LncRNA SPRY4-IT1 is upregulated and promotes the proliferation of prostate cancer cells under hypoxia *in vitro*

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Received May 22, 2022; Accepted December 21, 2022

DOI: 10.3892/ol.2023.13724

Abstract. The incidence and mortality rate of prostate cancer are among the highest for all cancers worldwide; this disease has a high cancer mortality rate in males, following lung cancer. Sprouty4-intron 1 (SPRY4-IT1) has been shown to play a variety of roles in tumors. Our previous study demonstrated that SPRY4-IT1 sponges microRNA-101-3p to promote the proliferation and metastasis of bladder cancer cells by upregulating enhancer of zeste homolog 2 expression; however, the role of SPRY4-IT1 in prostate cancer has not been fully established. In the present study, the expression levels, effects and mechanism of action of SPRY4-IT1 were investigated in prostate cancer tissues and cell lines using reverse transcription-quantitative PCR, western blotting, Cell Counting Kit-8 and flow cytometry assays. The results indicated that SPRY4-IT1 expression was upregulated in prostate cancer tissues and cell lines. Furthermore, hypoxia increased the expression levels of SPRY4-IT1 in prostate cancer cells. Knockdown of SPRY4-IT1 expression led to S-phase arrest, decreased expression levels of the cell cycle-associated proteins CDK2 and cyclin D1. AKT phosphorylation was also reduced by SPRY4-IT1 knockdown. In summary, the findings indicate the elevation of SPRY4-IT1 expression in prostate cancer. Under hypoxic conditions *in vitro*, SPRY4-IT1 overexpression promoted prostate cancer cell proliferation via a mechanism involving regulation of the cell cycle and the PI3K/AKT signaling pathway. Therefore, it may provide a basis for the development of targeted therapies.

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Key words: long noncoding RNA, SPRY4-IT1, prostate cancer, hypoxia, S phase

Introduction

The incidence and mortality rates of prostate cancer are among the highest for all cancers worldwide (1). In males, prostate cancer currently has the second highest cancer mortality rate after lung cancer. It is highly heterogeneous with a disease-specific mortality of approximately one in seven cases, and its incidence is expected to rise as a result of lifestyle changes and the aging of the global population (2). Long non-coding RNAs (lncRNAs) are heterogeneous transcripts, several of which function as master regulators of gene expression and contribute to biological processes, including carcinogenesis (3). Numerous lncRNAs have demonstrated an association with the development of diverse types of cancer in genome-wide association studies (4). The presence of mutations and the aberrant expression of lncRNAs play important roles in tumorigenesis and metastasis. Notably, numerous lncRNAs have been linked with the occurrence and progression of prostate cancer (5).

The lncRNA sprouty4-intron 1 (SPRY4-IT1) has been shown to have proto-oncogenic or anticancer activity, which varies according to tumor type (6). Our previous study showed that SPRY4-IT1 sponges microRNA (miR)-101-3p to promote the proliferation and metastasis of bladder cancer cells via upregulation of the expression of enhancer of zeste homolog 2 (7). Previous studies have confirmed that the expression of SPRY4-IT1 is upregulated in primary human prostatic adenocarcinomas and is higher in the PC3 prostatic cancer cell line than in normal prostate epithelial cells (8). In addition, the knockdown of SPRY4-IT1 expression has been shown to reduce the proliferation and invasive ability of PC3 cells and promote their apoptosis (9); however, the underlying mechanism is unclear. The receptor tyrosine kinase-mediated PI3K/AKT signaling pathway could be involved in the regulation of prostate cancer cell proliferation and differentiation, since in cancer, the activation of this pathway is known to promote cell proliferation, survival, invasion and metastasis (10).

Tumor cells actively proliferate *in vivo* and it is generally considered that hypoxia plays a vital role in this process (11). A hypoxic microenvironment facilitates tumor invasiveness and reduces the sensitivity of tumors to chemotherapy (12,13). Hypoxia is common in human prostate cancer, in which it is associated with disease progression and treatment resistance (14). Various types of solid tumors, including prostate

cancer, contain substantial hypoxic regions due to their tortuous and undeveloped vasculature (15).

In the present study, the role of SPRY4-IT1 in the development of prostate cancer was investigated. In particular, the expression levels of SPRY4-IT1 in prostate cancer tissues and cell lines were compared with the corresponding expression in pair-matched benign adjacent prostate tissues and in an immortalized non-cancerous prostatic epithelial cell line, respectively. Furthermore, the expression levels of SPRY4-IT1 were evaluated in the prostate cancer cells following culture under hypoxic conditions. To simulate the hypoxic microenvironment *in vivo*, a hypoxia incubator was used. The changes in the cell cycle and in the expression levels of cell cycle-associated proteins and PI3K/AKT signaling pathway components were investigated. The viability of SPRY4-IT1-overexpressing prostate cell lines was also monitored under hypoxic conditions.

Materials and methods

Clinical samples. A total of 36 pairs of fresh prostate cancer tissues and matched benign adjacent prostate tissues were collected from patients with prostate cancer at the Department of Urology of Shanghai Pudong Hospital affiliated with Fudan University (Shanghai, China) between May 2018 and November 2020. The protocols used in the present study were approved by the Shanghai Pudong Hospital Ethics Review Committee and written informed consent to participate was obtained from all patients prior to surgery. The specimens were classified according to the 2016 World Health Organization criteria and the TNM staging system (16). The size and Gleason score of each tumor was recorded (17). The clinicopathological features of the patients are shown in Table I. The inclusion criteria were as follows: Aged between 50-79 years; pathologically confirmed prostate cancer; accepted prostatectomy; and willing to participate in the study. The exclusion criteria were as follows: Aged <50 or >79 years; another active malignancy, with the exception of non-melanoma skin cancer, in addition to prostate cancer; did not accept prostatectomy; and unwilling to participate in the study.

Cell culture. The PC3, DU145 and LNCaP human prostatic cancer cell lines and the RWPE-1 human immortalized non-cancerous prostatic epithelial cell line were obtained from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. The PC3 cells were cultured in F12K medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.). The DU145 cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) in the presence of 10% FBS. The LNCaP cells were cultured in RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.) in the presence of 10% FBS. The RWPE-1 cells were cultured in keratinocyte serum-free medium (Gibco; Thermo Fisher Scientific, Inc.). All cell media were supplemented with 1% streptomycin/penicillin. The incubator temperature was set to 37˚C. An anaerobic environment was created in a hypoxic incubator in the presence of 1% O_2. The durations of hypoxia were 1, 6, 12 and 24 h.

Overexpression and short hairpin RNA (shRNA) plasmids. An overexpression vector targeting SPRY4-IT1 was purchased from GeneChem, Inc., and an empty PLVX vector was used as a control. Two shRNAs targeting SPRY4-IT1 and a negative control shRNA (shNC) with no specific target were synthesized by GeneChem, Inc. The following shRNA sequences were used: sh-SPRY4-IT1-1, GGTGGTTGAAAGGAA TCCT; sh-SPRY4-IT1-2, GCCTGTGAATGCCAACATC; and shNC, ATCGACTAGCCACTCAGAC. PC3 or DU145 cells were seeded in a 6-well plate for 24 h to reach a density of 30-50%, after which they were transfected with 2.5 μg overexpression vector, shRNA or respective control using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C according to the manufacturer's instructions. The transfected cells were harvested at 48 h following transfection. Stable cell lines were selected by treatment with 0.5‰ puromycin for 3 days, and the concentration used for maintenance was 0.1‰ puromycin

Hypoxic culture. A hypoxic environment was established using an anaerobic incubator with a 1% O_2 concentration. The cells were plated in a 6-well plate 24 h prior to the induction of hypoxia at 70-80% confluence. The time periods for hypoxic induction were set at 1, 6, 12 and 24 h.

Cell viability. Cell viability was assessed using a Cell Counting Kit (CCK)-8 assay (Dojindo Laboratories, Inc.) following the manufacturer's instructions. The cells were seeded in a 96-well plate at a density of 5,000 cells per well. Following the induction of hypoxia for 24 h, the CCK-8 solution was added to every well and the cells were cultured at 37˚C in an incubator in the dark under normal conditions for 2 h. The absorbance was detected at 450 nm using a microplate reader.

Flow cytometry analysis. For analysis of the cell cycle using flow cytometry, a Cell Cycle Staining Kit (CCS012; MultiSciences Biotech Co., Ltd.) was used. All steps were performed following the manufacturer's specifications. The analysis was performed using the BD FACSCalibur™ Flow Cytometer (BD Biosciences). The flow cytometry data were analyzed using FlowJo v10 software (FlowJo LLC).

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR) assays. Total RNA was isolated from cells using a SteadyPure Universal RNA Extraction Kit (cat. no. AG21017; Accurate Biology) according to the manufacturer's instructions. Complementary DNA was synthesized with random primers using the Evo M-MLV RT Kit with gDNA Clean for qPCR (Accurate Biology). The RT temperature protocol included gDNA removal at 42˚C for 2 min, and reverse transcription at 37˚C for 15 min and 85˚C for 5 sec. qPCR was carried out using the SYBR® Premix Ex Taq™ kit (Takara Bio, Inc.). The qPCR thermocycling conditions included initial denaturation at 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. The primer set for SPRY4-IT1 was as follows: Forward, 5'-AGCCACATAAAT TCAGCAGA-3' and reverse, 5'-CGATGTAGTAGGATTCCT TTCA-3'. Primers for β-actin were obtained from Accurate Biology (cat. no. AG11722) and had the following sequences: Forward, 5'-TATTTTGAATGATGAGCCTTCGT-3' and reverse, 5'-TGCACTTTTATTCAACTGGTCT-3'. All data analyses were performed using the StepOnePlus Real-Time

Table I. Associations between SPRY4-IT1 expression and the clinicopathological features of patients with prostate cancer.

		SPRY4-IT1 expression		
Parameters	Total	High	Low	P value
Age (years)				0.99
<69	12	9	3	
≥69	24	19	5	
Gleason score				0.04
6 or 7	11	6	5	
7-10	25	22	3	
Tumor stage				0.69
T2	12	10	2	
T3 or T4	24	18	6	
Tumor size (cm)				0.05
<0.6	8	4	4	
≥0.6	28	24	4	

SPRY4-IT1, sprouty4-intron 1.

PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The expression levels of SPRY4-IT1 were normalized against those of β-actin as the reference gene. The data were analyzed using the $2^{-\Delta\Delta Cq}$ method (18).

Western blot assays. Following the induction of hypoxia, the cells were quickly collected and lysed using RIPA protein extraction reagent (Epizyme, Inc.; Ipsen) supplemented with protease inhibitor and phosphatase inhibitor cocktails (both Epizyme. Inc.; Ipsen). The concentrations of the protein samples were detected using a BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). Protein extracts (15 μg/lane) were separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride membranes. The membranes were blocked for 25 min at room temperature in protein-free blocking buffer (Epizyme, Inc.; Ipsen) and incubated with primary antibodies at 4˚C for 12 h. Antibodies targeting CDK2 (cat. no. A0094), cyclin D1 (cat. no. A19038), AKT1 (cat. no. A20799), phosphorylated-AKT (cat. no. AP1172) and β-actin (cat. no. AC026) were used. All primary antibodies were diluted 1:1,000. All the primary antibodies were acquired from ABclonal Biotech Co., Ltd. Membranes were incubated with Anti-rabbit IgG, HRP-linked Antibody (dilution, 1:5,000; cat. no. 7074; Cell Signaling Technology, Inc.) at room temperature for 60 min. Immobilon Western HRP Substrate (WBKLS0050) was purchased from MilliporeSigma. The membrane was exposed to an autoradiography film and autoradiograms were quantified by densitometry using Quantity One software 4.4.6 (Bio-Rad Laboratories, Inc.).

Statistical analysis. All experiments were repeated three times. Patients were divided into high and low SPRY4-IT1 groups based on the Cq value of the RWPE-1 cells and clinicopathological data were analyzed according to using Fisher's exact test. Comparisons between the prostate cancer and normal adjacent tissues was performed using paired Student's t-test, and other comparisons between two groups were performed using unpaired Student's tests. One-way ANOVA with Tukey's post hoc test was used to determine the significance of differences among multiple groups. P<0.05 was considered to indicate a statistically significant result. All data were analyzed using Excel 2019 (Microsoft Corporation) and SPSS Statistics 21 (IBM Corp.).

Results

SPRY4-IT1 is highly expressed in prostate cancer tissues and cell lines. RT-qPCR was used to detect the expression levels of SPRY4-IT1 in prostate cancer tissues and cell lines. The expression levels of SPRY4-IT1 were significantly higher in prostate cancer tissues than in the pair-matched normal adjacent tissues (P<0.05; Fig. 1A). SPRY4-IT1 expression was associated with the Gleason score of patients with prostate cancer (P<0.04; Table I), and was independent of patient age, tumor stage and tumor size. In addition, SPRY4-IT1 was expressed at higher levels in the DU145 and PC3 prostate cancer cell lines than in the RWPE-1 human immortalized non-cancerous prostate epithelial cell line (P<0.01). However, SPRY4-IT1 expression was not upregulated in LNCaP cells (Fig. 1B).

SPRY4-IT1 expression is upregulated under hypoxic conditions. A hypoxic microenvironment is common in prostate cancer. To assess the effect of hypoxia on the expression of SPRY4-IT1, DU145 and PC3 cells were cultured in an anaerobic incubator for various time periods. Following 24 h of cell culture under hypoxia, the expression levels of SPRY4-IT1 were significantly increased in the DU145 and PC3 cells compared with those cultured under normoxic conditions (P<0.01). However, the expression levels of SPRY4-IT1 did not change significantly in DU145 and PC3 cell lines following 1, 6 and 12 h of culture under hypoxic conditions (Fig. 1C and D).

Knockdown of SPRY4-IT1 suppresses prostate cancer cell viability. DU145 and PC3 cells were transfected with SPRY4 overexpression vector or shRNA, and the transfection efficiency is presented in Fig. S1. To effectively simulate the hypoxic microenvironment *in vitro*, the transfected cells were cultivated in an anaerobic incubator for 24 h and the cell viability was evaluated using CCK-8 assays. The results demonstrated that the cell viability was significantly lower in DU145 and PC3 cells transfected with sh-SPRY4-IT1 compared with the corresponding control cells transfected with shNC (P<0.01). Overexpression of SPRY4-IT1 in DU145 and PC3 cells increased resistance to the hypoxic environment (P<0.05). This result indicates that SPRY4-IT1 had a protective effect on prostate cancer cell viability under hypoxic conditions (Fig. 2).

Knockdown of SPRY4-IT1 leads to S-phase arrest in prostate cancer cells. Flow cytometry was used to evaluate the cell cycle following the knockdown of SPRY4-IT1 expression in DU145 and PC3 cells. The results indicated that the knockdown of SPRY4-IT1 expression led to S-phase arrest in prostate cancer cells (P<0.05; Fig. 3).

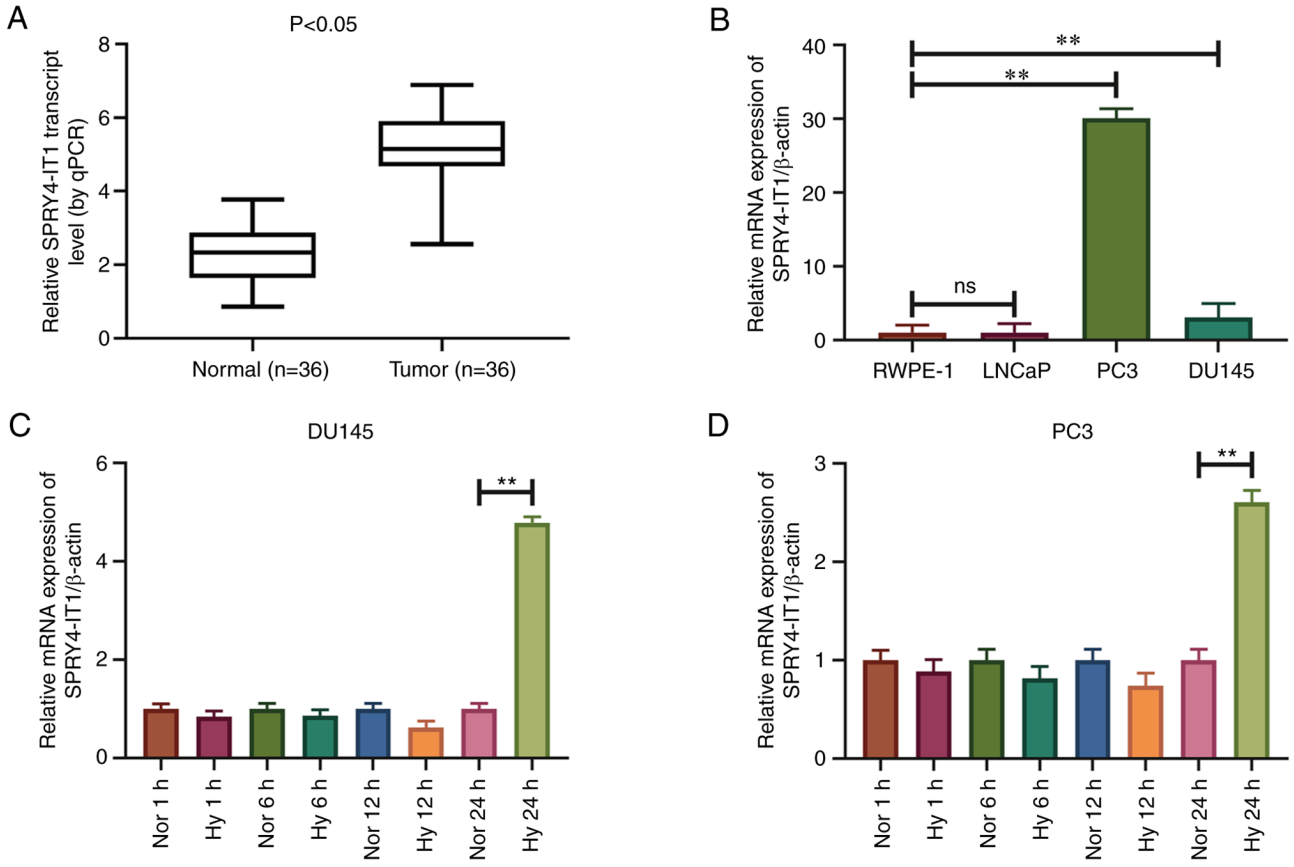

Figure 1. Expression levels of SPRY4-IT1 in prostate cancer tissues and cell lines. Expression of SPRY4-IT1 was higher in (A) prostate cancer tissues than normal adjacent tissues and (B) PC3 and DU145 prostate cancer cell lines than in RWPE-1 prostate epithelial cells. Expression levels of SPRY4-IT1 in (C) DU145 and (D) PC3 prostate cancer cell lines. After 24 h culture under hypoxic conditions, the expression level of SPRY4-IT1 was upregulated in the prostate cancer cells. Values are presented as the mean and SD. **P<0.01. SPRY4-IT1, sprouty4-intron 1; Nor, normoxia; Hyp, hypoxia; qPCR, quantitative PCR; ns, no significance.

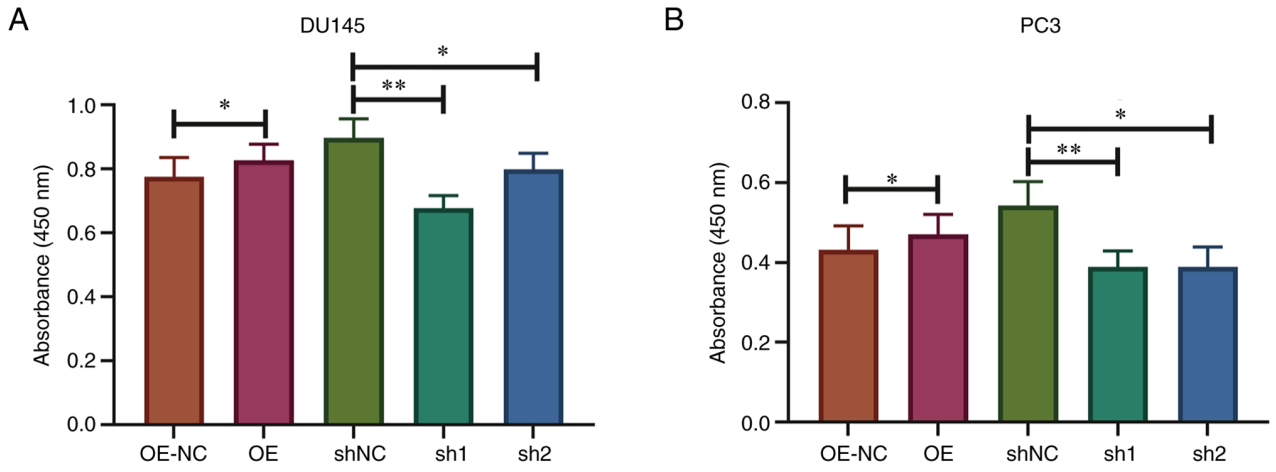

Figure 2. Cell Counting Kit-8 cell viability assay results. The knockdown of SPRY4-IT1 in (A) DU145 and (B) PC3 prostate cancer cell lines reduced cell viability following culture under hypoxic conditions for 24 h. *P<0.05, **P<0.01. SPRY4-IT1, sprouty4-intron 1; OE, overexpression; OE-NC, OE negative control (empty vector); shNC, short hairpin negative control; sh1, sh-SPRY4-IT1-1; sh2, sh-SPRY4-IT1-2.

Knockdown of SPRY4-IT1 inhibits cell cycle-associated protein expression and AKT phosphorylation. The transfected DU145 and PC3 cell lines were cultured in an anaerobic incubator for 24 h and the total protein was then rapidly extracted for western blot analysis. After SPRY4-IT1 was overexpressed in the DU145 cell line, the expression levels of CDK2 and cyclin D1 were increased (P<0.05). In SPRY4-IT1-overexpressing PC3 cells, cyclin D1 expression was upregulated (P<0.05), while no significant difference in CDK2 expression was observed. The expression levels of CDK2 and cyclin D1 were lower in DU145 and PC3 cells transfected with sh-SPRY4-IT1 compared with cells transfected with shNC (P<0.01), indicating that

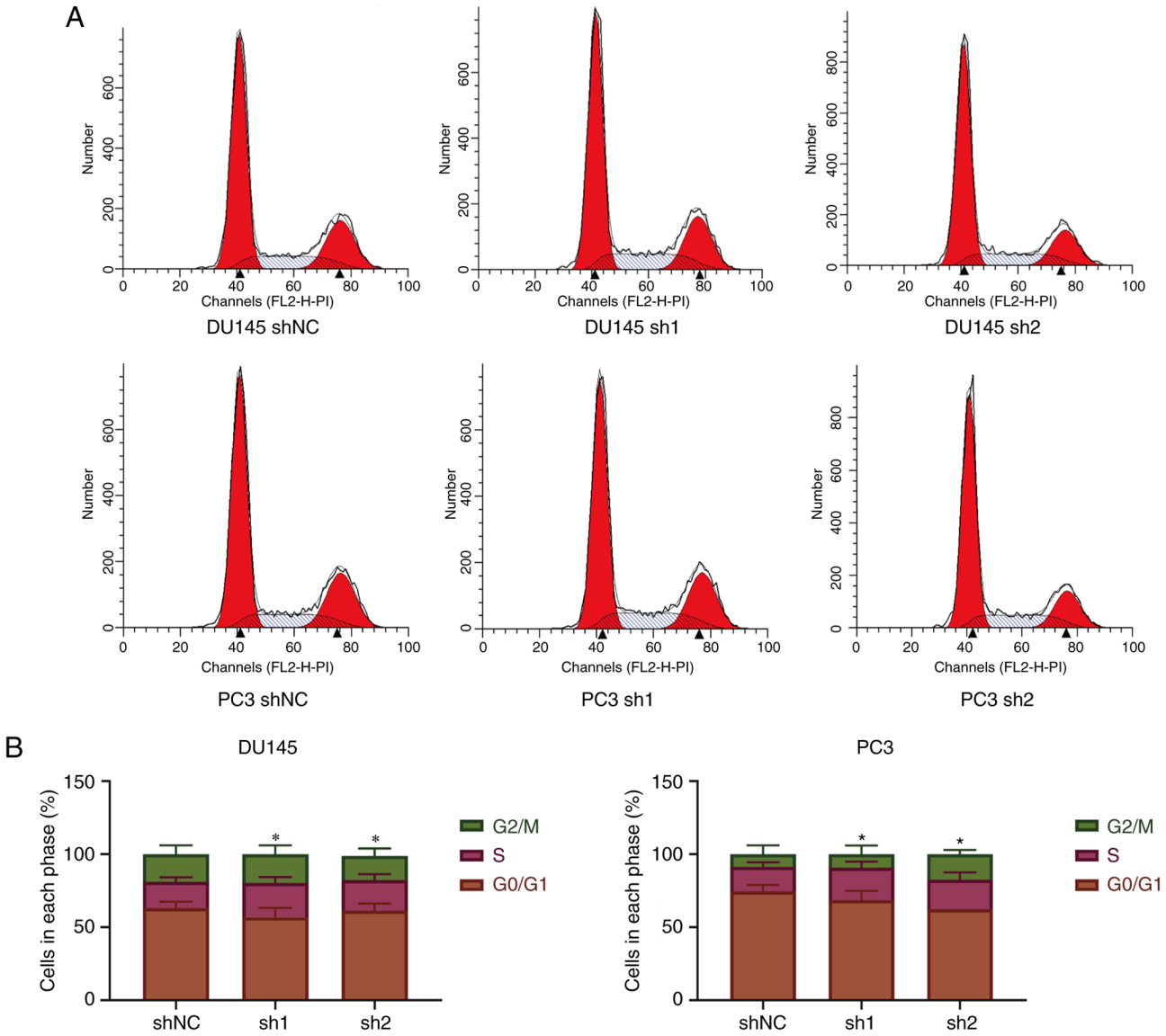

Figure 3. Flow cytometry results for DU145 and PC3 cells following the knockdown of SPRY4-IT1. (A) Representative flow cytometry plots and (B) cell cycle analysis. Results show that the knockdown of SPRY4-IT1 in the prostate cancer cell lines led to S phase arrest under a hypoxic microenvironment. *P<0.05, **P<0.01. SPRY4-IT1, sprouty4-intron 1; shNC, short hairpin negative control; sh1, sh-SPRY4-IT1-1; sh2, sh-SPRY4-IT1-2.

downregulation of SPRY4-IT1 expression affected the cell cycle progression of prostate cancer cells. This is consistent with the flow cytometry results. After overexpression of SPRY4-IT1, the phosphorylation levels of AKT in PC3 cells were increased (P<0.05), while those in DU145 cells were not significantly altered. AKT phosphorylation was also reduced following the knockdown of SPRY4-IT1 expression (Fig. 4).

Discussion

The present study revealed that the expression levels of SPRY4-IT1 expression were higher in prostate cancer tissues and cell lines than in pair-matched adjacent benign prostate tissues and the RWPE-1 human immortalized non-cancerous prostate epithelial cell line, respectively. SPRY4-IT1 has been reported to be highly expressed in prostate cancer tissues and the PC3 cell line (19). However, to the best of our knowledge, the expression levels of SPRY4-IT1 in the DU145 cell line have not been previously reported. The present study further confirmed that SPRY4-IT1 was highly expressed in two prostate cancer cell lines. SPRY4-IT1 expression was significantly associated with the Gleason score in prostate cancer but not with age, tumor stage or tumor size. These results suggest that SPRY4-IT1 is potentially involved in the progression of prostate cancer. However, the sample size of the present study was relatively small and the relationships between SPRY4-IT1 and the clinical parameters require further examination in subsequent studies.

Adaptation to hypoxic stress is pivotal in tumor progression and malignancy (20). The current study is a preliminary analysis demonstrating the effect of hypoxia on SPRY4-IT1 expression in prostate cancer. At the beginning of the hypoxic culture period, the expression of SPRY4-IT1 exhibited a slight reduction. However, following 24 h of cell culture under hypoxic conditions, the expression levels of SPRY4-IT1 in the DU145 and PC3 prostatic cancer cells were increased;

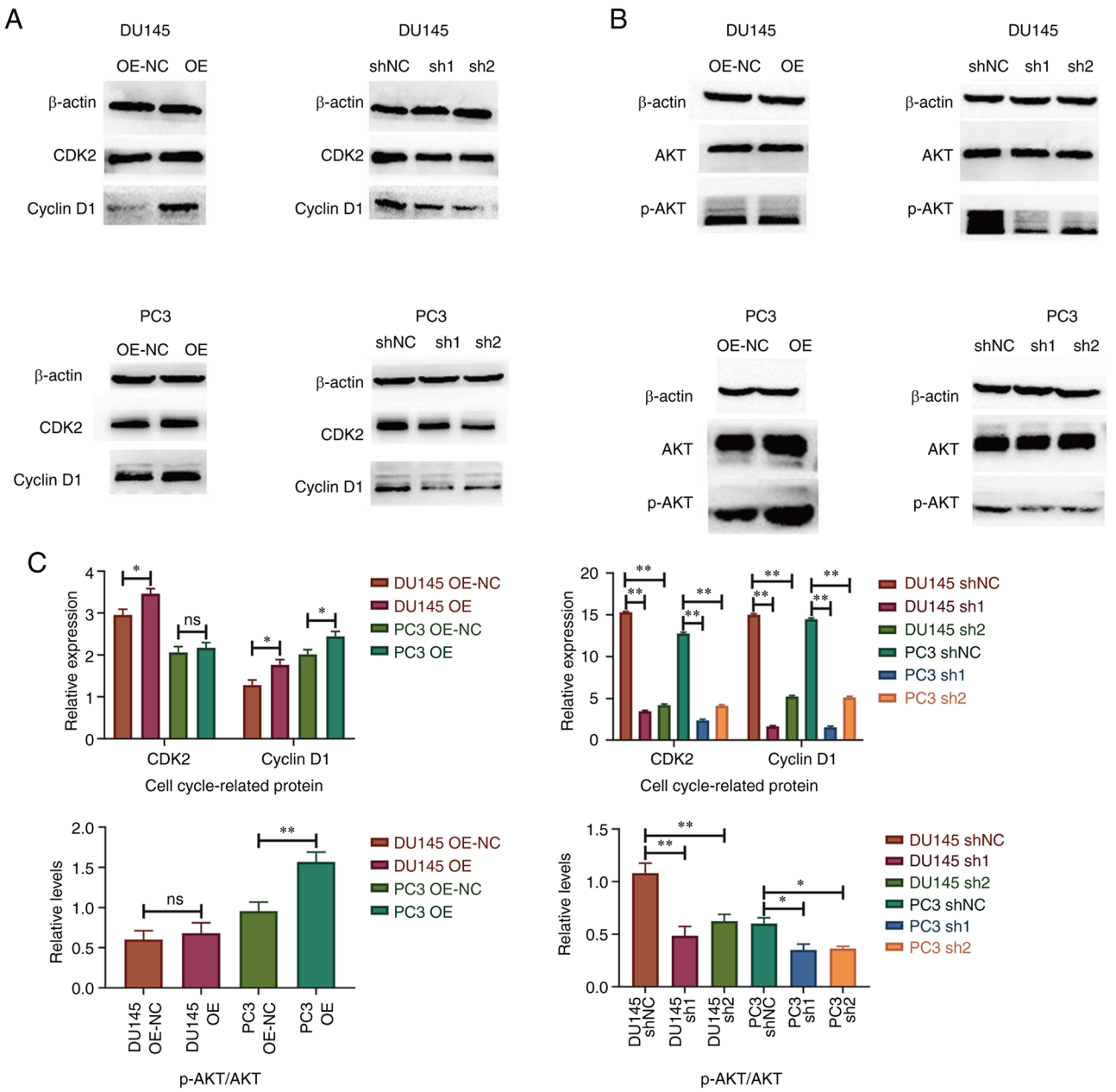

Figure 4. Western blotting results of cell cycle-associated proteins and AKT phosphorylation. Representative blots for (A) CDK2 and cyclin D1 and (B) AKT phosphorylation. (C) Quantification of the western blot results. Knockdown of SPRY4-IT1 in DU145 and PC3 cell lines led to reductions in CDK2 and cyclin D1 expression and AKT phosphorylation. *P<0.05, **P<0.01, ns, no significance. SPRY4-IT1, sprouty4-intron 1; OE, overexpression; OE-NC, OE negative control (empty vector); shNC, short hairpin negative control; sh1, sh-SPRY4-IT1-1; sh2, sh-SPRY4-IT1-2; p-, phosphorylated.

thus, during adaptation to hypoxia, the expression levels of SPRY4-IT1 in the prostate cancer cell lines were increased. The knockdown of SPRY4-IT1 expression in DU145 and PC3 cells resulted in a reduction in cell viability following cell culture under hypoxic conditions for 24 h. This finding indicates that SPRY4-IT1 promotes prostate cancer cell viability when the cells are exposed to hypoxia. Therefore, it appears that SPRY4-IT1 plays an important role in the adaptation of prostate cancer cells to hypoxic stress and in the maintenance of cell activity in a hypoxic environment. Considering hypoxia is common in human prostate cancer, we hypothesize that SPRY4-IT1 plays the same role *in vivo*.

The present study revealed that the knockdown of SPRY4-IT1 expression led to S-phase arrest in prostate cancer cells under hypoxic conditions. This result is in accordance with a study performed on human melanoma, in which the suppression of SPRY4-IT1 impaired cell proliferation and invasion (20). The present study also demonstrated that following the knockdown of SPRY4-IT1 expression in prostate cancer cells, the expression levels of the cell cycle-associated proteins CDK2 and cyclin D1 were decreased. The aberrant expression of CDK2 has been detected in a variety of tumors and is associated with the proliferation of tumor cells (21), while the upregulation of cyclin D1 expression has been shown to accelerate cell cycle progression and lead to tumor cell proliferation (22). Furthermore, CDK2 and cyclin D1 have both been shown to drive cell cycle progression through the S phase (23,24).

The flow cytometry results and western blot assay results were consistent regarding the effects of SPRY4-IT1 on the cell cycle. Knockdown of sprouty4 and SPRY4-IT1 expression in tumor cells has been reported to potently inhibit AKT phosphorylation (6), with the latter increasing the expression levels of cyclin D1 (25). Changes in the total and phosphorylated levels of AKT, a member of the PI3K/AKT signaling pathway, following the knockdown of SPRY4-IT1 expression were investigated in prostate cancer cells using western blot analysis. The knockdown of SPRY4-IT1 expression in DU145 and PC3 cells inhibited AKT phosphorylation under hypoxic conditions. The AKT pathway promotes cell survival via AKT phosphorylation and the subsequent inhibition of apoptosis (26); this mechanism of action may explain the decreased viability of prostate cancer cells following the knockdown of SPRY4-IT1 expression.

The present study verified that SPRY4-IT1 expression was elevated in prostate cancer tissues and the DU145 and PC3 cell lines compared with pair-matched adjacent benign prostate tissues and the non-cancerous prostate epithelial cell line RWPE-1. This finding improves our understanding of SPRY4-IT1 expression in prostate cancer. Based on these results, it is suggested that SPRY4-IT1 is likely to be involved in the progression of prostate cancer. Furthermore, the results indicated that SPRY4-IT1 expression was upregulated under hypoxic conditions and could regulate the viability of prostate cancer cells, possibly via the regulation of CDK2, cyclin D1 and AKT phosphorylation. The results suggest the underlying mechanism by which SPRY4-IT1 functions in a hypoxic microenvironment. Finally, the results suggest a novel application for SPRY4-IT1 in the clinical diagnosis and treatment of prostate cancer. Prostate-specific antigen screening and ultrasound-guided prostate puncture are the main methods of prostate cancer diagnosis, which often lead to excessive medical treatment. Detection of the expression level of SPRY4-IT1 in the urine of prostate cancer patients is potentially an auxiliary means for the diagnosis of prostate cancer, which may reduce the risk of over-diagnosis to some extent. To explore the feasibility of urine SPRY4-IT1 levels in the diagnosis of prostate cancer, SPRY4-IT1 levels in the urine of patients with prostate cancer should be detected in the future. The results of the present study also suggested that SPRY4-IT1 may be associated with a worse prognosis in prostate cancer patients. Therefore, the measurement of SPRY4-IT1 levels in patients with prostate cancer could potentially be used to select a more suitable treatment at an early stage to reduce mortality. However, further mechanistic studies of SPRY4-IT1 are required to fully understand its role in the pathogenesis of prostate cancer. Additional studies investigating the molecular interactions of SPRY4-IT1 with other genes may also improve our comprehension of the mechanisms underlying prostate cancer occurrence and development.

In summary, the present study demonstrates that the lncRNA SPRY4-IT1 is upregulated in prostate cancer tissues and cell lines. It also suggests that the upregulation of SPRY4-IT1 promotes the proliferation of prostate cancer cells under hypoxia *in vitro*. Therefore, SPRY4-IT1 may be a potential target for the diagnosis and treatment of prostate cancer.

lncRNAs play a variety of roles in tumor progression. Previous studies have shown that the lncRNA SPRY4-IT1 can bind to a variety of molecules, including miRs and proteins. A limitation of the present study is that it did not explore the potential molecules to which SPRY4-IT1 may bind. In addition, the number of cases included was relatively small and a higher number of cases are required to support the conclusions of the study.

Acknowledgements

Not applicable.

Funding information

The study was supported by the Natural Science Foundation of China (project no. 81802547) and the Science and Technology Development Fund of Shanghai Pudong New Area (grant no. PKJ2018-Y34).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WS and DL were responsible for the design and completion of the experiment, while RZ and MG were responsible for the data analysis and article writing. All authors read and approved the final version of the manuscript. MG and WS confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by Shanghai Pudong Hospital Ethics Review Committee. The patients provided written informed consent to participate.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Brandão A, Paulo P and Teixeira MR: Hereditary predisposition to prostate cancer: From genetics to clinical implications. Int J Mol Sci 21: 5036, 2020.
2. Wilson KM, Giovannucci EL and Mucci LA: Lifestyle and dietary factors in the prevention of lethal prostate cancer. Asian J Androl 14: 365-374, 2012.
3. Peng WX, Koirala P and Mo YY: LncRNA-mediated regulation of cell signaling in cancer. Oncogene 36: 5661-5667, 2017.
4. Peng Y, Tang D, Zhao M, Kajiyama H, Kikkawa F and Kondo Y: Long non-coding RNA: A recently accentuated molecule in chemoresistance in cancer. Cancer Metastasis Rev 39: 825-835, 2020.
5. Du Z, Fei T, Verhaak RG, Su Z, Zhang Y, Brown M, Chen Y and Liu XS: Integrative genomic analyses reveal clinically relevant long noncoding RNAs in human cancer. Nat Struct Mol Biol 20: 908-913, 2013.
6. Das MK, Furu K, Evensen HF, Haugen ØP and Haugen TB: Knockdown of SPRY4 and SPRY4-IT1 inhibits cell growth and phosphorylation of Akt in human testicular germ cell tumours. Sci Rep 8: 2462, 20186.

7. Liu D, Li Y, Luo G, Xiao X, Tao D, Wu X, Wang M, Huang C, Wang L, Zeng F, *et al*: LncRNA SPRY4-IT1 sponges miR-101-3p to promote proliferation and metastasis of bladder cancer cells through up-regulating EZH2. Cancer Lett 388: 281-291, 2017.
8. Li Z, Shen J, Chan MTV and Wu WKK: The long non-coding RNA SPRY4-IT1: An emerging player in tumorigenesis and osteosarcoma. Cell Prolif 51: e12446, 2018.
9. Lee B, Mazar J, Aftab MN, Qi F, Shelley J, Li JL, Govindarajan S, Valerio F, Rivera I, Thurn T, *et al*: Long noncoding RNAs as putative biomarkers for prostate cancer detection. J Mol Diagn 16: 615-626, 2014.
10. Regad T: Targeting RTK signaling pathways in cancer. Cancers (Basel) 7: 1758-1784, 2015.
11. Zhang T, Guo S, Zhu X, Qiu J, Deng G and Qiu C: Alpinetin inhibits breast cancer growth by ROS/NF-κB/HIF-1α axis. J Cell Mol Med 24: 8430-8440, 2020.
12. Xie C, Ji N, Tang Z, Li J and Chen Q: The role of extracellular vesicles from different origin in the microenvironment of head and neck cancers. Mol Cancer 18: 83, 2019.
13. Wu X, Zhu Y, Huang W, Li J, Zhang B, Li Z and Yang X: Hyperbaric oxygen potentiates Doxil antitumor efficacy by promoting tumor penetration and sensitizing cancer cells. Adv Sci (Weinh) 5: 1700859, 2018.
14. Krishnamachary B, Mironchik Y, Jacob D, Goggins E, Kakkad S, Ofori F, Dore-Savard L, Bharti SK, Wildes F, Penet MF, *et al*: Hypoxia theranostics of a human prostate cancer xenograft and the resulting effects on the tumor microenvironment. Neoplasia 22: 679-688, 2020.
15. Zhang W, Fan W, Rachagani S, Zhou Z, Lele SM, Batra SK and Garrison JC: Comparative study of subcutaneous and orthotopic mouse models of prostate cancer: Vascular perfusion, vasculature density, hypoxic burden and BB2r-targeting efficacy. Sci Rep 9: 11117, 2019.
16. Buyyounouski MK, Choyke PL, McKenney JK, Sartor O, Sandler HM, Amin MB, Kattan MW and Lin DW: Prostate cancer-major changes in the American joint committee on cancer eighth edition cancer staging manual. CA Cancer J Clin 67: 245-253, 2017.
17. Epstein JI, Allsbrook WC Jr, Amin MB, Egevad LL and ISUP Grading Committee: The 2005 international society of urological pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma. Am J Surg Pathol 29: 1228-1242, 2005.
18. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
19. Melana JP, Mignolli F, Stoyanoff T, Aguirre MV, Balboa MA, Balsinde J and Rodríguez JP: The hypoxic microenvironment induces stearoyl-CoA desaturase-1 overexpression and lipidomic profile changes in clear cell renal cell carcinoma. Cancers (Basel) 13: 2962, 2021.
20. Khaitan D, Dinger ME, Mazar J, Crawford J, Smith MA, Mattick JS and Perera RJ: The melanoma-upregulated long noncoding RNA SPRY4-IT1 modulates apoptosis and invasion. Cancer Res 71: 3852-3862, 2011.
21. Sheng Y, Han C, Yang Y, Wang J, Gu Y, Li W and Guo L: Correlation between LncRNA-LINC00659 and clinical prognosis in gastric cancer and study on its biological mechanism. J Cell Mol Med 24: 14467-14480, 2020.
22. Li K, Xu X, He Y, Tian Y, Pan W, Xu L, Ma Y, Gao Y, Gao J, Qi Y, *et al*: P21-activated kinase 7 (PAK7) interacts with and activates Wnt/β-catenin signaling pathway in breast cancer. J Cancer 9: 1821-1835, 2018.
23. Zhang X, Bai Q, Xu L, Kakiyama G, Pandak WM, Zhang Z and Ren S: Cytosolic sulfotransferase 2B1b promotes hepatocyte proliferation gene expression in vivo and in vitro. Am J Physiol Gastrointest Liver Physiol 303: G344-G355, 2012.
24. Jin H, Zhang X, Su J, Teng Y, Ren H and Yang L: RNA interference-mediated knockdown of translationally controlled tumor protein induces apoptosis, and inhibits growth and invasion in glioma cells. Mol Med Rep 12: 6617-6625, 2015.
25. Ito Y, Kaji M, Sakamoto E and Terauchi Y: The beneficial effects of a muscarinic agonist on pancreatic β-cells. Sci Rep 9: 16180, 2019.
26. Razak NA, Abu N, Ho WY, Zamberi NR, Tan SW, Alitheen NB, Long K and Yeap SK: Cytotoxicity of eupatorin in MCF-7 and MDA-MB-231 human breast cancer cells via cell cycle arrest, anti-angiogenesis and induction of apoptosis. Sci Rep 9: 1514, 2019.

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