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LncRNA UNC5B-AS1 promotes malignant progression of prostate cancer by competitive binding to caspase-9

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Abstract. – **OBJECTIVE:** This study aims to investigate the expression of LncRNA UNC5B-AS1 in prostate cancer (PCa) and to further investigate whether it can prompt malignant progression of PCa via regulating caspase-9.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was conducted to examine UNC5B-AS1 expression in 50 pairs of tumor tissue specimens and paracancerous ones collected from PCa patients, and the interplay between UNC5B-AS1 expression and clinical indicators of PCa was also analyzed. Meanwhile, UNC5B-AS1 levels in PCa cell lines were also further verified by qRT-PCR. In addition, UNC5B-AS1 knockdown model was constructed using lentivirus in PCa cell lines, and cell counting kit-8 (CCK-8), 5-Ethynyl-2'-deoxyuridine (EdU), transwell and flow cytometry assays were performed to figure out the impact of UNC5B-AS1 on the biological function of PCa cells. Finally, cell recovery experiment was conducted to explore the underlying mechanism and the association between UNC5B-AS1 and caspase-9.

RESULTS: QRT-PCR results suggested that UNC5B-AS1 expression in PCa tissue samples was remarkably higher than in adjacent ones, with a statistically significant difference. Compared with patients with low expression of UNC5B-AS1, patients with highly-expressed UNC5B-AS1 had a higher incidence of distant metastasis and more advanced pathological stage. At the same time, proliferation and invasion, as well as migration ability of cells in sh-UNC5B-AS1 group, was conspicuously attenuated while cell apoptosis ability was conversely enhanced. Furthermore, qRT-PCR results revealed that caspase-9 and UNC5B-AS1 showed a negative correlation in gene expression level in PCa tissues. The results of the luciferase reporter gene experiment demonstrated that UNC5B-AS1 can be targeted by caspase-9 through their binding site. Additionally, cell recovery experiment indicated that UNC5B-AS1 and caspase-9 can be mutually regulated, which then together affect the malignant progression of PCa.

CONCLUSIONS: UNC5B-AS1 expression was found remarkably increased in both PCa tissues and cell lines, which was remarkably associated with pathological stage and incidence of distant metastasis of PCa patients. In addition, UNC5B-AS1 was able to accelerate the malignant progression of PCa by modulating caspase-9 expression.

Key Words:
UNC5B-AS1, Caspase-9, PCa, Malignant progression.

Introduction

Prostate cancer (PCa) is one of the most common malignant tumors in male worldwide, ranking the second in morbidity and the sixth in mortality, seriously threatening the health of elderly men[1-3]. Although the incidence of PCa in China is lower than in European and American countries, its growth rate is very rapid. It is expected that by 2020, the incidence of PCa will be close to the average level in European and American developed countries, reaching 401.1 million male population[4]. Due to the concealed symptoms, domestic patients with PCa have been in advanced stage at the time of diagnosis, and more than 50% of patients have had local or distant metastasis. However, in the United States, more than 80% of PCa patients are diagnosed at an early stage. Apart from the fact that PSA screening has not been widely spread in China, racial difference is also an important reason[5,6]. Data from the Surveillance, Epidemiology, and End Results (SEER)database from 2006 to 2012 showed that the 5-year survival rate of localized PCa was 100%, while that of metastatic PCa was only 29.3%[7,8]. There-

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fore, it is of great significance to explore early diagnostic markers of PCa to improve the diagnosis and treatment of PCa[9,10].

The results of the Human Genome Project show that only about 19,000 genes encode proteins, accounting for 1-2% of the entire genome, and the remaining 99.99% are non-coding sequences[11]. With the maturity of Nextgeneration sequencing (NGS), long non-coding RNA (lncRNA), initially considered as transcription noise, gradually shows its important role[12]. LncRNAs make genomic regulation unusually complex and give full play to cell biological functions[13]. LncRNA is a kind of RNA with a transcript length of more than 200 bases and does not encode proteins. It has a broad tissue expression profile, strong tissue, and cell expression specificity, and can regulate gene expression and protein function at multiple levels such as epigenetic, transcriptional, and post-transcriptional[14,15]. LncRNA can regulate gene function through multiple pathways, thereby affecting the biological function of tumor cells and playing a role in promoting or inhibiting tumor[13-16]. At the same time, a large number of lncRNAs are also used for the early diagnosis and identification of PCa molecular typing[17,18]. At present, domestic and foreign literatures rarely report lncRNA UNC5B-AS1 in the development process of tumor[19]. Therefore, this investigation comprehensively analyzed the expression and biological effect of UNC5B-AS1 in PCa and preliminarily discussed the molecular mechanism of its tumor regulatory effect.

In recent years, many studies have found abnormal expression of caspase-9 in tumor tissues, which is closely related to the occurrence, development, metastasis, and prognosis of tumors[20,21]. LncRNA complements with the 3 ‘non-coding region of the mRNA to degrade or silence the target mRNA after transcription and inhibit its protein synthesis, thus negatively regulating the post-transcriptional level of the target gene. Therefore, this work discusses whether UNC5B-AS1 can mediate the malignant progression of PCa by modulating caspase-9 to provide an experimental basis for its clinical application.

Patients and Methods

Patients and PCa Samples

50 pairs of tumor tissue specimens and paracancerous ones from PCa patients were collected. According to the 8th edition of UICC/AJCC PCa tumor, node and metastasis (TNM) staging criteria, all patients were diagnosed as PCa by postoperative pathological analysis and did not preoperatively receive anti-tumor treatment such as radiotherapy or chemotherapy. This investigation has been approved by the Ethics Oversight Committee. Patients and their families had been fully informed that their specimens would be used for scientific research and then signed relevant informed consent.

Cell Lines and Reagents

Four human PCa cell lines (PC-3, DU-145, 22RV1, Lncap) and one human normal prostate matrix immortalized cell (WPMY-1) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). F-12k, 1640 medium, and fetal bovine serum (FBS) were purchased from Life Technologies (Gaithersburg, MD, USA). Cells were cultured at 37°C with 5% CO_2 in F-12k or Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA).

Transfection

The negative control sequence (shRNA) and the lentivirus containing the UNC5B-AS1 knockdown sequence (sh-UNC5B-AS1) were purchased from Shanghai Jima Company (Shanghai, China). Cells were plated in 6-well plates and grown to a cell density of 40%, and then transfection was performed according to the manufacturer’s instructions. After 48 h, the cells were collected for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis and cell function experiments.

Cell Proliferation Assay

After 48 h of transfection, the cells were resuspended and plated into 96-well plates at 2000 cells per well. They were cultured for 24 h, 48 h, 72 h, and 96 h respectively, and then added with THE cell counting kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) reagent. After incubation for 2 h, the optical density (OD) value of each well was measured in the microplate reader at 490 nm.

5-Ethynyl-2′-Deoxyuridine (EdU) Proliferation Assay

To test the ability of cell proliferation, the EdU proliferation assay (RiboBio, Nanjing, China) was performed according to the manufacturer’s requirements. After transfection for 24

h, the cells were incubated with 50 μm EdU for 2 h, then stained with AdoLo and 4',6-diamidino-2-phenylindole (DAPI), and the number of EdU-positive cells was detected by fluorescence microscopy. The display rate of EdU positive was shown as the ratio of the number of EdU positive cells to the total DAPI chromogenic cells (blue cells).

Flow Cytometry Analysis of the Cell Apoptosis

The renal cell carcinoma cells in logarithmic growth phase were seeded into 6-well plates. After 24 h, the cells were collected, washed twice with phosphate-buffered saline (PBS), and resuspended in the binding solution. Once incubated at room temperature for 15 min in the dark, 5 μL of AnnexinV -FITC (fluorescein isothiocyanate) and Propidium Iodide (PI) was added and mixed gently, and cell apoptosis rate was determined through flow cytometry (Partec AG, Arlesheim, Switzerland).

Transwell Cell Migration and Invasion Assay

After transfection for 48 h, the cells were trypsinized and resuspended in serum-free medium. After cell counting, the diluted cell density was adjusted to 2.0×10^5/mL, and the transwell chamber with or without Matrigel was placed in a 24-well plate. 200 μL of cell suspension was added to the upper chamber, and 500 μL of the medium containing 10% FBS was added to the lower chamber. After 48 h, the transwell chamber was taken out, washed 3 times with 1xPBS, and placed in methanol for cell fixation for 15 min. After that, the chamber was stained in 0.2% crystal violet for 20 min, and the cells on the upper surface of the chamber were carefully wiped off with a cotton swab. The perforated cells stained in the outer layer of the basement membrane of the chamber were observed under the microscope, with 5 fields of view randomly selected.

QRT-PCR

After the cells were treated accordingly, 1 mL of TRIzol (Invitrogen, Carlsbad, CA, USA) was used to lyse the cells, and the total RNA was extracted. The initially extracted RNA was treated with DNase I to remove genomic DNA and repurify the RNA. RNA reverse transcription was performed according to the Prime Script Reverse Transcription Kit (TaKaRa, Otsu, Shiga, Japan) instructions, Real Time PCR was performed according to the SYBR® Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan) kit instructions, and the PCR reaction was performed using the StepOne Plus Real Time PCR System (Applied Biosystems, Foster City, CA, USA). The primers used were as follows: UNC5B-AS1: F: 5'-GATCCTGCCTCAGGGAAA-3', R: 5'-GCTCAAGAGGTTGGGACT-3'; caspase-9: F: 5'-ACCACCCAATACCACAGGAA-3', R: 5'-CATTGGGAGCTGATGAGGAT-3'; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH): F: 5'-GGTCGGAGTCAACGGATTTG-3', U6: F: 5'-CTCGCTTCGGCAGCACATAT-3', R: 5'-TTGCGTGTCATCCTTGCG-3'. The Bio-Rad PCR instrument was used to analyze and process the data with the software iQ5 2.0 (Bio-Rad, Hercules, CA, USA). The GAPDH and U6 genes were used as internal parameters, and the gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

Dual-Luciferase Reporter Assay

A reporter plasmid was constructed in which a specific fragment of the target promoter was inserted in front of the luciferase expression sequence. The transcription factor expression plasmid to be detected was co-transfected with the reporter gene plasmid into 22RV1 and Lncap kidney cancer cells or other related cell lines. A specific luciferase substrate was added, and luciferase reacted with the substrate to generate fluorescence. By measuring the intensity of the fluorescence, the luciferase activity could be determined to figure out whether the transcription factor interacted with the target promoter fragment.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA). Statistical differences between the two groups and multiple groups were analyzed using Student's *t*-test and one-way analysis of variance (ANOVA), respectively. Independent investigations were repeated at least three times for each experiment and data were expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant.

Results

UNC5B-AS1 was Highly Expressed in PCa Tissues and Cell Lines

To determine the role of UNC5B-AS1 in PCa, we detected the expression of UNC5B-AS1 in 50 pairs of PCa tumor tissues and adjacent tissues by

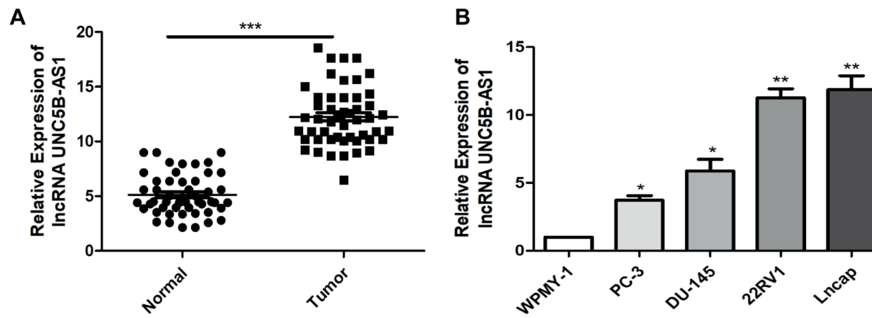

Figure 1. UNC5B-AS1 was highly expressed in prostate cancer tissues and cell lines. **A,** QRT-PCR was used to detect the difference in expression of UNC5B-AS1 in prostate cancer tissues and adjacent tissues. **B,** QRT-PCR was used to detect the expression level of UNC5B-AS1 in thyroid cancer cell lines. Data are mean ± SD, $*p<0.05$, $**p<0.01$, $***p<0.001$.

qRT-PCR. The results showed that UNC5B-AS1 expression was higher in the former than in the latter (Figure 1A), suggesting that UNC5B-AS1 might act as an oncogene in PCa. In addition, UNC5B-AS1 expression was found remarkably higher in PCa cell lines compared to the human normal prostate matrix immortalized cell line (WPMY-1), and the difference was statistically significant (Figure 1B).

UNC5B-AS1 Expression Was Correlated With Pathological Staging and Distant Metastasis In PCa Patients

Based on the mRNA levels of UNC5B-AS1 in 50 pairs of PCa tumor and paracancerous tissues, we divided them into high expression group and low expression group, and analyzed the relation between UNC5B-AS1 expression and age, clinical stage, and lymph node or distant metastasis of PCa patients. As shown in Table I, the high expression of UNC5B-AS1 was positively correlated with the pathological stage and the incidence of distant metastasis of PCa patients.

Knockdown of UNC5B-AS1 Inhibited Cell Proliferation, Metastasis, and Promoted Cell Apoptosis

To investigate the function of UNC5B-AS1 in PCa, we constructed a UNC5B-AS1 knockdown model through a lentiviral vector. After transfection of the UNC5B-AS1 knockdown model in the 22RV1 and Lncap cell lines, qRT-PCR was performed to verify the interference efficiency (Figure 2A). Subsequently, CCK-8, EdU, transwell, and flow cytometry assays were used to detect cell proliferation, invasion, migration, and apoptosis abilities of 22RV1 and Lncap cell lines, respectively. The results showed that the prolifera-

Table I. Association of lncRNA UNC5B-AS1 and caspase-9 expression with clinicopathologic characteristics of prostate cancer.

Parameters	Number of cases	UNC5B-AS1 expression			Caspase-9 expression		
		Low (%)	High (%)	*p*-value	Low (%)	High (%)	*p*-value
Age (years)				0.166			0.413
	20	12	8		7	13	
	30	12	18		14	16	
T stage				0.008			0.007
	30	19	11		8	22	
	20	5	15		13	7	
Lymph node metastasis				0.059			0.084
	33	19	14		11	22	
	17	5	12		10	7	
Distance metastasis				0.048			0.035
	30	19	12		9	21	
	20	7	14		12	8	

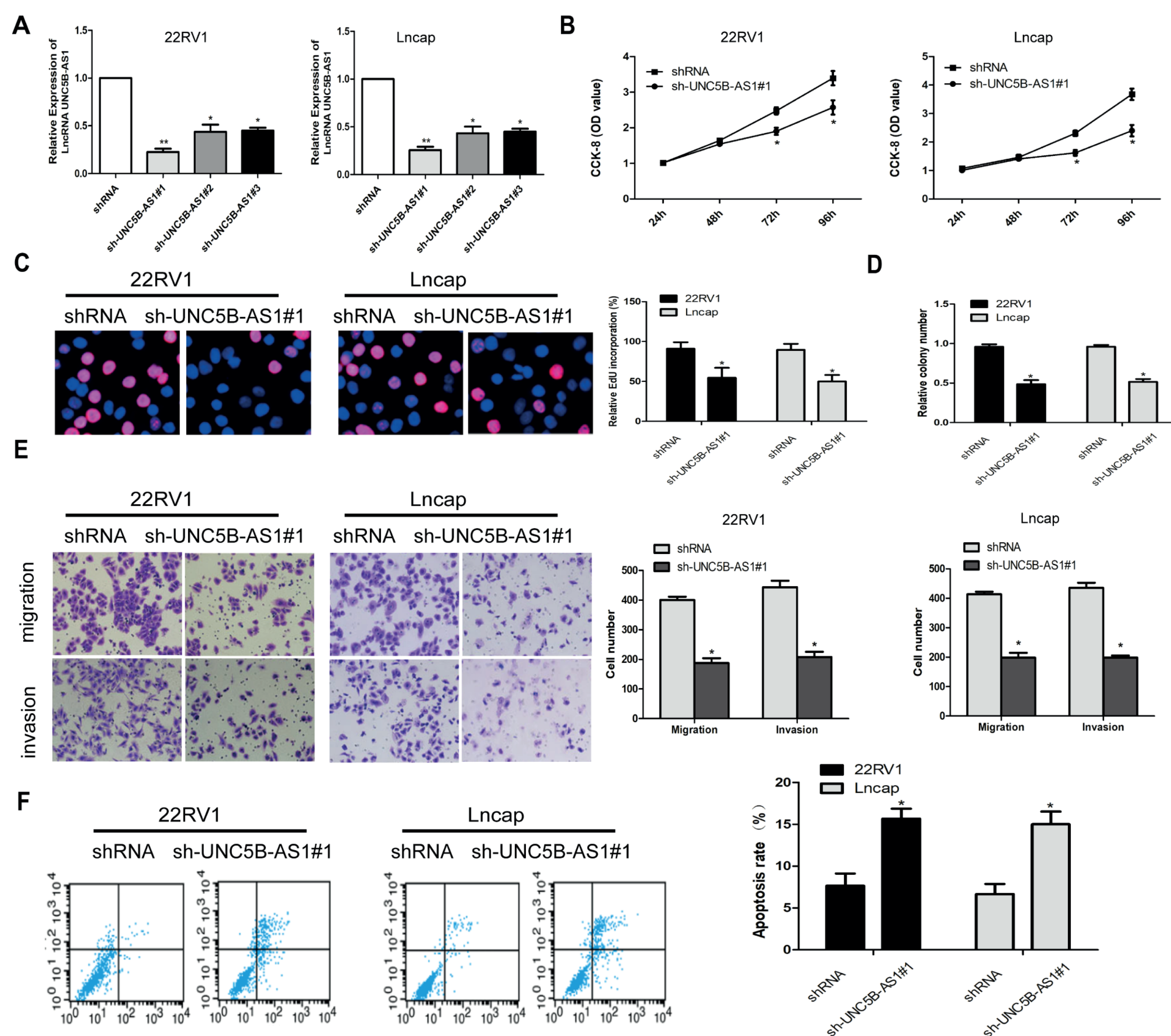

Figure 2. Silencing UNC5B-AS1 inhibited malignant progression of prostate cancer cells. **A,** QRT-PCR verified the interference efficiency of UNC5B-AS1 after transfection of the UNC5B-AS1 knockout vector in the 22RV1 and Lncap cell lines. **B,** CCK-8 assay detected the effect of silencing UNC5B-AS1 on proliferation of prostate cancer cells in 22RV1 and Lncap cell lines. **C-D,** EdU experimental assay detected the ability of UNC5B-AS1 to silence prostate cancer cells in the 22RV1 and Lncap cell lines (magnification: 200×). **E,** Transwell assay detected the effect of silencing UNC5B-AS1 on invasion and migration of prostate cancer cells in 22RV1 and Lncap cell lines (magnification: 40×). **F,** Flow cytometry assay was performed to detect the effect of UNC5B-AS1 on apoptosis of prostate cancer cells in 22RV1 and Lncap cell lines. Data are mean ± SD, *$p<0.05$.

tion, as well as invasion and migration abilities of cells in sh-UNC5B-AS1 group, was remarkably attenuated compared to the control shRNA group (Figures 2B-2E); on the contrary, cell apoptotic ability was found enhanced (Figure 2F).

UNC5B-AS1 Bound to Caspase-9

As shown in Figure 3A, to further verify the targeting of caspase-9 to UNC5B-AS1, a luciferase reporter gene assay was performed. The results showed that overexpression of caspase-9 remarkably attenuated the luciferase activity of the wild-type UNC5B-AS1 vector ($p<0.05$), further demonstrating that UNC5B-AS1 could be targeted by caspase-9 *via* this binding site.

Caspase-9 Was Lowly Expressed In PCa Tissues and Cell Lines

The qRT-PCR result showed that the expression level of caspase-9 in tumor tissues was remarkably lower than in PCa adjacent tissues (Figure

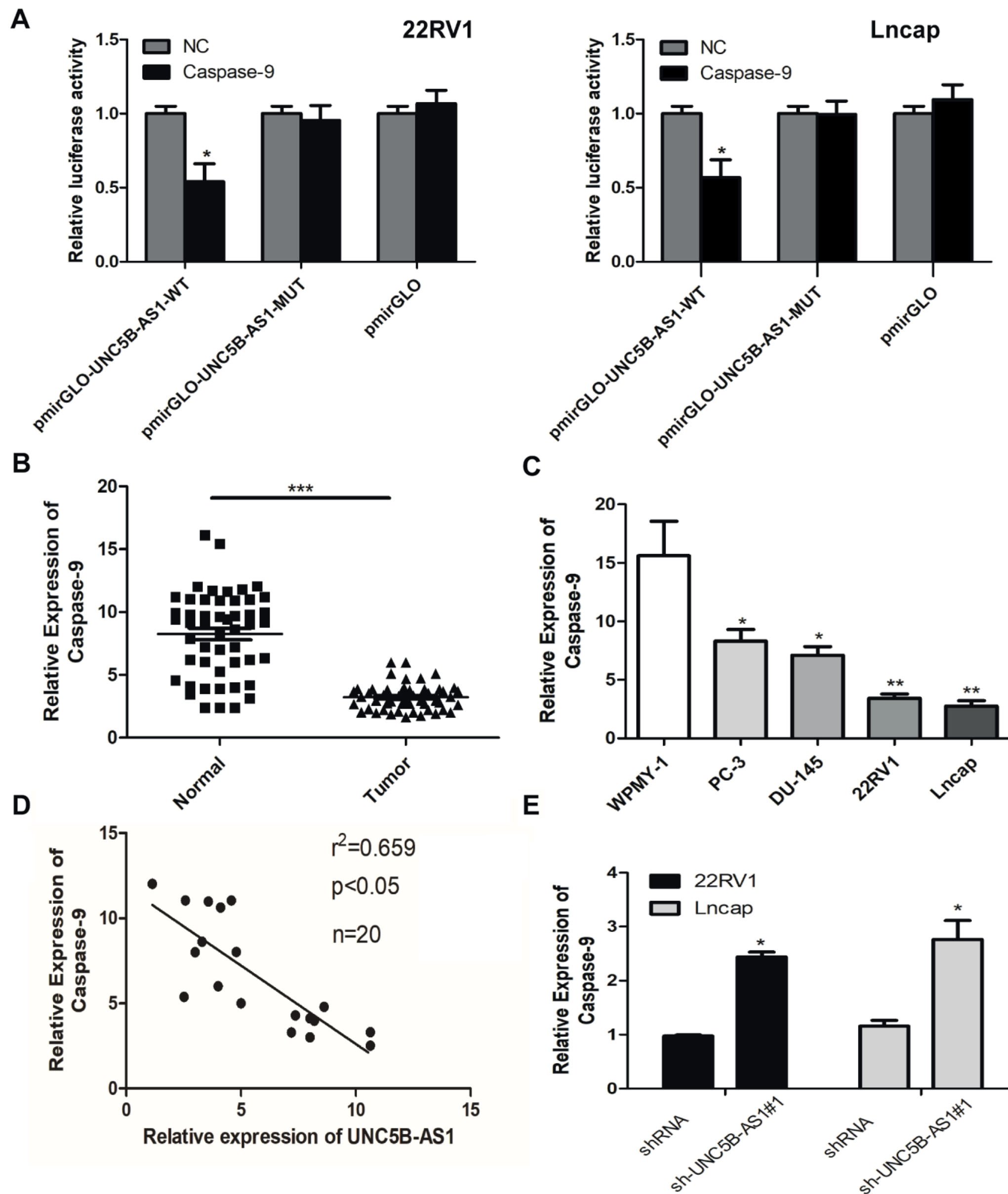

Figure 3. Direct targeting of caspase-9 by UNC5B-AS1. **A,** Dual-Luciferase reporter gene assay verified the direct targeting of UNC5B-AS1 and caspase-9. **B,** QRT-PCR was used to detect the difference in expression of caspase-9 in prostate cancer tissues and adjacent tissues. **C,** QRT-PCR verified the mRNA expression level of caspase-9 after transfection of UNC5B-AS1 in prostate cancer cell lines. **D,** Significant negative correlation between UNC5B-AS1 and caspase-9 expression levels in prostate cancer tissues. **E,** Silencing UNC5B-AS1 expression significantly increased caspase-9 expression. Data are mean ± SD, **p*<0.05, ***p*<0.01, ****p*<0.001.

3B). And a similar result was observed in PCa cell lines (Figure 3C). Therefore, we selected 20 pairs of PCa tissue samples and detected the expression of UNC5B-AS1 and caspase-9 in them. The results indicated that the expression level of the above two genes was negatively correlated (Figure 3D). Subsequently, after UNC5B-AS1 was down-regulated in PCa cells, caspase-9 expression was detected as remarkably increased, and the difference was statistically significant (Figure 3E). At the same time, as shown in Table 1, low expression of caspase-9 was found positively correlated with pathological stage and distant metastasis of PCa, but not with age and lymph node

meastasis.

Overexpression of Caspase-9 Inhibited Cell Proliferation, Metastasis, and Promoted Cell Apoptosis

To investigate the function of caspase-9 in PCa, we constructed a caspase-9 overexpression vector. After transfection of the vector in 22RV1 and Lncap cell lines, qRT-PCR was performed to verify the transfection efficiency (Figure 4A). CCK-8, EdU, and transwell assay were used to detect cell proliferation, as well as invasion and migration abilities of PCa cell lines. Results showed that the overexpression of caspase-9 significantly inhibited these cell functions (Figures 4B-4E).

UNC5B-AS1 Modulated Caspase-9 Expression in PCa Tissues and Cell Lines

To further explore the ways in which UNC5B-AS1 promotes the malignant progression of PCa, we found a possible relation between UNC5B-AS1 and caspase-9 through relevant bioinformatics analysis. In addition, to further explore the interaction between UNC5B-AS1 and caspase-9 in PCa cells, we down-regulated caspase-9 in the PCa cell line that had UNC5B-AS1 silenced and confirmed the transfection efficiency using qRT-PCR assay (Figure 5A). Lastly, we used EdU and transwell assay to find that caspase-9 could counteract the effects of UNC5B-AS1 on PCa cell proliferation (Figures 5B-5C).

Discussion

The advanced PCa progresses rapidly with a poor prognosis, while the five-year survival rate

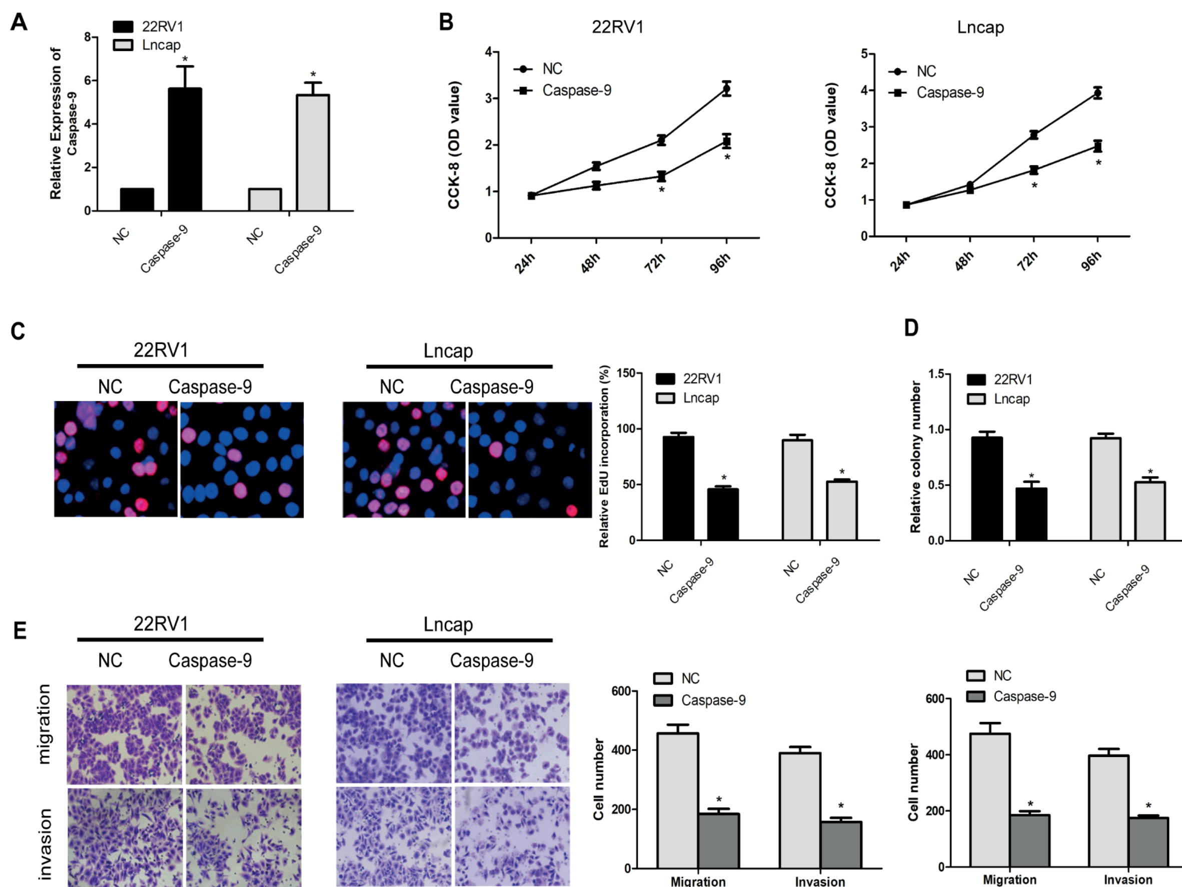

Figure 4. Overexpression of caspase-9 inhibited malignant progression of prostate cancer cells. **A,** QRT-PCR verified the interference efficiency of caspase-9 after transfection of caspase-9 overexpression vector in 22RV1 and Lncap cell lines. **B,** CCK-8 assay detected the effect to overexpress caspase-9 on proliferation of prostate cancer cells in 22RV1 and Lncap cell lines. **C-D,** EdU experimental assay detected the ability of overexpressing caspase-9 to proliferate prostate cancer cells in 22RV1 and Lncap cell lines (magnification: 200×). **E,** Transwell assay detected the effect of overexpression of caspase-9 on invasion and migration of prostate cancer cells in 22RV1 and Lncap cell lines (magnification: 40×). Data are mean ± SD, $*p<0.05$.

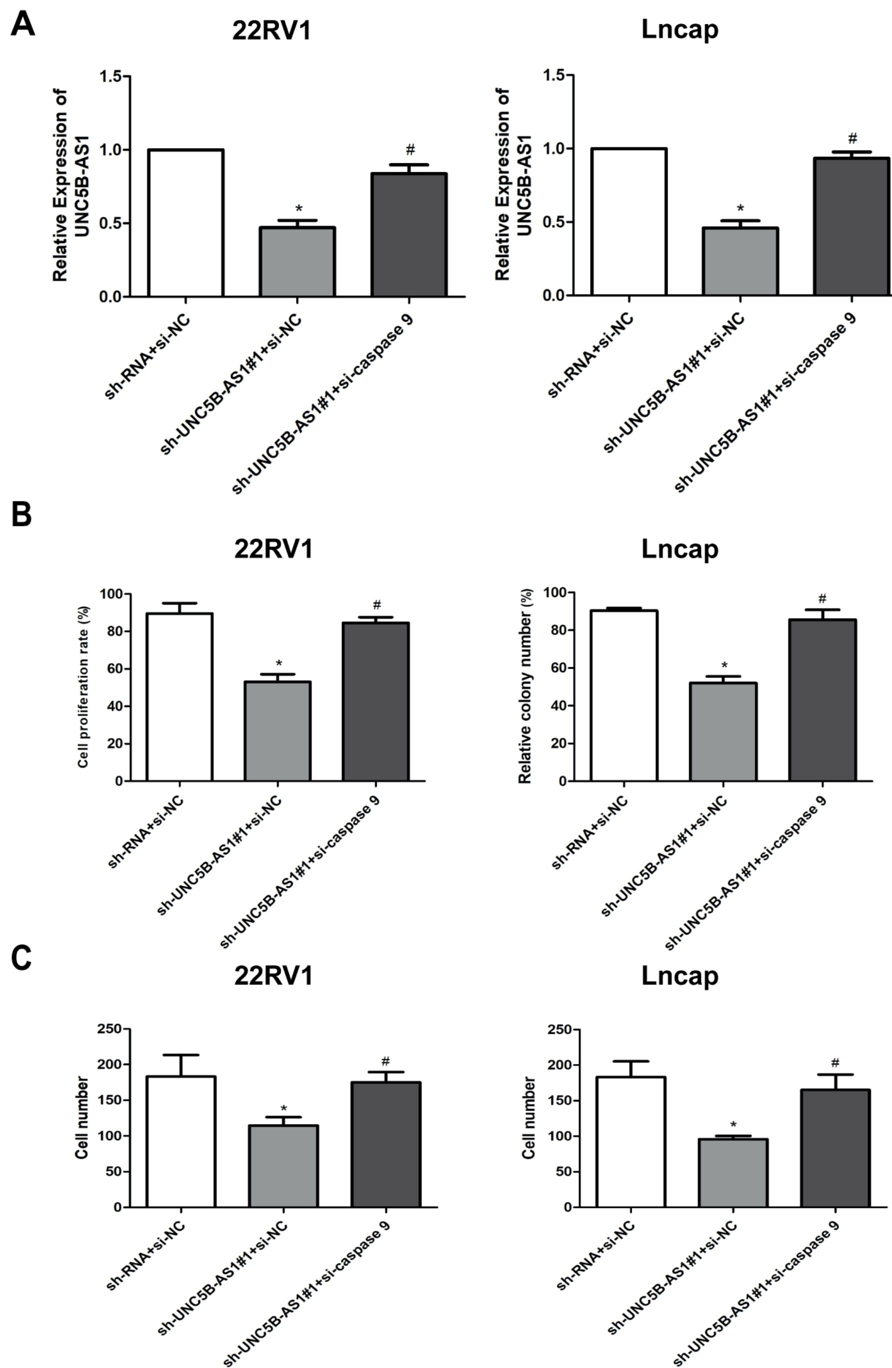

Figure 5. UNC5B-AS1 regulated the expression of caspase-9 in prostate cancer tissues and cell lines. **A,** UNC5B-AS1 expression levels in UNC5B-AS1 and caspase-9 co-transfected cell lines were detected by qRT-PCR. **B,** EdU assay detected the effect of UNC5B-AS1 and caspase-9 co-transfection on the regulation of prostate cancer cell proliferation. **C,** Transwell assay detected the effect of UNC5B-AS1 and caspase-9 co-transfection on the invasion and migration of prostate cancer cells. Data are mean ± SD, $*p<0.05$.

of early PCa is close to 100%[1-4]. Therefore, it is the current consensus to optimize existing diagnostic strategies to find early PCa[5]. Serum PSA is an important biomarker for PCa screening and early detection; however, this marker is still not very suitable for that it is an organ-specific indicator but not tumor-specific, and the presence of inflammation, hyperplasia, or invasive procedures in the prostate can also cause an increase in serum PSA[4-7]. These defects have led to a large

number of unnecessary punctures and excessive treatment of PCa patients, which have brought a huge psychological burden to patients and a significant economic and social burden to society[8,9]. Therefore, it is a hot issue in this field to find ways to make up for the defects of PSA, especially to improve the diagnostic indicators of "grey area" of the PSA diagnosis[8-10].

Long non-coding RNA can be stable in body fluids, timely reflect changes in tumor cells, and can be detected continuously and stably, which has a broad application prospect as a new tumor diagnostic marker[11-13]. UNC5B-AS1 is remarkably highly expressed in lung cancer[19], and its high expression is associated with the malignant progression and poor prognosis of patients with thyroid cancer. In this work, UNC5B-AS1 was selected as a candidate lncRNA associated with the malignant progression of PCa, and PCa cell lines with overexpression and knockdown of UNC5B-AS1 were established by lentiviruses to analyze the relation between UNC5B-AS1 and the occurrence and development of PCa. Up-regulated UNC5B-AS1 was confirmed to be able to promote the development of the malignant progression of PCa. Through tissue verification, we found that the expression of UNC5B-AS1 in PCa tissue was remarkably higher than in paracancer tissue, and was positively correlated with pathological staging and distant metastasis of PCa. Therefore, we indicated that UNC5B-AS1 might play a role in promoting cancer in PCa. To further study the molecular mechanism of UNC5B-AS1 during the development of PCa, *in vitro* experiments were conducted on cell lines. Subsequently, CCK-8, EdU, transwell, and flow apoptosis assay were performed. It was found that silencing UNC5B-AS1 could inhibit the malignant progression of PCa.

LncRNA can competitively bind miRNA and thus affect the regulation of its target gene[22,23]. Previous studies predicted the possible interaction between caspase-9 and UNC5B-AS1 through bioinformatics analysis. As a key molecule in the tumor pathway, caspase-9 was found to be less expressed in PCa tissues and to inhibit PCa malignant progression. In this research, we used bioinformatics analysis to find that UNC5B-AS1 sequence contains a caspase-9 binding site. Moreover, the Dual-Luciferase reporter gene assay confirmed that UNC5B-AS1 could directly bind to the downstream caspase-9. Furthemore, the expression levels of these two genes were found just negatively correlated. Meanwhile, we found that caspase-9 could offset the effects of UNC5B-AS1 on PCa cell biological functions. In summary, this study suggested that UNC5B-AS1 could inhibit the expression of caspase-9, thereby promoting the malignant progression of PCa.

Conclusions

Briefly, we revealed that the expression of LncRNA UNC5B-AS1 in PCa was found remarkably increased, which was correlated with the pathological staging and distant metastasis of PCa tissues. Furthermore, UNC5B-AS1 might promote the malignant progression of PCa by regulating caspase-9.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Smith ZL, Eggener SE, Murphy AB. African-American prostate cancer disparities. Curr Urol Rep 2017; 18: 81.
2) Kretschmer A, Tilki D. Biomarkers in prostate cancer - current clinical utility and future perspectives. Crit Rev Oncol Hematol 2017; 120: 180-193.
3) DE Nunzio C, Presicce F, Giacinti S, Bassanelli M, Tubaro A. Castration-resistance prostate cancer: what is in the pipeline? Minerva Urol Nefrol 2018; 70: 22-41.
4) Reulen RC, de Vogel S, Zhong W, Zhong Z, Xie LP, Hu Z, Deng Y, Yang K, Liang Y, Zeng X, Wong YC, Tam PC, Hemelt M, Zeegers MP. Physical activity and risk of prostate and bladder cancer in China: the South and East China case-control study on prostate and bladder cancer. PLoS One 2017; 12: e0178613.
5) Shi SJ, Wang LJ, Han DH, Wu JH, Jiao D, Zhang KL, Chen JW, Li Y, Yang F, Zhang JL, Zheng GX, Yang AG, Zhao AZ, Qin WJ, Wen WH. Therapeutic effects of human monoclonal PSMA antibody-mediated TRIM24 siRNA delivery in PSMA-positive castration-resistant prostate cancer. Theranostics 2019; 9: 1247-1263.
6) Park Y, Park HJ, Jang WI, Jeong BK, Kim HJ, Chang AR. Long-term results and PSA kinetics after robotic SBRT for prostate cancer: multicenter retrospective study in Korea (Korean radiation oncology group study 15-01). Radiat Oncol 2018; 13: 230.
7) Abdel-Rahman O. Comparison of outcomes of radiotherapy-treated localized prostate cancer patients within a clinical trial setting versus real-life setting. Future Oncol 2019; 15: 1269-1277.
8) Hoag JR, Andemariam B, Wang X, Gregorio DI, Jones BA, Sporn J, Salner AL, Swede H. Serious

adverse events in African-American cancer patients with sickle cell trait and inherited haemoglobinopathies in a SEER-Medicare claims cohort. Br J Cancer 2019; 120: 861-863.
9) Valentino A, Reclusa P, Sirera R, Giallombardo M, Camps C, Pauwels P, Crispi S, Rolfo C. Exosomal microRNAs in liquid biopsies: future biomarkers for prostate cancer. Clin Transl Oncol 2017; 19: 651-657.
10) Saini S. PSA and beyond: alternative prostate cancer biomarkers. Cell Oncol (Dordr) 2016; 39: 97-106.
11) Lingadahalli S, Jadhao S, Sung YY, Chen M, Hu L, Chen X, Cheung E. Novel lncRNA LINC00844 regulates prostate cancer cell migration and invasion through AR signaling. Mol Cancer Res 2018; 16: 1865-1878.
12) Miano JM, Long X, Lyu Q. CRISPR links to long noncoding RNA function in mice: A practical approach. Vascul Pharmacol 2019; 114: 1-12.
13) Huarte M. The emerging role of lncRNAs in cancer. Nat Med 2015; 21: 1253-1261.
14) Valadkhan S, Gunawardane LS. LncRNA-mediated regulation of the interferon response. Virus Res 2016; 212: 127-136.
15) Wu Z, Liu X, Liu L, Deng H, Zhang J, Xu Q, Cen B, Ji A. Regulation of lncRNA expression. Cell Mol Biol Lett 2014; 19: 561-575.
16) Worku T, Bhattarai D, Ayers D, Wang K, Wang C, Rehman ZU, Talpur HS, Yang L. Long non-coding RNAs: the new horizon of gene regulation in ovarian cancer. Cell Physiol Biochem 2017; 44: 948-966.
17) Misawa A, Takayama KI, Inoue S. Long non-coding RNAs and prostate cancer. Cancer Sci 2017; 108: 2107-2114.
18) Wu HF, Ren LG, Xiao JQ, Zhang Y, Mao XW, Zhou LF. Long non-coding RNA LINP1 promotes the malignant progression of prostate cancer by regulating p53. Eur Rev Med Pharmacol Sci 2018; 22: 4467-4476.
19) Hou S, Lin Q, Guan F, Lin C. LncRNA TNRC6C-AS1 regulates UNC5B in thyroid cancer to influence cell proliferation, migration, and invasion as a competing endogenous RNA of miR-129-5p. J Cell Biochem 2018; 119: 8304-8316.
20) Kim B, Srivastava SK, Kim SH. Caspase-9 as a therapeutic target for treating cancer. Expert Opin Ther Targets 2015; 19: 113-127.
21) Yilmaz SG, Yencilek F, Yildirim A, Yencilek E, Isbir T. Effects of caspase 9 gene polymorphism in patients with prostate cancer. In Vivo 2017; 31: 205-208.
22) He JH, Han ZP, Zou MX, Wang L, Lv YB, Zhou JB, Cao MR, Li YG. Analyzing the lncRNA, miRNA, and mRNA regulatory network in prostate cancer with bioinformatics software. J Comput Biol 2018; 25: 146-157.
23) Chen Y, Lin Y, Bai Y, Cheng D, Bi Z. A Long noncoding RNA (lncRNA)-associated competing endogenous RNA (ceRNA) network identifies eight lncRNA biomarkers in patients with osteoarthritis of the knee. Med Sci Monit 2019; 25: 2058-2065.