

LncRNA UBE2R2-AS1, as prognostic marker, promotes cell proliferation and EMT in prostate cancer

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Summary. Background. Long noncoding RNA ubiquitin-conjugating enzyme E2 R2 antisense RNA 1 (UBE2R2-AS1) has been recently reported to participate in the progression of tumors, including glioma and liver cancer. However, the roles of UBE2R2-AS1 in prostate cancer (PC) remained poorly understood.

Methods. The expression of UBE2R2-AS1 was determined in tumor tissues and paired adjacent tissues from PC patients using quantitative reverse transcription PCR analysis. Correlation between UBE2R2-AS1 expression and clinicopathological parameters and overall survival were investigated by Chi-square test and Kaplan-Meier method analysis. The *in vitro* experiments, including CCK-8 assay, colony formation, flow cytometry and transwell assay were performed to investigate the functional role of UBE2R2-AS1 knockdown or overexpression on PC cell lines (PC-3 and DU145). Related protein expression levels were measured by western blot analysis.

Results. Our data showed that UBE2R2-AS1 expression was significantly upregulated in PC tissues compared with that in adjacent tissues. The high levels of UBE2R2-AS1 were associated with high Gleason score, advanced clinical T stage, lymph node metastasis and poor prognosis. Knockdown of UBE2R2-AS1 suppressed cell proliferation, migration and invasion, induced cell cycle G0/G1 arrest and apoptosis in PC cells, along with decreased expression of PCNA, CDK4, Cyclin D1, Bcl-2, N-cadherin and Vimentin, and increased E-cadherin expression. Overexpression of UBE2R2-AS1 obtained the opposite results in PC cells.

Conclusions. Our findings suggest that UBE2R2-AS1 might be a potential diagnostic and/or therapeutic target in PC.

Key words: Prostate cancer, UBE2R2-AS1, Prognosis, Proliferation, Migration

Introduction

Prostate cancer (PC) is known as the most commonly diagnosed malignancy for males, accounting for 1,276,106 new cases and 358,989 deaths worldwide in 2018 (Bray et al., 2018). In the past decades, great progress has been made in conventional therapeutic approaches, including surgery, androgen deprivation and chemotherapy (Yuan et al., 2014; Hoang et al., 2017), but the prognosis of patients with advanced PC remains unfavorable (Crehange et al., 2016). Therefore, it is urgently needed to investigate the molecular mechanisms underlying PC cell malignant behaviors for improving early diagnosis and therapy of PC.

Long non-coding RNAs (lncRNAs) are a type of non-protein-coding transcripts with a length of more than 200 nucleotides (Kornienko et al., 2013), which have been demonstrated to participate in various biological processes, such as cell proliferation, apoptosis, differentiation and migration (Ponting et al., 2009; Jandura and Krause, 2017). Accumulating evidence has indicated that lncRNAs represent important molecular targets for the research of cancer pathogenesis (Spizzo et al., 2012; Bhan et al., 2017; Renganathan and Felley-Bosco, 2017). Recently, a newly discovered lncRNA ubiquitin-conjugating enzyme E2 R2 antisense RNA 1 (UBE2R2-AS1) has been reported to be involved in the development and progression of tumors (Zhou et al., 2018). For example, Xu et al. (2019) demonstrated that UBE2R2-AS1 suppressed glioblastoma cell growth, migration and invasion, as well as promoting apoptosis. Subsequently, Xu et al. (2021) further manifested that

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Abbreviations. UBE2R2-AS1, ubiquitin-conjugating enzyme E2 R2 antisense RNA 1, PC, prostate cancer; ATCC, American Type Culture Collection; FBS, fetal bovine serum; NC, negative control; CCK-8, Cell Counting kit-8; SD, standard deviation



UBE2R2-AS1 overexpression inhibited the growth of tumors in nude mice, which may be a good marker and treatment target for the clinical detection of glioma. Similarly, Liu et al. (2020) also demonstrated the suppressive role of UBE2R2-AS1 in cervical cancer by reporting UBE2R2-AS1 suppressing cell proliferation, migration and invasion. On the contrary, Wu et al. (2020) not only observed that UBE2R2-AS1 was increased in hepatocellular carcinoma (HCC) tissues and cell lines, but also demonstrated that increased UBE2R2-AS1 predicted poor survival of HCC patients and promoted cell growth and metastasis through *in vitro* and *in vivo* experiments. However, the expression pattern and biological role of UBE2R2-AS1 in PC have not been reported yet.

The current study aimed to investigate the role of UBE2R2-AS1 in PC. We first detected the expression of UBE2R2-AS1 in PC and matched normal tissues. Then, we evaluated the clinical significance of UBE2R2-AS1 in PC patients. Finally, we explored the biological function role of UBE2R2-AS1 in PC cells, including proliferation, cell cycle progression, apoptosis, migration and invasion *in vitro*.

Materials and methods

Patients and tissue samples

The paired tumor tissues and matched adjacent tissues were collected from PC patients at the Department of Urology, Heilongjiang Hospital (Harbin city, China) and immediately stored at -80°C before RNA extraction. None of the patients had received anti-tumor therapy before tissue collection. Written informed consents were signed by patients. The clinicopathological parameters were recorded and summarized. The date of prostatectomy was considered as the beginning of the follow-up period. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of First Affiliated Hospital of Heilongjiang Hospital (Harbin city, China).

Cell culture and transfection

Two human PC cell lines (PC-3 and DU145) and prostatic epithelial cell line (RWPE-1) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). All cell lines were cultured in the RPMI-1640 medium (Gibco BRL, Carlsbad, CA, USA) with 10% FBS at 37°C with 5% CO_2 . Small interfering RNA (siRNA) that targeted UBE2R2-AS1 (si-UBE2R2-AS1) and scrambled negative control (si-NC) was purchased from GenePharma (Shanghai, China). The full-length of UBE2R2-AS1 was subcloned into pcDNA3.1 to generate UBE2R2-AS1 overexpression plasmid, with empty pcDNA3.1 serving as control. The overexpression plasmid and pcDNA3.1 were bought from GenePharma Co., Ltd (Shanghai, China).

Subsequently, PC-3 or DU145 cells were severally transfected with the above plasmids for 48h via Lipofectamine 3000 (Invitrogen).

Quantitative reverse transcription PCR

Total RNA was extracted from tissues and cell lines by using TRIzol reagent (Invitrogen) and cDNA was synthesized with a reverse transcription kit (Takara, Dalian, China) according to the manufacturer's instructions. Quantitative reverse transcription PCR was performed with SYBR Green Master Mix (Takara) on an ABI StepOnePlus instrument (Applied Biosystems, Carlsbad, CA, USA) under the following conditions: an initial denaturation step of 10 min at 95°C , followed by 50 cycles of denaturation at 95°C for 30s, 95°C for 5s and 61°C for 30s and final annealing extension at 72°C for 5 min. The primer sequences used in this study were as follows: UBE2R2-AS1, forward 5'-GTCTGGGTAGTCAGCTGTGAGG-3', reverse 5'-TCTCCAGAGGCA GTGTTCCTC'-3'; GAPDH, forward 5'-AGCCACATCGCTCAGACAC-3', reverse 5'-GCCCAATACGACCAAATCC-3'. The relative expression of UBE2R2-AS1 was calculated by $2^{-\Delta\Delta\text{Ct}}$ method and normalized relative to GAPDH. Each experiment was performed in triplicate.

CCK-8 assay

Cell viability was assessed every 24h by the Cell Counting Kit-8 (CCK-8, Dojindo Molecular technologies, Inc., Kyushu, Japan) according to the manufacturer's instructions. Briefly, transfected cells were seeded into the wells of 96-well plates at a density of 3,000 cells per well. After incubation with 10 μl CCK-8 solution for 2h at 37°C , the absorbance at 450 nm was determined using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Colony formation assay

After being plated into six-well plates (500 cells per well), transfected cells were cultured for 14 days until forming colonies. Next, colonies were fixed with methanol for 10 min and stained with 0.1% crystal violet (Beyotime, Shanghai, China) for 15 min. Finally, the number of visible colonies were counted under the fluorescent microscope (Olympus, Tokyo, Japan).

Flow cytometry analysis

After transfection, cells were digested with trypsin and centrifuged at 1000 r/min for 5 min. Afterwards, cells were washed with pre-cold PBS twice and fixed with 75% ethanol for 4h at 4°C . For cell cycle assay, cells were incubated with 500 μL binding buffer containing iodide (PI, 40%, KeyGen Biotech Co. Ltd., Nanjing, China) at room temperature for 15 min in the dark. Then, cell cycle distribution was analyzed using

The role of UBE2R2-AS1 in prostate cancer

flow cytometry (FACS Calibur, BD Biosciences, Franklin Lakes, NJ, USA). For cell apoptosis assay, fixed cells were washed twice with PBS, followed by staining with Annexin V-FITC and PI (KeyGen Biotech Co. Ltd.) in the dark. Subsequently, the percentage of apoptotic cells was quantified using flow cytometry (FACS Calibur, BD Biosciences) following the manufacturer's instructions.

Transwell assay

Cell migration and invasion were evaluated with a transwell chamber coated without and with Matrigel (pore size, 8.0 μm ; BD Biosciences), respectively. In brief, transfected 2.5×10^4 cells with 200 μL of serum-free medium were seeded in the upper chambers, while 200 μL of the medium containing 20% FBS as a chemoattractant was added into the lower chambers. After 24h of incubation at 37°C, cells on the lower chambers were washed twice with PBS and fixed with 95% ethanol for 10 min. Following staining with 0.1% crystal violet for 10 min, the number of migratory or invasive cells were calculated at five randomly chosen views (200 \times magnification) under a microscope.

Western blot analysis

Total protein was extracted from transfected cells RIPA buffer (Beyotime, Shanghai, China) and protein concentration was determined by the Bradford protein assay (Bio-Rad, Hercules, CA, USA). An equal amount of protein was separated by 10-12% sodium dodecyl sulfate polyacrylamide-gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat milk and incubated with specific primary antibodies against PCNA, CDK4, Cyclin D1, Bcl-2, E-cadherin, N-cadherin, Vimentin and GAPDH (Abcam, Cambridge, USA) overnight at 4°C. The membranes were subsequently incubated with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology, Danvers, MA, USA) for 1 h at room temperature. Protein bands were visualized using an electrochemiluminescence (ECL) substrate

(Thermo Fisher Scientific).

Statistical analysis

Statistical analysis was performed with GraphPad Prism 7.0 (GraphPad Software). The correlation between UBE2R2-AS1 expression and clinical characteristics was examined by chi-square test. The Kaplan-Meier method was utilized to plot survival curves which were compared by log-rank test. The Cox proportional hazards regression model was used for univariate and multivariate analyses to obtain survival data. Data were expressed as the mean \pm standard deviation (SD) of at least three independent experiments. Student's t-test determined differences between the groups. Values of $P < 0.05$ were considered to be statistically significant.

Results

Levels of UBE2R2-AS1 expression were increased in PC tissues and cell lines

To investigate the clinical significance of UBE2R2-AS1 in the development of PC, the expression level of UBE2R2-AS1 was examined by quantitative reverse transcription PCR in 74 pairs of PC tissues and matched adjacent tissues. As shown in Figure 1A, UBE2R2-AS1 expression was significantly upregulated in PC tissues compared with adjacent tissues. Moreover, we conducted quantitative reverse transcription PCR to confirm UBE2R2-AS1 expression status in PC cell lines. As expected, UBE2R2-AS1 showed a dramatically higher expression level in PC cell lines (PC-3 and DU145) compared with normal human prostate epithelial cell line RWPE-1 (Fig. 1B).

Increased expression of UBE2R2-AS1 was associated with poor prognosis of PC patients

PC samples were divided into high expression group (n=38) and low expression group (n=36) in accordance with the median UBE2R2-AS1 expression level in all PC samples. As summarized in Table 1, high UBE2R2-AS1 expression was strikingly associated with high

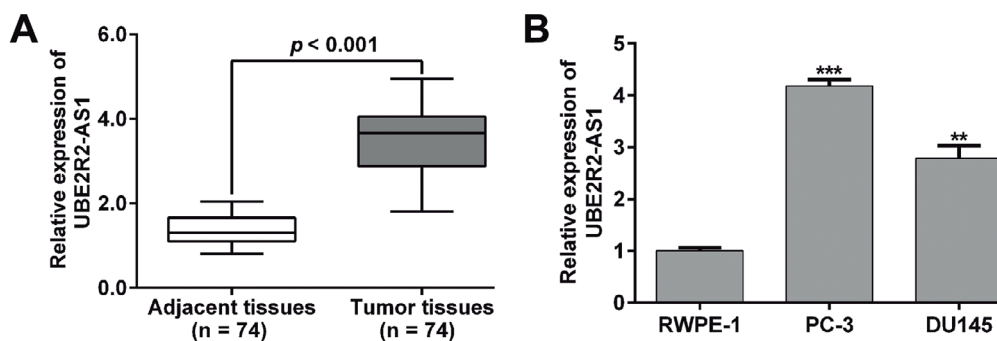


Fig. 1. Levels of UBE2R2-AS1 expression were increased in prostate cancer tissues and cell lines. **A.** Relative expression of UBE2R2-AS1 in 74 pairs of prostate cancer tissues and adjacent non-tumor tissues by quantitative reverse transcription PCR analysis. **B.** Upregulation of UBE2R2-AS1 in prostate cancer cells (PC-3 and DU145) compared with normal prostate epithelial cells RWPE-1. ** $P < 0.01$; *** $P < 0.001$, compared with RWPE-1

Gleason score (≥ 7 vs. < 7 , $P = 0.016$), advanced clinical T stage (T2 vs. T3–T4; $P = 0.035$) and lymph node metastasis (negative vs. positive; $P = 0.002$). The Kaplan-Meier analysis showed that PC patients in the higher expression levels of UBE2R2-AS1 group exhibited significantly poorer survival rates than those in the lower expression levels of UBE2R2-AS1 group (Fig. 2, $P = 0.001$). Univariate multivariate analysis revealed that Gleason score and UBE2R2-AS1 expression were independent prognostic factors for overall survival of patients with PC (Table 2).

UBE2R2-AS1 promoted cell proliferation, cell cycle progression and suppressed apoptosis of PC cells

To determine the potential biological role of UBE2R2-AS1 in PC cells, we first performed loss-of-

function assays in PC-3 and DU145 cells by transfection with si-UBE2R2-AS1. Quantitative reverse transcription PCR assay showed that PC-3 and DU145 cells transfected with si-UBE2R2-AS1 expressed lower levels of UBE2R2-AS1 than control cells transfected with si-NC (Fig. 3A). The results of CCK-8 assay indicated that downregulation of UBE2R2-AS1 expression dramatically inhibited cell viability in PC-3 and DU145 cells (Fig. 3B). Similarly, knockdown of UBE2R2-AS1 significantly decreased the number of colonies in both PC-3 and DU145 cells (Fig. 3C). In addition, PC-3 and DU145 cells were transfected with UBE2R2-AS1 overexpression plasmid to achieve UBE2R2-AS1 overexpressing cell lines. A significant increase in UBE2R2-AS1 expression was confirmed by quantitative reverse transcription PCR in PC-3 and DU145 cells (Fig. 3D), which markedly promoted cell viability (Fig. 3E)

Table 1. The relationship between UBE2R2-AS1 expression and clinicopathological characteristics of PC patients.

Characteristics	Cases (n=74)	UBE2R2-AS1 expression		P-values
		High (n=38)	Low (n=36)	
Age (year)				0.166
< 65	39	23	16	
≥ 65	35	15	20	
Preoperative PSA				0.168
< 10 ng/ml	29	12	17	
≥ 10 ng/ml	45	26	19	
Gleason score				0.016*
< 7	23	7	16	
≥ 7	51	31	20	
Clinical stage				0.035*
T2	51	22	29	
T3-T4	23	16	7	
Lymph node metastasis				0.002*
Negative	56	23	33	
Positive	18	15	3	
Biochemical recurrence				0.936
Negative	49	25	24	
Positive	25	13	12	

* $p < 0.05$; Abbreviations: PSA, prostate specific antigen; PC, prostate cancer.

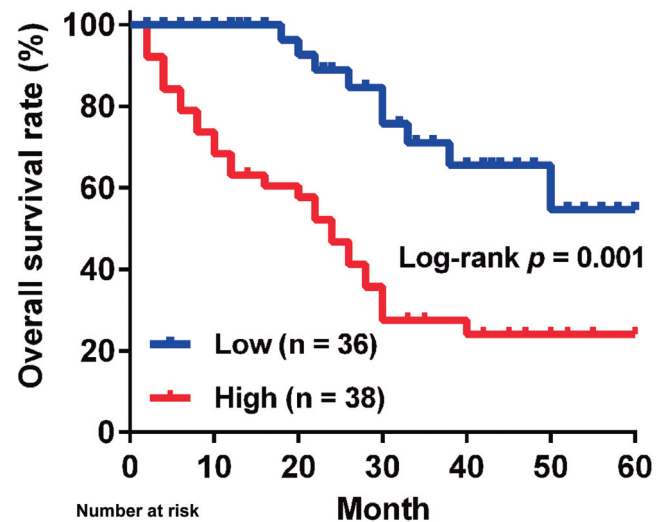


Fig. 2. High expression of UBE2R2-AS1 predicted a poor prognosis in prostate cancer patients. Kaplan-Meier survival curves for patients with prostate cancer tissues expressing low and high levels of UBE2R2-AS1. Prostate cancer patients with high UBE2R2-AS1 expression (n=38) had shorter overall survival than patients with low UBE2R2-AS1 expression (n=36).

Table 2. Cox proportional-hazard model for overall survival in patients with PC.

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (year; ≥ 65 vs. < 65)	1.19 (0.563-2.312)	0.675	NA	NA
Preoperative PSA (ng/ml; ≥ 10 vs. < 10)	1.875 (0.923-3.612)	0.092	NA	NA
Gleason score (≥ 7 vs. < 7)	3.152 (1.452-5.365)	0.006*	2.865 (1.215-5.526)	0.026*
Clinical stage (T3-T4 vs. T2)	1.258 (0.635-2.635)	0.489	NA	NA
Lymph node metastasis (Positive vs. Negative)	1.365 (0.712-2.579)	0.423	NA	NA
Biochemical recurrence (Positive vs. Negative)	0.799 (0.406-1.563)	0.503	NA	NA
UBE2R2-AS1 expression (High vs. Low)	6.256 (1.953-12.13)	0.002*	3.652 (1.175-10.256)	0.023*

* $p < 0.05$; Abbreviations: HR, hazard ratio; CI, confidence interval; PSA, prostate specific antigen; PC, prostate cancer.

The role of UBE2R2-AS1 in prostate cancer

and colony formation ability (Fig. 3F) in these two types of PC cells. Next, we investigated whether UBE2R2-AS1 had an effect on the regulation of cell cycle distribution and cell apoptosis. On the one hand, we observed that knockdown of UBE2R2-AS1 significantly

increased the percentage of cells at G0/G1 phase, but accordingly decreased cells at S phase in PC-3 and DU145 cells, indicating G0/G1 phase arrest induced by UBE2R2-AS1 depletion (Fig. 4A). What's more, downregulation of UBE2R2-AS1 resulted in a

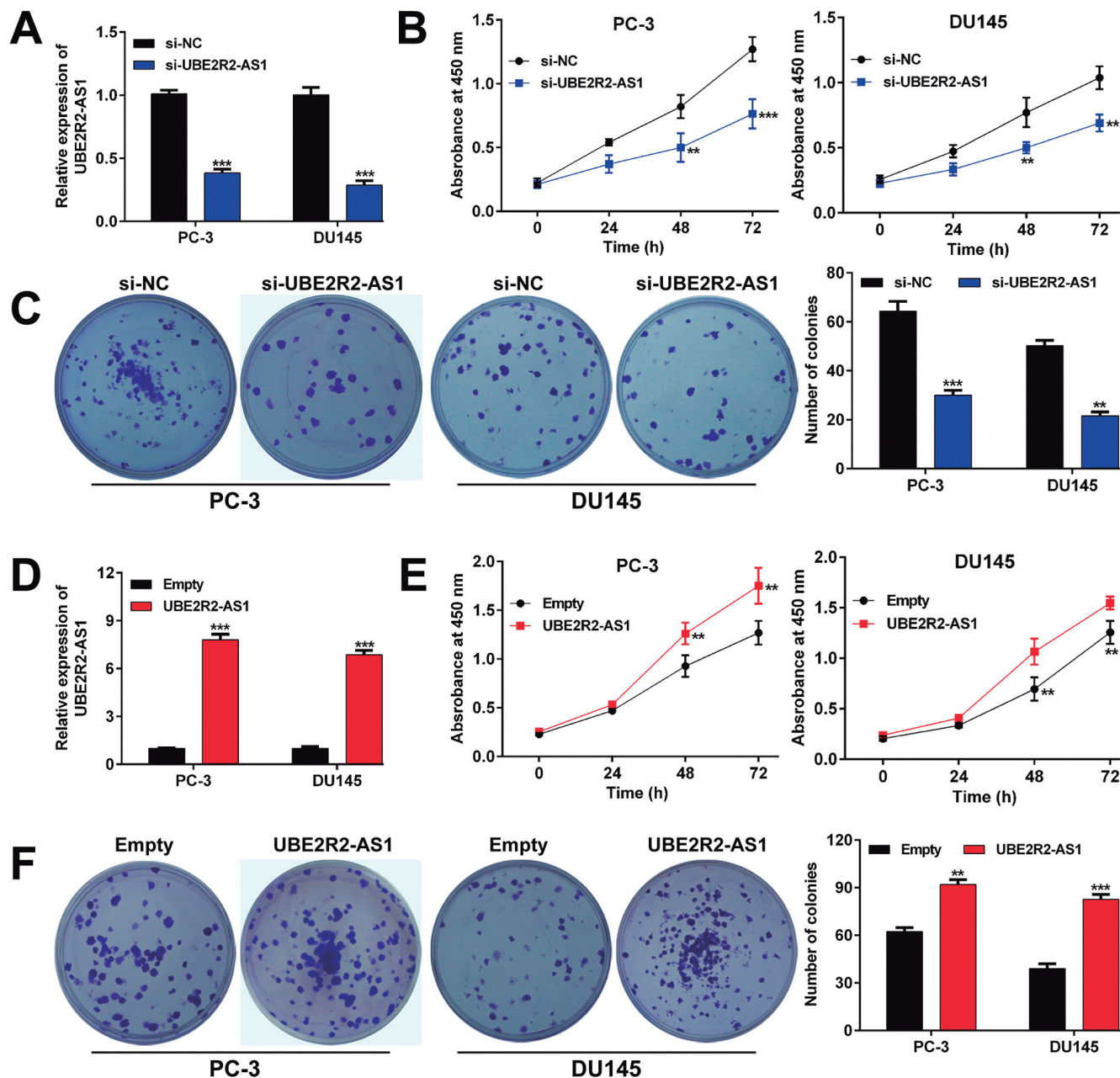


Fig. 3. Effects of UBE2R2-AS1 on the cell proliferation of prostate cancer cells. **A.** The expression level of UBE2R2-AS1 was determined in PC-3 and DU145 cells after transfection with si-UBE2R2-AS1 by quantitative reverse transcription PCR analysis. CCK-8 assay (**B**) and colony formation (**C**) was used to analyze cell proliferation in PC-3 and DU145 cells after transfection with si-UBE2R2-AS1. **D.** The expression level of UBE2R2-AS1 was determined in PC-3 and DU145 cells after transfection with UBE2R2-AS1 overexpression plasmid by quantitative reverse transcription PCR analysis. CCK-8 (**E**) assay and colony formation (**F**) was used to analyze cell proliferation in PC-3 and DU145 cells after transfection with UBE2R2-AS1 overexpression plasmid. Data were expressed as the mean \pm SD. ** $P < 0.01$; *** $P < 0.001$, compared with si-NC or empty vector.

significantly higher proportion of apoptotic cells in both PC-3 and DU145 cells (Fig. 4B). On the other hand, forced expression of UBE2R2-AS1 obviously promoted the cell cycle G1/S transition (Fig. 4C) and suppressed cell apoptosis (Fig. 4D) in both PC-3 and DU145 cells.

UBE2R2-AS1 promoted cell migration and invasion in PC cells

Next, we evaluated the role of UBE2R2-AS1 in PC cell migration and invasion, which are critical factors in cancer metastasis. Transwell migration assays revealed that the number of migratory cells was significantly reduced in si-UBE2R2-AS1-transfected PC-3 and DU145 cells, compared with si-NC transfected cells (Fig. 5A). Similarly, knockdown of UBE2R2-AS1 suppressed the invasive capacities of PC-3 and DU145 cells (Fig. 5B). In contrast, overexpression of UBE2R2-

AS1 dramatically increased the number of migratory cells (Fig. 5C) and invasive cells (Fig. 5D) in both PC-3 and DU145 cells.

UBE2R2-AS1 affected related protein expression levels in PC cells

To confirm the regulatory role of UBE2R2-AS1 in PC cells, we analyzed the effects of UBE2R2-AS1 on protein markers associated with cell proliferation, G1/S transition, apoptosis and EMT process in PC cells. As shown in Figure 6A, knockdown of UBE2R2-AS1 obviously downregulated the protein expression of PCNA, CDK4, Cyclin D1, Bcl-2, N-cadherin and Vimentin, while it upregulated E-cadherin expression in PC-3 cells. Furthermore, we observed that overexpression of UBE2R2-AS1 obtained the opposite results in these protein markers in DU145 cells (Fig. 6B).

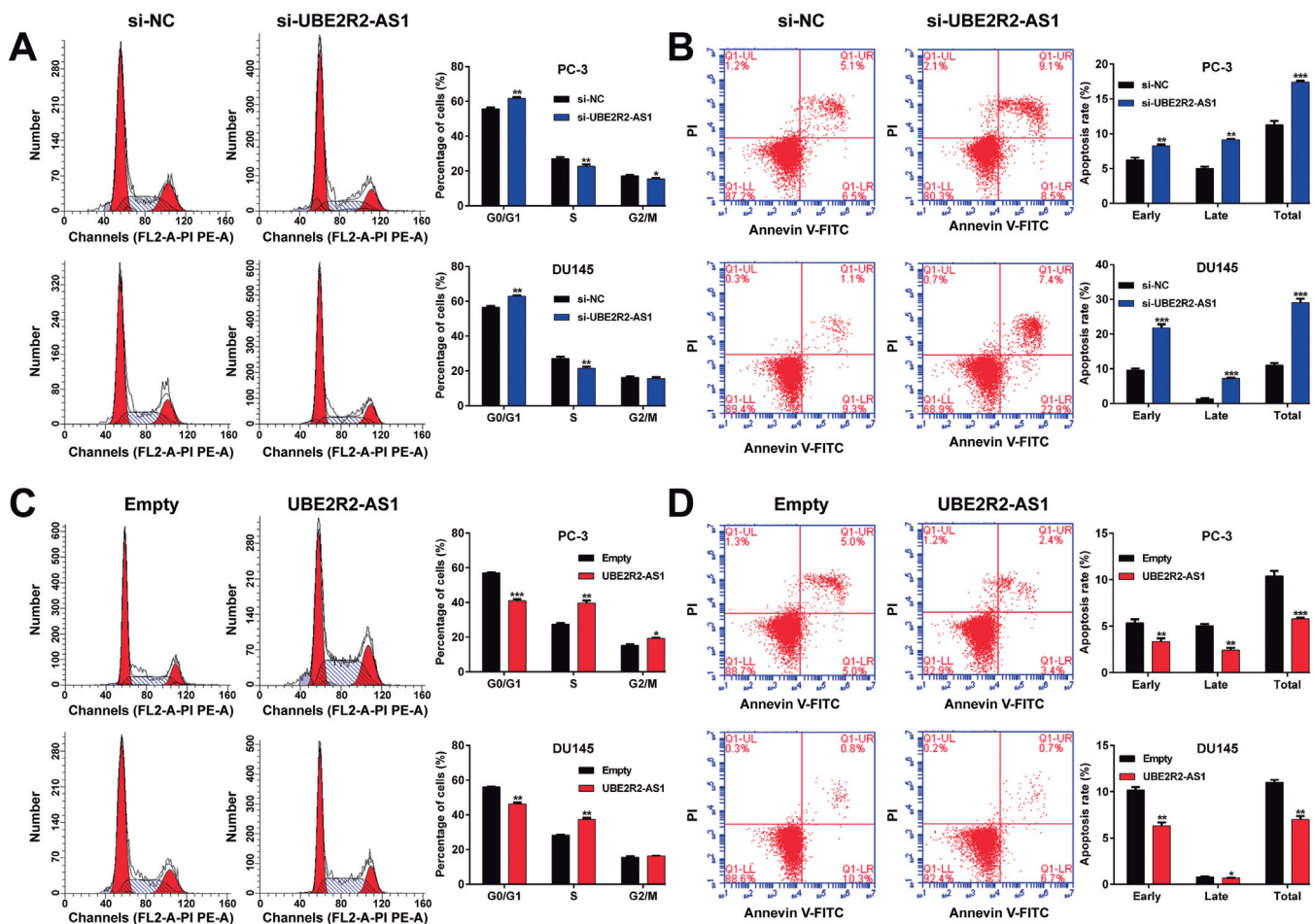


Fig. 4. Effects of UBE2R2-AS1 on the cell cycle progression and apoptosis of prostate cancer cells. Cell cycle distribution (A) and apoptotic rate (B) were measured in PC-3 and DU145 cells after transfection with si-UBE2R2-AS1. Cell cycle distribution (C) and apoptotic rate (D) were measured in PC-3 and DU145 cells after transfection with UBE2R2-AS1 overexpression plasmid. Data were expressed as the mean \pm SD. * P <0.05, ** P <0.01; *** P <0.001, compared with si-NC or empty vector.

Discussion

In recent years, lncRNAs have attracted significant attention from researchers for their roles in almost all the hallmarks of cancers, including sustaining proliferative signaling, resisting cell death and activating invasion/metastasis (de Oliveira et al., 2019). UBE2R2-AS1 has been identified to be a tumor suppressor in glioma (Xu et al., 2021) and cervical cancer (Liu et al., 2020), but an oncogene in HCC (Wu et al., 2020). Nevertheless, the biological roles of UBE2R2-AS1 in PC remain unclear. Therefore, we enrolled PC patients into this study and examined the expression of UBE2R2-AS1. The results showed that UBE2R2-AS1 expression was upregulated in PC tissues compared with adjacent tissues. Increased UBE2R2-AS1 was associated with high Gleason score, advanced clinical T stage, lymph node metastasis and poor prognosis in PC patients. Prostate-specific antigen (PSA) is a useful biomarker for detection of PC and for risk classification in addition to TNM classification and Gleason score (Iwamoto et al., 2019). However, the results from Table 1 showed that increased UBE2R2-AS1 expression did not correlate with PSA level, which might be ascribed to limited sample size. Consistent with our data, upregulated expression of UBE2R2-AS1 is associated with large tumor size, multiple tumor number, advanced TNM stage, and poor survival of HCC patients (Wu et al., 2020). Contrary to our results, UBE2R2-AS1 expression was dramatically downregulated in glioma compared with normal tissues (Xu et al., 2019). Compared with adjacent normal tissues, UBE2R2-AS1 expression was significantly suppressed in cervical cancer tissues

correlated with the increasing stage (Liu et al., 2020). The different expression levels of UBE2R2-AS1 in tumor tissues might be explained by different tumor types.

To examine the biological roles of UBE2R2-AS1 in PC, loss-of and gain-of functional experiments were conducted in PC cell lines. As expected, si-UBE2R2-AS1 transfection decreased, while the pcDNA3.1-UBE2R2-AS1 increased UBE2R2-AS1 expression in PC cell lines. Functional assays revealed that knockdown of UBE2R2-AS1 was able to decrease PC cell proliferation, migration and invasion, and induced cell cycle G0/G1 arrest and apoptosis. Not surprisingly, overexpression of UBE2R2-AS1 caused exactly the opposite effects of PC cell behaviors. Therefore, we confirmed that lncRNA UBE2R2-AS1 was able to promote PC progression. In fact, the regulatory role of UBE2R2-AS1 in tumor cell behaviors has been elucidated in the following articles. UBE2R2-AS1 suppressed cell proliferation and enhanced apoptosis, as well as decreased cell invasion and wound healing in cervical cancer (Liu et al., 2020). UBE2R2-AS1 suppressed cell growth, migration, and invasion, as well as promoting cell apoptosis in glioblastoma (Xu et al., 2019). These two studies indicated the tumor suppressive effects of UBE2R2-AS1. In line with our data, knockdown UBE2R2-AS1 inhibited HCC growth and metastasis through the in vitro and in vivo experiments (Wu et al., 2020). The opposite results of UBE2R2-AS1 in tumor cells might be ascribed to different tissue resources, tumor types and specific molecular mechanisms underlying the upstream and downstream signaling of UBE2R2-AS1.

Next, we investigated the expression of related

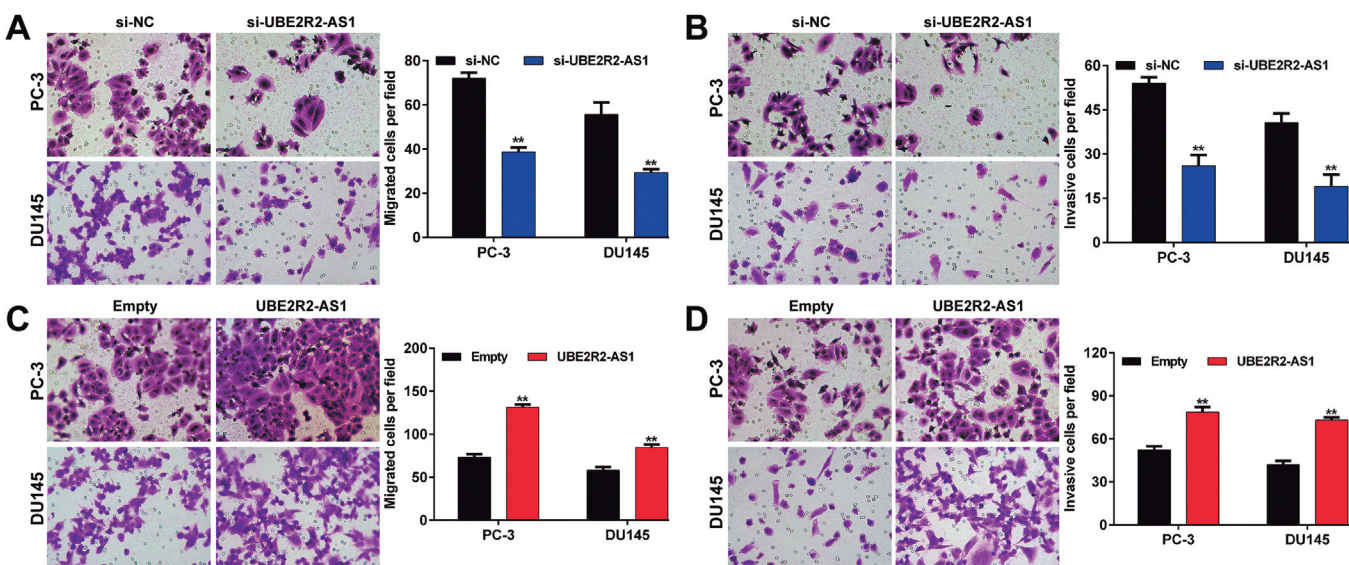


Fig. 5. Effects of UBE2R2-AS1 on cell migration and invasion in prostate cancer cells. Transwell assay was applied to assess cell migration (A) and invasion (B) in PC-3 and DU145 cells after transfection with si-UBE2R2-AS1. Transwell assay was applied to assess cell migration (C) and invasion (D) in PC-3 and DU145 cells after transfection with UBE2R2-AS1 overexpression plasmid. Data were expressed as the mean \pm SD. * P <0.05, compared with si-NC or empty vector.

proteins to confirm the regulatory role of UBE2R2-AS1 in PC cell proliferation, migration and invasion. Western blot assays showed that PCNA, CDK4, Cyclin D1, Bcl-2, N-cadherin and Vimentin were downregulated, while E-cadherin was upregulated following knockdown of UBE2R2-AS1 in PC cells. Overexpression of UBE2R2-AS1 obtained the opposite results. Proliferating cell nuclear antigen (PCNA) functions as a processivity factor for DNA polymerase delta, which is expressed at high levels in growing normal and tumor cells (Fukami-Kobayashi and Mitsui, 1999). The downregulation of PCNA after UBE2R2-AS1 knockdown further confirmed the suppressive effects of UBE2R2-AS1 knockdown on PC cell proliferation. As a binding partner and activator of the cyclin-dependent kinases CDK4 and CDK6, cyclin D1 plays a crucial role in G1-S transition (Sherr, 1994). Here, UBE2R2-AS1 knockdown induced downregulation of CDK4/Cyclin D1 and UBE2R2-AS1 overexpression induced upregulation of CDK4/Cyclin D1, further demonstrating that UBE2R2-AS1 promoted cell cycle G1/S transition in PC cells. The BCL-2 family modulates the checkpoint of apoptosis and has two major category members: apoptosis members like Bax and anti-apoptosis members like BCL-2 (Siddiqui et al., 2015). Our data showed that BCL-2 was downregulated following knockdown of UBE2R2-AS1, while it was upregulated following overexpression of UBE2R2-AS1, which further supported that UBE2R2-AS1 decreased cell apoptosis in

PC cells. Epithelial-mesenchymal transition (EMT) is a process of dedifferentiation and transformation of epithelial cells into mesenchymal cells (Luo et al., 2016). During EMT, the expression of E-cadherin is decreased, while the expression of vimentin, fibronectin, N-cadherin, and matrix metalloproteinases is up-regulated in malignant tumor cells (Kim et al., 2016; Yoshinaga et al., 2016). Here, the inhibition of EMT process induced by UBE2R2-AS1 knockdown might explain the suppressive effects of UBE2R2-AS1 knockdown on PC cell migration and invasion. Similar to our data, lncRNA SNHG7 promotes PC migration and invasion by modulating EMT (Han et al., 2019). In addition, lncRNA CHRFB promoted mesenchymal transition (EMT), showing down-regulation of E-cadherin and up-regulation of N-cadherin, vimentin and ZEB1 in PC-3 cells (Liu et al., 2019). Based on this evidence, we thus confirmed that UBE2R2-AS1 was able to promote PC progression. Of course, there were some limitations in our study as follows: 1) Lack of experimental validation on the overexpression of UBE2R2-AS1 on normal RWPE-1 cells; 2) Western blot analysis should be performed on the role of UBE2R2-AS1 overexpression on PC-3 and knockdown on DU145 cells in regulating related protein markers; 3) Lack of *in vivo* experiments to validate the oncogenic role of UBE2R2-AS1 in PC tumorigenesis.

Taken together, we provide evidence demonstrating that UBE2R2-AS1 predicted poor prognosis and promoted cell proliferation, migration and invasion in PC cells. This observation suggests a promising new therapeutic strategy for the treatment of PC, which might eventually lead to clinical trials targeting the UBE2R2-AS1 pathway.

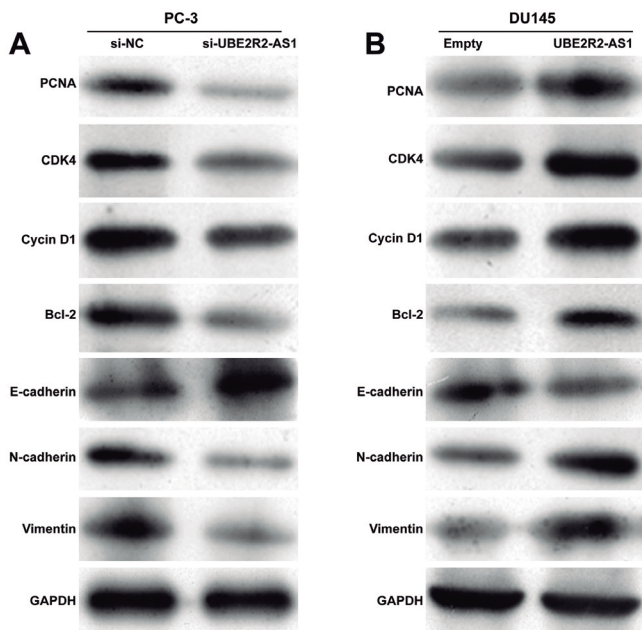


Fig. 6. UBE2R2-AS1 affected related protein expression levels in prostate cancer cells. **A.** The protein levels of PCNA, CDK4, Cyclin D1, Bcl-2, E-cadherin, N-cadherin and Vimentin were measured in PC-3 cells after transfection with si-UBE2R2-AS1. **B.** The protein levels of PCNA, CDK4, Cyclin D1, Bcl-2, E-cadherin, N-cadherin and Vimentin were measured in DU145 cells after transfection with UBE2R2-AS1 overexpression plasmid.

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Ethics Statement. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of First Affiliated Hospital of Heilongjiang Hospital (Harbin city, China).

Author contributions. DZP conceived and designed the experiments. WF and ZM performed the experiments. JYH performed the proliferative ability analysis. XSL and SDP performed cell cycle and apoptosis analysis. SDP and ZDH performed cell migration and invasion assay. WF and ZM revised figures and wrote the manuscript. All authors read and approved the final manuscript.

Availability of data and materials. All data generated or analyzed during this study are included in this article.

Patient consent for publication. Not applicable.

Competing interests. The authors declare that they have no competing interests.

References

- Bhan A., Soleimani M. and Mandal S.S. (2017). Long noncoding RNA and cancer: A new paradigm. *Cancer Res.* 77, 3965-3981.
- Bray F., Ferlay J., Soerjomataram I., Siegel R.L., Torre L.A. and Jemal A. (2018). Global cancer statistics 2018: Globocan estimates of

The role of UBE2R2-AS1 in prostate cancer

- incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 68, 394-424.
- Crehange G., Izaguirre A., Weinberg V., Hsu C.C., Gottschalk A.R., Hsu I.C., Shinohara K., Carroll P. and Roach M. 3rd (2016). Long-term outcomes following radiation therapy for prostate cancer patients with lymph node metastases at diagnosis treated with and without surgery. *Am. J. Clin. Oncol. Cancer* 39, 167-172.
- de Oliveira J.C., Oliveira L.C., Mathias C., Pedrosa G.A., Lemos D.S., Salviano-Silva A., Jucoski T.S., Lobo-Alves S.C., Zambalde E.P., Cipolla G.A. and Gradia D.F. (2019). Long non-coding RNAs in cancer: Another layer of complexity. *J. Gene. Med.* 21, e3065.
- Fukami-Kobayashi J. and Mitsui Y. (1999). Overexpression of proliferating cell nuclear antigen in mammalian cells negates growth arrest by serum starvation and cell contact. *Jpn. J. Cancer Res.* 90, 286-293.
- Han Y., Hu H. and Zhou J. (2019). Knockdown of lncRNA SNHG7 inhibited epithelial-mesenchymal transition in prostate cancer through miR-324-3p/WNT2B axis *in vitro*. *Pathol. Res Pract.* 215, 152537.
- Hoang D.T., Iczkowski K.A., Kilari D., See W. and Nevalainen M.T. (2017). Androgen receptor-dependent and -independent mechanisms driving prostate cancer progression: Opportunities for therapeutic targeting from multiple angles. *Oncotarget* 8, 3724-3745.
- Iwamoto H., Izumi K., Kadono Y. and Mizokami A. (2019). Prognosis of patients with prostate cancer and middle range prostate-specific antigen levels of 20-100 ng/ml. *Int. Braz. J. Urol.* 45, 61-67.
- Jandura A. and Krause H.M. (2017). The new RNA world: Growing evidence for long noncoding rna functionality. *Trends Genet.* 33, 665-676.
- Kim C.W., Hwang K.A. and Choi K.C. (2016). Anti-metastatic potential of resveratrol and its metabolites by the inhibition of epithelial-mesenchymal transition, migration, and invasion of malignant cancer cells. *Phytomedicine* 23, 1787-1796.
- Kornienko A.E., Guenzl P.M., Barlow D.P. and Pauler F.M. (2013). Gene regulation by the act of long non-coding RNA transcription. *BMC Biol.* 11, 59.
- Liu C., Li H. and Yin Q. (2020). The lncRNA UBE2R2-AS1 suppresses cervical cancer cell growth *in vitro*. *Open Med Warsaw.* 15, 1184-1192.
- Liu S., Wang L., Li Y., Cui Y., Wang Y. and Liu C. (2019). Long non-coding RNA CHRF promotes proliferation and mesenchymal transition (EMT) in prostate cancer cell line PC3 requiring up-regulating microRNA-10b. *Biol. Chem.* 400, 1035-1045.
- Luo W., Zhu X., Liu W., Ren Y., Bei C., Qin L., Miao X., Tang F., Tang G. and Tan S. (2016). Myc associated zinc finger protein promotes the invasion and metastasis of hepatocellular carcinoma by inducing epithelial mesenchymal transition. *Oncotarget* 7, 86420-86432.
- Ponting C.P., Oliver P.L. and Reik W. (2009). Evolution and functions of long noncoding RNAs. *Cell.* 136, 629-641.
- Renganathan A. and Felley-Bosco E. (2017). Long noncoding RNAs in cancer and therapeutic potential. *Adv. Exp. Med. Biol.* 1008, 199-222.
- Sherr C.J. (1994). G1 phase progression: Cycling on cue. *Cell.* 79, 551-555.
- Siddiqui W.A., Ahad A. and Ahsan H. (2015). The mystery of BCL2 family: Bcl-2 proteins and apoptosis: An update. *Arch. Toxicol.* 89, 289-317.
- Spizzo R., Almeida M.I., Colombatti A. and Calin G.A. (2012). Long non-coding RNAs and cancer: A new frontier of translational research? *Oncotargets Ther.* 12, 4577-4587.
- Wu Z., Wei Z.H. and Chen S.H. (2020). LncUBE2R2-AS1 acts as a microRNA sponge of miR-302b to promote HCC progression via activation EGFR-PI3K-AKT signaling pathway. *Cell Cycle* 19, 2426-2435.
- Xu W., Hu G.Q., Da Costa C., Tang J.H., Li Q.R., Du L., Pan Y.W. and Lv S.Q. (2019). Long noncoding RNA UBER2R2-AS1 promotes glioma cell apoptosis via targeting the miR-877-3p/TLR4 axis. *Oncotargets Ther.* 12, 3467-3480.
- Xu W., Che D.D., Chen L., Lv S.Q., Su J., Tan J., Liu Q. and Pan Y.W. (2021). UBER2R2-AS1 inhibits xenograft growth in nude mice and correlates with a positive prognosis in glioma. *J. Mol. Neurosci.* 71, 1605-1613.
- Yoshinaga T., Uwabe K., Naito S., Higashino K., Nakano T., Numata Y. and Kihara A. (2016). AM251 suppresses epithelial-mesenchymal transition of renal tubular epithelial cells. *PLoS One* 11, e0167848.
- Yuan X., Cai C., Chen S., Chen S., Yu Z. and Balk S.P. (2014). Androgen receptor functions in castration-resistant prostate cancer and mechanisms of resistance to new agents targeting the androgen axis. *Oncogene* 33, 2815-2825.
- Zhou M., Zhang Z., Zhao H., Bao S., Cheng L. and Sun J. (2018). An immune-related six-lncrna signature to improve prognosis prediction of glioblastoma multiforme. *Mol. Neurobiol.* 55, 3684-3697.

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