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Hsa_circ_0070440 mediates the prognosis and progress of human prostate cancer

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Summary. Background. This study was designed to explore whether hsa_circ_0070440 was dysregulated in prostate cancer (PCa), and assess the effects of hsa_circ_0070440 alteration on PCa prognosis and cell function.

Methods. The expression levels of hsa_circ_0070440 were assessed in PCa tissues and cell lines. After the classification of patients with PCa based on mean hsa_circ_0070440 level in 138 cases, Chi-square test and survival analyses (Kaplan-Meier method and multivariable Cox proportional hazards analysis) were performed to assess the predictive value of hsa_circ_0070440 in treatment failure (TTF), time to PSA progression (TTPP) and overall survival time. To examine the function of hsa_circ_0070440 in PCa cells, 22Rv1 and C4-2B cells were used for CCK-8 proliferation and Transwell migration assays. Hsa_circ_0070440- and TXNDC5-specific bindings with miR-382/383-5p were validated by bioinformatic analysis and luciferase gene reporter assay.

Results. An increased expression of hsa_circ_0070440 was found in PCA tissues and cell lines, associated with clinical T stage (p=0.021) and lymph node metastasis. Hsa_circ_0070440 predicted poor overall survival, TTPP, and TTF, acting as independent prognostic factors for overall survival, TTPP, and TTF in patients with PCa. Knockdown of hsa_circ_0070440 inhibited cell proliferation and migration *in vitro*. Furthermore, hsa_circ_0070440 could sponge miR-382/383-5p. *TXNDC5* was a common target gene for miR-382/383-5p in PCa cells.

Conclusion. This study demonstrated that hsa_circ_0070440 can predict the prognosis of PCa patients. Hsa_circ_0070440 can facilitate the proliferation and migration of PCa cells, possibly by sponging miR-382/383-5p.

Key words: Prostate cancer, Hsa_circ_0070440, Prognosis, Cell function

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Introduction

Prostate cancer (PCa) is one of the most common malignant tumors among men worldwide and the second leading cause of cancer-related deaths in men (Siegel et al., 2023). According to the statistics of GLOBOCAN 2020, the number of new cases of PCa worldwide exceeded 1.4 million, ranking second in male malignant tumors. At the same time, the number of deaths caused by PCa was about 370000, and the death rate of male malignant tumors was fifth (Wang et al., 2022b). In Asia, although the incidence rate of PCa is far lower than that in Western countries, it has been on the rise, and the growth rate is faster than that in Western countries (Zhu et al., 2021). In recent years, due to the aging of the population, the incidence rate of PCa in China is getting higher and higher, and it currently ranks first in the incidence rate of male malignant tumors (Wang et al., 2022a). In recent years, the age-standardized five-year survival rate of PCa has significantly improved (Culp et al., 2020). However, the initial symptoms of PCa are not obvious. Once patients have hematuria, acute urinary retention, urinary incontinence, and other symptoms, the disease has often progressed to the middle and late stages, and the five-year survival rate of these people is still low (Matti and Zargar-Shoshtari, 2021). Castration-resistant prostate cancer (CRPC) refers to PCa that continues to progress after initial continuous androgen deprivation treatment (Davies et al., 2019). Research shows that approximately 10-20% of patients will

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progress to CRPC within five years (Chism et al., 2014). For advanced PCa, although initial endocrine therapy may have good effects, it almost always progresses to CRPC over time (Sekhoacha et al., 2022). At present, once it becomes CRPC, treatment means are still relatively scarce, and chemotherapy, radiotherapy, immunotherapy, targeted therapy, and other treatments have limited improvement in its prognosis (Wade and Kyprianou, 2018). Discovering and predicting new disease-related biomolecules can help to further understand the mechanisms of disease development, and contribute to the exploration of disease biomarkers and therapeutic targets. Therefore, it is necessary to explore a factor that can predict the occurrence of PCa.

A circular RNA (circRNA) is a type of non-coding RNA that forms a covalent closed loop structure through reverse splicing and does not have a 5' end cap or 3' poly(A) tail (Huang et al., 2020). Unlike linear RNAs, circRNAs lack free terminal structures. This unique structure enhances their stability and protects them from degradation by RNA Exonuclease, resulting in more stable expression (Patop et al., 2019). Given the circular and stable characteristics, RNA Hydrolase (RNaseR) cannot bind and degrade circRNAs, so they have great potential as new molecular markers of human diseases (Zhang et al., 2018). Mounting results have proved the important relationship between circRNAs and a series of complex human diseases, such as diabetes, heart failure, and the occurrence and development of cancers such as PCa (Wang et al., 2022c). Numerous experiments have shown that circRNAs are involved in many important physiological processes, including their main functions as sponges or bait (Qi et al., 2015). CircRNA, as a competitive endogenous RNA (ceRNA), plays a molecular sponge role in regulating gene expression by binding to microRNAs (Cheng et al., 2015). Hsa_circ_0070440 is significantly upregulated in lung adenocarcinoma cells and promotes both apoptosis and ferroptosis of cells by sponging miR-485-5p and upregulating SLC7A11 expression (Zhao et al., 2023). However, its role in PCa lacks information.

This study aimed to investigate the expression level of hsa_circ_0070440 in PCa and its correlation with the adverse characteristics of PCa, analyzing its correlation with CRPC progression and prognosis. Moreover, this study used PCa cells to study the changes in cell proliferation and migration ability after knocking down hsa_circ_0070440 and to explore possible mechanisms.

Materials and methods

Tissue acquisition

This study selected biospecimens and clinical data from 138 patients who underwent laparoscopic radical prostatectomy at Sinopharm Dongfeng General Hospital, Hubei University of Medicine from June 2015 to December 2017. This study was approved by the Medical Ethics Committee of our hospital.

Inclusion criteria: (1) Patients diagnosed with PCa through prostate system biopsy; (2) Patients undergoing laparoscopic radical prostatectomy in our hospital.

Exclusion criteria: (1) Patients who underwent preoperative or postoperative radiotherapy and chemotherapy; (2) Patients undergoing preoperative neoadjuvant endocrine therapy; (3) Patients with incomplete follow-up data.

The diagnostic criteria of CRPC refer to the 2014/2019 Chinese Urology and Andrology Disease Diagnosis and Treatment Guidelines, which should meet the following conditions: 1) serum testosterone reaches the castration level (T<50 ng/dl or < 1.7 nmol/L); 2) The PSA value increases continuously three times with a week's interval, and is more than 50% higher than the lowest value; 3) Imaging progression, clinical symptoms, or bone related events occur.

Cell lines and transient transfections

All cell lines were from the Shanghai Chinese Academy of Sciences cell bank (China) and tested for the presence of Mycoplasma with the Mycoplasma PCR detection kit (ScienCell, USA). 22Rv1 and C4-2B cells were cultured in RPMI 1640 complete medium (Hyclone, USA); PC-3 were cultured in DMEM/F12; and DU 145 cells were cultured in DMEM. The normal prostate cell line RWPE-1 was cultured in Keratinocyte-SFM (Thermo Fisher, USA). All cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere.

22Rv1 and C4-2B cells were suspended in lipofectamine RNAiMAX reagent (Thermo Fisher Scientific, USA) and hsa_circ_0070440 siRNA (siCIRC) or control siRNA (siNC) from Dharmacon(China) for 48 hours of transfection.

RNA isolation and real-time quantitative RT-PCR (qRT-PCR)

Total RNA was isolated from tissue samples frozen in Tissue-Tek O.C.T. Compound (Sakura, USA) or cell lines with RNA STAT-60 (Tel-Test, USA), followed by purification with RNeasy Mini Kit (Qiagen). The purity and yield of the RNA were assessed on a NanoDrop 2000 (Thermo Fisher Scientific, USA). First-strand cDNA was synthesized with 1 μg RNA using a Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific, USA). PCR was performed using 200 μL cDNA and Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, USA) on ABI 7300 (Applied Biosystems, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control for hsa_circ_0070440 and TXNDC5, while U6 was for miR-382-5p and miR-383-5p. The primer used in this study were listed in Table 1. The relative expression levels of RNAs in each sample can be calculated according to the $2^{-\Delta\Delta Ct}$ formula. In expression level assays after transfection, data were first normalized to the corresponding reference genes, and then expressed

as fold increases relative to untreated or negative controls.

Cell proliferation (CCK-8) experiment

Cell proliferation was detected using the CCK-8 kit (Dojindo laboratories, Japan). In brief, after cell counting, 2500 cells per well for 22Rv1 cells and 2000 cells per well for C4-2B cells were added to the 96 well plates and incubated for five time points: 0, 24, 48, 72, and 96h. Each sample was equipped with five composite pores at each time point. At the set time point, 10 μL of CCK-8 were added to each well, and cells were incubated for 2 hours. The absorbance values at 450nm were detected with a microplate reader.

Cell migration (Transwell) experiment

After cell counting, an appropriate number of cells based on their migration ability (10×10^4 22Rv1 cells, 5×10^4 C4-2B cells) were used to prepare cell suspensions in a serum-free culture medium. The cell suspension was added to the upper chamber, and 600 μL complete medium was added to each well of the 24-well plate below the chamber. The transwell system was incubated in an incubator for 24 hours. Then, the membrane-penetrating cells were fixed and dyed in crystal violet solution for 20 min, six fields of view for each well were randomly selected to observe under a microscope and count the number of cells.

Bioinformatics analysis

The Circular RNA Interactome (Dudekula et al., 2016) online tool was used for the prediction of hsa_circ_0070440-targeting miRNAs. ENCORI was searched for the prediction of miR-382-5p/miR-383-5p-targeting genes (Li et al., 2013).

Luciferase reporter assay

The interaction between miR-382-5p (or miR-383-5p) and hsa_circ_0070440 or TXNDC5 was evaluated using the Renilla-Firefly Luciferase Dual Assay Kit (MedChemExpress, USA). 22Rv1 or C4-2B cells were transfected with wild-type hsa_circ_0070440 (wt-circ) or mutant hsa_circ_0070440 (mut-circ), together with miR-382-5p (or miR-383-5p) mimic plasmids or negative control (NC1 or NC2), for 48h using Lipofectamine 3000 (ThermoFisher) following manufacturer's instructions. The luciferase dual assay was performed using the Firefly Luciferase and Renilla Luciferase Working Solution successively. The luminescence values were subtracted from the corresponding background values and then the ratio of luminescence was calculated from the ratio of Firefly luciferase to Renilla luciferase. The luciferase activity was then normalized to mimicNC1 (control).

Statistical analyses

For each cell experiment, data were pooled from three independent experiments. Data were compared using the Mann-Whitney U test or t-test or analysis of variance. Patients were dichotomized with hsa_circ_0070440 expression levels into two groups. Clinicopathological parameters in these two groups were compared using the Chi-square test. The endpoints for survival analyses were the time to treatment failure (TTF), time to PSA progression (TTPP), and overall survival time. Survival curves were generated using the Kaplan-Meier method (log-rank test). Multivariable Cox proportional hazards analysis was conducted to determine the clinical significance of the main clinicopathological parameters. p-values <0.05 were considered significant.

Results

Quantification of hsa_circ_0070440 expression

To get insights into the expression of hsa_circ_0070440 in PCa, 138 paired PCa and non-cancerous tissues were opted for qRT-PCR assay. In all patients' datasets, we found that hsa_circ_0070440 was highly expressed in PCa tissues compared with non-cancer tissues (Fig. 1A). We next compared hsa_circ_0070440 expression in CRPC cases with non-CRPC cases. As shown for hsa_circ_0070440 expression levels in Figure 1B, CRPC cases have higher levels of hsa_circ_0070440 than non-CRPC cases. The hsa_circ_0070440 expression levels in the PCa cell lines were higher compared with

Table 1. Sequences of primers used for qRT-PCR in this study.

RNA ID	Forward (5'-3')	Reverse (5'-3')
Hsa_circ_0070440	GAAGGACGGTGATGTCTGCT	CGCTGCTTTACTTTGCCTGA
MiR-382-5p	ATCCGTGAAGTTGTTCGTGG	TATGGTTGTAGAGGACTCCTTGAC
MiR-383-5p	GGGAGATCAGAAGGTGATTGTGGCT	CAGTGCGTGTCGTGGAGT
TXNDC5	TGAGCCCACGGGTGACAAGG	GCCACACCACGGAGCATAGAAC
GAPDH	GAACGGGAAGCTCACTGG	GCCTGCTTCACCACCTTCT
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

normal prostate RWPE-1 cells (Fig. 1C). Altogether, these findings demonstrate the upregulated pattern of hsa_circ_0070440 in PCa, especially CRPC.

Clinical relevance of hsa_circ_0070440 expression

To assess the clinical significance of hsa_circ_0070440 expression in PCa, survival analyses were performed using Kaplan-Meier and Cox multivariable analyses. The mean expression of hsa_circ_0070440 (2.45) was determined for the dichotomization of patients into low and high hsa_circ_0070440 groups. Chi-square tests showed that the clinical T stage (p=0.021) and lymph node metastasis (p=0.018) differed significantly across the two groups (Table 2). Kaplan-Meier analyses showed that high hsa_circ_0070440 levels showed a significant trend for worse overall survival (p=0.012; Fig. 2A). Notably, high hsa_circ_0070440 levels showed trends for worse TTPP (p=0.00074; Fig. 2B) and TTF (p=0.00085; Fig. 2C). Multivariate analysis showed hsa_circ_0070440 as an independent factor for a lower overall survival (HR=3.24; 95%CI 1.40-7.46; p=0.006), TTPP (HR=2.78; 95%CI 1.45-5.31; p=0.002) and TTF (HR=2.25; 95%CI 1.25-4.06; p=0.007) (Table 3).

The positive role of hsa_circ_0070440 in PCa proliferation and migration

Based on the dysregulation and prognostic significance of hsa_circ_0070440 in PCa, it may therefore play roles in PCa cell function. To examine this possibility, we analyzed the proliferation and migration of cultured PCa cells. Using targeting siRNA, hsa_circ_0070440 was decreased significantly (Fig. 3A). Using the CCK-8 kit, we found a significant decrease in proliferation after hsa_circ_0070440 knockdown, compared with the control (Fig. 3B,C). Next, cell migration was assessed using an *in vitro* transwell assay. Under hsa_circ_0070440-knockdown conditions, PCa cells (22Rv1 and C4-2B) displayed a reduced number of

Table 2. Clinicopathological characteristics of patients with prostate cancer and associations with hsa_circ_0070440 expression

Variables	Number (n=138)	hsa_circ_0070440 expression Low (n=66)	High (n=72)	p values
Age				0.186
≤62 years	75	32	43	
>62 years	63	34	29	
PSA				0.625
<8 ng/mL	66	33	33	
≥8 ng/mL	72	33	39	
Gleason score				0.064
4-6	66	37	29	
7-10	72	29	43	
Clinical T stage				0.021
I/II	105	56	49	
III/IV	33	10	23	
Surgical margin				0.071
negative	101	53	48	
positive	37	13	24	
Size				0.072
0-20 mm	79	43	36	
>20 mm	59	23	46	
lymph node metastasis				0.018
negative	112	59	53	
positive	26	7	19	
Lactate dehydrogenase				0.928
≤1×ULN	58	28	30	
>1×ULN	80	39	42	
Alkaline phosphatase				0.360
<106 U/L	115	57	58	
≥106 U/L	23	9	14	

PSA, prostate-specific antigen.

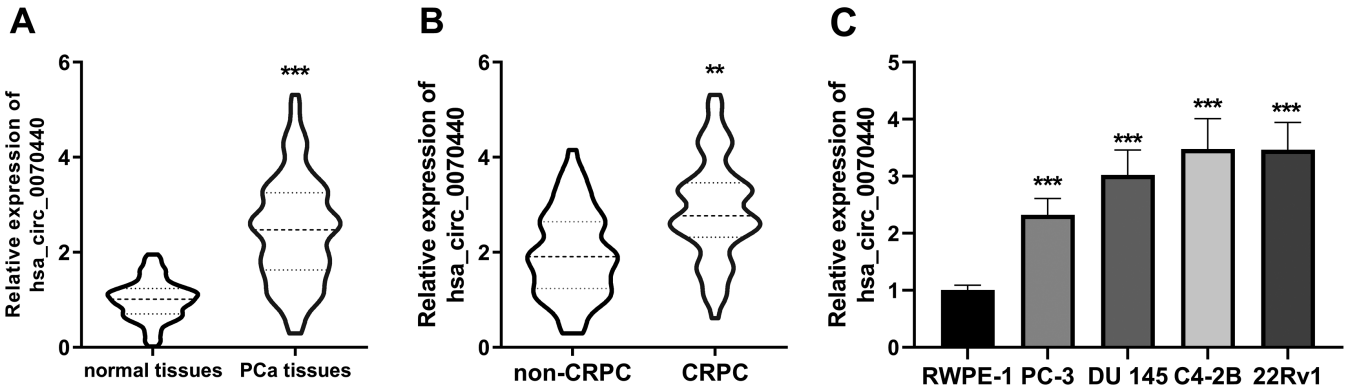

Fig. 1. Hsa_circ_0070440 was upregulated in prostate cancer (PCa). **A.** Detection of hsa_circ_0070440 in PCa and adjacent normal tissues by qRT-PCR. **B.** Detection of hsa_circ_0070440 in tissues with CRPC progression by qRT-PCR. **C.** Detection of hsa_circ_0070440 in PCa cell lines (22Rv1, C4-2B, PC-3, and DU 145) and the normal prostate cell line RWPE-1 by qRT-PCR. **p<0.01, ***p<0.001, using two-tailed t-tests.

migrated cells (Fig. 3D), compared with the negative control (siNC). These findings demonstrate that hsa_circ_0070440 can promote the proliferation and migration of PCa cells.

Prediction of hsa_circ_0070440-targeting miRNAs

Among the target miRNAs for hsa_circ_0070440 predicted by Circular RNA Interactome, 18 miRNAs reached context+ score percentiles > 80. Among these 18 miRNAs, miR-382-5p and miR-383-5p were reported as downregulated in PCa, and inhibitors in PCa cell proliferation and metastasis (Zhang et al., 2016; Bucay et al., 2017). Figure 4A shows that hsa_circ_0070440 had complementary nucleotide sequences with miR-382-5p and miR-383-5p. In our PCa case set, the expression level of hsa_circ_0070440 was negatively correlated with that of miR-382-5p (Fig. 4B). When the expression level of hsa_circ_0070440 decreased, it would cause an increase in the expression level of miR-382-5p (Fig. 4C). Moreover, the luciferase activity of PCa cells with wild-type hsa_circ_0070440 was significantly decreased after co-transfection with miR-382-5p; the luciferase activity of PCa cells with the mutation of the binding site had no significant change (Fig. 4D). For miR-383-5p, the hsa_circ_0070440 expression level was negatively correlated with its expression level (Fig. 4E), hsa_circ_0070440 inhibition can increase its expression level (Fig. 4F), and cells with wild-type hsa_circ_0070440 displayed a reduction in luciferase activity when co-transfected with miR-383-5p (Fig. 4G).

Prediction of miR-382/383-5p-targeting genes

To gain insight into the target genes of miR-382-5p and miR-383-5p, we retrieved the ENCORI database and

Table 3. Pathological characteristics and survival: multivariate analysis.

Variable	Overall survival			TTPP			TTF		
	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
hsa_circ_0070440	3.24	1.40-7.46	0.006	2.78	1.45-5.31	0.002	2.25	1.25-4.06	0.007
Age	1.89	0.91-3.92	0.09	1.26	0.73-2.15	0.41	1.08	0.65-1.78	0.77
PSA	1.87	0.91-3.84	0.09	1.07	0.63-1.84	0.79	1.14	0.69-1.87	0.61
Gleason score	2.52	1.24-5.32	0.01	2.02	1.17-3.48	0.01	1.81	1.09-2.99	0.02
Clinical T stage	1.98	0.98-4.00	0.06	1.30	0.73-2.29	0.37	1.67	1.00-2.78	0.05
Surgical margin	2.17	1.07-4.41	0.03	1.72	0.98-3.04	0.06	1.91	1.14-3.20	0.01
Size	2.03	0.90-4.60	0.09	1.33	0.75-2.37	0.34	1.14	0.67-1.94	0.62
lymph node metastasis	1.33	0.61-2.91	0.48	1.31	0.70-2.43	0.40	1.33	0.75-2.38	0.33
Lactate dehydrogenase	2.16	1.07-4.35	0.03	1.30	0.76-2.22	0.34	1.31	0.80-2.15	0.28
Alkaline phosphatase	1.02	0.41-2.61	0.97	1.27	0.64-2.54	0.50	1.15	0.58-2.25	0.69

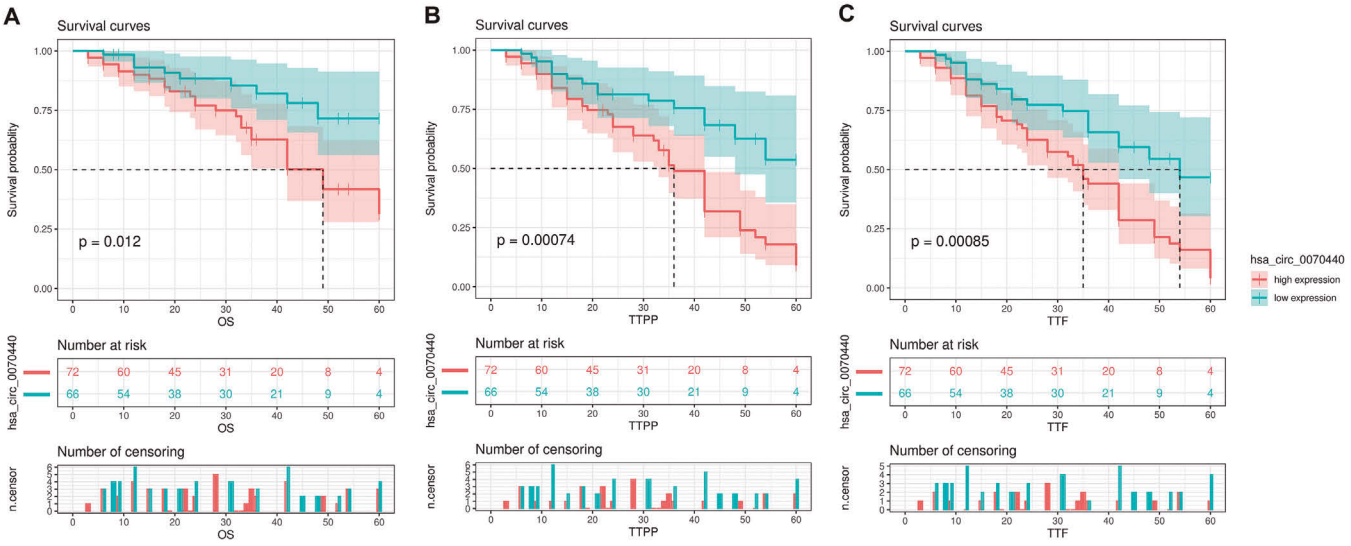

Fig. 2. Prognostic significance of hsa_circ_0070440 in PCa. Kaplan-Meier analysis of the efficacy of hsa_circ_0070440 according to overall survival (OS) **(A)**, time to PSA progression (TTPP) **(B)**, and time to treatment failure (TTF) **(C)**.

obtained the common target between miR-382-5p and miR-383-5p. Among the targets, TXNDC5 has been previously identified to regulate PCa cell death signaling and promote castration resistance in PCa (Wang et al., 2015). The binding sites for miR-382-5p and miR-383-5p in the 3'UTR of TXNDC5 are shown in Figure 5A. The expression level of TXNDC5 mRNA was negatively related to those of miR-382-5p and miR-383-5p (Fig. 5B). In the absence of miR-382-5p or miR-383-5p mimic, PCa cells displayed a decrease in TXNDC5 mRNA expression levels, compared with negative controls (Fig. 5C). The luciferase activity of the plasmids bearing the miRNA-binding sites under miR-382-5p or miR-383-5p overexpression decreased significantly compared with that of cells treated with mimic control (Fig. 5D,E).

Discussion

Currently, there is increasing research into prognostic factors for PCa and the proliferation and metastasis activity of cancer cells. CircRNAs are not easily degraded by nucleic acid exonuclease and have the characteristics of stability, tissue specificity, and conservatism. Moreover, this type of RNAs can participate in the regulation process of related genes and play a role. CircRNA can act as a sponge molecule of miRNA to regulate the expression of its target gene, thus affecting the biological behavior of PCa cells. Abnormally expressed circRNAs can serve as potential prognostic biomarkers and therapeutic targets for PCa. In the present study, we analyzed the expression level of hsa_circ_0070440 in PCa tissues and cells. The upregulation of hsa_circ_0070440 in PCa displayed the potential of its effects on PCa progression and its predictive capacity for survival. Using survival analyses (Kaplan-Meier method and multivariable Cox proportional hazards analysis), we identified hsa_circ_0070440 as an independent predictor of overall survival, TTPP, and TTF. Besides, our cell experimental results have shown that hsa_circ_0070440 may promote PCa progression via miR-382/383-5p/TXNDC5.

Biomarkers based on circRNAs have shown promising prospects in predicting the prognosis of various tumors. Hsa_circ_0070440 (circFAM13A) has been developed in a five-circRNA signature to successfully stratify CRPC patients into high-risk and low-risk groups (Tao et al., 2023). In this study, we conducted a survival analysis to assess the relationship of hsa_circ_0070440 with OS, TTPP, and TTF in PCa patients. Then, a multivariate Cox regression was conducted to determine hsa_circ_0070440 as independent prognostic factors for OS, TTPP, and TTF in PCa patients. These findings indicate that hsa_circ_0070440 may provide a theoretical basis for the individualized prognosis of PCa patients.

Due to the changes in the tumor microenvironment, dysregulated circRNA can play a role as a carcinogen or tumor suppressor gene in different cancers (Kristensen et al., 2022). Hsa_circ_0070440 has been reported as upregulated in lung adenocarcinoma and metastatic CRPC patients (Tao et al., 2023; Zhao et al., 2023). Herein, we found hsa_circ_0070440 to be upregulated in PCa patients, and also CRPC patients. Hsa_circ_0070440 has been identified to contribute to the malignant progression of lung adenocarcinoma by sponging miR-485-5p and upregulating SLC7A11 expression. In the present study, hsa_circ_0070440 was found to promote PCa cell proliferation and migration, possibly via miR-382/383-5p/TXNDC5. miR-382-5p has been proposed as a PCa suppressor by Zhang et al. (Zhang et al., 2016). miR-382 is significantly decreased

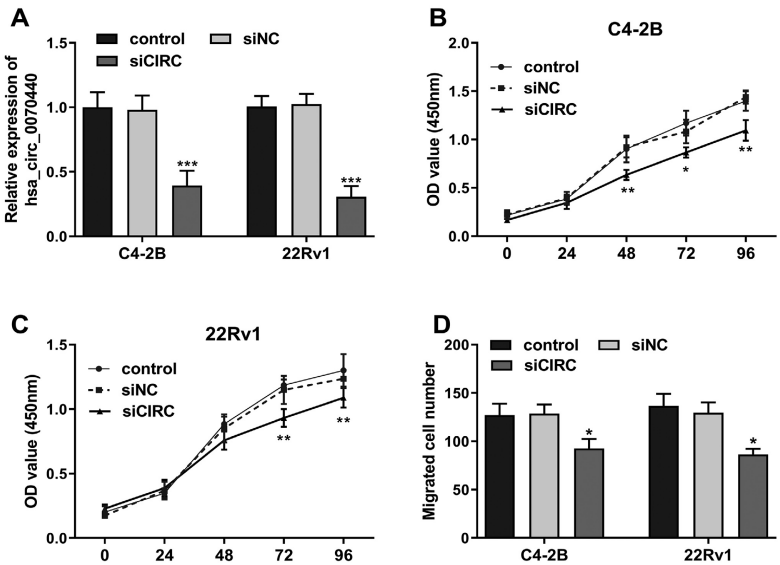

Fig. 3. Effect of hsa_circ_0070440 knockdown on PCa cells. **A.** Relative expression of hsa_circ_0070440 in C4-2B and 22Rv1 cells after transfection of hsa_circ_0070440 siRNA (siCIRC) or control siRNA (siNC). **B, C.** Cell proliferation of C4-2B cells after transfection of hsa_circ_0070440 siRNA (siCIRC) or control siRNA (siNC). **D.** The *in vitro* migration assay used C4-2B and 22Rv1 cells with hsa_circ_0070440 knockdown or not. *p<0.05, **p<0.01, ***p<0.001.

in PCa and inhibits PCa cell proliferation, migration, and invasion (Zhang et al., 2016). MicroRNA-383-5p is a tumor suppressor (Jafarzadeh et al., 2022). Ectopic expression of miR-383-5p inhibited the tumor-initiating and metastatic capacity of PCa cells (Bucay et al., 2017). TXNDC5 is up-regulated in CRPC cases compared with hormone-naive PCa (Wang et al., 2015). Functionally, TXNDC5 can promote the growth of both androgen-dependent PCa and CRPC xenografts *in vitro* and *in vivo* (Wang et al., 2015). Taken together, this study suggests that hsa_circ_0070440 inhibits PCa cell proliferation and metastasis through sponging miR-382/383-5p,

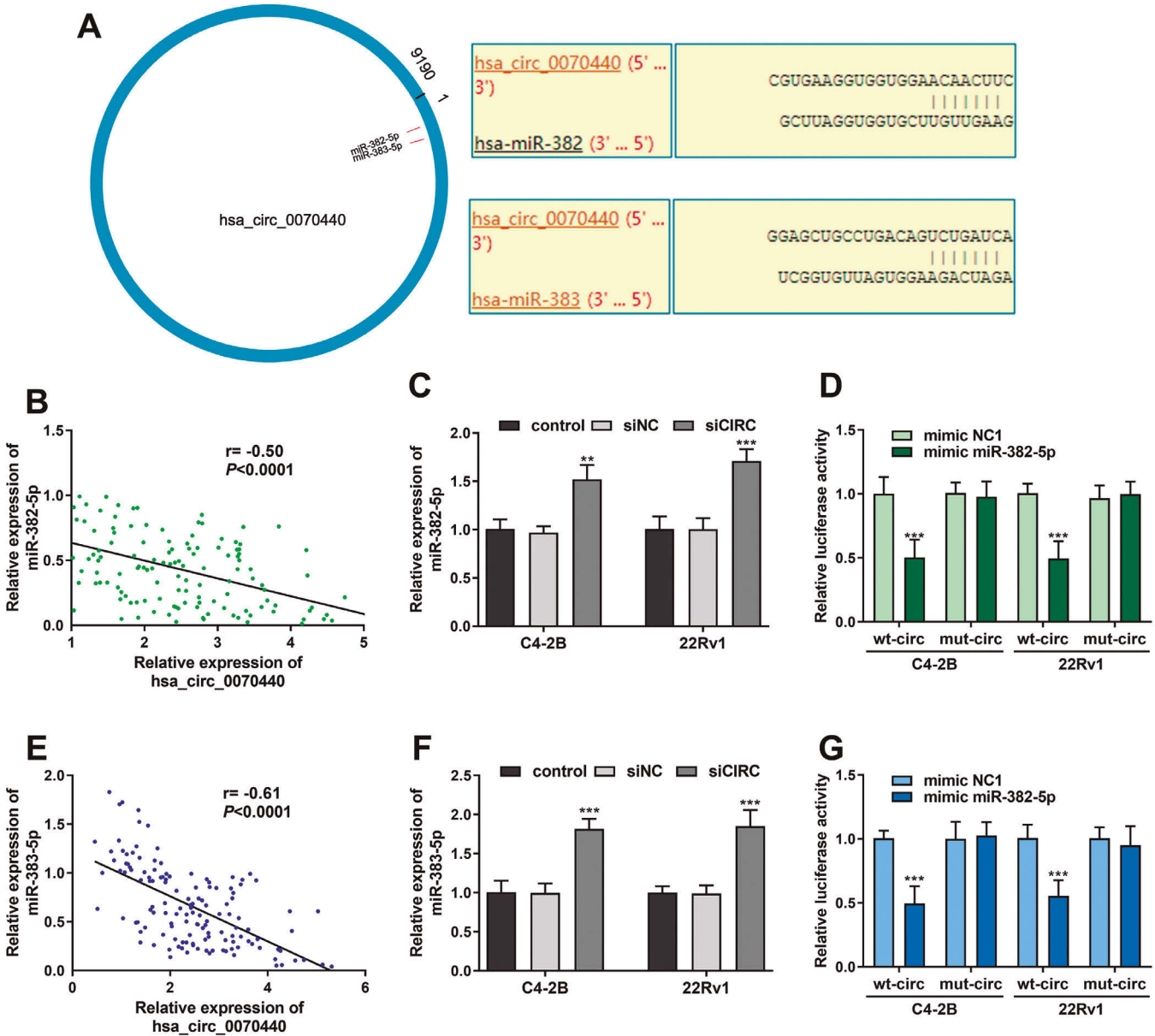

Fig. 4. MiR-382/383-5p were downstream miRNAs for hsa_circ_0070440 in prostate cancer (PCa) cells. **A.** The binding sites of miR-382/383-5p with hsa_circ_0070440. **B.** The negative correlation between expression levels of hsa_circ_0070440 and miR-382-5p. **C.** qRT-PCR analysis of the expression levels of miR-382-5p after transfection with hsa_circ_0070440 siRNA in C4-2B and 22RV1 cell lines. **D.** Assessment of luciferase activities of wild-type (wt) or mutant (mut) hsa_circ_0070440 after co-transfection with miR-382-5p mimic in C4-2B and 22RV1 cells. **E.** The negative correlation between expression levels of hsa_circ_0070440 and miR-383-5p. **F.** qRT-PCR analysis of the expression levels of miR-383-5p after transfection with hsa_circ_0070440 siRNA in C4-2B and 22RV1 cell lines. **G.** Assessment of luciferase activities of wild-type (wt) or mutant (mut) hsa_circ_0070440 after co-transfection with miR-383-5p mimic in C4-2B and 22RV1 cells. **p<0.01, ***p<0.001.

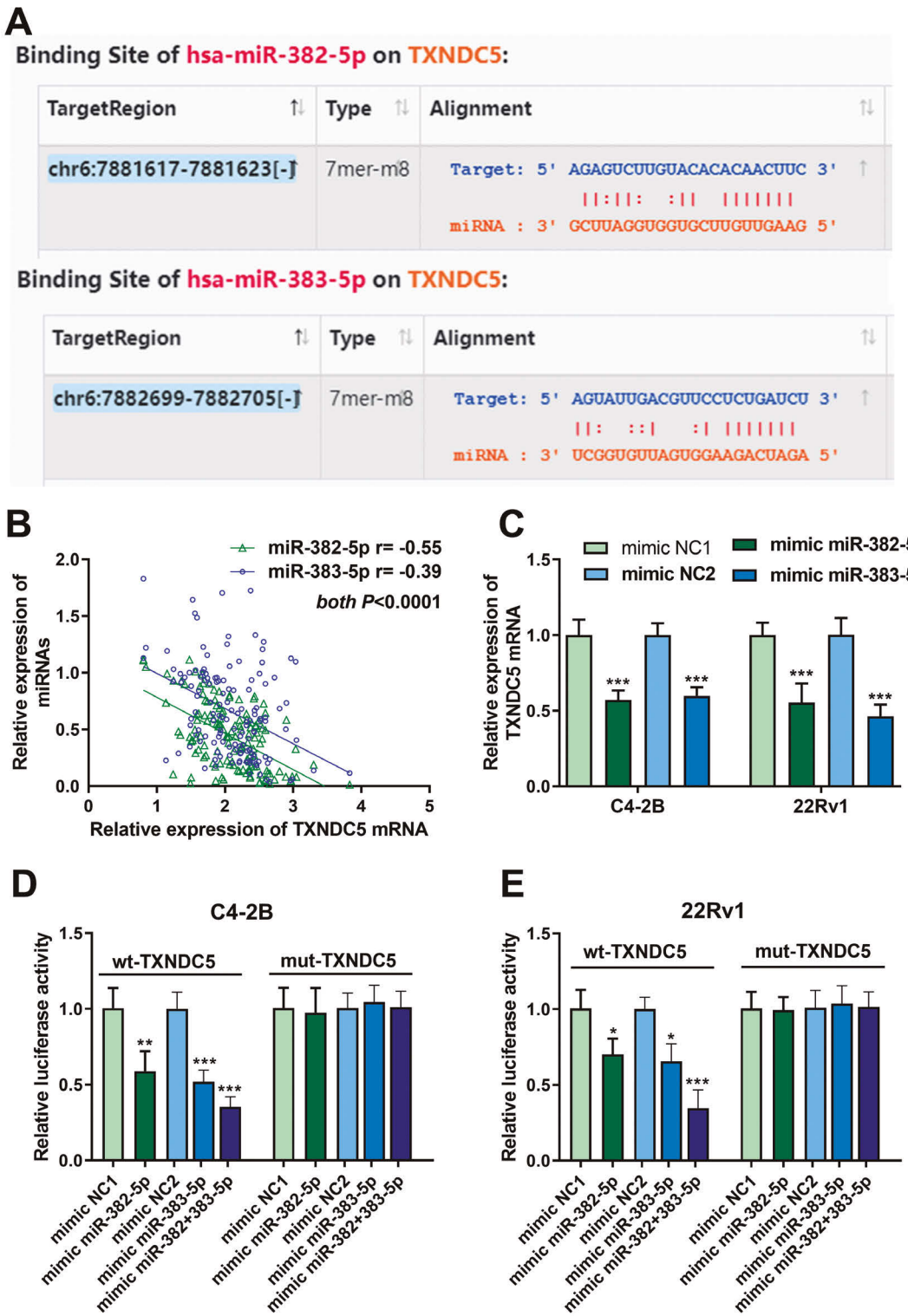

Fig. 5. TXNDC5 was a common target gene for miR-382/383-5p, which were downstream miRNAs for hsa_circ_0070440 in prostate cancer (PCa) cells. **A.** The binding sites of miR-382/383-5p with TXNDC5. **B.** The negative correlation between expression levels of TXNDC5 mRNA and miR-382/383-5p. **C.** qRT-PCR analysis of the expression levels of TXNDC5 mRNA after transfection with miR-382/383-5p mimics in C4-2B and 22RV1 cell lines. **D, E.** Assessment of luciferase activities of wild-type (wt) or mutant (mut) TXNDC5 after co-transfection with miR-382/383-5p mimic in C4-2B and 22RV1 cells. *p<0.05, **p<0.01, ***p<0.001.

representing a novel therapeutic target candidate for PCa therapy.

Overall, hsa_circ_0070440 was highly expressed in PCa and had prognostic potential. Knocking down hsa_circ_0070440 can reduce tumor cell proliferation and migration activity. Hsa_circ_0070440 can serve as a sponge molecule for miR-382/383-5p. Hsa_circ_0070440 may be a potential target and prognostic predictor of targeted therapy for PCa.

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Conflict of Interests. There was no conflict of interest in this study.

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