

Sox2 is involved in paclitaxel resistance of the prostate cancer cell line PC-3 via the PI3K/Akt pathway

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Abstract. Prostate cancer is the most commonly diagnosed type of cancer and the second leading cause of cancer-associated mortality in males. The efficacy of prostate cancer chemotherapy is frequently impaired by drug resistance; however, the underlying mechanisms of this resistance remain elusive. Sex determining region Y-box 2 (Sox2) is of vital importance in the regulation of stem cell proliferation and carcinogenesis. In the present study, using MTT, clone formation, cell cycle and apoptosis assays, over-expression of Sox2 was demonstrated to enhance the paclitaxel (Pac) resistance of the PC-3 prostate cancer cell line, promoting cell proliferation and exhibiting an anti-apoptotic effect. Western blot analysis revealed that the phosphoinositide 3-kinase/Akt signaling pathway was activated in cells overexpressing Sox2, and by targeting cyclin E and survivin, Sox2 promoted G1/S phase transition and prevented apoptosis under Pac treatment. The present study provided an understanding of Pac resistance in prostate cancer and may indicate novel therapeutic methods for chemoresistant prostate cancer.

Introduction

Prostate cancer is the most commonly diagnosed cancer and the second leading cause of cancer-associated mortality in males, having remained unchanged for >20 years in the USA (1). Androgen ablation therapy has been shown to be effective at the initial stages of prostate cancer; however, almost all patients progress to an androgen-independent stage

or hormone-refractory prostate cancer (HRPC), which is unresponsive to hormone deprivation (2). Currently, the standard treatment of patients with HRPC is with docetaxel, a paclitaxel (Pac; also known as taxol) derivative-based chemotherapeutic (3). However, the efficiency of this therapy is frequently impaired by drug resistance, a notable cause of mortality in this type of cancer (4). Certain genes, including octamer-binding transcription factor 4 (*OCT4*), have been demonstrated to be of vital importance in the formation of drug-resistant cells in prostate cancer (5,6). Therefore, since it is difficult to find novel drugs for chemotherapy, identifying the molecules involved in drug resistance and applying targeted methods may improve the efficacy of prostate cancer chemotherapy.

Sex determining region Y-box 2 (Sox2), a member of the SOX family of transcription factors (7-9), is critical in the self-renewal of embryonic stem cells (10,11), maintenance of pluripotency, generation of induced stem cells (12-14) and apoptosis (15-17). Aberrant overexpression of Sox2 has been reported in neural (16), respiratory (18,19), reproductive (20,21) and digestive system tumors (17,22). In gastric and colorectal cancer stem-like cells, Sox2 enhanced tumorigenicity and chemoresistance (23,24). It has been demonstrated that Sox2 promotes esophageal carcinoma growth by regulating the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway, which is key in the cell survival process (25). In addition, expression of Sox2 has been shown to be significantly increased in prostate cancer tissue compared with normal and hyperplastic tissues. As an androgen receptor-repressed gene, Sox2 promotes the formation of HRPC (26). Thus, targeted therapy against Sox2 may improve the efficiency of chemotherapy in patients with drug-resistant HRPC.

Pac and its derivatives are a wide class of well-known microtubule stabilizers and have been utilized as front-line chemotherapeutic agents for several types of cancer, including prostate cancer (3). However, these drugs frequently induce drug resistance. The molecular mechanism of Pac resistance has not been clarified, although a large amount of evidence has revealed that the PI3K/Akt signaling pathway is key in the formation of drug resistance in cancer via promotion of the expression of genes imperative for cell survival, consequently providing protection against apoptosis (27-30). In ovarian cancer cells, the constitutively activated PI3K/Akt signaling

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pathway conferred resistance to Pac, which was reversed by the PI3K/Akt inhibitor LY294002 (31). As a tumor suppressor gene, phosphatase and tensin homolog (PTEN) acts as a negative regulator of the PI3K/Akt signaling pathway and its loss of function is associated with the progression and aggressive behavior of numerous types of cancer (32,33). Regulation of the PI3K/Akt signaling pathway through PTEN has been reported to overcome sunitinib resistance in prostate cancer cells (34). For this reason, clarifying the roles of certain genes in the PI3K/Akt signaling pathway may be a rational way to approach drug-resistant cancer.

In the present study, the impact of Sox2 on the effects of Pac treatment, which include induction of apoptosis and inhibition of cell proliferation, were investigated in a prostate cancer cell line. In addition, the underlying mechanism of the PI3K/Akt signaling pathway was analyzed. Targeted therapy against Sox2 administered as a co-treatment with Pac may be a promising therapy in drug-resistant HRPC.

Materials and methods

Materials. Pac and LY294002 were supplied by Sigma (St. Louis, MO, USA). Sox2 primary antibody (ab97959, rabbit, polyclonal, 1:500) was obtained from Abcam (Cambridge, MA, USA) and antibodies against Akt (9272s, rabbit, polyclonal, 1:500) and phosphorylated (p)-Akt (4058s, rabbit, monoclonal, 1:200) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Antibodies against cyclin E (sc-198, rabbit, polyclonal, 1:1,000), were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Antibody to α -tubulin (mouse, monoclonal, 1:1,000), and anti-rabbit (goat, 1:2,000) and anti-mouse (goat, 1:2,000) secondary antibodies were obtained from Beyotime Institute of Biotechnology (Haimen, China). The MTT Cell Viability Detection kit, lactate dehydrogenase (LDH) Assay kit, Annexin V-fluorescein isothiocyanate (FITC) & propidium iodide (PI) Double Staining Apoptosis Detection kit for flow cytometry (FCM), Cell Mitochondria Isolation kit, Propidium Iodide, Caspase-3/9 Activity Assay kit and JC-1 probe were purchased from Beyotime Institute of Biotechnology.

Cell culture and transfection. PC-3 human prostate cancer cell lines were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) and 100 U/ml penicillin/streptomycin (Invitrogen Life Technologies, Carlsbad, CA, USA), at 37°C in a humidified atmosphere (5% CO₂/95% air). The human Sox2-coding-sequence was cloned into a pcDNA3.0 vector (Invitrogen Life Technologies) and termed as pcDNA3.0 Sox2; transfection was performed using Lipofectamine® 2000 (Invitrogen Life Technologies). A total of 850 µg/ml G418 (Calbiochem, San Diego, CA, USA) was applied to select the G418-resistant cells. The cells were treated with Pac or dimethyl sulfoxide (DMSO) as a vehicle (Veh) for 48 h, and in several cases, LY294002 was added 2 h prior to Pac treatment.

Cell viability analysis. The empty vector-transfected (PC-3 Mock) and pcDNA3.0 Sox2-transfected (PC-3 Sox2) cells (2x10⁵ cells/ml in 96-well plates) were treated with DMSO

(1:1,000) or 5 µM Pac for 48 h. Cell viability was measured using the MTT Cell Viability Detection kit, following the manufacturer's instructions. Absorbance was read at 450 nm on a microplate spectrophotometer (Spectra Max M3; Molecular Devices, Sunnyvale, CA, USA).

Clone formation assay. The PC-3 Mock and PC-3 Sox2 cells were seeded at 500 cells/well in six-well culture plates and treated with DMSO (1:1,000) or 5 µM Pac for 48 h. Following 10 days of incubation, the cell colonies were stained with crystal violet, counted and images were captured by a digital camera (BioSpectrum 810 Imaging System; UVP, Upland, CA, USA) (35). The ratio of clone formation was calculated using the following equation: Rate of clone formation (%) = (clone quantity/500) x 100. The relative clone formation ratio was normalized to the PC-3 Mock DMSO group.

LDH measurement. Leakage of LDH into the cell culture medium indicated cell membrane damage. The PC-3 Mock and PC-3 Sox2 cells were exposed to DMSO (1:1,000) or 5 µM Pac for 48 h, then the culture medium was centrifuged at 250 g for 10 min, and the supernatant was transferred to a 96-well culture plate to determine the quantity of LDH according to the manufacturer's instructions. The LDH activity was reported as the percentage relative to the control level (36). Absorbance was measured at 450 nm on the SpectraMax M3 microplate spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA, USA).

Cell cycle and apoptosis analysis. A total of 1x10⁶ cells were seeded into a 60 mm dish 24 h before treatment, then Pac (5 µM)/Veh was added for 48 h (in several cases, LY294002 was added 2 h before Pac treatment). For the cell cycle analysis, cells were harvested, fixed with 70% ethanol and stored at 4°C overnight, then incubated with RNase (25 µg/ml) at 37°C for 30 min, followed by staining with PI (50 µg/ml) for 30 min in the dark. For the apoptosis analysis, Annexin V-FITC and PI staining was performed according to the manufacturer's instructions (Beyotime Institute of Biotechnology). The stained cells were counted using a FACSCalibur Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA). All data were analyzed and visualized by FlowJo® software Ver. 7.6.1 for Windows (TreeStar, Inc., Ashland, OR, USA).

Mitochondrial membrane potential assay. The JC-1 probe was used to measure mitochondrial depolarization in the cells. Briefly, the mitochondria were separated from the cells following the indicated treatments using the Cell Mitochondria Isolation kit, were then incubated with 1 ml JC-1 staining-solution (5 µg/ml) for 20 min at 37°C and rinsed twice with phosphate-buffered saline. The mitochondrial membrane potentials were measured using the relative quantities of dual emissions from mitochondrial JC-1 monomers or aggregates using the Spectra Max M3 microplate spectrophotometer. The excitation wavelength was set at 485 nm. Fluorescence intensity was detected at 525 nm for monomers and 590 nm for aggregates. Mitochondrial depolarization was indicated by an increase in the 525/590 nm fluorescence intensity ratio.

Caspase-3 and -9 activity measurement. Caspase activity was determined using the Caspase-3/9 Activity Assay kit (Beyotime

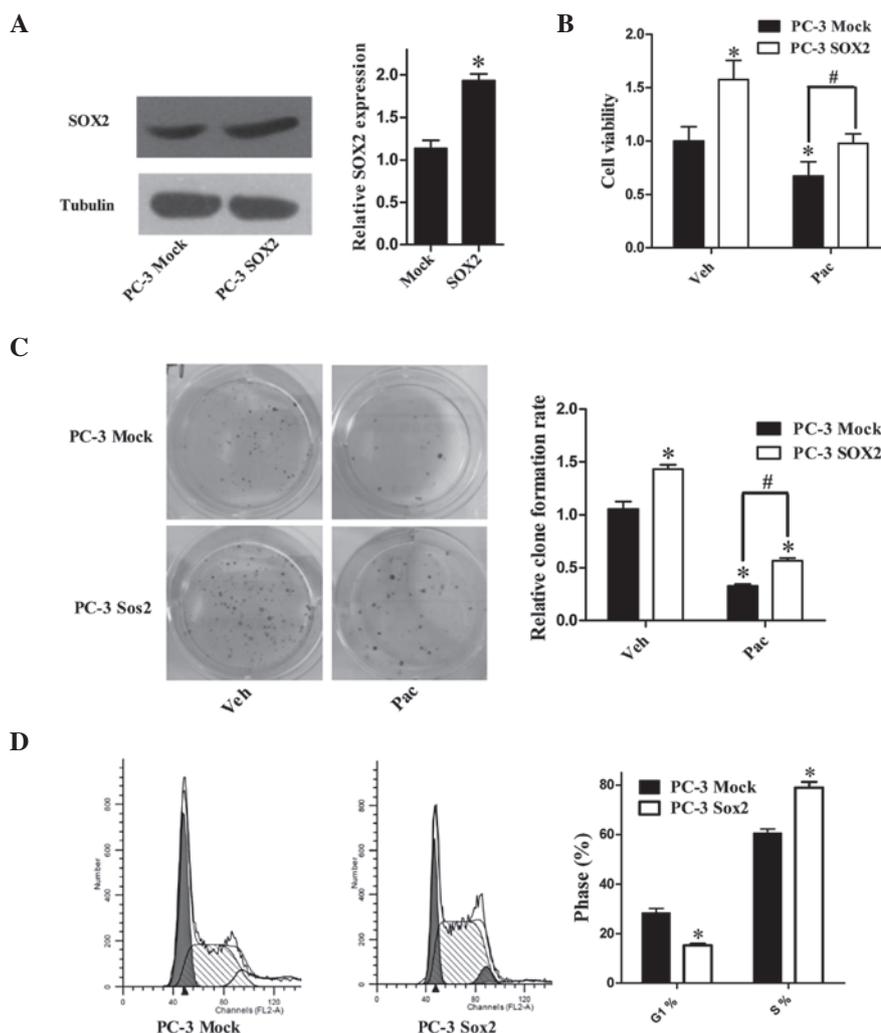


Figure 1. Sox2 promotes cell proliferation and impairs cell cycle arrest under paclitaxel treatment in the PC-3 prostate cancer cell line. (A) Expression levels of Sox2 in the empty vector (PC-3 Mock) and Sox2 over-expression (PC-3 Sox2) prostate cancer cells at the protein level, normalized to α -tubulin. (B) Cell viability of PC-3 Mock and PC-3 Sox2 cells under Pac or Veh treatment, measured by MTT assay. (C) Colony images and clone formation rate graphs of PC-3 Mock and PC-3 Sox2 cells with Pac or Veh treatment. (D) Cell cycle analysis of PC-3 Mock and PC-3 Sox2 under Veh, measured by flow cytometry. All data were analyzed by one-way analysis of variance and are presented as the mean \pm standard error of the mean of three independent experiments. * $P < 0.05$ vs. PC-3 Mock with Veh; # $P < 0.05$ vs. PC-3 Sox2 with Pac. Sox2, sex determining region Y-box 2; Pac, paclitaxel; Veh, vehicle (dimethylsulfoxide).

Institute of Biotechnology) following the manufacturer's instructions. The present study used 96-well microplates for incubating $10 \mu\text{l}$ cell lysate in $80 \mu\text{l}$ reaction buffer containing $10 \mu\text{l}$ caspase substrate (2 mM). The lysates were incubated at 37°C for 4 h. Data were collected using the SpectraMax M3 microplate reader at an absorbance of 405 nm. Caspase activity was expressed as the ratio of treated to vehicle control cells.

Transmission electron microscopy observation. The cells were fixed according to previous methods (37). The ultrastructure of the cells was examined by transmission electron microscopy (Hitachi H-600; Hitachi, Ltd., Tokyo, Japan).

Western blot analysis. Whole-cell lysate preparation and western blot analysis were conducted as described previously (38).

Statistical analysis. Each experiment was repeated in triplicate. Statistical analyses were performed using SPSS 20.0

software for Windows (IBM, Armonk, NY, USA). $P < 0.05$ was considered to indicate a statistically significant difference and the results are expressed as the mean \pm the standard error of the mean.

Results

Overexpression of Sox2 promotes cell proliferation and impairs the cell cycle arrest induced by Pac. To verify the effects of Sox2 on PC-3 cells, cells were stably transfected with Sox2-expressing vector and termed PC-3 Sox2, in contrast to the empty vector PC-3 Mock cell line (Fig. 1A). To examine whether Sox2 impacted the effect of Pac on cell proliferation, MTT and clone formation assays were performed to measure the cell proliferation status (Fig. 1B and C). The vehicle-treated PC-3 Sox2 cells exhibited increased proliferation as compared with the mock-transfected cells, and the decreases in cell growth following Pac-treatment were significantly attenuated in PC-3 Sox2 cells as compared with the

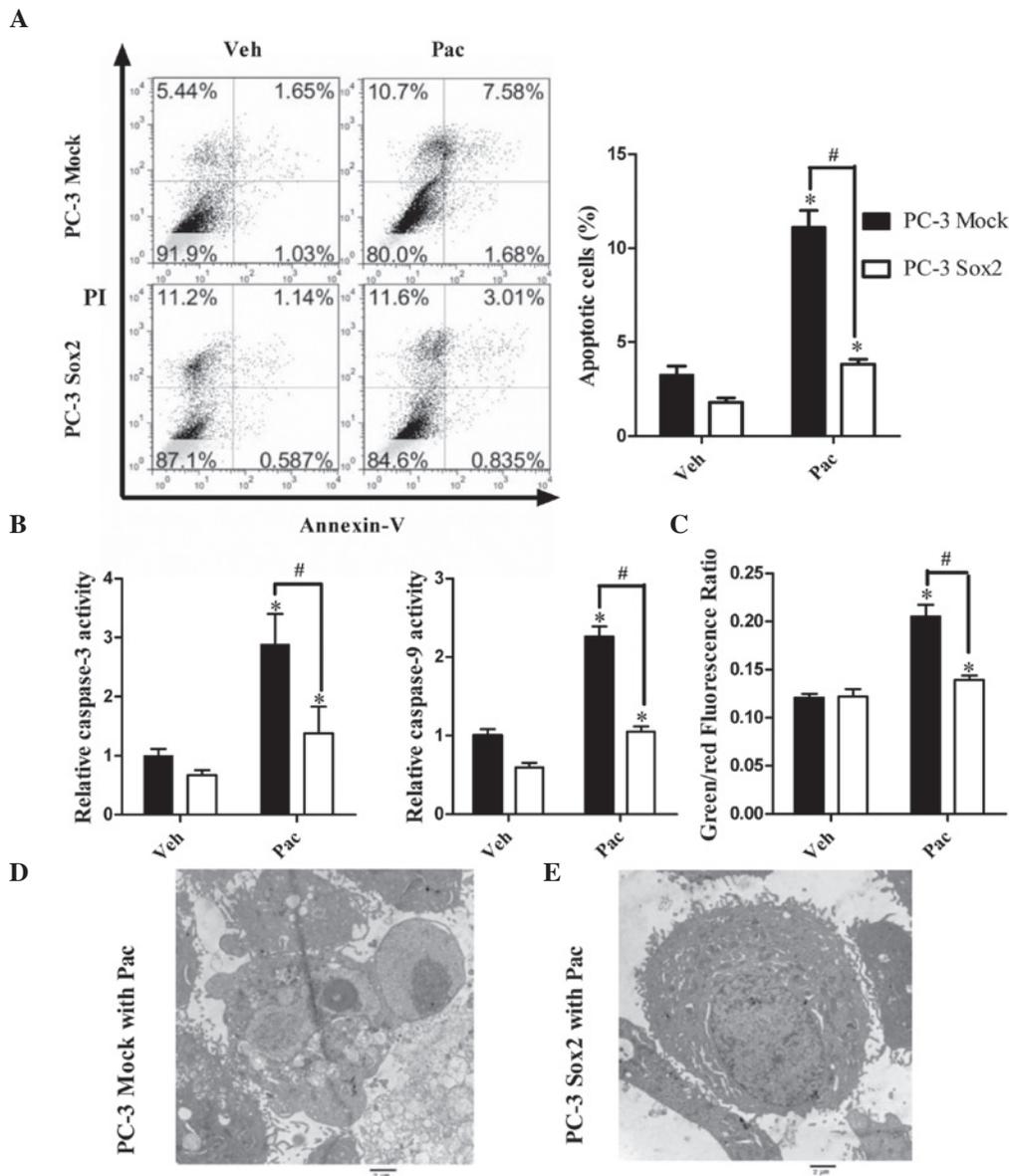


Figure 2. Sox2 inhibits the apoptotic effect induced by Pac treatment. (A) Apoptotic cell ratio of empty vector (PC-3 Mock)/Sox2 overexpression cells (PC-3 Sox2) under Pac or Veh treatment, measured by flow cytometry-based Annexin V-fluorescein isothiocyanate and PI double staining analysis. (B) Relative activity of caspase-3, 9 in PC-3 Mock/PC-3 Sox2 cells treated with Pac or Veh. (C) Mitochondrial membrane potential of PC-3 Mock and PC-3 Sox2 cells under Pac or Veh treatment, measured by JC-1 probe. An increase in the green/red ratio indicated depolarization of the mitochondrial membrane. (D and E) Transmission electron microscopy of the morphological changes of apoptosis induced by Pac. (D) PC-3 Mock cells treated with Pac (magnification, x8,000). (E) PC-3 Sox2 cells treated with Pac (magnification, x8,000). Results were representative of five independent experiments. All data were analyzed by one-way analysis of variance and are presented as the mean \pm standard error of the mean of three independent experiments. * $P < 0.05$ vs. PC-3 Mock with Veh; # $P < 0.05$ vs. PC-3 Sox2 with Pac. PI, propidium iodide; Sox2, sex determining region Y-box 2; Pac, paclitaxel; Veh, vehicle (dimethylsulfoxide).

mock-transfected group. An FCM-based cell cycle assay was conducted to detect the cell cycle distribution of PC-3 Mock and PC-3 Sox2 cells (Fig. 1D). The PC-3 Sox2 cells exhibited an increased percentage of cells in S-phase as compared with the mock-transfected cells. This implies that the resistance of Sox2-overexpressing cells may be based on their upregulation of proliferation and DNA synthesis. In conclusion, the data revealed that in the Sox2-overexpressing cell line, the cell growth inhibition caused by Pac was partly attenuated.

Sox2-expression leads to evasion of apoptosis induced by Pac treatment. To examine the impact of Sox2 on Pac-induced apoptosis, Annexin V/PI double staining and FCM analysis

were used to measure the apoptotic rate of the cells (Fig. 2A). In the Mock-transfected group, the apoptotic rate was increased following incubation with Pac, which was significantly attenuated in the Sox2-overexpressing cell line. The activities of caspase-3 and caspase-9 increased following 48 h Pac treatment in the PC-3 cells (Fig. 2B), which was significantly attenuated in the Sox2-overexpressing cells, while caspase-8 activity was not significantly induced (data not shown). JC-1 aggregated in normal mitochondria and exhibited red fluorescence. Exposure of the cells to Pac for 48 h also resulted in dissipation of the inner mitochondrial membrane potential, which was shown as an increased green/red fluorescence ratio. By contrast to mock-transfected cells, Sox2-overexpression

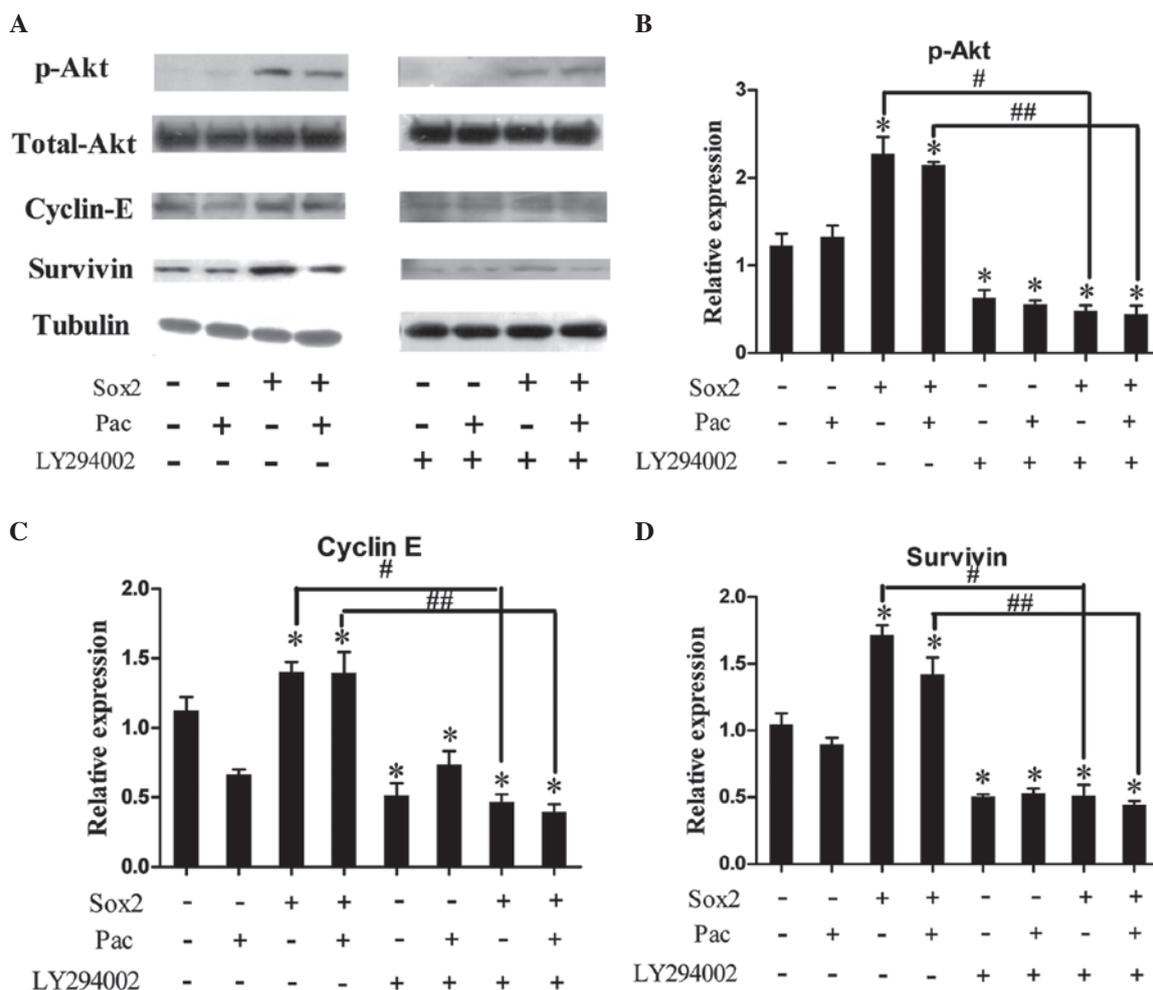


Figure 3. Expression levels of cell cycle- and apoptosis-associated proteins in PC-3 prostate cancer cell lines treated with Pac. (A) Expression levels of p-Akt, Akt, cyclin E and survivin in empty vector (PC-3 Mock; Sox2, -) and Sox2 overexpression (PC-3 Sox2; Sox2, +) cells under Pac treatment (Pac, +) or dimethyl sulfoxide (Pac, -), with or without pretreatment with the phosphoinositide 3-kinase/Akt pathway inhibitor LY294002. (B-D) Relative p-Akt, cyclin E and survivin expression levels in the different treatment groups. Data were analyzed by one-way analysis of variance and are presented as the mean \pm standard error of the mean of three independent experiments. * P <0.05, vs. PC-3 Mock without Pac; # P <0.05, vs. PC-3 Sox2 without Pac; ## P <0.05, vs. PC-3 Sox2 with Pac. Sox2, sex determining region Y-box 2; Pac, paclitaxel; p, phosphorylated.

completely inhibited the depolarization of the mitochondrial membrane following Pac treatment (Fig. 2C). These results indicated that Sox2 prevented Pac-induced apoptosis. In addition, photomicrographs of PC-3 cells exposed to Pac captured by transmission electron microscopy revealed typical apoptotic morphology with chromatin condensation and formation of apoptotic bodies (Fig. 2D and E).

An LDH assay was also performed to measure the cytotoxic effect of Pac on cells; however, no significant difference was observed between the PC-3 Mock (121.4 \pm 9.8%) and PC-3 Sox2 groups (119.7 \pm 8.6%).

The PI3K/Akt signaling pathway is involved in the Sox2-mediated anti-apoptotic and cell proliferation-promoting effect during Pac treatment. To investigate the underlying mechanism of Sox2 on Pac-treated cells, several signaling pathways and apoptosis- and proliferation-associated proteins were examined. p-Akt, cyclin E and survivin expression levels were found to be upregulated in PC-3 Sox2 cells as compared with levels in mock-transfected cells (Fig. 3), while B-cell lymphoma-2 and p21 expression levels remained unchanged

(data not shown). As cyclin E and survivin have been reported as Akt-regulated proteins, a PI3K inhibitor, LY294002, was used to suppress the PI3K/Akt signaling pathway activity of each group, respectively. Inhibition of the PI3K/Akt signaling pathway reduced the expression levels of p-Akt, cyclin E and survivin (Fig. 3), and partially attenuated the effects of Sox2 on preventing apoptosis and promoting cell proliferation under Pac treatment (Fig. 4).

Discussion

Prostate cancer is the most commonly diagnosed cancer and the second leading cause of cancer-associated mortality in males (1). In the clinic, the standard therapy for patients with HRP is docetaxel, a Pac derivative-based chemotherapeutic. However, the efficiency of this drug is frequently impaired due to drug resistance (4). Several genes, including *OCT4*, have been reported to be involved in the development of drug resistance in prostate cancer cells (5,6). Identifying novel molecular mechanisms underlying this drug resistance may achieve more effective chemotherapy for prostate cancer patients.

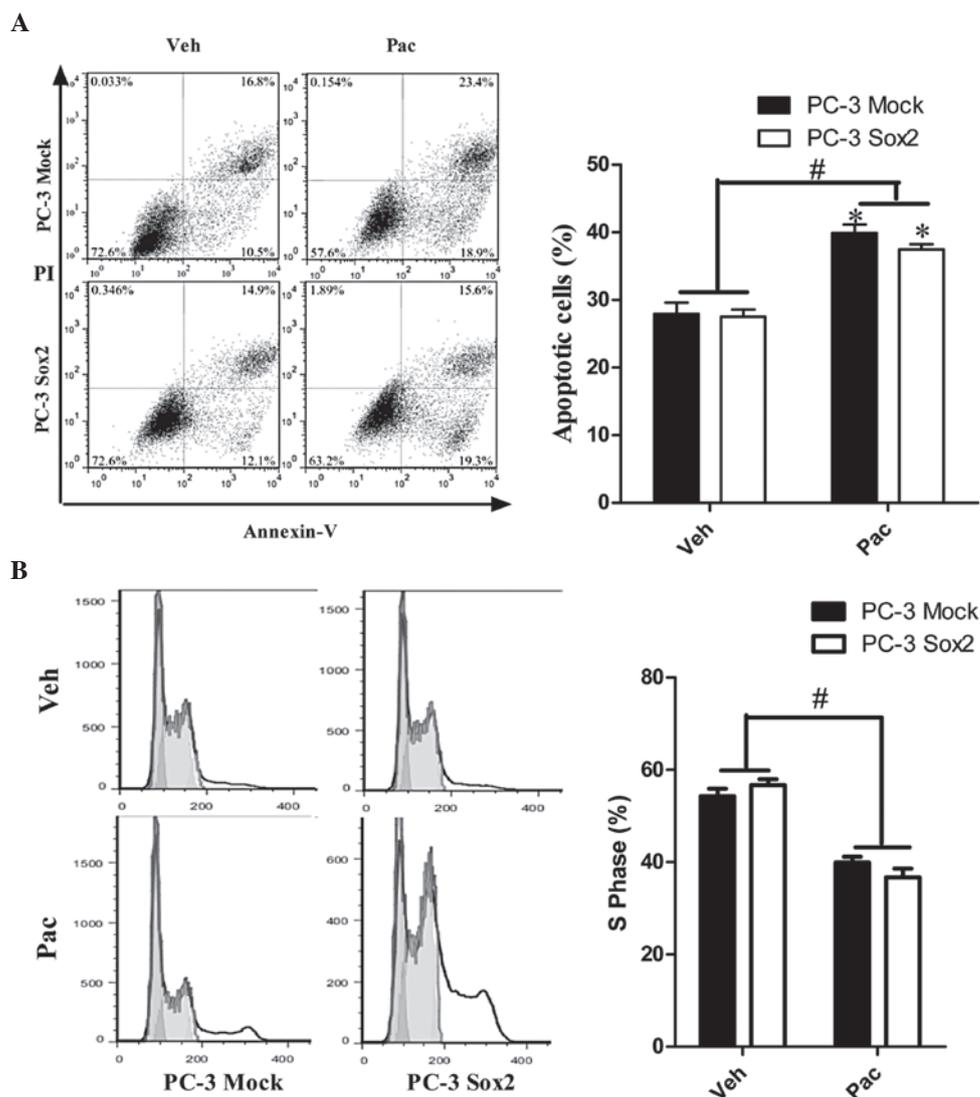


Figure 4. The PI3K/Akt pathway is involved in the Sox2-mediated anti-apoptotic and cell proliferation-promoting effects during paclitaxel treatment. (A) Apoptotic cell ratio of empty vector (PC-3 Mock)/Sox2 over-expression (PC-3 Sox2) cells under Pac and Veh treatment, with 2 h pre-treatment with 30 μ M LY294002, measured by FCM-based Annexin V-fluorescein isothiocyanate and PI double staining analysis. (B) Cell cycle analysis of PC-3 Mock and PC-3 Sox2 cells under Pac or Veh, with 2 h pretreatment with 30 μ M LY294002, measured by FCM. Data were analyzed by one-way analysis of variance and are presented as the mean \pm standard error of the mean of three independent experiments. * P <0.05, vs. PC-3 Mock with Veh; # P <0.05, PC-3 Mock and PC-3 Sox2 with Pac vs. PC-3 Mock and PC-3 Sox2 with Veh. FCM, flow cytometry; PI, propidium iodide; Sox2, sex determining region Y-box 2; Pac, paclitaxel; Veh, vehicle (dimethylsulfoxide).

Pac (taxol) has been used as a chemotherapeutic agent since the 1990s. The induction of apoptosis and cell growth inhibition by Pac have been demonstrated, independent to the microtubule stabilizing effect. However, for the majority of chemotherapeutic agents, including Pac, drug resistance frequently emerges following usage. To overcome this, a higher dose may be administered; however, this inevitably induces severe cytotoxicity in normal tissues. In this regard, a therapeutic strategy involving dual agents, particularly targeted drugs, has been evaluated to reach higher therapeutic efficacy (39). The mechanism of Pac resistance is not well-characterized; however, a number of mechanisms independent to microtubule stabilization function have been suggested (40). A large body of evidence has demonstrated that the PI3K/Akt signaling pathway, which regulates a series of cell survival- and proliferation-associated genes, is key in the development of chemoresistance (27-30).

Sox2, an important component of the 'induced pluripotent stem cell cocktail', is a member of the SOX family of transcription factors, and is critical in self-renewal of embryonic stem cells, maintenance of pluripotency, generation of induced stem cells and apoptosis. Aberrant over-expression of Sox2 has been reported in several types of tumor (16-22). Expression of Sox2 was found to be significantly increased in the prostate cancer tissues compared with normal and hyperplastic tissues (41). Sox2 is an androgen receptor-repressed gene and promotes the formation of HRPC. In a previous study, Sox2 was shown to be involved in transforming growth factor- α -induced cell proliferation and exhibit an anti-apoptotic effect in prostate cancer cells, mediated by cyclin E, p27 and survivin (38). In the present study, the results revealed that Sox2 serves as a 'safe guard' for maintaining cell proliferation, which characterizes tumor cells. In both the PC-3 Mock and PC-3 Sox2

cell groups, Pac inhibited cell growth, but the MTT and clone formation assay results revealed that, following Pac treatment, overexpression of Sox2 in the PC-3 Sox2 cells significantly promoted cell growth in comparison with the PC-3 Mock group. Furthermore, the FCM cell cycle assay indicated that overexpression of Sox2 increased the percentage of cells in S phase, which suggested that Sox2 promoted G1 to S phase progression. The G1/S checkpoint protein regulated by Sox2 was thus analyzed. In accordance with previous studies (38), the expression levels of cyclin E, which combines with cyclin-dependent kinase 2 and is essential for DNA replication, and G1/S transition were examined (42). The results revealed that cyclin E expression was upregulated in PC-3 Sox2 cells, which explains the cell cycle-promoting effects of Sox2.

In addition, the apoptosis-inducing effect of Pac was inhibited by Sox2 overexpression, as measured by an FCM apoptosis assay. To verify that this decrease in the apoptotic rate was not due to decreases in necrosis, the LDH assay was also performed, and the data did not reveal significant differences between the PC-3 Mock and Sox2 groups. This finding indicated that Sox2 affected the apoptosis-inducing effect of Pac, but not cell necrosis; and that at this concentration of Pac (5 μ M), necrosis induced by Pac was not evident.

By contrast to the PC-3 Mock cells, the phosphorylation levels of Akt were found to be significantly upregulated in the PC-3 Sox2 cells, which suggested that the cell proliferation and anti-apoptotic effects of Sox2 following Pac treatment may be mediated by activation of the PI3K/Akt signaling pathway. Survivin, a member of the inhibitor of the apoptosis-mediating protein family, is capable of regulating cell proliferation and apoptosis (43), and has been proven to be downstream of the PI3K/Akt signaling pathway (44). In the present study, survivin expression levels were detected at the protein level, and results indicated that the expression levels of survivin were significantly increased in PC-3 Sox2 cells, which may account for the anti-apoptotic effect of Sox2.

To confirm the impact of Akt activation on cell behavior, the PC-3 cells were pre-treated with LY294002, a PI3K/Akt inhibitor. The results revealed that LY294002 inhibited the upregulation of cyclin E and survivin as well as the phosphorylation of Akt induced by Sox2 over-expression. In addition, LY294002 inhibited the anti-apoptotic and G1/S transition promoting effects of Sox2 following Pac treatment. All results support the hypothesis that the effects of Sox2 are mediated by the PI3K/Akt signaling pathway. Notably, as determined by FCM, no significant differences in the percentage of apoptotic cells or the percentage of cells in S phase were identified between PC-3 Mock and PC-3 Sox2 cells receiving the same treatment (Veh or Pac), which indicated that LY294002 completely inhibited the effects of Sox2. However, markedly different S-phase distribution and apoptotic percentages were observed between Veh and Pac treatment in the same cell types (PC-3 Mock or PC-3 Sox2). This suggested that Pac impacted the cell cycle distribution and cell apoptosis in multiple ways.

In conclusion, the present study indicated that: (i) Overexpression of Sox2 exerted a drug-resistance function in PC-3 cells and may antagonize the effects of Pac; (ii) the Pac-resistance effect of Sox2 is mediated by continuous activation of the PI3K/Akt signaling pathway; and (iii) under Pac treatment, the PI3K/Akt signaling pathway promotes cell

proliferation and antagonizes apoptosis via targeting cyclin E and survivin. These results may indicate novel therapeutic methods for chemoresistant prostate cancer.

Acknowledgements

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