Mechanistic Study of Long Non-Coding RNA SNHG3 in Promoting Prostate Cancer Proliferation and Invasion

Keywords
prostate cancer, proliferation, invasion, lncRNA SNHG3

Abstract

Introduction

Background:SNHG3 (small nucleolar RNA host gene 3) is a long non-coding RNA (lncRNA) that plays a pivotal role in cellular regulation. It is intricately involved in modulating the cell cycle, enhancing cell proliferation, and inhibiting apoptosis. These functions position SNHG3 as a significant contributor to oncogenic processes. Given its substantial influence on tumorigenesis, elucidating the exact molecular mechanisms through which SNHG3 operates is paramount. Comprehensive understanding of SNHG3's interactions and pathways is not only critical for advancing our knowledge of cancer biology but also holds immense potential for identifying novel therapeutic targets. By targeting the regulatory networks associated with SNHG3, new strategies can be developed for the effective treatment of malignancies where SNHG3 is implicated.

Material and methods

Methods:We investigated the role of lncRNA SNHG3 in promoting prostate cancer (PCa) proliferation and invasion. Using TCGA data, we assessed SNHG3 expression in cancer/adjacent tissue, Gleason score, biochemical recurrence, and overall survival. SNHG3 expression was manipulated in four cell types via siRNA interference/over-expression. Various experiments were conducted to confirm SNHG3's role in PCa, including clone ability, apoptosis, migration, Transwell invasion, and subcutaneous tumor experiments in nude mice.

Results

Results:In PCa, SNHG3 was highly expressed, especially in Gleason score >7 patients, correlating with shorter overall survival. Interfering with SNHG3 using a plasmid reduced proliferation, increased apoptosis, and decreased migration and tumor growth. Conversely, SNHG3 overexpression yielded opposite results.

Conclusions

Conclusion:The lncRNA SNHG3 promotes PCa proliferation and invasion while inhibiting cell apoptosis.

Mechanistic Study of Long Non-Coding RNA SNHG3 in Promoting Prostate Cancer Proliferation and Invasion

Zebin Shi[1#], Heyun Sun[1#],Wei Zhang[2], Wenyuan Zhuang[1], Lei Zhang[1],Xiaokai Shi[1 3], Shenglin Gao[1 3],Lifeng Zhang[1 3]

[1] Department of Urology, Changzhou Second People's Hospital, 68 Gehu Middle Road, Wujin District, Changzhou 213000, China
[2]Department of Oncology, The Affiliated Taizhou People's Hospital of Nanjing Medical University 318000,China

[3]Changzhou Second People's Hospital, Changzhou Medical Center, Nanjing Medical University

[#] **Equal contributors.**
Address correspondence to: Lei Zhang, Department of Urology, Changzhou Second People's Hospital, Changzhou 213000, China. E-mail: 15250961196@163.com. Xiaokai Shi, Department of Urology, Changzhou Second People's Hospital, Changzhou 213000, China. E-mail: shixiaokai@njmu.edu.cn. Lifeng Zhang, Department of Urology, Changzhou Second People's Hospital, Changzhou 213000, China. E-mail: nj-likky@aliyun.com.

Funding: This study was supported by Basic research project of Changzhou Medical Center (CMCB202313; CMCB202319). Top Talent of Changzhou 'The 14th Five-Year Plan' High-Level Health Talents Training Project (Grant number: 2022CZBJ057 and 2022CZBJ058). Changzhou Health Committee Youth Science and Technology Program (No. QN20227).

Abstract

Background:SNHG3 (small nucleolar RNA host gene 3) is a long non-coding RNA (lncRNA) that is thought to be closely involved in regulating the cell cycle, promoting cell proliferation, and inhibiting apoptosis. Consequently, understanding its precise mechanisms of action and potential therapeutic targets is of critical significance.**Methods:**We investigated the role of lncRNA SNHG3 in promoting prostate cancer (PCa) proliferation and invasion. Using TCGA data, we assessed SNHG3 expression in cancer/adjacent tissue, Gleason score, biochemical recurrence, and overall survival. SNHG3 expression was manipulated in four cell types via siRNA interference/over-expression. Various experiments were conducted to confirm SNHG3's role in

PCa, including clone ability, apoptosis, migration, Transwell invasion, and subcutaneous tumor experiments in nude mice. **Results**: In PCa, SNHG3 was highly expressed, especially in Gleason score >7 patients, correlating with shorter overall survival. Interfering with SNHG3 using a plasmid reduced proliferation, increased apoptosis, and decreased migration and tumor growth. Conversely, SNHG3 overexpression yielded opposite results. **Conclusion**:The lncRNA SNHG3 promotes PCa proliferation and invasion while inhibiting cell apoptosis.

Keywords:prostate cancer,lncRNA SNHG3,proliferation,invasion

Introduction

In 2023, the incidence of PCa in the United States still ranks first, and the mortality rate is the second[1]. The latest epidemiological study in China has indicated that the incidence of PCa is the sixth in men, and the mortality is the tenth[2]. PCa has become a common urinary system tumor among men in China. In China, about 30% of PCa patients are middle/late stages, which may affect the overall cure rate. At present, although there are some schemes including endocrine therapy, chemotherapy, immunotherapy, targeted therapy, radionuclide therapy, etc., a large proportion of patients still progress from hormone-sensitive PCa (HSPC) to castration-resistant PCa (CRPC). At this stage, the average survival time of patients is less than 36 months, which is the main population that causes the death of PCa[3-5]. Therefore, clinical basic research is mainly to delay the above process, or to open up new target drugs to cure CRPC, and to improve the overall survival and quality of life. Based on our previous study, we carry out to identify novel targets and clarify the mechanism of PCa.

long noncoding RNAs are a class of noncoding RNAs that do not encode proteins[6, 7]. There is evidence suggesting that lncRNAs participate in genome regulation at the transcriptional, translational, and epigenetic levels[6, 8]. The regulatory functions of lncRNAs encompass gene activation and silencing, recruitment of epigenetic factors, modification of RNA interactions, transcription and post-transcriptional modifications, mRNA decay, and protein recruitment[9-14]. The regulatory functions of lncRNAs are dynamically modulated in a cell-, tissue-, development-, and context-specific manner[15]. LncRNAs in the cytoplasm can act as sponges, stabilizing mRNA and regulating mRNA translation, thereby modifying the expression of downstream target genes[16]. LncRNAs located in the cell nucleus may exert "cis-acting" or "trans-acting" functions[17, 18]. Recently, extensive research has shown that lncRNAs play functional roles in various cancers, especially in tumorigenesis and cancer progression[19]. LncRNAs regulate multiple malignant activities, including tumor advancement, proliferation, apoptosis, migration, invasion, chromatin remodeling, and metabolism[20-22].

SNHG3 plays a crucial regulatory role in the occurrence,development and progression of cancers, and the formation of tumor-related microenvironments. Literature reports have shown upregulated expression of SNHG3 in various tumor tissues, including liver cancer, gastric cancer, cervical cancer, papillary thyroid carcinoma, and acute myeloid leukemia[24-27]. Additionally, the expression level of SNHG3 is closely associated with clinical pathological parameters such as tumor staging and distant metastasis. Moreover, experimental evidence has demonstrated the tumor-promoting effects of SNHG3 in vivo, and at the cellular level, SNHG3 has been shown to

facilitate tumor proliferation and invasion in various cancers, participating in multiple signaling pathways,leading to poor patient prognosis[28，29]. In summary, SNHG3 presents itself as a novel target for tumor diagnosis and treatment. In this study, we initially analyze the correlation between SNHG3 expression levels and clinical characteristics of prostate cancer, aiming to identify new diagnostic markers and establish a foundational basis for subsequent investigations into its functional mechanisms.To further elucidate the biological function of SNHG3, cellular functional experiments and animal studies were employed to confirm its role in promoting carcinogenesis.

Materials and methods

Cell culture

The human prostate cancer cell lines PC3, DU145, LNCaP, and 22RV1 were obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences.These cells were cultured in a humidified environment at 37°C with 5% CO2, in Roswell Park Memorial Institute 1640 (RPMI 1640) medium supplemented with 10% fetal bovine serum(Gibco, USA).

Cytoplasmic plasmid transfection

For cytoplasmic plasmid transfection, the SNHG3 plasmid interference sequence (GGGGGATCATCTAGAAGGTAA) was used. 1 ml of medium containing plasmids was added to the culture flask [800 μL complete medium + 100 μL complete medium containing polybrene (diluted 1:200) + 100 μL complete medium containing plasmids]. Gentamicin was added to PC-3 and 22RV1 cells at a final concentration of 2 g/ml and to LNCaP and DU-145 cells at a final concentration of 4 g/ml, and the cells were continuously cultured for 2 weeks to establish stable knockdown cell lines.

RT-qPCR analysis of SNHG3

Cellular total RNA was extracted using the RNAiso Plus extraction kit (TaKaRa, Japan), following the instructions provided, and dissolved in DEPC water for final concentration determination. The total mRNA was reverse transcribed into cDNA using a reverse transcription kit (TaKaRa, Japan). The primer sequences for SNHG3 RNA were as follows: Forward (5′ to 3′): CAGCCGTTAAGCCATTTGGAACTTG; Reverse (5′ to 3′): CAACCCTGACCTCAACACCTTGG. Finally, SYBR Premix Ex TaqTM II (TaKaRa, Japan) was used to calculate the relative mRNA expression level.

Cloning experiment

Cells (800 cells per well, 2 ml per well) were seeded in triplicate in a 6-well plate. After 3 days of infection, plates were seeded and the medium replaced every 3 days. Continuous culture lasted for 14 days. Before termination, fluorescent microscopy images were captured. Cells were washed with PBS, fixed with 1 ml of 4% paraformaldehyde per well for 60 minutes, stained with 500 μL of crystal violet per well for 30 minutes, washed with ddH2O, air-dried, and photographed and counted for statistical analysis.

Flow cytometry

Briefly, prostate cancer cells in logarithmic growth were collected, washed with chilled PBS,

followed by 1× binding buffer. After centrifugation at 1300 rpm for 3 minutes, cells were resuspended in 200 μL of 1× binding buffer and treated separately with PI and Annexin V-FITC. After 15 minutes in the dark at room temperature, 400-800 μL of 1× binding buffer was added based on cell quantity. Apoptosis analysis was performed using a flow cytometer (BD, USA/Accuri C6).

Wound healing assay

After plasmid transfection, 3×10^4 cells were seeded per well in a 6-well plate. Upon reaching full growth, a vertical line was drawn using a yellow pipette tip. After washing with PBS, images were captured as the 0-hour control. Cells were gently rinsed thrice with serum-free medium, and low-serum culture medium was added. Images were captured again after 48 hours, and migration rates were calculated based on photographic data.

Transwell assay

Place Transwell chambers (Corning, USA) with 500 μl serum-free medium in both chambers in a 24-well plate, incubate at 37°C for 2 hours. Add 50 μl diluted matrix gel to the upper chamber, then seed approximately 5×10^4 cells (500 μl suspension) in the upper chamber and 500 μl complete medium in the lower chamber. After 48 hours at 37°C, invert the chambers, remove medium, clear upper surface cells, immerse the lower chamber in methanol for 30 minutes, then in crystal violet for 5 minutes, rinse, and air-dry. Randomly choose fields, count and photograph under an inverted microscope, calculate migration data, and compare statistically.

Subcutaneous tumorigenesis in nude mice

Used four-week-old female BALB/c nude mice (SYXK (Su) 2022-0053, Jiangsu Jicui Yaokang Biotechnology Co., Ltd.). PC3 cells mixed with basement gel formed a 1 ml cell suspension, injected bilaterally under mice's armpits (left: control, right: interference). Tumor size was recorded every 4 days, and after five weeks, tumors were surgically removed, with weights recorded and analyzed.

Statistical analysis

Statistical differences between the two groups were analyzed using t-tests, while one-way and two-way analysis of variance (ANOVA) were employed to test statistical differences among two and multiple groups, respectively. All statistical analyses were conducted in the R language, $p < 0.05$ was considered statistically significant.

Results

The expression of SNHG3 is upregulated in PCa

(Figure 1A) depicts information about the SNHG3 gene from the GeneCards database, including its chromosomal localization, expression levels in human normal tissues (Figure 1B), and expression in common human organs (Figure 1C). TCGA data included 52 adjacent normal tissue cases and 499 cancerous tissue cases for SNHG3 expression in PCa. Results show significantly elevated SNHG3 expression in PCa (Figure 2A, $P < 0.01$). Patients with Gleason score >7 exhibited higher SNHG3 expression than ⩽7 (Figure 2B, $P = 0.003$). SNHG3 expression showed no significant difference in post-radical prostatectomy biochemical recurrence

(Figure 2C, P = 0.956). Elevated SNHG3 expression was associated with shorter survival duration in PCa patients (Figure 2D, P = 0.02).

We further to explore the correlation between lncRNA SNHG3 expression and clinicopathological factors.The result in (table 1) show that the expression levels of SNHG3 in PCa patients, as well as its association with patient age, Gleason score, and TNM stage. The results suggest a higher proportion of SNHG3 overexpression in patients with a Gleason score greater than 7 (P = 0.02). There was no statistical significance observed in the subgroups of age and TNM stage analysis (both P > 0.05). We also performed univariate and multivariate analysis of the correlation between LncRNA SNHG3 expression and clinical characteristics as well as biochemical recurrence in PCa. The univariate analysis indicates a positive correlation between LncRNA SNHG3 expression and PSA and Gleason score (table 2,P = 0.036 and 0.007).

Knockdown of SNHG3 inhibits the growth and invasion of PCa cells

Using quantitative PCR, SNHG3 expression was significantly increased in the SNHG3 overexpression group and notably decreased in the SNHG3 knockdown group compared to the control (P < 0.01, Figure 3A). Clonogenic assays showed reduced colony formation in DU-145 and PC-3 cells post-SNHG3 knockdown (Figure 3B). Cell apoptosis assays revealed higher rates of apoptosis following SNHG3 interference in both cell lines (Figure 3C). Transwell invasion assays indicated reduced invasion capabilities after SNHG3 knockdown (Figure 3D). Scratch healing assays showed diminished scratch closure abilities post-SNHG3 knockdown (Figure 3E). In nude mice xenograft experiments, tumor weight substantially decreased after SNHG3 knockdown compared to the control (P < 0.001, Figure 3F).

SNHG3 overexpression promotes the growth and invasion of PCa cells

Clone formation assays showed that SNHG3 overexpression notably increased colony-forming capacity in 22RV1 and LNCaP cells compared to the control group (Figure 4A). Cell apoptosis assays revealed a significant decrease in apoptosis rates after SNHG3 overexpression in both cell lines (Figure 4B). Transwell invasion experiments indicated that SNHG3 overexpression significantly enhanced invasive abilities (Figure 4C). Scratch healing experiments demonstrated increased migration capabilities following SNHG3 overexpression (Figure 4D). In nude mice xenograft experiments, SNHG3 overexpression substantially increased tumor weight compared to the control (P < 0.001, Figure 4E), indicating a promotional role in tumor growth.

Discussion

Globally, prostate cancer (PCa) is the second most common cancer in men and the fifth leading cause of cancer-related deaths. While PCa incidence in China has historically been lower than in Western countries, it has gradually increased over the past two decades due to factors like population aging, dietary changes, and Westernized lifestyles[40-43]. According to a 2015 epidemiological survey in China, PCa ranked sixth among male cancers, accounting for 3.35% of male malignancies and the tenth leading cause of cancer-related male deaths at 2.1%[44, 45]. Notably, PCa has become the most diagnosed malignancy in the male genitourinary system in China, surpassing bladder cancer[44, 45].

According to predictions from the International Agency for Research on Cancer (IARC), a division of the World Health Organization (WHO), in 2020, the incidence rate of PCa in China was approximately 15.6 cases per 100,000 individuals. It was estimated that there were around

110,000 new cases and approximately 50,000 deaths due to PCa in that year[46]. Notably, the incidence of PCa is higher in major cities, with rates of 19.30 per 100,000 individuals in Beijing, 32.23 per 100,000 individuals in Shanghai, and 17.57 per 100,000 individuals in Guangzhou [47,48]. Projections from the Global Cancer Observatory website suggest that by 2040, China could see up to 200,000 new cases of PCa and 120,000 deaths from the disease [4]. This underscores the growing threat of PCa to men's health in China, emphasizing the need for widespread attention and awareness.

In China, a significant proportion of PCa patients are diagnosed at an intermediate to advanced stage during their initial assessment. This is primarily because a relatively low percentage of patients seek medical attention due to elevated PSA levels discovered during routine check-ups, which differs significantly from Western countries[5]. In the United States, for instance, approximately 90% of newly diagnosed PCa patients have an early stage of the disease (localized prostate cancer or pelvic lymph node involvement), while only around 6% have distant or metastatic disease at the time of diagnosis[49]. In contrast, in China, only about 30% of newly diagnosed PCa patients have early-stage disease, and a higher proportion presents with intermediate to advanced stages. Among these, approximately 30% already have bone metastases, contributing to the lower overall 5-year survival rate of PCa in China compared to Western countries[50].

In China, various treatment approaches are available for intermediate to advanced PCa patients, including hormone therapy represented by bicalutamide, chemotherapy represented by docetaxel, immunotherapy represented by PD-1/PD-L1 inhibitors, targeted therapy represented by olaparib, and radium-223 therapy as a representative of radiopharmaceutical treatment[51]. However, there is still a significant proportion of patients who progress from HSPC to CRPC. During this phase, the average survival period for patients typically does not exceed three years, making this the predominant group of individuals who succumb to the disease[3-5].

Therefore, clinical research in China focused on PCa primarily aims to delay this disease progression, develop new drug targets for managing castration-resistant PCa, or identify molecular biomarkers for early PCa detection that are more effective than PSA. Ultimately, the goal is to enhance the overall survival rate and quality of life for PCa patients.

Given these objectives, this research project aims to identify novel and effective biomarkers or target inhibitors using existing experimental data. The goal is to provide a theoretical foundation for early PCa diagnosis and inhibition of tumor proliferation and metastasis, contributing to the improvement of overall survival and quality of life for PCa patients in China.

It's important to note that PCa has well-defined risk factors, including age, ethnicity, and geography. PCa incidence significantly increases with age, with over 75% of cases diagnosed after the age of 65[52, 53]. African American men have a 58% higher incidence rate of PCa compared to European men, with a 144% higher mortality rate[53]. Globally, different countries and ethnic groups exhibit significant variations in PCa incidence and mortality rates, with Asian men generally having lower PCa incidence rates[53]. Genetics also play a role in PCa risk, as men with a family history of PCa are at a significantly higher risk. Moreover, several susceptibility genes, including BRCA2, CHEK2, HOXB13, NBS1, RNASEL, ELAC2, MSR1, OGG1, PON1, GDF15, and single nucleotide polymorphisms (SNPs), are associated with an increased risk of PCa[52, 53]. Additionally, urinary tract infections, sexually transmitted infections, and prostatitis can induce inflammation in the prostate, promoting the development of malignant prostate tumors[53].

Studies on immigrants have demonstrated significant variations in cancer incidence rates among patients, indicating the role of external risk factors. Factors such as smoking, as well as dietary components like fat, red meat, and vitamin D, can increase the risk of developing cancer to varying degrees[52, 53]. Dietary habits may influence PCa susceptibility by altering responses to oxidative stress, leading to the release of inflammatory factors, activation of carcinogenic genes, and disruption of the cell growth cycle. Other lifestyle factors, including physical activity, alcohol consumption, and sexual activity, are still subjects of ongoing research to determine their potential impact on PCa risk[52, 53].

PSA was identified as a diagnostic marker for PCa in 1986 due to its significant elevation in the serum of PCa patients. Screening for prostate cancer based on PSA has shown excellent clinical outcomes and created conditions for the early detection of PCa. However, subsequent clinical data revealed a certain level of inconsistency between PSA-based testing and actual clinical findings. Moreover, the specificity of PSA elevation has limitations, as it can also increase in cases of benign prostatic hyperplasia, prostatitis, prostate massage, or cystoscopy. Assuming that patients with elevated PSA levels all undergo biopsies can lead to excessive testing and treatment. One study suggested that PSA's predictive accuracy is approximately 25%[54]. Dahm and others have also shown that PSA-based screening may not improve the overall survival of PCa patients, but improvements in cancer detection and treatment methods have contributed to a decline in mortality rates in some regions of the world[55, 56]. Therefore, due to the limited specificity and sensitivity of PSA, both domestic and international efforts encourage research into other potential PCa biomarkers, with lncRNAs emerging as a recent hotspot in research.

lncRNAs as current research shows, are transcripts that do not encode proteins, but they play a role in almost all biological processes, including gene expression, cell cycle regulation, protein synthesis, and cellular transport[57]. It has only been recently discovered that lncRNA SNPs are associated with cancer and may be another risk factor for PCa[58]. On the one hand, lncRNAs promote metastasis and proliferation in various cancers, while in other cancers, they have inhibitory effects. They have the potential to serve as biomarkers for PCa and as targets for subsequent treatment.

Firstly, potential lncRNAs with diagnostic and therapeutic potential were identified through the TCGA database. It was found that lncRNA SNHG3 is highly expressed in PCa tissues and positively correlated with Gleason scores and overall survival, making it an effective biomarker for the clinical diagnosis of PCa. To elucidate the biological function of SNHG3, further cell functional experiments and animal experiments were conducted to confirm its pro-cancer effects. Overexpression/knockdown experiments confirmed that SNHG3 promotes PCa colony formation, scratch healing, invasion ability, and apoptosis.Xie et al. found that SNHG3 is highly expressed in non-muscle-invasive bladder cancer and is involved in tumor growth and metastasis through the regulation of the c-MYC/BMI1 signaling pathway[59]. Zhang et al. revealed that high expression of SNHG3 is associated with poor prognosis in liver cancer and can mediate liver cancer malignant proliferation through the transcription factor E2F1 regulating NEIL3[60]. Li et al. demonstrated that SNHG3 is significantly upregulated in gastric cancer and can target miR-448/DNMT1 to regulate SEPT9 methylation, affecting gastric cancer recurrence and progression[61]. The above literature collectively suggests that SNHG3 plays a pro-cancer role in malignant tumors.

CONCLUSION

This study confirms that the LncRNA SNHG3 can promote proliferation and invasion in PCa while inhibiting cell apoptosis. This provides new biomarkers for PCa diagnosis and potential inhibitors for PCa treatment.

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Figure 1: Gene Information for SNHG3 from GeneCards Database

A: SNHG3 Chromosomal Localization. B: Expression Levels of SNHG3 in Human Normal Tissues. C: Expression Levels of SNHG3 in Common Human Organs from the NCBI Database.

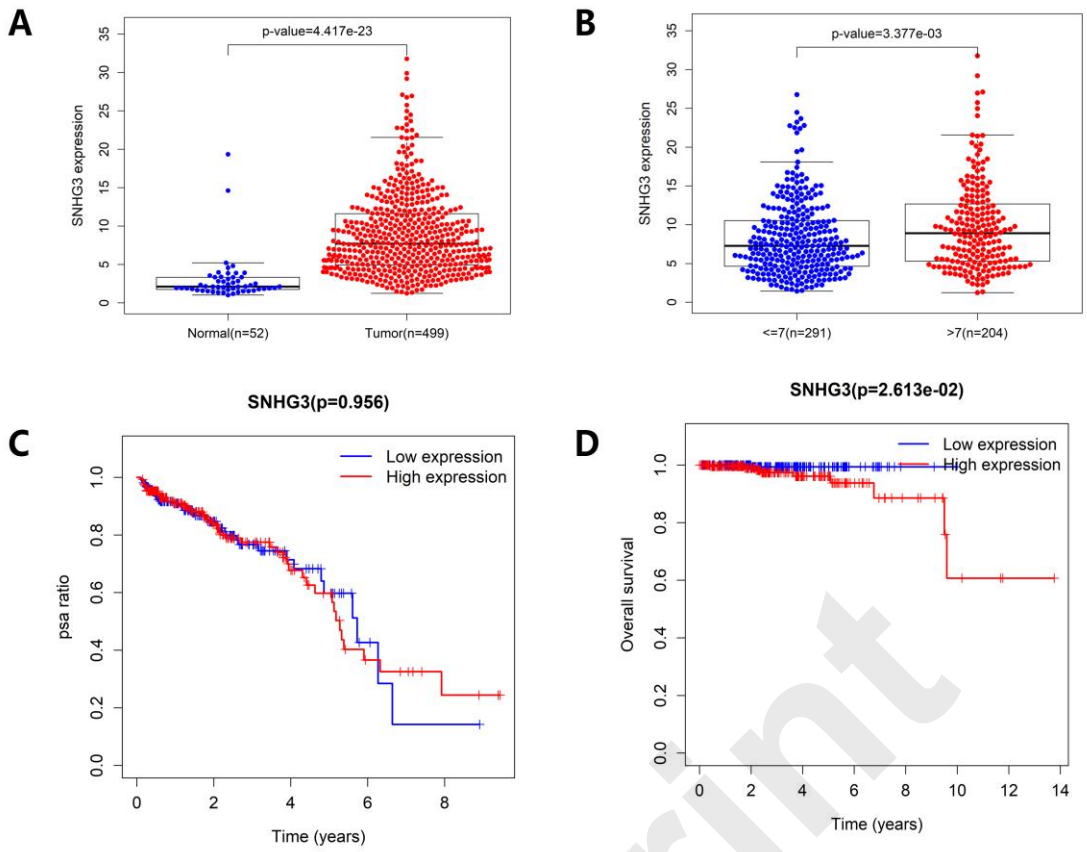


Figure 2: Analysis of the Correlation between SNHG3 Expression and Clinical Characteristics in the TCGA Database.A：Data from TCGA was utilized, encompassing SNHG3 expression data in 52 adjacent non-cancerous prostate tissues and 499 cancerous tissues. B: The correlation between SNHG3 expression and patients with a Gleason score greater than 7. C: The association between SNHG3 expression levels and biochemical recurrence after radical prostatectomy in PCa. D:The relationship between SNHG3 expression levels and the survival period of PCa.

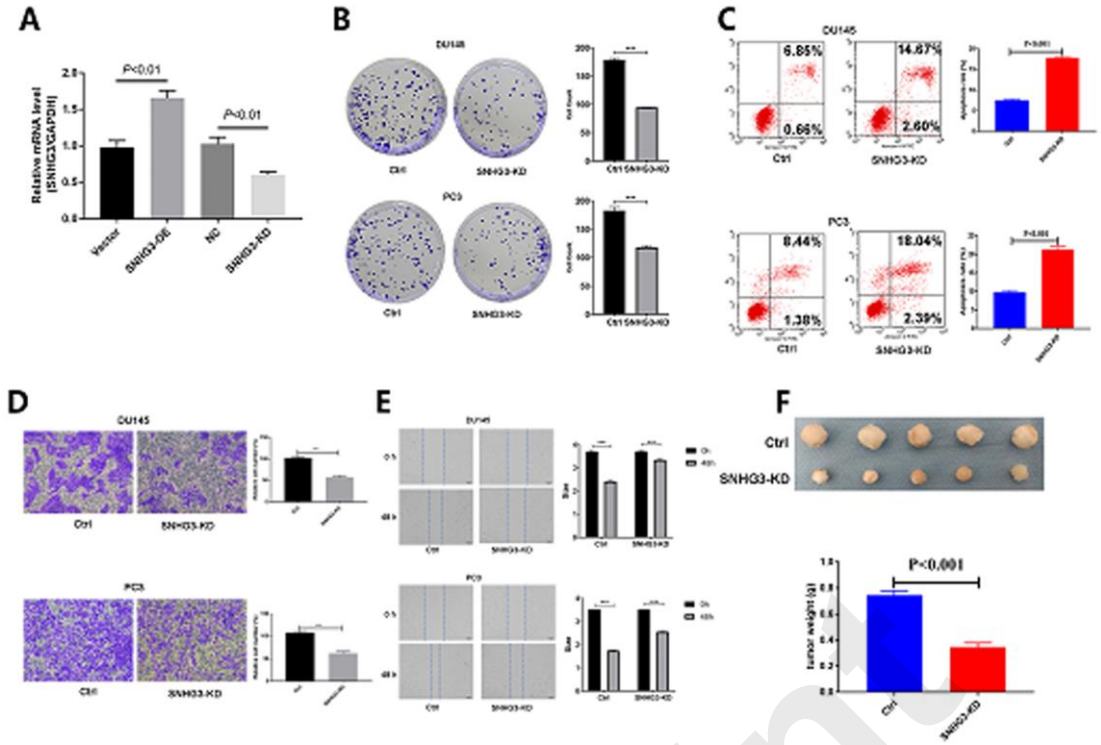


Figure 3:Knockdown of SNHG3 suppresses the growth and invasion of PCa cells.(A):RT-qPCR was conducted to detect the expression of SNHG3.(B):Clonogenic assay to assess the impact of SNHG3 knockdown on the proliferation ability of PCa cells.(C):Transwell invasion assay to assess the impact of SNHG3 knockdown on the invasion ability of PCa cells.(D):Transwell invasion assay to assess the impact of SNHG3 knockdown on the invasion ability of PCa cells.(E):Scratch healing experiment to assess the impact of SNHG3 knockdown on the migration ability of PCa cells.(F):The xenograft experiment in nude mice was conducted to observe the impact of SNHG3 knockdown on tumor growth.

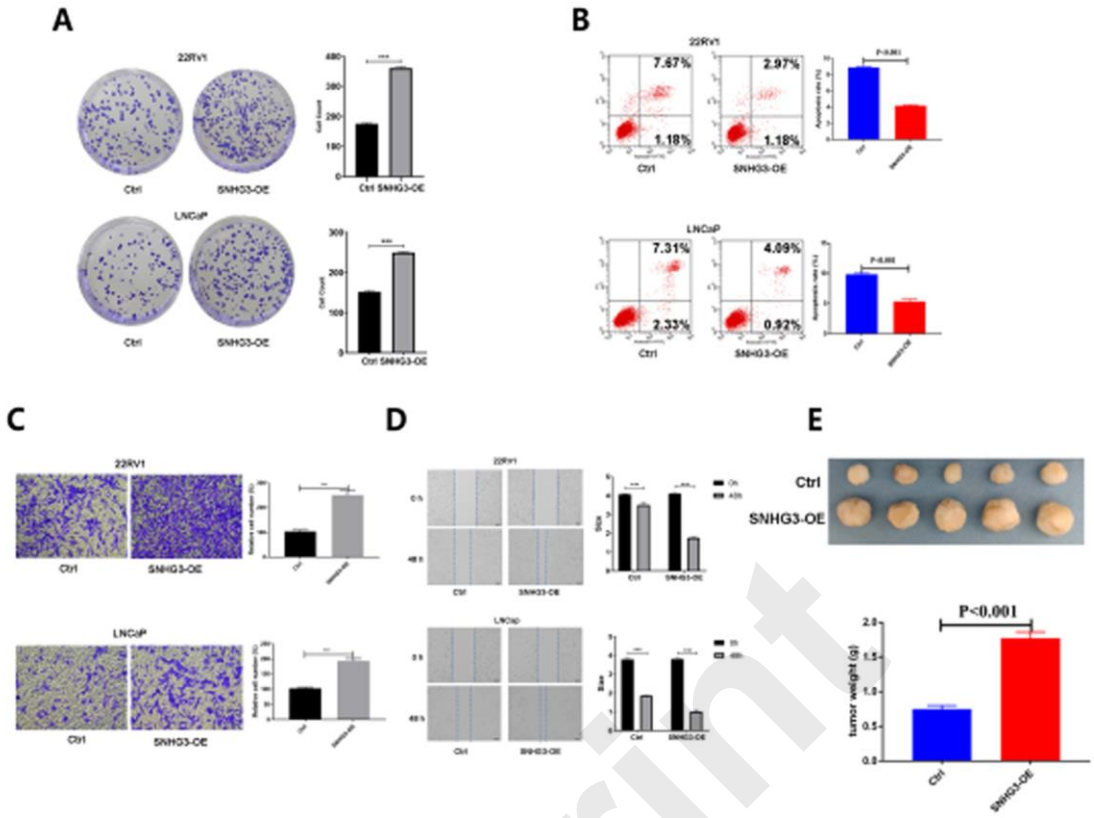


Figure 4:SNHG3 overexpression increases the proliferation and invasion of PCa cells.(A):Clonogenic assay to assess the impact of SNHG3 overexpression on the proliferation ability of PCa cells.(B):Flow cytometry analysis of apoptosis to assess the impact of SNHG3 overexpression on apoptosis in PCa cells.(C):Transwell invasion assay to assess the impact of SNHG3 overexpression on the invasion ability of PCa cells.(D):Scratch healing experiment to assess the impact of SNHG3 overexpression on the migration ability of PCa cells.(E):The xenograft experiment in nude mice was performed to observe the effect of SNHG3 overexpression on tumor growth.

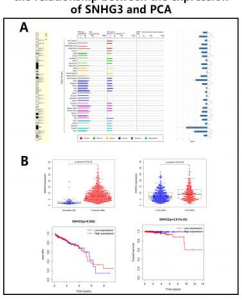
Covariates	Total(%)	LncRNA SNHG3		chi-square	*P* value
		High(%)	Low(%)		
Age					
<=65	352(71.11%)	167(67.61%)	185(74.6%)	2.6092	0.1062
>65	143(28.89%)	80(32.39%)	63(25.4%)		
Gleason score					
<=7	291(58.79%)	132(53.44%)	159(64.11%)	5.3848	0.0203
>7	204(41.21%)	115(46.56%)	89(35.89%)		
T					
T1	177(43.81%)	84(42.21%)	93(45.37%)	0.5384	0.9104
T2	172(42.57%)	86(43.22%)	86(41.95%)		
T3	53(13.12%)	28(14.07%)	25(12.2%)		
T4	2(0.5%)	1(0.5%)	1(0.49%)		
M					
M0	453(99.34%)	226(99.12%)	227(99.56%)	0	1
M1	3(0.66%)	2(0.88%)	1(0.44%)		
N					
N0	344(81.52%)	173(81.22%)	171(81.82%)	0.0011	0.9739
N1	78(18.48%)	40(18.78%)	38(18.18%)		

Table 1 Correlation between lncRNA SNHG3 expression and clinicopathological factors.

Variables	Univariate Analysis		Multivariate Analysis	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age	1.054 (0.956-1.162)	0.291	1.053 (0.938-1.180)	0.381
PSA	1.062 (1.004-1.124)	0.036	1.040 (0.979-1.105)	0.204
Gleason Score	2.952 (1.339-6.506)	0.007	2.033 (0.903-4.576)	0.087
SNHG3	1.053 (0.951-1.165)	0.321	0.985 (0.865-1.122)	0.822

Table 2: Univariate and Multivariate Analysis of the Correlation Between LncRNA SNHG3 Expression and Clinical Characteristics as well as Biochemical Recurrence in PCa

The gene information of SNHG3 and the relationship between the expression of SNHG3 and PCA



Association between SNHG3 expression with clinical features and pathological factors of PCA

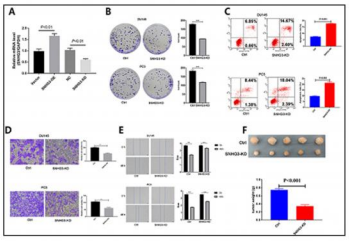
A

Covariate s	Total(%)	LncRNA SNHG3 High(%)	Low(%)	chi-squar e	P value
Age					
<=65	352(71.11%)	167(67.61%)	185(74.6%)	2.6892	0.1062
>65	143(28.89%)	80(32.39%)	63(25.4%)		
Gleason score					
<=7	291(58.79%)	132(53.44%)	159(64.11%)	5.3848	0.0203
>7	204(41.21%)	115(46.56%)	89(35.89%)		
T					
T1	177(43.81%)	84(42.21%)	93(45.37%)	0.5384	0.9104
T2	172(42.57%)	86(43.22%)	86(41.95%)		
T3	53(13.12%)	28(14.07%)	25(12.2%)		
T4	2(0.5%)	1(0.5%)	1(0.49%)		
M					
M0	453(99.34%)	226(99.12%)	227(99.56%)	0	1
M1	3(0.66%)	2(0.88%)	1(0.44%)		
N					
N0	344(81.52%)	173(81.22%)	171(81.82%)	0.0011	0.9739
N1	78(18.48%)	40(18.78%)	38(18.18%)		

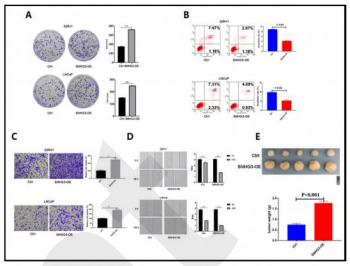
B

Variables	Univariate Analysis HR (95%CI)	P	Multivariate Analysis HR (95%CI)	P
Age	1.004 (0.990-1.018)	0.591	1.013 (0.998-1.029)	0.101
PSA	1.002 (1.000-1.004)	0.030	1.000 (0.998-1.002)	0.904
Gleason Score	2.102 (1.720-2.566)	0.007	2.033 (1.663-2.486)	0.007
SNHG3	1.013 (0.998-1.028)	0.091	0.995 (0.980-1.010)	0.522

Knockdown of SNHG3 suppresses the growth and invasion of PCa cells



Overexpression of SNHG3 increases the proliferation and invasion of PCa cells

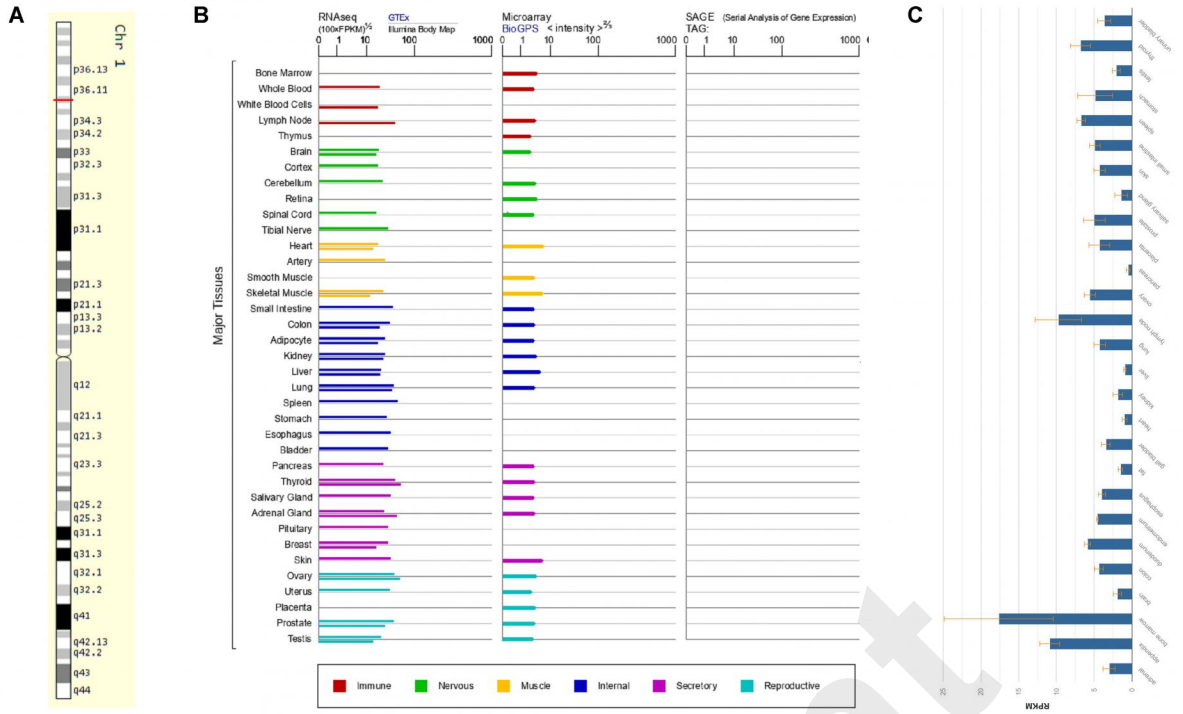


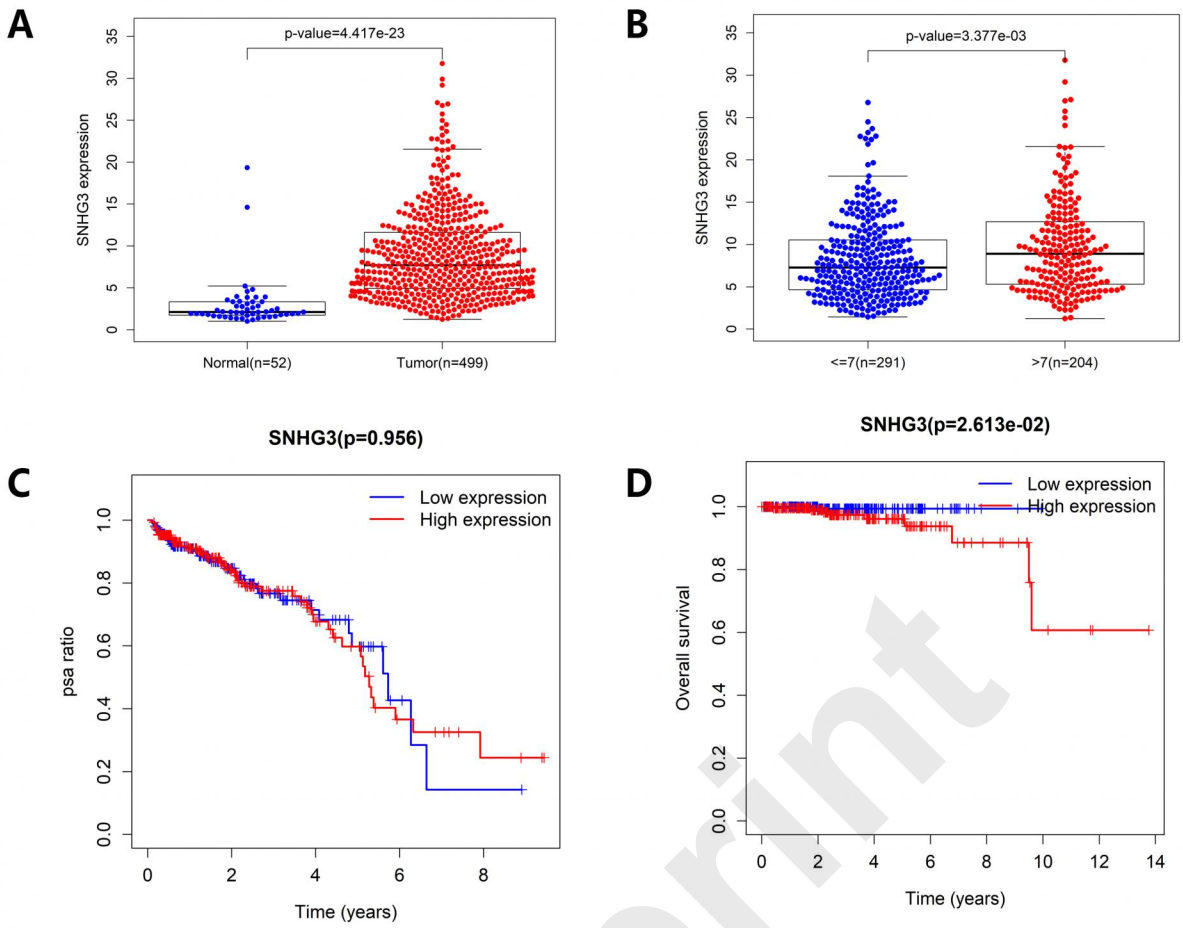
Covariates	Total(%)	LncRNA SNHG3		chi	*P* value
		High(%)	Low(%)		
Age					
<=65	352(71.11%)	167(67.61%)	185(74.6%)	2.6092	0.1062
>65	143(28.89%)	80(32.39%)	63(25.4%)		
Gleason score					
<=7	291(58.79%)	132(53.44%)	159(64.11%)	5.3848	0.0203
>7	204(41.21%)	115(46.56%)	89(35.89%)		
T					
T1	177(43.81%)	84(42.21%)	93(45.37%)	0.5384	0.9104
T2	172(42.57%)	86(43.22%)	86(41.95%)		
T3	53(13.12%)	28(14.07%)	25(12.2%)		
T4	2(0.5%)	1(0.5%)	1(0.49%)		
M					
M0	453(99.34%)	226(99.12%)	227(99.56%)	0	1
M1	3(0.66%)	2(0.88%)	1(0.44%)		
N					
N0	344(81.52%)	173(81.22%)	171(81.82%)	0.0011	0.9739
N1	78(18.48%)	40(18.78%)	38(18.18%)		

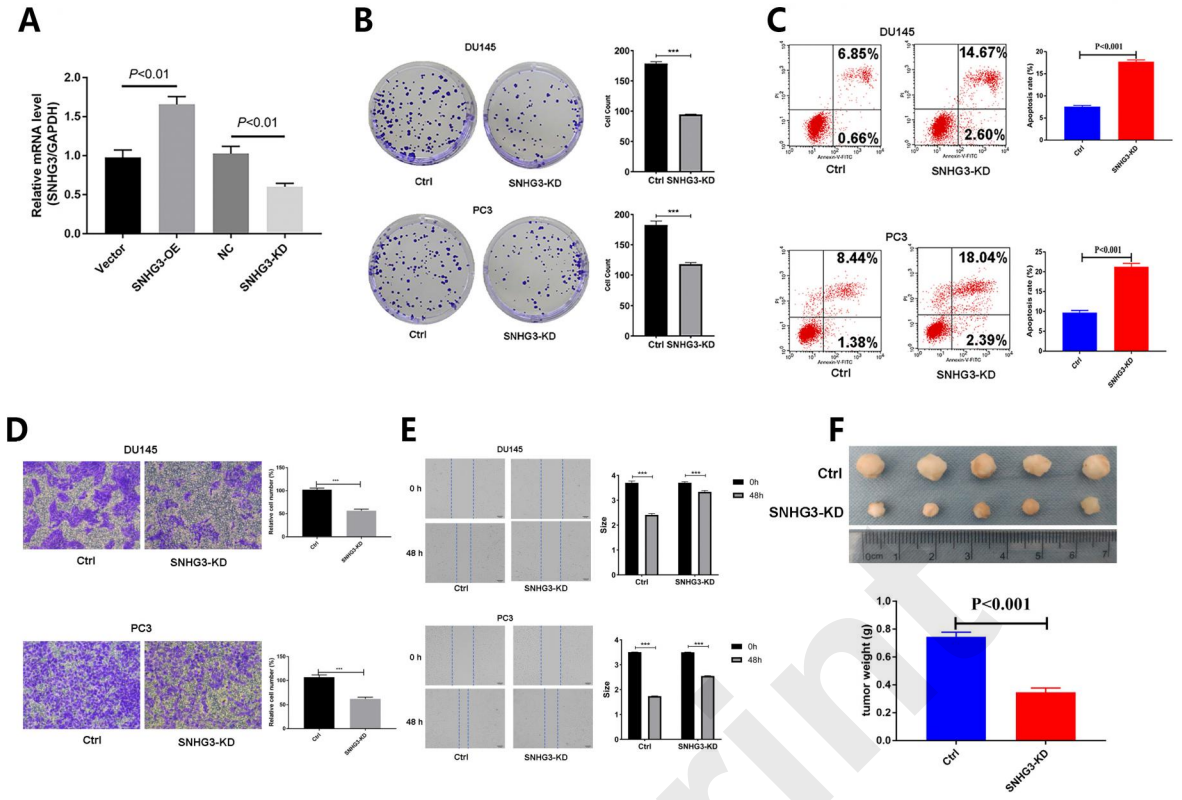
Table 1 Correlation between lncRNA SNHG3 expression and clinicopathological factors.

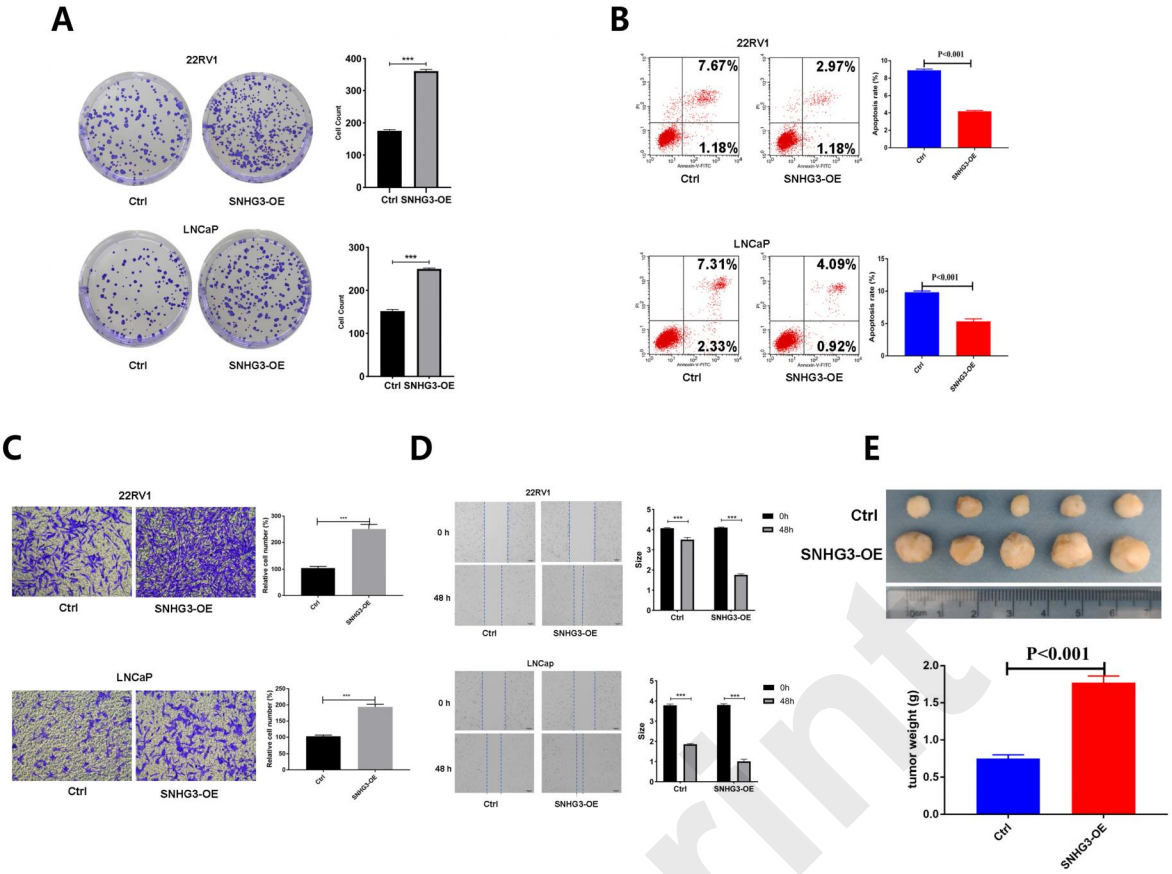
Variables	Univariate Analysis		Multivariate Analysis	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age	1.054 (0.956-1.162)	0.291	1.053 (0.938-1.180)	0.381
PSA	1.062 (1.004-1.124)	0.036	1.040 (0.979-1.105)	0.204
Gleason Score	2.952 (1.339-6.506)	0.007	2.033 (0.903-4.576)	0.087
SNHG3	1.053 (0.951-1.165)	0.321	0.985 (0.865-1.122)	0.822

Table 2: Univariate and Multivariate Analysis of the Correlation Between LncRNA SNHG3 Expression and Clinical Characteristics as well as Biochemical Recurrence in PCa


A
Chr 1
p36.13
p36.11
p34.3
p34.2
p33
p32.3
p31.3
p31.1
p21.3
p21.1
p13.3
p13.2
q12
q21.1
q21.3
q23.3
q25.2
q25.3
q31.1
q31.3
q32.1
q32.2
q41
q42.13
q42.2
q43
q44
B
RNAseq
(100+FPKM)1/2
GTEx
Illumina Body Map
Microarray
BioGPS
< intensity >1/2
SAGE
TAG:
(Serial Analysis of Gene Expression)
0 1 10 100 1000
Major Tissues
Bone Marrow
Whole Blood
White Blood Cells
Lymph Node
Thymus
Brain
Cortex
Cerebellum
Retina
Spinal Cord
Tibial Nerve
Heart
Artery
Smooth Muscle
Skeletal Muscle
Small Intestine
Colon
Adipocyte
Kidney
Liver
Lung
Spleen
Stomach
Esophagus
Bladder
Pancreas
Thyroid
Salivary Gland
Adrenal Gland
Pituitary
Breast
Skin
Ovary
Uterus
Placenta
Prostate
Testis
Immune
Nervous
Muscle
Internal
Secretory
Reproductive
C
RPKM


A
p-value=4.417e-23
SNHG3 expression
Normal(n=52)
Tumor(n=499)
B
p-value=3.377e-03
SNHG3 expression
<=7(n=291)
>7(n=204)
C
SNHG3(p=0.956)
Low expression
High expression
psa ratio
Time (years)
D
SNHG3(p=2.613e-02)
Low expression
High expression
Overall survival
Time (years)


A
P<0.01
P<0.01
Relative mRNA level (SNHG3/GAPDH)
2.0
1.5
1.0
0.5
0.0
Vector
SNHG3-OE
NC
SNHG3-KD
B
DU145
Ctrl
SNHG3-KD
Cell Count
Ctrl SNHG3-KD
PC3
Ctrl
SNHG3-KD
Cell Count
Ctrl SNHG3-KD
C
DU145
6.85%
0.66%
14.67%
2.60%
Ctrl
SNHG3-KD
P<0.001
Apoptosis rate (%)
Ctrl
SNHG3-KD
PC3
8.44%
1.38%
18.04%
2.39%
Ctrl
SNHG3-KD
P<0.001
Apoptosis rate (%)
Ctrl
SNHG3-KD
D
DU145
Ctrl
SNHG3-KD
Relative cell number (%)
Ctrl
SNHG3-KD
PC3
Ctrl
SNHG3-KD
Relative cell number (%)
Ctrl
SNHG3-KD
E
DU145
0 h
48 h
Ctrl
SNHG3-KD
Size
Ctrl
SNHG3-KD
0h
48h
PC3
0 h
48 h
Ctrl
SNHG3-KD
Size
Ctrl
SNHG3-KD
0h
48h
F
Ctrl
SNHG3-KD
P<0.001
tumor weight (g)
1.0
0.8
0.6
0.4
0.2
0.0
Ctrl
SNHG3-KD


A
22RV1
Ctrl
SNHG3-OE
Cell Count
Ctrl SNHG3-OE
LNCaP
Ctrl
SNHG3-OE
Cell Count
Ctrl SNHG3-OE
B
22RV1
7.67%
1.18%
2.97%
1.18%
Ctrl
SNHG3-OE
P<0.001
Apoptosis rate (%)
LNCaP
7.31%
2.33%
4.09%
0.92%
Ctrl
SNHG3-OE
P<0.001
Apoptosis rate (%)
C
22RV1
Ctrl
SNHG3-OE
Relative cell number (%)
LNCaP
Ctrl
SNHG3-OE
Relative cell number (%)
D
22RV1
0 h
48 h
Ctrl
SNHG3-OE
Size
0h
48h
LNCap
0 h
48 h
Ctrl
SNHG3-OE
Size
0h
48h
E
Ctrl
SNHG3-OE
P<0.001
tumor weight (g)
Ctrl
SNHG3-OE