Original Article

DOI: 10.5582/bst.2016.01045

Knockdown of prostaglandin reductase 1 (PTGR1) suppresses prostate cancer cell proliferation by inducing cell cycle arrest and apoptosis

Li Xue*, Zhen Zhu*, Ziming Wang, Hecheng Li, Peng Zhang, Zhenlong Wang, Qi Chen, Haiwen Chen, Tie Chong**

Department of Urology, The Second Affiliated Hospital, Xi'an Jiaotong University, Xi'an, Shannxi, China.

Summary **Chemoresistance is a serious problem for the treatment of androgen-independent prostate cancer (PC). The underlying molecular mechanisms by which androgen-independent PC cells acquire the capacity to proliferate remain largely unclear. The aim of this study was to investigate the biological role of prostaglandin reductase 1 (PTGR1) in prostate cancer. Data from the Oncomine database showed that PTGR1 is commonly upregulated in PC tissue in comparison to corresponding normal controls. Two PTGR1-specific short hairpin RNA (shRNA) sequences were used to block the expression of PTGR1 *via* a lentivirus-mediated system in the androgen-independent PC cell lines DU145 and PC 3. Functional analysis revealed that knockdown of PTGR1 significantly inhibited proliferation and colony formation by PC cells. The inhibition of cell proliferation was related to arrest of the cell cycle in the G0/G1 phase and increased apoptosis in response to PTGR1 knockdown as indicated by flow cytometry. PTGR1 silencing was found to mechanically enhance the expression of p21, caspase 3, and cleaved PARP and to decrease the level of cyclin D1. In conclusion, PTGR1 plays an essential role in PC cells and may be a potential therapeutic target for PC.**

Keywords: PTGR1, prostate cancer, proliferation, apoptosis, cell cycle

1. Introduction

Prostate cancer (PC) is the second most common cancer worldwide (*1*) and has the highest incidence among cancers affected men (*2*). Androgen deprivation therapy (ADT) is the primary treatment for men with PC. However, almost all patients eventually develop an androgen-independent form of PC that is highly metastatic (*3*). Androgen-independent PC is usually highly chemoresistant to conventional chemotherapeutic agents (*4*), hampering the development of effective approaches to treat chemoresistant PC. How PC acquires the capacity to proliferate despite androgen deprivation remains largely unknown. Therefore, new molecules need to be identified to overcome limitations in chemoresistance.

Human prostaglandin reductase 1 (PTGR1) is a highly inducible enzyme with enone reductase activity and can transform prostaglandins by enone reduction (*5,6*). A key activating enzyme, PTGR1 has been reported to significantly influence the activity of acylfulvenes, a class of antitumor agents (*7,8*). Moreover, overexpression of PTGR1 can improve the efficacy of antitumor chemotherapeutic agents (*9*). In addition, PTGR1 is the top-ranked protein and it possesses dual activity in tumor samples (*10*). Recent studies have reported that PTGR1 is overexpressed in liver carcinogenesis (*11*) and bladder cancer (*12*). Dick *et al*. indicated that overexpressed PTGR1 has a positive effect on cell viability (*13*). A recent study found that PTGR1 may play a role in the progression of gastric cancer and the study deemed PTGR1 to be a potential biomarker (*14*). However, the molecular

Released online in J-STAGE as advance publication April 30, 2016.

*These authors contributed equally to this works.
***Address correspondence to:*
Dr. Tie Chong, Department of Urology, The Second Affiliated Hospital, Xi'an Jiaotong University, Xi'an, 710004, China.
E-mail: chongtie123@126.com

mechanism by which PTGR1 acts in PC has rarely been investigated.

To investigate the biological function of PTGR1 in the progression of PC, the expression of PTGR1 was first determined using the Oncomine database. PTGR1 was then silenced in two stable cell lines, DU 145 and PC 3 (both are androgen-independent PC cell lines). The effects of PTGR1 knockdown on cell proliferation, cell cycle progression, and apoptosis were subsequently determined.

2. Materials and Methods

2.1. *Analysis of gene expression using the Oncomine database*

PTGR1 gene expression was analyzed using microarray gene expression datasets from the Oncomine database (*http://www.oncomine.org*). To determine the differences in expression of PTGR1 in PC and normal tissue, a combined filter was used to display the corresponding datasets. Briefly, the Cancer Type was defined as PC, the Data Type was mRNA, and the Analysis Type was Cancer versus Normal Analysis.

2.2. *Construction of lentiviral vectors*

Two PTGR1 short hairpin RNAs (shRNAs) were designed and cloned in pLV-GV115-lentiviral vectors (Shanghai Genechem Biotechnology, Shanghai, China) between the *Age*I and *Eco*RI restriction sites. The sequences of shRNA were as follows: shPTGR1#1: CCAGGTCTTTCACTGAACCAT and shPTGR1#2: GCAGACACACTGACTTCTCGA. A scrambled plasmid was used as a negative control (referred to as scrambled).

2.3. *Cell culture and infection*

The human PC cell lines PC 3, DU 145, and LNCap were cultured in the laboratory and maintained in RPMI-1640 culture medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) in a humidified chamber at 37°C in 5% CO_2. For lentiviral infection, PC 3 and DU 145 cells were cultured in 6-well plates and a lentivirus expressing PTGR1 shRNA (shPTGR1#1 or shPTGR1#2) or a scrambled lentivirus was added at a multiplicity of infection (MOI) of 20 for 96 h. The knockdown efficiency of PTGR1 was subsequently determined with quantitative real-time PCR and Western blot analysis.

2.4. *RNA extraction and real-time PCR*

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Approximately 500 ng of total RNA was reverse transcribed to cDNA using High-Capacity cDNA Reverse Transcription Kits (RR036A, TaKaRa). Primers for PTGR1 and beta-actin were designed as follows: PTGR1 forward, 5'-TCCTCCTGTGACCCTTTCGG-3' and reverse, 5'-GAAGGCGGCTGGGACTGC-3'; beta-actin forward, 5'-ACCGAGCGCGGCTACAGC-3' and reverse, 5'-CTCATTGCCAATGGTGAT-3'. Real-time PCR was carried out in a Bio-Rad CFX 96 Real-time PCR system. All samples were examined in triplicate. The relative level of PTGR1 expression was normalized to beta-actin using the comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method.

2.5. *Western blot analysis*

Cell were collected after treatment and lysed with lysis buffer (10 mM EDTA, 100 mM Tris-HCl, 4% SDS, and 10% Glycine). Each sample protein concentration was determined with a Bradford assay (Beyotime, China). Proteins were then subjected to polyacrylamide-sodium dodecyl sulfate (SDS-PAGE) electrophoresis and transferred onto PVDF membranes (Millipore, Bedford, MA, USA). Protein blots were probed with the indicated primary antibodies, including anti-PTGR1 (1:1,000, #ap5941c, Abgent), anti-p21 (1:1,000, SC-397, Cell Signaling Technology, USA), anti-Cyclin D1 (1:1,000, #6542, SAB), anti-caspase 3 (1:500, #9661, Cell Signaling Technology, USA), anti-PARP (1:1,000, #9542, Cell Signaling Technology, USA), and appropriate secondary antibodies (anti-GAPDH, 1:3,000, Proteintech, Chicago, IL, USA). Protein bands were visualized with an ECL kit (Pierce) according to the manufacturer's instructions. GAPDH was used as an internal control.

2.6. *CCK-8 assay*

The effect of PTGR1 on cell growth was determined using a CCK-8 assay. Briefly, cells were plated on 96-well plates at a density of 2,000 cells/well after transfection for 96 h. Ten µl of 10 mg/mL CCK-8 solution was added to each well 24, 48, 72, 96, and 120 h after transfection, and plates were incubated at 37°C for 2 h. The absorbance of each well was measured using a microplate reader (Bio-Rad, Hercules, CA, USA) at 450 nm. The CCK-8 assay was performed 3 times for all samples.

2.7. *Analysis of colony formation*

Cells were seeded in six-well plates at a density of 500 cells/well and cultured for 10 days. Colonies were fixed with 10% formaldehyde for 5 min followed by staining with 1.0% crystal violet for 30 s. Single colonies containing > 50 cells were considered to be viable and the number of those colonies was counted. Colony formation was determined 3 times for all samples.

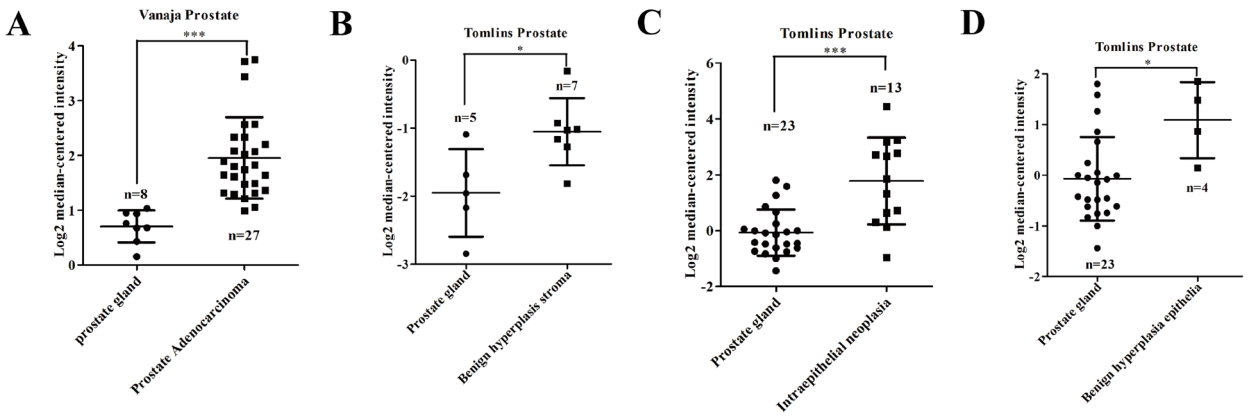

Figure 1. PTGR1 expression is upregulated in prostate cancer. The expression of PTGR1 mRNA in Oncomine datasets including Vanaja Prostate **(A)** and Tomlins Prostate **(B-D)**. *$p < 0.05$, ***$p < 0.001$ compared to normal control tissue.

2.8. *Analysis of the cell cycle*

Cells were harvested, washed using cold phosphate buffer saline (PBS), and then fixed in 70% ethanol for 30 min at 4°C. Then cells were resuspended in PBS containing 2 μg/mL RNAse and incubated at 37°C for 30 min. After extensive washing, the cells were incubated with 50 μg/mL PI for 1 h at room temperature and then subjected to flow cytometric analysis using a FACSCalibur (BD Biosciences, Bedford, MA, USA). The cell cycle was analyzed 3 times for all samples. The results are presented as the percentage (%) of cells in each phase of the cell cycle (G0/G1, S, and G2/M phase).

2.9. *Analysis of apoptosis*

Apoptosis of cells was analyzed with flow cytometry using Annexin V-PE/7-AAD double staining. Briefly, cells were harvested and seeded in 6-cm dishes at a density of 1×10^6 cells. After culturing for 48 h, cells were collected and subjected to Annexin V-PE/7-AAD double staining according to the manufacturer's instructions (eBioscience, San Diego, CA, USA). Flow cytometry analysis was performed as mentioned above. Apoptosis was analyzed 3 times for all samples.

2.10. *Statistical analysis*

Quantitative data from each experiment were analyzed using SPSS Version 10.0 Software (SPSS Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation (SD) of three independent experiments. Graphical representations were rendered using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, California, USA) software. The Student's *t*-test (two-tailed) was used to evaluate the differences between groups. $p < 0.05$ was considered statistically significant.

3. Results

3.1. *PTGR1 expression is upregulated in PC tissue*

To investigate the pattern of PTGR1 expression in PC, the difference in expression of PTGR1 mRNA in PC tissue and normal tissue was determined by analyzing microarray gene expression datasets from the Oncomine database. The expression of PTGR1 increased significantly in PC tissue compared to corresponding normal prostate tissue (Figures 1A and 1C, $p < 0.001$). Of particular note is the fact that the level of PTGR1 mRNA was markedly higher in hyperplastic prostate tissue compared to the normal prostate (Figures 1B and 1D, $p < 0.05$). These findings indicated a close relationship between the expression of PTGR1 and PC.

3.2. *shRNA-mediated knockdown of PTGR1 was effective in PC cells*

To determine the potential oncogenic role of PTGR1 in PC, the expression of PTGR1 was determined in three PC cell lines. The level of PTGR1 protein was higher in PC3 and DU145 cells than that in LNCap cells (Figure 2A). Lentiviral shRNA was successfully constructed and transfected into PC3 and DU145 cells, as verified by both real-time PCR and Western blotting. As shown in Figure 2B, the level of PTGR1 mRNA decreased significantly by 93.1% in DU145 cells infected with shPTGR1#1 and 82.9% in DU145 cells infected with shPTGR1#2 ($p < 0.001$). Moreover, the level of PTGR1 protein expression in DU145 cells decreased as a result of infection with shPTGR1#1 and shPTGR1#2 (Figure 2C). In addition, both the levels of PTGR1 mRNA and protein decreased markedly in PC3 cells infected with a lentivirus expressing PTGR1 shRNA compared to the control group (Figure 2C, $p < 0.001$). Compared to shPTGR1#2, shPTGR1#1 may have greater ability to downregulate PTGR1 expression in DU145 and PC3 cells.

3.3. *Inhibition of PTGR1 suppressed cell growth and colony formation*

To assess the role of PTGR1 in regulating cell

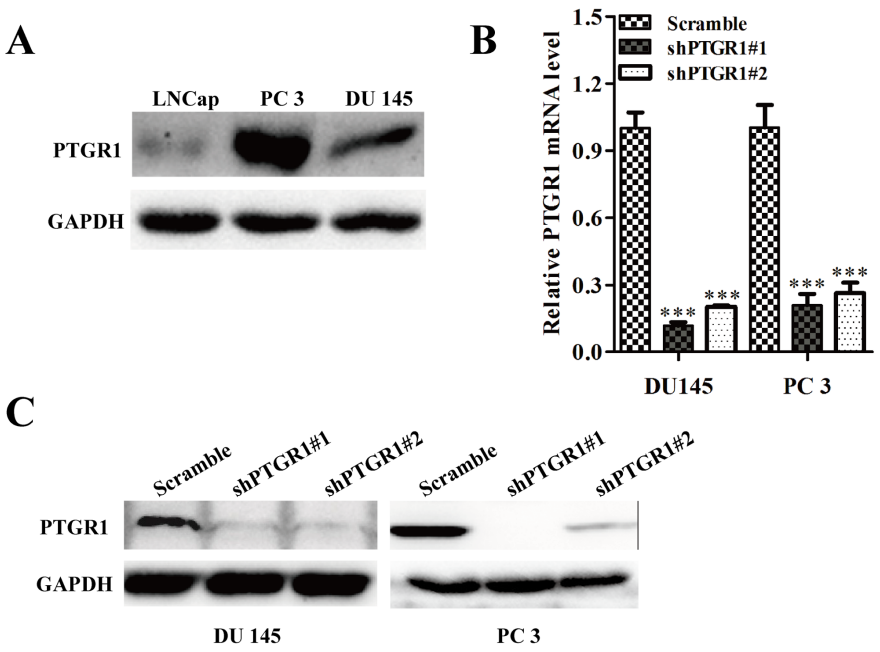

Figure 2. Silencing of PTGR1 expression in DU145 and PC3 cells by shPTGR1#1 or 2. (A) The expression of PTGR1 in PC cell lines, including DU145, PC3, and LNCap. **(B)** Real-time PCR analysis of PTGR1 mRNA levels in DU145 and PC3 cells following shPTGR1#1 or 2 infection. Values are presented as the mean ± standard deviation (SD). ***$p < 0.001$ compared to scrambled cells. **(C)** Western blot analysis of PTGR1 protein levels in DU145 and PC3 cells following shPTGR1#1 or 2 infection.

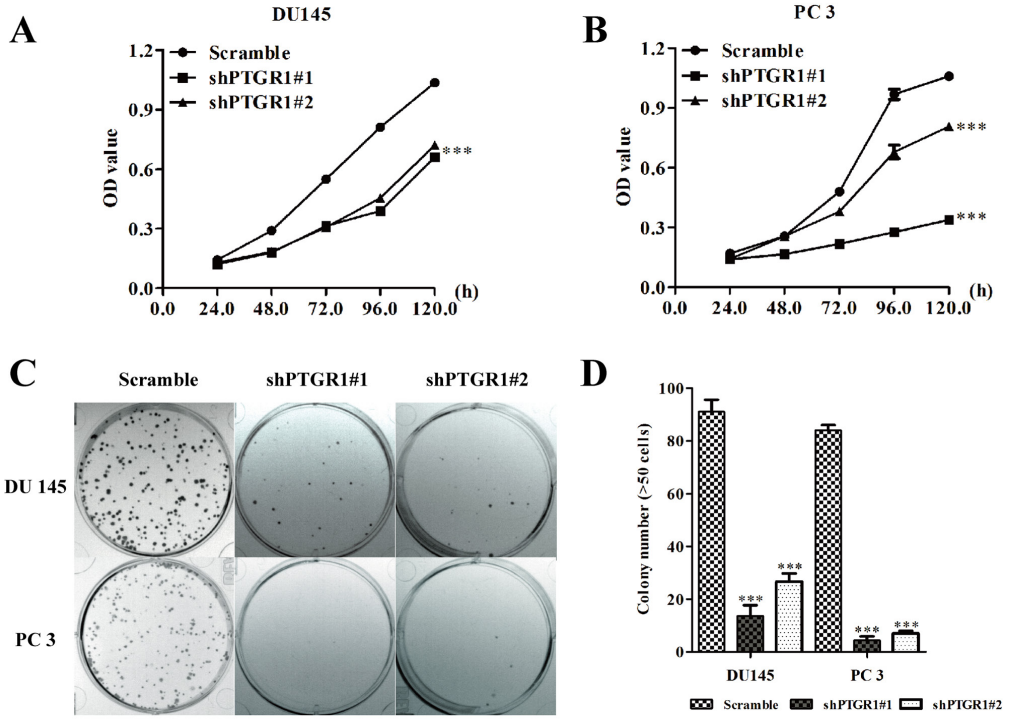

Figure 3. Knockdown of PTGR1 inhibited prostate cancer cell proliferation. (A and **B)** A CCK-8 assay revealed that PTGR1 silencing suppressed cell growth in DU145 and PC3 cells following shPTGR1#1 or 2 infection. A colony formation assay revealed that knockdown of PTGR1 impaired colony formation. Representative micrographs **(C)** and quantification **(D)** of colonies (> 50 cells per colony). Values are presented as the mean ± standard deviation (SD). ***$p < 0.001$ compared to scrambled cells.

proliferation, a CCK-8 assay was performed on DU145 and PC3 cells following lentiviral infection for 96 h. As shown in Figures 3A and 3B, the number of viable cells was much lower in cells infected with shPTGR1#1 and shPTGR1#2 ($p < 0.001$) than in scrambled cells. Moreover, a colony formation assay was performed to provide additional insight into the effects of PTGR1 on cell proliferation. Representative images of colony size and colony numbers are shown in Figure 3C. Smaller and fewer colonies were seen in cells infected with shPTGR1#1 and shPTGR1#2. Statistical analysis suggested that knockdown of PTGR1 significantly impaired colony formation in both DU145 and PC3 cells (Figure 3D, $p < 0.001$).

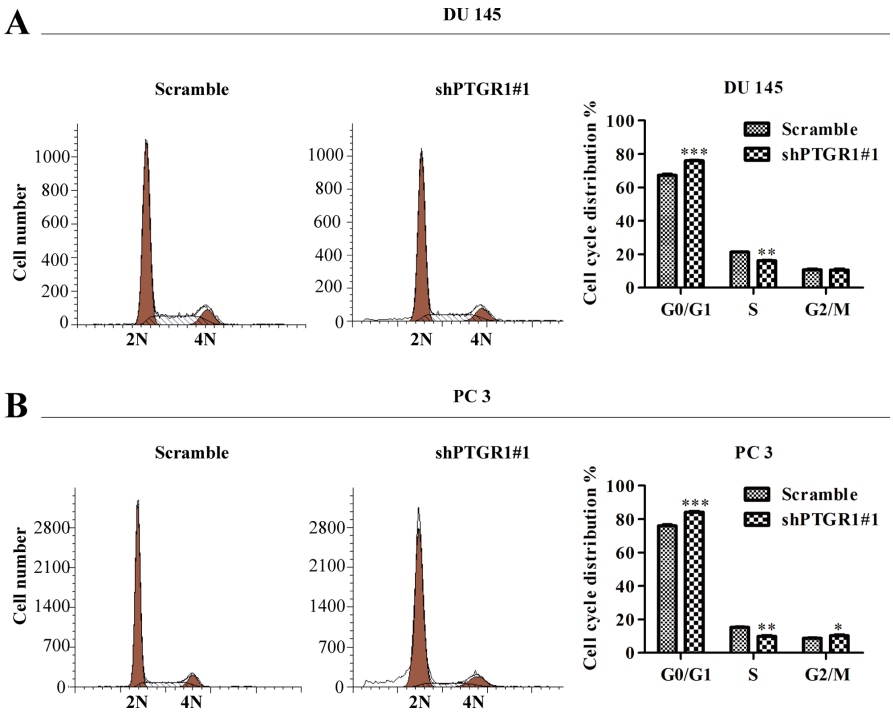

Figure 4. PTGR1 silencing blocked cell cycle progression in prostate cancer cells. Flow cytometric analysis of cell cycle distribution revealed that PTGR1 silencing led to an increase of cells in G0/G1 phase in DU 145 **(A)** and PC 3 cells **(B)**. Values are presented as the mean ± standard deviation (SD). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to scrambled cells.

3.4. *Knockdown of PTGR1 induced G0/G1 cell cycle arrest and apoptosis*

Cell cycle regulation is known to play an important role in cell proliferation. To investigate the mechanism involved in the inhibition of cell proliferation mediated by shPTGR1 in DU145 and PC3 cells, flow cytometry was used to study the effects of shPTGR1#1-mediated downregulation of PTGR1 on cell cycle progression. As shown in Figure 4A, flow cytometry analysis revealed that knockdown of PTGR1 induced a marked effect on cell cycle progression. Data show that the percentage of cells in the G0/G1 phase increased from 65.58 ± 0.66 in scrambled cells to 78.22 ± 0.63 in cells infected with shPTGR1#1 ($p < 0.001$). The percentage of cells in the S phase decreased from 25.18 ± 0.71 in scrambled cells to 17.8 ± 0.93 in DU145 cells infected with shPTGR1#1 ($p < 0.01$). Similar results were also apparent in PC3 cells (Figure 4B, $p < 0.001$, $p < 0.01$). Data revealed that PTGR1 contributed to the transition from the G0/G1 phase to the S phase and that PTGR1 plays an important role in cell cycle progression.

Whether shPTGR1#1 has any effect on apoptosis was also determined. Flow cytometry was used to assay the apoptosis of DU145 and PC3 cells. As shown in Figures 5A and 5C, shPTGR1#1 did cause significant changes in the profile of Annexin V-stained cell populations in DU145 and PC3 cells. Annexin V-PE *vs*. 7-AAD plots from gated cells showed that the populations corresponded to early (Annexin V+/7-AAD-) and late apoptotic (Annexin V+/7-AAD+) cells. Statistical analysis revealed that knockdown of PTGR1 increased the number of apoptotic cells, including early and late apoptosis, by nearly 2-fold compared to scrambled DU145 (Figure 5B, $p < 0.001$) and PC3 cells (Figure 5D, $p < 0.001$). Knockdown of PTGR1 can suppress the proliferation of PC cells through arrest of the cell cycle and apoptosis.

3.5. *Signs of the loss of cell cycle regulation and apoptosis due to PTGR1 knockdown*

To explain the arrest of the cell cycle and apoptosis caused by the knockdown of PTGR1, the expression of key proteins involved in these processes was determined to verify the findings obtained thus far. As shown in Figure 6, P21, a key cell cycle inhibitor, was upregulated in PC3 cells after PTGR1 knockdown. The level of expression of cyclin D1, which is associated with the G1/S transition, decreased in cells infected with shPTGR1#1. The level of expression of cleaved-PARP and caspase 3 increased in PC 3 cells following shPTGR1#1 infection. These findings suggest that knockdown of PTGR1 induced arrest of the cell cycle and apoptosis by regulating key proteins.

4. Discussion

PTGR1 is a nitroalkene reductase that has been found to enhance the susceptibility of cancer cells to chemotherapeutic agents and that has been found to be involved in cancer progression. Mounting evidence indicates that androgen-independent PC is less sensitive to chemotherapeutics (*15,16*). The current study used

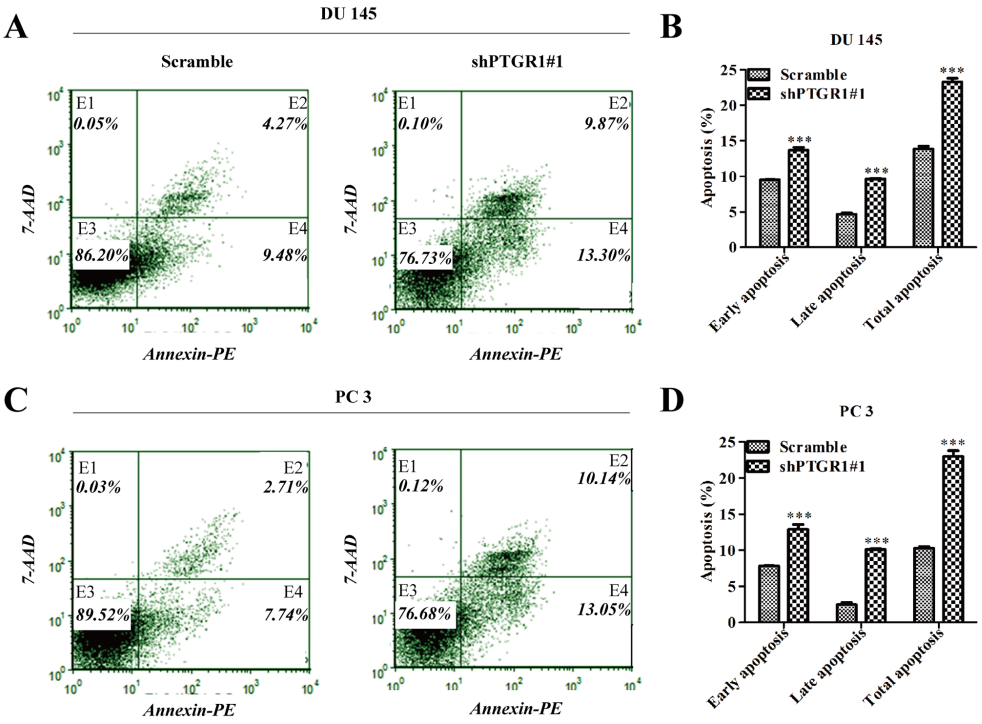

Figure 5. Knockdown of PTGR1 enhanced cell apoptosis in prostate cancer cells. (A and **C)** Representative images showing annexin V-PE staining in the scrambled cells transfected with shRNA and cells infected with shPTGR1#1 lentivirus. **(B** and **D)** Quantification of A and C. Values are presented as the mean ± standard deviation (SD). ***$p < 0.001$ compared to scrambled cells.

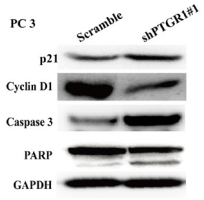

Figure 6. PTGR1 regulated key regulators associated with cell cycle regulation and apoptosis. Western blot analysis of p21, cyclin D1, cleaved-PARP, and caspase 3 protein levels in PC 3 cells following shPTGR1#1 infection.

the Oncomine database to determine the expression of PTGR1 in PC. Results revealed that the expression of PTGR1 mRNA was significantly upregulated in PC tissue compared to normal tissue. PTGR1 was closely associated with prostatic hyperplasia (Figure 1A). Two different PTGR1 shRNAs were selected to specifically knockdown the endogenous expression of PTGR1 in human androgen-independent PC cell lines DU145 and PC3 in order to further investigate the role of PTGR1 in PC. Functional analysis indicated that knockdown of PTGR1 suppressed the proliferative ability of DU145 and PC3 cells, causing G0/G1 arrest and inducing the apoptosis of PC cells.

To the extent known, cell cycle deregulation has been acknowledged as a hallmark of cancer progression in most malignant tumors (*17*). The cell cycle consists of three distinct sequential phases (G0/G1, S and G2/M) (*18*) and is regulated by cyclins and cyclin-dependent kinase inhibitors (CDKIs) (*19*). Cyclins are closely related to cell cycle progression and provide domains that are essential for enzymatic activity (*20*). Cyclin D1 expression is essential for the G1/S transition. A CDKI, p21 has been reported to inhibit the expression of CDKs, leading to arrest of the cell cycle G1-S transition (*21,22*). The current results revealed that knockdown of PTGR1 reduced the expression of cyclin D1 and enhanced the expression of p21. Therefore, one could reasonably assume that the mechanisms by which silencing of PTGR1 arrests the G1/S transition may be by partly regulating cyclin D1 and p21.

Previous studies have reported that PTGR1 is closely associated with gastric cancer (*14*) and liver carcinogenesis (*11*). Consistent with these reports, the current results revealed that knockdown of PTGR1 induced the apoptosis of androgen-independent PC cells. Moreover, this mechanism involved activation of caspase 3 and subsequent amplification of PARP cleavage. Apoptosis is the process of programmed cell death, which plays a crucial role in cancer cell proliferation (*23*). The caspase cascade is a central component of cell apoptosis and can be regulated by caspase 3, which is the key enzyme required for the caspase cascade (*24*). A specific substrate, PARP can be cleaved by activated caspase 3, resulting in cell apoptosis (*25*). This is why PARP is one of the diagnostic tools most commonly used to detect apoptosis (*26,27*). Based on these findings, the silencing of PTGR2 is presumed to induce apoptosis at least partially *via* a caspase-3-mediated pathway.

In summary, the current results provided evidence that PTGR1 plays an important role in promoting the survival and proliferation of androgen-independent PC.

Knockdown of PTGR1 by lentivirus-mediated shRNA may provide a novel therapeutic approach for the treatment of androgen-independent PC. Furthermore, these findings have also provided an experimental basis for future investigations.

References

1. Jernal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61:69-90.
2. Bjurlin MA, Rosenkrantz AB, Beltran LS, Raad RA, Taneja SS. Imaging and evaluation of patients with high-risk prostate cancer. Nat Rev Urol. 2015; 12:617-628.
3. Van Brussel JP, Mickisch GH. Multidrug resistance in prostate cancer. Onkologie. 2003; 26:175-181.
4. Sugahara R, Sato A, Uchida A, Shiozawa S, Sato C, Virgona N, Yano T. Annatto tocotrienol induces a cytotoxic effect on human prostate cancer PC3 cells *via* the simultaneous inhibition of Src and Stat3. J Nutr Sci Vitaminol (Tokyo). 2015; 61:497-501.
5. Vitturi DA, Chen CS, Woodcock SR, Salvatore SR, Bonacci G, Koenitzer JR, Stewart NA, Wakabayashi N, Kensler TW, Freeman BA. Modulation of nitro-fatty acid signaling: Prostaglandin reductase-1 is a nitroalkene reductase. J Biol Chem. 2013; 288:25626-25637.
6. Yu X, Egner PA, Wakabayashi J, Wakabayashi, J, Yamamoto M, Kensler TW. Nrf2-mediated induction of cytoprotective enzymes by 15-deoxy-delta12,14-prostaglandin J2 is attenuated by alkenal/one oxidoreductase. J Biol Chem. 2006; 28:26245-26252.
7. Gong J, Neels JF, Yu X, Kensler TW, Peterson LA, Sturla SJ. Investigating the role of stereochemistry in the activity of anticancer acylfulvenes: synthesis, reductase-mediated bioactivation, and cellular toxicity. J Med Chem. 2006; 49:2593-2599.
8. Gong J, Vaidyanathan VG, Yu X, Kensler TW, Peterson LA, Sturla SJ. Depurinating acylfulvene-DNA adducts: characterizing cellular chemical reactions of a selective antitumor agent. J Am Chem Soc. 2007; 129:2101-2111.
9. Yu X, Erzinger MM, Pietsch KE, Cervoni-Curet FN, Whang J, Niederhuber J, Sturla SJ. Up-regulation of human prostaglandin reductase 1 improves the efficacy of hydroxymethylacylfulvene, an antitumor chemotherapeutic agent. J Pharmacol Exp Ther. 2012; 343:426-433.
10. Ho DW, Yang ZF, Yi K, Lam CT, Ng MN, Yu WC, Lau J, Wan T, Wang X, Yan Z, Liu H, Zhang Y, Fan ST. Gene expression profiling of liver cancer stem cells by RNA-sequencing. PLoS One. 2012; 7:e37159.
11. Sanchez-Rodriguez R, Torres-Mena JE, De-la-Luz-Cruz M, Bernal-Ramos GA, Villa-Trevino S, Chagoya-Hazas V, Landero-Lopez L, Garcia-Roman R, Rouimi P, Del-Pozo-Yauner L, Melendez-Zajgla J, Perez-Carreon JI. Increased expression of prostaglandin reductase 1 in hepatocellular carcinomas from clinical cases and experimental tumors in rats. Int J Biochem Cell Biol. 2014; 53:186-194.
12. Tapak L, Saidijam M, Sadeghifar M, Poorolajal J, Mahjub H. Competing risks data analysis with high-dimensional covariates: an application in bladder cancer. Genomics Proteomics Bioinformatics. 2015; 13:169-176.
13. Dick RA, Kwak MK, Sutter TR, Kensler TW. Antioxidative function and substrate specificity of NAD(P)H-dependent alkenal/one oxidoreductase. A new role for leukotriene B4 12-hydroxydehydrogenase/15-oxoprostaglandin 13-reductase. J Biol Chem. 2001; 276:40803-40810.
14. Yang S, Luo F, Wang J, Mao X, Chen Z, Wang Z, Guo F. Effect of prostaglandin reductase 1 (PTGR1) on gastric carcinoma using lentivirus-mediated system. Int J Clin Exp Pathol. 2015; 8:14493-14499.
15. Berthold DR, Sternberg CN, Tannock IF. Management of advanced prostate cancer after first-line chemotherapy. J Clin Oncol. 2005; 23:8247-52.
16. Chen KC, Peng CC, Peng RY, Su CH, Chiang HS, Yan JH, Hsieh-Li HM. Unique formosan mushroom *Antrodia camphorata* differentially inhibits androgen-responsive LNCaP and -independent PC-3 prostate cancer cells. Nutr Cancer. 2007; 57:111-121.
17. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57-70.
18. Suryadinata R, Sadowski M, Sarcevic B. Control of cell cycle progression by phosphorylation of cyclin-dependent kinase (CDK) substrates. Biosci Rep. 2010; 30:243-55.
19. Li JP, Yang YX, Liu QL, Pan ST, He ZX, Zhang X, Yang T, Chen XW, Wang D, Qiu JX, Zhou SF. The investigational Aurora kinase A inhibitor alisertib (MLN8237) induces cell cycle G2/M arrest, apoptosis, and autophagy *via* p38 MAPK and Akt/mTOR signaling pathways in human breast cancer cells. Drug Des Devel Ther. 2015; 9:1627-1652.
20. Malumbres M. Cyclin-dependent kinases. Genome Biol. 2014; 15:122.
21. Gartel AL, Radhakrishnan SK. Lost in transcription: p21 repression, mechanisms, and consequences. Cancer Res. 2005; 65:3980-3985.
22. Wang J, Wang X, Wu G, Hou D, Hu Q. MiR-365b-3p, down-regulated in retinoblastoma, regulates cell cycle progression and apoptosis of human retinoblastoma cells by targeting PAX6. FEBS Lett. 2013; 587:1779-1786.
23. Denault JB, Boatright K. Apoptosis in biochemistry and structural biology. 3-8 February 2004, Keystone, CO, USA. IDrugs. 2004; 7:315-317.
24. Fan TJ, Han LH, Cong RS, Liang J. Caspase family proteases and apoptosis. Acta Biochim Biophys Sin (Shanghai). 2005; 37:719-727.
25. Yu SW, Andrabi SA, Wang H, Kim NS, Poirier GG, Dawson TM, Dawson VL. Apoptosis-inducing factor mediates poly(ADP-ribose) (PAR) polymer-induced cell death. Proc Natl Acad Sci U S A. 2006; 103:18314-18319.
26. Decker P, Muller S. Modulating poly (ADP-ribose) polymerase activity: potential for the prevention and therapy of pathogenic situations involving DNA damage and oxidative stress. Curr Pharm Biotechnol. 2002; 3:275-283.
27. Bressenot A, Marchal S, Bezdetnaya L, Garrier J, Guillemin F, Plenat F. Assessment of apoptosis by immunohistochemistry to active caspase-3, active caspase-7, or cleaved PARP in monolayer cells and spheroid and subcutaneous xenografts of human carcinoma. J Histochem Cytochem. 2009; 57:289-300.

(Received March 23, 2016; Revised April 5, 2016; Accepted April 10, 2016)