Differential Expression of the Estrogen Receptor Beta (ERβ) in Human Prostate Tissue, Premalignant Changes, and in Primary, Metastatic, and Recurrent Prostatic Adenocarcinoma

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BACKGROUND. Estrogen signaling mediated by the estrogen receptor beta (ERβ) has potential implications in normal and abnormal prostate growth. Few studies have addressed this issue in human prostate tissue leaving conflicting results on the immunolocalization of the ERβ in benign and neoplastic lesions.

METHODS. Using a new monoclonal antibody, the current study reports on the differential expression of the ERβ in tissue sections from 132 patients with prostate cancer.

RESULTS. The prostatic epithelium expressed the ERβ extensively in secretory luminal cell types and at lower levels in basal cells. Atrophic changes of the peripheral zone (PZ) were more immunoreactive than hyperplastic lesions of the transition zone (TZ). When compared with glandular tissue of the PZ, high-grade prostatic intraepithelial neoplasia (HGPIN) revealed decreased levels of the ERβ in 30 of 47 cases and was unreactive in six lesions. In informative cases with suitable internal controls, all primary tumors (n = 60), lymph node (n = 7), and bone metastases (n = 5) expressed the ERβ at variable degree. No correlation was found between the ERβ status, the primary Gleason grade (P = 0.254), and the pathological stage (P = 0.157). Recurrent adenocarcinoma revealed markedly decreased levels in 15 of 40 cases and was ERβ negative in five recurrent lesions.

CONCLUSIONS. The secretory epithelium is a major target of ERβ-mediated estrogen signaling in the human prostate. Its downregulation in HGPIN is consistent with chemopreventive effects that the ERβ may exert on the prostatic epithelium. The continuous expression of the receptor protein at significant levels in untreated primary and metastatic adenocarcinoma indicates that these tumors can use estrogens through an ERβ-mediated pathway. The partial loss of the ERβ in recurrent tumors after androgen-deprivation may reflect the androgen-dependence of ERβ gene expression in human prostate cancer. Prostate 54: 79–87, 2003.

KEY WORDS: ERβ; prostate; prostatic intraepithelial neoplasia; prostate cancer; immunohistochemistry

INTRODUCTION

Estrogen signaling pathways have regained considerable attention in contemporary prostate cancer research. Epidemiological and experimental studies suggest that estrogens may have carcinogenic and chemopreventive effects on the prostatic epithelium [1–3]. Neonatal estrogenization or long-term treatment of adult animals with androgens and estrogens leads to dysplasia and invasive cancer [4–6]. On the other hand, diets rich in phytoestrogens known for their chemo-

preventive properties in animal models are associated with a low risk of prostate cancer in epidemiological

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studies [1–3]. These divergent effects that estrogens can exert on the prostatic epithelium may reflect the presence of two distinct receptors, the estrogen receptor alpha (ERα) and beta (ERβ), which can homodimerize or heterodimerize to form a signaling dimeric complex [7,8]. Although both receptors bind estradiol with high affinity, they may have different functions. For example, in the immature rat uterus the ERα stimulates transcription and cell proliferation, while the ERβ restrains ERα activation [9]. Interestingly, the ERβ binds to several phytoestrogens and activates chemoprotective detoxification enzymes such as glutathione-S-transferase [1,3].

Recent studies performed in benign human prostate tissue have shown convincingly that ERα expression is restricted to prostatic stromal cells and the basal cell layer, while secretory luminal cell types of the prostatic epithelium lack the ERα at the mRNA and protein level [10]. Conversely, high steady state levels of ERα mRNA have been detected in high-grade prostatic epithelial neoplasia (HGPIN), which is considered the most likely precursor of prostatic adenocarcinoma. At least 10% of these premalignant lesions have been reported to express the ERα at the protein level [10]. In apparent contrast with breast cancer, the presence of the ERα in prostatic malignancies appears to be a late event in tumor progression. The most significant levels have been detected in metastatic and androgen-insensitive lesions [10]. A remarkably similar tissue distribution has been described for the estrogen-inducible progesterone receptor (PR), which is one of the most important markers for a functional ERα pathway in human tissue. At least 30% of metastatic and hormone refractory tumors express the PR at significant levels indicating that these tumors harbor a functional ERα [11].

Referring to the ERβ, its precise localization in human prostate tissue is not well established. Using two different antibodies, we were unable to detect the ERβ in human prostate tissue in a previous study [10]. However, two recent studies have readdressed this issue with other antibodies and were successful in detecting the ERβ, but left conflicting results. For example, Leav and colleagues by using a polyclonal antibody, which identifies a post-transcriptionally modified form of the long-form ERβ, have immunolocalized the ERβ predominantly in basal cells and to lesser extend in prostatic stromal cells [12]. The same authors report that the ERβ is lost in HGPIN and in transition zone cancer, reappears in Gleason grade 3 peripheral zone cancer and in metastatic lesions but is markedly decreased in high grade Gleason grade 4/5 primary tumors [12]. Results from another study suggest that the ERβ is progressively lost in hyperplastic and neoplastic lesions, whereas tumors that retain ERβ expression have a higher rate of recurrence [13].

In the current study, we have used a new monoclonal antibody, which allows reliable detection of the ERβ in secretory luminal cells, a major target of ERβ signaling in the rat prostate. Our results suggest that the ERβ is retained in untreated (primary and metastatic) prostate cancer but is partially lost in HGPIN and androgen-insensitive stages of the disease.

MATERIALS AND METHODS

Tissue Selection

Formalin fixed, paraffin-embedded tissue sections were obtained from 132 prostate cancer patients during a period from 1991 to 2001. This included 75 radical prostatectomy and pelvic lymphadenectomy specimens, and 8 lymph node metastases from staging lymphadenectomy without subsequent prostatectomy (Table I). Forty-seven foci of high-grade prostatic intraepithelial neoplasia (HGPIN) present in the radical prostatectomy specimens were submitted for study. The extent of HGPIN was arbitrarily defined by one microscopic field at low power magnification (100×). The material further contained bone metastases from 7 patients without previous hormonal therapy, and palliative transurethral resection specimens from 42 patients with recurrent prostatic adenocarcinoma after orchietomy. The pathological stages and grading of the material submitted are summarized in Table I. The ERβ status was evaluated in at least one or two representative tissue blocks from each patient.

Immunohistochemical Analysis

Tissue sections were deparaffinized in xylene, rehydrated through graded alcohol, and subsequently incubated in H2O2 (0.3% in deionized water) for 15 min to block endogenous peroxidase. For heat-induced antigen retrieval, sections were incubated in target retrieval solution pH 6.1 (Dako, Hamburg, Germany) for 1 hr at 94°C. After pretreatment, the sections were incubated for 30 min in a normal rabbit serum (Dako). The mouse monoclonal antibody MCA1974 (clone number PPG5/10) recognizes the peptide CSPAEDSKSKEGSQNPQSQ corresponding to the published C terminus of the human oestrogen receptor beta isoform 1 (Serotec, Kidlington, Oxford, UK) [14,15]. In Western blot experiments, the ERβ antibody detects the long (~59 kDa) and the short (~53 kDa) forms of the receptor and does not crossreact with the ERα [14,15]. In our immunohistochemical (IHC) analyses, the antibody is applicable in a dilution of 1:50 (in PBS).

Sections were incubated overnight in a humid chamber at 4°C. After incubation with the secondary biotinylated rabbit antimouse immunoglobulin (Dako)
for 30 min, the horseradish peroxidase-labeled avidin–biotin complex (ABC-HRP) method (Dako) was performed according to the manufacturer’s instructions. A signal amplification method based on the deposition of biotinylated tyramine was used to enhance the immunodetection. Preparation of the biotinylated tyramine reagent was recently described in greater detail [16]. After precipitation of the biotinylated tyramine (10 min at room temperature) through the enzymatic action of HRP and H2O2 (0.1%), the biotin precipitate was detected with an additional application of the HRP-labeled avidin–biotin complex (Dako) for 30 min in a humid chamber. The peroxidase reaction was developed by 3,3'diaminobenzidine (Sigma; Deisenhofen, Germany) leaving a brown colored endproduct.

Negative controls were performed on consecutive sections by replacing the primary antibody with a non-immune mouse serum.

### Quantitative Analysis of Staining

Immunostained slides were scored as previously described for the routine evaluation of the ER status in breast cancer [17]. First, a proportion score (PS) was assigned, which represents the estimated proportion of positive cells in each individual lesion present in the entire slide, for example, normal acini, HGPIN, and cancerous lesions with different Gleason patterns. The PS included six categories ranging from 0 to 5 (0: none; 1: <1%; 2: 1–10%; 3: 10–33%; 4: 33–66%; 5: >66%). Next, an intensity score (IS) was assigned which represents the average intensity of positive cells (0: none; 1: weak; 2: intermediate; 3: strong) in a particular lesion when compared with the ERβ immunoreactivity of host cells (e.g., fibroblasts, endothelial cells). The PS and IS were then added to obtain a total score (TS) (range, 0–8). For statistical analysis, the TS was subdivided in four categories including negative (TS, 0–2), weak (TS, 3–4), moderate (TS, 5–6), and strong (TS, 7–8).

Contingence table and $\chi^2$ analyses were used to study the relation between the ERβ-IHC score, the primary Gleason grade and the pathological stage of primary adenocarcinomas, recurrent disease and metastases (i.e., Kolmogorov–Smirnov-test; the likelihood-quotient-$\chi^2$, and Spearman’s coefficient of correlation for the statistical computation of significance). Statistical analyses were performed with the SPSS-software (SPSS ASC GmbH, Erkrath, Germany). $P < 0.05$ was regarded as statistically significant.

### RESULTS

The antibody used in the current study yield distinct nuclear staining in prostate tissue and other human tissue known for the presence of the ERβ including breast, ovarian granulosa cells, and testis. The results obtained in benign prostate tissue, HGPIN, and prostatic adenocarcinoma are summarized in Tables II and III.

### Benign Prostate Tissue

In well-fixed prostatectomy specimens the ERβ was detectable in subsets of stromal cells and other mesenchymal cells including adipocytes, endothelial cells, and vascular smooth muscle cells, which served as internal positive controls. Informative cases with suitable internal positive controls throughout the entire specimen revealed ERβ expression in virtually all benign glandular structures (Fig. 1) (Table II). The strongest staining intensities were recorded in

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**TABLE I. Pathological Stages and Grades**

<table>
<thead>
<tr>
<th>n</th>
<th>Stages</th>
<th>Primary Gleason grades</th>
<th>HGPIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>pT3b, pN1</td>
<td>5 (n = 6)</td>
<td>4 (n = 11)</td>
</tr>
<tr>
<td>20</td>
<td>pT3b, pN0</td>
<td>5 (n = 11)</td>
<td>4 (n = 13)</td>
</tr>
<tr>
<td>4</td>
<td>pT3a, pN1</td>
<td>4 (n = 4)</td>
<td>3 (n = 1)</td>
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<td>15</td>
<td>pT3a, pN0</td>
<td>4 (n = 8)</td>
<td>3 (n = 8)</td>
</tr>
<tr>
<td>11</td>
<td>pT2b, pN0</td>
<td>5 (n = 1)</td>
<td>4 (n = 5)</td>
</tr>
<tr>
<td>14</td>
<td>pT2a, pN0</td>
<td>4 (n = 4)</td>
<td>3 (n = 10)</td>
</tr>
<tr>
<td>8</td>
<td>pTx, pN1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Bone metastases</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Recurrent carcinomas</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

*The primary Gleason grades refer to the cancerous lesions obtained from 75 radical prostatectomy specimens. The number of primary Gleason grades submitted for study is indicated in parentheses. Lymph node and bone metastases, and recurrent lesions were not graded.

n, number of patients.
ND, not determined.
atrophic acini of the peripheral zone (PZ) (Fig. 2). Conversely, hyperplastic changes of the transition zone (TZ) revealed decreased ERβ levels depending on the degree of stromal hyperplasia present within the hyperplastic nodules (Fig. 3). In general, florid hyperplastic lesions with a prominent stromal component showed the lowest levels of ERβ detectable in prostatic epithelial cells. Weak to moderate staining intensities were observed in the central zone (CZ) and in seminal vesicles epithelial cells. In any case, secretory luminal cells were consistently more immunoreactive than basal cells irrespective of their zonal origin or hyperplastic changes (Fig. 1). Apoptotic cells present in luminal spaces were ERβ negative. Table II summarizes the results obtained in benign prostate tissue.

### Premalignant Lesions

Profound changes in the ERβ status were found in HGPIN when compared with normal or atrophic acini of the peripheral zone. At least 11 of 47 cases of HGPIN investigated revealed levels of ERβ comparable to those encountered in normal glandular structures of the peripheral zone (TS, 7–8). The remaining 64% (30 of 47 cases) showed decreased (n = 11) and markedly decreased levels (n = 19) in basal and luminal cell types (Fig. 4). Six cases were ERβ unreactive (13%). Statistical analysis showed that the ERβ status of HGPIN differs significantly from that of normal glandular tissue in the peripheral zone (P < 0.001).

### Primary Prostatic Adenocarcinoma

From 75 prostatectomy specimens submitted for study, 15 cases were excluded because prostatic stromal cells and pre-existing glandular tissue lacked convincing ERβ immunoreactivity. In informative cases with suitable internal positive controls the ERβ was detectable in all cancerous lesions including TZ cancer and intraductal spread. Eighty-seven percent (52 of 60 cases) had the same IHC score as benign glandular tissue (TS, 7–8) (Fig. 5). In the remaining eight cases (13%), the IHC score ranged from 5–6 (Table III). No correlation was found between the IHC score, the Gleason grade (P = 0.254), or the pathological stage (P = 0.157).

### Lymph Node and Bone Metastases

One lymph node and two bone metastases lacked ERβ immunoreactivity in host cells (e.g., fibroblasts, endothelial cells) and were excluded from evaluation. This reflects fixation artifacts after frozen section examination of lymph nodes and decalcification for examination of bone tissue. All lymph node and bone metastases with internal positive controls revealed ERβ expression in the metastatic lesion (Figs. 6 and 7) (Table III). Due to the limited number of informative cases, no statistical analysis was performed.

### Recurrent Prostatic Adenocarcinoma

Most of palliative transurethral resection (TUR) specimens resected for recurrent tumors after androgen-deprivation therapy revealed ERβ positivity in host cells and were suitable for ERβ evaluation (40 of 42 cases). In apparent contrast with untreated tumors, 50% of recurrent lesions (20 of 40 cases) lacked the ERβ or revealed low levels, while only 13% (5 of 40 cases) expressed the ERβ at high levels (TS, 7–8). The remaining 15 cases (38%) ranged within an intermediate group (TS, 5–6) (Fig. 8). Referring to their ERβ

### TABLE II. Differential Expression of the Estrogen Receptor Beta (ERβ) in Benign Prostate Tissue*

<table>
<thead>
<tr>
<th>Cell type/tissue</th>
<th>Intensity score (IS)</th>
<th>Proportion score (PS)</th>
<th>Total score (TS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostatic stromal cells</td>
<td>2–3</td>
<td>2–3</td>
<td>4–6</td>
</tr>
<tr>
<td>Secretory luminal cells</td>
<td>2–3</td>
<td>5</td>
<td>7–8</td>
</tr>
<tr>
<td>Basal cells</td>
<td>1–2</td>
<td>5</td>
<td>6–7</td>
</tr>
<tr>
<td>Apoptotic cells in luminal spaces</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PZ</td>
<td>2–3</td>
<td>5</td>
<td>7–8</td>
</tr>
<tr>
<td>TZ</td>
<td>1–3</td>
<td>5</td>
<td>6–8</td>
</tr>
<tr>
<td>CZ</td>
<td>1–2</td>
<td>5</td>
<td>6–7</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td>1–2</td>
<td>5</td>
<td>6–7</td>
</tr>
<tr>
<td>Atrophy</td>
<td>3</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Glandular hyperplasia</td>
<td>1–2</td>
<td>5</td>
<td>6–7</td>
</tr>
<tr>
<td>Basal cell hyperplasia</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

*The ERβ immunohistochemical (IHC) score was evaluated by a grading system ranging from 0 to 8.

PZ, peripheral zone; TZ, transition zone; CZ, central zone.
status, the difference between primary and recurrent tumors was statistically highly significant ($P < 0.001$).

**DISCUSSION**

To understand the exact contribution of the ERβ to normal and malignant prostatic cell biology, it is important to have precise information about its distribution in human prostate tissue. Two recent studies have addressed this issue leaving conflicting results. Using an antibody raised against a post-transcriptionally modified form of the ERβ, Leav et al. have immunolocalized the ERβ predominantly in basal cells of the prostatic epithelium [12]. This is an apparent contrast with the tissue distribution in the rat and murine prostate expressing the ERβ at high levels in luminal cells [18,19]. In the current study, we have used a well-characterized monoclonal antibody, which recognize the long and short form of the ERβ isoform 1 [14,15]. With this antibody, we were able to identify the ERβ predominantly in secretory luminal cell types and at a lesser extend in basal cells. These divergent results on the immunolocalization of the ERβ in benign human tissue may reflect the specificity of the primary antibodies used in both studies, and may explain why our results in premalignant changes and prostate cancer differ significantly from the data reported by Leav and colleagues [12]. In particular, the GC-17 antibody used by Leav et al. identifies a post-transcriptionally modified form of the long-form ERβ [12], while the monoclonal antibody used here recognizes the long and the short form of the ERβ [14,15].
Results of the current study show that the ERβ is differentially expressed in two functional compartments of the prostatic epithelium. The basal cell layer (with lower ERβ levels) is considered androgen-independent, maintains cell proliferation [20], and most likely houses prostatic stem cells [21]. Conversely, secretory luminal cells with high ERβ levels constitute the differentiation compartment, which is androgen-dependent, but has a limited proliferation capacity [20,21]. The biological effects that estrogens may exert on these functional compartments may also depend on the interplay between both steroid receptors (ERα, ERβ) to form homo- or heterodimeric signaling complexes. In secretory luminal cells, the ERβ most likely forms homodimers (ERβ/ERβ) because secretory luminal cells lack the ERα at the mRNA and protein level [10]. On the other hand, the ERβ can heterodimerize with the ERα in basal cells which express the ERα at the mRNA and protein level [10]. Given the differential expression of the ERβ in prostatic epithelial cells and its potential to homo- or heterodimerize, it is likely that the ERβ has different biological functions in basal and secretory luminal cells. Coexpressed with the nuclear androgen receptor (AR) in luminal cells, the ERβ may interact with the AR to control differentiation as described recently in the ventral rat prostate [19]. In the basal cell layer, the ERβ may interfere with cell proliferation by interacting with the ERα or other nonsteroidal growth factor pathways. Experimental data obtained in the ventral prostate suggest that the ERβ mediates antiproliferative effects on the prostatic epithelium [19].

The most consistent and extensive expression of the ERβ detectable in benign prostate tissue submitted in the current study was observed in glandular atrophy of the peripheral zone. Atrophic lesions have been
reported to express high levels of the carcinogen-detoxification enzyme glutathion-S-transferase, which can be activated by the ERβ [22]. Whether the high rates of ERβ expression reflect the cause or effect of atrophic changes, however, is currently unknown. Interestingly, glandular hyperplasia of the transition zone revealed lower rates of ERβ expression when compared with atrophic changes. It has been shown that ERβ knockout mice develop benign prostatic hyperplasia with age indicating that a functional ERβ protects the prostatic epithelium from hyperplastic changes [23]. In the light of this information, it is likely that the reduced levels of ERβ observed in the transition zone play a role in the development of glandular hyperplasia.

Profound changes in the ERβ status occur in HGPIN, which is the most likely precursor of prostate cancer [24]. A hallmark of HGPIN is the extension of proliferative activity to secretory luminal cells in the differentiation compartment of the prostatic epithelium [20]. Results of the current study have shown that secretory luminal cells with high ERβ expression in normal conditions tend to lose the ERβ in HGPIN. Although 23% of HGPIN retained high levels of ERβ, decreased (23%) or markedly decreased (40%) levels were observed in the majority of these premalignant changes. These data clearly differ from the results reported by Leav et al. suggesting that the ERβ is absent in HGPIN [12]. The decreasing levels of ERβ in HGPIN support the concept that ERβ signaling exerts preventive (antiproliferative) effects on the prostatic epithelium, which are partially lost in HGPIN. Conversely, the classical ERα is upregulated in HGPIN and may mediate cancerogenic events [10]. Restricted to stromal and basal cells in normal conditions, ERα mRNA expression extends to luminal cells with detectable levels of the ERα protein in at least 10% of HGPIN [10]. Thus, HGPIN appears to be heterogeneous in their ERα and ERβ expression. Both receptors can form homo- or heterodimeric signaling complexes depending on their receptor status. It is likely that such interactions are critical for the biological functions that estrogens exert in early phases of prostatic cancerogenesis. Further studies are required to define the differential expression of the ERα and ERβ and its implications on growth properties of HGPIN.

The evaluation of the ERβ status in prostate cancer tissue requires suitable internal positive controls to avoid false negative results. In the current study, a number of prostatectomy specimens (n = 15), metastatic lesions (n = 3), and TUR specimens (n = 2) were excluded from evaluation because a suitable internal positive control could not be obtained. Beside the specificity and sensitivity of the primary antibody, tissue processing and fixation are other important factors that can seriously interfere with a proper evaluation of the

| TABLE III. Comparative Evaluation of the Estrogen Receptor Beta (ERβ) Status in High-Grade Prostatic Intraepithelial Neoplasia (HGPIN), Primary Prostatic Adenocarcinoma (Primary Gleason Grades 3 to 5), Distant Metastases, and Recurrent Carcinoma After Hormonal Therapy (Recurrent PCA) |
|-----------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | HGPIN                           | Primary          | Primary          | Primary          | Metastases       | Recurrent       | PCA             |
|                 | None (TS = 0–2)                 | Primary          | Primary          | Primary          | Metastases       | Recurrent       | PCA             |
|                 | 6/47 (12.8%)                    | 0/17 (0%)        | 0/29 (0%)        | 0/14 (0%)        | 0/12 (0%)        | 5/40 (12.5%)    |                 |
|                 | Weak (TS = 3–4)                 | 19/47 (40.4%)    | 0/17 (0%)        | 0/29 (0%)        | 0/14 (0%)        | 1/12 (8.3%)     | 15/40 (37.5%)   |
|                 | Moderate (TS = 5–6)             | 11/47 (23.4%)    | 1/17 (5.9%)      | 6/29 (20.7%)     | 1/14 (7.1%)      | 5/12 (41.7%)    | 15/40 (37.5%)   |
|                 | Strong (TS = 7–8)               | 11/47 (23.4%)    | 16/17 (94.1%)    | 23/29 (79.3%)    | 13/14 (92.9%)    | 6/12 (50%)      | 5/40 (12.5%)    |

The ERβ immunohistochemical (IHC) score was evaluated by a grading system ranging from 0 to 8. Distant metastases included seven lymph node and five bone metastases.
ERβ in prostate cancer tissue. These parameters most likely explain why the results of the current study differ from the data recently published in this field. For example, Horvath et al. have reported a significant loss of the ERβ in primary prostatic adenocarcinoma with only 11% of cases retaining ERβ expression [13]. Conversely, the data from Leav et al. show markedly decreased levels of ERβ in Gleason grade 4/5 tumors and its absence in transition zone cancer [12]. When the above-mentioned tissue requirements were complied (internal positive controls), the current study identifies the ERβ in virtually all-primary prostatic adenocarcinoma including transition zone cancer. Eighty-seven percent of primary tumors retained the high-level expression of the ERβ encountered in benign glandular tissue, whereas the remaining cases revealed lower rates. Although the portion of tumors with lower levels slightly increased with the histological grade, there was no statistical correlation with the Gleason grade ($P = 0.254$) or the pathological stage ($P = 0.157$). In addition, all informative cases of lymph node and bone metastases submitted for study expressed the ERβ at high and moderate levels. This indicates that untreated primary and metastatic prostatic adenocarcinoma retains the ERβ, albeit more heterogeneously and sometimes at lower levels when compared with the benign prostate epithelium. In apparent contrast with untreated tumors, recurrent and androgen-insensitive lesions revealed markedly decreased rates of ERβ expression in at least 38% of cases and were unreactive in 13% of cases. Nevertheless, 13% of these tumors retained the ERβ at high levels. It is tempting to speculate that the partial loss of the ERβ in hormone-refractory tumors is related to the markedly decreased levels of bioavailable androgens after androgen-deprivation. Androgen-cycling experiments in the ventral rat prostate have shown that androgen deprivation decreases ERβ mRNA expression indicating that the ERβ is an androgen-regulated gene [1]. In the light of this information, the partial loss of the ERβ may be caused by the lack of bioavailable androgens. On the other hand, prostate cancer cells with continuous expression of the ERβ may have an hypersensitive androgen receptor (AR) retaining ERβ expression in an androgen-deprived milieu. Further studies are required to elucidate whether the presence of the ERβ at high levels is associated with AR gene amplification or point mutations able to maintain transcription activity of androgen-regulated genes after androgen withdrawal therapy [25].

Another important issue refers to the interactions between the ERβ and the classical ERα in prostate cancer tissue. It has been shown that primary prostatic adenocarcinoma rarely expresses significant amounts of the ERα and the estrogen-inducible progesterone receptor (PR). The most significant levels of these steroid receptors were detected in metastatic and androgen-insensitive lesions, indicating that ERα signaling pathways emerge in late stages of the disease [10,11]. Taking together these data, it is likely that the ERβ forms homodimeric signaling complexes (ERβ/ERβ) in primary tumors, whereas the ERβ can interact with the ERα to form heterodimers (ERα/ERβ) in subsets of metastatic and hormone refractory tumors. Keeping in mind that these dimeric signaling complexes may regulate different subsets of genes, it will be important to study the portion of tumor cells expressing both steroid receptors and define its relation to cell proliferation and apoptosis. From these study, we can expect more insight in the role of the ERβ in prostate cancer progression.

**CONCLUSION**

Results of the current study immunolocalize the ERβ predominantly in secretory luminal cell types indicating that the differentiation compartment is the major target of ERβ signaling in the human prostate. Its partial loss in HGPIN supports the concept that the ERβ exerts chemopreventive effects on the prostatic epithelium. There is a great need for standardization and reporting ERβ immunoreactivity in human prostate cancer tissue. In the current study, untreated primary and metastatic tumors generally retained ERβ expression without any clear correlation with the histological grade or the pathological stage, indicating that these tumors can use estrogens through a ERβ-mediated process. The partial loss of the ERβ observed in hormone refractory tumors may reflect the androgen-dependence of ERβ gene expression. Whether the sustained ERβ expression in subsets of androgen-insensitive prostate cancer cells involves a hypersensitive androgen receptor, needs to be established.

**REFERENCES**


