Involvement of estrogen receptor β in androgen receptor-induced growth inhibition in prostate cancer PC-3 cells

LONG XIAO[1], MINHUI XIAO[1], LINBO GAO[2] and WANCHAO XU[1]

[1]Department of Urology, The First People's Hospital of Yunnan Province, Kunming University of Science and Technology, Kunming, Yunnan 650041; [2]Laboratory of Molecular and Translational Medicine, West China Institute of Women and Children's Health, West China Second University Hospital, Sichuan University, Chengdu, Sichuan 610041, P.R. China

Received October 11, 2016; Accepted December 21, 2016

DOI: 10.3892/ol.2017.6544

Abstract. Previous studies have suggested that changes in sex hormone receptor expression may be associated with the initiation and progression of prostate cancer (PCa). Therefore, the present study aimed to investigate the association and possible pathways between two sex hormone receptors and PCa by measuring the expression levels of the androgen receptor (AR) and the estrogen receptor subtypes alpha (ERα) and beta (ERβ) in prostatic cancer PC-3 cell lines. The pcDNA3.1-hERβ plasmid was transfected into PC-3 cell lines. The expression levels of AR, ERα and ERβ were detected at the mRNA level by reverse transcription-polymerase chain reaction (RT-PCR) and quantitative PCR (qPCR). The results demonstrated that the expression levels of AR, ERβ and ERα were downregulated to different degrees: ERβ test group vs. PC-3 cell group (P=0.000; 95% confidence interval: 0.9803-1.6331). ERβ and AR expression was detected continuously in the PC-3 cells, but the expression of ERα was not. AR expression levels exhibited an upward trend whilst the expression of ERβ demonstrated a marked downward trend. There is a correlation between the expression levels of ERβ and the incidence of PCa, and ERβ may inhibit the growth of PC-3 cell lines by regulating the expression levels of AR. ERβ may provide a novel target for PCa therapies.

Introduction

Prostate cancer (PCa) is a common, hormone-dependent, type of malignant tumor regularly observed in men >60 years in the united states and european countries (1,2). However, the pathogenesis and mechanisms of progression remain unclear. Previous studies have suggested that interactions between the estrogen and androgen receptors and corresponding substrates may be the pathogenic factors and risk factors in the origin and progress of pca tumor formation (3-5). Estrogen receptor beta (ERβ) is highly expressed within the differential layer cells, and performs a secretory function. Due to high levels of ERβ expression, the prostate is more likely to be affected by environmental estrogen (6,7), which appears to explain the correlation between the low incidence rate of pca and the high dietary phytoestrogens in asian countries (8).

There is a marked correlation between ERβ expression levels and the formation of PCa (8). Results from previous studies have revealed that the expression levels of ERβ and androgen receptors (AR) exhibited a downward and upward trend, respectively, in PCa tissues with increasing degrees of malignancy (3,9-13). It is hypothesized that ERβ may curb the abnormal differentiation of prostate epithelial cells via the downregulation of AR expression levels. During PCa progression, the decreasing expression of ERβ suggests that ERβ is involved in the inhibition of cell proliferation. Therefore, the changing expression of ERβ, ERα and AR may serve an important role in the pathogenesis of PCa (14). Previous studies have revealed that PCa tissues are often accompanied by a variation in the expression of ER and AR (9,10,15). Conversely, Royuela *et al* (16) demonstrated that ERβ expression is increased in normal prostate, prostate hyperplasia and PCa tissue. Therefore, the present study aimed to detect the changes in the expression levels of AR, ERβ and ERα in the PCa PC-3 cell lines, to investigate the pathogenesis of PCa.

Materials and methods

Cell culture. The human PCa PC-3 cell line was obtained from the Pathology department of the West China Hospital of Sichuan University (Chengdu, China). The. PC-3 cells were grown and maintained in high glucose Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.), 100 units/ml penicillin, and 100 μg/ml streptomycin. The cell lines were maintained in humidified incubators with 5% CO_2 at 37˚C. Subsequent to cell adhesion to the base of cell culture dish, cells were subcultured into three equal dishes.

Correspondence to: Dr Wanchao Xu, Department of Urology, The First People's Hospital of Yunnan Province, Kunming University of Science and Technology, 157 Jinbi Road, Xishan, Kunming, Yunnan 650041, P.R. China
E-mail: doclongxiao@126.com

Key words: prostatic cancer, androgen receptor, estrogen receptor β, estrogen receptor α

Cell counting and seeding. The PC-3 cells were seeded in 6-well tissue culture dishes at a density of 175,000 cells/well in high glucose DMEM supplemented with 10% FBS, and were maintained in humidified incubators with 5% CO_2 at 37˚C for 48 h. Once the cells covered between 80-90% of the base of the dish, the media were discarded and the cells were washed twice with phosphate buffered saline (PBS), A total of 2 ml/well medium, without antibiotics and without FBS was then added to each well. All the assays were carried out in triplicate.

Cell grouping and treatment. According to the different reagents added, the cells were divided into pcDNA3.1-hERβ plasmid, the plasmid, Lipofectamine® 2000 and PC-3 cells control groups. The pcDNA3.1-hERβ plasmid, the blank plasmid and Lipofectamine® 2000 were purchased from the Shanghai GenePharma Company (Shanghai GenePharma Co.Ltd.,Shanghai,China). A total of 115 μl/well of the configured mixture of pcDNA3.1-hERβ plasmid-Lipofectamine® 2000 was added into the treated cell groups to induce transfection, following the protocol of the manufacturer. Lipofectamine® 2000, blank plasmids and DMEM were added to the remaining 3 groups. All of the groups were maintained in humidified incubators with 5% CO_2 at 37˚C for 4 h. The media were removed and the cells were washed twice with PBS, then 2 ml/well high glucose DMEM supplemented with 10% FBS was added. The cells were cultured for an additional 48 h and harvested. All cell groups were prepared in triplicate, and images were captured using an inverted phase contrast microscope (TS100; Nikon Corporation, Tokyo, Japan).

RNA isolation. The total RNA from all groups were isolated using TRIzol reagent and (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the protocol of the manufacturer. RNA samples were treated with DNase I (50 U/μl) (Thermo Fisher Scientific, Inc.) prior to analysis. The content and purity of the RNA was assayed with the DU 730 nucleic acid protein analyzer (Beckman Coulter Inc., Brea, CA, USA), and measured between 1.6 and 1.9 at A260/A280 nm. The amount of RNA was estimated from the optical density at 260 nm. The total RNA was then isolated and was used for qPCR analysis. The total RNA was isolated from the PC-3 cells treated with the pcDNA3.1-hERβ plasmid, the plasmid, Lipofectamine® 2000 and medium for 4 h, and reverse-transcribed into complementary (c)DNA. The cDNA was used for TaqMan analysis according to the protocol of the manufacturer. The PCR primers and TaqMan probes for AR, ERα and ERβ were purchased from the Shanghai GenePharma Co., Ltd. GAPDH was used as the internal control for normalization. The quantification cycle (Cq) values were evaluated by the relative standard curve method and normalized using the respective values of the internal control.

RNA reverse transcribed into cDNA. The total RNA was reverse transcribed into cDNA using SuperScript III First-Strand Synthesis SuperMix for RT-qPCR (Invitrogen; Thermo Fisher Scientific, Inc.). Personal protective equipment was used for all lab experiments to prevent from RNA enzyme contamination. All cDNA samples were treated with diethyl pyrocarbonate, and all reactions were prepared in PCR tubes without the RNase enzyme. Table I lists the components of the reaction mix used for reverse transcription (10 μl total) to generate cDNA using the Eppendorf Mastercycler nexus PCR instrument (Eppendorf, Hamburg, Germany). The reaction was incubated at 42˚C for 30 min; the reverse transcriptase was inactivated at 85˚C for 10 min, and cooled to 5˚C. When the reverse transcription was completed, the configured cDNA products were stored at -20˚C for qPCR detection.

Table I. Reverse transcription polymerase chain reaction.

Reaction reagent	Application amount (μl)
Total RNA	1
5x reverse transcription buffer	2
Random hexamers (50 μm)	0.5
Oligo dT Primer (100 μm)	0.5
PrimeScript™ RT Enzyme Mix I	0.5
DEPC water	5.5
Total volume	10

DEPC, diethlypyrocarbonate.

Table II. Real-time fluorescent quantitative PCR.

Reaction reagent	Application amount (μl)
Primers	0.8
2x quantitative PCR Master Mix	10
Fluorescent probe (10 μm)	0.4
cDNA template	2
Taq DNA polymerase (5 u/μl)	0.4
Double distilled water	6.4
Total volume	20

PCR, polymerase chain reaction; cDNA, complementary DNA.

Fluorescence qPCR. The cDNA was quantified by fluorescence qPCR using the ABI 7300 real-time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The PCR primers and fluorescence probes for AR (Gene ID: 367), ERα and ERβ (assay ID: Hs00174860_ml and Hs00230957_ml, respectively) were purchased from Shanghai GenePharma Co., Ltd. All sequences are summarized in Table I. The GAPDH (assay ID: No. 4352934E; Shanghai GenePharma Co., Ltd.) was used as the internal control for data normalization. The fluorescence qPCR reaction system was configured according to the protocol of the manufacturer, as summarized in Table II. Table III demonstrates the sequence information of the oligonucleotide primers and probes used. The PCR reaction was carried out according to the protocol of the manufacturer. The cycles were as follows: 95˚C for 1 min for prior degeneration; 95˚C for 12 sec and 62˚C for 40 sec to measure fluorescence, for 40 cycles. Each incident of mRNA expression of the target genes was indicated with a standardized ΔΔCq value (17). The obtained quantification cycle values of the interest gene were evaluated by the relative standard curve method and

Table III. Sequence information of oligonucleotides and probes.

Gene	Oligonucleotides	Sequence	Finished product bp
hERA	F Primer	GCAATGACTATGCTTCAGGCTAC	131
	R Primer	TTTATCAATGGTGCACTGGTTG	
	Probe	ATGGAGTCTGGTCCTGTGAGGGCTG	
hERB	F Primer	CAAGCTCATCTTTGCTCCAGA	150
	R Primer	GCCTTGACACAGAGATATTCTTTG	
	Probe	CTTGTTCTGGACAGGGATGAGGGGA	
hAR	F Primer	CATGTGGAAGCTGCAAGGTC	99
	R Primer	TTCGGAATTTATCAATAGTGCAATC	
	Probe	TCAAAAGAGCCGCTGAAGGGAAACA	
GAPDH	F Primer	CGACCACTTTGTCAAGCTCA	203
	R Primer	AGGGGAGATTCAGTGTGGTG	
	Probe	TCATCAGCAATGCCTCCTGCACCA	

F, forward; R, reverse; hERα, estrogen receptor α; hERβ, estrogen receptor β.

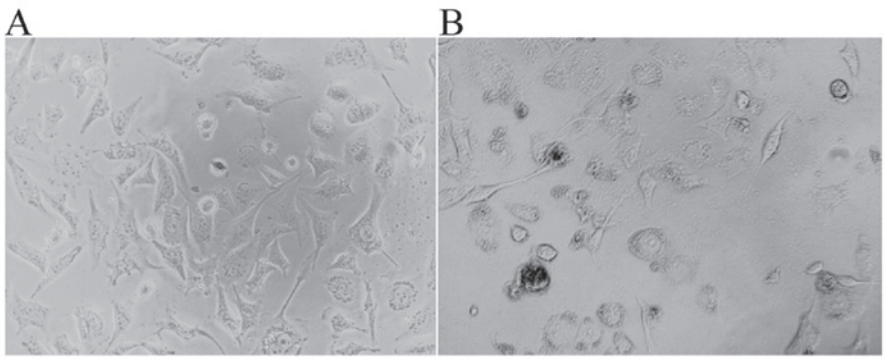

Figure 1. (A) PC-3 cells prior to transfection with the pcDNA3.1-hERβ-plasmid examined using inverted phase contrast microscopy (magnification, x200). Cells were seeded in 6 orifice plates and grown for 48 h. Cells are distributed over ~80-90% of the bottom of the dish. The cells are fusiform, in orderly rows, a medium and uniform size with larger nuclei and less cytoplasm. Silk phase separation was observed occasionally. (B) PC-3 cells subsequent to pcDNA3.1-hERβ plasmid transfection at magnification, x200. Rates of cell apoptosis were marked. Contraction of nuclei, less cytoplasm, few synapses and poor rates of growth were observed.

normalized using the respective values of the internal control GAPDH.

Statistical analysis. IBM SPSS 19.0 (Armonk, NY, USA) for Windows was used to establish the database and to conduct the statistical analysis. All results are reported as the mean ± standard deviation, using the independent samples t-test to detect the differences in each test group and the control group. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Cell culture and plasmid transfection. The PC-3 PCa cells cultured in high glucose DMEM supplemented with 10% FBS are illustrated in Fig. 1A. Once the pcDNA3.1-hERβ-plasmid had been transfected into the PC-3 cells, the cell-growth exhibited a very poor status, namely a high level of apoptosis, small nuclei, a reduced level of cytoplasm and few synapses were all observed (Fig. 1B).

Expression of AR, ERβ and ERα. Through the fluorescent qPCR, the patterns of expression levels of the mRNA of ERβ, ERα and AR were assayed in the recombinant plasmid, the empty plasmid, lipofectamine-2000 and the control groups. As demonstrated in Fig. 2, the results suggest that AR mRNA was expressed in all cell samples and expression of AR could be detected in all samples when compared with the blank plasmid group and blank cells. No difference was observed in the levels of expression of AR (pcDNA3.1-hERβ-plasmid transfection vs. PC-3 cell group, $P=0.889$) between all groups.

As illustrated in Fig. 3, the results also suggest that ERβ was expressed in all cells, and the expression of ERβ positive rate was 100%. The difference in the levels of ERβ expression was statistically significant in the pcDNA3.1-hERβ-plasmid transfection group compared with the PC-3 cell group ($P<0.0001$). A statistically significant difference was identified between all groups ($P<0.0001$), as illustrated in Fig. 3.

A statistically significant difference was observed between the levels of expression of ERβ, as measured by

Table IV. ΔΔCq value of AR, ERβ, ERα.

	AR	ERβ	ERα
Test	1.179±1.277	0.824±0.186	1.055±0.964
Blank plamid	0.670±0.328	1.406±0.218	0.807±0.758
Lipofect-2000	0.722±0.393	1.771±0.071	1.643±0.811
Control cell	1.263±0.209	1.790±0.032	1.202±1.119

AR, test group vs. control cell group, P=0.889; ERβ, test group vs. control cell group, P<0.0001; ERα, test group vs. control cell group, P=0.79; ERβ, blank plasmid group vs. control cell group, P<0.0001; ERβ, Lipofectamine® 2000 vs. control cell group, P<0.0001. AR, androgen receptor; ERβ, estrogen receptor β; ERα, estrogen receptor α.

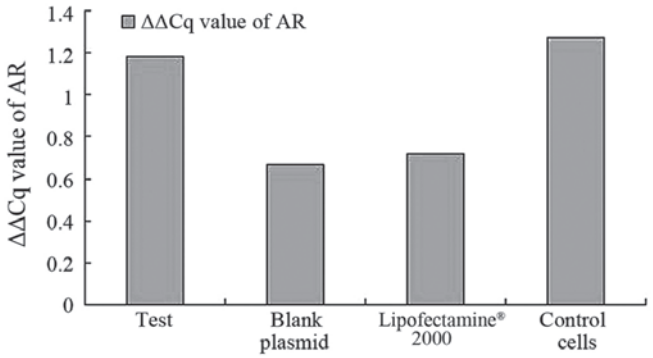

Figure 2. The ΔΔCq value of AR. Test, ΔΔCq value of AR in the test group; Lipofectamine® 2000, ΔΔCq value of AR in the Lipofectamine® 2000-transfected group; Blank plasmid, ΔΔCq value of AR in the blank plasmid-transfected group; Control cell, ΔΔCq value of AR in the control groups; AR, androgen receptor.

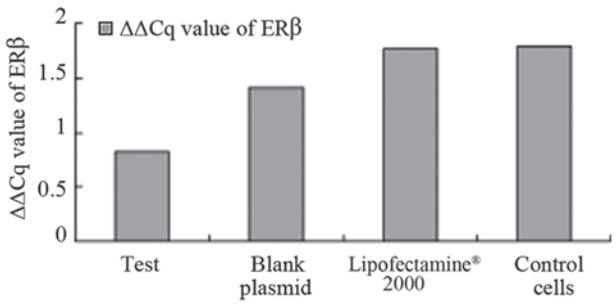

Figure 3. The ΔΔCq value of ERβ. Test, ΔΔCq value of ERβ in the test group; Lipofectamine® 2000, ΔΔCq value of ERβ in the Lipofectamine® 2000-transfected group; Blank plasmid, ΔΔCq value of ERβ in the blank plasmid-transfected group; Control cell, ΔΔCq value of ERβ in the control groups; ERβ, estrogen receptor β.

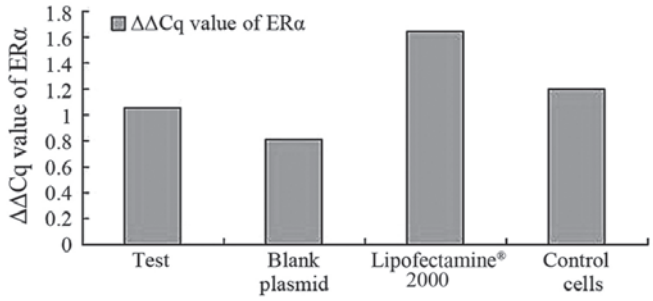

Figure 4. The ΔΔCq value of ERα. Test, ΔΔCq value of ERα in the test group; Lipofectamine® 2000 ΔΔCq value of ERα in the Lipofectamine® 2000-transfected group; Blank plasmid, ΔΔCq value of ERα in the blank plasmid-transfected group; Control cell, ΔΔCq value of ERα in the control groups; ERα, estrogen receptor α.

ΔΔCq value, in the pcDNA3.1-hERβ + plasmid transfection group compared with the control blank PC-3 cell group, 0.824±0.186 vs. 1.790±0.032, (P=0.000, 95% confidence interval (CI), 0.9803-1.6331), as demonstrated in Table IV. A statistically significant difference was also observed in the pcDNA3.1-hERβ + plasmid transfection group compared with the blank plasmid control group and lipofect2000 control group, (P<0.0001; 95% CI, -1.200-0.7066) and (P<0.0001; 95% CI, -0.8545-0.3093), respectively, suggesting that the pcDNA3.1-hERβ + plasmid, blank plasmid and Lipofectamine® 2000 treated PC-3 cell lines exhibited alterations in the expression levels of ERβ. The most marked effect was observed in the pcDNA3.1-hERβ + plasmid transfected cell lines.

When considering ERα, the results suggest that the expression level of ERα was lower compared with the expression level of AR and ERβ in the PC-3 cells, and that the positive rate of ERα expression was only 56%. There was no statistically significant difference observed in the levels of ERα expression in the pcDNA3.1-hERβ-plasmid group compared with blank cells group (P=0.79). When the rates of all groups were compared, there was no significant difference observed (P>0.05), as demonstrated in Fig. 4.

Expression levels of AR, ERα and ERβ in human PCa PC-3 cell lines transfected with the pcDNA3.1-hERβ plasmid. In the present study, qPCR detected the patterns of ERβ, ERα and AR mRNA expression levels in the recombinant plasmid, the empty plasmid, Lipofectamine® 2000 and normal PC-3 cells groups. As illustrated in Fig. 5, the expression of AR and ERβ was detected in all cells, with the AR and ER positive expression rates at 100%. However, the expression of ERα was only observed in 61% of the samples.

Subsequent to the transfection of the PC-3 cells with the pcDNA3.1-hERβ+plasmids, the expression levels of the 3 receptors were lower compared with the blank/control cell group: AR1.179±1.277 vs. 1.263±0.209; ERα: 1.055±0.964 vs. 1.202±1.119 and ERβ: 0.824±0.186 vs. 1.790±0.032, as demonstrated in Table IV and Fig. 6. Of these, the decrease was most marked in the expression levels of ERβ. There was also a marked increase in the level of apoptosis in the transfected cells, and the ratio of ERβ/AR was <1 (0.824±0.186/1.179±1.277 <1) compared with the control group in which the ERβ/AR ratio was >1 (1.790±0.032/1.263±0.209 >1). This demonstrates that ERβ exhibited a downward trend in expression levels, and AR expression levels exhibited an upward trend in the PCa PC-3 cell lines. When the ΔΔCq value of the expression level of ERα between the pcDNA3.1-hERβ+plasmid transfection, 1.055±0.964, and the control blank PC-3 cells groups, 1.202±1.119, was compared, no statistical significance was observed (P=0.079, 95% CI, 0.079-1.0166). This suggests that

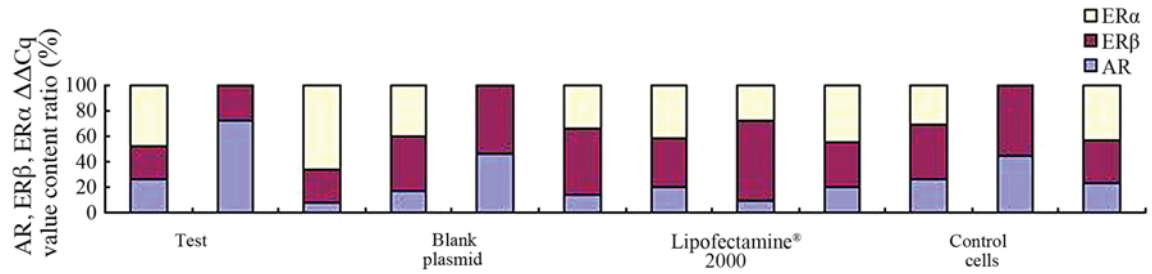

Figure 5. AR, ERβ, ERα ΔΔCq value content ratio diagram. The proportion of AR, ERβ and ERα in each group is demonstrated. AR, androgen receptor; ERβ, estrogen receptor β; ERα, estrogen receptor α.

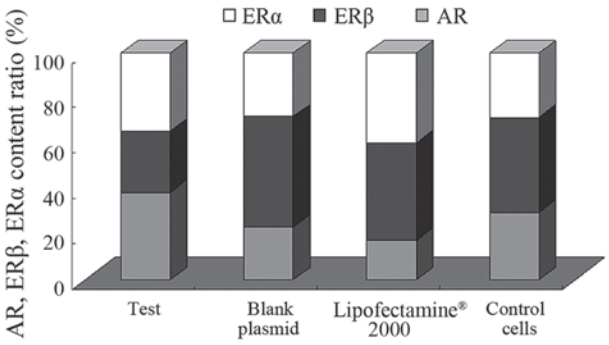

Figure 6. Value of AR, ERβ, ERα Content Ratio Diagram. The proportion of AR, ERβ and ERα in each group is demonstrated. AR, androgen receptor; ERβ, estrogen receptor β; ERα, estrogen receptor α.

the changing ERβ expression exhibited little effect on the expression of ERα.

Discussion

PCa is the most type of common hormone-dependent tumor in incidence after breast cancer, and the effect of the changes in the levels of sex hormones on the etiology and mechanism of the disease are significant (11,18). Current research on PCa pathogenesis demonstrates that ERs or ARs, particularly ERβ, are associated with the origin and development of prostatitis and PCa (5). However, the molecular mechanisms of this association remain unknown. Imbalances in the levels of circulating estrogen and androgens in males that occur as they age may cause changes in the levels of expression of AR in prostatic cells (19). Although the levels of androgen-dihydrotestosterone (DHT) in the prostate tissue do not increase, the changes in AR expression may also cause changes in the expression levels of numerous growth factors secreted by the gland and basal cells of the prostatic epithelium, which may stimulate proliferation and suppress the apoptosis of these cells, eventually leading to PCa (20). However, the mechanism of interaction between ERs and ARs in the process of PCa development, whether changes in the ER cause changes in the AR, remains unknown.

In present study, the pcDNA3.1-hERβ-plasmid was successfully transfected into the PC-3 cell lines using the eukaryotic cell transfection technique. The levels of expression of AR, ERβ and ERα were measured using RT-PCR and fluorescence qPCR, which demonstrated that AR and ERβ are constantly expressed in PC- 3 cell lines. However, the ERα were only expressed in ~50% of the PC-3 cell lines. These results suggest that the PC-3 cell line was an ideal cell model and may indicate the interaction between sex hormones and corresponding receptors. Therefore, PC-3 cell lines may be used to investigate the interaction between the hormone and its receptors *in vitro*. DHT serves an important role in the AR-mediated regulation of the development of the prostate (21). Mutations in the AR receptor may lead to an attenuated ligand-binding ability, which may cause complete or partial androgen resistance. Partially mutated AR may be activated by antagonists, which may lead to the development of hormone refractory PCa (21).

It has been hypothesized that the expression of AR is exhibited throughout PCa tissue: In castration-resistant PCa, AR is still expressed (22). In contrast, Suryavanshi *et al* (23) demonstrated that there was a significant loss in AR expression in certain cases of late hormone refractory PCa. As the level of expression of ER remains the same, Kleb *et al* (24) suggested that small cell PCa does not express AR or respond to hormonal therapies, as the expression of AR does not demonstrate the corresponding decline or deficiency. The current study indicates that there is an association between expression levels of AR and the progress of PCa, and demonstrated that the expression level of AR was constant in all groups, although no significant difference between the pre- and post-transfection cells was observed. However, as levels of ERβ expression exhibited a downward trend, the level AR expression exhibited an upward trend in the PC-3 cell lines.

The prostate gland is not a classical target organ of estrogen and exhibits low or undetectable expression of ERα. Simultaneously, the prostate gland demonstrates a significant expression of ERβ (14). Weihua *et al* (25) and McPherson *et al* (26) reported the ERα was mainly expressed in the stromal cells of the prostate, whereas ERβ expression levels were marked in the luminal epithelial cells. However, Lau *et al* (27) detected the expression of ERβ and ERα in the androgen-independent PC-3 cell lines, and revealed that estrogen and anti-estrogen negatively regulate PC-3 cell growth. The present study demonstrated that unlike the significant levels of expression of AR and ERβ, ERα exhibited no constant expression levels.

The loss of ERα expression at the mRNA level was identified in ~50% of the cell groups, comparing the transfection and control groups. The expression of ERα also exhibited no significant change, which suggests that ERα may not serve a major role in PC-3 cell growth, and demonstrates that ERα

was not the predominant ER subtype in the PC-3 cell line. It also suggests that the prostate gland is not a classical estrogen target organ.

Cell proliferation in early PCa is attributed to the inhibition of apoptosis of cells (28). Previous studies report that ERβ may promote apoptosis via the downward regulation of the protein kinase B signaling pathway (7). This is an important signaling pathway that may promote the growth of tumor cells and blood vessels and enhance the metastatic efficacy of the tumor cells. Concurrently, ERβ may promote the expression levels of B-cell lymphoma-2-like protein 4 and the apoptosis promoter protein-caspase-3 (28). A previous study reported that ERβ may serve an anti-proliferative role via downregulating the expression levels of androgen receptors (25). In the present study, it was demonstrated that PC-3 cells may consistently express ERβ. Furthermore, when the PC-3 cells were transfected the pcDNA3.1-hERβ-plasmid, the expression of ERβ exhibited a marked downward trend, which was evidently different compared with the control groups. The PC-3 cells illustrated a clear inhibition of proliferation, which indirectly suggests an association between the lack of ERβ with the levels of apoptosis of the PC-3 cells, and also indirectly confirms that the ERβ recombinant plasmid was successfully transfected into the PC-3 cell lines.

With a marked downward regulation of the expression levels of ERβ, the expression levels of AR and ERα were compared, and it was demonstrated that although the expression levels of AR were also downregulated, the degree was less compared with ERβ. Concurrently, the respective proportional upward and downward trends of expression levels of AR and ER-β exhibited when the transfection and blank control groups were compared provides data to suggest that ERβ may regulate PCa cell growth via the expression of AR.

To the best of our knowledge, the present study is the first to demonstrate the hypothesis that ERβ may regulate the expression of AR to inhibit the growth of PCa PC-3 cells. It was difficult to determine the association between ERα and AR, as the expression levels of ERα were not consistent between the PC-3 cell lines.

In the present study, subsequent to pcDNA3.1-hER-plasmid transfection, it was demonstrated that the expression levels of the three receptors AR, ERβ and ERα were lower in the transfection group compared with the control group. There were different degrees of downregulation, in particular the expression of ERβ was markedly decreased, which suppressed PCa PC-3 cell lines growth *in vitro*. These data support the hypothesis that ERβ performs the opposite regulatory action to cell growth: ERβ may serve a role in the direct suppression of PC-3 cell proliferation. However, the data do not fully describe and analyze the interaction between ERs and ARs due to the low number of cell samples, use of a singular cell line and the lack of confirmation of results through crosschecking analysis between other cell lines, such as Dul45 and LNCaP. Therefore, additional studies are required to investigate the molecular mechanisms underlying the suppression of the growth of prostate cancer cells by hormone receptors.

In conclusion, in PCa PC-3 tissues, the expression of AR demonstrated an upward or variant trend, and ERβ expression was downregulated. Therefore, it is hypothesized that the variation in expression levels of ER and AR may serve an important role in the pathogenesis of PCa. At an mRNA level, the PCa PC-3 cell line constantly expressed AR and ERβ, whereas the level of ERα expression was inconsistent. These results support the hypothesis that ERβ is a candidate gene: Increasing the ERβ expression level in PCa cells may be an effective therapeutic strategy to treat PCa.

Acknowledgements

This study was funded by the KunMing University of Science and Technology (grant no. kksy201460021).

References

1. Benjamins MR, Hunt BR, Raleigh SM, Hirschtick JL and Hughes MM: Racial disparities in prostate cancer mortality in the 50 Largest US Cities. Cancer Epidemiol 44: 125-131, 2016.
2. James N, Pirrie S, Pope A, Barton D, Andronis L, Goranitis I, Collins S, McLaren D, O'Sullivan J, Parker C, *et al*: TRAPEZE: A randomised controlled trial of the clinical effectiveness and cost-effectiveness of chemotherapy with zoledronic acid, strontium-89, or both, in men with bony metastatic castration-refractory prostate cancer. Health Technol Assess 20: 1-288, 2016.
3. Slusarz A, Jackson GA, Day JK, Shenouda NS, Bogener JL, Browning JD, Fritsche KL, MacDonald RS, Besch-Williford CL and Lubahn DB: Aggressive prostate cancer is prevented in ERαKO mice and stimulated in ERβKO TRAMP mice. Endocrinology 153: 4160-4170, 2012.
4. Levakov AF, Kaćanski MM, Vucković N, Zivojinov M, Amidzić J and Sabo JI: The expression and localization of estrogen receptor beta in hyperplastic and neoplastic prostate lesions. Vojnosanit Pregl 72: 906-913, 2015.
5. Wang L, Zhang P, Meng X, Chen X, Xiang Z, Lin X, Liu Y, Gan W, Han X and Li D: Correlation between the germline methylation status in ERβ promoter and the risk in prostate cancer: A prospective study. Fam Cancer 15: 309-315, 2016.
6. Weihua Z, Warner M and Gustafsson JA: Estrogen receptor beta in the prostate. Mol Cell Endocrinol 193: 1-5, 2002.
7. Kumar R, Verma V, Sharma V, Jain A, Singh V, Sarswat A, Maikhuri JP, Sharma VL and Gupta G: A precisely substituted benzopyran targets androgen refractory prostate cancer cells through selective modulation of estrogen receptors. Toxicol Appl Pharmacol 283: 187-197, 2015.
8. Mahmoud AM, Al-Alem U, Ali MM and Bosland MC: Genistein increases estrogen receptor beta expression in prostate cancer via reducing its promoter methylation. J Steroid Biochem Mol Biol 152: 62-75, 2015.
9. Asgari M and Morakabati A: Estrogen receptor beta expression in prostate adenocarcinoma. Diagn Pathol 6: 61, 2011.
10. Attia DM and Ederveen AG: Opposing roles of ERα and ERβ in the genesis and progression of adenocarcinoma in the rat ventral prostate. Prostate 72: 1013-1022, 2012.
11. Medeiros R, Vasconcelos A, Costa S, Pinto D, Morais A, Oliveira J and Lopees C: Steroid hormone genotypes ARStuI and ER325 are linked to the progression of human prostate cancer. Cancer Genet Cytogenet 141: 91-96, 2003.
12. Muthusamy S, Andersson S, Kim HJ, Butler R, Waage L, Bergerheim U and Gustafsson JÅ: Estrogen receptor β and 17β-hydroxysteroiddeh ydrogenase type 6, a growth regulatory pathway that is lost in prostate cancer. Proc Natl Acad Sci USA 108: 20090-20094, 2011.
13. Signoretti S and Loda M: Estrogen receptor beta in prostate cancer: brake pedal or accelerator? Am J Pathol 159: 13-16, 2001.
14. Megas G, Chrisofos M, Anastasiou I, Tsitlidou A, Choreftaki T and Deliveliotis C: Estrogen receptor (α and β) but not androgen receptor expression is correlated with recurrence, progression and survival in post prostatectomy T3N0M0 locally advanced prostate cancer in an urban Greek population. Asian J Androl 17: 98-105, 2015.
15. Lombardi AP, Pisolato R, Vicente CM, Lazari MF, Lucas TF and Porto CS: Estrogen receptor beta (ERβ) mediates expression of β-catenin and proliferation in prostate cancer cell line PC-3. Mol Cell Endocrinol 430: 12-24, 2016.

16. Royuela M, de Miguel MP, Bethencourt FR, Sánchez-Chapado M, Fraile B, Arenas MI and Paniagua R: Estrogen receptors alpha and beta in the normal, hyperplastic and carcinomatous human prostate. J Endocrinol 163: 447-454, 2001.
17. 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.
18. Bosland MC: Sex steroids and prostate carcinogenesis: Integrated, multifactorial working hypothesis. Ann N Y Acad Sci 1089: 168-176, 2006.
19. Thapa D and Ghosh R: Chronic inflammatory mediators enhance prostate cancer development and progression. Biochem Pharmacol 94: 53-62, 2015.
20. Alcaraz A, Hammerer P, Tubaro A, Schröder FH and Castro R: Is there evidence of a relationship between benign prostatic hyperplasia and prostate cancer? Findings of a literature review. Eur Urol 55: 864-873, 2009.
21. Veldscholte J, Ris-Stalpers C, Kuiper GG, Jenster G, Berrevoets C, Claassen E, van Rooij HC, Trapman J, Brinkmann AO and Mulder E: A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. Biochem Biophys Res Commun 173: 534-540, 1990.
22. Heinlein CA and Chang C: Androgen receptor in prostate cancer. Endocr Rev 25: 276-308, 2004.
23. Suryavanshi M, Mehta A, Jaipuria J, Sharma AK, Rawal S and Seth N: Weaker ERG expression in patients with ERG-positive prostate cancer is associated with advanced disease and weaker androgen receptor expression: An Indian outlook. Urol Oncol 33: 331.e9-15, 2015.
24. Kleb B, Estécio MR, Zhang J, Tzelepi V, Chung W, Jelinek J, Navone NM, Tahir S, Marquez VE, Issa JP, *et al*: Differentially methylated genes and androgen receptor re-expression in small cell prostatecarcinomas. Epigenetics 11: 184-193, 2016.
25. Weihua Z, Ekman J, Almkvist A, Saji S, Wang L, Warner M and Gustafsson JA: Involvement of androgen receptor in 17beta-estradiol-induced cell proliferation in rat uterus. Biol Reprod 67: 616-623, 2002.
26. McPherson SJ, Ellem SJ, Simpson ER, Patchev V, Fritzemeier KH and Risbridger GP: Essential role for estrogen receptor beta in stromal-epithelial regulation of prostatic hyperplasia. Endocrinology 148: 566-574, 2007.
27. Lau KM, LaSpina M, Long J and Ho SM: Expression of estrogen receptor (ER)-alpha and ER-beta in normal and malignant prostatic epithelial cells: Regulation by methylation and involvement in growth regulation. Cancer Res 60: 3175-3182, 2000.
28. Cheng J, Lee EJ, Madison LD and Lazennec G: Expression of estrogen receptor beta in prostate carcinoma cells inhibits invasion and proliferation and triggers apoptosis. FEBS Lett 566: 169-172, 2004.