Original Article

The inhibition effect of psoralen on prostate cancer PC3 cells via down-regulation of long non-coding RNA ENST00000510619

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Background: New medications are needed to improve outcomes of castration-resistant prostate cancer (CRPC). Psoralen has been reported to have anti-cancer properties for various tumors, but there are limited reports about psoralen treatment in prostate cancer (PCa). This study aimed to investigate the effect of psoralen on PC3 cells and to investigate potential underlying mechanisms of action.

Methods: The effect of psoralen on the proliferation and cell cycle progression of PC3 cells was determined using Cell Counting Kit-8 (CCK-8) test and flow cytometry, respectively. The differential gene profiles in PC3 cells treated with psoralen were determined with microarray analyses. The effect of psoralen on long non-coding RNA (lncRNA) ENST00000510619 expression in PC3 cells was detected by real-time quantitative polymerase chain reaction (RT-qPCR). The effect of psoralen and transfection of small interfering lnc-RNA (si-lncRNA) ENST00000510619 on cell viability, invasion ability, and migratory activity of PC3 cells were evaluated using the CCK-8 test, transwell assay, and wound healing, respectively.

Results: Psoralen significantly inhibited PC3 cells in a concentration- and time-dependent manner and caused G1 phase and G2/M phase cycle arrests. When screened with a fold change (FC) of ≥2 and a P value of <0.05, 1,716 lncRNAs and 1,160 messenger RNAs (mRNAs) were significantly up-regulated, whereas 3,269 lncRNAs and 3,263 mRNAs were significantly down-regulated in PC3 cells after psoralen treatment. Among the differentially down-regulated lncRNAs in which the signal of the probe showed significant differences compared to the background, lncRNA ENST00000510619 had the highest FC. The expression of lncRNA ENST00000510619 was shown to be down-regulated by psoralen in a concentration-dependent manner. CCK-8 assay, wound healing, and transwell assay showed that both psoralen and si-lncRNA ENST00000510619 transfection significantly inhibited the activity, invasion, and migration of PC3 cells (P<0.01 for all).

Conclusions: Psoralen was confirmed to inhibit proliferation and block the cell cycle in PC3 cells in this *in vitro* study. The molecular mechanism involves multiple differentially expressed lncRNAs and mRNAs and is related to the down-regulation of lncRNA ENST000000510619 expression. This study provides the experimental basis for the development of psoralen as a novel anti-CRPC drug and for the consideration of lncRNA ENST00000510619 as a potential clinical target for CRPC.

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Keywords: Psoralen; prostate cancer (PCa); long non-coding RNA (lncRNA); microarray analysis; lncRNA ENST00000510619

Submitted Aug 28, 2024. Accepted for publication Oct 16, 2024. Published online Oct 28, 2024.
doi: 10.21037/tau-24-457
View this article at: https://dx.doi.org/10.21037/tau-24-457

Introduction

Prostate cancer (PCa) is the second most common cancer in men, affecting millions of men worldwide (1). Radical prostatectomy, external-beam radiotherapy, and brachytherapy are available curative treatments for localized PCa, but some patients present with metastatic PCa and forfeit the opportunity for curative treatment (2). Endocrine therapy for advanced PCa is effective, but the disease eventually progresses to castration-resistant prostate cancer (CRPC) and becomes refractory to treatment (2,3). New treatment techniques or medications are needed to improve outcomes and quality of life in patients with CRPC.

Psoralen, one of the major compounds of fructus psoraleae, has been reported to have anti-cancer properties for various tumors, including breast cancer, mucoepidermoid cancer, and bladder cancer, and can reverse multi-drug resistance of cancer cells (4-7). It has been demonstrated to induce anti-proliferation, cell cycle arrest, apoptosis, and differentiation of cancer cells *in vitro* (5), and is therefore viewed as a promising anti-tumor drug (6). Scaffidi *et al.* (8) reported that an X-ray-activated anticancer "nanodrug" composed of yttrium oxide (Y^2O^3) nanoscintillators and psoralen could yield concentration-dependent reductions in cell numbers of PC3, a human androgen-independent prostate carcinoma cell line. However, there are limited reports about psoralen treatment in the field of PCa and the anti-tumor mechanism of psoralen has not been fully elucidated.

Long non-coding RNAs (lncRNAs) are transcripts with a length of at least 200 nucleotides and no or low protein translation (9). They comprise the majority part of transcripts in the mammalian transcriptome and are considered to widely participate in the cellular biological functions, including metabolism, proliferation, and apoptosis (10,11). Accumulating evidence has revealed the close relationship of lncRNAs with cancer development and progression (12). Several lncRNAs have been demonstrated to play important biological roles in tumor development and progression of PCa (13-15). Some of these lncRNAs act as PCa growth inhibitors or promoters, while the functions of others remain unknown (15-18). Existing researches indicate that lncRNAs may serve as promising therapeutic targets of PCa in the future, but only a few have been sufficiently validated (13,19). Further investigations are required to characterize the critical lncRNAs involved in PCa treatment, and to explore their functional role and molecular mechanisms.

In order to explore the effect of psoralen on PC3 cells

Highlight box

Key findings

- Psoralen could inhibit PC3 cells in a concentration- and time-dependent manner and had a cell cycle blocking effect on PC3 cells.
- The microarray analysis results revealed the complex molecular mechanism of psoralen involving multiple differentially expressed long non-coding RNAs (lncRNAs) and messenger RNAs (mRNAs).
- Psoralen could down-regulate the expression of lncRNA ENST00000510619 in a concentration-dependent manner.
- The inhibition effect of psoralen on the malignant biological behaviors of PC3 cells might be achieved via the down-regulation of lncRNA ENST00000510619.

What is known and what is new?

- Psoralen, one of the major compounds of fructus psoraleae, has been reported to have anti-cancer properties for various tumors. To date, there have been limited reports regarding psoralen's potential anti-prostate cancer (PCa) properties. LncRNA ENST00000510619 is a novel lncRNA located on Chromosome 11: 35,212,550-35,214,007 forward strand. The function of this lncRNA remains unknown.
- Psoralen can inhibit androgen-independent PCa cells *in vitro*. This is the first exploration of the functionality of lncRNA ENST00000510619, and the findings suggest its role in the inhibition of PCa by psoralen.

What is the implication, and what should change now?

- This study provides the experimental basis for the development of psoralen as a novel anti-PCa drug and for the consideration of lncRNA ENST00000510619 as a potential clinical target for PCa.
- *In vivo* experiments on psoralen in the treatment of PCa and the target genes of lncRNA ENST00000510619 as well as its specific function need to be further studied.

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and the potential underlying mechanisms, we observed the inhibition of psoralen on PC3 cell proliferation and performed microarray analysis to identify differentially expressed lncRNAs and messenger RNAs (mRNAs), so as to reveal the potential functional involvement of lncRNAs in psoralen treatment for PCa. Furthermore, based on the results of microarray analysis, down-regulation of lncRNA ENST00000510619 and psoralen on the malignant biological behaviors of PC3 cells were detected by Cell Counting Kit-8 (CCK-8) test, transwell assay and wound healing. The aim of this study was to find a novel potential antitumor drug and potential targets for the treatment of CRPC. We present this article in accordance with the MDAR reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-24-457/rc).

Methods

Cell culture and chemicals

Human PC3 cells were provided by Prof. Yinghao Sun (Center of Prostate Disease, Naval Military Medical University, China). PC3 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, USA) in a humidified atmosphere with 5% carbon dioxide at 37 ℃. Psoralen (Xiya, Shandong, China) was dissolved in 100% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 33.33 mg/mL before being diluted with cell culture medium to achieve various concentrations.

Cell proliferation assay

Exponentially growing PC3 cells (3,000 cells) were trypsinized and seeded in 96-well plates and allowed to adhere to the plate overnight. To determine the influence of psoralen on PC3 cells, the experimental groups (each group contained 6 wells) were treated with 0 (containing 0.3% DMSO), 10, 30, 50, and 100 μg/mL psoralen for 24, 48, 72, and 96 hours, respectively. To determine the influence of down-regulation of lncRNA ENST00000510619 expression in the proliferation inhibition of prostate on PC3 cells, the experimental groups included PC3 cells and PC3 cells with small interfering lncRNA (si-lncRNA) ENST00000510619. After the treatment, CCK-8 (MedChemExpress, Monmouth Junction, NJ, USA) was used to assess cell viability. The cell culture medium of each well was replaced with 100 μL fresh medium containing 10 μL CCK-8 solution for 1 hour. Absorbance at 450 nm was measured using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). A total of three independent experiments were performed. Cell viability was calculated as the percentage of the absorbance of the experimental group to the absorbance of the control group.

Cell cycle analysis

PC3 cells were cultured with 0, 10, 30, 50, and 100 μg/mL psoralen for 24 hours. Approximately 2×10^6 cells per dish were collected, fixed in 70% ethanol at 4 ℃ for 2 hours, then washed with phosphate-buffered saline (PBS), stained with 50 μg/mL propidium iodide (PI; Sigma, USA) in the dark for 30 min, and finally detected by FACSAria flow cytometry [Becton, Dickinson, and Co. (BD), Franklin Lakes, NJ, USA].

Microarray analysis

PC3 cells were seeded in 90 mm dishes and allowed to attach overnight. The psoralen group was stimulated with 50 μg/mL psoralen for 48 hours and the control group was stimulated with 0.15% DMSO for 48 hours. SBC Human (4×180 k) lncRNA microarrays (V6.0, Shanghai Biotechnology Corporation, Shanghai, China) were used in this research to detect 29,857 coding transcripts and 91,007 lncRNAs. The microarray scan data were extracted with Agilent Feature Extraction Software v10.7.3 (Agilent Technology, Santa Clara, CA, USA). Raw data were normalized with the quantile algorithm in the Gene Spring software (Agilent Technology, USA).

Bioinformatics analysis

All differentially expressed mRNAs were selected for Gene Ontology (GO) analysis (http://www.geneontology.org) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses (http://www.genome.ad.jp/kegg/) to explore the potential functions and pathways involved in the inhibition of psoralen on PC3 cells. GO analysis was used in functional enrichment studies to categorize the roles of mRNAs into three domains: cellular component (CC), molecular function (MF), and biological process (BF). KEGG analysis was used to reveal potential biological pathways associated with differentially expressed genes

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Table 1 Primers used for RT-qPCR

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
lncRNA		
NR-104586	CGGATTGTGAGGGTGAAACT	ACAGTATGCCTCGTGTGCAG
NR-027632	TTTCAGAGCTGGCATTTCCT	TCCATCAACGGCAGTCATTA
NR-125375	ATGGCCACATACAGGAGGAG	GAGCTGAACCTGAAGGATGC
NR-036512	GTCCCTGTGTGAGCAGAGGT	CCAACTCGCAAACCTCAAGT
NR-024399	TTGGTTGATGCATTTGGAGA	AGGAAAGATGGCAGCACTGT
ENST00000510619	ACATGCTTGGCCTCATTTCTCTG	5CTAAGGAGTGTAAGGGGCTTTGTC
mRNA		
NM-015392	GACTCAGAAGGCCGACTACG	CGTGAAGTCTCCGTCCTCAT
NM-198075	AGGGACAGACTTGGAGAGCA	CAGCTTCAGTTGGTCCAGGT
NM-001142522	TGAGGTTGCCAAGACATTGA	GAGGAGCTTGCCATCTGAAC
NM-001288653	AACCGCCTTGCAGAGTTAGA	TTTCCTAGCCCCATCAACAG
NM-016343	GTGGCAACAGAAGCTGACAA	TCTTCTGTGTCGATGCCAAG
β-actin	GTTGTCGACGACGAGCG	GCACAGAGCCTCGCCTT

RT-qPCR, real-time quantitative polymerase chain reaction; lncRNAs, long non-coding RNAs; mRNAs, messenger RNAs.

(DEGs). The function of differentially expressed lncRNAs were predicted via cis- or trans-regulatory effects using the GO and KEGG databases.

Cell transfection

si-lncRNA ENST00000510619 (5'-GUUCUGCUCUCA UUUAUUATT-3') and si-NC (5'-UUCUCCGAA CGUGUCACGUTT-3') were synthesized by Sangon Biotech (Shanghai, China). A population of 1×10^6 PC-3 cells was cultivated in 6-well plates without antibiotics for 24 hours. Hieff Trans® Liposomal Transfection Reagent (Yeasen Biotechnology, Shanghai, China) was used for transfection. si-lncRNA ENST00000510619 and the blank control (si-NC) were separately transfected into the PC-3 cell lines, and a fresh culture medium was provided after 6 hours. Cell collection for subsequent experiments occurred 48 h after the transfection.

Real-time quantitative polymerase chain reaction (RT-qPCR)

There were three parts of experiments using RT-qPCR for the measurement of target genes in each group. (I) Microarray data validation: five differentially expressed lncRNAs and five differentially expressed mRNAs were randomly selected and determined. PC3 cells were treated and grouped as "Microarray analysis". (II) The effect of psoralen on the expression of lncRNA ENST000000510619 in PC3 cells: PC-3 cells were cultured and exposed to various concentrations of psoralen (0, 10, 30, 50, and 100 μg/mL) for 48 hours. Subsequently, the expression levels of lncRNA ENST00000510619 in PC-3 cells were determined. (III) Transfection efficiency evaluation of si-lncRNA ENST000000510619: PC3 cells were divided into the control group (si-NC) and the transfection group (si-lncRNA ENST000000510619). Total RNA was extracted from the cells with TransZol Up and RNA Extraction Agent (TransGen Biotech, Beijing, China). The complementary DNA (cDNA) synthesis involved 5× HiScript II qRT SuperMix II (Vazyme Biotech, Nanjing, China). The qRT-PCR was performed by Hieff UNICON® Universal Blue qPCR SYBR Green Master Mix (Yeasen Biotechnology, Shanghai, China) to estimate the expression of target genes. Relative expressions of target genes were normalized to the β-actin mRNA level using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used are listed in *Table 1*.

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Transwell assay and wound healing

The experimental groups included PC3 cells, PC3 cells with si-lncRNA ENST00000510619, and PC3 cells treated with 50 μg/mL psoralen. The transwell assay was conducted with a 24-well transwell chamber with 8-μm pore size (Corning, Corning, NY, USA) and Basement Membrane Matrix (Phenol Red; Thermo Fisher, USA) to assess cellular invasion in PC-3 cells from different groups. Specifically, a population of 1×10^5 PC3 cells of each group was seeded in the top chamber with serum-free medium, while the medium containing 10% FBS was added to the lower chamber. After a 24-hour incubation period, the migrated cells were counted with a microscope. For wound healing assay, the cellular monolayer was delicately abraded with pipette tips to create distinct wound gaps. Sequential images were captured at the intervals of 0 and 12 hours with an inverted microscope.

Statistical analysis

We independently performed three biological replicates for all experiments. All data were analyzed using SPSS 17.0 (IBM Corp., Chicago, IL, USA), ModFit LT 9verity software House, Bedford, MA, USA) and R software 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria). Differences between the groups were analyzed using one-way analysis of variance (ANOVA) or Student's *t*-test, as appropriate. In the microarray analysis, fold changes (FCs) of >2 and P values <0.05 were used to determine statistical significance in comparisons of the differential expressions of lncRNAs and mRNAs between the two groups. The pathways with a P value <0.05 were defined as those significantly enriched in DEGs.

Results

Effect of psoralen on PC3 cell proliferation and cell cycle in vitro

CCK-8 test indicated that the growth of PC3 cells was significantly inhibited by psoralen in a concentration- and time-dependent manner (*Figure 1A*). Cell viability of the PC3 cells gradually decreased as the psoralen concentrations increased and the culture time extended. Flow cytometry test demonstrated that psoralen caused G0/G1 phase and G2/M phase cycle arrests in PC3 cells, and this inhibitory effect gradually became evident with increasing psoralen concentration (*Figure 1B*, *Table 2*).

Differentially expressed lncRNAs and mRNAs

Based on the results of the cell proliferation assay, microarray analysis was used to identify the differentially expressed lncRNAs and mRNAs involved in PC3 cells treated with 50 μg/mL psoralen for 48 hours. Hierarchical clustering was applied to group lncRNAs and mRNAs based on their expression levels (*Figure 2A,2B*). In the scatter plot and volcano plot, 91,007 lncRNAs and 29,857 mRNAs were represented. When screened with a FC of ≥2.0 and a P value of <0.05, 1,716 lncRNAs and 1,160 mRNAs were significantly up-regulated (red plots), whereas 3,269 lncRNAs and 3,263 mRNAs were significantly down-regulated (blue plots) (*Figure 2*). The top 10 downregulated lncRNAs in which the signal of the probe showed significant differences compared to the background are shown in *Table 3*. Among them, lncRNA ENST00000510619 had the highest FC and was selected for further investigation.

Bioinformatics analysis of differentially expressed mRNAs and lncRNAs

GO analysis showed that a total of 663 items were correlated with the differentially expressed mRNAs, which were classified into three categories: BPs (468 items), CCs (88 items), and MF (107 items). The top 30 enriched GO items of differentially expressed mRNAs are shown in *Figure 3A*. As shown in *Figure 3B*, the KEGG results showed that the differentially expressed mRNAs were enriched in 22 pathways.

We used GO database data and KEGG database to predict target genes of differentially expressed lncRNAs, including cis-target genes and trans-target genes. In the cis-target gene prediction, a total of 705 items were found, including 495 BP-related items, 94 CC-related items, and 116 MF-related items. The trans-target gene prediction results showed 181 items, including 40 BP-related items, 19 CC-related items, and 122 MF-related items. KEGG pathway enrichment analysis showed that 18 cis-target pathways were predicted, including homologous recombination, cell cycle, endocytosis, and so on. The trans-target pathway predicts 15 pathways, including lysosome, D-glutamine and glutamic acid metabolism, sphingolipid biosynthetic spheres, heterologous spheres, and so on. The KEGG enriched cis-/trans-target signaling pathways are shown in *Figure 4A,4B*.

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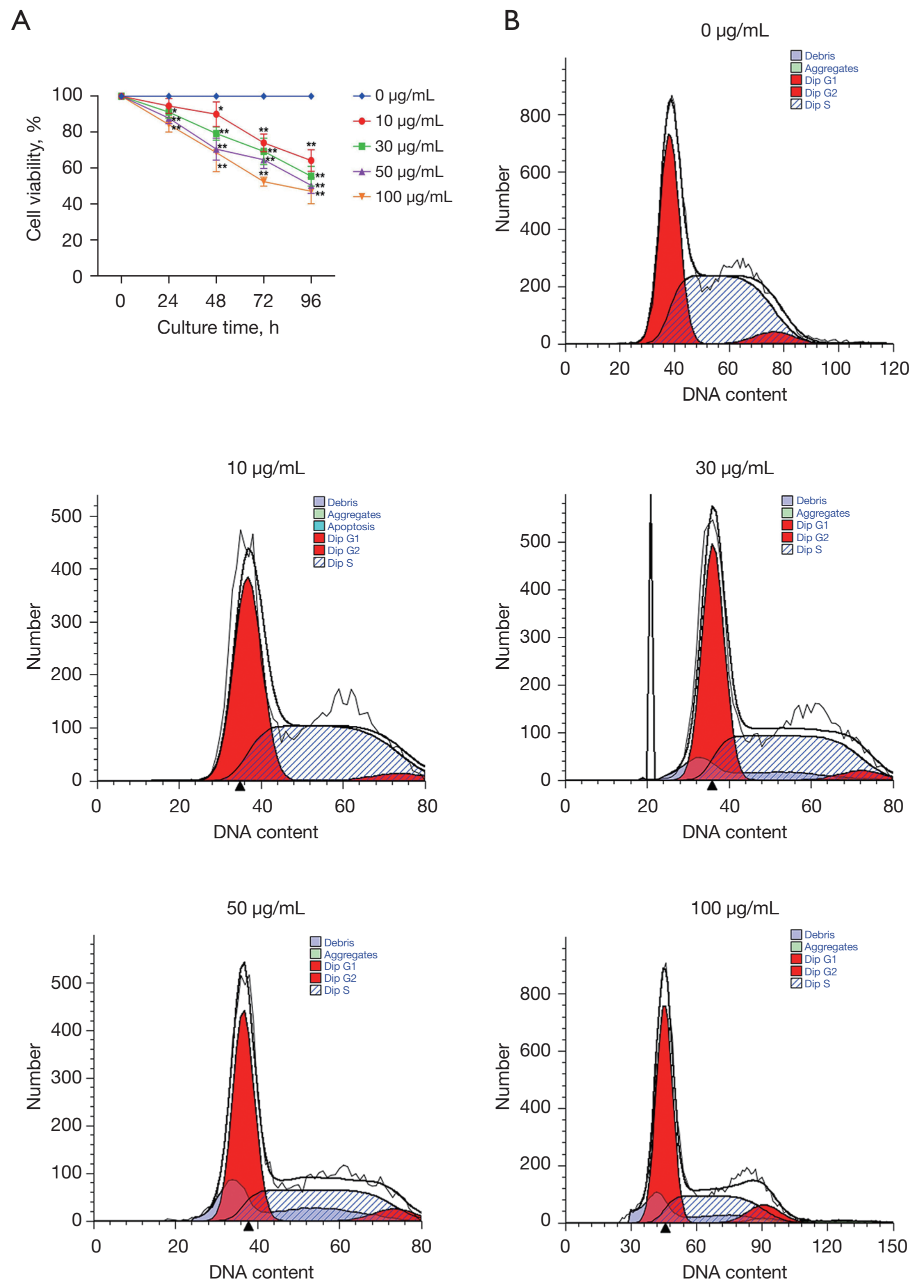

Figure 1 Effect of psoralen on PC3 cell proliferation and cell cycle. (A) Survival rate of PC3 cells after treatment with different concentrations of psoralen determined by CCK-8 test. *, P<0.05 *vs.* the control group (0 μg/mL psoralen); **, P<0.01 *vs.* the control group (0 μg/mL psoralen). (B) Numbers of PC3 cells in the G0/G1, S, and G2/M phases when treated with different concentrations of psoralen determined by flow cytometry. CCK-8, Cell Counting Kit-8.

Table 2 Effect of psoralen on PC3 cell cycle (%)

Phase	Psoralen concentration					F value	P value
	Control	10 μg/mL	30 μg/mL	50 μg/mL	100 μg/mL		
G0/G1 phase	39.10±2.82	45.84±1.60	48.03±1.19	52.80±2.65	56.80±1.43	32.696	<0.001
S phase	58.34±3.09	50.59±1.21	48.02±0.29	40.42±4.51	32.31±1.35	44.746	<0.001
G2/M phase	2.57±1.92	3.56±1.84	3.95±1.44	6.77±1.95	10.89±2.50	8.894	0.002

Data presented as mean ± standard deviation.

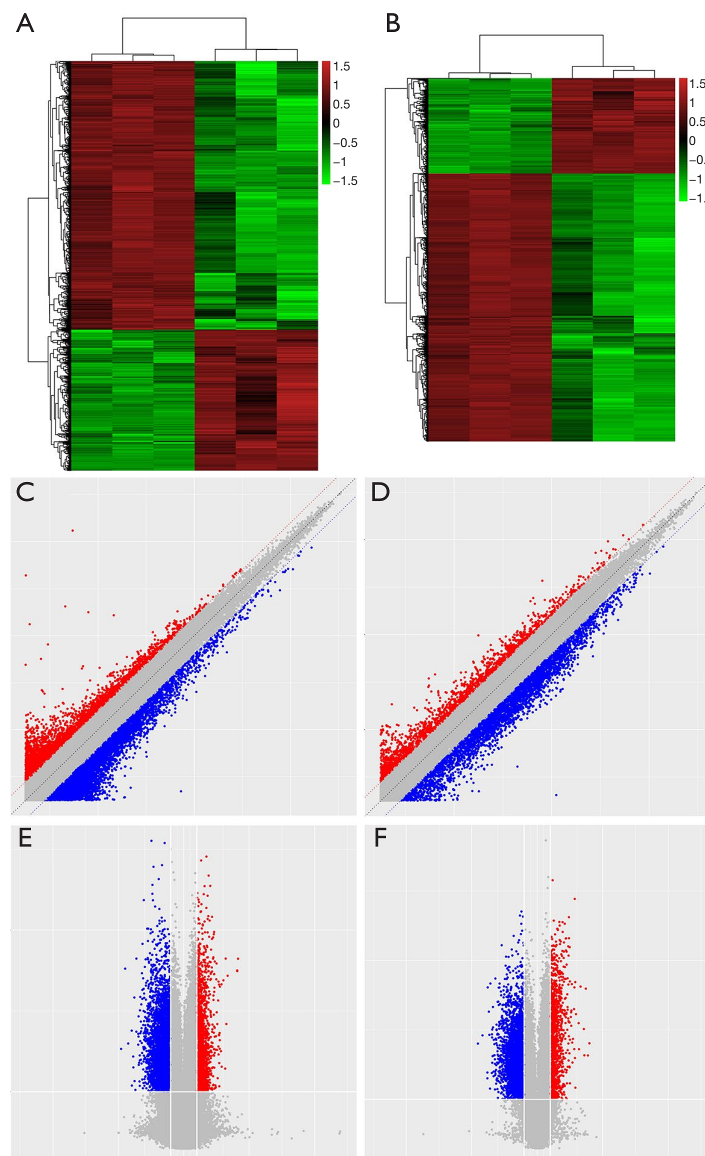

Figure 2 Differentially expressed lncRNAs and mRNAs in PC3 cells treated with 50 μg/mL psoralen for 48 h. (A,B) lncRNAs and mRNAs grouped by Hierarchical Clustering. (C,D) The scatter plot of differentially expressed lncRNAs and mRNAs. (E,F) The volcano plot of differentially expressed lncRNAs and mRNAs. Red spots represent up-regulated lncRNAs and blue spots represent down-regulated lncRNAs. Grey spots represent lncRNAs and mRNAs with no significant differences. lncRNAs, long non-coding RNAs; mRNAs, messenger RNAs.

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RT-qPCR validation of the microarray analysis and determination of the influence of psoralen on lncRNA ENST00000510619 expression in PC3 cells

We randomly selected five differentially expressed lncRNAs (NR-104586, NR-027632, NR-125375, NR-036512, and NR-024399) and five differentially expressed mRNAs (NM-015392, NM-198075, NM-001142522, NM-001288653, and NM-016343) for RT-qPCR to verify the reliability of the results of the microarray data. The RT-qPCR results showed the same trend as the microarray data (*Figure 5A,5B*). Besides, the expression of lncRNA ENST00000510619 in PC-3 cells was shown to be down-regulated by psoralen in a concentration-dependent manner, as determined by RT-qPCR (*Figure 5C*).

Table 3 The top 10 downregulated lncRNAs in PC3 cells treated with 50 μg/mL psoralen for 48 hours

Name	P values	Fold change	Regulation
ENST00000510619	0.001	9.690960827	Down
lnc-GLI3-4:1	<0.001	8.588433743	Down
lnc-TSPY2-5:1	0.004	8.512423262	Down
lnc-C3orf80-2:1	<0.001	8.386712135	Down
lnc-PCDH11X-1:1	0.003	8.159451492	Down
lnc-KIAA1524-2:1	0.002	7.95494635	Down
lnc-C3orf80-3:1	<0.001	7.417104105	Down
lnc-PDHX-4:1	0.003	6.98756201	Down
lnc-MC5R-1:2	0.001	6.840549068	Down
lnc-MTF2-3:1	0.004	6.764208274	Down

lncRNAs, long non-coding RNAs.

Effects of psoralen and down-regulation of lncRNA ENST00000510619 on the malignant biological behaviors of PC3 cells

As determined by RT-qPCR, the expression of lncRNA ENST00000510619 in PC3 cells was significantly down-regulated after the transfection of si-lncRNA ENST00000510619, which indicated the successful construction of si-lncRNA ENST00000510619 PC-3 cells through the si-RNA technology (*Figure 6A*). CCK-8 test showed that the cell viability of PC3 cells with si-lncRNA ENST00000510619 transfection was inhibited when compared with normal PC3 cells (*Figure 6B*). Transwell assay and wound healing were conducted to assess the invasion ability and migratory ability of PC3 cells, respectively. The invasion ability (*Figure 6C*) and migration

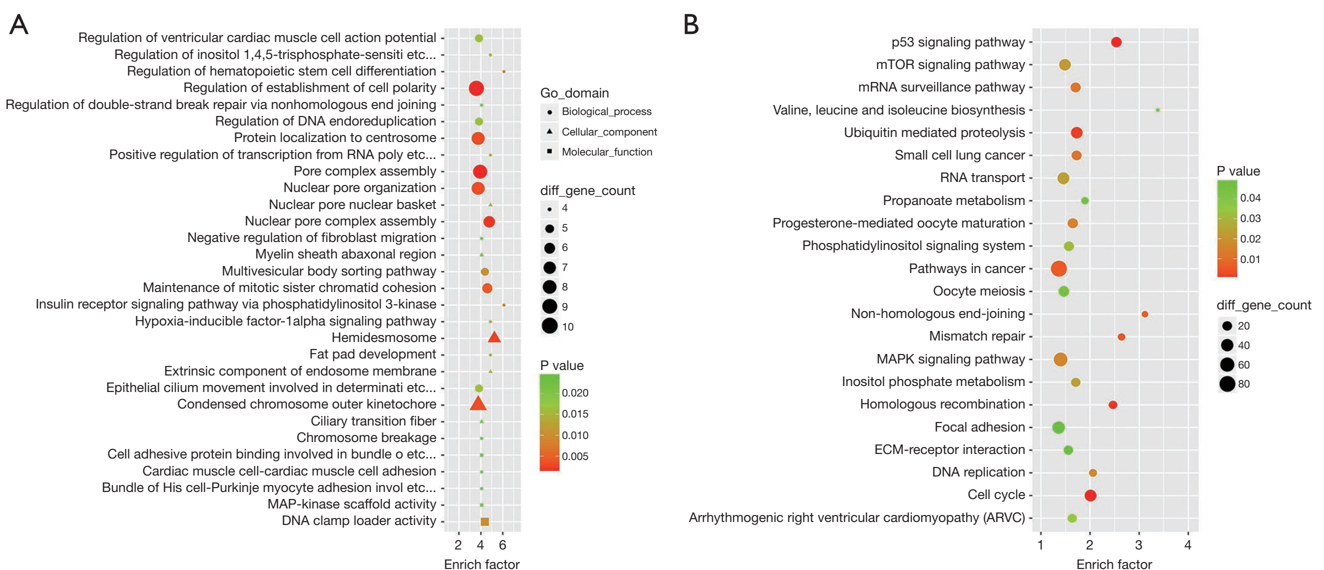

Figure 3 GO and KEGG analysis of the differentially expressed mRNAs in PC3 cells treated with 50 μg/mL psoralen for 48 h. (A) The top 30 of GO analysis of differentially expressed mRNAs. (B) The 22 KEGG pathway enrichment of differently expressed mRNAs. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; mRNAs, messenger RNAs; MAP, mitogen-activated protein; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; ECM, extracellular matrix.

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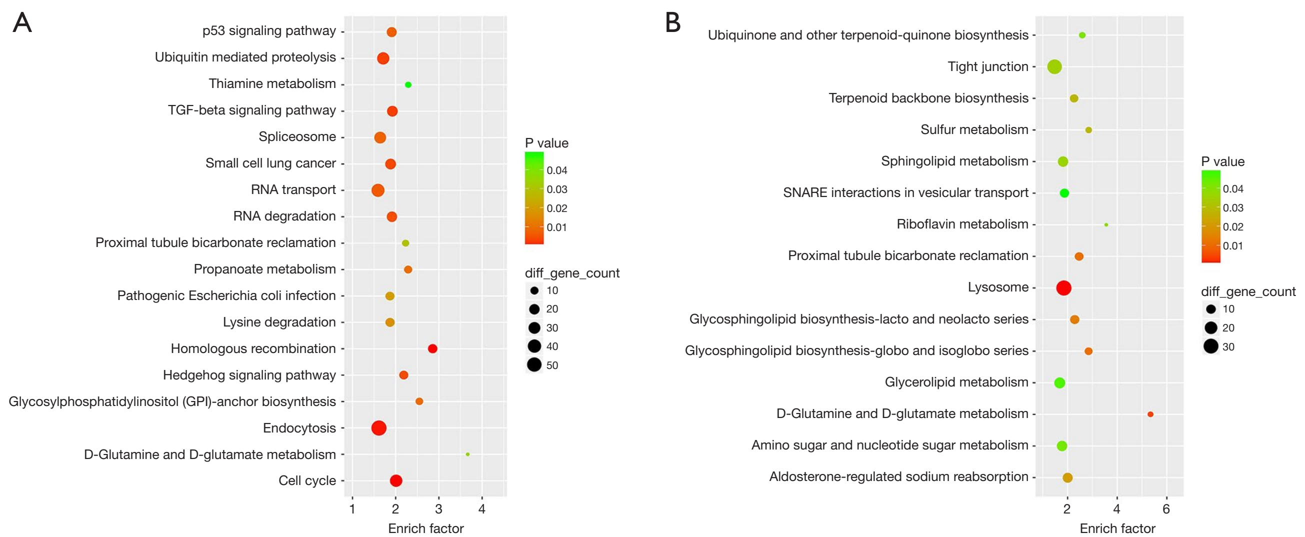

Figure 4 KEGG pathway analysis of cis-target genes (A) or trans-target genes (B) of differentially expressed lncRNAs in PC3 cells treated with 50 µg/mL psoralen for 48 h. KEGG, Kyoto Encyclopedia of Genes and Genomes; lncRNAs, long non-coding RNAs; TGF, Transforming growth factor; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor.

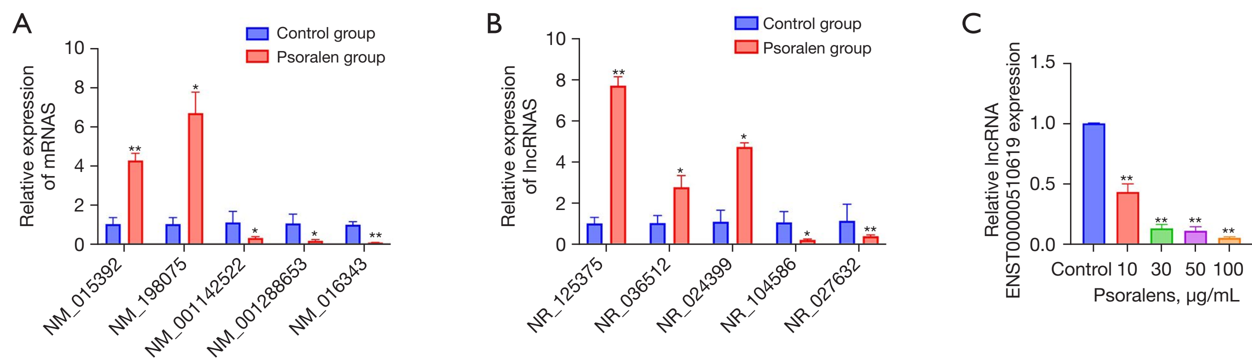

Figure 5 RT-qPCR validation of 5 differentially expressed mRNAs (A) and lncRNAs (B) in PC3 cells treated with 50 µg/mL psoralen for 48 h. (C) Effect of psoralen on the expression of lncRNA ENST00000510619 in PC3 cells. *, P<0.05 *vs.* the control group; **, P<0.01 *vs.* the control group. RT-qPCR, real-time quantitative polymerase chain reaction; lncRNAs, long non-coding RNAs; mRNAs, messenger RNAs.

activity (*Figure 6D*) of PC3 cells were both significantly inhibited by psoralen and si-lncRNA ENST00000510619 transfection.

Discussion

In this study, psoralen was confirmed to have an inhibitory effect on the proliferation of PC3 cells. The CCK-8 experiments demonstrated that the inhibition of psoralen on PC3 cells was both time- and concentration-dependent. The flow cytometry test demonstrated that psoralen had a cell cycle blocking effect on PC3 cells in a concentration-dependent manner. Psoralen was observed to cause G1 phase and G2/M phase cycle arrest in PC3 cells.

To further elucidate the molecular mechanism of psoralen's inhibition on PC3 cell proliferation, microarray analysis was conducted to compare the comprehensive lncRNA and mRNA expression profiles between PC3 cells and PC3 cells treated with psoralen. Based on results of the CCK-8 experiment and the flow cytometry test, the concentration of 50 µg/mL of psoralen and the action time of 48 hours were chosen for the subsequent microarray analysis. The results showed that a total of 4,985 lncRNAs and 4,423 mRNAs were significantly differentially expressed

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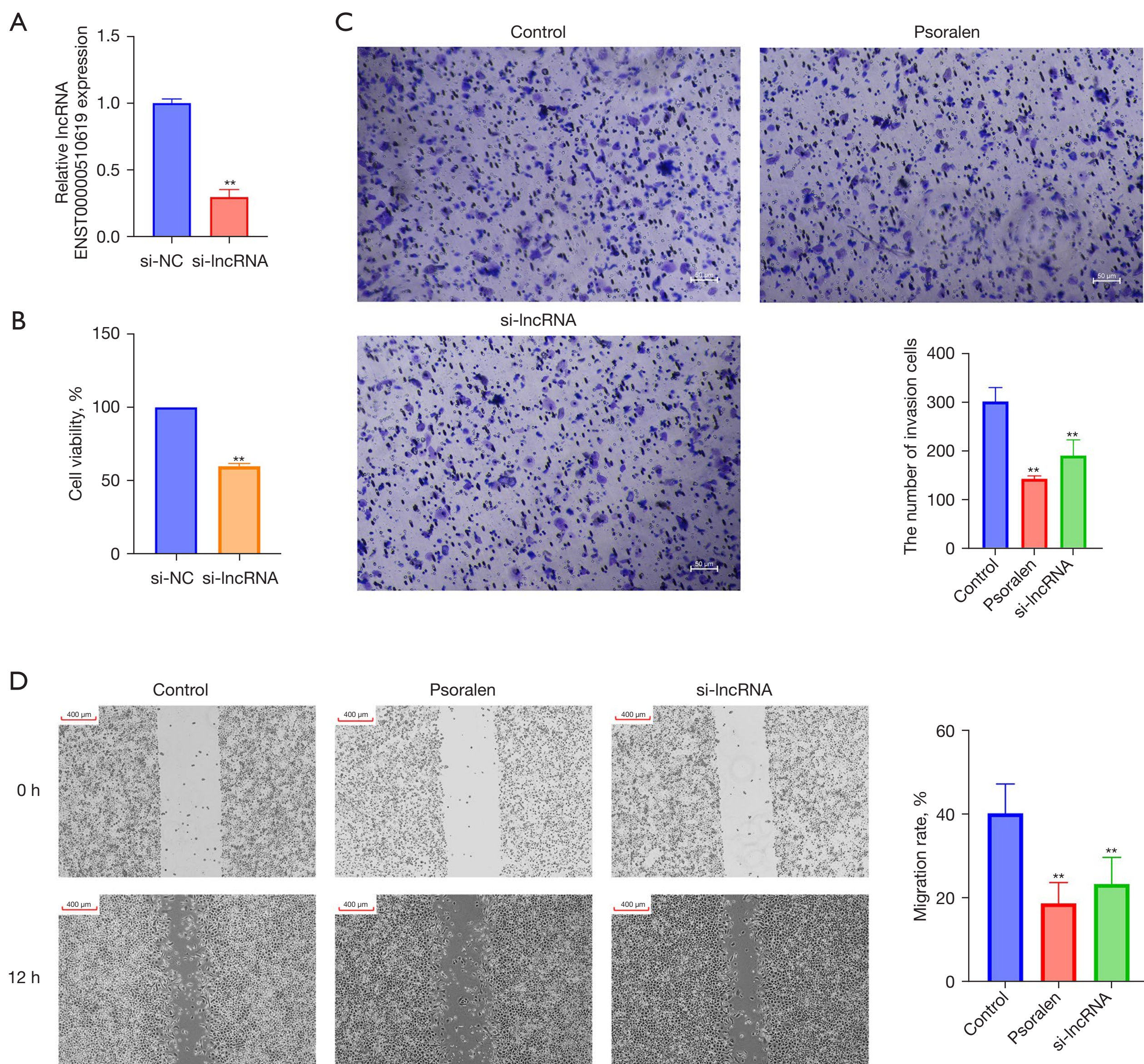

Figure 6 Effects of psoralen and down-regulation of lncRNA ENST00000510619 on the malignant biological behaviors of PC3 cells. (A) RT-qPCR validation of si-lncRNA ENST00000510619 transfection in PC3 cells. (B) Effect of si-lncRNA ENST00000510619 transfection on PC3 cell proliferation tested by CCK-8. (C) Effect of psoralen (50 µg/mL psoralen) and si-lncRNA ENST00000510619 transfection on PC3 cell invasion tested by transwell assay. Cells were stained with 0.1% crystal violet. (D) Effect of psoralen (50 µg/mL) and si-lncRNA ENST00000510619 transfection on PC3 cell migration tested by wound healing test. **, P<0.01 *vs.* the si-NCl group (A,B) or the control group (C,D). si-NC, siRNA-negative control; si-lncRNA, siRNA-lncRNA ENST00000510619; RT-qPCR, real-time quantitative polymerase chain reaction; si-lncRNA, small interfering long non-coding RNA; CCK-8, Cell Counting Kit-8.

between the two groups with a difference of more than two-fold. KEGG pathway enrichment analysis of differentially expressed mRNAs showed 22 significantly related signaling pathways, including the p38 MAPK signaling pathway, mTOR pathway, p53 pathway, and cell cycle pathway, which have been previously reported to be associated with the development and the treatment of PCa (20-26). Previous studies concerning psoralen on other tumors showed that the anti-tumor mechanisms of psoralen include induction of tumor cell apoptosis and inhibition of tumor

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cell proliferation and migration (5,27,28). Taken together, our findings indicated that the underlying mechanism of action of psoralen on PC3 cell inhibition may be complex and involve multiple pathways.

LncRNAs are a large cluster of regulators widely expressed with diverse functions, and their cellular localizations vary distinctly (9-11). Accumulating reports have revealed that lncRNAs play critical role in tumorigenesis and tumor development through cis- and trans-regulation of other genes (29,30). LncRNAs can exert function through different mechanisms, such as transcriptional interference and epigenetic silence of gene clusters (12,31). Some lncRNAs were demonstrated as titrating miRNAs through acting as a sponge, resulting in the post-transcriptional alteration of target proteins (32). We used the GO database to predict cis-target genes and trans-target genes of differentially expressed lncRNAs. The results showed 705 items in the cis-target gene prediction and 181 items in the trans-target gene prediction. KEGG pathway enrichment analysis showed that 18 cis-target pathways and 15 trans-target pathways were predicted. Previously, there has been no study on the role of lncRNAs in psoralen's anti-tumor. Our results indicate that lncRNAs are of great importance in the course of psoralen inhibiting the proliferation of PC3 cells. The differentially expressed lncRNAs identified in the present study can provide potential targets for further functional study of lncRNAs in the inhibition of psoralen on PCa.

Among the differentially down-regulated lncRNAs in which the signal of the probe showed significant differences compared to the background, lncRNA ENST00000510619 had the highest differential expression FC. According to the database of Ensembl (https://asia.ensembl.org/index.html), lncRNA ENST00000510619 is a novel lncRNA located on Chromosome 11: 35,212,550-35,214,007 forward strand. The function of this lncRNA remains unknown. In the present study, we further explored the relationship between lncRNA ENST00000510619 and psoralen's inhibition on PCa, as well as the effect of lncRNA ENST00000510619 on PC3 cells. The RT-qPCR results showed psoralen could down-regulate the expression of lncRNA ENST00000510619 in a concentration-dependent manner, which indicated the potential underlying role of lncRNA ENST00000510619 for PC3 cell inhibition by psoralen. We then used siRNA technology to reduce the expression of lncRNA ENST00000510619 in PC3 cells. The CCK-8 test results showed that transfection of si-lncRNA ENST00000510619 could significantly decrease the viability of PC3 cells. The transwell test and the wound healing test showed that both psoralen and transfection with si-lncRNA ENST00000510619 could significantly inhibit cell invasion ability and migration activity. These results together indicate that effects of psoralen on the malignant biological behavior of PC3 cells may be achieved by down-regulating the expression of lncRNA ENST00000510619. This is the first exploration of the functionality of the novel lncRNA, and the findings suggested its role in the inhibition of psoralen in PCa.

Conclusions

Psoralen can inhibit PC3 cells in a concentration- and time-dependent manner and has a cell cycle blocking effect on PC3 cells. This study provides the experimental evidence for the development of psoralen as a novel anti-CRPC drug. The microarray analysis revealed the complex molecular mechanism of psoralen involving multiple differentially expressed lncRNAs and mRNAs. Psoralen can down-regulate the expression of lncRNA ENST00000510619 in a concentration-dependent manner. The inhibition effect of psoralen on the malignant biological behaviors of PC3 cells may be achieved via the down-regulation of lncRNA ENST000005106l9. This is the first exploration of the function of lncRNA ENST00000510619, and the results provide the experimental basis for the consideration of lncRNA ENST00000510619 as a potential clinical target for CRPC. Otherwise, there are limitations in this study. First, this is an *in vitro* study, and there is a lack of *in vivo* experiments. Second, the results were obtained from the bioinformatics analysis only. The DEGs and their roles need to be further confirmed. Third, the target genes of lncRNA ENST00000510619 and its specific function need to be further studied.

Acknowledgments

Funding: The present study was supported by the Natural Science Foundation of Fujian Province (No. 2020J011138) and Joint Funds for the Innovation of Science and Technology, Fujian Province (No. 2021Y9223).

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-24-457/rc

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Data Sharing Statement: Available at https://tau.amegroups.com/article/view/10.21037/tau-24-457/dss

Peer Review File: Available at https://tau.amegroups.com/article/view/10.21037/tau-24-457/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-24-457/coif). R.K.S. receives consulting fees from Johnson & Johnson, outside the submitted work. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Chen S, Weng M, Lin R, Wei J, Zhu L, Ju Z, Lin Z, Zhan B, Pathak RA, Sayyid RK, Ge R. The inhibition effect of psoralen on prostate cancer PC3 cells via down-regulation of long non-coding RNA ENST00000510619. Transl Androl Urol 2024;13(10):2294-2306. doi: 10.21037/tau-24-457

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