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ORIGINAL ARTICLE

Knockdown of zinc finger protein X-linked inhibits prostate cancer cell proliferation and induces apoptosis by activating caspase-3 and caspase-9

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Zinc finger protein X-linked (*ZFX*) is a highly conservative mammalian gene with related functions in cell survival and proliferation. However, there are limited reports on regulation of ZFX as a therapeutic target in cancer treatment. In this study, the expression of ZFX in prostate cancer with matched normal adjacent tissues ($n = 45$) and benign prostatic hyperplasia (BPH) tissues ($n = 16$) were observed by immunohistochemical analysis. The effect of lentiviral siRNA (small interference RNA)-mediated dysfunction of ZFX on the proliferation of prostate cancer cells was studied. ZFX mRNA and protein expression levels in prostate cancer cells (PC-3 and DU145) were analyzed by western blotting and real-time polymerase chain reaction (RT-PCR). The effects of siRNA targeting ZFX on growth, cell cycle and apoptosis of PC-3 cells were evaluated by MTT assay and flow cytometry. We also investigated the effect of ZFX deletion on the activation of caspase-1, -3 and -9 by western blotting and colorimetric assay. Prostate cancer specimens appeared significantly higher (42.2% of cases being positive) than that observed in normal adjacent tissues (11.8% of cases being positive) and BPH tissues (12.5% of cases being positive). Repression of ZFX in the prostate cancer cells effectively suppressed the cellular proliferation and colony-formation ability, and led to G1 phase cell cycle arrest. Moreover, inhibition of ZFX-induced cell apoptosis by activating caspase-1, -3 and -9. In conclusion, ZFX represents the prominent role in the progression of prostate cancer and may be a promising therapy target for prostate cancer.

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Keywords: prostate cancer; zinc finger protein X-linked; proliferation; apoptosis

INTRODUCTION

Prostate cancer is one of the most frequently diagnosed non-cutaneous malignancy and the sixth leading cause of cancer death in males, accounting for 14% (903 500) of the total new cancer cases and 6% (258 400) of the total cancer deaths in males in 2008.[1] Incidence and mortality rates are rising in Asian countries. Older age, race and family history remain the only well-established risk factors and there are no established preventable risk factors for prostate cancer.[2] Mutations of tumor suppressor genes in individuals suffering from prostate cancer may lead its specificity among selected group of individuals. For example, it has been found that the putative tumor suppressor gene (*PTEN/MMAC1)* on 10q23 is one of the most frequently deleted chromosomal regions in human prostate cancer.[3,4] Therefore, genome-based therapies are potentially ideal options for prostate cancer therapy.

Basic and applied research has gained new insights owing to the possibilities in site-specific manipulation of the mammalian genome. This also can be effectively used in cancer therapy. Zinc finger proteins are among the most abundant proteins in eukaryotic genomes with over 800 relevant proteins predicted in human.[5] They are involved in multiple functions, such as DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly and lipid binding.[6] There are a number of studies reporting diverse structures of zinc finger proteins corresponding to novel topologies.[6,7] Zinc finger protein X-linked (ZFX) is a member of the krueppel C_2H_2-type zinc-finger protein family and is on the X chromosome. This is structurally similar to a related gene on the Y chromosome, zinc finger protein Y-linked (*ZFY*). The X-linked ZFX and Y-linked ZFY C_2H_2-type genes are located at the very end of non-recombining regions of sex chromosomes in humans.[8] The full-length ZFX protein contains an acidic transcriptional activation domain, a nuclear localization sequence and a DNA-binding domain consisting of 13 C_2H_2-type zinc fingers.[9] Despite many years of relevant research, the physiological role of ZFX remains obscure. However, Luoh *et al.*[10] have found that in mouse embryonic and adult hematopoietic stem cells, ZFX is required as a transcriptional regulator for self-renewal in both stem cell types. Further research has found that deletion of ZFX abolished the self-renewal and decreased survival of hematopoietic stem cells, causing a severe block in cell development.[11] This evidence suggests that ZFX regulates the cell survival and activity, and it could be used as a therapeutic target to treat human diseases. Therefore, in this study, we investigated the effect of ZFX deletion on prostate cancer using cellular prostate cancer models: PC-3 and DU145.

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MATERIALS AND METHODS

Immunohistochemistry

From 2006 to 2011, 45 prostate cancer samples were obtained from radical prostatectomy paired with adjacent normal tissues, which received detailed pathological assessment and regular follow-up. These samples were from a group of patients with prostate cancer in T1c-T2b, Gleason score 6–8 and mean prostate-specific antigen $15.3 \pm 8.9\,ng\,ml^{-1}$. In addition, 16 human benign prostatic hyperplasia (BPH) tissues were obtained by transurethral resection as normal control. The study was conducted according to the regulations of the Ethics Committee of the hospital. For immunohistochemical analysis, tissue specimens were fixed in 10% formalin and embedded in paraffin. Tissue sections (4-μm) were deparaffinized and rehydrated. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol. Following antigen retrieval, the sections were blocked with 5% bovine serum albumin for 20 min at room temperature and then probed with Rabbit Anti-ZFX Polyclonal Antibody (1:300 dilution; Sigma-Aldrich, Cat # HPA003877, St Louis, MO, USA) at 4 °C overnight. After the incubation, the sections were treated with biotinylated goat anti-rabbit immune-globulins at room temperature for 1 h, and visualized using the peroxidase-conjugated streptavidin and diaminobenzidine. Then the sections were counter stained with Mayer's haematoxylin. The results were evaluated with the help of two pathologists blinded to all clinical data. Immunopositivity was scored according to the percentage of positive cells in four distinct categories: 0 for 5%, 1 for 5–10%, 2 for 10–50% and 3 for 50%. The staining intensity was then scored where 0 for negative, 1 for weak staining, 2 for intermediate staining and 3 for strong staining. Both scores were added together, resulting in a maximum staining score of 6 for any tissue score. 0–1, 2–3, 4–5 or 6 were considered negative (−), weakly positive (+), positive (++) or hadro-positive (+++) staining, respectively.

Cell culture and cell proliferation analysis

Prostate cancer cells (PC-3 and DU145) and human embryonic kidney (HEK) 293T cell line were obtained from American Type Culture Collection. Cells were maintained in Dulbecco's modified Eagle medium (Gibco, Cambrex, MD) supplemented with 10% heat-inactivated fetal bovine serum and penicillin/streptomycin at 37 °C in humidified atmosphere of 5% CO_2.

The cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Briefly, cells were washed with phosphate-buffered saline and suspended at a final concentration of 2×10^4 per ml in an assay medium and dispensed into 96-well plates. The plates were incubated at 37 °C for 1–5 days in a humidified CO_2 incubator. After the treatment time, 100 μl of MTT ($5\,mg\,ml^{-1}$) was added to each well, the plates were incubated at 37 °C for 4 h, and then 10% dimethylsulfoxide (100 μl) was added to each well. The absorbance at 490 nm was measured using a 96-well plate reader. Experiments were performed in triplicate.

Construction of ZFX short harpin RNA-expressing lentivirus

The non-silencing small interference RNA (siRNA) (si-CTRL, 5′-TTCTC CGAACGTGTCACGT-3′), which was used as the control, and ZFX siRNA (5′-GTCGGAAATTGATCCTTGTAA-3′) were inserted into the pFH-L plasmid (Hollybio, Shanghai, China), respectively. The lentivirus-based short harpin RNA-expressing vectors were constructed, confirmed by DNA sequencing and named as pFH-L-si-ZFX or pFH-L-si-CTRL. For the transfection, HEK293T cells (1×10^7) were seeded in 10-cm dishes and cultured for 24 h to reach 80% confluence. At 2 h before transfection, the medium was replaced with fetal bovine serum-free Dulbecco's modified Eagle medium. The plasmid mixture containing pFH-L-si-ZFX (or pFH-L-si-CTRL) and pVSVG-I/pCMV□R8.92 packaging vectors, as well as Lipofectamine 2000 (Invitrogen, Carlsbad, CA) were added to the cells. At 5 h after incubation, the medium was replaced with fetal bovine serum-containing Dulbecco's modified Eagle medium. Lentiviral particles (Lv-si-ZFX or Lv-si-CTRL) were harvested at 48 h after transfection and purified by ultra centrifugation according to previous reports.[12,13] As the lentivirus carries green fluorescence protein (GFP), the viral titer was determined by counting GFP-expressing cells under fluorescence microscopy 96 h after infection as described in previous reports.[14]

RNA extraction and real-time PCR analysis

Total RNA of lentivirus-infected cells was prepared using RNeasy Midi Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The first-strand complementary DNA was synthesized from total RNA (5 μg) using Super ScriptII reverse transcriptase (Invitrogen). ZFX mRNA expression was evaluated by real-time PCR using SYBR Green Master Mix Kit on DNA Engine Opticon System (MJ Research, Waltham, MA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was applied as the input reference. The primers used for ZFX detection was 5′-GGCAGTCCACAGCAAGAAC-3′ and 5′-TTGGTATCCGAGAAAGTCAGAAG-3′. The primers used for GAPDH detection was 5′-TGACTTCAACAGCGACACCCA-3′ and 5′-CACCCTGTTGCTG TAGCCAAA-3′. For relative quantification, $2^{-\Delta\Delta CT}$ was calculated and used as an indication of the relative expression levels, which was calculated by subtracting CT values of the control gene from the CT values of ZFX.[15]

Western blot

Total protein was isolated from cells that infected with lentivirus for 96 h. Cells were washed with ice-cold phosphate-buffered saline and lysed in a radioimmunoprecipitation assay buffer containing phenylmethyl sulfonylfluoride and protease inhibitors on ice for 30 min. The lysates were clarified by centrifugation at 12 000 r.p.m. for 30 min at 4 °C. The total protein was quantified by bovine serum albumin protein analysis method. Protein (20 μg) was loaded onto a 10% SDS-PAGE and transferred to polyvinylidene difluoride membrane following electrophoresis (Millipore, Billerica, MA, USA). The proteins levels of ZFX, caspase-1, -3, -9 were detected by respective antibodies using ECL kit (Amersham, Piscataway, NJ, USA) and exposed to X-ray film. GAPDH was used as loading control and detected by an anti-GAPDH antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Bands on X-ray films were quantified with an ImageQuant densitometric scanner (Molecular Dynamics, Piscataway, NJ, USA). The antibodies used were as follows: anti-ZFX (1:800 dilution; Sigma-Aldrich); anti-GAPDH (1:2000 dilution; Abcam, Cambridge, MA, USA); anti-caspase-1, anti-caspase-3, and anti-caspase-9 (1:1000 dilution; all from Abcam).

Formation of colonies

Lv-si-ZFX- or Lv-si-CTRL-infected cells (total of 800 cells per well) were seeded in six-well plates. The cells were grown by exchanging medium every 3 days until 2 weeks of culture. Then the cells were washed with phosphate-buffered saline and treated with Giemsa stain. After 30 min of staining, the cells were rinsed with distilled water. Colonies with more than 50 cells were counted under fluorescence microscopy.

Fluorescence-activated cell sorting analysis

The DNA contents of cell cycle phases can be reflected by varying propidium iodide (PI) fluorescent intensities. Therefore, cell cycle distribution of Lv-si-ZFX- or Lv-si-CTRL-infected cells was analyzed by flow cytometry assay following PI staining as described.[16] In brief, PC-3 cells were collected 96 h after infection with lentivirus containing si-ZFX and seeded in six-well plates (1×10^6 cells per well). Cells were allowed to attach overnight and collected. After washing with ice-cold phosphate-buffered saline, cells were suspended in about 0.5 ml of 70% cold alcohol and kept at 4 °C for 30 min. The cells were then treated with $100\,\mu g\,ml^{-1}$ of DNase-free RNase and incubated for 30 min at 37 °C. PI ($50\,\mu g\,ml^{-1}$; Sigma-Aldrich) was added directly to the cell suspension. The suspension was filtered through a 50-mm nylon mesh, and total of 10 000 stained cells were analyzed by a flow cytometer (FACS Cali-bur, BD Biosciences).

Annexin V-APC Apoptosis Detection Kit (BioVision, Mountain View, CA) was used to detect cell apoptosis following 5 days of lentivirus infection. In brief, 5×10^5 cells were collected by centrifugation. The cells were re-suspended in 500 μl of binding buffer. A total of 5 μl of Annexin V-APC and 5 μl of PI were added and incubated at room temperature for 5 min in the dark. Finally, cells were analyzed by FACScan (Becton-Dickinson, Franklin Lakes, NJ).

Detection of the activation of caspase-3 and -9

To detect the activation levels of caspase-3 and -9 in Lv-si-ZFX or Lv-si-CTRL-infected cells, caspase-3/caspase-9 ELISA kits from Nanjing kaiji Bio-Tek Corporation, Nanjing, China, were used. Cells (3×10^6) were harvested, lysed and treated with 50 μl of 2× reaction buffer followed by the treatment with 5 ml of caspase-3 or caspase-9 substrate. After incubation for 4 h, the samples were assayed using an ELISA reader (Bio-Rad, Hercules, CA) at 405 nm.

Statistical analysis

All data were expressed as mean ± s.d. of three independent experiments, in which each assay was performed in triplicate. The Student's *t*-test was

Table 1. Expression pattern of ZFX in prostate cancer tissues and normal tissues

Type of tissues	*Number of cases*	*ZFX-negative*	*ZFX-positive*			P-*value*[a]
		−	+	++	+++	
Cancer tissues	45	26 (57.8%)	15 (33.3%)	3 (6.7%)	1 (2.2%)	0.035
Adjacent tissues	34	30 (88.2%)	4 (11.8%)	0 (0%)	0 (0%)	
Normal tissues	16	14 (87.5%)	2 (12.5%)	0 (0%)	0 (0%)	

[a]Based on Fisher's exact test.

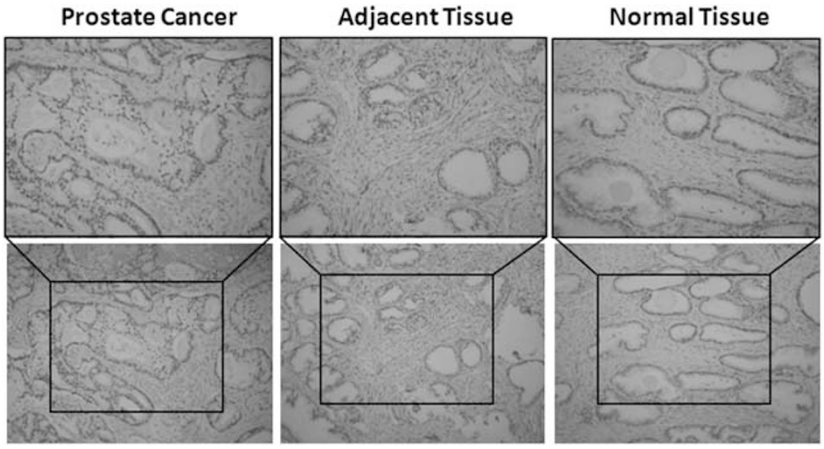

Figure 1. Zinc finger protein X-linked (ZFX) expression levels were higher in prostate cancer tissues. Immunohistochemical analysis of ZFX expression levels in prostate cancer tissue, adjacent tissue and benign prostatic hyperplasia (BPH) tissue. Images were taken following immunohistochemical staining at $\times$ 100 (upper panel) and $\times$ 200 (lower panel) magnification. Data are representatives of 45 human prostate cancer specimens and 16 BPH tissues.

used to evaluate the differences and a *P*-value of <0.05 was considered statistically significant. Fisher's exact test was performed to compare the expression of ZFX in tumor, normal and BPH tissues.

RESULTS

ZFX expression levels in prostate cancer tissues

ZFX expression levels in prostate cancer tissues, adjacent tissues and BPH tissues were observed by immunohistochemical staining and data were shown in Table 1 and Figure 1. Of the 45 cases of prostate cancer tissues, 19 cases (42.2%) showed positive ZFX immunopositivity. Whereas in adjacent tissues and BPH tissues, only 11.8 and 12.5% specimens showed ZFX-positive expression, where no positive staining or hadro-positive staining was seen in the non-tumorous tissues adjacent to the tumors. Notably, the prostate cancer tissues exhibited significantly higher ZFX expression than BPH and adjacent tissues. The χ^2 value was 10.967 and the significance of bilateral exact test is 0.035 by Fisher exact test. Therefore, it is clear that ZFX is positively contributing to the pathogenesis and progression of prostate cancer.

Expression of ZFX was suppressed by infection with Lv-si-ZFX in prostate cancer cells

ZFX is a highly conserved gene in human cells. ZFX protein expression levels in prostate cancer and HEK293 cells were analyzed by western blotting. As depicted in Figure 2a, all three cell lines expressed ZFX protein, whereas the highest expression level was observed in PC-3 prostate cancer cells followed by DU145 prostate cancer cells. To suppress ZFX expression level, lentivirus that expresses ZFX-specific siRNA or control siRNA was infected to PC-3 and DU145 prostate cancer cells (multiplicity of infection 50). Successful infection was observed after 96 h, and more than 90% of cells in both cell lines expressed GFP (Figure 2b). Following lentivirus infection, expression levels of ZFX mRNA and protein were analyzed. ZFX mRNA levels in Lv-si-ZFX-infected cells were significantly suppressed corresponding to 70% and 65% suppression in PC-3 and DU145 cells ($P<0.01$), respectively, compared with control cells (Figure 2c). As expected, the protein expression levels of ZFX in both cell lines were significantly suppressed due to Lv-si-ZFX-infection ($P<0.01$, Figure 2d). Therefore, it is evident that siRNA targeting ZFX is effective in silencing *ZFX* gene in prostate cancer cells (PC-3 and DU145).

Infection with Lv-si-ZFX suppressed cell proliferation in prostate cancer cells

The effect of Lv-si-ZFX infection on the cell proliferation was investigated in prostate cancer cells by using MTT assay. The cell proliferation was observed 5 days after infection and the ZFX deletion has significantly reduced the cell proliferation compared with control lentivirus-infected cells ($P<0.01$, Figures 3a and b). Furthermore, the lentivirus infection with Lv-si-ZFX significantly reduced the colony numbers accounting for 45% reduction in PC-3 cells (Figure 3c) and 55% reduction in DU145 cells (Figure 3d). Therefore, it can be concluded that ZFX knocking down effectively suppresses the cellular proliferation in prostate cancer cells.

ZFX knocking down affects the cell cycle progression in PC-3 cells

ZFX was found to be an effective contributor in cell survival.[17] Then, the effect of siRNA-induced downregulation of ZFX on the cell cycle progression of PC-3 cells was analyzed using FACS

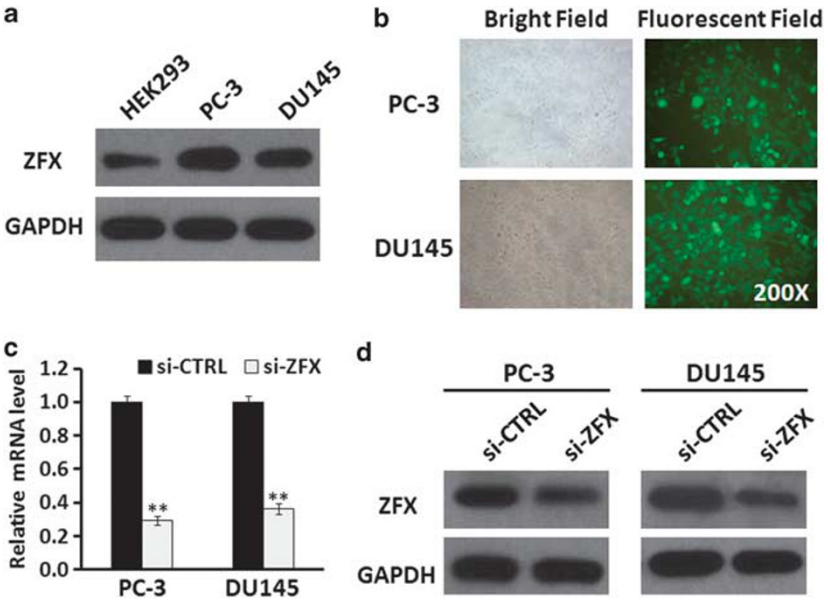

Figure 2. Zinc finger protein X-linked (ZFX) small interference RNA (siRNA) suppressed the ZFX mRNA and protein expression levels in prostate cancer cells. (**a**) ZFX expression levels in non-infected prostate cancer cells (PC-3 and DU145) and HEK293 cells. (**b**) Green fluorescence protein (GFP) fluorescence levels of PC-3 and DU145 cells 96 h after infection with lentivirus containing si-ZFX at the magnification of $\times 200$. (**c**) Quantitative real-time PCR data of the ZFX mRNA levels following the infection with Lv-si-ZFX compared with non-Lv-si-CTRL-infected cells. Data were expressed as the fold changes. (**d**) Western blotting data of ZFX protein expression levels following Lv-si-ZFX infection compared with Lv-si-CTRL-infected cells. The values represent the mean from three independent experiments. $^{**}P < 0.01$ in comparison with control.

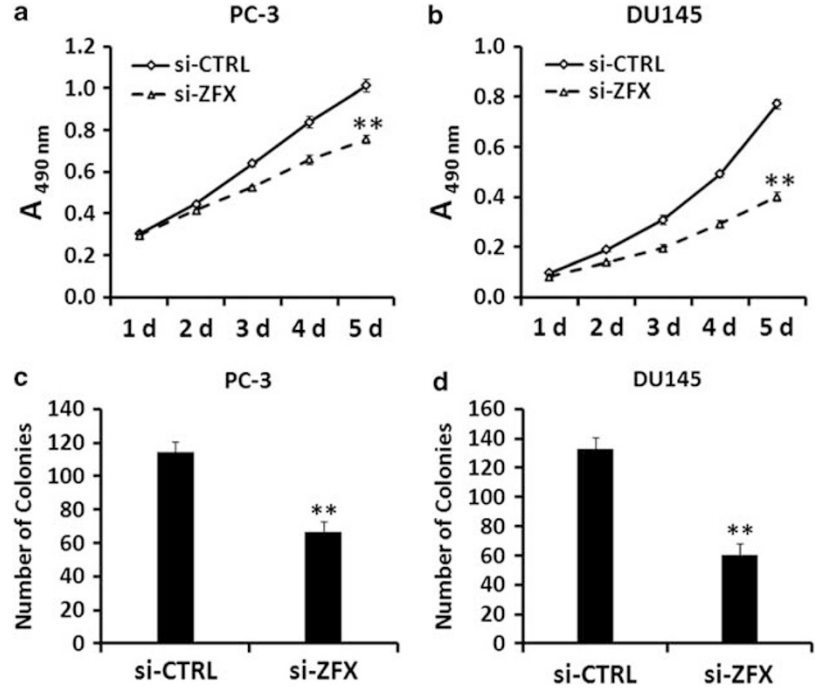

Figure 3. The proliferation of PC-3 and DU145 cells were suppressed by Lv-si-ZFX infection. The cell growth was measured using MTT assay. (**a**) Cellular proliferation pattern of Lv-si-ZFX infected and control PC-3 cells. (**b**) Cellular proliferation pattern of Lv-si-ZFX-infected and control DU145 cells. (**c**) Quantitative analysis of colony formation of Lv-si-ZFX-infected and control PC-3 cells. (**d**) Quantitative analysis of colony formation of Lv-si-ZFX-infected and control DU145 cells. The values represent the mean from three independent experiments. $^{**}P < 0.01$ in comparison with control.

(Figure 4a). In the control group, the G1 and S phase accounted for cell percentages of 51% and 34%, respectively. Whereas following Lv-si-ZFX infection, the corresponding cell percentages of G1 and S phases were 59% and 26%, respectively (Figure 4b). Apparently, infection with ZFX siRNA-expressing lentivirus has arrested the cell cycle progression in G1 the phase compared with the control cell group. It indicates that the higher numbers of cells arrested in the G1 phase may contribute to the suppression of cell proliferation.

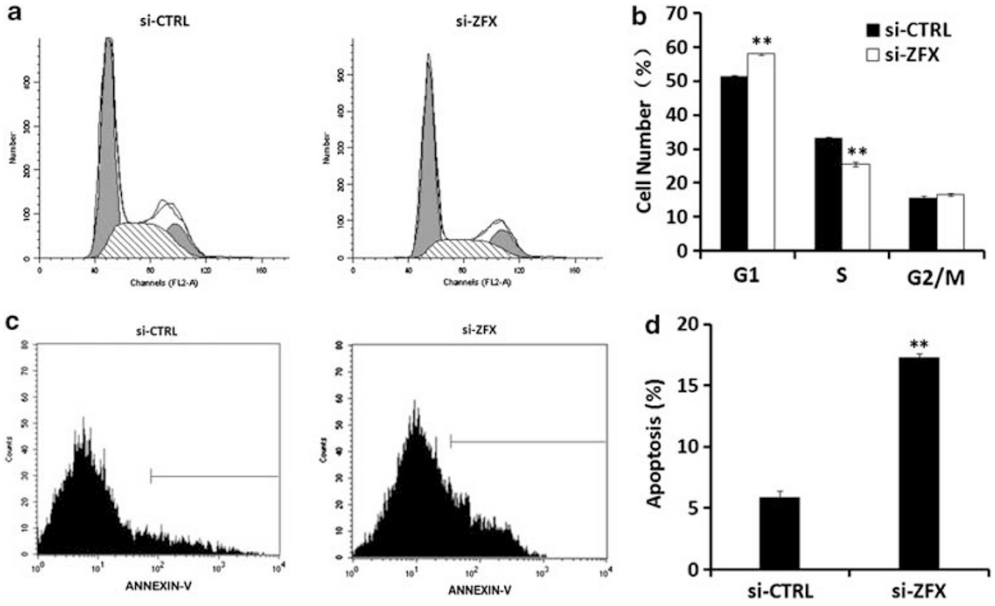

Figure 4. Zinc finger protein X-linked (ZFX) knockdown induced cell cycle arrest at G1 phase and induced apoptosis. (**a**) The flow cytometric analysis of cell cycle of control PC-3 cells or ZFX knockdown PC-3 cells 96 h after transient infection. (**b**) The percentages of PC-3 cells at different phases. (**c**) FACS analysis of Annexin V-APC-stained control cells or ZFX knockdown cells 96 h after transient infection. (**d**) The percentage of apoptotic cells in ZFX knockdown group and control group. The values represent the mean from three independent experiments. $^{**}P<0.01$ in comparison with control.

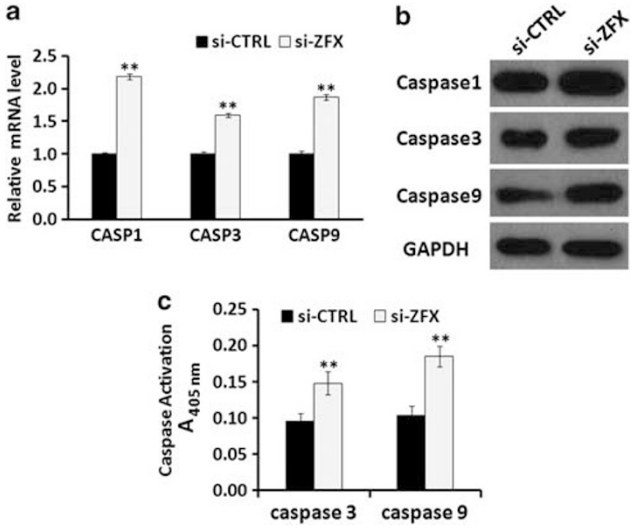

Figure 5. Zinc finger protein X-linked (ZFX) knockdown induces the expression and activity of effector caspases. (**a**) Upregulation of caspase-1, -3, -9 mRNA expression levels in ZFX knocked-down PC-3 cells. (**b**) Western blotting data of upregulation of caspase-1, -3 and -9 protein expression levels in ZFX knocked-down PC-3 cells. (**c**) The activation levels of caspase-3 and -9 in ZFX siRNA-infected and control PC-3 cells. The values represent the mean from three independent experiments. $^{**}P<0.01$ in comparison with control.

ZFX depletion enhanced the apoptosis rates in PC-3 cells

To further confirm that knockdown of ZFX reduces the cell survival rates, the presence of apoptotic cells with or without ZFX knocking down were compared in PC-3 cells by FACS analysis and followed by Annexin VPC/PI staining. As shown in Figures 4c and d, deletion of ZFX has significantly increased the number of apoptotic cells ($P<0.01$), by 11% higher level compared with the control. It clearly shows that the ZFX knocking down leads to apoptosis, which may be another reason to repress cell proliferation.

ZFX repression induces the expression of effector caspases

Effector caspases are activated in the onset of apoptosis. It is found that when the ZFX is repressed, the level of apoptosis is increased in PC-3 cells. Caspases are important proteins involved in apoptosis, as it cleave the cellular components and lead to cell death process. Therefore, the effects of ZFX repression on the apoptosis by the activation of caspases are evaluated. As shown in Figures 5a and b, both mRNA and protein levels of effector caspases-1, -3, -9 were increased in ZFX-deleted group compared with the control group. Furthermore, the activation levels of caspase-3 and caspase-9 were significantly increased with Lv-si-ZFX infection ($P<0.01$, Figure 5c). These findings suggest that the ZFX repression lead to apoptosis via activation of caspases in PC-3 cells.

DISCUSSION

The ZFY- and ZFX-related zinc finger genes comprise a highly conserved vertebrate gene family.[18] These are incorporated into sex chromosomes.[19] However, there have been limited studies related to ZFX. ZFX as a transcription factor would promote the transcription of downstream target genes such as growth factors or oncogenes.[20] Therefore, ZFX is believed to have an important role in tumor progression and pathogenesis. With the light of past research, this study was planned to find the effects of ZFX knockdown in prostate cancer cells as a therapeutic target. However, ZFX silencing as a therapeutic target was less studied except the resent report on its functions in human laryngeal squamous cell carcinoma.[21]

Initially, the transcriptional levels of ZFX in prostate cancer tissues were examined, while comparing it with normal tissues. The immunohistochemical data revealed that the ZFX levels were increasing in prostate cancer tissues compared with the BPH tissues and adjacent tissues. The excessive activation of ZFX in cancer tissue would attribute to the similar phenomenon where

the, ZFX analog, ZFY transcription activates in human prostate cancer cells by deregulation of other regulatory genes.[16] Therefore, we hypothesized whether the elevated expression level of ZFX would have an effective role in caner progression. To confirm the hypothesis, the study was continued with analysis of the biological effects after deletion of ZFX in prostate cancer cells. Both PC-3 and DU145 prostate cancer cells showed higher expression levels of ZFX. However, PC-3 cells showed slightly higher levels of ZFX expression at normal levels and less expression levels after knocking down ZFX. Hence, PC-3 cells were used for further investigations about the underlying molecular mechanisms of which ZFX regulates prostate cell proliferation.

To suppress the expression of *ZFX* gene, prostate cancer cells were treated with GFP-containing lentivirus that expresses ZFX siRNA and the successful infection was confirmed as shown in Figure 2b. Lentivirus is widely applied in transfecting genes, as it integrates with the host genome effectively and is considered safe for humans.[22] The PC-3 cell survival rates were significantly reduced with Lv-si-ZFX infection. This reduction of cell proliferation was attributed to the cell cycle arrest at the G1 phase. Furthermore, infection with ZFX siRNA induced the apoptosis of infected PC-3 cells. In further investigation, we found that the deletion of ZFX led to upregulation of apoptosis-related downstream signaling. The effector caspases, which have a major role in apoptosis, were significantly activated when ZFX was repressed in prostate cancer cells.

In conclusion, this study reveals that ZFX is a critical transcription factor in prostate cancer cell survival and when ZFX was deleted, the cancer cell survival rates were significantly reduced. Therefore, knocking down of ZFX would be an ideal therapeutic approach to treat prostate cancer. Furthermore, to the best of our knowledge, this is the first study to examine the effect of ZFX in prostate cancer. Further investigation regarding the functional role of ZFX may help to better understand prostate cancer progression.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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