Long non-coding RNA SNHG17 enhances the aggressiveness of C4-2 human prostate cancer cells in association with β-catenin signaling

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Abstract. Long non-coding (lnc) RNAs have emerged as important regulators of cancer development and progression. Several lncRNAs have been reported to be associated with prostate cancer (PCa); however, the involvement of lncRNA SNHG17 in PCa remains unclear. In the present study, the mRNA expression level of SNHG17 in 58 pairs of PCa tumor samples and adjacent non-tumor tissues, as well as in PCa tumor cell lines was analyzed. The regulatory effect of SNHG17 on the oncogenic phenotypes of the C4-2 tumor cell line was also investigated. The clinicopathological analysis revealed that SNHG17 mRNA expression level was increased in the PCa tumor samples, and its high expression levels were associated with poor patient outcomes, indicating that SNHG17 may act as a biomarker for the prognosis of PCa. SNHG17 mRNA expression level was also increased in different PCa tumor cell lines. Functionally, SNHG17 increased C4-2 tumor cell growth and aggressiveness by stimulating tumor cell proliferation, survival, invasion and resistance to chemotherapy. Furthermore, SNHG17 promoted *in vivo* tumor growth in a xenograft mouse model. Notably, the SNHG17-induced *in vitro* and *in vivo* oncogenic effects were associated with activation of the β-catenin pathway. The results from the present study revealed that lncRNA SNHG17 could be an important regulator in the oncogenic properties of human PCa and may; therefore, represent a potential PCa therapeutic target.

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Abbreviations: GSK-3β, glycogen synthase kinase-3β; HPrEC, human prostate epithelial cells; lncRNAs, long non-coding RNAs; LEF, lymphoid enhancer factor; PCa, prostate cancer; TCF, T cell factor

Key words: prostate cancer, lncRNA, SNHG17, Wnt

Introduction

Prostate cancer (PCa) is the most common type of cancer among men, worldwide (1.6 million cases in 2015) (1). Patients with localized PCa can achieve long-term survival when treated with surgery, radiotherapy and androgen-deprivation therapy. However, a large proportion of tumors may progress to castration-resistant prostate cancer (CRPC), which is an aggressive cancer type and typically results in metastasis (2,3). Therefore, it is imperative to investigate novel molecular targeted therapies for PCa that are based on an in-depth knowledge of the signaling pathways underlying prostate carcinogenesis.

Long non-coding (lnc)RNAs are a type of non-coding RNA, that are >200 nucleotides in length. lncRNAs have been associated with numerous cellular activities, including proliferation, apoptosis, survival, differentiation and cell cycle control (4). Both increased and decreased expression levels of lncRNAs have been demonstrated to play an important role in human tumorigenesis (4-6). For example, in breast cancer, upregulation of ARNALI and lnc015192 or downregulation of PDCD4-AS1 and ANCR has been implicated in tumor progression (6). High-throughput RNA sequencing has discovered a variety of novel lncRNAs in the past 10 years, which are also aberrantly regulated in PCa; however, only a few have been functionally examined. For example, the expression level of lnc01296 was associated with the preoperative blood level of prostate-specific antigen (PSA), lymph node metastasis, and tumor stage (7). Petrovics *et al* (8) reported that the overexpression of PCGEM1 in tumor cells promoted proliferation and colony formation, and the increased expression level was associated with a higher risk of developing PCa. In addition, Linc00963 was associated with androgen-independent PCa, and Linc00963 knockdown inhibited LNCaP and C4-2 cell viability, motility and invasiveness (9).

lncRNA SNHG17 is located on 20q11.23 and is known as an unfavorable prognostic factor in colorectal cancer, and the overexpression of SNHG17 stimulated tumor cell proliferation by epigenetically silencing P57 (10). SNHG17 is also

overexpressed by non-small cell lung cancer and gastric cancer tissues, and promoted oncogenic phenotypes in the two types of cancer (11,12). Microarray analysis found SNHG17 to be one of four lncRNAs that was significantly upregulated in metastatic and androgen-independent C4-2 tumor cells compared with that in parental non-metastatic, androgen-dependent LNCaP cells (9). This suggested that SNHG17 functions as an important regulator in human PCa. However, to the best of our knowledge, the associations between SNHG17 and the growth and aggressiveness of PCa have not been reported.

Wnt/β-catenin signaling is crucial for cell polarity, tissue development and homeostasis. The binding of Wnt ligands to the receptor Frizzled and the co-receptor low-density lipoprotein receptor-related protein-5 or 6 activated Dishevelled, which in turn leads to the inhibition of glycogen synthase kinase-3β (GSK-3β). The inhibition of GSK-3β repressed β-catenin phosphorylation to prevent its degradation by the GSK-3β-adenomatous polyposis coli-axin multi-protein complex. As a result, the stabilized β-catenin could translocate to the nucleus and interact with T cell factor/lymphoid enhancer factor (TCF/LEF) to initiate the transcription of target genes (e.g., *c-myc* and *cyclin D1*), which are involved in the control of cellular processes, such as growth, differentiation, and metabolism (13,14). Hyperactive aberrant Wnt/β-catenin signaling has been associated with different types of cancer (14), including PCa (15).

In the present study, the SNHG17 mRNA expression levels in PCa tumors and adjacent non-tumor tissue samples were analyzed. The cellular and molecular mechanisms that underlie SNHG17-mediated oncogenic properties in the C4-2 human PCa cell line were also investigated. It was found that SNHG17 could be an important regulator of tumor aggressiveness and that this oncogenic effect was associated with β-catenin signaling activity.

Materials and methods

Acquisition of human tissues. The present study was approved by the Human Ethics Committee of Qingdao Municipal Hospital (QMH;l Qingdao, China). The patients were recruited from QMH and verbal informed consent was provided by each patient. The diagnosis of PCa was performed using both MRI radiography and prostate biopsy. Only patients displaying multiple positive results in the biopsy were included in the study. Patients that died from diseases other than PCa were excluded from the study. Based on the criteria, a total of 58 patients (mean age, 68.5±4.2 years; range, 56.2-85.6 years) were selected for the study. Paired PCa tumor samples and matched adjacent non-tumor tissues (3-mm between tumor and non-tumor tissue) were obtained from each patient undergoing radical prostatectomy between January 2013 and December 2018 at QMH. Clinical factors (age, sex, tumor diameter and tumor grade) from these patients were used for clinicopathological analysis. All specimens were immediately stored at -80˚C prior to RNA isolation. The integrity of the RNA was confirmed using 1% agarose gel electrophoresis before the reverse transcription-quantitative (RT-q)PCR assay. Based on the mean value of the *SNHG17* mRNA expression level (3.412±1.66) in the tumor samples, the patients were divided into two groups: The high- and the low-expression level groups.

Cell culture. The human prostate cancer cell lines LNCaP [clone FGC; CRL-1740; American Type Culture Collection (ATCC)] and C4-2 (CRL-3314; ATCC), and the normal human prostate epithelial cell line, HPrEC (PCS-440-010; ATCC) were cultured in DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% FCS (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C in a humidified incubator with 5% CO_2. For SNHG17 knockdown, recombinant lentivirus-mediated SNHG17-short hairpin (sh)RNA vectors (Lv-SNHG17-shRNA; Sangon Biotech, Co., Ltd.) were prepared. These vectors were ready-to-use viral particles, which contained a pool of three expression constructs, each encoding target-specific shRNA. The three SNHG17 shRNA sequences are shown in Table SI. When the C4-2 cells reached 80% confluency, they were transduced with Lv-SNHG17-shRNA viral particles at a multiplicity of infection of 25 in the presence of polybrene (8 μg/ml; Sigma-Aldrich; Merck KGaA). After 16 h at 37˚C, the virus-containing medium was removed and fresh complete medium was added. The transduced cells were then split at a ratio of 1:5 and treated with puromycin (5 μg/ml; Thermo Fisher Scientific, Inc.) for 2 weeks at 37˚C in a humidified incubator with 5% CO_2. The cells transduced with negative control (NC) shRNA lentivirus harboring a scrambled shRNA sequence were used as the control. For the overexpression of SNHG17, cDNA encoding the human SNHG17 gene was amplified using PCR and subcloned into the pcDNA3.1 expression vector (Invitrogen; Thermo Fisher Scientific, Inc.). When the C4-2 cells had reached 80% confluency they were transfected with pcDNA-SNHG17 (2 μg/100 μl medium) using FuGENE reagent (Roche Diagnostics) at 37˚C for 24 h, according to the manufacturer's protocol. The C4-2 cells transfected with pcDNA3.1 empty vector were used as the control. After 24 h, the cells were used for the subsequent experimentation. The cell lines were routinely tested for contamination.

Proliferation, viability, and apoptosis assays. The proliferation of SNHG17-knockdown, SNHG17-overexpressing, or control C4-2 tumor cells was assessed using an MTT assay kit (Abcam) according to the manufacturer's protocol. Dimethyl sulfoxide was used to dissolve the formazan product. The absorbance at 570 nm was measured. Cells were cultured and measured every 24 h for 5 days. For treatment, SNHG17-overexpressing cells were cultured in DMEM-10% FCS and treated with ICG001 (Selleck Chemicals) at a dosage of 10 μM.

Cell viability was determined using the CellTiter-Glo assay kit (Promega Corporation). Briefly, the SNHG17-knockdown or control C4-2 cells were seeded at $1x10^4$, $5x10^4$ and $1x10^5$ cells/well in one 96-well plate, then the assay reagent was added to each well to induce cell lysis, and the luminescence signal was measured using a microplate reader. The viability was evaluated based on the output of relative luminescence units (RLU), which correlates with viable cell numbers.

For the apoptosis assay, the SNHG17-knockdown, SNHG17-overexpressing, or control C4-2 cells were cultured under normal (DMEM; 10% FCS) or serum starvation conditions (DMEM; 0.5% FCS) for 48 h. Caspase 3 activity was analyzed with a colorimetric assay kit (Novus Biologicals) and the absorbance at 405 nm was recorded using a microplate reader (Synergy HTX, BioTek Instruments Inc.). Comparison

of the absorbance allows the determination of the fold change in caspase 3 activity. For treatment, SNHG17-overexpressing cells were cultured in normal or serum-starved medium and treated with ICG001 at 10 μM for 48 h.

Immunofluorescence (IF) and immunocytochemistry (ICC). The SNHG17-knockdown or control C4-2 tumor cells were fixed in methanol for 10 min at room temperature (RT) and blocked with 10% normal horse serum (Sigma-Aldrich; Merck KGaA) for 1 h at RT. After washing with PBS, the cells were incubated overnight at 4˚C with a rabbit antibody against human β-catenin (1:100, cat. no. 8480, Cell Signaling Technology, Inc.). The cells were then washed with PBS and further incubated with FITC-conjugated secondary antibody (1:200, cat. no. ab97063, Abcam) for 45 min at RT. The cells were then incubated in DAPI-containing mounting medium for nuclear staining (Abcam) for 5 min at RT. Cells were monitored using a fluorescence microscope (Carl Zeiss, x200). For ICC, the SNHG17-knockdown or control C4-2 cells were incubated with a rabbit antibody against human TCF1 (1:100, cat. no. 2203, Cell Signaling Technology, Inc.) at 4˚C overnight. After washing with PBS, cells were treated with biotinylated donkey anti-rabbit secondary antibody (1:500, cat. no. ab208000, Abcam) for 1 h at RT. Cells were counterstained with eosin solution (Sigma-Aldrich) for 30 sec at RT. Nuclear staining of TCF1 was monitored using a light microscope (Olympus, x200).

Invasion assay. The SNHG17-knockdown or control C4-2 tumor cell invasion was evaluated using the Boyden chamber system in 24-well plates. The filter inserts, with 8-μm pore size, were pre-coated with Matrigel for 30 min at 37˚C. The tumor cells were cultured in DMEM with 10% FCS at 37˚C. After 5 h, the cells were cultured in fresh DMEM, with 1% FCS for 24 h. The cells were then harvested and plated on the upper chamber of the Matrigel-coated 24-well Transwell filters ($2x10^5$ cells/filter) and incubated in DMEM with 1% FCS at 37˚C. DMEM-20% FCS medium was added to the lower chamber. After 24 h, unmigrated cells from the upper chamber were removed and the inserts were stained with eosin for 30 sec at RT. The invading cells were counted under a light microscope (Olympus, x200).

Docetaxel sensitivity assay. For the chemotherapeutic resistance assay, the SNHG17-knockdown or control C4-2 cells were plated into three 96-well plates at $5x10^4$ cells/well. After 6 h, the cells were treated with docetaxel at dosages of 0, 1, 2.5, 5 or 10 nM for 1, 3 or 5 days. The cell viability was determined using a Cell Counting Kit-8 kit (Sigma-Aldrich; Merck KGaA). The CCK-8 solution provided from the kit was added to each well and incubated at 37˚C for 2 h. The absorbance at 450 nm was measured for each well.

TCF reporter assay. The SNHG17-knockdown and control C4-2 cells were co-transfected with TOPflash (Sigma-Aldrich; Merck KGaA) and pRL (Promega Corporation), or FOPflash (Sigma-Aldrich; Merck KGaA) and pRL, using FuGENE reagent (Roche Diagnostics), according to the manufacturer's protocols. TOPflash is a β-catenin-responsive firefly luciferase reporter plasmid, which contains three copies of the TCF binding site upstream of the thymidine kinase minimal promoter and luciferase open reading frame. FOPflash is a non β-catenin-responsive plasmid that contains mutated TCF binding site (16). pRL is a constitutively expressing *Renilla* luciferase plasmid used as an internal control for transfection efficiency (17). The medium was removed and replaced with fresh medium containing 5 mM LiCl (or NaCl as a control) 24 h post-transfection. Cell lysates were extracted using reporter lysis buffer (Promega Corporation) 24 h after treatment. Luciferase activity was detected using dual-luciferase assay kits (Promega Corporation). Both firefly and *Renilla* luminescence signals were recorded. The firefly luciferase activities of TOPflash and FOPflash were normalized to the *Renilla* luciferase activity of pRL, respectively. The ratio of TOPflash activity to FOPflash activity (TOPflash/FOPflash) was calculated for the measurement of TCF reporter activity.

RT-qPCR and western blot analysis. Total RNA was extracted from the specimens cells using TRIzol® (Thermo Fisher Scientific, Inc.). The RNA was then reverse transcribed into cDNA using the SuperScript III reverse transcriptase kit (Thermo Fisher Scientific, Inc.) at 50˚C for 50 min. qPCR was conducted using SYBR green master mix (Thermo Fisher Scientific, Inc.). The cycling conditions were: 50˚C for 2 min; 95˚C for 10 min; then 95˚C for 15 sec and 60˚C for 1 min for 40 cycles. PCR products were analyzed with the ABI PRISM 7900HT Sequence Detections System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The relative mRNA level of the target gene was determined using ABI software (RQ Manager, version 1.2). The threshold cycle of the target gene was normalized to that of the endogenous GAPDH transcript (ΔΔCt). The fold change was determined using the formula $2^{-\Delta\Delta Cq}$ method (18). The designed primers for PCR are listed in Table I.

For the western blot analysis, the cell lysates were extracted from SNHG17-knockdown or control C4-2 cells with RIPA lysis buffer (Sigma-Aldrich; Merck KGaA) and 25 μg of each protein sample was separated using 8% SDS-PAGE. After electrophoresis, the proteins were transferred to a PVDF membrane (Bio-Rad Laboratories, Inc.), then washed with TBS/0.1% Tween-20 (TBST) and blocked with 1% BSA (Sigma-Aldrich; Merck KGaA) for 30 min at RT. Subsequently, the membrane was incubated with rabbit anti-human β-catenin (1:1,000, cat. no. 9562, Cell Signaling Technology, Inc.) and rabbit anti-human β-tubulin antibody (1:1,000, cat. no. 2146, Cell Signaling Technology, Inc.) overnight at 4˚C. The PVDF membrane was then incubated with an HRP-conjugated secondary antibody (1:3,000, cat. no. 7074, Cell Signaling Technology, Inc.) for 1 h at RT. Protein expression was detected using an enhanced chemiluminescence kit (Bio-Rad Laboratories, Inc.). Densitometry of the bands was measured with ImageJ software (v1.51j8; National Institutes of Health).

Xenograft mouse model. A total of 14 BALB/c female nude mice (8-10 weeks old; weight, 21.18±1.23 g) were used in the present study. The C4-2 tumor cells were washed with PBS and mixed with PBS/Matrigel (1:1) at a density of $10^4/\mu$l. The animals were anesthetized using an intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). Next, $1x10^6$ (100 μl) SNHG17-knockdown or control C4-2 tumor cells (7 animals/group) were subcutaneously injected

Table I. Primers designed for quantitative PCR.

Gene	Forward (5'-3')	Reverse (5'-3')
SNHG17	TGCTTGTAAGGCAGGGTCTC	ACAGCCACTGAAAGCATGTG
CTNNB1	TCTTGCCCTTTGTCCCGCAAATCA	TCCACAAATTGCTGCGTCCCA
TCF1	CGGGACAGAGGACCATTACA	CCACCTGCCTCGGCCTGCCAAAGT
TCF4	CTGCCTTAGGGACGGACAAAG	TGCCAAAGAAGTTGGTCCATTTT
LEF1	CTTTATCCAGGCTGGTCTGC	TCGTTTTCCACCATGTTTCA
c-myc	TTCGGGTAGTGGAAAACCAG	AGTAGAAATACGGCTGCACC
Cyclin D1	TCTGGCATTTTGGAGAGGAAG	CATCTACACCGACAACTCCATC
Axin2	CAAGGGCCAGGTCACCAA	CCCCCAACCCATCTTCGT
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

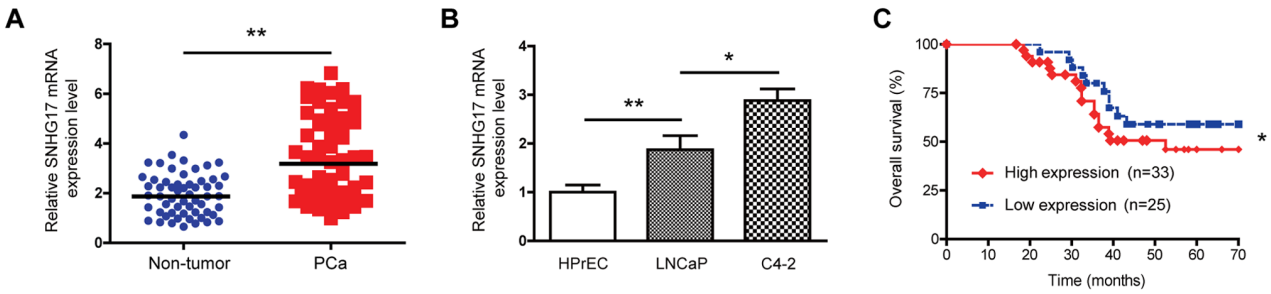

Figure 1. Upregulation of lncRNA SNHG17 is associated with poor outcomes in patients with PCa. (A) Reverse transcription-quantitative PCR was performed to determine the SNHG17 mRNA expression level in 58 paired tumor and non-tumor tissues, and (B) HPrEC, LNCaP and C4-2 cell lines (n=3). (C) Kaplan-Meier survival curve indicating that patients with high SNHG17 mRNA expression levels had poorer overall survival rates compared with patients with low expression levels. The data are presented as the mean ± SD. *P<0.05, **P<0.01. PCa, prostate cancer; lnc, long non-coding.

into the flanks of the mice. Local tumor growth was examined using a caliper every 5 days. The tumor volume (mm^3) was calculated using the 'width^2 x length/2' formula. At 50 days after tumor cell injection, the mice were sacrificed using CO_2, with a fill rate of 30% of the chamber volume/min, to the existing air inside the chamber to minimize the animals' distress. The death of mice was confirmed by examining the respiratory rate and cardiac arrest. All tumor samples were harvested immediately following the sacrifice of the animals at day 50. During the entire experiment, all the animals were maintained in a standard mouse facility maintained at 22-24˚C, 50-60% humidity, a 12-h light/dark cycle, and free access to water and food. The animal study was reviewed and approved by the Animal Care Committee of Qingdao University.

Immunohistochemistry (IHC). Tumors were harvested and fixed in 4% paraformaldehyde at 4˚C for 2 days. The samples were embedded in paraffin and 5-μm thick sections were made. For IHC analysis, tumor sections were deparaffinized in xylene and were rehydrated via graded alcohol (100%, 95%, 90%, 80% and 70%) and distilled water. After washing with PBS, sections were incubated with proteinase K (Dako) for 10 min at RT for antigen retrieval. Sections were washed with PBS and blocked with horse serum (Sigma-Aldrich; Merck KGaA) for 30 min at RT. Sections were then incubated with rabbit anti-Ki67 (1:200, cat. no. ab16667, Abcam), rabbit anti-β-catenin, (1:100, cat. no. 8480, Cell Signaling Technology, Inc.), or rabbit anti-TCF1 (1:100, cat. no. 2203, Cell Signaling Technology, Inc.), respectively, overnight at 4˚C. Sections were washed with PBS and incubated with biotinylated donkey anti-rabbit secondary antibody (1:1,000, cat. no. ab208000, Abcam) for 1 h at RT. After washing with PBS, sections were incubated with DAB substrate (Vector Lab) for 5 min at RT, and were counterstained with Mayer's hematoxylin solution (Sigma-Aldrich; Merck KGaA) for 10 sec at RT. Positive stains were monitored under a light microscope (Olympus, x100).

Statistical analysis. A two-tailed paired Student's t-test or one-way ANOVA followed by Tukey's post hoc test was used for comparisons between two groups or among multiple groups, respectively. A χ^2 test was used to analyze the clinicopathology characteristics. A log-rank test was used to analyze the Kaplan-Meier survival curve. Experiments were performed in triplicate and repeated three times. Data are presented as the mean ± standard deviation. All statistical analyses were conducted using the GraphPad Prism software (v5.0; GraphPad Software, Inc.).

Results

SNHG17 upregulation is associated with poor outcomes in patients with PCa. To investigate the role of SNHG17 in prostate carcinogenesis, the expression level of SNHG17 was determined in 58 pairs of human PCa tumor and matched adjacent non-tumor tissues using RT-qPCR. As shown in Fig. 1A, the expression level of *SNHG17* mRNA was higher in

Table II. Analysis of the clinicopathological characteristics.

Characteristic	Number	Expression level of SNHG17		P-value
		Low (n=25)	High (n=33)	
Mean age, years				
<65	16	6	10	0.594
≥65	42	19	23	
Tumor diameter, cm				
<2.0	27	13	14	0.469
≥2.0	31	12	19	
Gleason score				
<6	26	12	14	0.672
≥7	32	13	19	
Histological grade				
II+III	30	18	12	0.007
IV	28	7	21	
Tumor stage				
T2	18	13	5	0.003
T3+T4	40	12	28	
Lymph node metastasis				
Yes	22	4	18	0.003
No	36	21	15	
Distant metastasis				
Yes	19	4	15	0.018
No	39	21	18	

the PCa samples compared with that in the non-tumor tissues, suggesting that this lncRNA was upregulated in PCa tumors. The mRNA expression level of SNHG17 was also compared between tumor and epithelial cells, and the results showed that the mRNA expression level of *SNHG17* was elevated in the LNCaP and C4-2 tumor cells compared with that in the normal HPrEC cells (Fig. 1B). Notably, the aggressive and metastatic C4-2 tumor cells exhibited a 1.54-fold increase in the *SNHG17* mRNA expression level compared with that in the parental less aggressive, non-metastatic LNCaP cells, suggesting that high SNHG17 expression level might be associated with tumor aggressiveness. These findings indicated that SNHG17 was upregulated in human PCa.

Next, the association between the SNHG17 mRNA expression level and survival time in patients with PCa was also analyzed. Based on the mean SNHG17 mRNA expression level (3.412±1.66), the patients were divided into high-(n=33) and low-expression level groups (n=25). As shown in Fig. 1C, the Kaplan-Meier survival curve indicated that compared with that in those with low expression levels, patients with high SNHG17 mRNA expression levels exhibited poor overall survival time. The clinicopathological analysis showed that most patients suffered from advanced PCa. For example, among the 58 patients, 32 patients displayed a Gleason score of ≥7; 40 patients had T3+T4 stage PCa; 22 patients had lymph node metastasis, and 19 patients developed distant metastasis. Notably, high mRNA expression levels of *SNHG17* were associated with advanced histological grade, tumor stage and metastasis. However, no association was found between the SNHG17 mRNA expression level and age, tumor size or Gleason score (Table II). These findings suggested that increased SNHG17 expression levels in tumors may be a predictor of poor patient outcomes.

SNHG17 increases the proliferation, viability but reduces apoptosis in the C4-2 tumor cells. To investigate the role of SNHG17 in modulating PCa oncogenic properties, a knockdown approach was used in the C4-2 tumor cells to determine the function of SNHG17 at the cellular level. RT-qPCR analysis showed that knockdown of *SNHG17* resulted in a 68% reduction in *SNHG17* mRNA expression level (Fig. 2A). The MTT assays showed that knockdown of SNHG17 significantly ($P<0.01$) decreased the proliferation of the C4-2 cells (Fig. 2B). Tumor cell viability was also decreased in cells treated with *SNHG17* shRNA (Fig. 2C). Subsequently, caspase 3 activity was measured and it was found that SNHG17 knockdown only slightly increased caspase 3 activity in cells cultured under normal conditions (10% FCS). However, SNHG17 knockdown significantly stimulated caspase 3 activity in the cells cultured under serum starvation conditions (0.5% FCS) (Fig. 2D). These data suggested that SNHG17 increases the proliferation and viability, but reduces apoptosis in PCa tumor cells.

SNHG17 increases invasion and chemotherapeutic resistance in the C4-2 tumor cells. Invasive ability is an essential marker of tumor cell aggressiveness (19). Therefore, a Transwell

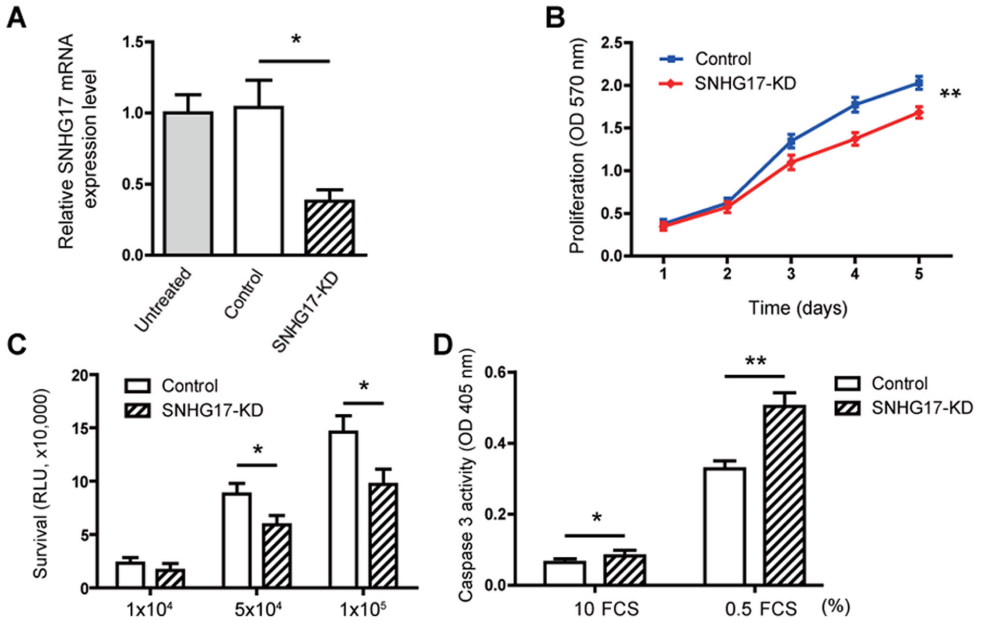

Figure 2. KD of SNHG17 inhibits the proliferation and survival but increases caspase 3 activity of the C4-2 tumor cells. (A) Reverse transcription-quantitative PCR was used to determine the mRNA expression level of SNHG17 in untransduced, SNHG17-KD and control cells (n=3). (B) MTT assay was used to determine cell proliferation in cells transfected with SNHG17 or control shRNA (n=4). (C) Viability assay was used to measure cell survival cells transduced with SNHG17 or control shRNA (n=3). (D) Caspase 3 activity was measured in cells transduced with SNHG17 or control shRNA (n=3). The data are presented as the mean ± SD. *P<0.05, **P<0.01. FCS, fetal calf serum; KD, knockdown; OD, optical density; sh, short hairpin; RLU, relative luminescence units.

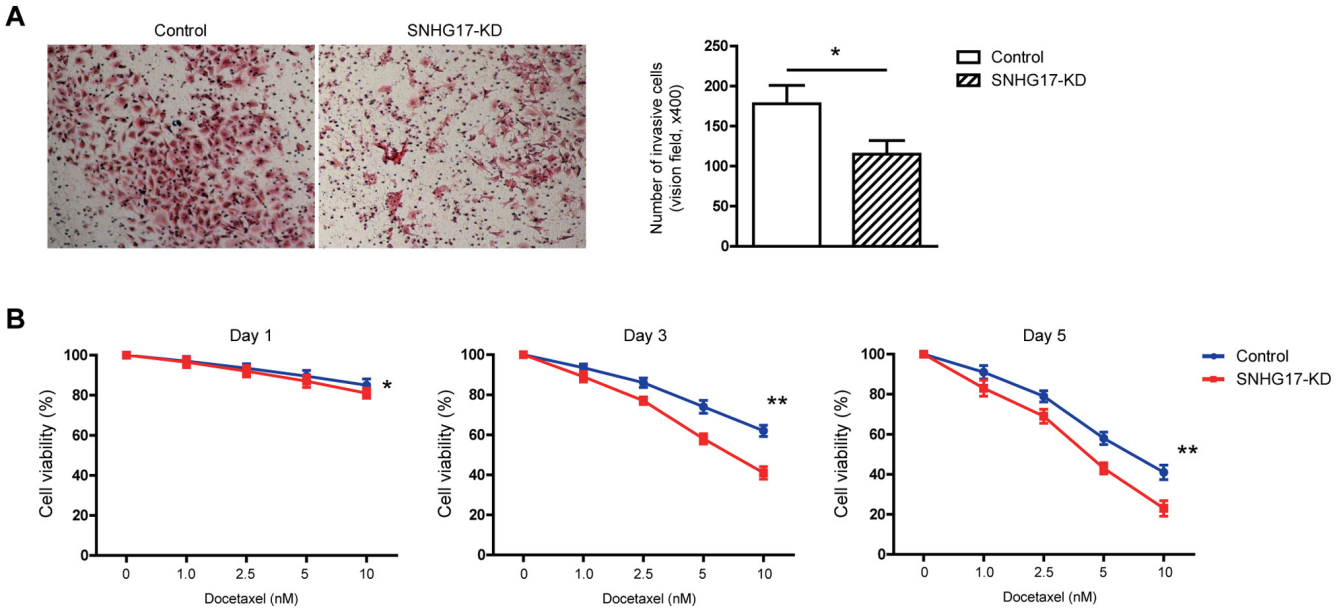

Figure 3. KD of SNHG17 suppresses the invasion and chemotherapeutic resistance of the C4-2 tumor cells. (A) Invasion assay showing that KD of SNHG17 reduces the number of invasive cells (n=3); magnification x400. (B) Viability assay in control and SNHG17-KD tumor cells, treated with docetaxel (0, 1, 2.5, 5 and 10 nM) for 1, 3 and 5 days, respectively. (n=4). The data are presented as the mean ± SD. *P<0.05, **P<0.01. KD, knockdown.

chamber was used to investigate how altering the SNHG17 mRNA expression level in tumor cells affected their invasive ability. It was found that SNHG17 knockdown significantly decreased the number of invading C4-2 tumor cells compared with that in the control group (Fig. 3A), suggesting that SNHG17 increased tumor cell invasion.

Since 2004, docetaxel has been used as an important chemotherapeutic agent for treating patients with PCa and metastatic CRPC (20); however, most patients with CRPC, who are treated with docetaxel, eventually become refractory, due to the development of drug resistance (21). Therefore it was subsequently investigated whether SNHG17 was associated with chemotherapeutic resistance in the PCa tumor cells, by treating androgen-independent C4-2 cells with various concentrations of docetaxel over different time points. It was found that docetaxel reduced the viability of the tumor cells in a dose-dependent manner. Notably, this reduction was further enhanced by the knockdown of SNHG17 (Fig. 3B). These

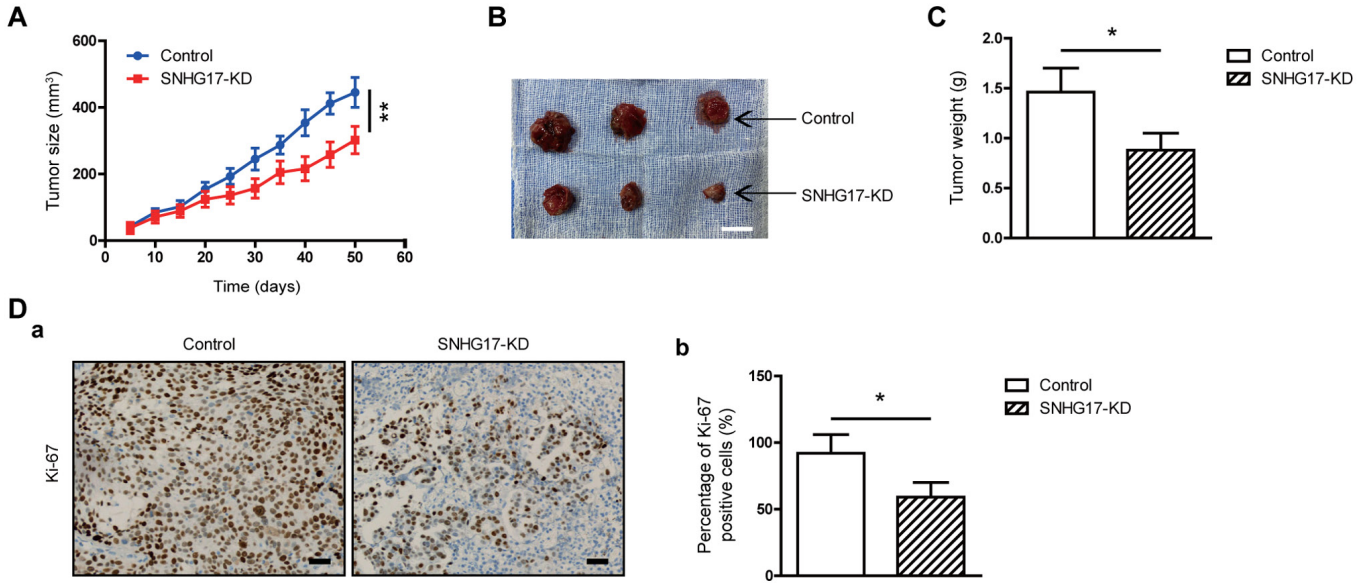

Figure 4. KD of SNHG17 suppresses *in vivo* tumor growth. A total of 14 BALB/c nude mice were divided into two groups (7 mice/group) and were injected subcutaneously with $1x10^6$ control or SNHG17-KD C4-2 tumor cells. (A) Mean tumor size. (B) Images of the tumor samples harvested at day 50 following implantation. Scale bar, 1 cm. (C) Mean tumor weight. (D-a) Immunohistochemistry Ki-67 staining in tumors. Scale bar, 100 μm (D-b) Quantification of the Ki-67 positive cells. The data are presented as the mean ± SD. *P<0.05, **P<0.01. KD, knockdown.

observations suggested that SNHG17 decreased the cellular sensitivity of tumor cells in response to docetaxel treatment, leading to increased chemotherapeutic resistance.

SNHG17 stimulates in vivo tumor growth. As aforementioned it was found that SNHG17 increased tumor cell viability and invasion *in vitro*. To determine whether SNHG17 also increased tumor growth *in vivo*, a subcutaneous xenograft mouse model was used and C4-2 tumor cells were injected into the flanks of the BALB/c female nude mice. After 50 days, the tumor sizes in the mice injected with SNHG17-knockdown cells were smaller compared with that in mice that were injected with the control cells (Fig. 4A and B). The mean weight of the tumor samples was also lower in mice injected with the SNHG17-knockdown tumor cells compared with that in animals injected with the control cells (Fig. 4C). Immunostaining for Ki-67 was also performed to further confirm that SNHG17 knockdown inhibited tumor cell proliferation *in vivo*. As shown in Fig. 4D, the number of Ki-67-positive cells was reduced by ~36% in tumors, that had developed from SNHG17-knockdown cells compared with that in the control group. These results suggest that SNHG17 promotes *in vivo* tumor growth.

SNHG17 facilitates tumor growth via β-catenin activity. As hyperactivity of the Wnt/β-catenin pathway has been associated with human PCa (15), it was determined whether the SNHG17-induced tumor-promoting effect was associated with the activation of this pathway. Notably, SNHG17 knockdown decreased the expression level of β-catenin, at both the mRNA and protein levels in the C4-2 tumor cells (Fig. 5A and B). The IF analysis revealed that β-catenin fluorescence was reduced in the tumor cells following knockdown of SNHG17 (Fig. 5C). The immunocytochemistry results showed that there were fewer TCF1-positive cells in the SNHG17-knockdown group compared with that in the control group (Fig. 5D). Furthermore, the TCF reporter assay showed that SNHG17 knockdown not only inhibited baseline luciferase activity, but also LiCl-enhanced luciferase activity (Fig. 5E). In addition, the gene expression levels of several Wnt signal molecules, such as *TCF1*, *TCF4*, *LEF1*, *c-myc*, *cyclin D1* and *axin2*, were decreased in SNHG17-knockdown C4-2 cells (Fig. 5F). These data indicated that SNHG17 upregulated β-catenin/TCF transcription.

To further confirm that the SNHG17-induced oncogenic effect was mediated by its association with β-catenin, SNHG17 was also overexpressed in the C4-2 cells and treated them with ICG001, an inhibitor of the Wnt/β-catenin signaling pathway. As shown in Fig. 5G, transfection with pcDNA-SNHG17 resulted in a 3.78-fold increase of SNHG17 mRNA expression level compared to that in the control cells. The MTT assay showed that SNHG17 overexpression significantly increased tumor cell proliferation, whereas ICG001 alone only slightly reduced the proliferation rate (Fig. 5Ha). In contrast, ICG001 significantly attenuated SNHG17-accelerated proliferation (Fig. 5Hb). The overexpression of SNHG17 also reduced the caspase 3 activity level of C4-2 cells, whereas this reduction was reversed in the presence of ICG001 (Fig. 5I). To verify the association between SNHG17 and the β-catenin pathway during *in vivo* tumor growth, RT-qPCR analysis was performed and a reduction of β-catenin mRNA expression level in the tumors from SNHG17-knockdown cells was found compared with that in the control group (Fig. 5J). Immunohistochemistry analysis further revealed that the β-catenin staining intensity was decreased in tumors derived from SNHG17-knockdown cells compared with that in the control group (Fig. 5K). The population of TCF1+ cells was also notably decreased in tumors derived from SNHG17-knockdown cells (Fig. 5L). Taken together, these results suggested that SNGH17 could facilitate tumor growth via its association with β-catenin-mediated TCF-dependent transcriptional activity.

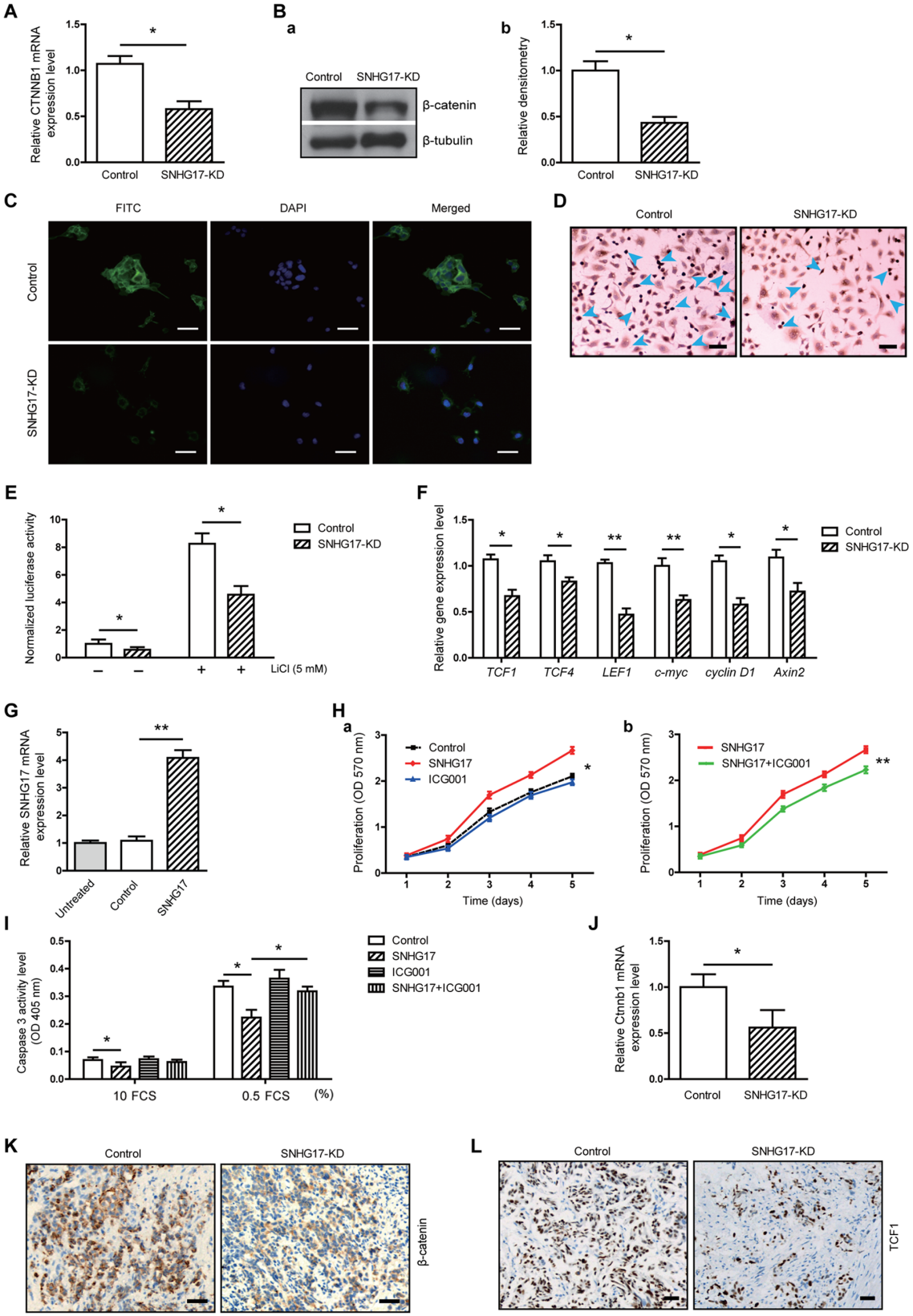

Figure 5. SNHG17 facilitates C4-2 tumor growth via β-catenin. (A) RT-qPCR and (B-a) western blot analysis revealed that β-catenin mRNA and protein expression level was decreased in the SNHG17-KD cells, respectively (n=3). (B-b) Densitometry analysis of β-catenin (normalized to β-tubulin using ImageJ software). (C) Immunofluorescence staining of β-catenin and (D) immunocytochemical staining of TCF1 in the control or SNHG17-KD tumor cells. (E) TCF reporter assay in the control or SNHG17-KD cells, treated with/without LiCl (5 mM) for 24 h (n=3). RT-qPCR was performed to determine the mRNA expression level of (F) different genes in the control or SNHG17-KD tumor cells and (G) SNHG17 in cells transfected with pcDNA-SNHG17 vector (n=3). MTT assay was used to determine cell proliferation in the (H-a) control, SNHG17-overexpressing and control cells treated with ICG001 (10 μM) and (H-b) in SNHG17-overexpressing cells treated with/without ICG001 (n=4). (I) Caspase 3 activity assay was measured in the control or SNHG17-KD cells, treated with/without ICG001 (10 μM) for 48 h (n=3). (J) RT-qPCR was used to measure the mRNA expression level of β-catenin in the tumor samples (n=3). Immunohistochemistry staining of (K) β-catenin and (L) TCF1 in the tumor samples. For C: Scale bar, 50 μm; For D, K and L: Scale bar, 100 μm. The data are presented as the mean ± SD. *P<0.05, **P<0.01. RT-qPCR, reverse transcription-quantitative PCR; KD, knockdown; OD, optical density.

Discussion

In the present study, the role of SNHG17 in human PCa was characterized. SNHG17 mRNA expression level was found to be increased in tumor tissues and its mRNA expression level was also associated with poor patient outcomes. Functionally, SNHG17 increased tumor aggressiveness by promoting cell proliferation, invasion and resistance to chemotherapy. Notably, this modulating effect was mediated via β-catenin signaling activity. The results from the present study suggested that SNHG17 could be a critical regulator of human PCa aggressiveness.

The role of lncRNA in human different types of cancer has received considerable attention in recent years. Several lncRNAs, such as PCA3, PCAT1, CCAT2, GASS, and Linc01296, have been found to play either an oncogenic or tumor-suppressive role in PCa (22). SNHG17 is also a novel lncRNA and has been associated with lung (11), breast (23) and gastric (12) cancers. Based on a microarray, SNHG17 was shown to be among the few lncRNAs, that were upregulated in metastatic and androgen-independent C4-2 tumor cells compared with that in the parental non-metastatic, androgen-dependent LNCaP cells (9). However, it is unclear whether SNHG17 is associated with PCa progression.

To investigate this issue, 58 pairs of PCa tumor and matched adjacent non-tumor tissues were analyzed. The mRNA expression level of SNHG17 was increased in the PCa samples compared with that in the non-tumor tissues, suggesting that SNHG17 may play a role in PCa. Notably, patients with high tumor mRNA expression levels of SNHG17 had a poorer overall survival rate compared with patients with lower SNHG17 mRNA expression levels. The clinicopathological analysis further showed that high SNHG17 mRNA expression level was associated with indicators of cancer aggressiveness, including advanced histological grade, tumor stage and metastasis. These results showed that increased SNHG17 mRNA expression level could be a good predictor of poor outcomes in patients with PCa. In the present study, the overall survival was relatively lower compared with that in reported statistics (24). This may be due to the fact that all the patients selected in the present study had advanced PCa. For example, among the 58 patients, 32 patients displayed a Gleason score of ≥7; 40 patients had T3+T4 stage PCa; 22 patients had lymph node metastasis, and 19 patients developed distant metastasis (Table II). In addition, all patients showed multiple positive results using prostate biopsy prior to radical prostatectomy. It should also be noted that only patients expressing high-level SNHG17 displayed a shortened overall survival rate, whereas patients expressing low-level SNHG17 significantly had a prolonged survival rate.

In addition, one limitation of the present clinicopathological study is that it did not include PSA doubling time (PSADT) data; PSADT has been found to be a useful marker to predict patient outcomes. Takeuchi *et al* (25) reported that patients with longer preoperative PSADTs (>24 months) displayed favorable pathological findings (80% of patients with T2 stage and 55% of patients with a Gleason score of 2-6), and a higher PSA non-recurrence rate compared with patients who had shorter preoperative PSADTs (<24 months). In androgen-independent PCa, patients with a mean PSADT of 12.7 months before deferred antiandrogen therapy showed an improved response compared with that in patients with a mean PSADT of 7.5 months (26). These studies suggest that PSADT is a strong predictor of patient outcomes. Therefore, the inclusion of PSADT data may provide a more creditable clinicopathological evaluation regarding the association between SNGH17 and PCa. Another limitation is that only 58 patients were included in the present study. This sample size could meet the requirement for meaningful statistical assessments in clinicopathological and survival analyses; however, a larger number of samples would be preferred for more reliable evaluation.

The SNHG17 mRNA expression level between tumor cell lines and normal epithelial cells was also compared in the present study. SNHG17 had higher mRNA expression levels in the LNCaP and C4-2 tumor cells compared with that in the normal HPrEC cells. Notably, SNHG17 mRNA expression level was significantly increased in the metastatic C4-2 cell line compared with that in the non-metastatic parental LNCaP tumor cell line. These *in vitro* and *in vivo* results indicated that SNHG17 was associated with the aggressiveness of human PCa. To clarify the mechanism by which SNHG17 regulated the oncogenic properties of PCa, a knockdown approach was used and the modulatory effect of SNHG17 on tumor cell behavior was investigated. SNHG17 has been shown to increase the proliferation of lung and breast cancer cells (11,23). Consistent with these studies, it was found that SNHG17 knockdown decreased cell proliferation and viability, but increased caspase 3 activity in the C4-2 cells, indicating that SNGH17 could exert a growth-promoting effect in the human PCa tumor cells. In a xenograft mouse model, it was also found that SNHG17 knockdown suppressed tumor growth *in vivo*. Ki-67 staining revealed that the inhibition of tumorigenic activity was associated with decreased cancer cell proliferation. Based on these results, SNHG17 could promote PCa progression, at least partly through its positive regulation of tumor cell growth.

Cancer cell invasion is a key factor that contributes to tumor aggressiveness, including metastasis (18). SNHG17 has been reported to induce tumor cell invasion in breast (23) and gastric (12) cancers. Consistent with the previous studies, it was found that SNHG17 knockdown reduced the invasive ability of the C4-2 tumor cells, suggesting that this lncRNA could also promote the invasion of prostate tumor cells. Resistance to chemotherapy is another key factor leading to poor prognosis in patients with cancer (27). Docetaxel has been an important chemotherapy agent to treat patients with PCa and metastatic CRPC (20); however, most patients with CRPC treated with docetaxel eventually develop drug resistance (19). In the present study, it was found that SNHG17 knockdown significantly reduced the viability of the C4-2 tumor cells following docetaxel treatment, suggesting that SNHG17 reduced the cellular sensitivity of PCa cells in response to docetaxel. Based on the results from the present study, it is possible that SNHG17 promoted the aggressiveness of prostate tumor cells by enhancing both their invasive ability and their resistance to chemotherapy.

Furthermore, it was found that SNHG17 facilitated tumor growth and progression in association with β-catenin signaling activity. Increasing evidence has indicated that

lncRNAs regulate various cellular processes through the activation/inactivation of signaling pathways. For example, the lncRNA PICART1 suppressed gastric tumor cell proliferation by positively regulating the PI3K/AKT and MAPK/ERK pathways (28). Rani *et al* (29) reported that LncND induced neuroblastoma cell proliferation and neuronal differentiation via its crosstalk with the Notch signaling pathway. The interaction between lncRNAs and the Wnt signaling pathway has also received increasing attention (30). Several lncRNAs, such as lncSOX4 and SLCO4A1-AS1, have been reported to facilitate tumor cell motility and metastasis through a β-catenin-dependent mechanism in osteosarcoma and colorectal cancer (31,32). Silencing of HOXA11-AS negatively regulated hepatocellular carcinoma stem cell growth and self-renewal via the inactivation of the Wnt signaling pathway (33). In the present study, it was found that SNHG17 knockdown inhibited the accumulation of β-catenin. Notably, the TCF reporter activity and the mRNA expression level of several key β-catenin signaling molecules, such as TCF1, TCF4, LEF1, axin2, c-myc, and cyclin D1, were downregulated in the SNHG17-knockdown tumor cells. These findings support our hypothesis that SNHG17 stabilized β-catenin and subsequently activated the TCF-dependent transcription of Wnt target genes. Notably, the SNHG17-induced tumor cell growth and SNHG17-reduced cell apoptosis were attenuated following treatment with the β-catenin inhibitor, ICG001. The animal study from the present study also showed that β-catenin and TCF1 protein expression level was inhibited in the tumor samples from mice treated with SNHG17-knockdown cells. Taken together, these results indicated that SNHG17 induced PCa tumor growth in association with β-catenin/TCF activity. The present study has identified the SNHG17/β-catenin/TCF axis in PCa tumor cells; however, the possibility that other mechanisms may also be involved cannot be excluded. For example, Kim *et al* (34) reported that pimozide repressed the proliferation of PCa tumor cell lines (PC3 and DU145) via the generation of reactive oxygen species (ROS). Takeuchi *et al* (35) also reported that ROS was associated with the proapoptotic activity of bladder cancer cells. Therefore, whether SNHG17 exerts its tumor-promoting effect by targeting ROS merits further investigation.

In conclusion, the results from the present study demonstrated that lncRNA SNHG17 mRNA expression level was increased in human PCa and, thus, represents a potential biomarker of poor prognosis. Mechanistically, SNHG17 increased cell proliferation, viability, invasion and resistance to chemotherapy. The SNHG17-induced oncogenic effect was mediated via β-catenin activity. The present study revealed that SNHG17 could be critically implicated in PCa and may be considered as a potential molecular target for PCa treatment.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author's contributions

HJZ and HZ designed the study. HJZ, HJD and PW performed experiments and collected the data. HJZ, HJD and HZ performed the data analysis. HJZ and HZ drafted the initial manuscript. HJZ, HJD, and HZ confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All animal procedures were reviewed and approved by the Animal Care Committee of Qingdao University (approval no. 201803233A). The present study was also approved by the Human Ethics Committee of Qingdao Municipal Hospital (approval no. 05243) and verbal informed consent was provided by each patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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