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Original article

Expression of Estrogen Alpha and Beta Receptors in Prostate Cancer and Hyperplasia: Immunohistochemical Analysis

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ABSTRACT

Objectives: Estrogen receptors are believed to play a significant role in the pathogenesis of prostate carcinoma (PCa). The aim of this study is to evaluate the expression of ER-α and ER-β in human benign and malignant prostatic tissue.

Patients and Methods: The archival materials of 100 prostatic specimens (65 PCa, 35 BPH) were collected from the Department of Pathology, King Abdul-Aziz University Hospital, Jeddah, Saudi Arabia. Seven PCa cases contained foci of high-grade prostate intraepithelial neoplasia (HGPIN). Immunohistochemistry was used to test the protein expression of ER-α and ER-β utilizing monoclonal mouse antihuman antibodies.

Results: Among the 65 cases of PCa, ER-α was expressed in 3 cases (4.6%) in epithelial cells and 4 cases (6.1%) in stromal cells. ER-α was not expressed in any of the HGPIN foci. Additionally, ER-α was not expressed in either luminal or basal cells in any of the 35 BPH cases. However it was expressed in 4 cases (11.4%) in stromal cells of BPH. In PCa, ER-β was expressed in 61 cases (93.8%) and 35 cases (53.8%) in the epithelial and stromal cells respectively. ER-β was expressed only in 2 cases (28.5%) out of 7 HGPIN foci. It was expressed in 33 cases (94.3%) of epithelial and stromal cells of BPH.

Conclusion: The majority of PCa and BPH exhibited nuclear immunoreactivity for ER-β in both tumor and stromal cells and they are usually negative for ER-α. There is probably partial loss of ER-β in HGPIN. ER-β may have a role in the process of prostatic hyperplasia and malignancy.

Key Words: ER-α, ER-β, prostate, hyperplasia, premalignant, cancer, immunohistochemistry

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INTRODUCTION

Prostate carcinoma (PCa) is a large global health problem. It is the second most commonly diagnosed cancer in men and is the second leading cause of cancer-related death in the United States[1-4]. Several studies have focused on the association between androgens and PCa risk, postulating that androgens are needed for prostate growth and differentiation[5-7]. Nevertheless, there is a growing body of evidence to suggest that estrogen signaling also plays a significant role in normal and abnormal growth of the prostate gland[8-11]. Estrogens directly target prostate tissue by specific estrogen receptors (ER). The human prostate is equipped with a dual system of ERs (ER-α and ER-β)

that undergoes profound remodeling during prostate cancer development and progression[12-14]. The evidence on the role of estrogens and ER in prostate carcinogenesis is largely obtained from experimental data reported in animal models[15]. In rat models, it was clear that estrogens are required for a maximal carcinogenic response to androgens. The question arises whether the carcinogenic effects of estrogens demonstrated in animal prostate cancer models are applicable to the biology of the human prostate, as few studies have addressed this issue in humans[16]. Additionally, the complex intraprostatic interactions of estrogens and ER as well as their combined effect remain to be ascertained. In this study we examined the expression of ER-α and ER-β in human PCa as well as benign prostatic hyperplasia (BPH) specimens, using immunohistochemistry techniques to localize ER expression in epithelial (luminal and basal) as well as stromal cells.

MATERIALS AND METHODS

Setting and specimens: The study was conducted in the Departments of Pathology and Urology at King Abdul-Aziz University Hospital, Jeddah, Saudi Arabia. The archived prostatic specimens (100) of 65 consecutive patients with PCa and 35 patients with BPH obtained from 2003 to 2008 were selected for the study. BPH was pathologically diagnosed as glandular and/or fibromuscular hyperplasia in transurethral resection of the prostate (TURP) or open prostatectomy specimens or transrectal ultrasound (TRUS) guided prostate biopsies. The study involved 40 TRUS guided biopsies, 45 TURP specimens and 15 open surgical prostatectomy specimens. The indications for biopsy were elevated serum PSA levels, abnormal findings on digital rectal examination, or both. Biopsy cores were obtained according to the standard sextant technique. The indications for surgical intervention in BPH patients included failed medical therapy with alpha-blockers and/or 5-alpha reductase inhibitors, refractory or repeated episodes of acute urinary retention (AUR), recurrent urinary tract infections and hematuria.

Immunohistochemical examination and interpretation

The specimens were previously fixed in 10% formalin solution and prepared for the immunohistochemical procedure using the avidin-biotin immuno-enzymatic technique (ABT). The principal steps are as follows: selected blocks were cut into 5 micron sections, deparaffinized in xylene, rehydrated in graded alcohol and rinsed in Tris-buffered saline (TBS). Antigen-retrieval was done using water bath microwave. The sections were incubated in 5% normal rabbit serum and incubated with monoclonal mouse antihuman antibodies for ER-α (dilution 1: 35; clone 1D5, Dako) and ER-β (dilution 1: 35; clone PPG5/10; Dako). The slides were visualized using 3, 3'-diaminobenzidine (DAB). Meyer's hematoxylin was used as counter stain. Positive and negative controls were used with each run of immunoassay. Positive control sections were obtained from mammary tissue known to be positive for the antibodies. Primary antibodies were omitted for negative controls. The case was only considered positive when both negative and positive controls were working, which avoids any false positive staining. After completion of the immunohistochemical staining, the cases were examined microscopically for the localization and intensity of the selected antibody. The degree of immunoreactivity in the targeted cells was evaluated. Positive immunohistochemical staining was defined as unequivocal nuclear staining of at least 10% of the cells. The intensity of staining was determined and scored in an ascending 1 to 3 scale[17].

Statistical analysis

Statistical analysis was performed with Fisher's exact test using GraphPad InStat, Version 4 (GraphPad Software Inc, La Jolla, CA, USA). Statistical significance was determined as $p<0.05$.

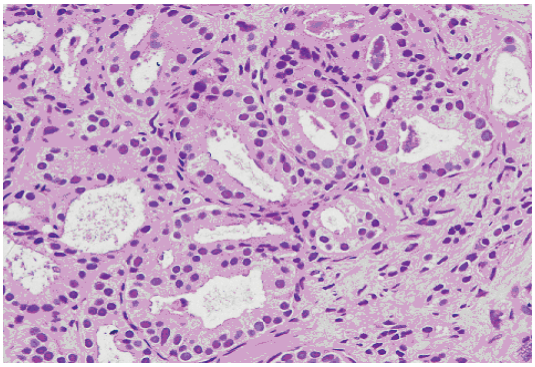

Fig. 1-A: Well differentiated prostate carcinoma (H&E X200)

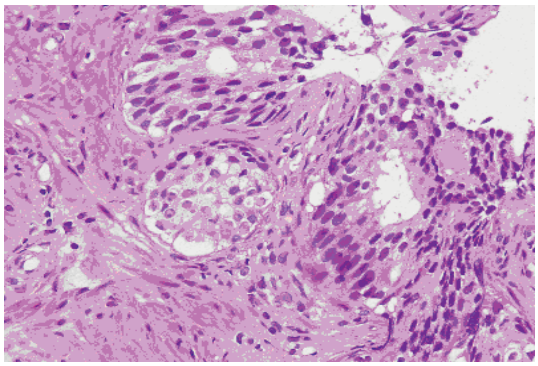

Fig. 1-B: A focus of high-grade prostate intraepithelial neoplasia (HGPIN) (H&E X200)

RESULTS

The histopathology findings in the 100 specimens (65 PCa and 35 BPH) are shown in Table 1. Foci of HGPIN were seen in 7 cases (10.8%) of PCa specimens (Fig. 1).

ER-α expression in PCa specimens

Out of the 65 PCa specimens, only 3 (4.6%) revealed nuclear immunoreactivity (Fig. 2), while 62 (95.4%) were negative for nuclear ER-α expression (Fig. 2 and Table 2). Two of the ER-α positive case were Gleason score 7 and one score 8. Four PCa specimens (6.2%) exhibited stromal cell nuclear immunoreactivity. Seven cases (10.8%) demonstrated weak non-specific cytoplasmic staining for ER-α without any nuclear positivity. The 2 specimens of SCC as well as the 7 foci of HGPIN were all negative for nuclear ER-α immunoexpression.

ER-α expression in BPH specimens

All BPH specimens were negative for ER-α immunoexpression in the epithelial cells (Fig. 2) although 4 BPH specimens (11.4%) showed nuclear stromal cell reactivity for ER-α (Table 2).

ER-β expression in PCa specimens

Among PCa, 61 (93.8%) of specimens showed diffuse ER-β nuclear immunoreactivity with variable degrees of intensity in cancer cells (Fig. 3). Among them 26 specimens (42.6%) showed strong ER-β immunoexpression with score (3+) reactivity, 24 (39.3%) were score (2+) and 11 (18.1%) showed score (1+). Two of the specimens labeled as negative showed only focal nuclear staining for ER-β (less than 10% of the cells) and the other 2 specimens lacked any nuclear immunoexpression. The ER-β negative cases were Gleason score 6 (2 cases) and 7 (2 cases). Similarly, the two specimens of SCC showed strong positive nuclear immunoreactivity for ER-β. Thirty five (53.8%) of PCa specimens showed stromal cell immunoexpression for ER-β (Table 3). ER-β was expressed only in 2 (28.5%) of 7 PIN foci. No cytoplasmic staining was seen in any of the specimens.

ER-β expression in BPH specimens

Thirty three of 35 BPH specimens demonstrated positive nuclear immunostaining in the secretory cell layers. As shown in Table 3, 18 specimens (51.4%) exhibited diffuse ER-β nuclear immunoreactivity, 15 (42.9%) showed focal ER-β nuclear immunopositivity, while the remaining 2 (5.7%) lacked any nuclear immunoexpression (Fig. 3). Among the 33 positive nuclear immunostaining BPH specimens, 11 (33.3%) revealed score (3+), 19 (57.6%) showed score (2+) and 3 (9.1%) were score (1+). Basal cell layer immunoexpression for ER-β was detected in 25 (71.4%) BPH specimens. All BPH specimens that revealed positive epithelial nuclear immunoreactivity also revealed stromal cell immunopositivity. The specimens which lacked epithelial nuclear immunoreactivity lacked stromal cell reactivity as well. No cytoplasmic staining was seen in any of the specimen.

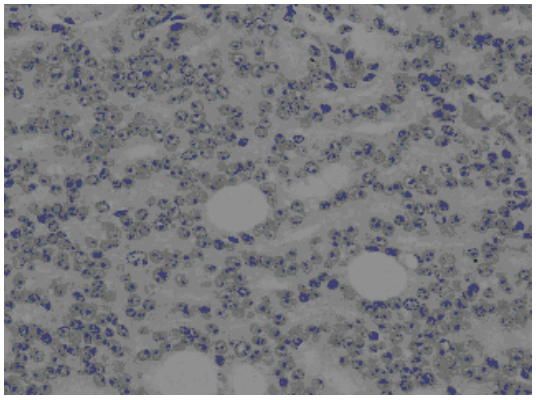

Fig. 2-A: Prostate cancer with immuno-negativity for ER-α in both tumor and stromal cells (DAB X 400)

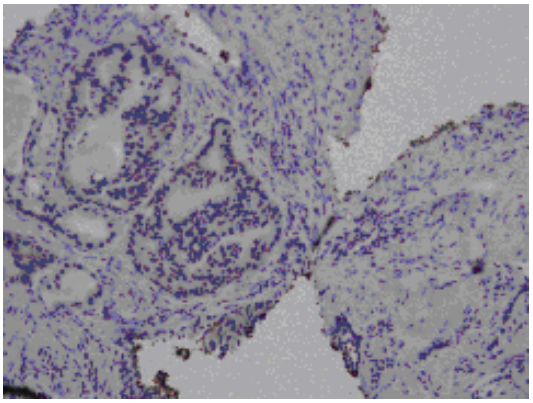

Fig. 2-D: Benign prostatic hyperplasia with negative nuclear immunostaining for ER-α in both glandular epithelium and stromal cells (DAB X200)

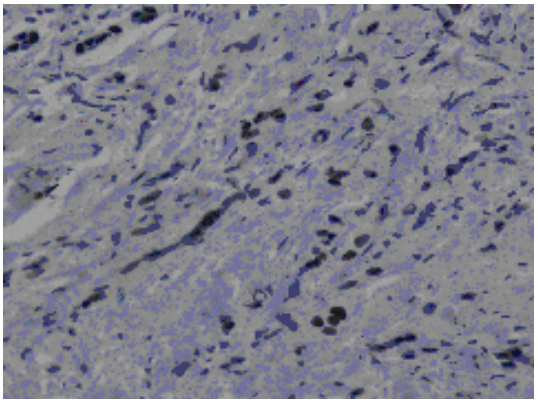

Fig. 2-B: Prostate cancer with positive immunoreactivity for ER-α in tumor cells (upper left corner) and negative immunostaining in stromal cells (DAB X 400)

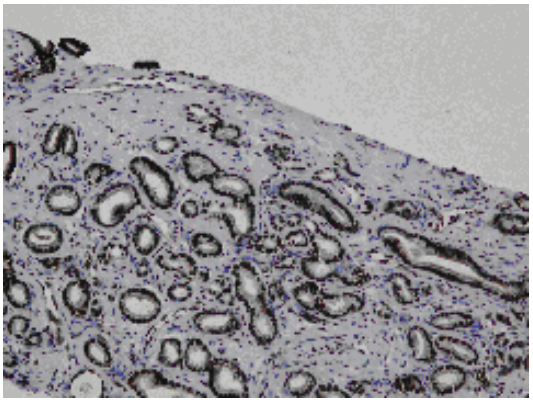

Fig. 3-A: Prostate cancer with strong and diffuse positive immunoreactivity for ER-β in tumor as well as stromal cells (DAB X100)

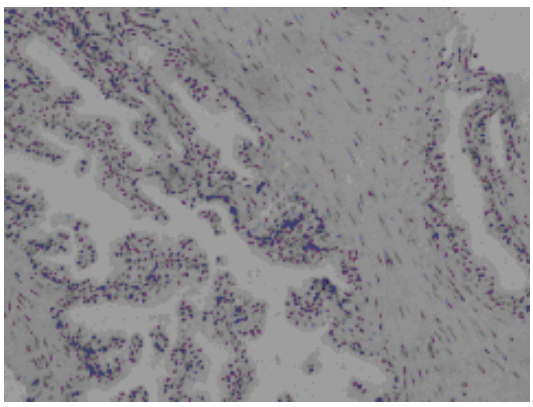

Fig. 2-C: Benign prostatic hyperplasia with negative immunostaining for ER-α in both epithelial and stromal cells (DAB X200)

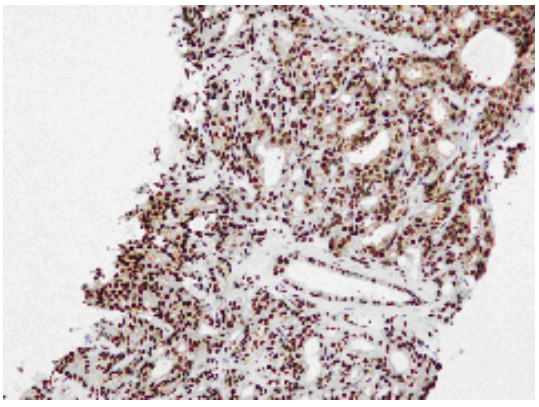

Fig. 3-B: Prostate cancer with positive immunostaining for ER-β in tumor (DAB X100)

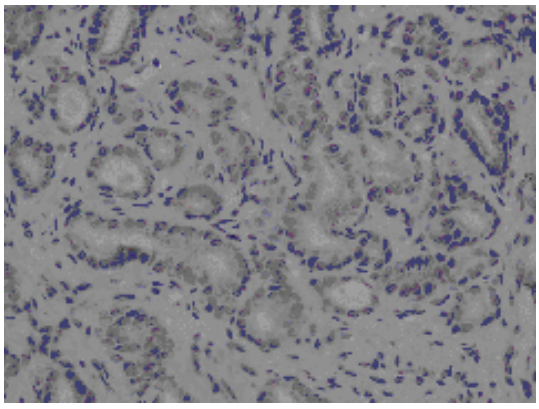

Fig. 3-C: Prostate cancer with negative immunoreactivity for ER-β in tumor and stromal cells (DAB X200)

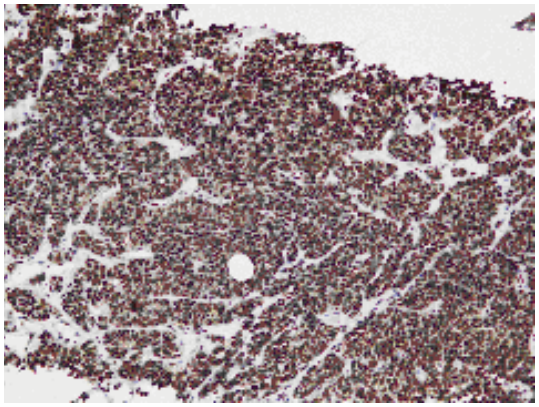

Fig. 3-E: Prostatic small cell carcinoma with strong and diffuse positivity for ER-β in tumor cells (DABX200)

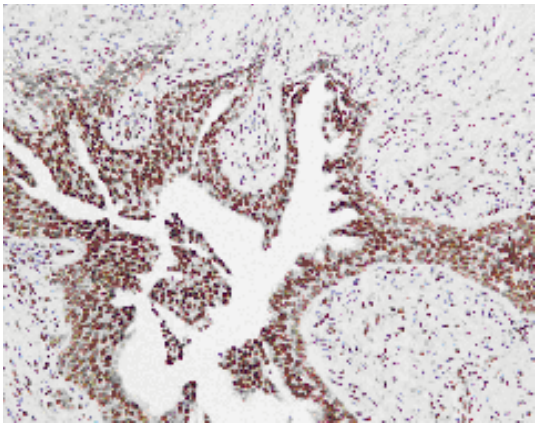

Fig. 3-D: Benign prostatic hyperplasia with positive immunostaining for ER-β in luminal, basal and stromal cells (upper right corner) (DAB X200)

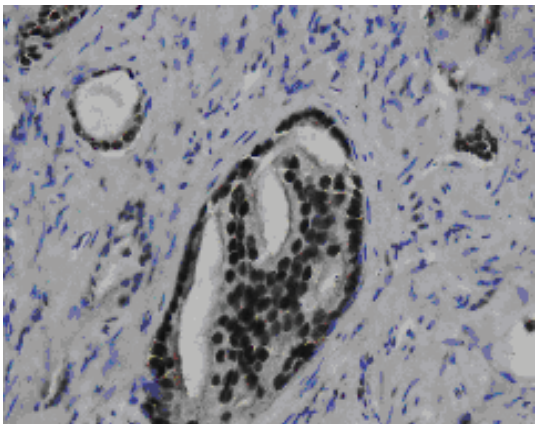

Fig. 3-F: High-grade prostatic intra-epithelial neoplasia (HGPIN) with positive nuclear immunostaining for ER-β in the dysplastic cells (DAB X200)

DISCUSSION

Elucidation of the mechanisms of action of estrogens in prostatic carcinogenesis will likely spur novel strategies in prostate cancer prevention and treatment. Probably, there will be a significant potential for the use of ER-α and ER-β modulators to prevent prostate cancer and delay disease progression[18]. It was reported that ER-β may play a significant role in prostate cell differentiation and proliferation and may modulate both the initial phases of prostate carcinogenesis and androgen-independent tumor growth. It remains to be etablished whether ER-β enhances or suppresses prostate cancer development and/or progression[19]. For prostatic cancer cell lines, LNCap expressed the mRNA of both receptors, but DU-145 and PC-3 only expressed ER-β mRNA[20,21]. Although ER-α and ER-β have similar ligand-binding domains and both bind estrogen, there is evidence that ER-α and ER-β demonstrate distinct and sometimes opposing transcriptional activities[22-23]. Also, upregulation of ER-β expression by certain drugs such as phytoestrogens and isoflavones has been found to be able to transform prostate cancer cells into a less malignant phenotype which can add more insights into the role of ER-β in prostate cancer management[24]. Ellem and Risbridger[25] suggested that ER-β appears to mediate its beneficial effects by preventing hyperplasia, inflammation and carcinogenesis. Additionally, ER-β may protect normal prostate epithelia from undergoing aberrant cell proliferation

and may have a role in enhancing survival of PCa cells[14,18,26-29]. In another study, Zhu et al demonstrated evidence of repression of ER-β in local PCa and re-expression of the gene within metastasis[30]. It has been suggested that there is a significant role of prostatic inflammation in the pathogenesis of PCa. However, the link between estrogen, inflammation and PCa requires further investigation[31-33].

Our study has confirmed previous reports[31-36] that ER-α is not usually seen in the tumor cells or epithelial cells of BPH and can be seen rarely in the stromal cells. There was no statistically significant difference between PCa and BPH regarding the expression of ER-α ($p = 0.54$). Similarly, our observation is in agreement with those mentioned by Horvath et al[18] who reported that none of the 39 radical prostatectomy specimens examined showed epithelial staining for ER-α, but 16 cases had stromal staining of ≥5% positive nuclei. In our study there was no relationship between the type of prostatic specimen examined and the immunopositivity for ER-α expression. There was also no relationship between the combined Gleason score and the degree of ER-α expression.

Most previous studies failed to show significant levels of ER-α immunostaining in the epithelial components, while immunostaining when found was confined to the stromal tissue[34,37]. However, a few previous studies[12,14,38] found positive ER-α immunostaining in the epithelia. They described this as immunolocalization within the basal cell layer. Yet, this staining was universally weak. ER-α was down regulated in PCa hormone refractory tumors[39,40]. Additionally, some authors reported that the level of ER-α expression and methylation was inversely correlated with the pathological grades of PCa[34,41]. Our findings are also in contrast with those reported by Bonkhoff and co-workers[12] who found immunostaining of ER-α in high-grade dysplasia and grade 4-5 PCa. Walton et al[38] performed a study on pure populations of benign and malignant prostatic epithelial cells after alteration of >99% of the stromal cells. They reported that median ER-α expression was 9.4 times more common than ER-β expression. This discrepancy may be due to the use of RT-PCR technique. Some authors[9,42] reported that higher levels of ER-α was observed in Hispanics and Asian men than in Caucasian and African-American men. In the Japanese population polymorphism in codon 10 ER-α was found to be associated with PCa [43].

In our study, all 7 foci of HGPIN were negative for ER-α immunoexpression. Leav et al[14] reported that ER-α expression was limited only to a small subset of dysplastic lesions. Bonkhoff et al suggested that ER-α in the human prostate acts as an oncogene, which is overexpressed during malignant transformation of the prostatic epithelium[12]. The complete absence of ER-α in basal and luminal cells in our study is not supportive of this conclusion.

Regarding the expression of ER-β in PCa, this study revealed that 93.8% of the cases were diffusely immunoreactive for ER-β, 3.1% were focally positive, while the remaining 3.1% were negative for ER-β immunoexpression. The majority of studied PCa specimens exhibited moderate to strong positive nuclear ER-β immunoreactivity. Additionally, 35 cases (53.8%) of PCa showed stromal cell immunostaining for ER-β. There was no statistically significant difference between PCa and BPH regarding the expression of ER-β ($p = 1.00$). Similar to ER-α, ER-β immunostaining was not affected by the type of prostatic specimen or by the Gleason score. We noticed also that ER-β immunoexpression was seen in 2 (28.5%) of the 7 HGPIN foci. About the immunoexpression of ER-β in BPH, this study revealed that 94.3% exhibited either diffuse or focal nuclear immunostaining in the secretory cell layers. However, immunoexpression of ER-β in the basal cell layer was detected in 71.4% of the cases. Additionally, stromal cell immunostaining was seen in all BPH specimens that revealed positive epithelial nuclear immunoreactivity (94.3%).

The results on ER-β expression in PCa are even more controversial and conflicting. Multiple previous publications reported complete loss of ER-β immunostaining in PCa[18,30,44-46]. Hormone-naive PCa was reported to retain high levels of ER-β expression even in lymph node and bone metastasis [13]. Gabal et

al reported ER-β nuclear immunoreactivity in 90% of the studied BPH cases. They showed that 53% of PIN cases were negative for ER-β expression and 82.8% of the studied PCa cases were negative[47]. However, a study done by Stettner et al[24] reported no change in the expression of ER-β in PCa. On the other hand, Walton et al[38] used quantitative RT-PCR technique and reported 4.5 fold increases in the expression of ER-β in PCa specimens compared with BPH. Additionally, they found a significant positive correlation between ER-β expression and androgen receptor-dependent PSA PCa. Torlakovic et al reported that ER-β was expressed in 93% of PCa and was positively associated with primary Gleason grade[48]. They suggested that ER-β, as detected by PPG5/10 antibody, may have a role in the process of dedifferentiation of PCa, with higher levels present in less differentiated tumors[48].

Our study, did not show any correlation between tumor Gleason score and ER-β expression. Lai et al[49] reported that ER-β was expressed in all 33 studied cases of osseous metastasis and in all 27 cases of non-osseous metastasis from PCa. They suggested that the use of selective estrogen modulators may be an effective method of treating advanced PCa. They speculated that the intense staining of ER-β in metastases may be due to changes of the methylation pattern of ER-β promoter in metastasic versus primary PCa. In our study we noticed a loss of ER-β immunoexpression in HGPIN. As the chemopreventive role of phytoestrogens depends on the presence and function of ER-β, it can be speculated that the dietary intake of phytoestrogens is beneficial in those patients with either no HGPIN or with HGPIN retaining high levels of ER-β expression[16].

The variation between different studies regarding ER-β status in PCa may due to the different techniques used, duration of fixation, fixative used, prolonged exposure of slides to room air or even using different cut-off values for positivity in the same technique. For example, some of the studies defined positive expression for ER as nuclear staining in >50% by immunohistochemistry techniques, while others used lower cut-off values. The problem with PCR techniques is the purity of the material and the inclusion of stromal and epithelial cells in the specimens. In this study we utilized the immunohistochemistry technique which is a powerful and generally accepted tool for assessment of ER status in breast cancer. We also used the generally accepted cut-off value for positivity, which is 10% of the cells with clear nuclear staining[50, 51].

CONCLUSIONS

In this study, the majority of PCa and BPH specimens were negative for nuclear immunostaining for ER-α in both tumor and stromal cells. ER-α was negative in both luminal and basal cells in the BPH cases. The majority of PCa and BPH specimens exhibited nuclear immunoreactivity for ER-β in both tumor and stromal cells. Also, ER-β was positive in the majority of luminal and basal cells in BPH. Although the number of HGPIN cases was small in this study, the results suggested partial loss of ER-β in HGPIN. Both ER-α and ER-β immunopositivity was not affected by the type of prostatic specimen or the grade of Pca. The predominance of ER-β in the prostate may offer new opportunities for designing therapies or intervention strategies based on understanding the biology of ER subtypes.

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