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Original Article

LncRNA LOC283194 regulates cell cycle and apoptosis in prostate cancer cell lines and predicts poor prognosis in PCa patients

Yuxiao Zheng[1*], Fade Liu[1*], Gong Cheng[1*], Qiang Cao[1], Yongsheng Pan[1], Haoxiang Xu[1], Yiren Gao[1], Feng Qi[1], Chao Qin[1], Shancheng Ren[2], Zengjun Wang[1], Lixin Hua[1], Haiwei Yang[1], Yinghao Sun[2]

[1]The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China; [2]Department of Urology, Shanghai Changhai Hospital, Second Military Medical University, Shanghai 200433, China. [*]Equal contributors.

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Abstract: Objective: To investigate the effect of the prostate cancer-up-regulated lncRNA LOC283194 on the cell function of prostate cancer (PCa) cell lines, and to analyze the clinical significance of measurement of LOC283194 content in cases of human PCa. Materials and methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to demonstrate the expression of LOC283194 in 64 pairs of PCa tumor and adjacent non-tumor tissues, and in PC3, DU-145 and WPMY-1 cell lines. Cell cycle and apoptosis were assessed using flow cytometry in PC3 and DU-145 cell lines following suppression of LOC283194 by siRNA. The influence of LOC283194 on the viability and proliferation these cell lines were evaluated with Cell Counting Kit-8 (CCK-8) assay and cell cloning experiments. The correlation of LOC283194 expression with clinic-pathologic characteristics and prognosis of PCa patients was assessed by Log-Rank test and Cox proportional hazards model. Results: The expression of LOC283194 was significantly higher in human PCa tissues compared with that in adjacent non-tumor tissues, as well as in PCa cell lines relative to a normal prostate cell line. We further demonstrated that LOC283194 increased the expression of cell cycle regulatory protein Cyclin D1, induced cell cycle arrest in S phase and reduced cell cycle arrest in G0/G1 phase. In addition, our study showed that knockdown of LOC283194 induced apoptosis of PCa cell lines. Besides, the high expression level of LOC283194 was associated with advanced features and poor prognosis of PCa. The multivariate analysis confirmed LOC283194 expression was an independent prognostic factor for PCa. Conclusion: LOC283194 induced cell cycle arrest in G0/G1 phase and promoted apoptosis of PCa cell lines, and it may represent a prognostic biomarker in PCa diagnosis and treatment.

Keywords: Prostate cancer, long non-coding RNA, apoptosis, prognosis

Introduction

Prostate cancer (PCa) is one of the most prevalent malignant tumors in western countries, and is the first leading cause of cancer death in men [1]. Although a large portion of the human genome is transcribed, protein coding genes account for < 2% of genomic sequence, with the remainder of the human transcriptome being comprised of largely of non-coding RNAs (ncRNAs) [2]. Long non-coding RNAs (lncRNAs) are untranslated transcripts longer than 200 nucleotides bearing many of the structural characteristics of mRNAs, but no conserved open reading frame [3]. In addition to the classic protein coding mRNAs, more recently, non-coding RNAs were characterized, such as microRNAs, small interfering RNAs and long non-coding RNAs, that encode for more than 98% of the genomic transcription products [4]. Different from originally thought to be transcriptional noise, lncRNAs have contributed significantly to the mechanistic, functional and translational aspects of cancer biology recently. Nowadays, lncRNAs are known to play important roles during cellular development and their misregulation has been shown in various types of cancers, such as breast cancer, PCa and hepatocellular carcinoma [5-7]. The role of lncRNA as a potential biomarker and therapeutic target is being gradually revealed [8]. According to recent studies, lncRNAs may play

the role such as proto-oncogenes, tumor suppressor genes, or drivers of metastatic transformation to affect epigenetic information and the process of tumor growth [9-11]. Similar to other types of malignancies, PCa is a complicated biological process characterized by a myriad spectrum of molecular abnormalities, and lncRNAs are widely involved in the development and progression of PCa. PCA3 was the first prominent lncRNA which initially described as a novel biomarker of PCa [12] and subsequently used as a promising urine test for it [13]. In recent years, researchers have begun to explore the potential of lncRNAs as a target for treatment of PCa and other malignancies [8]. Therefore, identification of cancer-associated lncRNAs and exploration of their clinical significance and functions may play a significant role in future diagnosis and treatment of cancer.

Our previous study [14] revealed that some lncRNAs are frequently over expressed in human PCa tissues compared to matched adjacent non-tumor tissues as demonstrated by RNA-Seq. In that study, we presented a comprehensive landscape of the transcriptome profles of 14 primary prostate cancers and their paired normal counterparts from the Chinese population using RNA-seq, revealing tremendous diversity across prostate cancer transcriptomes with respect to gene fusions, lncRNA, alternative splicing and somatic mutations. We found one of the lncRNAs was matched with LOC283194, which is located in chromosome 11 in the UCSC GenBank. The aim of this study was to investigate the expression of lncRNA LOC283194 in PCa tissues and matched normal tissues, and to explore the effect of LOC283194 on cell cycle and apoptosis of PCa cell lines.

Materials and methods

Tissue specimens and cell culture

Sixty-four pairs of PCa and matched adjacent non-tumor tissue specimens were obtained from the archives of Jiangsu Province Hospital with informed consent and with the approval of the institutional ethics committee. All specimens were obtained by the pathologists within 30 minutes after surgery, and were immediately frozen in liquid nitrogen and stored at -80°C until the extraction of total RNA.

Human PCa cell lines (PC3 and DU-145) and a normal myofibroblast stromal cell line (WPMY-1) were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). PC3 and DU-145 were maintained in F-12K Nutrient Mixture (GIBCO, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS), 1100 U/ml penicillin and 100 μg/ml streptomycin. While WPMY-1 was maintained in DMEM (GIBCO, Carlsbad, CA, USA) supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. All cell lines were cultured at 37°C in a humidified incubator with 5% CO_2.

RNA isolation, reverse transcription and qRT-PCR

Total RNA was extracted from frozen tissue specimens and cell lines using Trizol reagent (Life Technologies, Carlsbad, CA) and then reverse transcribed into cDNAs using PrimeScript RT reagent Kit (Perfect Real Time) (TaKaRa, DaLian, China) according to the manufacturer's instructions. LOC283194 expression level was measured by qRT-PCR in PCa tissues and matched adjacent non-tumor tissue specimens, and prostate cells lines using SYBR Premix Ex TaqTM (Perfect Real Time) (TaKaRa, DaLian, China) on ABI StepOne Plus instrument (Applied Biosystems, Foster City, CA). The primers used in this study were 5'-GCTACCGCTGACCTCTTACC-3' and 5'-ATGTGTGCAGTTGAGTGGGT-3' for LOC283194, and, 5'-ACTGGAACGGTGAAGGTGAC-3', and 5'-AGAGAAGTGGGGTGGCTTTT-3' for β-actin. All reactions were run in triplicate. The amplification profile was 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s, and annealing at 60°C for 30 s. LOC283194 expression in each sample was finally determined after correction with β-actin expression. The gene specific threshold cycle (Ct) for each sample was corrected by subtracting the Ct for the housekeeping gene β-actin (ΔCt).

Plasmid and siRNA transfection

A segment of the LOC2831943'-UTR and a mutated 3'-UTR of LOC283194 was cloned into the downstream of the luciferase gene in the pGL3-REPORT luciferase vector (Invitrogen). For luciferase assay, cells were cultured into 24-well plates and cultured for 24 h. Then, cells

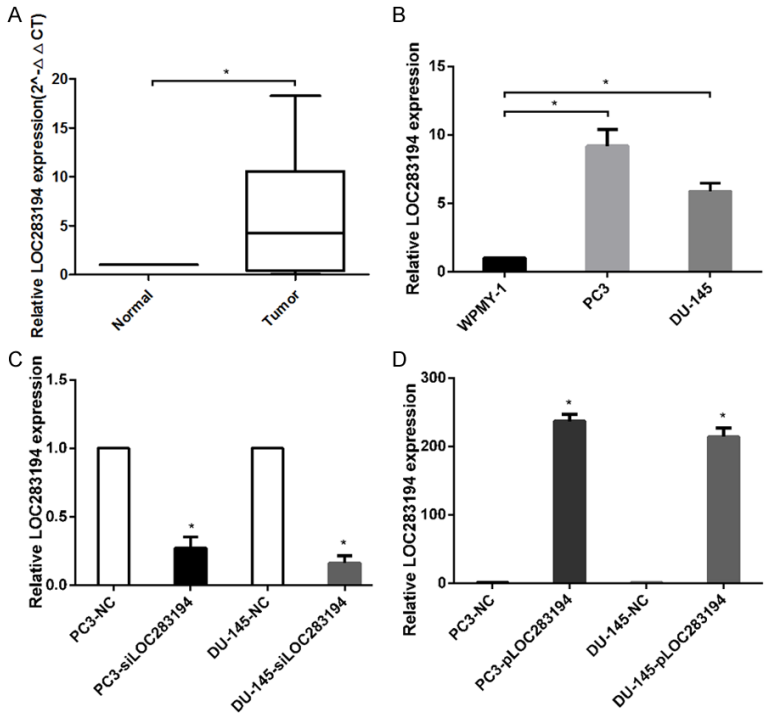

Figure 1. LOC283194 is over expressed in prostate cancer tissues and cells. LOC283194 was low expressed after silencing by siRNA and high expressed after plasmid transfection. (A) Quantitative RT-PCR analysis of LOC283194 expression in prostate cancer and matched normal prostatic tissues. (B) LOC283194 expression level is determined by qRT-PCR in PC-3, DU145 and WPMY-1 cell lines. LOC283194 expression level is determined by qRT-PCR in PC-3 and DU145 cell lines compared to negative control (NC) cells after transfected by siRNA (C) and plasmid (D). Data represent the mean ± SD from three independent experiments. *$P < 0.05$.

were co-transfected with pGL3-LOC283194-3'-UTR or control reporter plasmid, LOC283194 mimics or NC and LOC283194 activator or NC activator. Luciferase activities were measured by dual luciferase assays (Promega) after 48 hr after co-transfection. The results were normalized against the activity of the firefly/Renilla luciferase gene.

To promote siRNA transfection, PC3 and DU-145 cells were seeded at a density of 2.5×10^5 cells/well into a 6-well culture plate with F-12K Nutrient Mixture or DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. At the same time, PC3 and DU-145 cells were transfected with siRNA packaged by Lipofectamine 2000 transfection reagent (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. siRNAs were designed and synthesized by Shanghai GenePharma Co., Ltd., Shanghai, China. Forty-eight hours after transfection, the cells were harvested for further studies. The siRNAs used in this study were 5'-UCUUACCACCUGUGCCAAATT--3' and 5'-UUUGGCACAGGUGGUAAGATT-3' for siLOC283194, and, 5'-UUCUCCGAACGUGUCACGUTT-3' and 5'-ACGUGACACGUUCGGAGAATT-3' for control siRNAs.

Cell viability assays

Proliferation of PC3 and DU-145 cells was evaluated by the Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) assay according to the Manufacturer's instructions. This assay is based on the cleavage of the tetrazolium salt WST-8 by mitochondrial dehydrogenase in viable cells. 2×10^3 cells/well were incubated with 100 μL culture medium in 96-multiwell plates. Cells were cultured for 24, 48, 72, 96 hours before the addition of 20 μL CCK-8 (5 mg/ml) to the culture medium in each well. After a 2 hour incubation at 37°C, absorbance of each well was measured at 450 nm with a Thermomax microplate reader. Each experiment was repeated 3 times, and the data represent the mean of all measurements.

Colony formation assay

We used siLOC283194 or empty vector transfected prostate cancer cell lines PC3 and DU-145 stably. Then these cells were seeded into culture dishes at a density of 1500 cells per dish and cultured for 14 days. Methanol were performed to fix cells for 15 min and 0.1% crystal violet were used to stain cells for 30 min. The number of colonies containing > 50 cells was calculated with an inverted microscope.

Flow cytometry analysis of cell cycle and apoptosis

PC3 and DU-145 cells were seeded at 4.0×10^5/ml in 24-well plates in 500 μl media/well and transfected with siLOC283194, empty plasmids or scramble sequences by Lipofe-

Table 1. Association between LOC283194 expression and clinicopathological characteristics

Variable	N	LOC283194 expression		*P* value
		High (n=45)*	Low (n=19)*	
Age (years)				
< 65	14 (21.88%)	10 (71.43%)	4 (28.57%)	0.918
≥65	50 (78.12%)	35 (70.00%)	15 (30.00%)	
Tumor size (cm)				
< 2	18 (28.13%)	13 (72.22%)	5 (27.78%)	0.834
≥2	46 (71.87%)	32 (69.57%)	14 (30.43%)	
Gleason score				
< 8	43 (67.19%)	26 (60.47%)	17 (39.53%)	0.014
≥8	21 (32.81%)	19 (90.48%)	2 (9.52%)	
T stage				
T2	37 (57.81%)	22 (59.46%)	15 (40.54%)	0.026
T3-4	27 (42.19%)	23 (85.19%)	4 (14.81%)	
Lymph node stage				
Positive	22 (34.38%)	19 (86.36%)	3 (13.64%)	0.042
Negative	42 (65.62%)	26 (61.90%)	16 (38.10%)	
Multiple lesions				
Yes	24 (37.50%)	18 (75.00%)	6 (25.00%)	0.525
No	40 (62.50%)	27 (67.50%)	13 (32.50%)	

*LOC283194 expression was defined as 'high' or 'low' expression when it was twice or half as expression in normal tissue.

ctamine 2000 reagent in serum-free F-12K Nutrient Mixture for 6 hours. After transfection, 500 μl of the appropriate growth medium containing 10% FBS were added to each well. Cells were incubated for another 48 hours, then harvested, washed twice with PBS buffer. For cell cycle analysis, the cells was fixed with 70% ethanol, and treated with RNase A (1 mg/ml), then stained with PI (50 μg/ml) for 30 min at room temperature. After washed twice with PBS buffer, cells were analyzed on a FACS Aria flow cytometer with FACS Diva software (BD Biosciences). For apoptosis analysis, the cells were double-stained with FITC-conjugated annexin-V and propidium iodide (PI) solution (50 μg/ml). For each sample, data from approximately 10000 cells were recorded. Apoptosis and necrosis were analyzed by quadrant statistics on double negative, annexin-V-positive/PI-negative, annexin-V-negative/PI-positive, and double positive cells.

Western blot assay

Cell lines were lysed in RIPA buffer (KeyGene Biotech, Beijing, China) supplemented with protease inhibitors at 4°C for 1 hour. The protein samples were electrophoresed in 10% SDS-PAGE gels, transferred onto PVDF membranes (Millipore, Billerica, USA), and then blocked for 2 hours with 5% non-fat milk at room temperature. After being incubated with primary antibodies at 4°C overnight, the PVDF membranes were washed three times with TBST (20 nM Tris-HCl, PH 7.6, 137 mM NaCl, and 0.1% Tween 20) and then incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody at room temperature for 2 hours. The blots were detected by chemiluminescence (Thermo Scientific, Waltham, USA). Protein levels were determined by normalizing to GAPDH.

Statistical analysis

In this study, data were expressed as the mean ± standard deviation. Differences in LOC283194 expression between the PCa tumor tissue and the matched adjacent non-tumor tissue specimens were analyzed using Wilcoxon signed-rank test by SPSS software (version **). Patient survival was evaluated using the Kaplan-Meier method and compared using log-rank test. Univariate and multivariate Cox regression analyses were performed for survival data. The PCa cell viability was performed with analysis of variance. Statistical significance was defined as $P < 0.05$.

Results

LOC283194 expression was significantly up-regulated in PCa cell lines and tissues

We first detected LOC283194 expression in 64 pairs of PCa tumor tissue and matched adjacent non-tumor tissue specimens. LOC283194 expression was significantly up-regulated in PCa samples compared to adjacent non-tumor samples (median (IQR): 4.13 (0.35-10.56), $P < 0.05$, **Figure 1A**). Expression was further examined by qRT-PCR in PCa cell lines and a normal human myofibroblast stromal cell line WPMY-1. As shown in **Figure 1B**, the expression of LOC283194 was significantly higher in

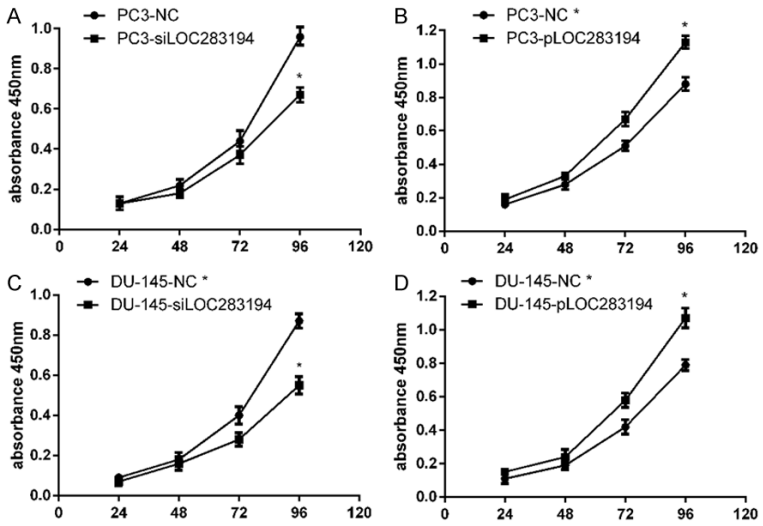

Figure 2. LOC283194 promotes the viability of prostate cell lines. Viability of PC3/DU145 was down-regulated when LOC283194 silenced by siRNA (A, C) and up-regulated when LOC283194 was over-expressed stably after the transfenction of plasmid (B, D). *$P < 0.05$.

DU-145 and PC3 than that in WPMY-1 (PC3: 9.44 ± 1.01 vs. 1, P < 0.05; DU145: 5.97 ± 0.60 vs. 1, P < 0.05). In addition , we found the expression of LOC283194 was suppressed in PC3 and DU-145 cell lines compared with negative control (NC) cells after silenced by siRNA (P < 0.05, **Figure 1C**) and was elevated after transfected with plasmids (P < 0.05, **Figure 1D**). In the 64 PCa cases, LOC283194 was highly expressed in 45 cases compared with adjacent non-tumor tissue. For further analysis, PCa tissue specimens were classified into high-expression group (n=45) which the expression of LOC283194 was more than 5 times that of adjacent non-tumor tissue; and low-expression group (n=19) which the expression of LOC283194 was less than 5 times that of adjacent non-tumor tissue. The association between LOC283194 expression and clinicopathological characteristics of PCa patients was shown in **Table 1**, and the result demonstrated that LOC283194 was positively associated with Gleason score (P=0.007), T stage (P=0.031), and lymph node stage (P=0.02) in PCa patients.

LOC283194 promotes viability of PCa cells

CCK-8 assay were performed to evaluate the effect of LOC283194 on viability in PCa cell lines. As shown in **Figure 2**, the viability of PC3/DU145 was down-regulated when LOC283194 silenced by siRNA (**Figure 2A**. PC3: 0.66 ± 0.03 vs. 0.96 ± 0.04, P < 0.05, **Figure 2C**. DU145: 0.56 ± 0.03 vs. 0.87 ± 0.03, P < 0.05) and up-regulated when LOC283194 was over-expressed stably after the transfection of plasmid (**Figure 2B**. PC3: 1.09 ± 0.04 vs. 0.88 ± 0.04, P < 0.05, **Figure 2D**. DU145: 1.07 ± 0.04 vs. 0.78 ± 0.02, P < 0.05).

LOC283194 promotes proliferation of PCa cells

To determine whether LOC283194 affects PCa cell proliferation in vitro, colony formation assays were performed and date showed that down-regulated LOC283194 suppressed the colony formation capacity of PCa cells (**Figure 3A** and **3B**). We then attempt to confirm this with flow cytometry analysis of cell cycle. As shown in **Figure 3C-H**, suppression of LOC283194 increased the proportion of cells in G0/G1 phase and decreased the proportion of cells in S phase compared with the cells of negative control group. In order to reversely verify our results, LOC283194 was up-regulated by plasmid and results showed that the G0/G1 phase cell rate declined and the S phase rate ascended (**Figure 3I-N**).

LOC283194 induces apoptosis of PCa cells

There is increasing evidence that decreased apoptosis are pivotal in the formation and progression of cancer. Our experiments have shown that LOC283194 did not influence cell proliferation for PC3 and DU-145 cell lines. To explore whether LOC283194 affect PCa cell apoptosis, we performed an apoptosis assay in PC3 and DU-145 cell lines. Knockdown of LOC283194 resulted in significant induction of apoptosis in PC3 and DU-145 cell lines, and also resulted in a significant increase in expression of cleaved-PARP in these two cell lines, suggesting that LOC283194 could inhibit apoptosis of PCa cells via cleaved-PARP (**Figure 4**).

LOC283194 promotes Bcl-2 and Cyclin D1 protein expression and inhibits Bax protein expression

To explore the mechanism by which LOC283194 induced growth arrest and apoptosis, western

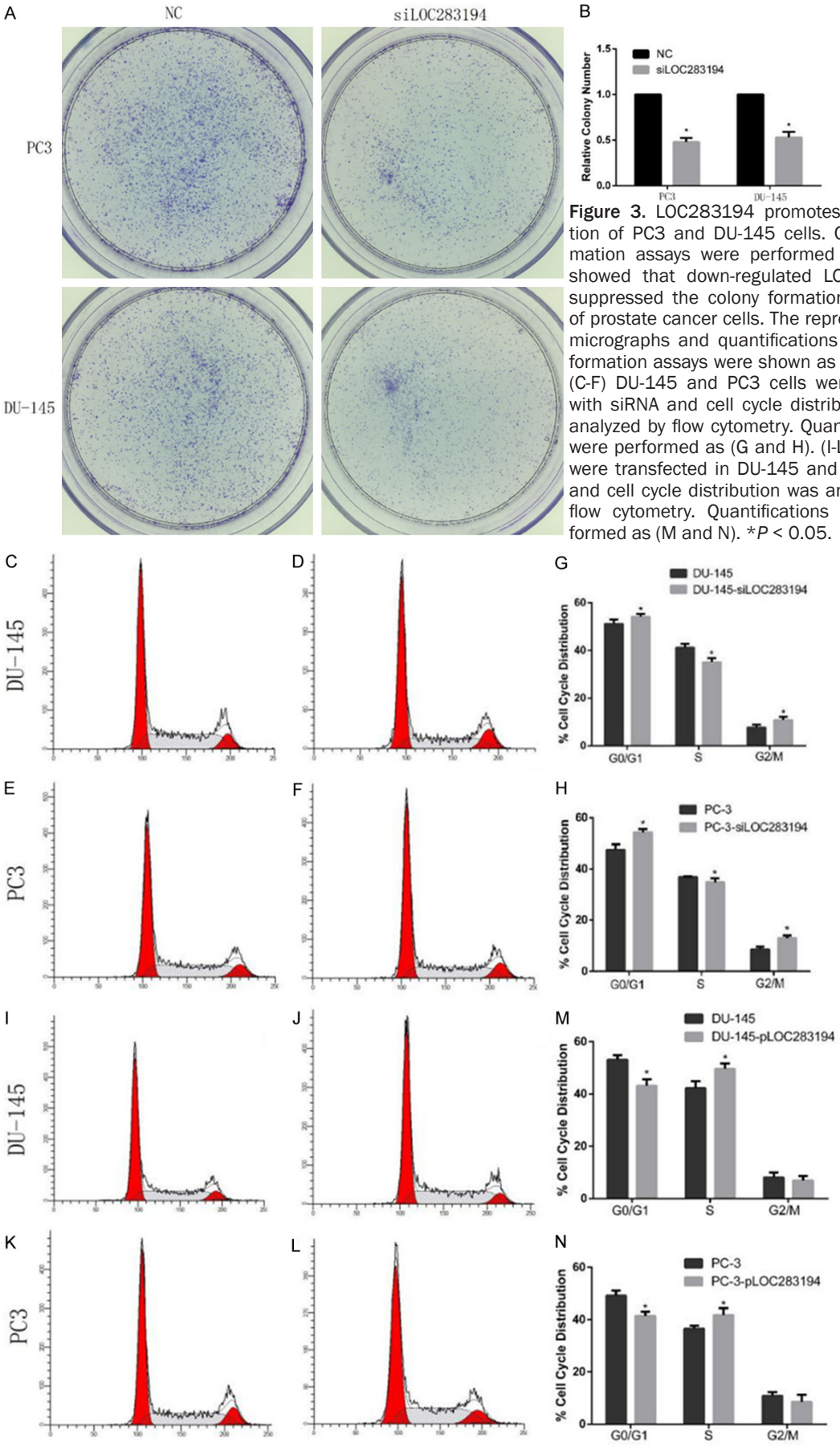

Figure 3. LOC283194 promotes proliferation of PC3 and DU-145 cells. Colony formation assays were performed and date showed that down-regulated LOC283194 suppressed the colony formation capacity of prostate cancer cells. The representative micrographs and quantifications of colony formation assays were shown as (A and B). (C-F) DU-145 and PC3 cells were treated with siRNA and cell cycle distribution was analyzed by flow cytometry. Quantifications were performed as (G and H). (I-L) Plasmid were transfected in DU-145 and PC3 cells and cell cycle distribution was analyzed by flow cytometry. Quantifications were performed as (M and N). $*P < 0.05$.

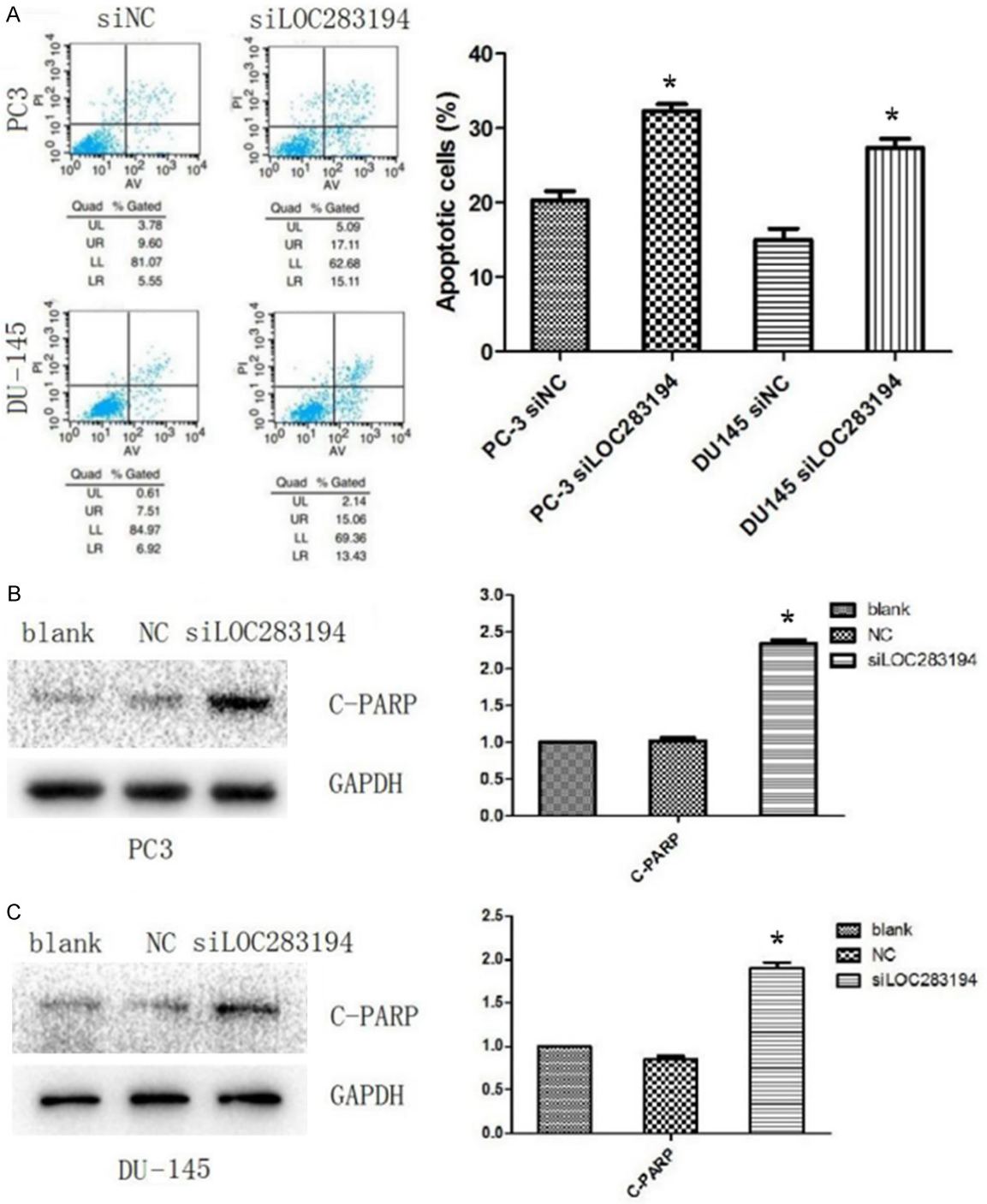

Figure 4. Suppression of LOC283194 induces apoptosis in PC-3 and DU145 cells. (A) Flow cytometric analysis demonstrated that knockdown of LOC283194 induced apoptosis in PC3 and DU-145 cell lines. (B) and (C) Western blotting assay showed cleaved-PARP was induced by LOC283194 in PC3 and DU145 cells. *$P < 0.05$.

blot assay was performed to examine the expression of cell cycle and apoptosis associated proteins, including Cyclin D1, Cyclin A2, Bax and Bcl-2. As shown in **Figure 5A**, Bax was up-regulated and Cyclin D1 and Bcl-2 were down-regulated in siLOC283194-transfected PCa cells compared with negative control cells, but there was no significant change in cyclin A2 expression. Besides, the extent of difference in expression of cyclin D1 in PC3 and DU145 cells was larger than that in Bcl-2 and BAX after transfection of siLOC283194. Next, qRT-PCR assay was performed to examine the expression of Cyclin D1, Cyclin A2, Bax and Bcl-2

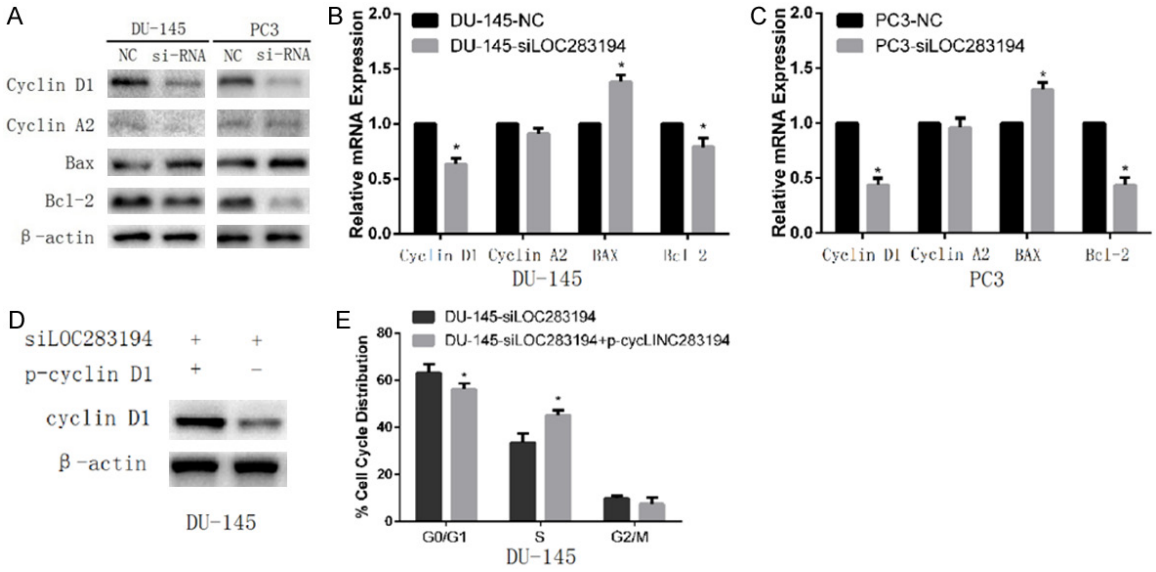

Figure 5. The expression of Cyclin D1, Cyclin A2, Bax and Bcl-2. (A) After down-regulation the expression of LOC283194 with siRNA transfection, the expression of Cyclin D1, Cyclin A2, Bax and Bcl-2 protein in DU-145 and PC3 cell lines was analyzed by western blot assay. (B, C) The mRNA expression level of Cyclin D1, Cyclin A2, Bax and Bcl-2 was analysized by qRT-PCR. (D) The expression of Cyclin D1 protein was detected in DU-145 cells co-transfected with siLOC283194 and pCDNA-cyclin D1, and the flow cytometry analysis of cell cycle was performed (E). *$P < 0.05$.

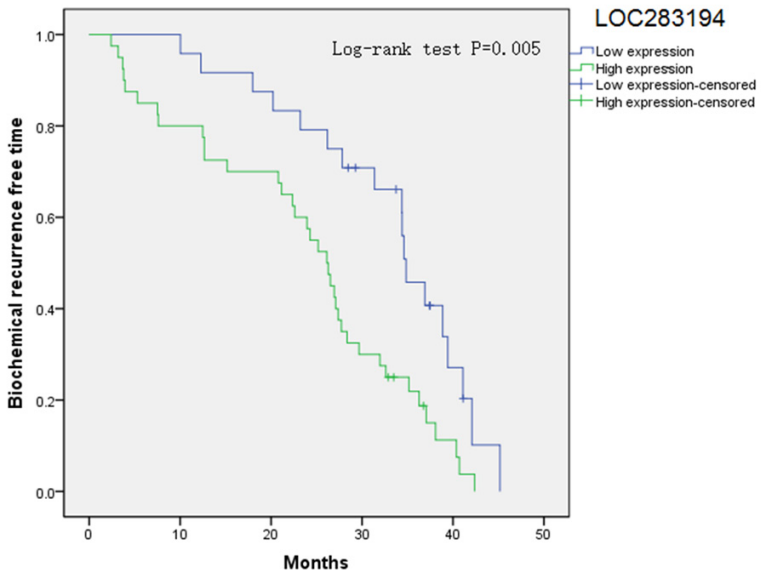

Figure 6. LOC283194 expression is associated with biochemical recurrence-free time in PCa patients. LOC283194 expression was defined as 'high' or 'low' expression when it was twice or half as expression in normal tissue. Patients with high and low expression of LOC283194 were 45 and 15, respectively. Patients with higher expression of LOC283194 showed shorter biochemical recurrence-free time compared to patients with lower expression of LOC283194 (P=0.005, log-rank test).

mRNA, and the results demonstrated that Cyclin D1 and Bcl-2 mRNA was down-regulated by siLOC283194, and BAX mRNA was up-regulated by siLOC283194 (**Figure 5B**, **5C**). After that, pCDNA-Cyclin D1 were co-transfected with siLOC283194 into DU-145 cells to confirm the role of Cyclin D1 in cell cycle changes. Compared to the DU-145 cells transfected siLOC283-194 singly, the expression of Cyclin D1 protein increased in cells co-transfected with pCDNA-Cyclin D1 and siLOC283-194. In addition, the results of flow cytometry analysis of cell cycle showed that the G0/G1 phase cell rate declined and the S phase rate ascended in co-transfection group (**Figure 5E**).

LOC283194 expression is associated with biochemical recurrence-free time in PCa patients

In order to assess the prognostic value of LOC283194 expression on PCa patients, we

Table 2. Univariate and multivariate analysis of clinicopathologic characteristics for biochemical recurrence-free time in 64 patients with PCa

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
Age (years)						
≥65 vs. < 65 (ref)	0.998	0.962-1.036	0.932			
Tumor size						
≥2 vs. < 2 (ref)	1.103	0.635-1.917	0.727			
Gleason score						
≥8 vs. < 8 (ref)	46.461	14.425-148.998	< 0.001	16.216	4.271-61.571	< 0.001
T stage						
T3-4 vs. T2 (ref)	8.78	4.617-16.697	< 0.001	3.027	1.192-7.685	0.02
Lymph node stage						
Positive vs. Negative (ref)	4.399	2.395-8.079	< 0.001	2.495	1.278-4.873	0.007
Multiple lesions						
Yea vs. No (ref)	1.209	0.724-2.017	0.468			
LOC283194						
High vs. Low (ref)*	4.070	2.132-7.769	< 0.001	2.861	1.449-5.651	0.002

*LOC283194 expression was defined as 'high' or 'low' expression when it was twice or half as expression in normal tissue.

investigated the association between LOC283194 expression levels and biochemical recurrence-free time in 64 PCa cases. As shown in **Figure 6**, we observed that LOC283194 expression was significantly associated with biochemical recurrence-free time of PCa patients (Log rank P=0.005). In addition, multivariate analysis showed that increased LOC283194 expression was an independent poor prognostic factor for PCa patients (P=0.002, **Table 2**).

Discussion

In recent years, the development of high-throughput chip technology contributes great help in lncRNA screening and research. In our previous study [8], several lncRNAs were detected frequently over expressed in human PCa tissues compared to matched adjacent non-tumor tissues by RNA-Seq. we got a lncRNA sequence from the analyzed result of RNA-Seq and found it matched with LOC283194, which is located in chromosome 11 in the UCSC GenBank. Thus, we retrospectively analyzed the expression of LOC400891 in 64 patients with PCa, and characterized that LOC283194 expression is higher in PCa tissues and cell lines compared to matched normal tissues and cell lines. Furthermore, the association between the expression of LOC283194 and clinicopathological characteristics in 64 PCa patients was explored and the result demonstrated that LOC283194 was positively associated with Gleason score, T stage and lymph node stage in PCa patients. In addition, high expression level of LOC283194 was significantly associated with a shorter biochemical recurrence-free time, indicating that high expression of LOC283194 correlates with malignant status and poor prognosis in PCa patients. In summary, expression level of LOC400891 may be an independent prognostic factor for PCa patients, and overexpression of LOC283194 may promote the development and progression of PCa.

In order to further elucidate the biological function of LOC283194 in prostate cancer, we investigated the effect of LOC283194 on the viability, proliferation, cell cycle and apoptosis of prostate cancer cells after down/up-regulated the expression level of LOC283194 transfected by siRNA/plasmid in vitro. The studies we have performed suggested that LOC283194 promote the viability, proliferation of prostate cancer cell lines, inhibit PCa cell apoptosis and induced cell cycle arrest in S phase and reduced cell cycle arrest in G0/G1 phase.

To explore the specific mechanism of LOC283194 regulation of progression in PCa, we investigated the cell cycle and apoptosis related protein cyclin D1, cyclin A2, BAX, and Bcl-2.

Cyclin D1 is a key regulator protein promotes the G1 phase to the S phase in the cell cycle [15]. The overexpression of cyclin D1 may lead to the shortening of G1 phase and the entire cell cycle. The cumulative effect of the reduction of cell genome mutation time may be the source of cell carcinogenesis [16]. Study have confirmed that cyclin D1 is overexpressed in tissue of prostate cancer, and involved in the development of prostate cancer and other processes [17]. The Bcl-2 protein plays an important role in preventing cancer cell apoptosis [18]. Bax is a pro-apoptotic protein wildly enrolled in apoptosis-related biochemical process [19]. Our results showed that LOC283194-induced apoptosis of PC3 and DU145 cells was associated with the down-regulation of the Bax and the up-regulation of the Bcl-2. Furthermore, we demonstrated that LOC283194 regulated cell cycle by increasing cyclin D1, and the rescue assay was performed to confirm this result. However, the effect of LOC283194 on cell cycle related protein cyclin A2 was not significant.

In conclusion, we demonstrated for the first time that LOC283194 may promote prostate cancer progression by regulating cell cycle and reducing apoptosis, and suggested that LOC283194 was positively associated with Gleason score, T stage and lymph node stage in PCa patients. In addition, we fund that LOC283194 was significantly associated with a shorter biochemical recurrence-free time. LOC283194 may be a potential biomarker in prostate cancer diagnosis and treatment.

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Disclosure of conflict of interest

None.

Address correspondence to: Lixin Hua and Haiwei Yang, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029 China. Tel: +8613072564303; E-mail: lixinhua@njmu.edu.cn (LXH); Tel: +8613338608666; E-mail: haiweiyang@njmu.edu.cn (HWY)

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