

Progesterone Receptor Expression in Human Prostate Cancer: Correlation With Tumor Progression

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BACKGROUND. The recent discovery of the classical estrogen receptor alpha (ER α) in metastatic and recurrent prostatic adenocarcinoma suggests that estrogens are implicated in prostate cancer progression.

METHODS. To get more insight into estrogen signaling in prostate cancer tissue, the current study has examined the immunoprofile of the estrogen-inducible progesterone receptor (PR), and evaluated its relation to ER α gene expression.

RESULTS. In primary tumors, the PR was detectable in 36% of primary Gleason grade 3 (5 of 14 cases), 33% of primary Gleason grade 4 (5 of 15 cases), and in 58% of primary Gleason grade 5 tumors (7 of 12 cases). None of the 41 primary tumors investigated revealed significant PR expression in more than 50% of tumor cells. Conversely, moderate to strong receptor expression was observed in 60% of metastatic lesions (9 of 15 cases), and in 54% of androgen-insensitive tumors (38 of 71 cases). Irrespective of grades and stages, the presence of the PR was invariably associated with high steady state levels of ER α mRNA, whereas the ER α protein was undetectable by immunohistochemistry (IHC) in a significant number of cases (58 of 97 cases).

CONCLUSIONS. The progressive emergence of the PR during tumor progression obviously reflects the ability of metastatic and androgen-insensitive tumors to use estrogens through a ER α -mediated pathway. The present data provide a theoretical background for studying the efficiency of antiestrogens and antigestagens in the medical treatment of advanced prostate cancer. *Prostate 48:285–291, 2001.* © 2001 Wiley-Liss, Inc.

KEY WORDS: progesterone receptor; estrogen receptor alpha; immunohistochemistry; in situ hybridization; prostate cancer

INTRODUCTION

Estrogen- and progesterone receptor factors are members of ligand-induced nuclear transcription which play a pivotal role in estrogen target cells and estrogen-dependent tumors. Although prostatic adenocarcinoma is considered a classical target of androgens, estrogens may also be involved in the natural history of these neoplasias. The recent discovery of the classical estrogen receptor alpha (ER α) in premalignant lesions, metastatic and androgen-insensitive tumors suggests that estrogens can affect prostatic cancerogenesis and tumor progression through a receptor-mediated process [1]. In contrast with breast cancer, ER α gene expression appears to be a late event in prostate cancer progression. The most significant

levels have been detected in metastatic and recurrent lesions after androgen deprivation therapy [1]. It has been suggested that prostate cancer cells can survive in an androgen-deprived milieu by using estrogens for their own growth [1]. Although these observations may have important clinical implications, it is currently unknown whether the ER α present in prostate tissue sections is functionally active and promotes transcriptional activity of estrogen-regulated genes. In breast

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cancer and other estrogen-regulated neoplasias, the progesterone receptor (PR) is a widely accepted marker of estrogen action, and has been shown to provide additional prediction power for likely response to endocrine therapy [2]. Conflicting results have been reported on the PR status in prostate cancer tissue. Most studies have demonstrated the PR exclusively in stromal compartments of primary and metastatic prostatic lesions [3–6]. At least one study using the monoclonal antibody NCL-PGR-1A6 and antigen retrieval located the PR in 12 of 26 cases, but failed to demonstrate any significant association with grade or stage [7]. In addition, the PR has not yet been detected in metastatic and androgen-insensitive lesions [6], although a significant number of these tumors has been reported to express the ER α [1].

The potential implications of estrogens in prostate cancer growth prompted us to evaluate the PR status, and define its relation to ER α gene expression in human prostate tissue. Results of the current study demonstrate the PR in a significant number of metastatic and recurrent lesions, and describe a close association with the ER α at least at the mRNA level.

MATERIALS AND METHODS

Tissue Selection

Formalin fixed, paraffin-embedded tissue sections were obtained from 152 prostate cancer patients during a period from 1991 to 2000. This included 48 radical prostatectomy and pelvic lymphadenectomy specimens and 11 lymph node metastases from staging lymphadenectomy without subsequent prostatectomy (Table I). Forty-eight 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 ($\times 100$). The material further contained bone metastases from eight patients without previous hormonal therapy, and palliative transurethral resection specimens from 85 patients with recurrent prostatic adenocarcinoma after orchiectomy. The pathological stages and grading of the material submitted are summarized in Table I. The PR status was evaluated in at least one or two representative tissue blocks from each patient. All specimens with detectable levels of PR in the cancerous lesion were examined for the presence of ER α by immunohistochemistry (IHC) and in situ hybridization (ISH) on adjacent section.

Immunohistochemical Analysis

Tissue sections were deparaffinized, rehydrated through graded alcohol, and subsequently incubated

TABLE I. Pathological stages and grades. The primary Gleason grades refer to the cancerous lesions obtained from 48 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

Pathological Stages and Grades				
n*	Stages	Primary Gleason Grades		
1	pT4; pN1	5 (n=1)		
8	pT3b; pN1	5 (n=4)	4 (n=3)	3 (n=1)
9	pT3b; pN0	5 (n=2)		3 (n=7)
1	pT3a; pN1			4 (n=1)
18	pT3a; pN0	5 (n=5)	4 (n=6)	3 (n=7)
10	pT2b; pN0			4 (n=5) 3 (n=5)
1	pT2a; pN0			4 (n=1)
11	pTx; pN1	ND		
8	bone metastases	ND		
85	recurrent carcinomas	ND		

* Number of patients

ND not determined

in H₂O₂ (0.3%) to block endogeneous peroxidase. For microwave-based antigen retrieval, sections were microwaved (750 W for 5 min and 450 W for 5 min) in 10 mM citrate-buffer, pH 6.0. After pretreatment, the sections were incubated for 30 min in a normal rabbit serum (Dako, Hamburg, Germany). The mouse monoclonal antibodies NCL-PGR-1A6 against the domains A and B of the progesterone receptor protein (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) and NCL-ER-6F11, directed against the full length ER molecule (Novocastra Laboratories Ltd) were used in a dilution of 1:200 and 1:50, respectively. Specificity of these antibodies was tested by Western blotting and by IHC on breast and other tissues [8]. Sections were incubated overnight in a humid chamber. After incubation with the secondary biotinylated rabbit anti mouse immunoglobulin (Dako) for 30 min, the horse-radish 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 [1]. 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 nonimmune mouse serum.

ISH Analysis

ISH for detection of ER α mRNA in tissue sections was performed as described recently [1]. A 24 base cDNA oligonucleotide antisense and sense probes (antisense: 5'-CTC CAG CTC GTT CCC TTG GAT CTG-3'; sense: 5'-CAG ATC CAA GGG AAC GAG CTG GAG-3') complementary to human ER α mRNA coding for amino acids 17–24 (GenBank accession number M12674) were synthesized by MWG-Biotech (Ebersberg, Germany). The oligonucleotides (antisense probe and sense probe for negative controls) were biotin-16-dUTP using the DNA tailing kit (Roche Diagnostics, Mannheim, Germany). The labeling procedure was performed according to the manufacturer's instructions (Roche Diagnostics). The efficiency of labeling reaction was checked on dot blot dilution series. Detection of ER α mRNA in tissue sections was achieved by a nonradioactive ISH procedure described recently [1]. To prove the specificity of the hybridization process, the following negative controls were performed in each case. The slides were hybridized with the corresponding sense probe. Secondly, the hybridization procedure was performed by omitting the sense and antisense probe. In addition, slides pretreated with RNase (Roche Diagnostics) were hybridized as described above.

Quantitative Analysis of Staining

The staining results obtained by IHC and ISH were classified into four categories ranging from 0 to 3:

- 0: 0–5% positive tumor cells
- 1: 6–20% positive tumor cells
- 2: 21–50% positive tumor cells
- 3: 51–100% positive tumor cells

The percentage of positive staining recorded in the entire cancerous lesion present in one or two tissue blocks was estimated at low power magnification ($\times 100$).

Statistical Analysis

Contingence table and χ -square analyses were used to study the relation between the PR-IHC score and the primary Gleason grade of primary adenocarcinomas, recurrent disease and metastases (i.e., the Kolmogorov–Smirnov test; the likelihood- χ -square, 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

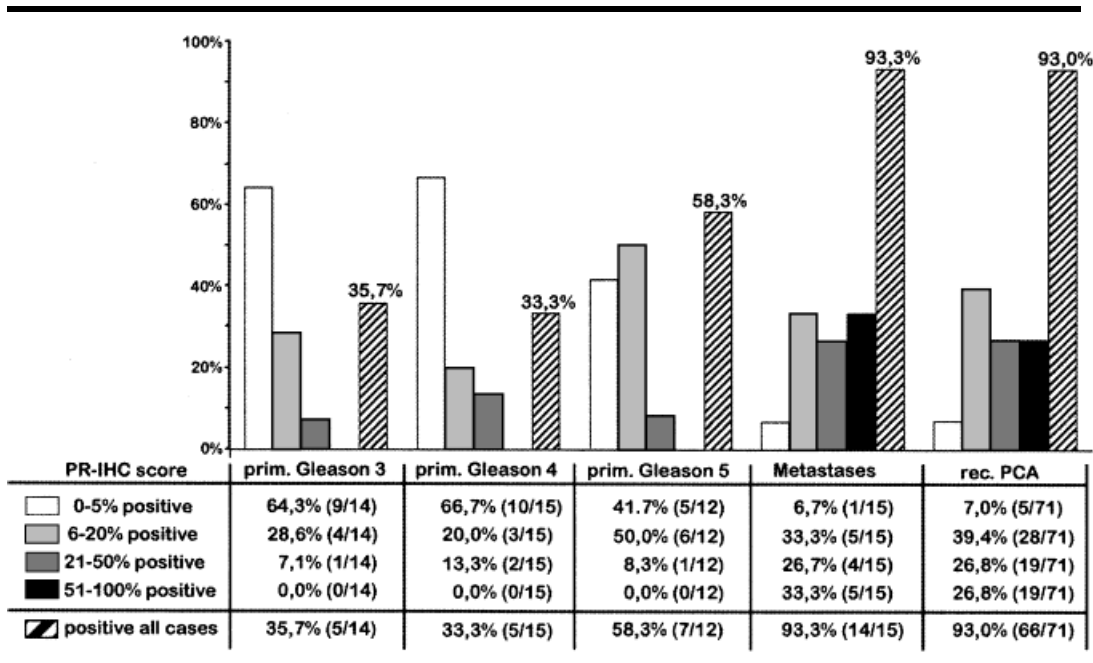
PR Status in Radical Prostatectomy Specimens

In well fixed prostatectomy specimens the PR was detectable in subsets of prostatic stromal cells and other mesenchymal cells including adipocytes, endothelial cells, and vascular smooth muscles which served as a internal positive control. From the 48 prostatectomy specimens submitted for study, seven cases were excluded because a suitable internal positive control could not be obtained. In informative cases benign acini revealed PR positivity only in basal cells. All 48 cases of HGPIN submitted for study were PR negative in the dysplastic epithelium. The results obtained in primary prostatic adenocarcinoma are summarized in Table II. Primary Gleason grade 3 tumors showed PR positivities in 36% of 14 cases evaluated. Among these intermediate grade adenocarcinomas, only one primary Gleason 3 tumor was identified which expressed the PR in approximately 30% of tumor cells. Similar findings were obtained in Gleason grade 4 tumors, although 2 of 15 cases revealed moderate PR expression (PR-IHC score: 2) (Fig. 1). PR positivities were found in 58% of primary Gleason grade 5 tumors but only one case showed PR expression in approximately 40% of tumor cells (Table II). None of the primary adenocarcinomas revealed significant PR expression (more than 50% of positive tumor cells). Although the amount of detectable PR slightly increased from low to high grade tumors, statistical analysis failed to demonstrate a significant correlation between primary Gleason grades and the PR status ($P = 0.324$).

PR Expression in Lymph Node and Bone Metastases

A number of cases submitted for study had to be excluded from evaluation because the PR was undetectable in host cells (e.g., fibroblasts, endothelial cells, adipocytes). This reflects fixation artifacts after frozen section examination of lymph nodes and decalcification procedures for examination of bone tissue. In informative cases with internal positive controls (11 of 21 cases), 10 lymph node metastases revealed PR positivities (91%). In contrast with primary adenocarcinomas, 36% of lymph node metastases (4 of 11 cases) showed significant PR positivities in more than 50% of tumor cells (Fig. 2). Among the eight bone metastases submitted for study, an internal positive control could be obtained in four specimens. In these informative cases, the PR-IHC score ranged from 3 ($n = 1$) (Fig. 3), 2

TABLE II. Comparative evaluation of the PR status in primary adenocarcinoma (primary Gleason grades 3 to 5), recurrent carcinoma after hormonal therapy (rec. PCA), and distant metastases. The PR immunohistochemical (IHC) score was evaluated by a grading system ranging from 0 to 3. Statistical analyses comparing high grade (primary Gleason grades 4 and 5) adenocarcinoma with metastatic and recurrent lesions revealed a significant correlation with the PR status ($P < 0.001$)



(n = 1) to 1 (n = 2). When comparing the PR-IHC score of primary (high grade Gleason grades 4 and 5) tumors with metastatic lesions (lymph node and bone metastases), a statistically highly significant difference between both groups was recorded ($P = 0.005$).

PR Expression in Recurrent Prostatic Adenocarcinoma

Most of palliative TUR specimens resected for recurrent and androgen-insensitive prostatic adenocarcinoma revealed PR positivities in stromal cells and were suitable for PR evaluation (71 of 85 cases). The PR-IHC score obtained in recurrent prostatic adenocarcinoma was nearly identical to that of metastatic lesions (Table II). In particular, 54% of recurrent tumor expressed the PR in more than 20% of tumor cells, including 26.8% of cases with significant PR expression (>50% of positive tumor cells) (Fig. 4). Recurrent lesions significantly differed in their PR status primary (untreated) adenocarcinoma ($P < 0,001$).

Relation to ERα Protein and mRNA Expression

All specimens with detectable levels of PR in the cancerous lesion revealed high steady state levels of ERαmRNA (ERα-ISH score 3) on adjacent sections

(Fig. 4). Conversely, 60% of PR positive lesions lacked ERα positivities by IHC (58 of 97 cases). These PR⁺/ERα⁻ tumors included primary Gleason grade 3 (3 of 5 cases), primary Gleason grade 4 (3 of 5 cases), primary Gleason grade 5 (2 of 7 cases), metastatic lesions (7 of 14 cases), and recurrent lesions (43 of 66 cases). On the other hand, all ERα positive lesions also expressed the PR at variable degrees (Fig. 1).

DISCUSSION

Estrogens have been widely used in the medical treatment of advanced prostate cancer to reduce the testicular output of androgens. The recent discovery of the ERα in metastatic and androgen-insensitive lesions, however, suggests that estrogens can also affect prostate cancer progression through a receptor-mediated process [1]. This newly recognized pathway raises the issue of downstream events of estrogen signaling in prostate cancer cells. Among the various estrogen-regulated proteins, the PR is one of the most important marker for estrogen-regulated growth in clinical studies [2]. To our knowledge, the current study is the first demonstrating the PR at significant levels in metastatic and androgen-insensitive adenocarcinomas. This observation clearly contradicts

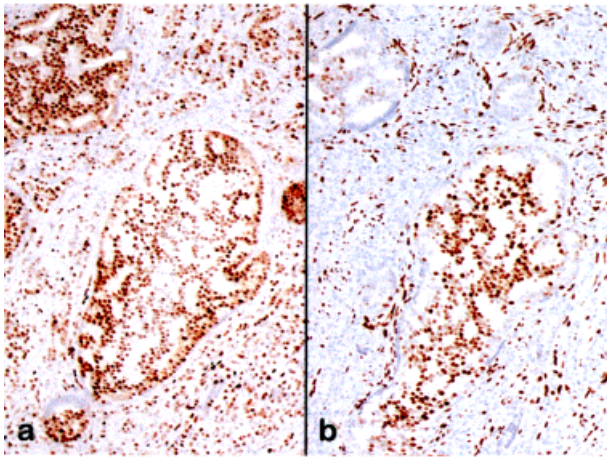


Fig. 1. Primary prostatic adenocarcinoma (primary Gleason grades 3 and 4). Parts of the tumor specimen reveal strong nuclear PR expression (a). On adjacent sections, ER α positivities are restricted to the cribriform (intraductal) lesion and to mesenchymal cells of the host tissue (b). Note that both markers are partially coexpressed. Other parts of this tumor lacked detectable levels of PR and ER α immunoreactivity in the neoplastic lesion. Original magnification, $\times 50$.

results of previous studies that failed to detect the PR in advanced stages of the disease [3,5,6]. Improvement of antigen retrieval methods in recent years, and the availability of new antibodies may account for this discrepancy. The monoclonal antibody NCL-PGR1A6 used here has a wide application in routine practice in the assessment of the PR status in breast cancer, and is directed against the domain A and B of the receptor protein, a site of predicted high antigenicity [8,9]. Tissue processing and fixation are other important factors which may seriously interfere with the immunolocalization of the PR, especially in archival tissue

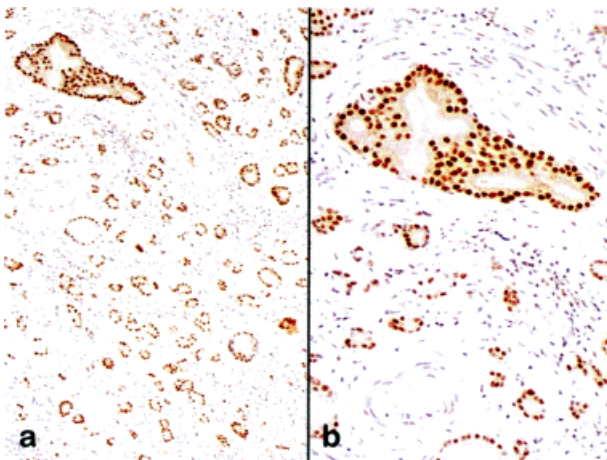


Fig. 2. Lymph node metastasis with extensive PR expression in the entire neoplastic lesion. Original magnification, a $\times 50$; b $\times 100$.

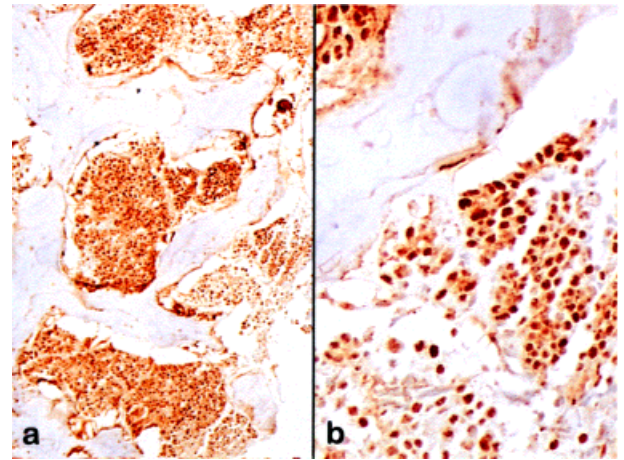


Fig. 3. Bone metastasis from a untreated prostatic adenocarcinoma showing extensive PR expression. Original magnification, a $\times 50$; b $\times 200$.

blocks. In each case, internal positive controls (e.g., immunoreactive stromal and endothelial cells) are required for proper evaluation of the PR status in prostate cancer tissue. For example, a significant number of lymph node (11 of 21 cases) and bone metastases (4 of 8 cases) submitted for study had to be excluded because a suitable positive control could not be obtained in archival paraffin blocks.

It is well established that transactivation of the PR gene is enhanced and maintained by estrogens through a ER-mediated process [10]. The presence of the PR in human tissue is therefore a widely accepted marker for a functional ER pathway. In the manage-

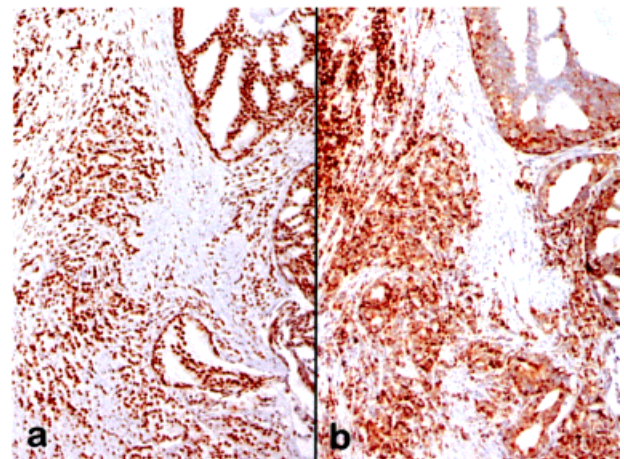


Fig. 4. Recurrent prostatic adenocarcinoma after androgen deprivation therapy. Strong PR expression is detectable in the neoplastic lesion and in subsets of stromal cells (a), ISH reveals high steady state levels of ER α mRNA in the neoplastic lesion and in subsets of stromal cells (b). Original magnification, a, b $\times 50$.

ment of breast cancer patients, the PR status is one of the most powerful predictor of response to antiestrogen therapy [2]. Results of the current study show that the presence of the PR in prostate cancer tissue is invariably associated with high steady state levels of ER α mRNA. This provides circumstantial evidence that upregulation of the PR in prostate cancer cells involves the ER α pathway. On the other hand, the levels of detectable PR were significantly higher than the ER α -IHC score may predict. In our series, 60% of lesions with established ER α mRNA and PR expression lacked detectable levels of the ER α protein (58 of 97 cases). This observation obviously reflects the difficulty to assess the ER α status in prostate cancer tissue by current immunohistochemical methods [1]. Thus, we can not exclude that upregulation of the PR may also be triggered by the novel characterized ER β which has been recognized the most prevalent ER variant in the rat prostatic epithelium [11–14]. In addition, recent RT-PCR analyses of prostate cancer cell lines suggest that PR gene expression in LNCaP and DU145 cells involves rather the ER β than the classical ER α [15]. Regardless of these divergent findings, there is currently no published evidence for the presence of the ER β at the protein level in human prostate cancer tissue [1]. More effective antibodies for detection of the ER β are probably required to address this issue. At present, the PR appears to be the most powerful immunohistochemical marker to assess estrogen signaling in human prostate cancer tissue.

In contrast with breast cancer, expression of the PR in prostatic adenocarcinoma is a late event in tumor progression. In primary tumors, most of informative cases were unreactive or expressed the PR in less than 20% of tumor cells. Although the PR IHC score slightly increased from low to high grade histology, a significant correlation with the primary Gleason grade could not be obtained. The most consistent and extensive PR expression observed in the current study was detected in androgen-insensitive and metastatic lesion, including bone and lymph node metastases. Moderate to strong PR expression (PR-IHC score 2 and 3) was identified in 60% of metastatic lesions (9 of 15 cases), and in 54% of recurrent tumors (38 of 71 cases). Statistical analysis comparing high grade (primary Gleason grades 4 and 5) adenocarcinoma with recurrent and metastatic lesions revealed a significant correlation with the PR status ($P < 0.001$). The progressive emergence of the PR during tumor progression obviously reflects the ability of metastatic and androgen-insensitive phenotypes to use estrogens through a ER α -mediated process. Whether the ER β may also be involved in estrogen signaling in human prostate tissue, warrants further investigations.

CONCLUSIONS

In summary, the occurrence of the PR in a significant number of metastatic and androgen-insensitive prostatic adenocarcinoma suggests that these tumors harbor a functional ER mediating downstream events of estrogen signaling. This supports the concept that prostate cancer cells can escape androgen deprivation by using estrogens for their growth and maintenance. Based on the present information, androgen-insensitive and metastatic prostatic adenocarcinomas are potential targets for antiestrogens and antigestagens, provided that the pertinent receptors are detectable at significant levels in the neoplastic lesion.

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