ORIGINAL ARTICLE

UDC: 616.65
DOI: 10.2298/VSP131022069F

# The expression and localization of estrogen receptor beta in hyperplastic and neoplastic prostate lesions

## Ekspresija i lokalizacija estrogenog receptora beta kod hiperplastičnih i neoplastičnih lezija prostate

**Aleksandra Fejsa Levakov*†, Mihaela Mocko Kaćanski*†, Nada Vučković*‡, Mirjana Živojinov*‡, Jelena Amidžić*†, Jelena Ilić Sabo*†**

***Pathology and Histology Center, Clinical Center of Vojvodina, Novi Sad, Serbia;**
**†Department for Histology and Embryology, ‡Department for Pathology, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia**

**Abstract**

**Background/Aim.** Benign acini in benign prostatic hyperplasia (BPH) are lined with pseudostratified cylindrical epithelium with a continuous basal cell layer. Adenocarcinoma of the prostate is the most common cancer in men. High gradus-prostatic intraepithelial neoplasia (HGPIN) lesions precede invasive cancer. Prostate adenocarcinoma (PCa) implies a complete absence of basal cells and stromal invasion by malignant acini. Estrogen receptor (ER) is located in nuclei of acinar basal and secretory cells and partially in stromal cells. The aim of this research was to demonstrate and localize ER in BPH and in PCa of different Gleason scores. Considering literature data for ER-betą expression in different morphologic prostate lesions, it is assumed that there is expression of ER-beta in most moderately differentiated PCa, and that the observed receptor expression is lost with increasing of the Gleason score. **Methods.** Four groups of patients were formed: the control with BPH and three experimental groups with PCa of different grades and scores, according to the Gleason grading system. The patients were male of various ages suspected of PCa, based on clinical and laboratory parameters. The study was conducted in a period 2010–2012. None of the patients received prior hormonal therapy. Sextant byopsies with BPH and PCa were treated for ER-beta (Novocastra). Localization and intensity of ER-betą expression is reported through the score: 0 = zero; 1 = < 1%; 2 = 1–10%; 3 = 11–33%; 4 = 34–66%; 5 – > 66%. Positive fibroblasts and endothelial cells are used for comparison. **Results.** ER-beta expression in acinar epithelial cells was the weakest in well-differentiated adenocarcinoma. A decline of ER-beta expression was noticed in malignant lesions of the prostate *vs* benign ones. Less differentiated adenocarcinomas showed a decrease of ER-beta expression in basal and in the secretory cells. ER-betą expression in basal cells was stronger than in secretory ones in BPH and well-differentiated adenocarcinoma. **Conclusion.** ER-beta expression was most pronounced in BHP samples and declined in malignant prostatic lesions. This finding supports statement on antiproliferative role of ER-beta in prostatic tissue.

**Key words:**
**prostatic neoplasms; adenocarcinoma; estrogen receptor beta.**

**Apstrakt**

**Uvod/Cilj.** Benigni acinusi kod benigne hiperplazije prostate (BHP) obloženi su pseudostratifikovanim cilindričnim epitelom sa kontinuiranim slojem bazalnih ćelija. Adenokarcinom prostate najčešći je karcinom kod muškaraca. Intraepitelne prostatične neoplazme visokog gradusa (HGPIN) su lezije koje prethode nastanku invazivnog karcinoma (Pca) i podrazumevaju kompletno odsustvo bazalnih ćelija i invaziju strome malignim acinusima. Estrogeni receptor (ER) beta nalazi se u jedrima bazalnih i sekretornih ćelija acinusa i delimično u stromalnim ćelijama. Cilj ovog istraživanja bio je da se prikaže i lokalizuje ER beta u BHP, kao i u PCa sa različitim Gleason skorom. S obzirom na podatke iz literature o ekspresiji ER beta u različitim morfološkim lezijama prostate, pretpostavlja se da je ekspresija ER beta prisutna u većini srednje diferentovanih PCa, i da se ekspresija posmatranog receptora gubi sa povećanjem Gleason scora. **Metode.** Ispitivane su četiri grupe bolesnika: kontrolna grupa sa BHP i tri eksperimentalne grupe sa PCa različitih gradusa i skorova prema Gleason-ovom sistemu. Bolesnici su bili muškarci različite starosti sa sumnjom na PCa, na osnovu kliničkih i laboratorijskih parametara. Studija je sprovedena u periodu 2010–2012. Nijedan od bolesnika nije prethodno

**Correspondence to:** Aleksandra Fejsa Levakov, Pathology and Histology Center, Clinical Center of Vojvodina 21 000 Novi Sad, Serbia. E-mail: aleksandralevakov@sbb.rs

primio hormonsku terapiju. Sekstant biopsije sa nalazom BHP i PCa bojene su na ER beta (Novocastra). Lokalizacija i intenzitet ER betą ekspresije prikazani su kroz skor: 0 = nula; 1 = < 1%; 2 = 1–10%; 3 = 11–33%; 4 = 34–66%; 5 = > 66%. Pozitivni fibroblasti i endotelne ćelije korišćene su za poređenje. **Rezultati.** Ekspresija ER beta u epitelnim ćelijama acinusa bila je najslabija u dobro diferentovanim adenokarcinomima. Smanjena ekspresije ER beta primećena je kod malignih lezija prostate naspram benignih lezija. Loše diferentovani adenokarcinomi prikazali su smanjenje ekspresije ER beta u bazalnim i sekretornim ćelijama. Kod BHP i dobro diferentovanih adeniokarcinomima bila je veća ekspresija ER betą u bazalnim ćelijama nego u sekretornim. **Zaključak.** Ekspresija Er-beta receptora B najizraženija je u uzorcima tkiva prostate bolesnika sa BHP i smanjuje se kod adenokarcinoma prostate. Ovaj nalaz podupire stanovište o antiproliferativnoj ulozi ER-beta u tkivu prostate.

**Ključne reči:**
**prostata, neoplazme; adenokarcinom; receptor, estrogenski, beta.**

## Introduction

### *Benign prostatic hyperplasia (BPH)*

Histologically, BPH shows proliferation of epithelial cells of acini and ducts, proliferation of smooth muscle cells and stromal fibroblasts in varying proportions [1]. Hyperplastic acini are lined with pseudostratified cylindrical epithelium with characteristic papillary hyperplasia [1]. Benign glands are surrounded by a continuous layer of basal cells positive for 34 beta E12. In chronic prostatitis, this layer may be discontinuous, and basal cells are progressively lost in prostatic intraepithelial neoplasia (PIN), as dysplasia becomes higher. Periuretheral glandular tissue is responsible for the development of BPH [1] and the true prostate tissue is the origin of prostate adenocarcinoma (PCa). Occult adenocarcinoma is found in 10% of surgical specimens with preoperative diagnosis of BPH.

It is possible that BPH is associated with a disturbed balance of androgen and estrogen in blood [1]. Generally accepted theory of the pathogenesis of BPH is associated with the effect of 5 alfa-dihydrotestosterone (DHT), androgen that controls normal prostate growth and proliferative disorders such as hyperplasia and Pca [1, 2]. DHT is synthesized in stromal cells by the conversion of circulating testosterone in the presence of 5 alfa-reductase. Once synthesized, DHT acts as autocrine agent on stromal cells as a paracrine hormone on the glandular epithelium, leading to its proliferation. In patients with BPH, relationship of circulating testosterone and DHT may be abnormally low. Reduced catabolism of DHT results in imbalance between losing and multiplication of epithelial and stromal cells [1].

### *Prostate adenocarcinoma*

The pathogenesis of PCa referred to genetic and environmental factors [3, 4]. The cause of PCa is still unknown, but endocrine effects are the center of researches. The chemical and physical trauma results in the damage of epithelial cells. Oxidative stress has the underlying causes: endogenous metabolites, inflammation, red meat and animal fat intake and circulating growth factors [1, 3–5]. Chronic inflammation caused by infectious agents of sexually transmitted diseases (STD) or environmental factors (nutrition) in 20% of all cases are the cause of PCa [3]. Most inflammatory lesions of the prostate are associated with focal atrophy of the epithelium [3, 4].

PIN represents the ducts lined with cytologically atypical luminal cells, and reduced in the number of the basal ones. High gradus-prostatic intraepithelial neoplasia (HGPIN) lesions precede invasive cancer [5, 6]. Histological definition of PCa is a complete absence of basal cells and local stromal invasion by malignant acini [4].

### *Estrogen receptors (ER): biological and tumorigenic role*

In men, androgens are generally responsible for proliferation, whereas estrogens have a dual action, both directly and indirectly they affect the proliferation and differentiation of epithelial cells. Estrogens indirectly suppress the release of pituitary luteinizing hormone (LH), decrease testicular androgen synthesis, also decrease systemic androgens and induce apoptosis in prostate epithelium and atrophy [7–11]. At the same time, a local-direct effect of estrogen through ER-alpha in the prostate stroma, stimulates aberrant epithelial differentiation and proliferation of basal layer, developing squamous metaplasia [7, 8]. This proliferation is opposite to proliferation under the androgen influence and can progress to inflammation and cancer [7, 8, 10]. Because of the stromal localization of ER-alpha, the estrogen effect on the epithelium is mediated by paracrine mechanisms [10–16]. Because of the ER-alpha localization in the prostatic epithelium, direct effect of estrogen is possible on the epithelial cells [10, 11, 17].

ER-alpha is required for differentiation and proliferation of epithelial cells, whereas ER-beta has an antiproliferative role [7, 8, 10, 11]. The effect of estrogen through ER-alpha leads to hyperplasia, dysplasia and neoplasia. Antiproliferative activity of estrogen can be explained by activation of epithelial ER-beta over ER-alpha. The loss of the local estrogen effect results in the reduced activation of ER-beta and consequently the increased proliferation and developing glandular hyperplasia [13].

The action of reductase on the testosterone metabolism results in formation of androgens (DHT) and with aromatase results in formation of estrogens. Androgen metabolites such as 5 alpha-androstane-3 beta, 17 beta-diol (3beta adiol) can act as ligands for ER-beta, but less effective than estrogen (E2) [7, 10]. Aromatase is present in the stroma, and ER in the prostatic epithelium. It is assumed that the effect of estrogen through ER-beta involves stromal-epithelial signaling impulses. Androgen acts *via* stromal receptors in induction of epithelial differentiation and proliferation. Lowering androgen

level would reduce proliferation and induce apoptosis of epithelial cells [13].

The amount of 3beta-Adiol in the prostate is 100 times higher than the amount of E2 [7]. As the ER affinity for 3beta-Adiol is 10 times lesser than for E2, its concentration in the prostate makes 3beta-Adiol endogenous ligand for ER. 3beta-Adiol binds to ER-alpha and with even greater affinity for ER-beta, but does not bind to androgen receptors (AR) [12]. DHT is biosynthetic precursor of 3beta-Adiol with antiproliferative role. Thus, the ER-beta is physiological regulator of epithelial growth and differentiation in the prostate [9, 10, 13–15]. Specific effects of estrogen in the tissue depends on the amount of estrogen and ER.

Kuiper et al. [18] found that ER-beta shows the highest affinity for 17 beta-estradiol (E2) > diethylstilbestrol (DES) > estriol (E3) > estrone (E1) > 5alpha-Androstane-3beta, 17 beta-diol (3beta-Adiol) >> testosterone = progesterone = corticosterone. In the absence of other ligands, E2 binds to ER-beta in 100%.

Low levels of circulating estrogens are present throughout life. With againg, circulating estrogen rises due to increase in body fat, which is a peripheral source of estrogen. In addition, the decline of bioavailable testosterone leads to an increase of effector cell (E) target cell (T) ratio and disrupting the balance in favor of estrogen, which can cause reactive growth and consequent neoplastic transformation [7, 10, 16]. The rise of estrogen increases the sensitivity of the prostate to androgens, by increasing the number of AR.

The paradox is that estrogen alone or in combination with androgens induce BPH, dysplasia and PCa [16].

Functional antagonism of ER-beta is reflected in the direct repression of some ER alpha conditional effects, including reduction of fat cell and proliferation in the prostate. ER-beta acts as a major regulator of the estrogen effects, its coexpression with ER-alpha leads to the reduced transcription caused by ER-alpha [8, 15–17]. Although E2 acts on both receptors, ER-alpha stimulates transcription and cell proliferation, and ER-beta inhibits the activity of ER-alpha and suppresses epithelial proliferation and enables the differentiation of the prostatic epithelium [10, 16]. As proliferation and differentiation opposite one another, cellular homeostasis and the overall proliferative response is the result of dynamic balance of ER-alpha and ER-beta [2, 8].

Adverse effects mediated through ER-alpha include unplanned proliferation, inflammation and malignancy. The beneficial effects in prevention of hyperplasia, inflammation and carcinogenesis are played through ER-beta [8] (Figure 1).

*ER-beta in prostate pathology*

Marked expression of ER-beta is registered in basal cells of NP [2, 9, 10, 11] this subpopulation have different biological characteristics from secretory epithelial cells, especially in their ability to proliferate [9, 10, 13], synthesize steroids and have the crucial role in prostate carcinogenesis. Weaker expression is found in stromal cells [9, 19]. Some authors found no presence of ER-beta in secretory cells [9], but other did [13, 14, 16]. Secretory cells are differentiated cells, androgen-dependent, but without proliferative capacity [13]. Secretory cells that still show positivity for ER-beta or Ki-67 represent incompletely differentiated intermediate cells with retained ability to proliferate throughout life.

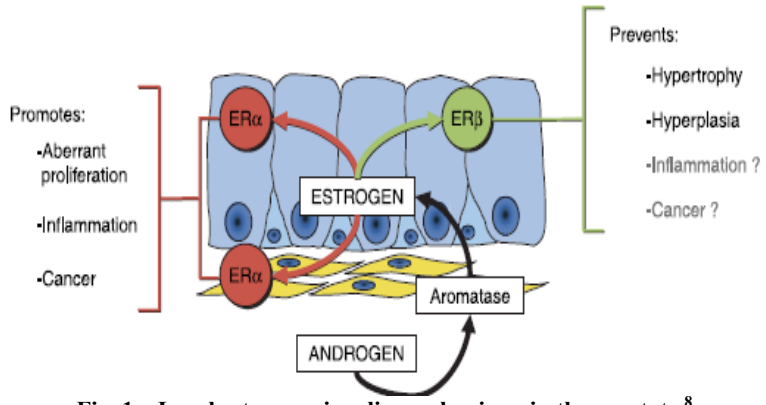


**Fig. 1 – Local estrogen signalig mechanisms in the prostate [8].**
**Testosterone is metabolized to estrogen by aromatase and acts *via* estrogen receptor (ER) alpha or ER-beta. Adverse effects via ER-alphą in stroma and epithelia include aberrant proliferation, inflammation and cancer. In contrast, estrogen also exerts beneficial effects *via* ER-betą in epithelia in preventing hyperplasia and hypertrophy, being antiproliferative and anticarcinogenic.**

The controversial results are in the expression of ER-beta in different morphologic lesions of the prostate. ER-beta is strongly present in normal prostate (NP), a progressive loss of expression is registered in hyperplastic epithelium, and even greater loss in invasive carcinoma [2, 10, 13–15, 17]. Pasquali et al. [11] show that the loss of ER-beta in conjunction with unknown molecular events may accelerate cell proliferation and possible carcinogenesis. It was interesting that in a few cases there was no change, or was an increase of ER-beta expression in tumors, compared to NP.

Some of them found ER-beta expression in low-grade prostatic intraepithelial neoplasia (LGPIN), possible as a response to proliferative stimuli in the early stages of dysplasia, and lacking of expression in HGPIN, which may contribute to the initial phase of carcinogenesis [9, 14]. The presence of ER-beta in secretory cells in LGPIN may represent a transi-

ent unsuccessful attempt to stop the growth of these cells. The disappearance of ER-beta positive basal cells in HGPIN can mean the continuous loss of inhibition for proliferation in these precursor lesions [9, 14, 17]. The mechanism of inactivation of ER-beta in PCa remains unknown [2, 14].

Finding all the three receptors (AR, ER-alpha and ER-beta in stroma indicates that the signal transduction of steroid hormones is mediated by paracrine mechanisms to epithelial cells. ER-beta acts as an inhibitor of ER-alpha transcription and leads to a reduction in overall cellular sensitivity to E2. ER-beta and ER-alpha present together in the stromal cells may play a key role in the regulation of paracrine signals to epithelial cells. Estrogens influence over ER- beta placed in the basal cells may directly affect their growth. Loss of ER-beta seen in neoplastic progression may represent receptor function impairment [9].

The following different actions of epithelial ER-beta in the prostate are so far confirmed: pro-differentory, antiproliferative, anti-inflammatory and induction of the antioxidative gene.

The major role of ER-beta is the anti-estrogenic growth inhibition [14, 15]. The result is the arrest in the G2 phase of the cell cycle. Loss of ER-beta leads to uncontrolled cell proliferation [17]. The cells with ER-beta expression undergo apoptosis and may contribute to the reduced proliferation. In cancer cells, ER-beta promotor is methylated, which explains the loss of ER-beta [17]. The ER-beta expression in PCa leads to inhibition of cell proliferation and invasion, and increased apoptosis. The tissues undergoing dysplastic transformation have decline in ER-beta expression that indicates about its 'guardian' role.

Immunohistochemical studies that use only one ER-beta antibody cannot specify which isoform is expressed. There are at least four isoforms of ER-beta mixed in the prostate (485aa, 495aa, 503A). These isoforms have different capacities for binding with estrogen and different effectś [12, 13]. Their existence significantly complicates the understanding of the ER-beta role.

## Methods

Four groups of patients were formed: the control with BPH and three experimental groups with PCa of different grades and scores, according to the Gleason grading system: group I – 10 prostate samples with signs of BPH; group II – 8 samples with well-differentiated PCa and Gleason score 2–4; group III – 10 samples with moderately differentiated PCa and Gleason score 5–7; group IV – 10 samples with poorly differentiated PCa and Gleason score 8–10.

A total of 39 specimens were sampled by transrectal ultrasound-guided biopsy. Bioptic samples were fixed in formalin, embedded in paraffin, cutted in 5μ slices and stained with hematoxylin-eosin. We determined histologic grades and Gleason scores in prostates with PCa, and BPH. Also, PSA levels were determined in all the patients.

Immunohistochemical staining for ER-beta was done with lyophilized mouse monoclonal antibody diluted 1 : 50–1 : 100 Novocastra. Staining was performed according to the manufacturer's instructions.

The intensity of ER-beta expression and localization was determnined in the control group with BPH, as well as in experimental groups with different scores of PCa. Counting was done at 40× and 63× magnification. Epithelial and stromal components were separately numbered. Approximately 100 cells on the average, were counted. Tumor cells with any nuclear positivity were specifically examined. ER-beta status was determined on the 2 immuno-slices. The score of six categories was applied, the number of positive cells was expressed in percentage: 0 – zero; 1 – < 1%; 2 – 1–10%; 3 – 11–33%; 4 – 34–66%; 5 – > 66%.

Positive and negative controls were used: external and internal. Epidermis of skin showed positivity for ER-beta. The slices incubated with nonimmune mouse serum instead of primary antibody showed immunoreactivity for ER-beta and represented negative control. Positivity of acinar epithelial cells was also observed in comparison with positively stained fibroblasts and endothelial cells. The results were analyzed in Excel.

## Results

The patients were male of various ages (in average (67.34 years) suspected of PCa, based on clinical and laboratory parameters, treated in our institution from 2010 to 2012 (Table 1).

**Table 1**

**Pathological changes and patient's age**

| Groups of patients | Values | | | | | | | | | | Mean values of GS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BHP | | / | | | | | | | | | |
| GS | 4 | 7 | / | / | / | / | 7 | / | / | / | 6 |
| age (years) | 66 | 72 | 65 | 69 | 68 | 68 | 72 | 61 | 70 | 53 | |
| GS 2–4 | | / | | | | | | | / | / | |
| GS | 4 | / | 4 | 4 | 4 | 4 | 4 | 4 | / | / | 4 |
| age (years) | 74 | | 74 | 74 | 61 | 61 | 61 | 61 | / | / | |
| GS 5–7 | | 5 | | | | | | | | | |
| GS | 5 | 63 | 7 | 7 | 6 | 7 | 5 | 5 | 5 | 5 | 5.9 |
| age (years) | 63 | | 72 | 74 | 82 | 69 | 65 | 65 | 63 | 63 | |
| GS 8–10 | | 9 | | | | | | | | | |
| GS | 9 | 77 | 8 | 9 | 9 | 9 | 8 | 9 | 9 | 9 | 8.8 |
| age (years) | 62 | 71 | 59 | 73 | 73 | 60 | 65 | 62 | 77 | 77 | |

**GS – Gleason score; BHP – benign prostatic hyperplasia.**

None of the patients received prior hormonal therapy.

The mean Gleason score was 6.35. In the experimental groups it was 6.17 (Tables 1 and 2). The mean PSA value was 22.39 ng/mL (24.19 ng/mL in the experimental groups) (Table 3). Out of the total of 39 biopsy samples, multiplied with 3 different functional sections, there were 117 observed parameters. The lack of ER-beta expression in any sections was in 9 cases, accounting for 7.69%. The overall positivity was 92.3% (Table 4). Pathological changes and biopsy localization are shown in Table 5.

In the group I (BPH) we found the strongest positivity in basal cells (mean 3.3), than in secretory cells (mean 3.1), and in stromal ones (mean 3.0). The overall positivity in the group I was 90% (Figure 2).

In the experimental groups (PCa) the overall positivity was 92.8% and in 7.2% was negative.

In the group II the positivity was strongest in the stromal cells (mean 2.37), and similar in basal and secretory ones (mean 1.87). The overall positivity in the group II was 87.5% (Figure 3).

In the group III the positivity was strongest in basal cells (mean 3.5), then in stromal cells (mean 3.4) and in

**Table 2**

**Pathological changes and Gleason grades**

| Group | Gleason grades | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| BHP | 2 + 2 | 3 + 4 | / | / | / | / | 3 + 4 | / | / | / |
| GS 2–4 | 2 + 2 | 2 + 2 | 2 + 2 | 2 + 2 | 2 + 2 | 2 + 2 | 2 + 2 | 2 + 2 | / | / |
| GS 5–7 | 2 + 3 | 3 + 4 | 3 + 4 | 4 + 3 | 3 + 3 | 4 + 3 | 2 + 3 | 3 + 2 | 3 + 2 | 3 + 2 |
| GS 8–10 | 4 + 5 | 4 + 5 | 4 + 4 | 4 + 5 | 4 + 5 | 4 + 5 | 4 + 4 | 4 + 5 | 4 + 5 | 4 + 5 |

**BHP- benign prostatic hyperplasia; GS – Gleason score.**

**Table 3**

**Pathological changes and prostate specific antigen (PSA) levels**

| Group | PSA levels (ng/mL) | | | | | | | | | | Mean values |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BHP | 9.78 | 5.83 | 11.95 | 9.20 | 10.20 | 10.20 | 5.83 | 12.55 | 6.47 | 6.64 | 8.86 |
| GS 2–4 | 5.76 | / | 5.76 | 5.76 | 7.21 | 7.21 | 7.21 | 7.21 | / | / | 6.58 |
| GS 5–7 | 7.91 | 16.5 | 16.5 | 14.42 | 15.65 | 1.8 | 5.67 | 5.67 | 7.91 | 7.91 | 9.99 |
| GS 8–10 | >100 | 2.4 | 15.2 | 66.29 | 66.29 | 28 | 19 | >100 | >100 | >100 | >56.01 |

**BHP – benign prostatic hyperplasia; GS – Gleason score; Normal: 0–4, category 1; Gray zone: > 4–10, category 2; Ca suspect: > 10 category, 3.**

**Table 4**

**Pathological changes and estrogen receptor (ER) beta expression**

| Group | ER beta expression | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| BHP | | | | | | | | | | |
| stromal cells | 4 | 4 | 4 | 3 | 4 | 3 | 3 | 2 | 3 | - |
| basal cells | 5 | 5 | 5 | 4 | 4 | 3 | 4 | 2 | 1 | - |
| secretory cells | 3 | 5 | 5 | 4 | 4 | 3 | 5 | 1 | 1 | - |
| GS 2–4 | | | | | | | | | | |
| stromal cells | 0 | 3 | 4 | 2 | 3 | 2 | 2 | 3 | | |
| basal cells | 0 | 3 | 2 | 1 | 3 | 2 | 2 | 2 | | |
| secretory cells | 0 | 2 | 3 | 1 | 4 | 1 | 2 | 2 | | |
| GS 5–7 | | | | | | | | | | |
| stromal cells | 3 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 2 | 3 |
| basal cells | 3 | 4 | 4 | 4 | 5 | 4 | 3 | 4 | 2 | 2 |
| secretory cells | 3 | 5 | 3 | 5 | 4 | 3 | 4 | 4 | 1 | 1 |
| GS 8–10 | | | | | | | | | | |
| stromal cells | 1 | 4 | 4 | 4 | 3 | 4 | 4 | 1 | 3 | 4 |
| basal cells | 0 | 4 | 5 | 4 | 5 | 3 | 4 | 1 | 3 | 4 |
| secretory cells | 0 | 4 | 5 | 4 | 4 | 3 | 4 | 0 | 2 | 3 |

**Marks for ER-beta positive cells: 0 – zero; 1 – < 1%; 2 – 1–10%; 3 – 11–33%; 4 – 34–66%; 5 – > 66% cells;**
**BHP- benign prostatic hyperplasia; GS – Gleason score.**

**Table 5**

**Pathological changes and biopsy localization**

| Group | Biopsy localization | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BHP | a.d. | s.l. | a.d. | s.l. | s.d. | a.d. | b.l. | a.l. | a.d. | a.d. | ad. sl |
| GS 2-4 | b.d. | b.l. | b.d. | a.d. | a.d. | a.d. | s.d | b.d. | / | / | bd. ad |
| GS 5-7 | s.l. | s.d. | b.d. | s.b.d. | s.d. | s.l. | b.l. | a.l. | a.l. | a.l. | al. sd |
| GS 8-10 | a.d. | b.l. | s.l. | s.d. | b.d. | a.d. | s.d. | b.l. | d. | l. | bl. sd |

**a – apex, s – middle, b – base; d – dexter (right), l – left; BHP- benign prostatic hyperplasia; GS – Gleason score.**

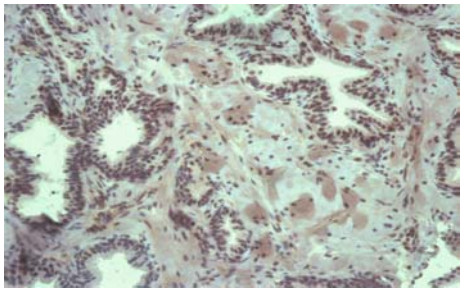

**Fig. 2 – Immunohistochemical staining for estrogen receptor (ER) beta in benign prostatic hyperplasia (the control group) showing positivity in stromal cells (mean 4), basal cells (mean 5) and in secretory ones (mean 5) (photomicrograph, ×20) Marks for ER-beta positive cells – 4: 34–66% positive cells; mark 5: > 66% positive cells.**

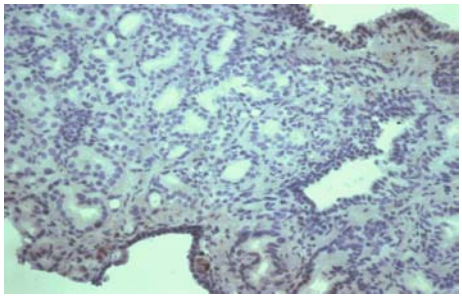

**Fig. 3 – Immunohistochemical staining for estrogen receptor (ER) beta in prostate adenocarcinoma (PCa) Gleason score 2–4, showing that stromal, basal and secretory cells were ER-beta negative (photomicrograph ×20).**

secretory ones (mean 3.3). The overall positivity in the group III was 100% (Figure 4).

In the group IV the positivity was strongest in basal cells (mean 3.3), then in stromal cells (mean 3.2), and in secretory ones (mean 2.9). The overall positivity in the group IV was 90% (Figure 5).

The strongest basal cell positivity was registered in the group III (mean 3.5), then in the group I and IV (mean 3.3), and weakest in the group II (mean 1.87). The strongest secretory cell positivity was registered in the group III (mean 3.3), then in the group I (mean 3.1), in the group IV (mean 2.9), and the weakest in the group II (mean 1.87). The strongest stromal positivity was registered in the group III (mean 3.4), then in the group IV (mean 3.2), in the group I (mean 3.0) and weakest in the group II (mean 2.37).

Our data showed that expression of ER-beta in epithelial cells of the prostate acini is the weakest in well-differentiated adenocarcinomas (Figure 6).

There was a decline of ER-beta expression in malignant compared to benign prostatic epithelial acini.

Less difernetiated adenocarcinomas showed a decrease in the expression of ER-beta in basal cells and in secretory ones (Figure 7).

The expression of ER-beta in basal cells was stronger, than in secretory ones in BPH (Figure 8) and in moderately differentiated adenocarcinomas (Figure 9).

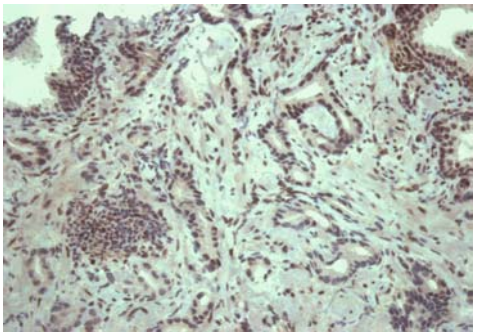

**Fig. 4 – Immunohistochemical staining for estrogen receptor (ER) beta, in prostate adenocarcinoma (PCa), Gleason score 5–7, showing ER-beta positivity in stromal cells (mark 3), basal cells (mark 3), and in secretory ones (mark 3) (photomicrograph ×20).**
**Mark for ER-beta positive cells – 3: 11–33% positive cells.**

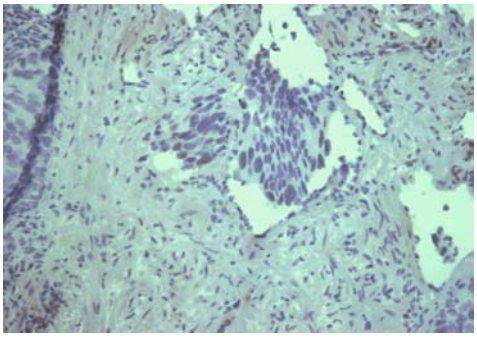

**Fig. 5 – Immunohistochemical staining for estrogen receptor (ER) beta, in prostate adenocarcinoma (Pca, Gleason score 8–10) showing ER-beta positivity in stromal cells (mark 1), basal cells (mark 0) and in secretory ones (score 0) (photomicrograph ×20). Marks for ER-beta positive cells – 0 positive cells: zero mark; 1: < 1% positive cells.**

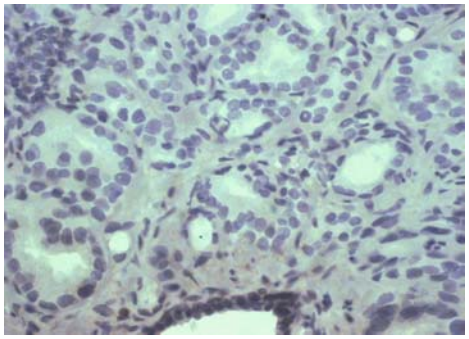

**Fig. 6 – Immunohistochemical staining for estrogen receptor (ER) beta, showing ER-beta expression in well-differentiated adenocarcinoma (photomicrograph × 40).**

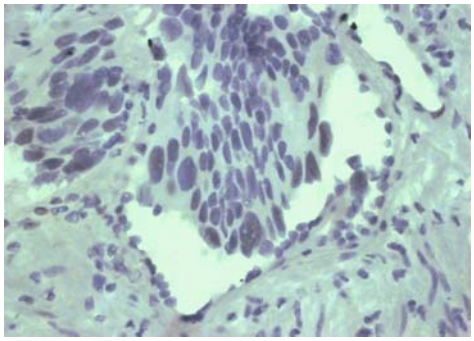

**Fig. 7 – Immunohistochemical staining for estrogen receptor (ER) beta, showing ER-beta expression in less-differentiated adenocarcinoma (photomicrograph × 40).**

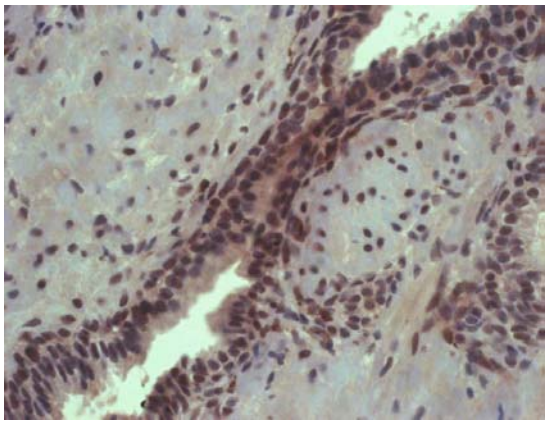

**Fig. 8 – Immunohistochemical staining for estrogen receptor (ER) beta, showing ER-beta expression in basal cells was stronger than in secretory ones in benign prostatic hyperplasia (BPH) (photomicrograph ×40).**

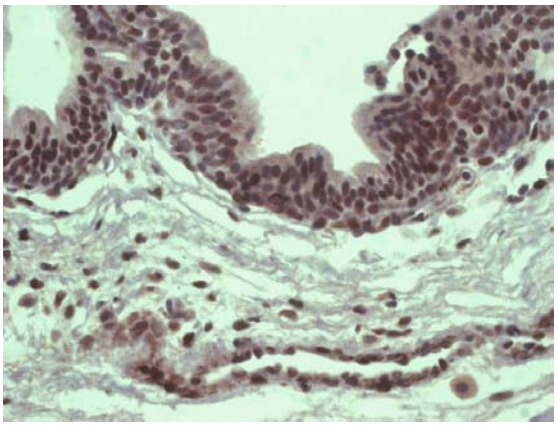

**Fig. 9 – Immunohistochemical staining for estrogen receptor (ER) beta, showing ER-beta expression in benign prostatic hyperplasia (BPH), with ER-beta positivity in the stromal cells (mark 3), basal cells (mark 4) and in secretory ones (mark 4) (photomicrograph ×40).**
**Marks for ER-beta positive cells – 3: 11–33% positive cells; mark 4: 34–66% positive cells.**

## Discussion

As noted above ER-beta was registered in the nuclei of basal cells of prostatic acini [2, 14, 20] as in stromal cells [21]. Stroma was positive in 20–60% of cases [14]. ER-beta was also located in secretory luminal cells of NP [13, 14]. ER-beta was located in the epithelium of NP, BPH and PCa. [2, 12, 20, 21].

Using antibodies for long isoform of ER-beta. Leav et al. [9] found the positivity in basal cells, mainly. This is in apparent contradiction with the distribution of ER-beta in the secretory cells of the prostate gland in rats. Fixemer et al. [13] used monoclonal antibodies to distinguish long from short isoform of ER-beta and identified positive secretory luminal cells and with lesser intense basal ones [13].

We also found the strongest positivity in basal cells in BPH and moderately differented adenocarcinoma.

The secretory luminal cells have high expression of ER-beta which is lost with transformation into HGPIN [13, 20].

A high ER-beta expression was found in 90% cases of BPH with PSA ≤ 10 ng/ml [2, 9, 10].

The presence of ER-beta in secretory cells of well- to moderately differentiated lesions may represent a transient abortive attempt to prevent their growth. In contrast, disappearance of ER-beta positive basal cells in HGPIN may involve the continuous loss of growth inhibitory functions mediated through ER-beta in these precursor lesions [2, 14]. ER-beta is very intense in BPH [2, 14].

ER-beta localization in secretory luminal cells showed that the differentiated section is the main target of estrogen action in human prostate. Partial loss of ER-beta in HGPIN supports the concept that ER-beta has hemopreventive effects on prostate epithelium [22, 23].

We also found a strong ER-beta expression in BPH.

We noticed the weakest ER-beta expression in well-differentiated PCa.

ER-beta have an important role in proliferation and differentiation of prostatic epithelium [19, 21] and can affect the initial phase of carcinogenesis in the prostate and in androgen independent tumors [13]. Localization of ER-beta in basal cells is related to the role in epithelial proliferation and renewal processes [19]. Estrogen stimulation of cell proliferation leads to the development of cancer [19]. Antiproliferative, anti-invasive and proapoptotic role of ER-beta is associated with the tumor suppressor function [17]. ER-alpha has the proliferative role restricted with the influence of ER-beta [15].

Horvath et al. [14] observed ER-beta in NP and registered positivity in more than 95% of epithelial nuclei and in 35% of stromal cells. In BPH near carcinoma ER-beta positivity fall to 24%, and to 11% in PCa. Progressive loss of ER-beta expression is registered in BPH, and even greater in Pca [14].

We found a progressive lost of ER-beta expression in malignant compared to benign prostate epithelium and a decrease of expression in less differented PCa. This findings are consistent with most of the actual works.

In BHP Horvath et al. [14] found stronger ER-beta expression in epithelial cells than in the stroma.

ER-beta expression was present in stromal and mesenchymal cells in the BPH group (adipocytes, endothelial cells, smooth muscle cells of blood vessels). Secretory luminal cells were more positive than the basal ones [13].

In BHP we found the overall positivity of 90%. Stronger positivity was in basal than in secretory cells and the least in stroma.

PCa expressed ER-beta in 87% of the cases, as in benign epithelium. In 13% of the cases there was a decreased ER-beta expression. There was no correlation between the degree of expression, grade or stage of the disease [13].

In the groups with PCa we found the positivity of 92.8%.

It seems that there is no explanation for the discrepancy between the published results. The lack of specificity of the antibody, or the difference in the primary antibody, or antigen retrival and inadequate tissue processing, or the presence of unknown ER isoforms may affect the results of immunohistochemical staining.

Metastases to lymph nodes and bones expressed ER-beta in 100% of the cases [10]. While the loss of ER-beta may

contribute to the progression of organ localized disease, the appearance of ER-beta re-expression in metastases suggests a possible role in androgen-independent progression [10].

Untreated primary and metastatic tumors retain expression of ER-beta. These tumors use estrogen through ER-beta regulated processes. Partial loss of ER-beta in hormone refractory tumors may represent an androgen-dependent gene expression of ER-beta [13].

Such observations may have clinical implications in cases where tumor cells showed expression of these receptors, potentially responsive to estrogen and survive in conditions of androgen deprivation and also for treatment with estrogen antagonists [24, 25].

## Conclusion

ER-beta expression was most pronounced in BHP samples and declined in malignant prostatic lesions. This finding supports statement on antiproliferative role of ER-beta in prostatic tissue.

## R E F E R E N C E S


1. *Rosai J.* Rosai and Ackerman's surgical pathology. 9th ed. Philadelphia: Mosby, Elsevier Inc; 2004.
2. *Gabal SM, Habib FM, Helmy DO, Ibrahim MF.* Expression of estrogen receptor-B (ER-B) in bengin and malignant prostatic epithelial cells and its correlation with the clinico-pathological features. J Egypt Natl Canc Inst 2007; 19(4): 239−48.
3. *De Marzo AM, Platz EA, Sutcliffe S, Xu J, Grönberg H, Drake CG,* et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer 2007; 7(4): 256−69.
4. *de Marzo AM, Nelson WG, Isaacs WB, Epstein JI.* Pathological and molecular aspects of prostate cancer. Lancet 2003; 361(9361): 955−64.
5. *Bostwick DG, Qian J.* High-grade prostatic intraepithelial neoplasia. Mod Pathol 2004; 17(3): 360−79.
6. *Montironi R, Mazzucchelli R, Lopez-Beltran A, Cheng L., Scarpelli M.* Mechanisms of Disease: high-grade prostatic intraepithelial neoplasia and other proposed preneoplastic lesions in the prostate. Nature Clin Pract Urol 2007; 4(6): 321−32.
7. *McPherson SJ, Ellem SJ, Simpson ER, Patchev V, Fritzemeier K, Risbridger GP.* Essential role for estrogen receptor beta in stromal-epithelial regulation of prostatic hyperplasia. Endocrinology 2007; 148(2): 566−74.
8. *Risbridger GP, Ellem SJ, McPherson SJ.* Estrogen action on the prostate gland: a critical mix of endocrine and paracrine signaling. J Mol Endocrinol 2007; 39(3): 183−8.
9. *Leav I, Lau KM, Adams JY, McNeal JE, Taplin ME, Wang J,* et al.. Comparative studies of the estrogen receptors beta and alpha and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. Am J Pathol 2001; 159(1): 79−92.
10. *Prins GS, Korach KS.* The role of estrogens and estrogen receptors in normal prostate growth and disease. Steroids 2008; 73(3): 233−44.
11. *Pasquali D, Rossi V, Esposito D, Abbondanza C, Puca GA, Bellastella A,* et al. Loss of estrogen receptor beta expression in malignant human prostate cells in primary cultures and in prostate cancer tissues. J Clin Endocrinol Metab 2001; 86(5): 2051−5.
12. *Weihua Z, Warner M, Gustafsson J.* Estrogen receptor beta in the prostate. Mol Cell Endocrinol 2002; 193(1−2): 1−5.
13. *Fixemer T, Remberger K, Bonkhoff H.* Differential expression of the estrogen receptor beta (ERbeta) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. Prostate 2003; 54(2): 79−87.
14. *Horvath LG, Henshall SM, Lee CS, Head DR, Quinn DI, Makela S,* et al. Frequent loss of estrogen receptor-beta expression in prostate cancer. Cancer Res 2001; 61(14): 5331−5.
15. *Stettner M, Kaulfuss S, Burfeind P, Schweyer S, Strauss A, Ringert R,* et al. The relevance of estrogen receptor-beta expression to the antiproliferative effects observed with histone deacetylase inhibitors and phytoestrogens in prostate cancer treatment. Mol Cancer Ther 2007; 6(10): 2626−33.
16. *Steiner MS, Raghow S.* Antiestrogens and selective estrogen receptor modulators reduce prostate cancer risk. World J Urol 2003; 21(1): 31−6.
17. *Cheng J, Lee EJ, Madison LD, Lazennec G.* Expression of estrogen receptor beta in prostate carcinoma cells inhibits invasion and proliferation and triggers apoptosis. FEBS Lett 2004; 566(1−3): 169−72.
18. *Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA.* Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A 1996; 93(12): 5925−30.
19. *Royuela M, de Miguel MP, Bethencourt FR, Sánchez-Chapado M, Fraile B, Arenas MI,* et al. Estrogen receptors alpha and beta in the normal, hyperplastic and carcinomatous human prostate. J Endocrinol 2001; 168(3): 447−54.
20. *Bonkhoff H, Fixemer T, Hunsicker I, Remberger K.* Estrogen receptor expression in prostate cancer and premalignant prostatic lesions. Am J Pathol 1999; 155(2): 641−7.
21. *Signoretti S, Loda M.* Estrogen receptor beta in prostate cancer: brake pedal or accelerator. Am J Pathol 2001; 159(1): 13−6.
22. *Hartman J, Ström A, Gustafsson JÅ.* Current concepts and significance of estrogen receptor β in prostate cancer. Steroids 2012; 77(12): 1262−6.
23. *Asgari M, Morakabati A.* Estrogen receptor beta expression in prostateadenocarcinoma. Diagn Pathol 2011; 6: 61.
24. *McPherson SJ, Hussain S, Balanathan P, Hedwards SL, Niranjan B, Grant M,* Risbridger GP. Estrogen receptor-beta activated apoptosis in benign hyperplasia and cancer of the prostate is androgen independent and TNFalpha mediated. PNAS 2010; 107(7): 3123−8.
25. *Vojinov S, Levakov I, Jeremić D, Zivojinov S, Marusić G.* Hormonal status in patients with advanced prostatic cancer on the therapy with androgen blockade. Vojnosanit Pregl 2011; 68(4): 321−6.


Received on October 22, 2013.
Revised on October 20, 2014.
Accepted on October 22, 2014.
Online First August, 2015.