

SMC1A* promotes growth and migration of prostate cancer *in vitro* and *in vivo

XIU-WU PAN^{1,2*}, SI-SHUN GAN^{1*}, JIAN-QING YE^{1*}, YING-HUI FAN^{3*}, YI HONG², CHUAN-MIN CHU¹,
YI GAO², LIN LI¹, XI LIU², LU CHEN², YI HUANG², HONG XU², JI-ZHONG REN²,
LEI YIN², FA-JUN QU¹, HAI HUANG¹, XIN-GANG CUI¹ and DAN-FENG XU²

¹Department of Urinary Surgery, Third Affiliated Hospital, Second Military Medical University, Shanghai 201805;

²Department of Urinary Surgery, Changzheng Hospital, Second Military Medical University, Shanghai 200003;

³Department of Anesthesiology, Renji Hospital, Shanghai Jiaotong University, Shanghai 200127, P.R. China

Received July 4, 2016; Accepted August 31, 2016

DOI: 10.3892/ijo.2016.3697

Abstract. Structural maintenance of chromosome 1 alpha (*SMC1A*) gene has been reported to be related to tumor development in some types of human cancers. However, the misregulation of *SMC1A* and its functions in castration-resistant prostate cancer (CRPC) have not been well understood. In the present study, we found that *SMC1A* was elevated in androgen-independent PCa cell lines PC-3 and DU-145 compared to androgen sensitive LNCap and 22RV1 cells by qPCR and western blot assay. Knockdown of *SMC1A* inhibited cell growth, colony formation and cell migration abilities of PC-3 and DU145 cells by MTT, colony formation and Transwell assays, and affected cell cycle progression in PC-3 and DU145 cells by flow cytometry. Moreover, *SMC1A* knockdown significantly reduced tumor growth *in vivo* in a nude mouse model. Additionally, we also found that the expression of *SMC1A* gene was higher in prostate cancer tissues than in the adjacent normal tissues by immunohistochemical staining, and was positively correlated to tumor metastasis and recurrence by Oncomine database mining. Taken together, the present study indicates that *SMC1A* may play an important role in malignant transformation of PCa under conditions of androgen deprivation and act as a new target for PCa diagnosis and treatment.

Introduction

Prostate cancer (PCa) is one of the most common cancers in male urogenital system and is the main risk factor to male health. Huggins *et al* (1) found that surgical castration and estrogen treatment could delay the metastatic PCa progression and first confirmed the reactivity of PCa to androgen deprivation in 1941 (1). Currently, androgen deprivation therapy has become the main treatment method in PCa. Unfortunately, most of PCa usually develop to an advanced stage acquiring castration resistance (CRPC) after 18 months of treatment. Androgen receptor (AR) signaling is a pivotal pathway regulating prostate development and malignant transformation and is therefore an anticancer drug target. Knockdown or suppression of AR signaling has been shown to upregulate certain oncogenic candidates including glucocorticoid receptor, which has been suspected to be a mechanism of CRPC (2,3). Although targeted therapy has been the focus of clinical research, several phase III clinical trials of target drugs such as bevacizumab, sunitinib have been shown to fail in significantly prolonging survival in patients with CRPC (4,5). Thus, the novel molecular targets are urgently needed to improve CRPC patient prognosis.

The structural maintenance of chromosome 1 alpha (*SMC1A*) gene is located in Xp11.22-p11.21, consisting of 25 exons and 24 introns. *SMC1A* gene encodes a core subunit of the cohesin complex, which is essential to sister chromatid cohesion. SMC1, SMC3, SCC1 (also known as MDC1 and RAD21) and SCC3 (also known as SA2 and STAG2) subunits could interact with each other and form a ring-shaped cohesin complex (6-8). As is known, central components of the cohesin and condensin complexes are required for conversion of interphase chromatin into mitotic-like condense chromosomes (9). Structural maintenance of chromosome (SMC) proteins are core component of the cohesin and condensin complex and essential for chromosome condensation during DNA replication and chromatid segregation of the genome in all organisms. They are also involved in checkpoint responses and epigenetic silencing of gene expression (10).

SMC1A gene plays a pivotal role in chromosome function, gene regulation and double-stranded DNA repair. Mutations

Correspondence to: Dr Dan-Feng Xu, Department of Urinary Surgery, Changzheng Hospital, Second Military Medical University, Shanghai 200003, P.R. China
E-mail: danfengxu_urology@163.com

Professor Xin-Gang Cui, Department of Urinary Surgery, Third Affiliated Hospital, Second Military Medical University, Shanghai 201805, P.R. China
E-mail: xingangcui@126.com

*Contributed equally

Key words: prostate cancer, *SMC1A*, cell proliferation, cell migration, nude mice

of *SMC1A* gene may cause the Cornelia de Lange syndrome (CdLS), which is dominantly a developmental disorder with multisystem abnormalities including slow growth before and after birth, characteristic facial features, upper extremity defects, hirsutism, gastroesophageal dysfunction and cognitive retardation (11,12). Eleven different *SMC1A* mutations in 14 unrelated patients have been reported, in which all patients had a mild to moderate CdLS phenotype (13-15).

Genes involving chromosome maintenance and DNA repair have been found to be responsible for the malignant transformation of tumors. Although the study of *SMC1A* focuses mainly on the CdLS, it has been reported that upregulation of *SMC1A* might be related to the development of glioblastoma, colon and lung cancer (16-18). However, the function of *SMC1A* in PCa and its correlation with CRPC has not been studied yet. In the present study, based on a lentiviral shRNA library screening, we identified *SMC1A* as a novel oncogenic candidate. We further performed relevant research to confirm the underlying roles of *SMC1A* in PCa cells, and the expression levels and clinical significance of *SMC1A* in PCa.

Materials and methods

Patient samples and immunohistochemical staining. All of the patient samples for immunohistochemical (IHC) analysis were obtained from the Department of Urinary Surgery, Shanghai Changzheng Hospital, Shanghai, China. This study was approved by the Clinical Research Ethics Committee of Shanghai Changzheng Hospital, and written informed consents were obtained from all the subjects.

For IHC analysis, tissue samples were paraffin-embedded, cut into 5- μ m-thick sections and pasted onto glass slides. After deparaffinizing in xylene and dehydration with graded ethanol washes, the specimens were sequentially incubated with blocking solution for 10 min and 1:100 dilution of anti-*SMC1A* antibody (SAB4300451; Sigma-Aldrich) at 4°C overnight, and stained using UltraSensitive™ SP (mouse/rabbit) IHC kit (KIT9730; Fuzhou Maixin Biotechnology, Co., Ltd., Fuzhou, China) according to the user's manual.

Oncomine database analysis. The clinical significance of *SMC1A* expression in prostate cancer were analyzed using the online Oncomine database (www.oncomine.org) consisting of previously published and publicly available microarray data. Welsh prostate dataset (19) and Singh prostate dataset (20), which have a total of 136 samples, were used to compare the differential expression of *SMC1A* between normal (59 cases) and cancerous tissue (77 cases). Glinsky prostate dataset (21) (79 cancer cases) was used to analyze the correlation of *SMC1A* expression level with cancer biochemical recurrence. Moreover, three independent datasets including Holzbeierlein prostate dataset (22), LaTulippe prostate dataset (23), and Chandran prostate dataset (24), which have a total of 31 cancer tissues, were used to explore the relationship between *SMC1A* expression level and distant metastasis.

Reagents and antibodies. Dulbecco's modified Eagle's medium (DMEM; cat. no.12430-054), F-12 (cat. no. 21127022) and Roswell Park Memorial Institute 1640 (RPMI-1640; cat. no. 11875-093) medium and fetal bovine serum (FBS;

cat. no. 10099-141) were purchased from Gibco (Grand Island, NY, USA). TRIzol reagent was purchased from Invitrogen (Carlsbad, CA, USA). Giemsa was from Chemicon International (Temecula, CA, USA). M-MLV reverse (cat. no. M5301) transcriptase was purchased from Promega (Madison, WI, USA). Oligo-dT₍₁₈₎ was synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Terra™ qPCR Direct SYBR® Premix (638318) was from Takara Bio (Shiga, Japan). Anti-*SMC1A* antibody (SAB4300451) was from Sigma-Aldrich (Munich, Germany). Mouse anti-GAPDH (sc-32233), goat anti-mouse IgG (sc-32233) and goat anti-rabbit IgG (sc-2030) were from Santa Cruz Biotechnology (Dallas, TX, USA). All the other chemicals were of analytical grade from Sangon Biotech.

Cell culture. Human embryonic kidney (HEK) 293T cells and human prostate cancer cell lines PC-3, DU145, LNCap and 22RV1 were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Science (Shanghai, China). 293T cells were cultured in DMEM containing 10% FBS. PC-3 and DU145 cells were maintained in F-12 medium supplemented with 10% FBS, 100 U/ml penicillin and 100 μ g/ml streptomycin. 22RV1 and LNCap cells were incubated with RPMI-1640 supplemented with 10% FBS, 100 U/ml penicillin and 100 μ g/ml streptomycin. All cells were cultured in a humidified incubator at 37°C with 5% CO₂.

RNA interference and recombinant lentivirus transduction. To silence the expression of *SMC1A* in PCa cell lines, the short hairpin (shRNA) sequence identified to target human *SMC1A* gene was 5'-TAGGAGGTTCTTCTGAGTACA-3'. The sequence of the negative control shRNA was 5'-TTCTCCGAACGTGTCACGT-3'. The oligos were annealed and ligated into pFH-L vector (Holly Lab, Shanghai, China) through *NheI*/*PacI* restriction sites to generate pFH-Lv-sh*SMC1A* and pFH-Lv-shCon. Finally, the sequencing was performed to confirm the results of construction.

Lentiviruses were generated by triple transfection with modified pFH-shRNA plasmid and pVSVG-I and pCMVΔR8.92 helper plasmids into HEK-293T cells using Lipofectamine 2000 according to the manufacturer's instructions. The lentiviral particles were then harvested by centrifugation, filtered through a 0.45 μ m filter and then stored at -80°C.

PC-3 or DU145 cells were seeded at the concentration of 5x10⁴ cell/well in 6-well plates. After 24 h of culture, lentivirus containing shRNA targeting *SMC1A* (sh*SMC1A*) or the negative control (shCon) were added at a multiplicity of infection (MOI) of 50 into F-12 basic medium. After 6-h incubation, PC-3 and DU145 cells were cultured in complete medium replacing the basic medium containing the lentivirus. Then, after 5 days post-transfection, the green fluorescent protein (GFP) expression was examined using fluorescent microscopy (Olympus; cat. no.CKX41) to assess the infection efficiency.

Quantitative real-time RT-PCR analysis. The total RNA was extracted using TRIzol reagent according to the manufacturer's instruction and synthesized complementary DNA (cDNA) by using M-MLV reverse transcriptase. Real-time PCR reactions using Terra™ qPCR Direct SYBR® Premix were run on Takara TP800 Thermal Cycler Dice™ real-time system. The

following primers were used: *SMC1A*: 5'-AGCGAAAGGCA GAGATAATGG-3' (forward) and 5'-GGTAGTCAAGAGGC AAGAAGG-3' (reverse); β -actin: 5'-GTGGACATCCGCAA AGAC-3' (forward) and 5'-AAAGGGTGTAACGCAACTA-3' (reverse). Thermal cycling conditions were as follows: initial denaturation 1 min at 95°C, followed by 40 cycles of denaturation for 5 sec at 95°C, extension for 20 sec at 60°C and absorbance value was read at the extension stage. The data were analyzed with Takara Thermal Dice Real-Time system software ver3.0. *SMC1A* relative mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method with normalization to β -actin.

Western blot analysis. Cells were washed twice with ice-cold PBS and lysed in 2X sodium dodecyl sulfate (SDS) sample buffer (2% mercaptoethanol, 20% glycerol, 4% SDS in 100 mM Tris-HCl buffer, pH 6.8), and incubated for 15 min on ice. The supernatants were collected by centrifugation at 12,000 \times g for 15 min at 4°C, and a BCA protein assay kit was used to measure the protein content. Equal amounts of protein samples (30 μ g) were loaded and separated in 10% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes. Whereafter, the membrane was blocked with TBST buffer containing 5% non-fat milk at room temperature for 1 h, and incubated with the primary antibodies in the blocking solution at 4°C overnight. After being washed three times with TBST, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5,000) at room temperature for 1 h. The objective bands were detected by Pierce ECL western blotting detection kit (Thermo Fisher Scientific, Waltham, MA, USA). GAPDH was used as an internal control.

MTT assay. To evaluate the effect of *SMC1A* in the proliferation of prostate cancer cells, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed. Four days after lentivirus infection, both PC-3 and DU145 cells were reseeded in 96-well plates at an ultimate density of 2000 cells/well and the number of active cells was measured for five consecutive days. Subsequently, MTT (10 μ l, 5 mg/ml) was added to each well at a fixed time-point. After incubation at 37°C for 4 h, the acidic isopropanol (150 μ l/well) was added and then incubated at 37°C. Optical density (OD) of each well was measured at 570 nm using an ELx808 Absorbance Reader (BioTek Instruments, Inc., Winooski, VT, USA).

Colony formation assay. To examine the effect of *SMC1A* in the colony formation of a single prostate cancer cell, the colony formation assay was executed. Four days after lentivirus infection, both PC-3 and DU145 cells were reseeded in 6-well plates at a density of 200 cells/well and cultured for 14 days in the humidified incubator at 37°C with 5% CO₂. Culture medium was replaced at 3-day intervals. Then, cells were washed in PBS, fixed in 4% paraformaldehyde for 30 min and stained with Giemsa for 15 min at room temperature. The stained colonies were washed with ddH₂O and air-dried. Finally, the ability of colony formation was observed through a light/fluorescence microscope and the colonies (>50 cells/colony) were counted.

Flow cytometric analysis. Cell cycle distribution was analyzed by propidium iodide (PI) staining. Briefly, both PC-3 and

DU145 cells were reseeded at a density of 1 \times 10⁵ cells/well in 6-cm dishes after lentivirus infection. After the incubation period cells were harvested and fixed in 70% ethanol overnight at 4°C. The next day, cells were washed thrice and resuspended in PBS containing 100 μ g/ml RNase A and 50 μ g/ml PI, and then incubated in the dark at room temperature for 30 min. Cells were analyzed by flow cytometry using a FACSCalibur flow cytometer (Becton-Dickinson, San Jose, CA, USA). The percentage of the cells in sub-G1, G0/G1, S and G2/M phases were analyzed using ModFit software (Verity Software House, Inc., Topsham, ME, USA).

Migration assay. To explore the effect of *SMC1A* in the migration of prostate cancer cells, a 24-well Transwell chamber with 8.0 μ m pore polycarbonate filter inserts (Corning; cat. no. #3422) was performed. After lentivirus infection, both PC-3 and DU145 cells were reseeded at a density of 1 \times 10⁵ cells/well in serum-free F-12 containing 0.2% BSA in the upper chamber of each Transwell. In addition, F-12 supplemented with 10% FBS was added in the lower chamber. Then, the migration installation was incubated at 37°C with 5% CO₂ overnight and the non-migrated cells on the upper surface of the filter were lightly removed using cotton buds. The migrated cells on the lower surface were fixed in 4% paraformaldehyde for 10 min, stained in crystal violet for 2 min, and counted (five random fields per well) under a bright-field microscope. Additionally, the migrated cells were dissociated by 33% acetic acid and quantified at 570 nm using the Epoch microplate spectrophotometer (BioTek Instruments).

Animal experiments. The impact of *SMC1A* silencing on the tumor development of prostate cancer *in vivo* was examined. DU145 (Con group), DU145-Lv-shCon (shCon group) or DU145 Lv-shSMC1A (shSMC1A group) at a density of 5 \times 10⁶ per mouse were injected subcutaneously into 4-week-old BALB/c nude mice (n=10 per group; Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai, China). The development and growth of solid tumors were monitored by measuring tumor size using a vernier caliper every three days for a 28-days period. The tumor volume was calculated using a standard formula: tumor volume (mm³) = width (mm)² \times length (mm) \times 0.5. At the end of the experiment, all mice were sacrificed and individual tumor weight was measured using an electronic balance. All the animal experiments were approved by the Animal Care Committee of the Second Military Medical University.

Statistical analysis. GraphPad Prism 5.0 software was used to perform the statistical analyses. Data are presented as mean \pm SD from at least three independent experiments. The Student's t-test was used to compare the differences between the groups. P<0.05 was considered to indicate a statistically significant result.

Results

SMC1A is upregulated in androgen-independent prostate cancer cells. Through a lentiviral shRNA library-based screening on PC-3 cells, we identified *SMC1A* as a novel oncogenic candidate. To validate and further explore the function of

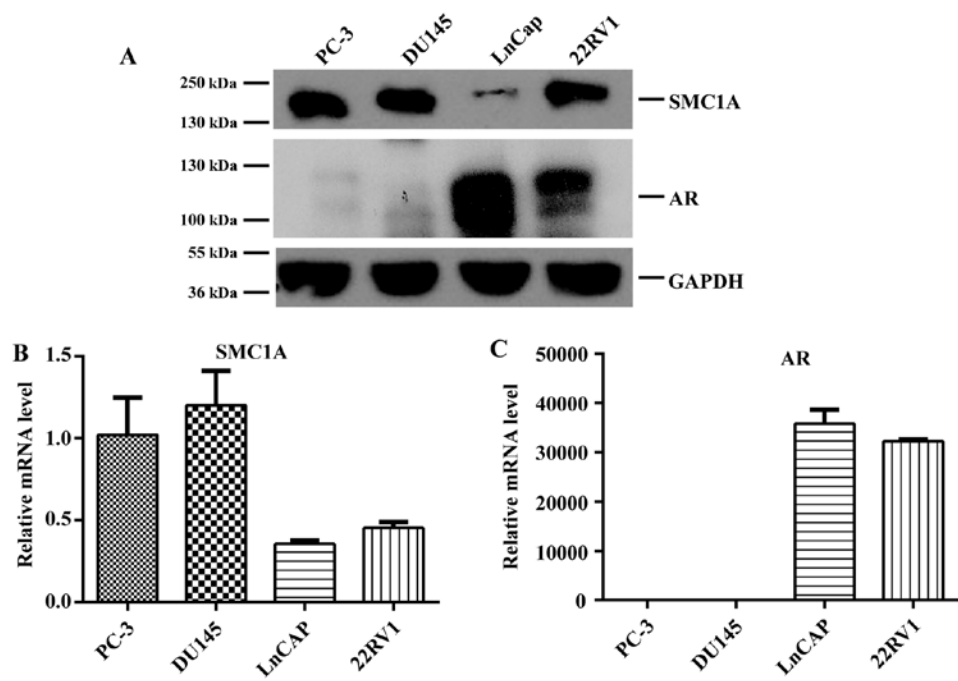


Figure 1. Expression of *SMC1A* is increased in androgen-independent prostate cancer cells. (A) Expression of *SMC1A* and AR was detected by western blot analysis in four prostate cancer cell lines. (B and C) *SMC1A* and AR expression was analyzed respectively by real-time PCR in four prostate cancer cell lines.

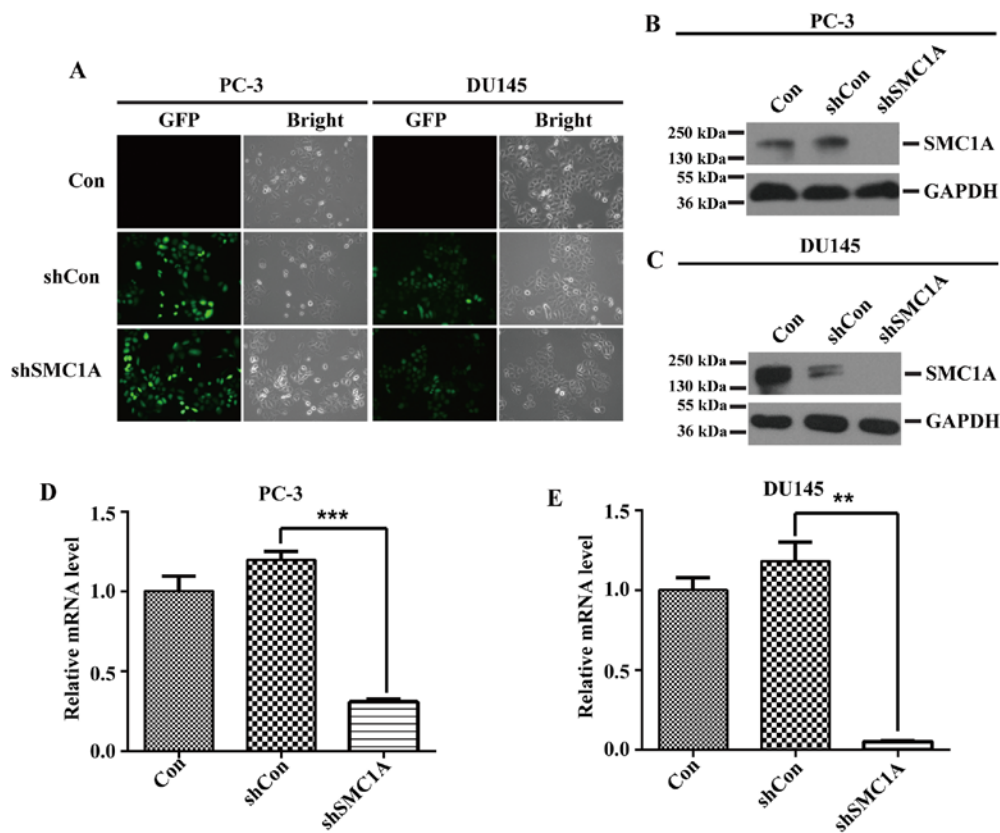


Figure 2. Lentivirus mediated knockdown of *SMC1A* in prostate cancer cells. (A) Representative images of PC-3 and DU145 cells infected with lentivirus expression with shCon or shSMC1A by observing GFP fluorescence. (B and D) Knockdown efficiency of *SMC1A* was analyzed by real-time PCR and western blot analysis in PC-3 cells. (C and E) Knockdown efficiency of *SMC1A* was analyzed by real-time PCR and western blot analysis in DU145 cells. Con, uninfected cells; shCon, cells infected with control shRNA; shSMC1A, cells infected with *SMC1A* shRNA; ** $P < 0.01$, *** $P < 0.001$.

SMC1A in prostate cancer, we first examined the expression of *SMC1A* in different PCa cell lines. Notably, we found that the expression of *SMC1A* was significantly increased in androgen-independent prostate cancer cell lines PC-3 and DU145

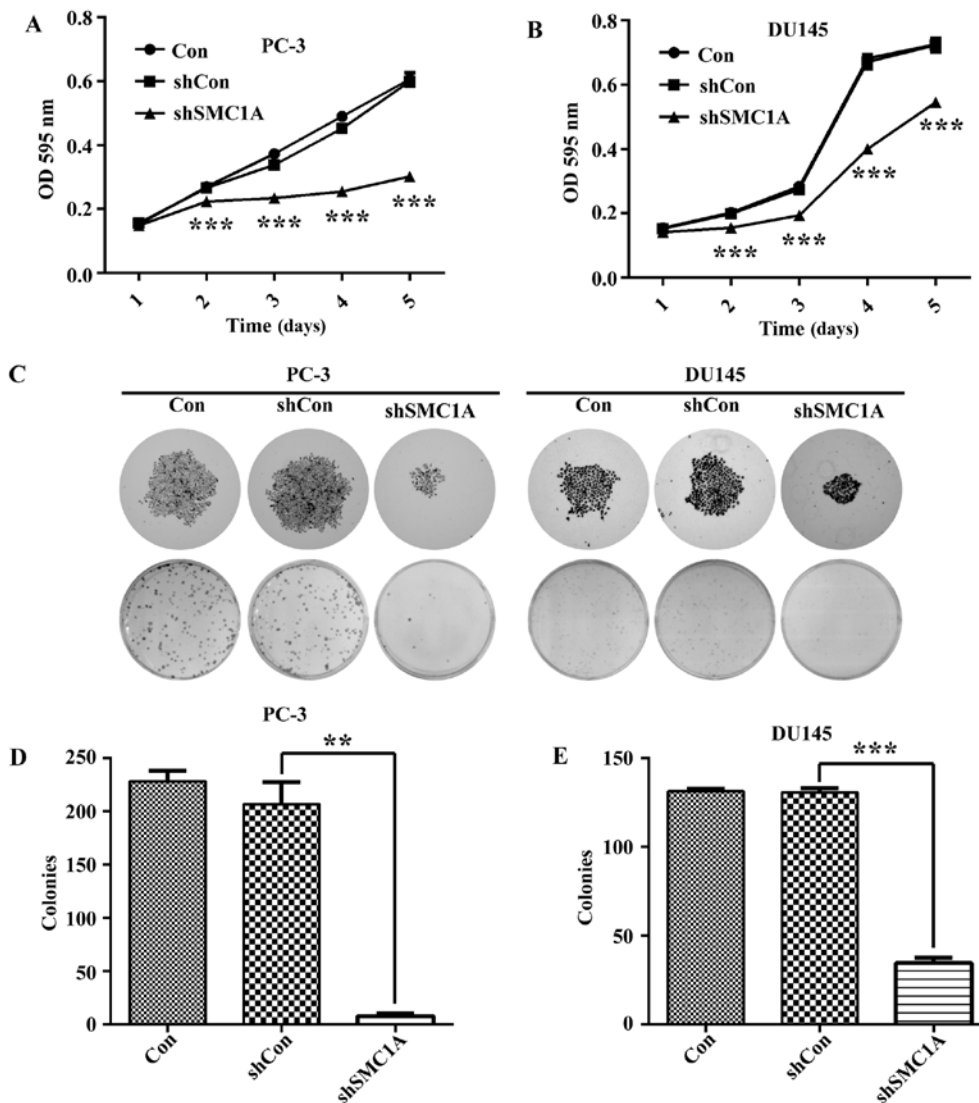


Figure 3. Downregulation of *SMC1A* inhibits cell proliferation and colony formation in prostate cancer cells. (A and B) Cell proliferation rates of PC-3 and DU145 cells were measured by MTT assay. (C) Representative pictures of the size and the number of colony formation in PC-3 and DU145 cells. (D and E) Statistical analysis of the number of colony formation in PC-3 and DU145 cells, respectively. ** $P < 0.01$, *** $P < 0.001$.

compared with androgen-sensitive cell lines LNCap and 22RV1, showed *SMC1A* expression was negatively correlated with the expression status of androgen receptor (AR) in both the protein and mRNA levels (Fig. 1A-C). Considering that *SMC1A* expression was much higher in androgen-independent prostate cancer cells PC-3 and DU145, they were used for further investigation.

Lentivirus mediated *SMC1A* silencing in prostate cancer cells. Both PC-3 and DU145 cells were untreated or transfected with shCon or shSMC1A. The transfection efficiencies were >90% in both cells confirmed by fluorescent microscope (Fig. 2A). Western blot analysis demonstrated that shSMC1A efficiently knocked down *SMC1A* expression in protein levels in PC-3 and DU145 cells (Fig. 2B and C). The results of real-time PCR indicated that *SMC1A* was downregulated >80 and 90% in mRNA levels in PC-3 and DU145 cells, respectively (Fig. 2D and E; $P < 0.001$, $P < 0.01$).

Downregulation of *SMC1A* inhibits cell proliferation and colony formation in prostate cancer cells. Effect of *SMC1A*

silencing on prostate cancer cell viability was assessed by MTT and colony formation assay. As shown in Fig. 3A and B, the growth rates of PC-3 and DU145 cells were significantly suppressed in shSMC1A group in comparison to the Con or shCon group ($P < 0.001$). Furthermore, *SMC1A* depletion significantly inhibited colony formation ability of PC-3 and DU145 cells in size and number compared to the Con or shCon group (Fig. 3C-E; $P < 0.01$, $P < 0.001$). The results suggested that both PC-3 and DU145 cells showed impaired cell proliferation and colony formation abilities after *SMC1A* knockdown, indicating a pivotal role of *SMC1A* in regulation of prostate cancer cell vitality.

Knockdown of *SMC1A* modulates cell cycle progression in prostate cancer cells. When culturing shSMC1A transfected PC-3 and DU145 cells, we noted that these cells showed more non-adherent cells during passages. We decided to confirm how *SMC1A* affected the proliferation of prostate cancer cells, and whether it functioned through the cell cycle distribution. To verify this hypothesis, PC-3 and DU145 cells were

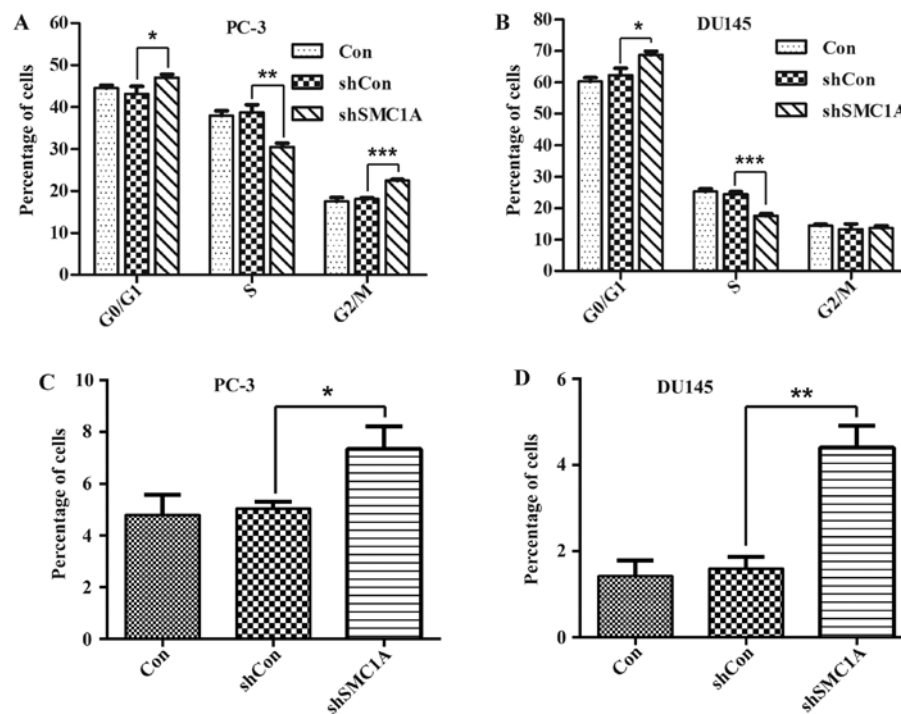


Figure 4. Knockdown of *SMC1A* modulated cell cycle progression in prostate cancer cells. (A and B) Graphical representation of the cell percentage in different phase of cell cycle in PC-3 and DU145 cells, respectively. (C and D) The proportions of Sub-G1 phase in PC-3 and DU145 cells were revealed. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

stained using PI and analyzed with FACS. As expected, the results showed that knockdown of *SMC1A* expression caused G0/G1 and G2/M-phase cell population increase ($P < 0.05$, $P < 0.001$) while S-phase cell population reduction ($P < 0.01$) that indicated cell cycle arrest at G2/M-phase in PC-3 cells (Fig. 4A). Moreover, *SMC1A* silencing presented G0/G1-phase cell population increase ($P < 0.05$) and S-phase cell population reduction ($P < 0.001$) that showed cell cycle arrest at S-phase in DU145 cells (Fig. 4B). In addition, the rate of cells in the Sub-G1 phase, representing apoptotic cells indirectly, was remarkably increased in shSMC1A group compared with Con and shCon groups in PC-3 and DU145 cells (Fig. 4C and D; $P < 0.05$, $P < 0.01$). These results indicated that *SMC1A* might be involved in cell apoptotic and cell cycle progression.

Knockdown of *SMC1A* represses cell migration ability of prostate cancer cells. PC-3 and DU145 cells were transfected with indicated lentivirus for 96 h, and then subjected to Transwell assay for the cell migration ability. As shown in Fig. 5, the cell numbers of PC-3 and DU145 cells in shSMC1A group which migrated to the lower chamber were less than the Con and shCon groups ($P < 0.01$, $P < 0.001$). These results indicated that knockdown of *SMC1A* significantly inhibited the migration ability of PC-3 and DU145 cells.

Knockdown of *SMC1A* represses tumor growth in a xenograft nude mouse model. To further study the function of *SMC1A* *in vivo*, DU145 cells were untreated, or transfected with shCon or shSMC1A, and subcutaneously injected into the nude mice to investigate the impact of *SMC1A* on tumor growth. As shown in Fig. 6A, knockdown of *SMC1A* inhibited subcutaneous tumor growth of DU145 cells. At day 28, the mice were euthanized

and the tumors were removed. At the end of the experiment, we found that the tumor volumes were significantly reduced in time-dependent manner, while the tumor weights were markedly decreased by *SMC1A* silencing (Fig. 6B and C; $P < 0.001$).

Upregulated *SMC1A* is related to biochemical recurrence and distant metastasis in prostate cancer patients. To illustrate the expression level of *SMC1A* and the clinical significance of *SMC1A* in PCa patients, we detected the specimens from patients and performed the data mining of the publicly available Oncomine datasets. In the present study, we found that the protein expression of *SMC1A* is significantly upregulated in PCa tissues by IHC staining and western blot assay (Fig. 7A and B). Similar results were also observed that the mRNA expression level of *SMC1A* was remarkably higher in PCa tissues than the normal tissues using Singh prostate (Fig. 7C; $n=102$, $P < 0.001$) and Welsh prostate databases (Fig. 7D; $n=34$, $P=0.015$). Notably, we found that the expression of *SMC1A* was obviously upregulated in the patients with postoperative biochemical recurrence (BCR) at 3 years by analyzing Glinsky prostate database (Fig. 7E; $n=79$, $P=0.0408$). Furthermore, we also detected that *SMC1A* expression was significantly and positively associated with distant metastasis in Holzbeierlein prostate database (Fig. 7F; $n=48$, $P=0.0307$). This finding was verified by other two independent databases including LaTulippe prostate ($n=32$) and Chandran prostate ($n=31$) databases. Specifically, comparing with primary site, *SMC1A* was visibly upregulated in three metastasis tissues (Fig. 7G, lymph node, $n=5$; bone, $n=2$, $P=0.0067$; soft tissues, $n=2$, $P=0.0267$; total metastasis, $P=0.0087$) in LaTulippe prostate database and also obviously elevated in lymph node ($n=13$, $P < 0.0001$), adrenal gland ($n=2$, $P=0.0303$), liver ($n=5$, $P=0.0047$) and lung

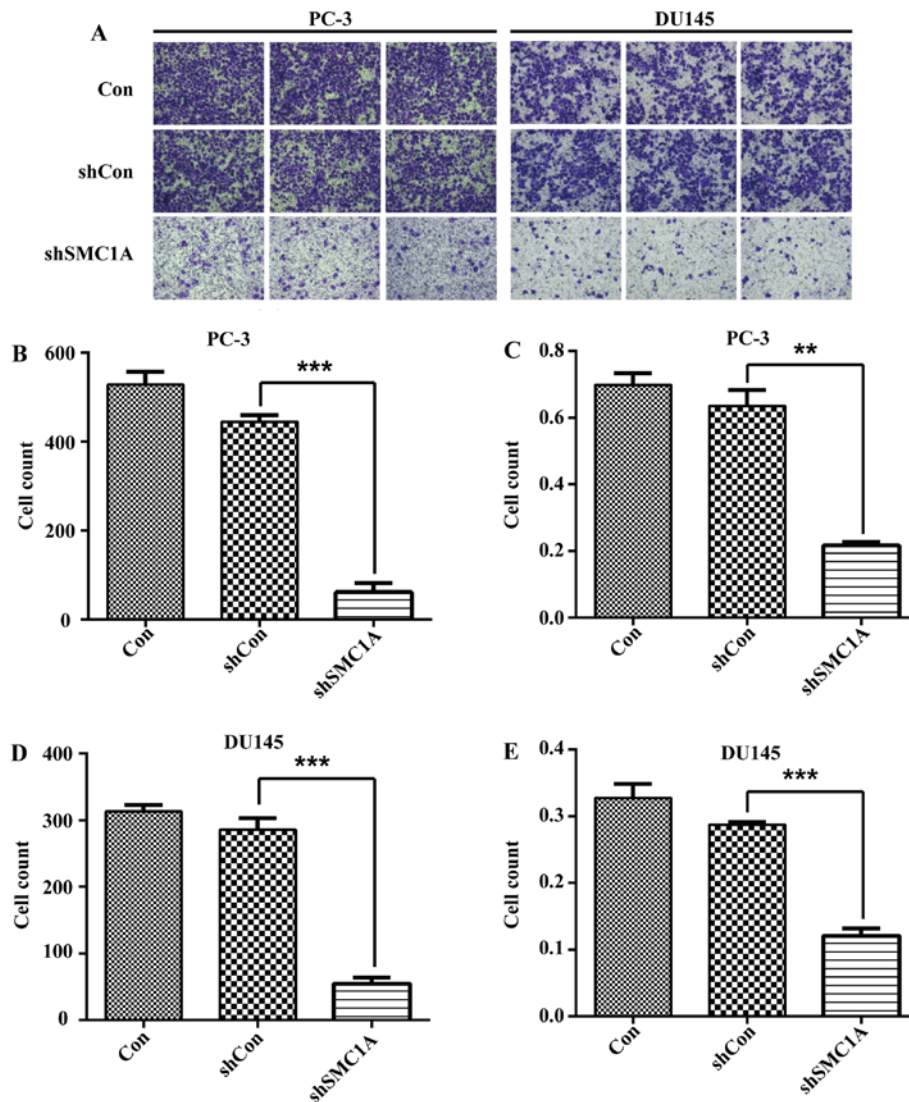


Figure 5. Knockdown of *SMC1A* suppresses cell migration ability of prostate cancer cells. (A) Representative images of the number of migration in PC-3 and DU145 cells. (B and C) Statistical analysis of the number of migrated cells in PC-3 and DU145 cells. (D and E) Quantitative analysis of migrated cells by decoloration and measurement of OD value at 570 nm. ** $P < 0.01$, *** $P < 0.001$.

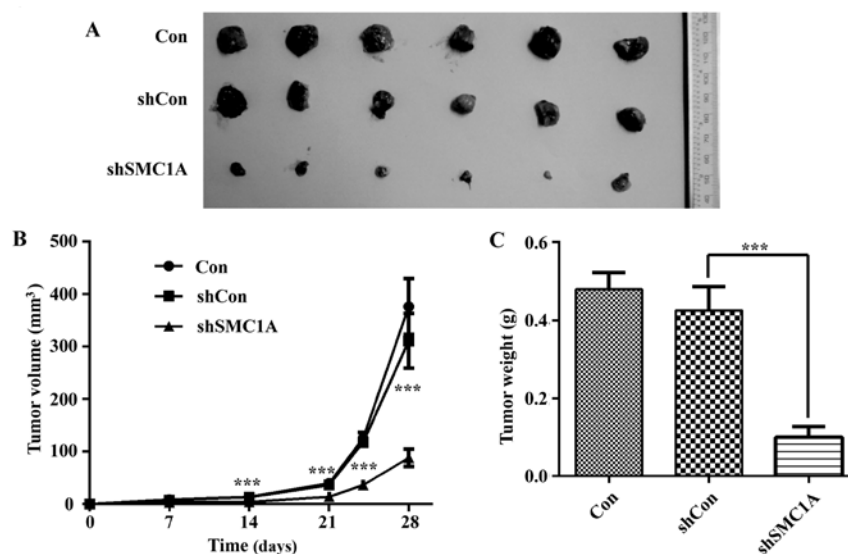


Figure 6. Knockdown of *SMC1A* represses tumor growth in a xenograft nude mouse model. (A) Representative images of the subcutaneous tumor sizes in different groups. (B) Statistical analysis of the subcutaneous tumor volume of different groups in different days. (C) Column graph represents the subcutaneous tumor weight. *** $P < 0.001$.

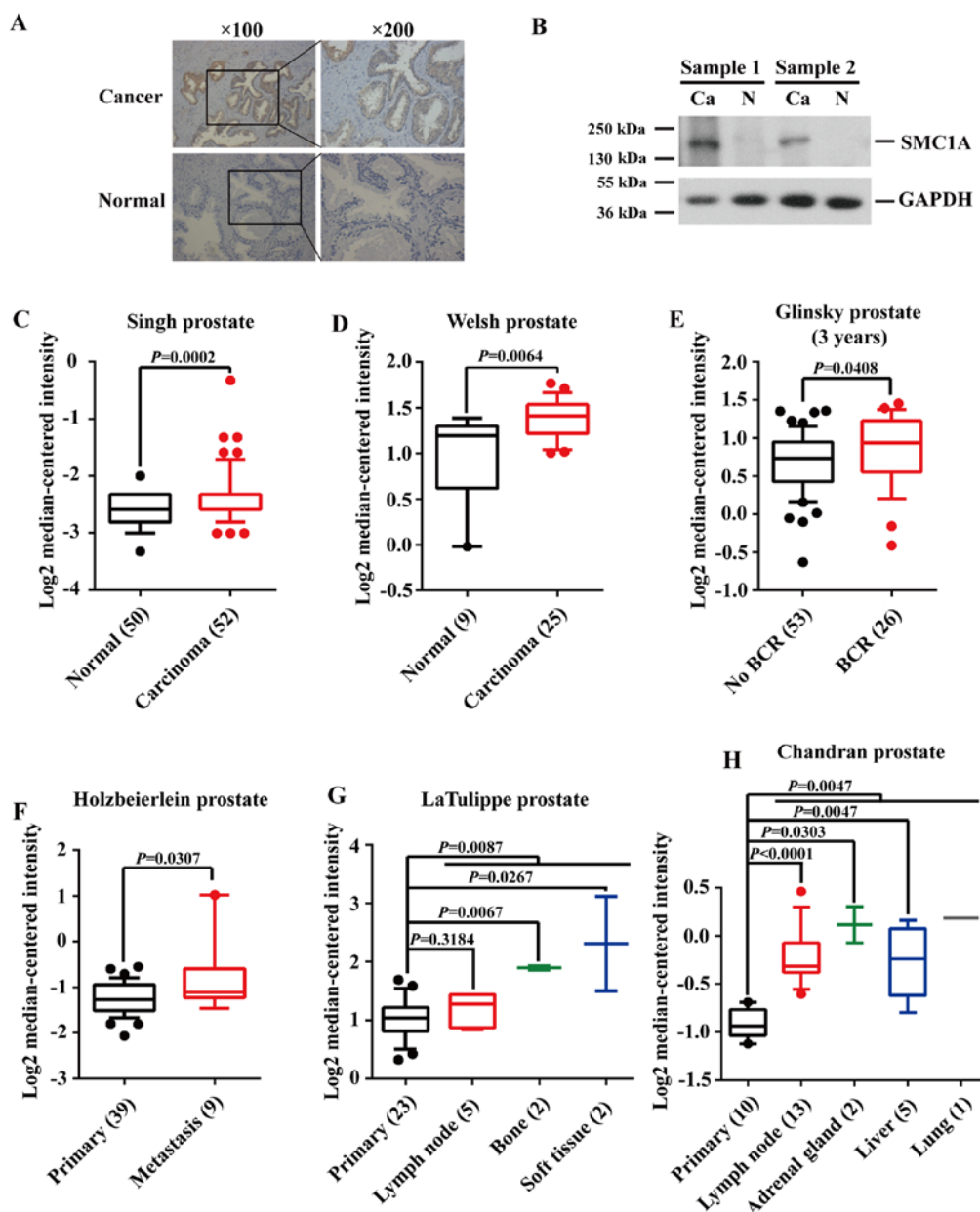


Figure 7. *SMC1A* is clinically correlated with prostate cancer. (A) Expression of *SMC1A* in the prostate cancer tissue (left panel, x10 magnification; right panel, x20 magnification). (B) Prostate cancer tissues (Ca) and adjacent normal tissues (N) were analyzed by western blot analysis. (C and D) *SMC1A* expressions in the normal and carcinoma tissues in the Singh prostate and Welsh prostate databases were exposed. (E) Column graph represents the correlation of *SMC1A* expression and biochemical recurrence in Glinsky prostate database. (F-H) The relationship between *SMC1A* expression and distant metastasis in Holzbeierlein prostate, LaTulippe prostate, and Chandran prostate databases is shown as a histogram, respectively.

(n=1) metastasis as revealed in Chandran prostate database (Fig. 7H; total metastasis, $P=0.0047$).

Discussion

The present study focused on *SMC1A*, whose clinical significance and potential biological functions in PCa are still unknown. We found that *SMC1A* was significantly upregulated in both PCa clinical tissues and CRPC cell lines. By silencing *SMC1A* expression and database mining, we confirmed that the expression *SMC1A* was closely related to the progression, metastasis and recurrence of human PCa.

Cohesin factors are involved in DNA repair and genome stability. Defects in cohesin-associated genes are emerging

as potential drivers of genomic instability and carcinogenic progression. In several tumor types, mutations of cohesin genes have been identified (25). Many tumors are either over-expressed or lowly expressed with cohesin gene (26-29). It has been shown that the loss of cohesin subunits would induce genomic instability in human cancers and the associated aneuploidy, as is observed in many cell lines which mutated in cohesin, resulting in further genomic instability (30-32). The above suggest that cohesin dysfunction may contribute to tumor development and progression.

SMC1A gene encoding a core component of the cohesin complex and cohesin-associated genes have been considered as potential drivers of tumor development and progression in many studies (17,18,26,28,33,34). Mannini *et al* (35) found

that *SMC1A* mutations were associated with the canonical role of cohesion. In fact, mutations affecting correct chromosome segregation lead to chromosome instability. Additionally, some studies demonstrated that *SMC1A* mutations may contribute to tumorigenesis by regulating the expression of oncogenes or suppressor genes.

The present study is the first revealing the potential role of *SMC1A* in PCa. In this study, we studied the expression of *SMC1A* in both PCa cell lines and clinical tissues. We found that the *SMC1A* expression was much higher in the androgen-independent cells PC-3, and DU145 than in the androgen-sensitive cells LNCap and 22RV1 and was negatively correlated with AR status. In clinical samples, we found that the protein and mRNA expressions of *SMC1A* were markedly upregulated in PCa tissues using IHC staining, western blot assay and Oncomine database mining. In addition, through analysis of clinical significance of *SMC1A* in PCa, we found that the expression level of *SMC1A* was correlated to biochemical recurrence and distant metastasis, suggesting that *SMC1A* could be a potential prognostic indicator.

Detection of the proliferation is one of the widely used methods to evaluate and measure the tumor responses to a new oncogene. Herein, we examined the proliferation-inducing effects of *SMC1A* silencing on prostate cancer cells *in vitro*. We found that knockdown of *SMC1A* by small interfering RNA could inhibit the growth and proliferation of CRPC cells PC-3, and DU145 by MTT and colony formation assay. Moreover, *SMC1A* silencing led to cell cycle arrest at G2/M phase in PC-3 cells while at S phase in DU145 cells. The difference of cell cycle arrest in PC-3 and DU145 cells may be due to the different cell sources that PC-3 cell is from a human prostatic adenocarcinoma metastatic to bone (36), while DU145 cell is from metastasis to the brain (37). Moreover, when *SMC1A* was silenced, the number of cells in the sub-G1 phase was increased significantly in both PC-3 and DU145 cells, indicating that knockdown of *SMC1A* could give rise to PCa cell apoptosis. In addition, the migration of tumor cells is one of the main risk factors for tumor progression. In this study, Transwell assay showed that depletion of *SMC1A* could reduce the migration of PC-3 and DU145 cells. However, the underlying molecular mechanism needs further investigation.

To further confirm the efficacy of the antitumor growth of *SMC1A* silencing *in vivo*, the xenograft nude mouse models were established by subcutaneous injecting the different treatments of DU145 cells. The results indicated that *SMC1A* silencing could significantly reduce tumor growth in xenograft models, which suggested that *SMC1A* may be a potential anti-tumor target for drug development.

In conclusion, the results have suggested that overexpression of *SMC1A* is a crucial molecule associated with PCa. It is involved in proliferation, cell cycle regulation, apoptosis and migration process of CRPC cells. The potential application of *SMC1A* targeted therapy will need further investigation in pre-clinical and clinical studies.

Acknowledgements

The present study was supported by grants from the National Natural Science Foundation of China for Youth (nos. 81001136 and 81202020), the National Natural Science Foundation of

China (nos. 30973006, 81170637 and 81572525), the Shanghai Committee of Science and Technology General Program for Medicine (no. 11JC1402302), the Key Project of Science and Innovation Foundation of Shanghai Ministry of Education (no. 14zz084) and the Military Fund for Health Care (no. CWS13BJ09).

References

1. Lytton B: Prostate cancer: A brief history and the discovery of hormonal ablation treatment. *J Urol* 165: 1859-1862, 2001.
2. Arora VK, Schenkein E, Murali R, Subudhi SK, Wongvipat J, Balbas MD, Shah N, Cai L, Efstathiou E, Logothetis C, *et al*: Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell* 155: 1309-1322, 2013.
3. Xie N, Cheng H, Lin D, Liu L, Yang O, Jia L, Fazli L, Gleave ME, Wang Y, Rennie P, *et al*: The expression of glucocorticoid receptor is negatively regulated by active androgen receptor signaling in prostate tumors. *Int J Cancer* 136: E27-E38, 2015.
4. Michaelson MD, Oudard S, Ou YC, Sengeløv L, Saad F, Houede N, Ostler P, Stenzl A, Daugaard G, Jones R, *et al*: Randomized, placebo-controlled, phase III trial of sunitinib plus prednisone versus prednisone alone in progressive, metastatic, castration-resistant prostate cancer. *J Clin Oncol* 32: 76-82, 2014.
5. Kelly WK, Halabi S, Carducci M, George D, Mahoney JF, Stadler WM, Morris M, Kantoff P, Monk JP, Kaplan E, *et al*: Randomized, double-blind, placebo-controlled phase III trial comparing docetaxel and prednisone with or without bevacizumab in men with metastatic castration-resistant prostate cancer: CALGB 90401. *J Clin Oncol* 30: 1534-1540, 2012.
6. Guacci V, Koshland D and Strunnikov A: A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of *MCD1* in *S. cerevisiae*. *Cell* 91: 47-57, 1997.
7. Losada A, Hirano M and Hirano T: Identification of Xenopus SMC protein complexes required for sister chromatid cohesion. *Genes Dev* 12: 1986-1997, 1998.
8. Michaelis C, Ciosk R and Nasmyth K: Cohesins: Chromosomal proteins that prevent premature separation of sister chromatids. *Cell* 91: 35-45, 1997.
9. Kimura K, Cuvier O and Hirano T: Chromosome condensation by a human condensin complex in Xenopus egg extracts. *J Biol Chem* 276: 5417-5420, 2001.
10. Harvey SH, Krien MJ and O'Connell MJ: Structural maintenance of chromosomes (smc) proteins, a family of conserved atpases. *Genome Biol* 3: Reviews3003, 2002.
11. Krantz ID, McCallum J, DeScipio C, Kaur M, Gillis LA, Yaeger D, Jukofsky L, Wasserman N, Bottani A, Morris CA, *et al*: Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of *Drosophila melanogaster* Nipped-B. *Nat Genet* 36: 631-635, 2004.
12. Tonkin ET, Wang TJ, Lisgo S, Bamshad MJ and Strachan T: NIPBL, encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins and fly Nipped-B, is mutated in Cornelia de Lange syndrome. *Nat Genet* 36: 636-641, 2004.
13. Borek G, Zarhrate M, Bonnefont JP, Munnich A, Cormier-Daire V and Colleaux L: Incidence and clinical features of X-linked Cornelia de Lange syndrome due to *SMC1L1* mutations. *Hum Mutat* 28: 205-206, 2007.
14. Deardorff MA, Kaur M, Yaeger D, Rampuria A, Korolev S, Pie J, Gil-Rodríguez C, Arnedo M, Loeys B, Kline AD, *et al*: Mutations in cohesin complex members SMC3 and SMC1A cause a mild variant of cornelia de Lange syndrome with predominant mental retardation. *Am J Hum Genet* 80: 485-494, 2007.
15. Musio A, Selicorni A, Focarelli ML, Gervasini C, Milani D, Russo S, Vezzoni P and Larizza L: X-linked Cornelia de Lange syndrome owing to SMC1L1 mutations. *Nat Genet* 38: 528-530, 2006.
16. Ma Z, Lin M, Li K, Fu Y, Liu X, Yang D, Zhao Y, Zheng J and Sun B: Knocking down SMC1A inhibits growth and leads to G2/M arrest in human glioma cells. *Int J Clin Exp Pathol* 6: 862-869, 2013.
17. Yang Y, Zhang Z, Wang R, Ma W, Wei J and Li G: siRNA-mediated knockdown of SMC1A expression suppresses the proliferation of glioblastoma cells. *Mol Cell Biochem* 381: 209-215, 2013.

18. Wang J, Yu S, Cui L, Wang W, Li J, Wang K and Lao X: Role of SMC1A overexpression as a predictor of poor prognosis in late stage colorectal cancer. *BMC Cancer* 15: 90, 2015.
19. Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, Frierson HF Jr and Hampton GM: Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. *Cancer Res* 61: 5974-5978, 2001.
20. Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C, Tamayo P, Renshaw AA, D'Amico AV, Richie JP, *et al*: Gene expression correlates of clinical prostate cancer behavior. *Cancer Cell* 1: 203-209, 2002.
21. Glinksy GV, Glinkii AB, Stephenson AJ, Hoffman RM and Gerald WL: Gene expression profiling predicts clinical outcome of prostate cancer. *J Clin Invest* 113: 913-923, 2004.
22. Holzbeierlein J, Lal P, LaTulippe E, Smith A, Satagopan J, Zhang L, Ryan C, Smith S, Scher H, Scardino P, *et al*: Gene expression analysis of human prostate carcinoma during hormonal therapy identifies androgen-responsive genes and mechanisms of therapy resistance. *Am J Pathol* 164: 217-227, 2004.
23. LaTulippe E, Satagopan J, Smith A, Scher H, Scardino P, Reuter V and Gerald WL: Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease. *Cancer Res* 62: 4499-4506, 2002.
24. Chandran UR, Ma C, Dhir R, Bisceglia M, Lyons-Weiler M, Liang W, Michalopoulos G, Becich M and Monzon FA: Gene expression profiles of prostate cancer reveal involvement of multiple molecular pathways in the metastatic process. *BMC Cancer* 7: 64, 2007.
25. Xu H, Yan M, Patra J, Natrajan R, Yan Y, Swagemakers S, Tomaszewski JM, Verschoor S, Millar EK, van der Spek P, *et al*: Enhanced RAD21 cohesin expression confers poor prognosis and resistance to chemotherapy in high grade luminal, basal and HER2 breast cancers. *Breast Cancer Res* 13: R9, 2011.
26. Ghiselli G and Iozzo RV: Overexpression of bamacan/SMC3 causes transformation. *J Biol Chem* 275: 20235-20238, 2000.
27. Hagemann C, Weigelin B, Schommer S, Schulze M, Al-Jomah N, Anacker J, Gergras S, Kühnel S, Kessler AF, Polat B, *et al*: The cohesin-interacting protein, precocious dissociation of sisters 5A/sister chromatid cohesion protein 112, is up-regulated in human astrocytic tumors. *Int J Mol Med* 27: 39-51, 2011.
28. Oikawa K, Ohbayashi T, Kiyono T, Nishi H, Isaka K, Umezawa A, Kuroda M and Mukai K: Expression of a novel human gene, human wings apart-like (hWAPL), is associated with cervical carcinogenesis and tumor progression. *Cancer Res* 64: 3545-3549, 2004.
29. Zhang N, Ge G, Meyer R, Sethi S, Basu D, Pradhan S, Zhao YJ, Li XN, Cai WW, El-Naggar AK, *et al*: Overexpression of Separase induces aneuploidy and mammary tumorigenesis. *Proc Natl Acad Sci USA* 105: 13033-13038, 2008.
30. Barber TD, McManus K, Yuen KW, Reis M, Parmigiani G, Shen D, Barrett I, Nouhi Y, Spencer F, Markowitz S, *et al*: Chromatid cohesion defects may underlie chromosome instability in human colorectal cancers. *Proc Natl Acad Sci USA* 105: 3443-3448, 2008.
31. Sheltzer JM, Blank HM, Pfau SJ, Tange Y, George BM, Humpton TJ, Brito IL, Hiraoka Y, Niwa O and Amon A: Aneuploidy drives genomic instability in yeast. *Science* 333: 1026-1030, 2011.
32. Solomon DA, Kim T, Diaz-Martinez LA, Fair J, Elkahoul AG, Harris BT, Toretsky JA, Rosenberg SA, Shukla N, Ladanyi M, *et al*: Mutational inactivation of STAG2 causes aneuploidy in human cancer. *Science* 333: 1039-1043, 2011.
33. Zhang YF, Jiang R, Li JD, Zhang XY, Zhao P, He M, Zhang HZ, Sun LP, Shi DL, Zhang GX, *et al*: SMC1A knockdown induces growth suppression of human lung adenocarcinoma cells through G1/S cell cycle phase arrest and apoptosis pathways in vitro. *Oncol Lett* 5: 749-755, 2013.
34. Hömme C, Krug U, Tidow N, Schulte B, Kühler G, Serve H, Bürger H, Berdel WE, Dugas M, Heinecke A, *et al*: Low SMC1A protein expression predicts poor survival in acute myeloid leukemia. *Oncol Rep* 24: 47-56, 2010.
35. Mannini L, Liu J, Krantz ID and Musio A: Spectrum and consequences of SMC1A mutations: The unexpected involvement of a core component of cohesin in human disease. *Hum Mutat* 31: 5-10, 2010.
36. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF and Jones LW: Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Invest Urol* 17: 16-23, 1979.
37. Stone KR, Mickey DD, Wunderli H, Mickey GH and Paulson DF: Isolation of a human prostate carcinoma cell line (DU 145). *Int J Cancer* 21: 274-281, 1978.