Effects of cisplatin on the LSD1-mediated invasion and metastasis of prostate cancer cells

ZHI-YUAN CHEN, HUI CHEN, TAO QIU, XIAO-DONG WENG, JIA GUO, LEI WANG and XIU-HENG LIU

Department of Urology, Renmin Hospital of Wuhan University, Wuhan, Hubei 430060, P.R. China

Received February 4, 2015; Accepted December 22, 2015

DOI: 10.3892/mmr.2016.5571

Abstract. Prostate cancer poses a major public health problem in men. Metastatic prostate cancer is incurable, and ultimately threatens the life of patients. Lysine-specific demethylase 1 (LSD1) is an androgen receptor-interacting protein that exerts a key role in regulating gene expression and is involved in numerous biological processes associated with prostate cancer. Cisplatin, also known as cis-diamminedichloroplatinum or DDP, is a standard chemotherapeutic agent used to treat prostate cancer; however, it has the disadvantage of various serious side effects. The present study aimed to investigate the effects of LSD1 knockdown, and the interplay between LSD1 and DDP, on prostate cancer cell proliferation, apoptosis and invasion, and, therefore, the potential of LSD1 as a target for prostate cancer therapy. Flow cytometric analysis, Cell Counting kit 8 assay, Transwell assay and western blotting results revealed that LSD1 knockdown, in combination with DDP treatment, exerted antiproliferative, proapoptotic and anti-invasive effects on PC3 prostate cancer cells. In addition, knockdown of LSD1 acted synergistically with DDP, thereby enhancing the induction of apoptosis, and the inhibition of proliferation and invasion in prostate cancer cells. These results indicated that LSD1 may serve as a potential therapeutic target, and may enhance the sensitivity of PC3 cells to DDP.

Introduction

Prostate cancer, which is characterized by the development of prostate epithelial malignant tumors, occurs solely in men and is associated with the highest rates of morbidity and mortality (1). Prostate cancer poses a major public health problem worldwide (1). The incidence of prostate cancer is particularly prevalent in older men (2). The therapeutic strategies currently used to treat prostate cancer include watchful waiting, surgery, radiotherapy, chemotherapy, hormone therapy and biotherapy (3). The androgen receptor (AR) has an important role in prostate cancer development and progression (3). Androgen-deprivation therapy (ADT) is an important means of treatment for patients with prostate cancer; however, one disadvantage is that the prostate cancer may develop resistance to ADT over time (4). At present, there is a dearth of effective treatment methods which are beneficial to those patients who have developed androgen resistance in prostate cancer. Therefore, prostate cancer therapy remains unsatisfactory, and there is an urgent requirement to identify novel therapeutic strategies to overcome resistance to androgens in patients with prostate cancer.

Lysine specific demethylase 1 (LSD1) is a histone demethylase, which exerts important roles in tumorigenesis (5-8). LSD1 has been reported to be highly expressed in various cancer cell types, particularly in prostate cancer (9). Previous studies have demonstrated that LSD1, as an AR-interacting protein, may promote AR-dependent gene expression, which subsequently leads to the constitutive maintenance of cancer cells via growth signals and an enhanced risk of tumor relapse (9,10). In addition, it has been suggested that histone modification patterns may be used to predict the risk of prostate cancer recurrence (11). Although LSD1 regulates the expression of a wide range of genes and is involved in the processes of prostate cancer progression and deterioration (9), the underlying molecular mechanisms remain to be fully elucidated. Therefore, the inhibition of LSD1 activity may provide a useful target for the treatment of prostate cancer.

Cisplatin, also known as cis-diamminedichloroplatinum or DDP, is a platinum-based drug commonly used in the clinic as a chemotherapeutic agent. It has numerous characteristic properties, including broad-spectrum anticancer activity and curative effects, which render it useful for the clinical treatment of various tumors (12). However, its use is associated with several side effects, which serve to limit the doses that may be administered, predominantly due to nephrotoxicity (13). Even so, it remains in use as a standard chemotherapeutic agent for the treatment of numerous types of cancer, including ovarian, cervical and prostate cancer (14-17). A previous study demonstrated that patients treated with DDP in combination with β-elemene were able to better tolerate the chemotherapy, which afforded an improved treatment for hormone-refractory prostate cancer (18).
Therefore, how to reduce the toxicity associated with DDP treatment is a keenly studied topic in cancer research. The present study aimed to provide important insights into the effects of LSD1 knockdown and its interplay with DDP on the proliferation, apoptosis and invasion of PC3 human prostate cancer cells. In addition, the present study revealed whether LSD1 knockdown could increase the sensitivity of DDP for the treatment of prostate cancer. The results may provide important implications for the development of novel therapeutic strategies.

**Materials and methods**

**Cell line and culture.** The PC3 human prostate cancer cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were grown in Gibco™ RPMI-1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS) and 2 mM glutamine (both Sigma-Aldrich, St. Louis, MO, USA) at 37°C in a humidified atmosphere containing 5% CO₂. The cell line was cultured for 24 h. The p53 and p21 gene expression levels were quantified using the Bicinchoninic Acid Protein Assay kit (Thermo Fisher Scientific, Inc.) and the mRNA was reverse transcribed into cDNA using the TIANScript RT kit (Tiangen Biotech. Co. Ltd., Shanghai, China) and the mRNA was reverse transcribed into cDNA using the ABI 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) and the mRNA was reverse transcribed into cDNA using the ABI 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.).

**Flow cytometric analysis.** The PC3 cells (5x10⁵ cells/ml) were inoculated into 6-well plates. Each group comprised three double wells on the plate. Following a 24 h incubation, the groups were generated by addition of the appropriate reagents to the cells, and the cells were incubated for a further 24 h. The flow cytometric analysis was performed according to the protocol of the Annexin V-Fluorescein Isothiocyanate Apoptosis Detection kit (Abcam, Cambridge, UK). The apoptotic rates were analyzed immediately using a FACScalibur™ flow cytometer (BD Biosciences, San Jose, CA, USA).

**Cell invasive capability measured using a Transwell assay.** Following the removal of the culture medium from each group, the cells were digested with trypsin and diluted to 1x10⁵/ml in serum-free Dulbecco's Modified Eagle's Medium containing 1% FBS (GE Healthcare Life Sciences, Logan, UT, USA). A total of 800 µl culture medium containing 10% FBS was added to the coated lower chambers of a Transwell system. Matrigel (BD Biosciences) was coated onto the upper chambers of the Transwell system, after which 200 µl cell suspension was added to the upper chambers at a density of 5x10⁴/well. The plate was cultured for 24 h, after which the cells on the upper layer were removed. The cells that had migrated to the lower layer were washed with PBS, fixed with methanol and stained with 1% crystal violet. The invasive cells were counted in five fields for each sample under an inverted microscope (BX51; Olympus Corporation, Tokyo, Japan), and the results were averaged. The experiments were repeated three times.

**Western blot analysis.** Total protein was extracted from the cell samples using radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology), and was quantified using the Bicinchoninic Acid Protein Assay kit (Thermo Fisher Scientific, Inc.). Subsequently, equal volumes of protein (30 µg) were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by immunoblotting onto a nitrocellulose membrane (EMD Millipore, Billerica, MA, USA) using an electrophoretic transfer cell (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membranes were blocked with 5% skimmed milk, followed by incubation overnight at 4°C with the following primary antibodies: Monoclonal anti-E-cadherin (#14472; 1:1,000), monoclonal anti-cyclo-oxygenase-2 (COX-2; #4842; 1:1,000), monoclonal anti-Smad2/3 (#8685; 1:1,000), monoclonal anti-phosphorylated (p)-Smad2/3 (#8828; 1:1,000), monoclonal anti-cyclo-D (D#2978; 1:1,000), monoclonal anti-p-Akt (#9271; 1:1,000), monoclonal anti-Akt (#9272; 1:1,000), monoclonal anti-extracellular signal-regulated kinase (ERK; #4695; 1:1,000), monoclonal anti-p-ERK (#4376; 1:1,000).
1:1,000) and monoclonal anti-GAPDH (#5174; 1:1,500) from Cell Signaling Technology, Inc. (Danvers, MA, USA), and polyclonal anti-transforming growth factor-β1 (TGF-β1; ab92486; 1:800) and anti-vascular endothelial growth factor (VEGF; ab46154; 1:1,000) from Abcam. Subsequently, the blots were washed three times with PBS and incubated with horseradish peroxidase-conjugated goat anti-mouse (A0216; 1:1,000) or goat anti-rabbit (A0208; 1:1,000; both Beyotime Institute of Biotechnology) secondary antibodies for 1 h at room temperature. The bands were detected by reaction with enhanced chemiluminescence detection system reagents (EMD Millipore) and exposure to X-ray film (Kodak, Rochester, NY, USA), which was subsequently developed and used to capture photographic images. GAPDH was used to normalize the protein expression. Band intensities were analyzed using ImageJ 1.49 software (https://imagej.nih.gov/ij/).

**Results**

Proliferation of PC3 cells is decreased following LSD1 knockdown and treatment with DDP. The present study hypothesized that LSD1 may have an important role in PC3 cells, which has not been previously investigated. Lentiviral-mediated RNA interference technology was used to establish a stably transfected LSD1 knockdown PC3 cell line, and the CCK-8 colorimetric assay was subsequently used to determine cell proliferation. As shown in Fig. 1A, the mRNA expression levels of LSD1 in the knockdown group were decreased to <60% of the control levels (P<0.01), whereas the mRNA expression levels of LSD1 in the mock group (i.e. control cells which were transfected with an irrelevant interference sequence) exhibited no significant changes. All of the groups contained similar cell numbers at the 0 h time point; however, proliferation of the LSD1 knockdown PC3 cells was significantly decreased after 24, 48 and 72 h, as compared with the mock group (P<0.01; Fig. 1B). Furthermore, compared with the mock group, DDP (at a concentration of 5 µg/ml) exerted a marked inhibitory effect on PC3 cell proliferation. Notably, the LSD1 knockdown + DDP group demonstrated a more marked inhibition on PC3 cell proliferation, as compared with the DDP or LSD1 knockdown groups (P<0.05). These findings indicate that the proliferative capability of the PC3 cells was decreased...
following LSD1 knockdown, thus suggesting that LSD1 may contribute to the proliferation of PC3 cells, and that siRNA interference may result in reduced cell growth. Furthermore, DDP had similar effects to LSD1 siRNA, and a combination of LSD1 knockdown and DDP treatment produced a synergistic effect.

**LSD1 knockdown and DDP treatment inhibit PC3 cell proliferation via regulation of the Akt and ERK signal transduction pathways.** The results of the present study revealed that cell proliferation was inhibited when LSD1 was knocked down and the cells were co-treated with DDP, as compared with the mock group. Since the Akt and ERK signal transduction pathways are reported to modulate cell proliferation and tumorigenicity (20,21), the protein expression levels of p-Akt and p-ERK were subsequently investigated. Furthermore, cyclin D has a crucial role in the Akt signal pathway as a regulatory protein of the cell cycle (22), and therefore the protein expression levels of cyclin D were also investigated. The protein expression levels of p-Akt, p-ERK and cyclin D1 were downregulated in the treatment groups (LSD1, mock + DDP, LSD1 + DDP), as compared with the mock group (Fig. 1C and D). These results suggest that LSD1 RNA interference and treatment with DDP may inhibit PC3 cell proliferation via regulation of the Akt and ERK signal pathways.

**Percentage of PC3 cells in the early apoptotic phase is increased following LSD1 knockdown and treatment with DDP.** In addition to regulating PC3 cell growth, LSD1 may be involved in regulating cell apoptosis. As shown in Fig. 2A and B the proportion of PC3 cells in the early apoptotic phase was significantly increased following LSD1 knockdown, as compared with the mock group. Furthermore, it was revealed that DDP contributes to the increased levels of apoptosis in the PC3 cells, and that treatment with DDP exerted a synergistic action on PC3 cell apoptosis when combined with LSD1 siRNA. These results suggest that a combination of LSD1 knockdown and DDP treatment may contribute to the increased percentage of PC3 cells in the early apoptotic stage.

**Invasive capability of PC3 cells is decreased following LSD1 knockdown and treatment with DDP via regulation of the TGF-β1/Smad2/3 signal transduction pathway.** Invasive capability is essential for the malignant progression of tumors. As shown in Fig. 3A and B, the invasive capability of the PC3 cells was decreased following LSD1 knockdown or treatment with DDP, as compared with the mock group. Furthermore, a combination of LSD1 knockdown and DDP treatment exerted a synergistic effect on the decline in invasive ability. These results suggest that LSD1 may have a crucial role in the invasion of PC3 cells.

TGF-β1 is a cytokine peptide, which is associated with various biological roles in cancer (23). The Smad proteins act as substrates of the TGF-β1 receptor, and their activation by phosphorylation propagates the TGF-β1 signal transduction. In particular, the TGF-β1/Smad2/3 signaling pathway is involved in mechanisms underlying the invasion and metastasis of prostate cancer cells, a process which is also regulated by tumor angiogenesis, the host immune system, the cells themselves and the surrounding matrix microenvironment (24-26). Consequently, the protein expression levels of TGF-β1, p-Smad2/3, VEGF, COX-2 and E-cadherin, all of which are associated with the TGF-β1/Smad2/3 signal transduction pathway, were investigated. The results revealed that the expression levels of TGF-β1, p-Smad2/3, VEGF and COX-2 in the DDP-treated cells were downregulated, whereas the protein expression levels of E-cadherin were upregulated.
Furthermore, the combination of LSD1 knockdown and treatment with DDP exerted a synergistic effect, as compared with either treatment taken in isolation or with the mock cells (Fig. 3C-E).

**Discussion**

Prostate cancer is a complex disease, and numerous controversies are associated with aspects of different treatment strategies (27). The identification of genetic and molecular events that may improve the early detection of prostate cancer, or that could be used as therapeutic targets, is a top priority in this line of study.

LSD1 is a flavin-dependent amine oxidase, which has been reported to interact with the AR (9). Previous studies have reported that LSD1 is overexpressed in prostate cancer (9,28). DDP is an inorganic compound that is widely used in cancer therapy (12). In the present study, the antiproliferative, proapoptotic and anti-invasive effects of LSD1 knockdown and DDP treatment, either in isolation or in combination, on PC3 prostate cancer cells were investigated. The results demonstrated that knockdown of LSD1, in combination with DDP, suppressed the invasion of prostate cancer cells.

A combination of LSD1 knockdown and DDP treatment effectively suppressed the proliferation of PC3 cells. The Akt and ERK signaling pathways have been reported to be important pathways closely associated with cell proliferation (29,30). In the present study, western blotting results suggested that the protein expression levels of the associated proteins, p-Akt, cyclin D1 and p-ERK, were markedly decreased in the LSD1 siRNA, DDP treatment and LSD1 siRNA + DDP cell groups, as compared with the mock group. These findings indicated that LSD1 knockdown and DDP treatment may effectively inhibit the proliferation of PC3 cells via regulation of the Akt and ERK signaling pathways. Flow cytometry was also performed to determine the extent of apoptosis in the prostate cancer cells. All of the treatments, i.e. LSD1 knockdown and treatment with DDP, either alone or in combination, resulted in an increased induction of PC3 cell apoptosis.

Tumor cell invasion and metastasis are complicated by the existence, and interplay, of several mechanisms (31). The effects of LSD1 knockdown on the invasive capability of the PC3 prostate cancer cells were examined in the present study. The results demonstrated that knockdown of LSD1, in combination with DDP, suppressed the invasion of prostate cancer cells. TGF-β is a cytokine peptide, which exerts various biological activities. A previous study demonstrated that TGF-β1 was able to promote the invasion and metastasis of prostate tumor cells (32). Smad proteins are the sole substrates of TGF-β1 identified in the TGF-β1 signal transduction pathway (33). In the present study, the protein expression levels of TGF-β1 and p-Smad2/3 were decreased, which implied that this signaling pathway was inhibited by LSD1 knockdown and DDP treatment.
pathway may be primarily responsible for the process by which LSD1 knockdown and DDP treatment inhibited the invasion of PC3 cells. TGF-β1/Smad2/3 signaling is known to promote specific mechanisms underlying the processes of cell invasion and metastasis in prostate cancer, via regulation of angiogenesis, the immune defense system, changes in the substrate microenvironment or alterations to the cells themselves (39-40).

The formation of blood vessels is crucial in tumor growth, invasion and metastasis (37). The present study revealed that VEGF, as one of the most important promoters of angiogenesis (38), was downregulated in the LSD1 siRNA group. In addition, epithelial-mesenchymal transition is considered to be a key step in the process of tumor metastasis. For example, E-cadherin is as a Ca++-dependent glycoprotein, which performs an essential role in cell invasion and migration (39,40). The results of the present study implied that LSD1 knockdown and DDP treatment inhibited the invasion of the PC3 cells by upregulating the expression levels of E-cadherin. COX-2, which is a rate-limiting enzyme in the prostaglandin biosynthesis pathway, is able to promote tumor angiogenesis (41). The western blotting results of the present study demonstrated that the protein expression levels of COX-2 were decreased in DDP-treated LSD1 knockdown cells, with a similar result obtained for VEGF. These results may verify the hypothesis that LSD1 knockdown inhibits the invasion of PC3 cells by regulating the TGF-β1/Smad2/3 signal pathway, and a combination of LSD1 siRNA and DDP treatment may lead to more pronounced effects.

In conclusion, the present study aimed to identify the proapoptotic, antiproliferative and anti-invasive effects of LSD1 knockdown, in combination with DDP treatment, on PC3 human prostate cancer cells, and to offer an explanation for the underlying mechanism. The results of the present study may provide novel insights into the molecular mechanism underlying the progression and pathogenesis of prostate cancer, and may be useful for the optimization of therapeutic interventions for the treatment of this disease.

References

1. Center MM, Jamal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O and Bray F: International variation in prostate cancer incidence and mortality rates. Eur Urol 61: 1079-1092, 2012.
2. Bechis SK, Carroll PR and Cooperberg MR: Impact of age at diagnosis on prostate cancer treatment and survival. J Clin Oncol 29: 235-241, 2011.
3. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG and Sawyer CL: Molecular determinants of resistance to antiandrogen therapy. Nat Med 10: 33-39, 2004.
4. Shahinian VB, Kuo YF, Freeman JL and Goodwin JS: Risk of fracture after androgen deprivation for prostate cancer. N Engl J Med 352: 154-164, 2005.
5. Lv T, Yuan D, Miao X, Lv Y, Zhan P, Shen X and Song Y: Over-expression of LSD1 promotes proliferation, migration and invasion in non-small cell lung cancer cells. Plos One 7: e35065, 2012.
6. Zhao ZK, Dong P, Gu J, Chen L, Zhuang M, Lu WJ, Wang DR and Liu YB: Overexpression of LSD1 in hepatocellular carcinoma: A latent target for the diagnosis and therapy of hepatoma. Tumor Biol 34: 173-180, 2013.
7. Qin Y, Zhu W, Xu W, Zhang B, Shi S, Ji S, Liu J, Long J, Liu C, Liu L, et al: LSD1 sustains pancreatic cancer growth via maintaining HIF1α-dependent glycolytic process. Cancer Lett 347: 225-232, 2014.
8. Kaufmann EC, Robinson BD, Downes MJ, Powell LG, Lee MM, Scherr DS, Gudas LJ and Mongan NP: Role of androgen receptor and associated lysine-demethylase coregulators, LSD1 and JMJD2A, in localized and advanced human bladder cancer. Mol Carcinog 50: 931-944, 2011.
9. Metzger E, Wissmann M, Yin N, Müller JM, Schneider R, Peters AH, Günther T, Buettner Rand Schüle R: LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. Nature 439: 746-749, 2006.
10. Kahl P, Gullotti L, Heukamp LC, Wolf S, Friedrichs N, Vorreuther R, Solleder G, Bastian PJ, Ellinger J, Metzger E, et al: Androgen receptor coactivators lysine-specific histone demethylase 1 and four and half LIM domain protein 2 predict risk of prostate cancer recurrence. Cancer Res 66: 11341-11347, 2006.
11. Seligson DB, Horvath S, Shi T, Yu H, Tze S, Grunstein M and Kurdistani SK: Global histone modification patterns predict risk of prostate cancer recurrence. Nature 435: 1262-1266, 2005.
12. Breit G and Coluccia M: Current status of trans-platinum compounds in cancer therapy. Coord Chem Rev 216: 383-410, 2001.
13. Miller RP, Tapagavadi RK, Ramesh G and Reeves WB: Mechanisms of Cisplatin nephrotoxicity. Toxins (Basel) 2: 2490-2518, 2010.
14. Florea AM and Büsselfberg D: Cisplatin as an anti-tumor drug: Cellular mechanisms of activity, drug resistance and induced side effects. Cancers (Basel) 3: 1351-1371, 2011.
15. Gumulec J, Balvan J, Szantalovich M, Raudenska M, Dvorakova V, Knopfova L, Polanska H, Hudcova K, Rutkay-Nedecky B, Babula P, et al: Cisplatin-resistant prostate cancer model: Different hypoxic tumor system, apoptosis and cell cycle. J Int Oncol 44: 923-933, 2014.
16. Rose PG, Sill MW, McMeeken DS, Ahmed A, Salani R, Yamada SD, Wolfson AH, Fusco N and Fracasso PM: A phase I study of concurrent weekly topotecan and cisplatin chemotherapy with whole body photoradiation therapy in locally advanced cervical cancer: A gynecologic oncology group study. Gynecol Oncol 158: 125-162, 2012.
17. Stordal B, Hamon M, McEnaney V, Roche S, Gillet JP, O’Leary JJ, Gottesman M and Clynes M: Resistance to paclitaxel in a cisplatin-resistant ovarian cancer cell line is mediated by P-glycoprotein. Plos One 7: e40717, 2012.
18. Li QQ, Wang G, Reed E, Huang L and Cuff CF: Evaluation of cisplatin in combination with β-elemene as a regimen for prostate cancer chemotherapy. Basic Clin Pharmacol Toxicol 107: 686-687, 2010.
19. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
20. Jin F, Irshad S, Yu W, Belakavadi M, Chekmareva M, Ittmann MM, Abate-Shen C and Fondell JD: ERK and AKT signaling drive MED1 overexpression in prostate cancer in association with elevated proliferation and tumorigenicity. Mol Cancer Res 11: 736-747, 2013.
21. Rick FG, Schally AV, Szalontay L, Block NL, Szepeshazi K, Nadji M, Zardani M, Hohla F, Buchholz S and Setz S: Antagonists of growth hormone-releasing hormone inhibit growth of androgen-independent prostate cancer through inactivation of ERK and Akt kinases. Proc Natl Acad Sci USA 109: 1655-1660, 2012.
22. Harashima N, Inao T, Imamura R, Okano S, Suda T and Harada M: Roles of the PI3K/Akt pathway and autophagy in TLR3 signaling-induced apoptosis and growth arrest of human prostate cancer cells. Cancer Immunol Immunother 61: 667-676, 2012.
23. Litvonen SK and Kähäri VM: Transforming growth factor-beta-signaling in cancer invasion and metastasis. Int J Cancer 121: 2119-2124, 2007.
24. Nicholson B and Theodorescu D: Angiogenesis and prostate cancer tumor growth. J Cell Biochem 91: 125-150, 2004.
25. Wikström P, Damber J and Bergh A: Role of transforming growth factor-beta in prostate cancer. Microsc Res Tech 52: 411-419, 2001.
26. Thiery JP: Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2: 442-454, 2002.
27. Denmeade SR and Isaacs JT: A history of prostate cancer chemotherapy. Basic Clin Pharmacol Toxicol 107: 868-876, 2010.
30. Kinkade CW, Castillo-Martin M, Puzio-Kuter A, Yan J, Foster TH, Gao H, Sun Y, Ouyang X, Gerald WL, Cordon-Cardo C and Abate-Shen C: Targeting AKT/mTOR and ERK MAPK signaling inhibits hormone-refractory prostate cancer in a preclinical mouse model. J Clin Invest 118: 3051-3064, 2008.

31. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. Cell 144: 646-674, 2011.

32. Danielpour D: Functions and regulation of transforming growth factor-beta (TGF-beta) in the prostate. Eur J Cancer 41: 846-857, 2005.

33. ten Dijke P and Hill CS: New insights into TGF-beta-Smad signalling. Trends Biochem Sci 29: 265-273, 2004.

34. Tuxhorn JA, McAlhany SJ, Yang F, Dang TD and Rowley DR: Inhibition of transforming growth factor-beta activity decreases angiogenesis in a human prostate cancer-reactive stroma xenograft model. Cancer Res 62: 6021-6025, 2002.

35. Donkor MK, Sarkar A, Savage PA, Franklin RA, Johnson LK, Jungbluth AA, Allison JP and Li MO: T cell surveillance of oncogene-induced prostate cancer is impeded by T cell-derived TGF-β1 cytokine. Immunity 35: 123-134, 2011.

36. Micalizzi DS, Farabaugh SM and Ford HL: Epithelial-mesenchymal transition in cancer: Parallels between normal development and tumor progression. J Mammary Gland Biol Neoplasia 15: 117-134, 2010.

37. Kirsch M, Schackert G and Black PM: Angiogenesis, metastasis, and endogenous inhibition. J Neurooncol 50: 173-180, 2000.

38. Ferrara N, Gerber HP and LeCouter J: The biology of VEGF and its receptors. Nat Med 9: 669-676, 2003.

39. Pàmies P: E-cadherin-guided migration. Nat Mater 13: 664, 2014.

40. Canel M, Serrels A, Frame MC and Brunton VG: E-cadherin-integrin crosstalk in cancer invasion and metastasis. J Cell Sci 126: 393-401, 2013.

41. Muraki C, Ohga N, Hida Y, Nishihara H, Kato Y, Tsuchiya K, Matsuda K, Totsuka Y, Shindoh M and Hida K: Cyclooxygenase-2 inhibition causes antiangiogenic effects on tumor endothelial and vascular progenitor cells. Int J Cancer 130: 59-70, 2012.