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# Suppressor of activator protein-1 regulated by interferon expression in prostate cancer tissues and cells

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ABSTRACT

*Purpose:* The aim of this study was to investigate the role of the suppressor of activator protein-1 regulated by interferon (SARI), in the development and progression of prostate cancer.
*Methods:* Sixty-seven prostate cancer tissue specimens and 20 benign prostatic hyperplasia specimens were used to investigate the correlation between SARI expression and clinicopathologic parameters. Immunohistochemistry was used to detect the SARI and E-cadherin protein expression in the prostate cancer and benign prostatic hyperplasia specimens, and their correlation was established. Quantitative PCR (qPCR) was used to determine the SARI mRNA expression in a normal prostate cell line (RWPE-1) and prostate cancer cell lines (LNCaP and PC3). Western blotting was used to detect the SARI protein expression in the RWPE-1, LNCaP, and PC3 cell lines.
*Results:* SARI protein expression did not correlate with the prostate cancer patients' age or serum Prostate-Specific Antigen value but did show a correlation with the tumor stage of prostate cancer and Gleason score. SARI and E-cadherin expression in the prostate cancer tissue was significantly lower than in the benign prostatic hyperplasia specimens, suggesting a positive correlation between the SARI and E-cadherin expression. SARI mRNA and protein were highly expressed in RWPE-1, the normal prostate cell line, but SARI mRNA and protein expression were reduced in the prostate cancer cell lines, LNCaP and PC3. Significant differences in the expression were found between the prostate cancer cell lines and the normal prostate cell line.
*Conclusion:* In this study, high SARI expression was found to be negatively correlated with the development and progression of prostate cancer.

## 1. Introduction

Prostate cancer is among the most common cancers reported in males, and the number of prostate cancer patients has been increasing continuously worldwide [1]. Prostate cancer has an insidious onset and is prone to metastasis in the bone and other organs. Prostate-Specific Antigen (PSA) is an important indicator for early screening of prostate cancer, but PSA has no tumor specificity. Prostatitis, benign prostatic hyperplasia, and prostate cancer can lead to an increase in total PSA levels (free PSA plus PSA), which also leads to prostate cancer. Early diagnosis becomes very difficult. In China, most patients are diagnosed only at an advanced stage of the disease, and fewer than 30% of these patients have the option to undergo surgery [2–4]. Patients with localized prostate cancer who undergo radical surgery supplemented by endocrine therapy can reach 100% 5-year survival. Patients with advanced prostate cancer tend to have an androgen-independent phenotype, and endocrine monotherapy results in only 32% 5-year survival [5]. Further study of the molecular mechanisms associated with the etiology and tumor progression of prostate cancer is necessary.

The suppressor of activator protein-1 regulated by interferon (SARI) gene, which has a leucine zipper as the basic gene structure. SARI binds to the AP-1 transcription factor dimer subunit through the alkaline leucine zipper domain, but since it lacks an activation domain, it cannot initiate transcription, and thus, it inhibits AP-1 activity and ultimately suppresses tumor cell growth [6,7]. The SARI gene is expressed in a variety of normal tissues and cells; however, the expression of the SARI gene is significantly lower in many solid tumors. The SARI gene is considered a new tumor suppressor gene, but its expression in the prostate tissue has not been thoroughly investigated.

Epithelial-mesenchymal transition (EMT) is a crucial step in tumor

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progression and has an important role during cancer invasion and metastasis [8]. In response to EMT-inducing signals, epithelial cells weaken or lose cell-cell adhesion, repress the expression of epithelial cell markers (including adhesion molecules such as E-cadherin), and activate mesenchymal genes (e.g., N-cadherin) [9]. These changes endow cells of epithelial origin with the increased migratory and invasive capacity of mesenchymal cells. E-cadherin is a well-known tumor suppressor protein, and the loss of its expression in tumor cells frequently occurs during tumor progression and metastasis [10]. The phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway plays an important role in regulating cell proliferation and maintaining the biological characteristics of malignant cells [11,12]. Furthermore, the PI3K/AKT signaling pathway has been linked to EMT in cancer [13]. AKT may have a role in the loss of E-cadherin expression as it has been shown that AKT activation leads to the downregulation of E-cadherin expression [14]. The extensive biological activity of AKT produces biological effects mainly through the phosphorylation of downstream substrates such as the mammalian target of rapamycin (mTOR) and Glycogensynthasekinase3β (GSK-3β). The PI3K/AKT/GSK-3β signaling pathway is involved in proliferation, apoptosis, and invasion of various cancer cells. For example, Yun [15] proposed that protein kinase D2 in hepatoma cells can contribute to TNF-α-induced EMT and invasion through the PI3K/GSK-3β/β-catenin pathway.

In this study, we detected SARI expression in prostate tissue and different prostate epithelial cell lines to explore the role of SARI in the development and progression of prostate cancer.

## 2. Materials and methods

### 2.1. Tissue samples and clinical data

A total of 87 prostate tissue specimens from the Department of Urological Surgery, the Third Xiangya Hospital, Central South University, Changsha, China, were collected from January 2010 to January 2017, including 67 cases of prostate cancer and 20 cases of benign prostate hyperplasia. All patients signed written informed consent forms before participating in this study, and the study protocol was approved by the Ethics Committee of the Third Xiangya Hospital, Central South University.

The mean age of the patients with prostate cancer was 63.56 ± 2.35 years old. All patients were histopathologically diagnosed with prostate cancer according to their surgical specimens and had not received any preoperative radiotherapy and chemotherapy. Twelve prostate cancer patients were ≤60 years old, and 55 prostate cancer patients were > 60 years old. Of the 67 prostate cancer patients, 10 cases had a serum PSA value ≤4 ng/mL; 13 cases had PSA ranging from > 4 ng/mL to ≤10 ng/mL; and 44 cases had PSA > 10 ng/mL. Tumor staging of the 67 prostate cancer patients showed 10 cases of T1, 12 cases of T2, 30 cases of T3, and 15 cases of T4.

### 2.2. Cell culture and transfection

LNCaP (androgen-dependent prostate cancer cells), PC3 (non-androgen-dependent prostate cancer cells), and RWPE-1 (normal prostate epithelial cells) were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China).

Prostate cancer cell lines (LNCaP and PC3) were cultured in RPMI 1640 medium containing 10% neonatal calf serum. The normal prostate cell line (RWEP-1) was cultured in K-SFM medium. All cells were incubated at 37 °C in an incubator with 5% $CO_2$ and saturated humidity. Culture medium was changed every 2–3 days.

Cells were transfected with SV40-EGFP-IRES-SARI according to the manufacturer's instructions; the cells transfected with SV40-EGFP-IRES were used as a negative control. The positive cells with ectopic expression of SARI or blank control were further sorted by GFP-mediated flow cytometry technology and expanded in culture. The cells were harvested for Western blot experiments.

### 2.3. Histopathological slide preparation and analysis

The prostate specimens were fixed in 10% formalin and embedded in paraffin. The tissues were sectioned into 3 μm sections and stained with hematoxylin and eosin (HE). The samples were then diagnosed according to the tumor classification standard reported by the World Health Organization (WHO; 2016 edition) [16].

### 2.4. Immunohistochemistry (IHC)

Three-micrometer-thick tissue sections were immunostained in an automated immunostainer using antibodies against SARI (Ab157466, 1:200), E-cadherin (MAB-0589, 1:200), and β-catenin (MAB-0259, 1:200).

### 2.5. IHC analysis

SARI protein expression was mainly localized in the nuclei of glandular epithelial cells, presenting brownish yellow color and granular form after IHC. E-cadherin protein expression was mainly localized in the glandular epithelial cell membranes, presenting brownish yellow color and granular form after IHC. A semi-quantitative scoring system was used to evaluate the IHC protein expression. IHC staining intensity of the samples was scored from 0 to 3, with 0 representing negative staining, score 1 representing weak positive staining, score 2 presenting moderate positive staining, and score 3 representing strong positive staining. In addition, the IHC extent of staining of each sample was scored from 0 to 4 according to the percentage of positive staining in the specimen: Score 0: 0% positive staining; Score 1: 1%–25% positive staining; Score 2: 26%–50% positive staining; Score 3: 51%–75% positive staining; and Score 4: > 75% positive staining. The results of staining intensity and the percentage of positive staining of each specimen were multiplied to obtain the final score. A final score of 0–5 represented low expression, and a final score of ≥6 represented high expression. The final scores of all specimens were used for statistical analysis [17]. In benign hyperplasia of prostate acinar epithelial cells, β-catenin is highly expressed in the cell membrane. In prostate cancer tissues, the expression of β-catenin was significantly decreased in the cell membrane, and a positive expression was observed in the cytoplasm, as well as in a few nuclei, which is ectopic expression [18].

### 2.6. Cell proliferation

The cells were counted and seeded in 96-well plates in a total amount of 100 μl of RPMI-1640 supplemented with 10% fetal calf serum at a concentration of $1 \times 10^3$ cells/well. RPMI-1640 supplemented with 10% fetal bovine serum. Ten μl of Cell Counting Kit-8 (CCK-8) detection reagent were added per well and cells were further incubated at 37 °C for 1–7 days. Absorbance was measured at a wavelength of 450 nm using a microplate reader.

### 2.7. Migration experiment

A Transwell migration assay was performed using Millipore transwell chambers (8 μm pore size, Millipore). PC3 cells ($1 \times 10^5$ in each well) were transfected with a SARI expression vector and vector controls and seeded in the upper chambers of the 12 well plate (Corning, USA) in 500 μl of serum-free medium. The lower chambers were filled with 1 ml of complete medium. The chamber was incubated at 37 °C for 24 h. At the end of incubation, the cells in the upper surface of the membrane were removed with a cotton swab. Cells in the lower chamber were fixed with methanol and stained with Giemsa (HiMedia Labs). The images were taken with an inverted microscope (CX41, Olympus) and analyzed using NIH imageJ software.

### 2.8. Invasion experiment

The tip, EP tube, Matrigel, and Transwell were pre-cooled at 4 °C overnight. Matrigel (2.5 mg/ml) was diluted in 40 μl of 1:10 serum-free DMEM medium to a final concentration of 50 μg/ml per well, and after incubation at 37 °C for 30 min the supernatant was aspirated. The lower chamber was placed in 600 μl of complete medium (10% FBS DMEM medium). The cells were digested with trypsin to make them into single cells, and the cells were then resuspended in serum-free medium to $1 \times 10^6$ cells/ml, and 100 μl of cells were added to each well. After incubation at 37 °C for 24 h, the upper chamber was removed, cells were placed in a new well containing PBS, the upper chamber was washed three times with PBS, and the upper chamber cells were wiped with a cotton ball. The mixture was mixed with methanol and acetone to prepare a fixing solution, fixed for 20 min, and the membrane was then removed and stained in 0.5% crystal violet for 5 min, washed 3–5 times, and dried naturally at room temperature. For each Transwell cell, fields of view were randomly selected to take 9 photos of ×100 photos and 9 photos of ×200. The ×200 photographs were eventually chosen to analyze the data, and the difference in invasive ability between the experimental group and the control group was compared.

### 2.9. Quantitative PCR

Total RNA was extracted from different groups of cells using Trizol reagent. The purity and content of the extracted RNA were determined by ultraviolet spectrophotometry, followed by agarose gel electrophoresis to determine the integrity. One microgram of total RNA was taken from each group of cells for reverse transcription into cDNA. cDNA products were stored at −20 °C for later use. The primers for PCR were: SARI forward, 5′-AGACCCCAAGGAGCAACA-3′, reverse, 5′-CAG GGCGAGGTTGTCTTT-3′; GAPDH forward, 5′-TGACTTCAACAGCGACA CCCA-3′, reverse, CACCCTGTTGCTGTAGCCAAA. The total volume of the amplification system for each group of genes was 20 μl (containing 10 μl L-SYBR Green, 0.5 μl of 10 pmol/μl upstream primer, 0.5 μl of 10 pmol/μl downstream primer, 7 μl $ddH_2O$, and 2 μl cDNA). Amplification conditions involved forty cycles of 95 °C denaturation for 10 s, 60 °C annealing for 20 s (during the first 10 cycles, there was a consecutive 0.5 °C reduction in the annealing temperature at each cycle), and 72 °C elongation for 20 s. Each group had three replicates. After the reaction, the average Ct value of each group of samples was calculated, and GAPDH served as an internal reference gene to calculate the relative mRNA expression of each target gene.

### 2.10. Western blot

LNCaP, PC3, and RWPE-1 were harvested in the logarithmic growth phase and incubated with 0.25% trypsin, followed by seeding at a density of $3 \times 10^4$ cells/well into 6-well plates. After 30 min incubation on the ice, cells were homogenized and lysed with RIPA lysis buffer to extract the total protein from each group. The protein concentration of each group was measured using the trace BCA protein assay. From each group, 150 μg of total protein were loaded into each lane and separated in 12% SDS-PAGE, followed by transferring the protein gel into PVDF membrane. The membrane was blocked overnight at 4 °C with TBST solution containing 5% skim milk, followed by incubation for 1 h with SARI primary antibody (1:1000). The protein blot was then incubated with the secondary antibody and subsequently developed using an ECL kit before scanning and imaging.

SARI was purchased from Abcam INC (Burlingame, CA). E-cadherin and β-catenin antibodies were obtained from THERMO (Waltham, MA, USA). Secondary antibodies and diaminobenzidine (DAB) chromogenic agent were obtained from Fuzhou Maixin Technology (Fuzhou, China). The qPCR primers used in the current study were synthesized by Shanghai Shenggong Biological Engineering (Shanghai, China). DEME was obtained from Gibco (CA, USA). GAPDH was purchased from Kaiji Biological (Nanjing, China). RPMI 1640 medium was purchased from Gibco (CA, USA). Enhanced chemiluminescence (ECL) kit was purchased from Amersham Corporation (Millipore, Shanghai, China).

### 2.11. Statistical analysis

SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis in this study. Measurement data were presented as mean ± standard deviation ($\bar{x} \pm s$). The correlation between two indices was analyzed by Spearman correlation. Comparison between groups was performed using the $\chi^2$-test. $P < 0.05$ was considered indicative of statistical significance.

## 3. Results

### 3.1. Histopathological analysis of the HE sections

Sixty-seven prostate cancer specimens were benign prostate adenocarcinoma and classified according to the prostate Gleason score of the 2016 Worth Health Organization (WHO) Classification of Tumors of the Urinary System and Male Genital Organs. The specific groupings of the 67 prostate cancer tissues were as follows: nine cases of Gleason scores in the 2–6 range; 30 cases of Gleason scores of 7; and 28 cases of Gleason scores ranging 8–10. The 20 cases of benign prostatic hyperplasia specimens were consistent with the pathological diagnosis.

### 3.2. SARI is expressed at low levels in prostate cancer

IHC results showed SARI protein to be localized in the nuclei of prostate glandular epithelial cells. As shown in Fig. 1A, the samples positive for SARI IHC stained brownish yellow in color and a small or granular matter were localized in the cell nuclei. The high expression rates of SARI in the prostate cancer tissue and benign prostatic hyperplasia specimens were 40.30% (27/67) and 75% (15/20), respectively. The expression of the SARI protein in the prostate cancer tissue was significantly lower than in the benign prostatic hyperplasia specimens ($\chi^2 = 7.428$, $P = 0.006$), as shown in Table 1. We then searched for the expression of SARI mRNA in prostate cancer in the TCGA database (http://ualcan.path.uab.edu/cgi-bin/ualcan-res.pl). Our experimental results are similar to those of the database (Fig. 1B).

### 3.3. The expression of SARI shows a positive correlation with E-cadherin in prostate cancer tissues, but a negative correlation with β-catenin

SARI facilitates EMT, leading to lung adenocarcinoma metastasis by regulation of E-cadherin expression [19]. Moreover, SARI functions as a critical protein in regulating EMT by modulating the (GSK)-3β-β-catenin signaling pathway. Thus, we measured the expression of E-cadherin and β-catenin at the same time. The IHC results revealed E-cadherin to be highly expressed in the prostate cancer tissues and benign prostatic hyperplasia tissues at rates of 73.13% (49/67) and 100% (20/20) in IHC results, respectively (Fig. 1C). We then searched the TCGA database (http://ualcan.path.uab.edu/cgi-bin/ualcan-res.pl) for the expression of E-cadherin mRNA in prostate cancer. Our experimental results are similar to those of the database (Fig. 1D). E-cadherin expression was lower in the prostate cancer tissue specimens than in the benign prostatic hyperplasia specimens ($\chi^2 = 6.775$, $P = 0.009$), as shown in Table 1. The IHC results revealed the expression of SARI and E-cadherin in prostate cancer tissues, as showed in Table 2. This study revealed a positive correlation between SARI and E-cadherin expression in the prostate cancer tissues ($r = 0.292$, $P = 0.017$).

β-catenin was found to be ectopic expressed in the prostate cancer tissues and benign prostatic hyperplasia tissues, at a rate of 67.2% (45/67) and 30% (6/20), respectively. (Fig. 1E). Ectopic expression of β-Catenin was significantly higher in the prostate cancer tissue specimens than in the benign prostatic hyperplasia specimens ($\chi^2 = 8.770$,

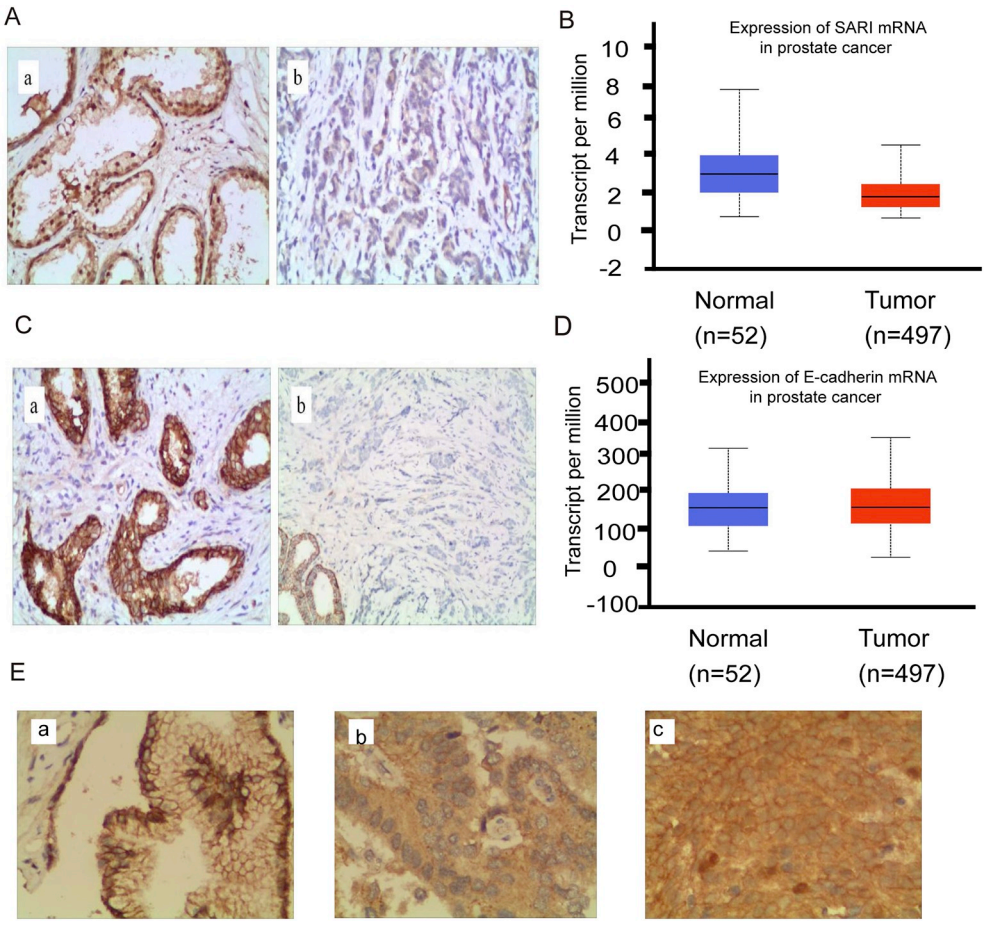


**Fig. 1.** (A) SARI protein expression of different tissues detected by IHC (Elivison ×200). Note: a. High SARI expression in the prostate acinar epithelium with benign hyperplasia; b. Low SARI expression in the prostate cancer tissue. (B) Analysis of SARI gene expression profile from TCGA; red represents the gene expression level of prostate cancer group, and blue represents the gene expression level of the normal group. $p < 0.05$. (C) E-cadherin protein expression in prostate tissues detected by IHC (Elivison ×200). Note: a. High E-cadherin expression was observed in the prostate epithelial cell membrane of the benign prostatic hyperplasia tissue; b. Low E-cadherin expression in the cell membrane of the prostate cancer tissues. The lower left corner shows several residual normal ductal epithelia with E-cadherin expression. (D) Analysis of E-cadherin gene expression profile from TCGA; red represents the gene expression level of prostate cancer group, and blue represents the gene expression level of the normal group. (E) The expression of β-catenin protein was detected by immunohistochemistry in different tissues (Elivison ×200). a. The expression of β-catenin in alveolar epithelium of benign prostatic hyperplasia was high in the cell membrane (Elivison ×400); b. The expression of β-catenin in the cell membrane of prostate cancer was significantly decreased, and the expression of β-catenin in the cytoplasm was positive (Elivison ×400); c. The expression of β-catenin in the cell membrane of prostate cancer was significantly decreased, the expression of β-catenin in the cytoplasm was positive, and a few nuclei were positive (Elivison ×400). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 1**
Expression difference of SARI, E-cadherin and β-catenin between prostate cancer and benign prostatic hyperplasia tissues.

| | Prostate cancer ($n$ = 67) | Benign prostatic hyperplasia ($n$ = 20) | $\chi^2$ | $P$ value |
|---|---|---|---|---|
| SARI high expression | 27 (40.3%) | 15 (81.5%) | 7.428 | 0.006 |
| E-cadherin high expression | 49(73.13%) | 20(100%) | 6.775 | 0.009 |
| β-Catenin ectopic expression | 45(67.2%) | 6(30%) | 8.770 | 0.003 |

**Table 2**
Expression difference of E-cadherin and β-catenin between high and low SARI expression subgroups in prostate cancer tissues.

| | Low SARI ($n$ = 40) | High SARI ($n$ = 27) |
|---|---|---|
| E-cadherin | | |
| Low expression | 15 (37.5%) | 3 (11.1%) |
| High expression | 25 (62.5%) | 24 (88.9%) |
| β-catenin | | |
| Cell membrane | 17 (42.5%) | 5 (18.5%) |
| Ectopic expression | 23 (57.5%) | 22 (81.5%) |

$P = 0.003$), as shown in Table 1. The IHC results revealed the expression of SARI and β-catenin in prostate cancer tissues, as showed in Table 2. This study revealed a negative correlation between SARI and β-catenin expression in the prostate cancer tissues ($r = -0.251$, $P = 0.040$).

### 3.4. Correlation between SARI protein expression and the clinicopathological parameters of prostate cancer

Correlation analysis showed SARI protein expression in the prostate cancer tissues to be unassociated with patients' age ($P = 0.457$) and serum PSA ($P = 0.583$), and to be associated with clinical staging ($r = -0.311$, $P = 0.010$) and the Gleason score of the prostate cancer ($r = -0.244$, $P = 0.047$; Table 3).

**Table 3**
Comparison of SARI protein expression with clinicopathological parameters of prostate cancer (n = 67).

| Clinicopathological parameters | | Low expression(n) | High expression(n) | High expression rate | P value |
|---|---|---|---|---|---|
| Age | < 60 | 6 | 6 | 50.0% | 0.457 |
| | ≥60 | 34 | 21 | 38.2% | |
| PSA (ng/mL) | < 4 | 6 | 4 | 40.0% | 0.583 |
| | 4–10 | 9 | 4 | 30.8% | |
| | > 10 | 25 | 19 | 43.2% | |
| Clinical stages | T1 | 3 | 7 | 70.0% | 0.010 |
| | T2 | 5 | 7 | 58.3% | |
| | T3 | 21 | 9 | 30.0% | |
| | T4 | 11 | 4 | 26.7% | |
| Gleason score | 2–6 | 3 | 6 | 66.7% | 0.047 |
| | 7 | 17 | 13 | 43.3% | |
| | 8–10 | 20 | 8 | 28.6% | |

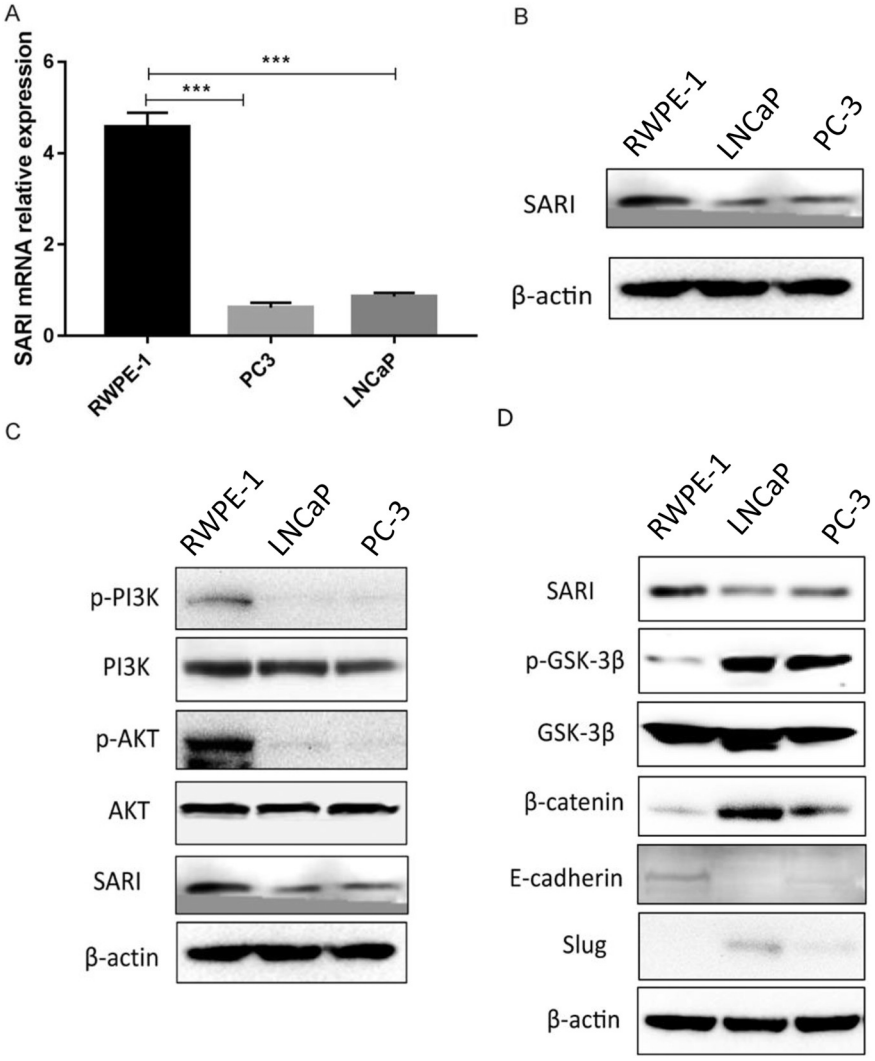


**Fig. 2.** (A) SARI mRNA expression in different prostate epithelial cell lines. (B) SARI protein expression in different prostate epithelial cell lines. (C) Verification of differential molecules by Western blot analysis. The expression of PI3K, p-PI3K, Akt, p-Akt and SARI genes in RWPE-1, PC3, and LNCaP cells. (D) Verification of differential molecules by Western blot analysis. The expression of GSK-3β, p-GSK-3β, β-catenin, E-cadherin, slug, and SARI genes in RWPE-1, PC3, and LNCaP cells by Western blot analysis.

### *3.5. SARI mRNA expression in different prostate epithelial cell lines*

SARI mRNA expression in the normal prostate epithelial line (RWPE-1) was significantly higher than in the prostate cancer cell lines (PC3 and LNCaP), as shown in Fig. 2A. Analysis of variance was used to compare the differences between the normal and the prostate cancer cell lines ($P < 0.05$).

### *3.6. SARI protein expression in different prostate epithelial cell lines*

In the Western blot analysis, protein bands at a molecular weight of 26 kDa were observed, indicating SARI protein expression in different prostate epithelial cell lines. However, the SARI protein quantities varied in different cell lines. SARI protein expression in RWPE-1, PC3, and LNCaP was 0.84 ± 0.11, 0.18 ± 0.05, and 0.30 ± 0.05, respectively. SARI protein expression in the normal prostate epithelial cell line (RWPE-1) was significantly higher in the prostate cancer cell lines (Figs. 2B and S1A). Grayscale analysis was performed using compass software of a fully automated protein blot quantitative analysis system (ProteinSimple, CA, USA) to measure the SARI protein levels in different bands of the protein blots. Significant differences in SARI protein expression were observed between the prostate cancer cell lines and the normal prostate epithelial RWEP-1 cell line ($P < 0.05$).

### *3.7. The PI3K/AKT signaling pathway is regulated by SARI*

The study found that the PI3K/AKT signaling pathway is regulated by SARI. Subsequently, we examined the expression of SARI and the levels of PI3K/AKT signal molecules at the above three cell lines. Western blot analysis showed that the protein levels of SARI in RWEP-1 cells were significantly higher than in the LNCaP and PC-3 cells, while the levels of p-PI3K and p-AKT were lower, indicating that the expression of SARI is downregulated in prostate cancer cells and thus leads to the activation of the PI3K/AKT signaling pathway, which is an important factor in the development of prostate cancer (Figs. 2C and S1B).

### *3.8. SARI inhibits GSK-3β/β-catenin/EMT signaling in prostate cancer cell lines*

To determine whether expression of SARI affects the expression levels of GSK-3β, p-GSK-3β, β-catenin, Slug, and E-cadherin, we used Western blot to detect the above molecules in the prostate normal epithelial cell line (RWPE-1) and prostate cancer cells (LNCaP, PC-3).

The results showed that SARI protein expression in RWPE-1 was higher than in the prostate cancer cell lines. In the prostate cancer cell lines, the expression level of the epithelial cell marker protein E-cadherin protein was significantly lower than in RWPE-1, and the levels of p-GSK-3β, β-catenin, and interstitial cell marker protein Slug were significantly higher than in RWPE-1. These results suggest that the low expression of SARI in prostate cancer cell lines leads to a decrease in the inhibition of the downstream GSK-3β/β-catenin-mediated EMT signaling pathway, which induces the development of prostate cancer (Figs. 2D and S1C).

### 3.9. Effect of the overexpression of SARI in PC3 cells

To examine the effect of SARI overexpression on the biological behavior of PC3 cells, both mRNA and protein of SARI were successfully overexpressed in PC3 cells. qRT-PCR and Western blot analysis confirmed that the levels of SARI mRNA (Fig. 3A) and protein (Fig. 3B and Fig. S1D) were significantly upregulated in the PC3 cells transfected with SARI-GV358 when compared with the levels in the cells transfected with GV358 (the control group) ($P < 0.05$).

### 3.10. Role of the overexpression of SARI in PC3 cells

To indicate the role of SARI in PC3 proliferation, migration, and invasion, two different groups of cells were examined by CCK8 and Transwell assays. The results revealed that upregulation of SARI expression significantly inhibited the proliferation (Fig. 3C). Transwell assay results are displayed in Fig. 3D, upregulation of SARI expression significantly inhibited the migration (Fig. 3E) and invasion (Fig. 3F) of the PC3 cells. Compared with the control group, the difference was statistically significant ($P < 0.05$).

## 4. Discussion

SARI expression was found to be downregulated in various tumors, including hepatocellular carcinoma, oral squamous cell carcinoma, colorectal cancer tissue, and the corresponding cancer cells. The low SARI expression in the tumor tissues is positively correlated with the degree of tumor differentiation [20–22]. Low SARI expression in pediatric medulloblastoma and non-small cell lung cancer is positively correlated with the tumor prognosis [23]. In addition, negative and downregulated SARI expression are positively correlated with poor tumor differentiation and lymph node metastasis [24]. To study the correlation between SARI expression and development of prostate cancer, we collected 67 prostate cancer tissues and 20 benign prostatic hyperplasia specimens and used IHC to determine the SARI protein expression in different groups. Our results showed that the SARI protein expression in the prostate cancer tissues is significantly lower than in

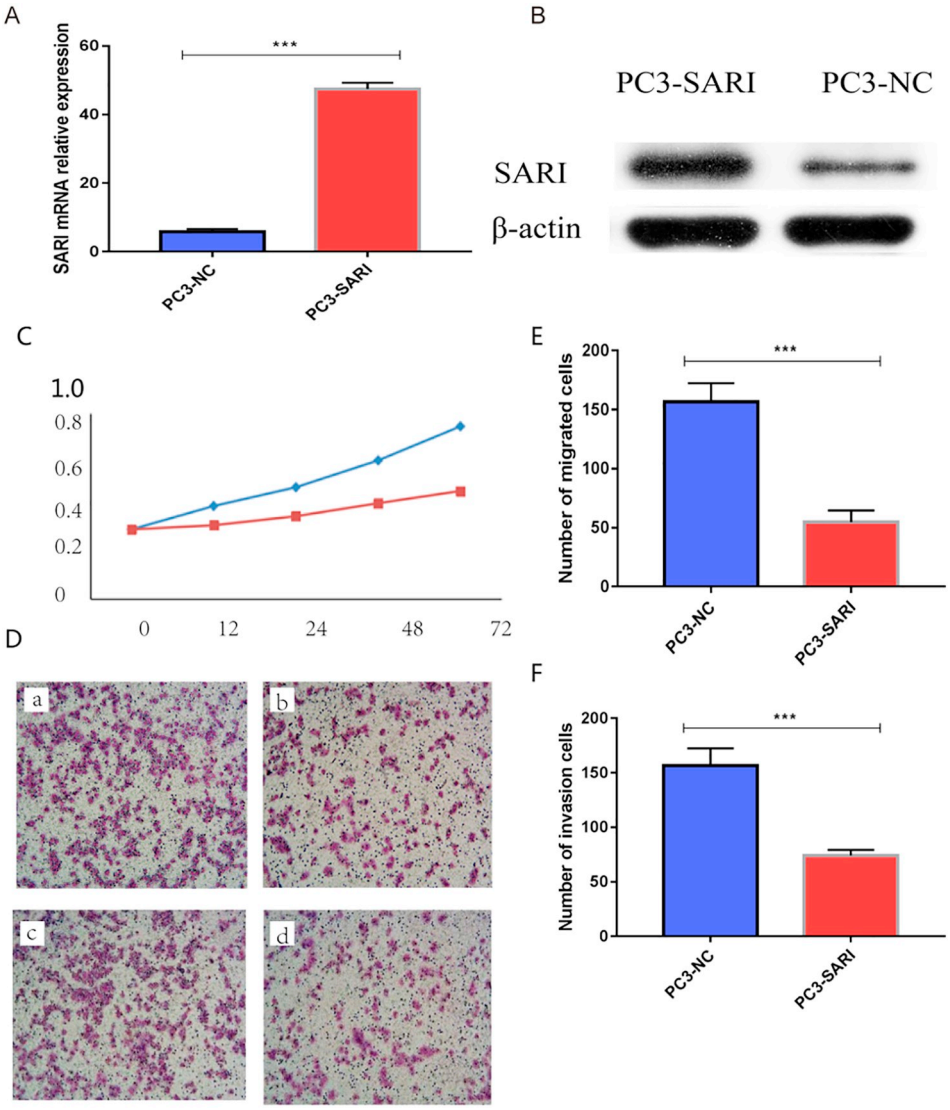


**Fig. 3.** (A) The expression of SARI mRNA. $^{***}P < 0.001$ (B) The expression of the SARI protein was significantly upregulated in the PC3 cells transfected with SARI-GV358 when compared with the levels in the cells transfected with GV358 (the control group). (C) The Cell Counting Kit-8 assay examined the proliferation of PC3/SARI cells and PC3/NC. The results demonstrated that overexpression of SARI can inhibit cell proliferation. Blue represents the PC3/NC, red represents the PC3/SARI. (D) The results show migration and invasion of PC3/SARI cells and PC3/NC in Transwell (×100). a represents migration of the PC3/NC; b represents the migration of the PC3/SARI; c represents invasion of the PC3/NC; d represents the invasion of the PC3/SARI. (E) Transwell assay of PC3/SARI and PC3/NC cell migration assay results. The results demonstrated that SARI could inhibit the migration ability of PC3 cells. The difference was statistically significant between the PC3/SARI and PC3/NC groups. (F) Transwell assay of PC3/SARI and PC3/NC cell invasion assay results. The results demonstrated that SARI could inhibit the invasion ability of PC3 cells. The difference was statistically significant between the PC3/SARI and PC3/NC groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

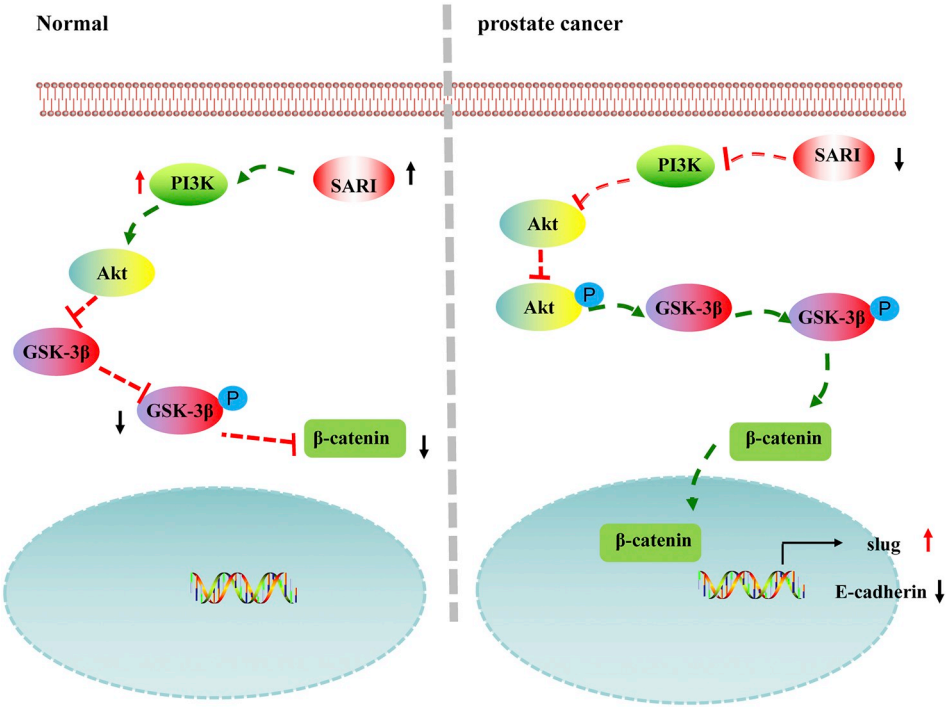


**Fig. 4.** SARI regulates the molecular mechanisms involved in the AKT/PI3K signaling pathway.

the benign prostatic hyperplasia specimens, suggesting that SARI expression was correlated with the development of prostate cancer.

We then used the prostate Gleason score of the 2016 WHO Classification of Tumors of the Urinary System and Male Genital Organs to review the 67 prostate cancer specimens and further analyzed SARI expression in different groups to evaluate its correlation with different clinicopathological parameters. The results showed that SARI expression had no correlation with the age or serum PSA value of the prostate cancer patients; however, SARI expression was found to be correlated with the clinical staging and Gleason score of prostate cancer, which further indicated that downregulated SARI expression was closely related to the malignant biological behavior and tumor progress of prostate cancer.

RWPE-1, LNCaP, and PC3 cell lines have been commonly used in in vitro studies of prostate cancer. RWPE-1 is a normal prostate-derived epithelial cell line. LNCaP contains endogenous androgen receptors, representing hormone-dependent prostate cancer cells. PC3 cells are derived from bone metastasis of human prostate cancer. The PC3 cell line is poorly differentiated and the cells do not contain the endogenous androgen receptors, making them androgen-independent prostate cancer cells [25]. To understand the SARI expression in different types of prostate epithelial cells in a more comprehensive way, in this study we used qPCR and Western blotting to detect SARI mRNA and protein expression in different prostate epithelial cell lines. The results showed that the normal prostate epithelial cell line (RWPE-1) had significantly higher SARI mRNA and protein expression than the prostate cancer cell lines (LNCaP and PC3). These results suggested that SARI mRNA and protein expression were closely associated with the development of prostate cancer.

EMT is a biological process of epithelial cells losing normal polarity and intercellular tight junction, and differentiating into mesenchymal cells, which is closely related to tumor cell invasion and metastasis [26,27]. E-cadherin and β-catenin are important markers of EMT. E-cadherin promotes the adhesion between epithelial cells and helps to maintain the normal polarity and morphological structure of epithelial cells [28]. Reduced E-cadherin expression suggests poor intercellular adhesion. In addition, E-cadherin expression is negatively correlated with the local invasion, distant metastasis, and disease progression of cancer [29,30]. This study used IHC to detect E-cadherin expression of 67 prostate cancer tissues and 20 benign prostatic hyperplasia specimens. Our results showed downregulated E-cadherin expression in the prostate cancer tissues to be significantly different from the E-cadherin expression in the benign prostatic hyperplasia specimens. β-Catenin is a multifunctional protein with a central role in physiological homeostasis. Its aberrant high expression leads to various diseases, including cancer. β-Catenin is also a key marker of EMT, mainly regulated by GSK-3β [31,32]. In the present study, we found that the expression level of β-catenin is higher in prostate cancer cells than in the normal prostate epithelial cells. These results showed that prostate cancer is accompanied by EMT occurrence. Next, we found that the SARI enhanced the activity of PI3K/AKT signaling pathway, while the up-regulation of SARI promoted the E-cadherin expression and inhibited the expression of GSK-3β, p-GSK-3β, and β-catenin. Our findings are consistent with reported results [11–15]. Further statistical analysis showed that SARI expression of the prostate cancer tissue to be positively correlated with the E-cadherin expression. This result further confirms the relationship between SARI and prostate cancer EMT (Fig. 4). In addition, after overexpression of SARI in PC3 cells, Transwell assay confirmed that the migration and invasion abilities of these cells were significantly decreased, further proved that SARI has the function of tumor suppressor, which is consistent with the report of SARI in other tumors [6,7].

In summary, SARI expression is downregulated in prostate cancer tissues and cells, and the high SARI expression is negatively correlated with the development and progression of prostate cancer.

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## Compliance with ethical standards

### *Conflict of interest*

The authors declare that they have no conflict of interest.

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*Ethical approval*

All procedures performed in studies involving human participants were in accordance with the ethical standards of The Third Xiangya Hospital research committee.

*Informed consent*

Informed consent was obtained from all individual participants included in the study.



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