European Review for Medical and Pharmacological Sciences 2018; 22: 8609-8615

FOSL1 enhances growth and metastasis of human prostate cancer cells through epithelial mesenchymal transition pathway

Y.-Z. LUO, P. HE, M.-X. QIU

Department of Urology, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, Chengdu, China

Abstract. – OBJECTIVE: To investigate the effect of highly-expressed FOSL1 on the tumorigenesis and metastasis of prostate cancer.

PATIENTS AND METHODS: Researches were carried out in human prostate cancer tissues and cell lines. In prostate cancer tissues, the expression of FOSL was detected by immunohistochemistry. *In vitro* cell line experiments, we constructed a prostate cancer cell model with FOSL1 stable knockdown and tested cell proliferation and metastasis before and after knockdown of FOSL1. Finally, the epithelial-mesenchymal transition (EMT) markers before and after interference of FOSL1 were also analyzed.

RESULTS: FOSL1 was confirmed to have a high expression in prostate cancer. Transwell experiments demonstrated that FOSL1 could enhance prostate cancer metastasis, while in vivo experiments revealed an accelerated progression of prostate cancer caused by FOSL1. In addition, Western blot analysis revealed an elevated level of N-cadherin and Snail1 and a reduced level of E-cadherin that was induced by FOSL1.

CONCLUSIONS: FOSL1 can promote the occurrence and progression of prostate cancer by altering the EMT process of cells.

Key Words:
FOSL1, Metastasis, Prostate cancer, EMT.

Introduction

Prostate cancer is one of the most common malignancies in men. In Europe and the United States, its incidence rate has been the highest among male malignant tumors for many years, and the mortality rate ranks second[1]. Although the prognosis of early prostate cancer is desirable, most patients are diagnosed with advanced cancer when they visit physicians, and the 5-year survival rate is only about 30%[2,3]. There are many emerging molecular methods for the diagnosis and treatment of prostate cancer, but clinical trials to date have not found superior efficacy over traditional methods.

Epithelial-mesenchymal transition (EMT) is an important pathological aspect in which tumor cells acquire exercise capacity and exhibit invasive growth characteristics. In the process of EMT, epithelial cell phenotypes are transformed into mesenchymal phenotypes, and tight junctions and adhesions between cells are weakened or even lost, and cells transformed into mesenchymal phenotypes acquire strong invasive properties[4]. E-cadherin is a marker molecule of epithelial phenotype cells that can promote cell-cell interactions, forms tight junctions, maintains cell-to-cell polarity and inhibits cell migration[5]. N-cadherin, Vimentin and Snail1 are markers of mesenchymal phenotype cells, which can promote the migration and movement of cells to adjacent tissues and be beneficial to the invasive growth of cells[6]. The FOSL1 gene encodes a protein in the human body as Fos-related antigen 1 (FAR1). FOSL1 has been shown to have multiple functions; for example, it plays a vital role as a transcriptional target of the c-Fos gene during osteoclast differentiation[7]. In tumors, patients with high levels of FOSL1 gene in lung cancer and pancreatic cancer have a poor prognosis[8,9]. FOSL1 is also essential in the pathogenesis of malignant glioma[10]. Previous studies have found that FOSL1 is closely associated with the malignancy of prostate carcinoma and the ability of cells to proliferate and invade[11]. However, the related molecular mechanisms underlying it still remain elusive. In this study, we first analyzed FOSL1 level in prostate tumor tissues and cells, and then further elucidated the function of FOSL1 in tumor cells and the mechanism of promoting EMT, thus providing a new molecular basis for the pathogenesis of prostate carcinoma.

Corresponding Author: Peng He, MD; e-mail: 1047594120@qq.com

Patients and Methods

Cell Lines and Reagents

The prostate cancer cell lines including PC3, DU145, LNCaP, VCaP and C4-2 were all from American Type Culture Collection (ATCC, Manassas, VA, USA). They were cultured in Dulbecco's modified eagle medium (DMEM, HyClone, South Logan, UT, USA) or Roswell Park Memorial Institute-1640 (RPMI-1640, HyClone, South Logan, UT, USA) in 10% fetal calf serum (FCS) and grown at 37°C, 5% CO_2 incubator. Antibodies included FOSL1, E-cadherin, N-cadherin, Snail1, and β-actin.

Tumor Sample

All tests in clinical samples were approved by the Ethics Committee of Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital. A total of 46 primary prostate cancer specimens were surgically removed, including the corresponding adjacent tissues. All tissue samples were fixed in 4% paraformaldehyde and embedded in paraffin for immunohistochemistry.

Immunohistochemistry

All paraformaldehyde-fixed prostate cancer tissue samples were embedded in paraffin and sectioned. The sections were deparaffinized in xylene for 20 minutes and then the endogenous peroxidase was quenched with 3% hydrogen peroxide. Slides were rehydrated with ethanol and antigen retrieval was performed in sodium citrate buffer. The tissue was blocked with blocking solution for 5 minutes at room temperature. After that, the sample was incubated with the FOSL1 antibody for 1 hour at room temperature. Then, it was incubated with HRP (horseradish peroxidase) secondary antibody; diaminobenzidine (DAB, Boster, Wuhan, China) dye was developed, and hematoxylin was counterstained. Semi-quantitative methods were used to analyze the staining results, including staining intensity (0 negative, 1-low, 2-moderate, 3-strong) and percentage of stained cells (0-0%, 1-1-25%), 2-25-50%, 3-50-100%), which were taken as the score of the pathological tissue.

shRNA

The shRNA sequence of FOSL1 is 5'-CCGGCCTCAGCTCATCGCAAGAGTACTCGAGTACTCTTGCGATGAGCTGAGGTTTTT-3'. The FOSL1-shRNA and NC-shRNA were individually packaged with lentivirus, then PC3, LNCaP cells were infected and a stable knockdown cell line was obtained by puromycin screening.

Cell Migration and Invasion Analysis

The cells were cultured with 600 μL of serum-free medium in the upper chamber of the transwell, while 600 μL of complete medium were added to the lower chamber. After cells were incubated at 37°C for 24 hours, the non-invasive cells in the upper chamber were gently removed with a cotton swab. The invading cells on the lower surface of the transwell membrane were stained with 0.1% crystal violet, and the cells were photographed.

Clonal Formation Assay

1×10^3 FOSL1 knockdown cells and control cells were respectively seeded in 12-well plates and cultured in complete medium for 2 weeks. After that, they were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Then we took a picture and counted the number of clones per well.

Xenograft Tumor Experiment

3 x 10^6 stably knockdown FOSL1 and control PC3 cells were injected subcutaneously into BALB/c male nude mice. The size of the tumor was recorded every 3-4 days. After 4 weeks, the nude mice were sacrificed and each tumor was photographed and weighed.

Statistical Analysis

The required statistical analysis was completed using Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA). The *t*-test is used for analysis of the classification data. p value < 0.05 was considered to be significantly different.

Results

High Expression of FOSL1 in Prostate Carcinoma Could Predict Undesirable Prognosis

To elucidate the potential effect of FOSL1 on prostate cancer, we extracted RNA from 20 prostate carcinoma tissues and adjacent tissues to detect the level of FOSL1 mRNA. RT-PCR results indicated that FOSL1 gene level in cancer tissues was strikingly higher than that in adjacent tissues (Figure 1A). Subsequently, we analyzed FOSL1 protein level in 46 pairs of cancer and

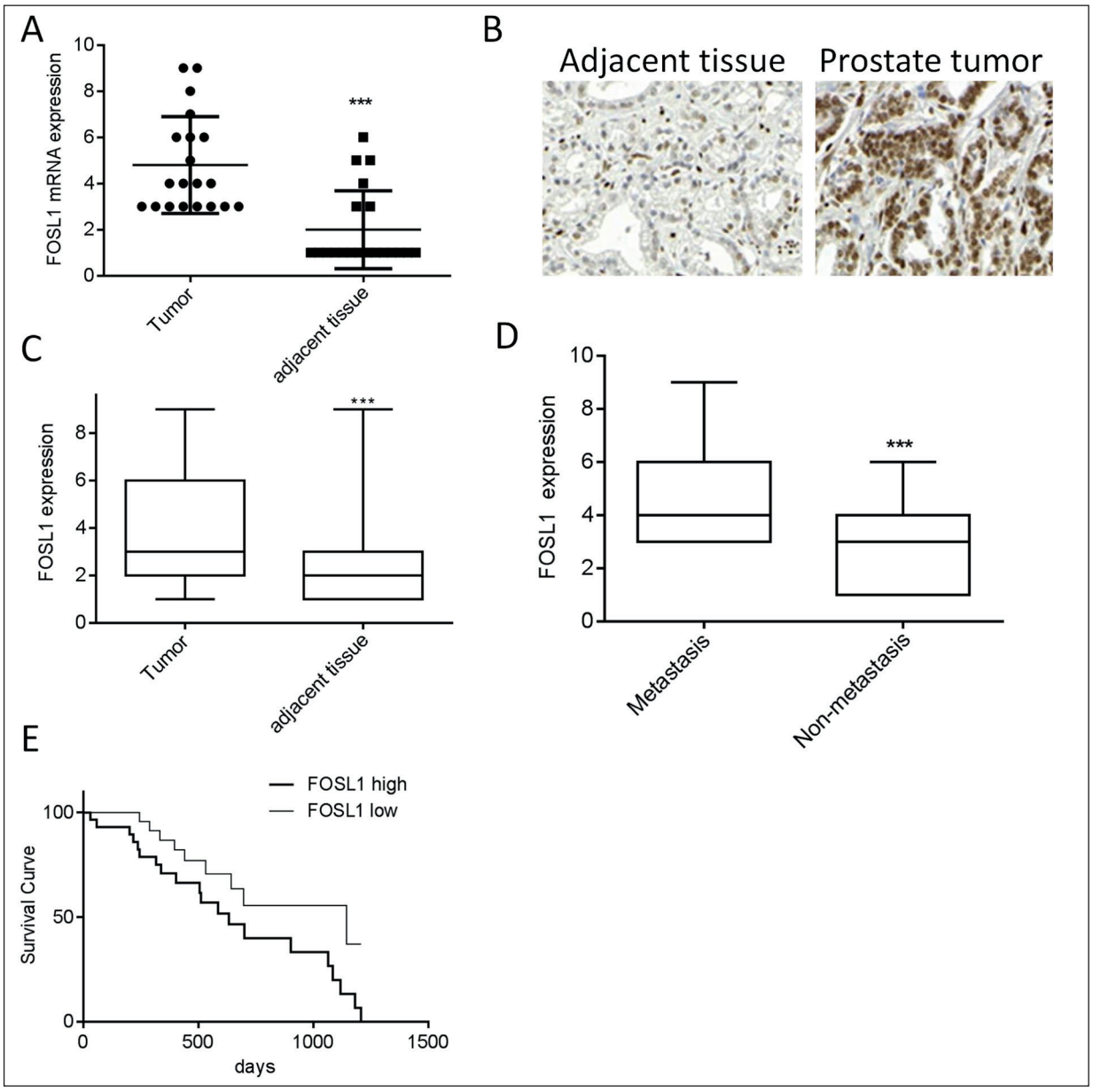

Figure 1. FOSL1 is highly expressed in prostate cancer. ***A,*** mRNA expression of FOSL1 in prostate cancer tissues and adjacent tissues. ***B,*** Low FOSL1 expression in normal paracancerous tissues (negative staining) and high FOSL1 expression in prostate cancer tissues (positive staining). ***C,*** Quantitative statistics of FOSL1 expression in prostate cancer and its adjacent normal tissues. ***D,*** Expression of FOSL1 in metastatic and non-metastatic prostate cancer tissues. ***E,*** Survival curves of patients with low expression of FOSL1 and high expression of FOSL1.

adjacent tissues by immunohistochemistry, and found that FOSL1 had a higher expression in prostate carcinoma tissues (Figure 1B and 1C). In addition, compared with non-metastatic prostate cancer, FOSL1 was found significantly elevated in metastatic one (Figure 1D). Kaplan-Meier analysis revealed that patients with higher FOSL1 expression had a shorter survival time (Figure 1E). These results indicated that FOSL1 might lead to the metastasis and malignant progression of prostate carcinoma, and its high expression could predict a poor prognosis.

High Expression of FOSL1 Promoted Proliferation of Prostate Carcinoma Cells

We examined FOSL1 level in five cell lines including PC3, DU145, LNCaP, VCaP and C4-2 by RT-PCR, and found that FOSL1 had a higher

expression in PC3 and LNCaP cells and relatively lower in other cell lines (Figure 2A). Therefore, we selected PC3 and LNCaP cells as subjects and constructed shRNA knockdown vectors to establish stable FOSL1 knockdown model in PC3 and LNCaP cell lines. FOSL1 protein levels in PC3 and LNCaP cells with FOSL1-shRNA knockdown were detected using Western blot (Figure 2B). The cells count and clone formation assays were then used to elucidate the effect of FOSL1 on proliferation of prostate carcinoma cells. It was found that the proliferation ability of cells in FOSL1-shRNA group was remarkably reduced compared to the NC group (Figure 2C and 2D). In

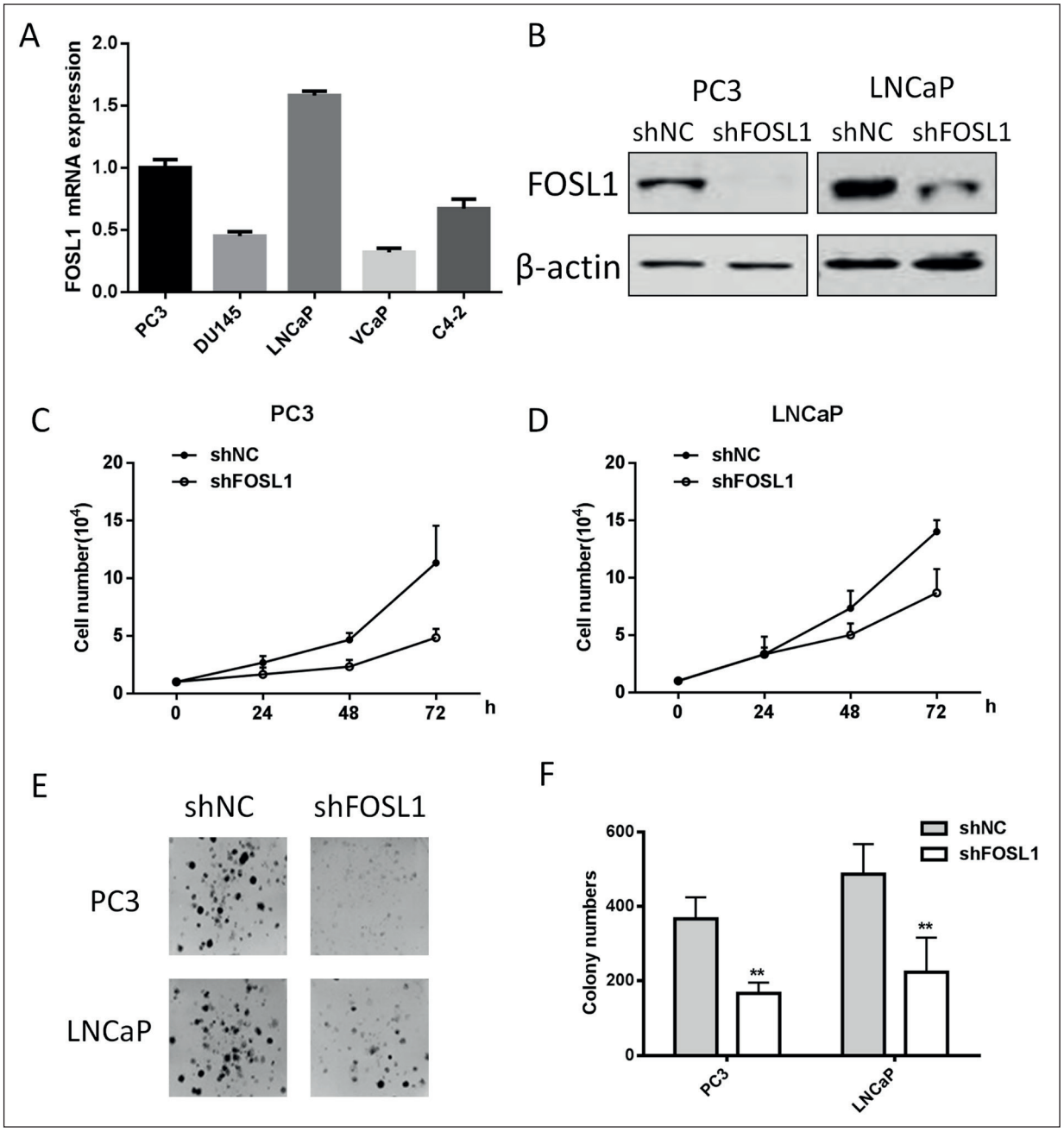

Figure 2. FOSL1 is highly expressed in prostate cancer cell lines and promotes prostate cancer cell proliferation. ***A,*** mRNA expression of 5 prostate cancer cell lines was detected by RT-PCR. ***B,*** FOSL1 was knocked down by shRNA in PC3 and LNCaP cells, and knockdown efficiency was detected by Western blot. ***C, D,*** Cell proliferation curve after FOSL1 shRNA knockdown in PC3 and LNCaP cells. ***E,*** Cloning of PC3 and LNCaP cells transfected with shNC and shFOSL1. ***F,*** Clone number statistics.

the clone formation assay, the number of clones in PC3 or LNCaP cells was significantly reduced when shRNA was knocked down by shRNA compared to NC-shRNA (Figure 2E and 2F). Therefore, we believed that knockdown of FOSL1 inhibited prostate carcinoma cell proliferation.

Promotion Effect of FOSL1 on Prostate Carcinogenesis in vivo

To figure out the role of FOSL1 in the development of prostate carcinoma *in vivo*, we used a xenograft tumor model for tumorigenesis experiments. PC3 cells stably expressing FOSL1-shRNA and shNC were subcutaneously injected into nude mice. The results indicated that the FOSL1-shRNA group markedly slowed tumorigenesis of prostate cancer cells compared to the control group (Figure 3A). In addition, the tumor size (Figure 3B) and weight (Figure 3C) formed by FOSL1-shRNA cells were also significantly smaller than the shNC group. Therefore, the role of FOSL1 in promoting prostate carcinoma *in vivo* was confirmed.

FOSL1 Accelerated Migration and Invasion of Prostate Carcinoma Cells by Regulating EMT

In the transwell assay, the invasive (Figure 4A and 4B) and migratory (Figure 4C) abilities of PC3 or LNCaP cells transfected with FOSL1 shRNA were remarkably reduced compared to the control cells, which was consistent with the analysis of data from patients with metastatic prostate cancer in Figure 1D. Since activation of EMT is common in prostate carcinoma and EMT is closely related to tumorigenesis and metastasis, we examined the potential relationship between FOSL1 expression and EMT activation by Western blot. Results revealed that FOSL1 knockdown in PC3 or LNCaP cells inhibited the expression of N-cadherin and Snail1 and promoted the expression of E-cadherin (Figure 4D). Therefore, we concluded that FOSL1 could accelerate the process of EMT of prostate tumor cells, which might be the reason for its promotion effect on cell proliferation and metastasis of prostate cancer.

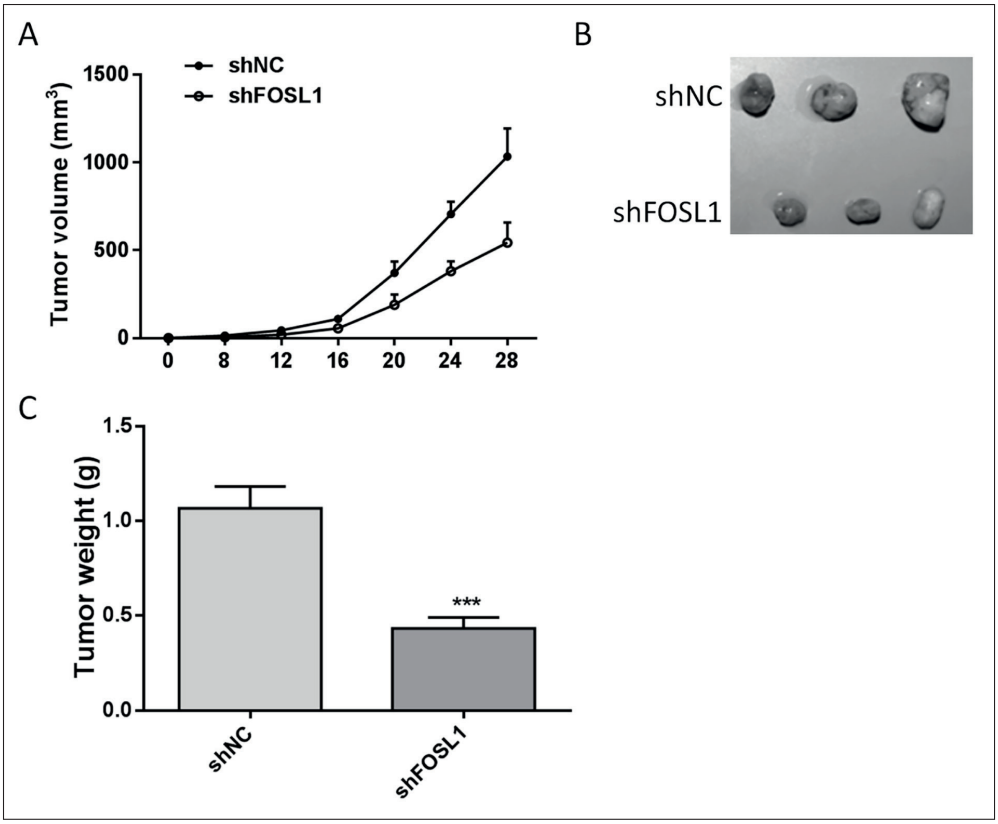

Figure 3. FOSL1 promotes prostate cancer tumorigenesis *in vivo*. ***A,*** After injecting PC3 cells transfected with shNC or shFOSL1 in nude mice, the tumor volume was meatured in tumor-bearing nude mice within 4 weeks. ***B,*** Tumors were collected four weeks later. ***C,*** Statistics of tumor weight after 4 weeks were analyzed.

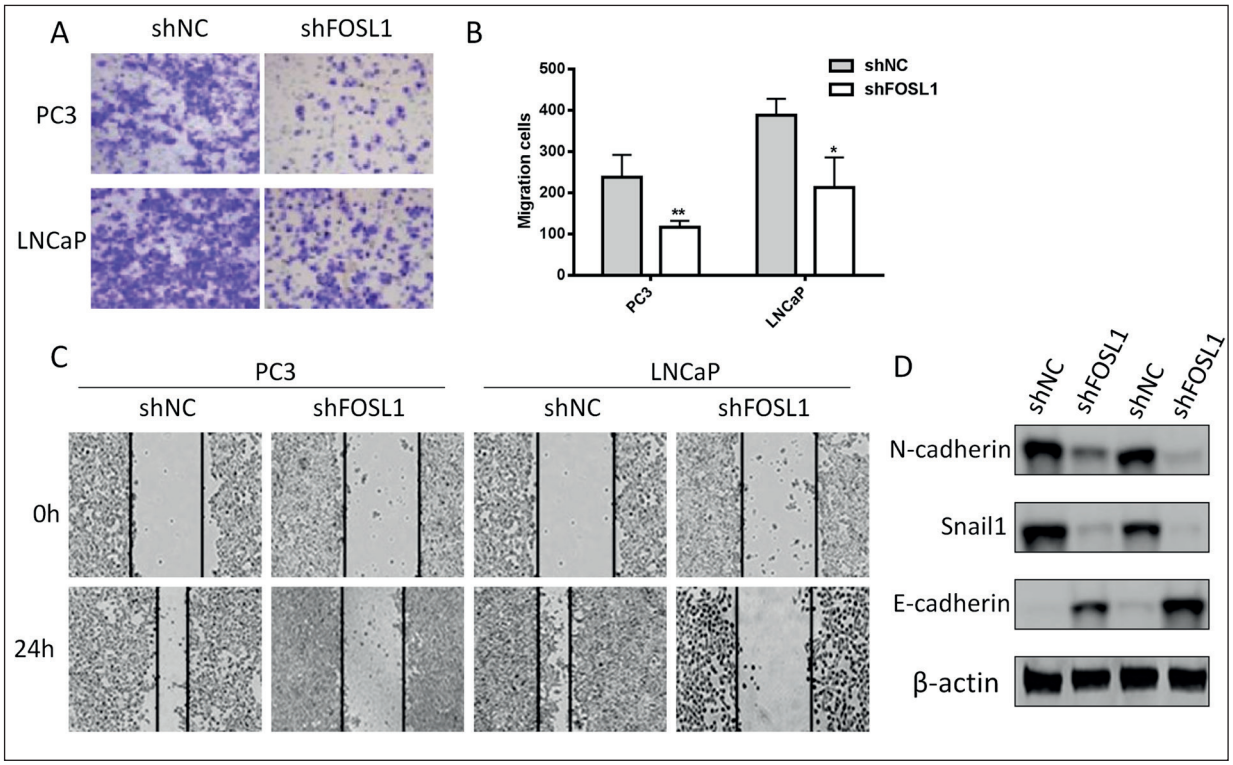

Figure 4. FOSL1 alters EMT to promote prostate cancer cell migration and invasion. ***A,*** PC3 and LNCaP cells transfected with shNC or shFOSL1 were seeded into transwell and the invading cells were photographed. ***B,*** Statistical chart of the number of invading cells was shown. ***C,*** In scratch experiments of PC3 and LNCaP cells after transfecting with shNC and shFOSL1, the cell migration status was observed after 24 h. ***D,*** Expression of EMT markers were detected in PC3 (two lanes on the left) and LNCaP (two lanes on the right) transfected with shNC or shFOSL1.

Discussion

FOSL1 belongs to the FOS family and is one of the subunits of the transcription factor AP-1. The protein encoded by the FOSL1 gene is FRA-1, and the leucine zipper structure region of the protein can form a heterodimer with the Jun family protein, thereby exerting a regulation function on downstream molecules[12,13]. Researchers[14] have shown that in addition to promoting tumor proliferation and metastasis, FOSL1 is also associated with tumor chemotherapy tolerance. In MCF7 cells, silencing FOSL1 will inhibit cell growth while increasing the sensitivity of this cell to toremifene. Studies[15] have reported that FOSL1 in breast cancer tissue is positively correlated with chemosensitivity. Down-regulation of FOSL1 can increase the chemotherapy tolerance of breast carcinoma cells to doxorubicin, while enhancing FOSL1 expression may make cells sensitive to chemotherapy. Although investigations on the function of FOSL1 in tumors are relatively scattered, it can be reflected that FOSL1 as an important molecule should be involved in tumor development and drug resistance. In our research, we found a differential expression of FOSL1 between prostate cancer and normal tissues, and demonstrated that FOSL1 might play an oncogene role in the development of prostate cancer. Activation of EMT can promote cell movement and invasion of prostate carcinoma. When EMT occurs, the expression of E-cadherin protein in prostate cancer cells is inhibited and the cell-to-cell junction is lost. At the same time, the phenotype of mesenchymal cells displayed, including up-regulation of N-cadherin and Snail[16]. Snail1 can be regulated by PPAR transcription and is highly expressed in prostate cancer. By regulating the expression of a series of genes such as PLC and twist, the effect of EMT on prostate cancer is achieved[17]. The occurrence of EMT can up-regulate the mRNA and protein expression of matrix metalloproteinase MMP-9, degrade the extracellular matrix, and promote the metastasis of prostate cancer cells[18]. Our experimental data indicated that FOSL1 activated EMT, which not only promoted the proliferation of pros-

tate tumor cells, but also participated in the cells invasion and metastasis by regulating N-cadherin or E-cadherin. Therefore, FOSL1 can serve as a phenotypic regulatory target for prostate cancer and provide an effective molecular direction for the diagnosis and treatment of prostate carcinoma.

Conclusions

This study explored the role and possible mechanism of FOSL1 in the development of prostate carcinoma. We found that FOSL1 could activate the EMT pathway to further accelerate the growth and metastasis of prostate tumor cells, thus providing a new target for the diagnosis and treatment of this cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Madalinska JB, Essink-Bot ML, de Koning HJ, Kirkels WJ, van der Maas PJ, Schroder FH. Health-related quality of life in patients with screen-detected versus clinically diagnosed prostate cancer preceding primary treatment. Prostate 2001; 46: 87-97.
2) Zhiping C, Shijun T, Linhui W, Yapei W, Lianxi Q, Qiang D. MiR-181a promotes epithelial to mesenchymal transition of prostate cancer cells by targeting TGIF2. Eur Rev Med Pharmacol Sci 2017; 21: 4835-4843.
3) Petrylak DP, Tangen CM, Hussain MH, Lara PJ, Jones JA, Taplin ME, Burch PA, Berry D, Moinpour C, Kohli M, Benson MC, Small EJ, Raghavan D, Crawford ED. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. N Engl J Med 2004; 351: 1513-1520.
4) Byles V, Zhu L, Lovaas JD, Chmilewski LK, Wang J, Faller DV, Dai Y. SIRT1 induces EMT by cooperating with EMT transcription factors and enhances prostate cancer cell migration and metastasis. Oncogene 2012; 31: 4619-4629.
5) Zhau HE, Odero-Marah V, Lue HW, Nomura T, Wang R, Chu G, Liu ZR, Zhou BP, Huang WC, Chung LW. Epithelial to mesenchymal transition (EMT) in human prostate cancer: lessons learned from ARCaP model. Clin Exp Metastasis 2008; 25: 601-610.
6) Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 2009; 28: 15-33.
7) Matsuo K, Owens JM, Tonko M, Elliott C, Chambers TJ, Wagner EF. Fosl1 is a transcriptional target of c-Fos during osteoclast differentiation. Nat Genet 2000; 24: 184-187.
8) Vallejo A, Perurena N, Guruceaga E, Mazur PK, Martinez-Canarias S, Zandueta C, Valencia K, Arricibita A, Gwinn D, Sayles LC, Chuang CH, Guembe L, Bailey P, Chang DK, Biankin A, Ponz-Sarvise M, Andersen JB, Khatri P, Bozec A, Sweet-Cordero EA, Sage J, Lecanda F, Vicent S. An integrative approach unveils FOSL1 as an oncogene vulnerability in KRAS-driven lung and pancreatic cancer. Nat Commun 2017; 8: 14294.
9) Vallejo A, Valencia K, Vicent S. All for one and FOSL1 for all: FOSL1 at the crossroads of lung and pancreatic cancer driven by mutant KRAS. Mol Cell Oncol 2017; 4: e1314239.
10) Debinski W, Gibo DM. Fos-related antigen 1 modulates malignant features of glioma cells. Mol Cancer Res 2005; 3: 237-249.
11) Zhang K, Myllymaki SM, Gao P, Devarajan R, Kytola V, Nykter M, Wei GH, Manninen A. Oncogenic K-Ras upregulates ITGA6 expression via FOSL1 to induce anoikis resistance and synergizes with alphaV-Class integrins to promote EMT. Oncogene 2017; 36: 5681-5694.
12) Wenzel A, Iseli HP, Fleischmann A, Hafezi F, Grimm C, Wagner EF, Reme CE. Fra-1 substitutes for c-Fos in AP-1-mediated signal transduction in retinal apoptosis. J Neurochem 2002; 80: 1089-1094.
13) Galvagni F, Orlandini M, Oliviero S. Role of the AP-1 transcription factor FOSL1 in endothelial cells adhesion and migration. Cell Adh Migr 2013; 7: 408-411.
14) Pennanen PT, Sarvilinna NS, Toimela T, Ylikomi TJ. Inhibition of FOSL1 overexpression in antiestrogen-resistant MCF-7 cells decreases cell growth and increases vacuolization and cell death. Steroids 2011; 76: 1063-1068.
15) Chen X, Zhao M, Huang J, Li Y, Wang S, Harrington CA, Qian DZ, Sun XX, Dai MS. microRNA-130a suppresses breast cancer cell migration and invasion by targeting FOSL1 and upregulating ZO-1. J Cell Biochem 2018; 119: 4945-4956.
16) Sethi S, Macoska J, Chen W, Sarkar FH. Molecular signature of epithelial-mesenchymal transition (EMT) in human prostate cancer bone metastasis. Am J Transl Res 2010; 3: 90-99.
17) Lin SJ, Yang DR, Li G, Chang C. TR4 nuclear receptor different roles in prostate cancer progression. Front Endocrinol (Lausanne) 2015; 6: 78.
18) Chen CM, Lin CL, Chiou HL, Hsieh SC, Lin CL, Cheng CW, Hung CH, Tsai JP, Hsieh YH. Loss of endothelial cell-specific molecule 1 promotes the tumorigenicity and metastasis of prostate cancer cells through regulation of the TIMP-1/MMP-9 expression. Oncotarget 2017; 8: 13886-13897.