lncRNA SNHG3 is upregulated in PCa tissues and cell lines. To evaluate SNHG3 expression in PCa, the mRNA data of 492 patients with PCa from TCGA dataset were analyzed. The results demonstrated that SNHG3 expression was significantly elevated in PCa tumor tissues compared with normal tissues (P<0.05; Fig. 1A). Kaplan-Meier survival analysis was subsequently performed to assess the cumulative survival time. As presented in Fig. 1B, patients with high SNHG3 expression had a shorter overall survival time than those with low SNHG3 expression (P=0.0022). To eliminate the possibility that the differential expression levels of SNHG3 in TCGA patients were caused by the uneven sample size, SNHG3 expression was also detected in 30 paired PCa tumor tissues and adjacent normal tissues. The results confirmed that SNHG3 expression was significantly upregulated in PCa (P<0.01; Fig. 1C). RT-qPCR analysis was performed to detect SNHG3 expression in PCa cell lines. The results demonstrated that SNHG3 expression was significantly upregulated in PCa cell lines compared with the normal human prostate epithelial cell line, RWPE-1 (P<0.05; Fig. 1D). Taken together, these results suggest that SNHG3 is an oncogene in the tumorigenesis of PCa, and is associated with a poor prognosis.

lncRNA SNHG3 knockdown inhibits PC-3 cell proliferation and migration in vitro. To further investigate the biological functions of SNHG3 in PCa, PC-3 cells were selected for subsequent experimentation as they express high expression levels of SNHG3 (Fig. 1D). PC-3 cells were transfected with si-SNHG3 and cell viability was assessed via the CCK-8 assay. The results demonstrated that SNHG3 expression significantly decreased following transfection compared with the control group (Fig. 2A). In addition, SNHG3 knockdown significantly inhibited the proliferation of PC-3 cells (Fig. 2B). The migratory and invasive abilities of PC-3 cells following transfection were subsequently assessed. SNHG3 knockdown significantly suppressed the migratory and invasive abilities of PC-3 cells (Fig. 2C and D). The expression levels of the protein markers of apoptosis (cleaved-PARP) and epithelial-to-mesenchymal transition [EMT; N-cadherin and matrix metalloproteinase (MMP)9] were detected following SNHG5 knockdown via western blotting. The results demonstrated that cleaved-PARP expression significantly increased following SNHG5

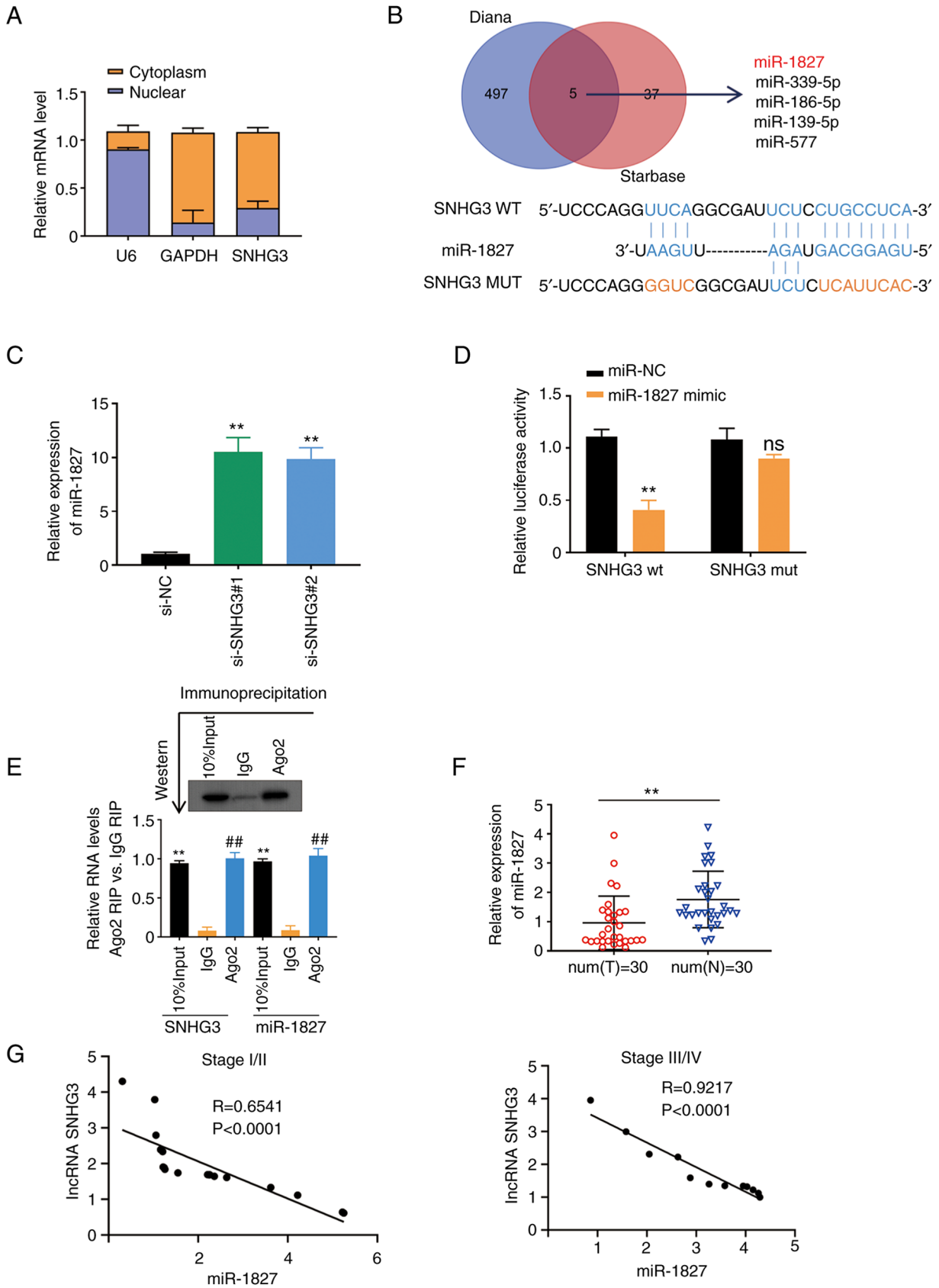

Figure 4. miR-1827 is a direct target of SNHG3. (A) Subcellular fraction assay revealed the subcellular location of SNHG3 in PC-3 cells. (B) Overlap of predicted downstream miRNAs of SNHG3 (upper panel) and predicted binding regions between miR-1827 and SNHG3 (lower panel) using the DIANA and StarBase databases. (C) RT-qPCR analysis was performed to detect miR-1827 expression in PC-3 cells transfected with SNHG3 siRNAs. (D) miR-1827 was validated as a direct target of SNHG3 via the dual-luciferase reporter assay in PC-3 cells. (E) The RNA immunoprecipitation assay confirmed the interaction between miR-1827 and SNHG3. (F) RT-qPCR analysis was performed to detect miR-1827 expression in PCa tumor tissues (n=30) compared with adjacent normal tissues (n=30). (G) Pearson's correlation analysis between SNHG3 and miR-1827 expression in tissues from stage I/II PCa (left) and stage III/IV PCa (right). Data are presented as the mean ± standard error of the mean (n≥3). **P<0.01 vs. control. $^{\#\#}$<P<0.01 vs. IgG group. miR, microRNA; SNHG3, small nucleolar RNA host gene 3; qPCR, reverse transcription-quantitative PCR; si, small interfering; WT, wild-type; MUT, mutant; NC, negative control; T, tumor; N, normal.

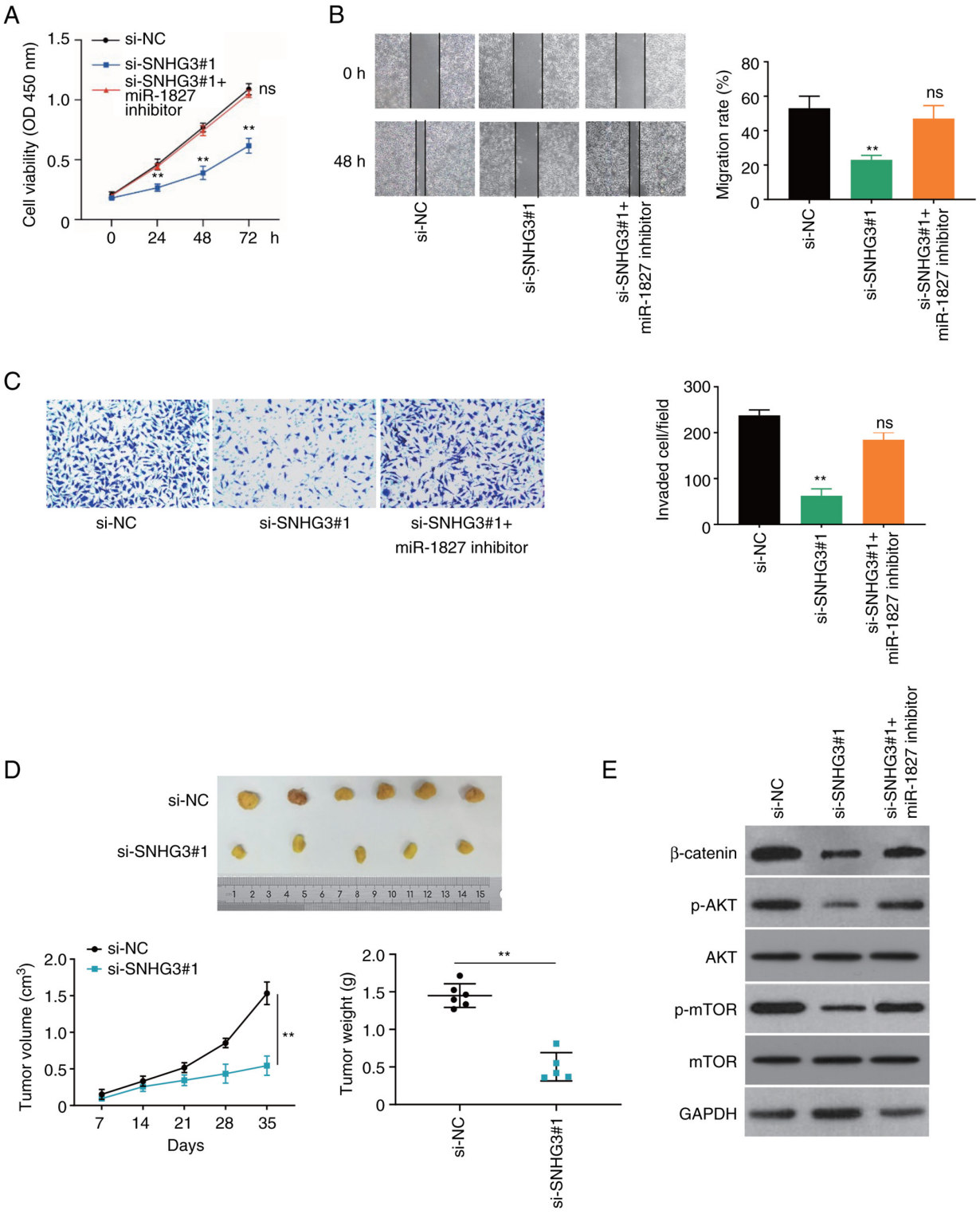

Figure 5. miR-1827 mediates the tumor-suppressive effects of SNHG3 knockdown. (A) The Cell Counting Kit-8 assay was performed to assess the proliferation of PC-3 cells transfected with SNHG3 siRNAs alone or a combination of SNHG3 siRNAs and miR-1827 inhibitor. (B) The wound healing assay was performed to assess cell migration following transfection with SNHG3 siRNAs alone or a combination of SNHG3 siRNAs and miR-1827 inhibitor. (C) The Transwell assay was performed to assess cell invasion following transfection with SNHG3 siRNAs alone or a combination of SNHG3 siRNAs and miR-1827 inhibitor. (D) Tumor volume and weights were measured at different time points. (E) Western blot analysis was performed to detect the protein expression levels of β-catenin, p-AKT and p-mTOR following transfection with si-SNHG3 or a combination of SNHG3 siRNAs and miR-1827 inhibitor. Data are presented as the mean ± standard error of the mean (n≥3). **P<0.01 vs. control. miR, microRNA; SNHG3, small nucleolar RNA host gene 3; si, small interfering; p, phosphorylated; NC, negative control; OD, optical density.

knockdown, which suggests that impaired cell proliferation may be induced by cell apoptosis (Fig. 2E). Conversely, the expression levels of N-cadherin and MMP9 decreased following SNHG5 knockdown (Fig. 2E), suggesting that EMT inhibits the migration of PCa cells. Collectively, these results suggest that lncRNA SNHG3 promotes malignancy of PCa *in vitro*.

Overexpression of lncRNA SNHG3 promotes DU145 cell proliferation and migration in vitro. DU145 cells were selected for the overexpression experiments as they express low levels of SNHG3 (Fig. 1D). SNHG3 expression was significantly upregulated in DU145 cells following transfection with SNHG3 overexpression plasmid (OE; Fig. 3A). In addition, transfection with SNHG3 OE promoted the proliferation of DU145 cells (Fig. 3B). The migratory and invasive abilities of DU145 cells were assessed following transfection with SNHG3 OE. The results demonstrated that overexpression of SNHG3 significantly promoted the migratory and invasive abilities of DU145 cells (Fig. 3C and D). The expression levels of protein markers of apoptosis and EMT were also investigated following overexpression of SNHG3. The results demonstrated that overexpression of SNHG3 increased the protein expression levels of N-cadherin and MMP9 but decreased cleaved-PARP expression (Fig. 3E). Taken together, these results suggest that overexpression of SNHG3 promotes the proliferation and EMT of PCa cells.

miRNA-1827 is a direct target of lncRNA SNHG3 in PC-3 cells. Having demonstrated the association between SNHG3 and PCa progression, the present study investigated the oncogenic mechanisms of lncRNA SNHG3. The results of the nuclear/cytoplasmic fractionation assay demonstrated that lncRNA SNHG3 was predominantly located in the cytoplasm of PC-3 cells (Fig. 4A). The potential interactive targets of lncRNA SNHG3 were identified using the DIANA and StarBase databases. A total of five overlapped downstream miRNAs were predicted (Fig. 4B). Among these overlapped targets, only miR-1827 expression increased following SNHG3 knockdown in PC-3 cells (Figs. 4C and S1). The dual-luciferase reporter and RIP assays were performed to verify the direct interaction between SNHG3 and miR-1827. The results of the dual-luciferase reporter assay demonstrated that overexpression of miR-1827 significantly inhibited the relative luciferase activity of SNHG3-WT, but had no effect on the SNHG3-MUT group (Figs. 4D), which confirms the direct association between SNHG3 and miR-1827. The results of the RIP assay demonstrated that SNHG3 and miR-1827 was enriched in Ago2-immunoprecipitated complexes, confirming the interaction between SNHG3 and miR-1827 in PC-3 cells (Fig. 4E). To further assess the interaction between SNHG3 and miR-1827 in PC-3 cells, the following experiments were performed. The present study first evaluated the effect of miR-1827 on SNHG3 levels in PC3. The results demonstrated that lncRNA SNHG3 was marginally altered following transfection with miR-1827 mimics or miR-1827 inhibitor (Fig. S2A-S2C). The effect of SNHG3 on miR-1827 in MCF-7 cells was also assessed. The results demonstrated that miR-1827 expression changed marginally following transfection of MCF-7 cells with si-SNHG3 (Fig. S2D).

miR-1827 expression was detected in 30 paired PCa tumor tissues and adjacent normal tissues. The results demonstrated that miR-1827 expression was significantly higher in adjacent normal tissues compared with tumor tissues (Fig. 4F). Pearson's correlation analysis demonstrated that SNHG3 expression was negatively correlated with miR-1827 expression in tissues from PCa patients with stage I/II or stage III/IV assessed by the tumor, node and metastasis (TNM) system (17) (Fig. 4G). Collectively, these results suggest that SNHG3 binds to miR-1827 and modulates miR-1827 expression in tissues from patients with PCa (Fig. 4G). Collectively, these results suggest that SNHG3 binds to miR-1827 and modulates miR-1827 expression.

miR-1827 mediates the tumor-suppressive effects of lncRNA SNHG3 knockdown on PC-3 cells. The rescue assay was performed to further investigate the association between SNHG3 and miR-1827 in PC-3 cells. Notably, the decreased cell viability of PC-3 cells following SNHG3 knockdown was recovered following inhibition of miR-1827 expression (Fig. 5A). In addition, the wound healing and Transwell assays indicated that the reduced migration and invasion of PC-3 cells following SNHG3 knockdown was also recovered following miR-1827 knockdown (Fig. 5B and C).

lncRNA SNHG3 knockdown inhibits PCa tumorigenesis in vivo. The promoting role of SNHG3 in tumorigenesis *in vivo* was verified. The results demonstrated that tumor volume and weight decreased following SNHG3 knockdown ($P<0.01$; Figs. 5D and S2E). These results suggest that SNHG3 is essential for tumor progression of PCa *in vivo*.

The present study investigated the Wnt/β-catenin and PI3K/AKT/mTOR signaling pathways in PCa cells transfected with si-SNHG3 or a combination of si-SNHG3 and miR-1827 inhibitor by western blotting. The expression levels of β-catenin, p-AKT and p-mTOR significantly decreased following transfection with si-SNHG3, which were partially restored following transfection with both si-SNHG3 and miR-1827 inhibitor (Fig. 5E). Taken together, these results suggest that lncRNA SNHG3 promotes PCa progression through miR-1827 via the Wnt/AKT/mTOR pathway.

Discussion

lncRNAs play crucial roles in several biological processes of PCa, including cell proliferation, migration, invasion and apoptosis (4,6,8,9). lncAMPC has been reported to promote metastasis and immunosuppression of PCa via the lncAMPC/LIF/LIFR axis (8). lncRNA FOXP4-AS1 has been demonstrated to promote the tumorigenesis and progression of PCa by regulating the miR-3184-5p/FOXP4 axis (9). The present study reported that a novel lncRNA, SNHG3, was significantly upregulated in PCa tissues, which was associated with a shorter survival time of patients. Furthermore, the results demonstrated that SNHG3 knockdown suppressed proliferation, migration and invasion of PCa cells *in vitro*. The oncogenic mechanisms of SNHG3 in PCa were investigated in the present study using both *in vitro* and *in vivo* experiments.

lncRNA SNHG3 is located at 1p36.1 (18). SNHG3 was first reported to dysregulate translational machinery and ribosome biogenesis during the neurodegeneration of Alzheimer's disease (18). Recently, lncRNA SNHG3 has been reported to be dysregulated in different types of cancer, with varied functional roles (11-13,15). In colorectal cancer, SNHG3 acts as a competing endogenous RNA molecule to promote cancer progression (15). In glioma, SNHG3 facilitates malignant behavior via the KLF2/p21 axis (13). However, in papillary thyroid carcinoma, SNHG3 acts as a tumor suppressor via the

AKT/mTOR/ERK pathway (12). Its diversity may be caused by inadequate research on SNHG3 expression patterns and functional mechanisms. A previous study suggested that SNHG3 can promote PCa progression via the miR-577/SMURF1 axis (14). However, this conclusion was drawn solely based on the experiments using cell lines, which, by no means, can accurately reflect the situation in clinical specimens. The present study investigated the expression pattern and functional role of SNHG3 using TCGA PCa patient cohort and PCa tissues. The results demonstrated that SNHG3 expression was significantly upregulated in tumor tissues compared with normal tissues from both TCGA cohort and hospitalized patients. In addition, high SNHG3 expression was associated with a shorter overall survival time. The present study further verified that SNHG3 knockdown significantly inhibited cell viability, proliferation, migration and invasion of PCa cells, the effects of which were reversed following overexpression of SNHG3 in PCa cells *in vitro*. Tumor growth in nude mice was investigated following SNHG3 knockdown. The results demonstrated that SNHG3 knockdown significantly decreased tumor volume and weight *in vivo*. Overall, the results of the present study are consistent with previous findings (14,19,20), that SNHG3 acts as an oncogene in PCa.

Increasing evidence suggest that lncRNAs can function as competitive endogenous RNAs in different types of cancer (3). The competitive endogenous RNA can reduce the stability of target miRNAs, thereby modulating the expression of the miRNA-targeted genes (21). For examples, SNHG3 can sponge miR-577 to regulate the SMURF1 expression to promote PCa progression (14). The results of the present study demonstrated that miR-1827 contains a putative binding site of SNHG3. miR-1827 is dysregulated in different types of cancer, including colorectal cancer and lung cancer, and exhibits varied biological functions (22,23). In colorectal cancer, miR-1827 acts as an oncogene and promotes the progression of colorectal cancer by elevating Wnt/β-catenin activity (22). In lung cancer, miR-1827 is downregulated in high invasive lung cancer cell lines, and suppresses cancer cell migration by targeting CRKL (23). However, the role of miR-1827 in PCa remains largely unknown. The results of the present study demonstrated that SNHG3 can directly interact with miR-1827. In addition, SNHG3 knockdown significantly decreased miR-1827 expression, while SNHG3 expression was not significantly altered following transfection with miR-1827 mimics or inhibitors. Notably, miR-1827 expression was downregulated in tumor tissues compared with normal tissues, and SNHG3 expression was negatively correlated with miR-1827 expression at different stages of PCa. Furthermore, decreased malignant phenotypes of PC-3 cells caused by SNHG3 knockdown were recovered following miR-1827 knockdown. These results confirm that miR-1827 is the downstream target of SNHG3 and is negatively regulated by SNHG3. The results of the present study also demonstrated that the expression levels of β-catenin, p-AKT and p-mTOR significantly decreased following transfection with si-SNHG3, which was partially reversed following transfection with a combination of si-SNHG3 and miR-1827 inhibitor. Collectively, these results suggest that lncRNA SNHG3 promotes PCa progression through miR-1827 via the Wnt/AKT/mTOR pathway.

The present study is not without limitations. First, the downstream targets of miR-1827 were not investigated. Thus, further studies are required to investigate the downstream targets of miR-1827 in PCa. Furthermore, the association between miR-1827 and AKT/mTOR requires further investigation.

In conclusion, the results of the present study demonstrated that lncRNA SNHG3 expression was significantly upregulated in PCa samples, which was associated with poor survival outcomes. Mechanistically, SNHG3 promoted cell proliferation, migration and invasion by inhibiting miR-1827 expression in the cytoplasm. The inhibited malignance of PCa cells induced by SNHG3 knockdown was recovered following miR-1827 knockdown. Taken together, these results suggest that lncRNA SNHG3 promotes PCa progression through miR-1827 via the Wnt/AKT/mTOR pathway. Thus, the lncRNA SNHG3/miR-1827/Wnt/AKT/mTOR axis may be used as a diagnostic biomarker for patients with PCa, and may serve as a promising therapeutic target for the treatment of PCa.

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Availability of data and materials

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Authors' contributions

HG designed the present study. MH, MR, XC, and MS performed the cell experiments. MH, ZZ and YY performed the *in vivo* experiments. MH and HG analyzed the data. HG and MH drafted the initial manuscript. MH and HG confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of Affiliated Nanhai Hospital, Southern Medical University (Foshan, China; IRB no. 2020-027-1) and written informed consent was provided by all patients prior to the study start. All animal experiments were reviewed and approved by the Animal Welfare Committee of Affiliated Nanhai Hospital, Southern Medical University (Foshan, China; IRB no. IRB-NY-2020-018).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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