·*Original Article*·

Androgen receptor expression in clinically localized prostate cancer: immunohistochemistry study and literature review

Yi-Qing Qiu[1,2], Ivo Leuschner[3], Peter Martin Braun[2]

[1]*Department of Urology, the Second Affliated Hospital of Zhejiang University, Hangzhou 310009, China*
[2]*Department of Urology and Pediatric Urology,* [3]*Department of Pathology, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Kiel 24105, Germany*

Abstract

Aim: To evaluate androgen receptor (AR) expression in clinically localized prostate cancer (PCa). **Methods:** Specimens were studied from 232 patients who underwent radical prostatectomy for clinically localized prostatic adenocarcinoma without neoadjuvant hormonal therapy or chemotherapy at our institution between November 2001 and June 2005. Immunohistochemical study was performed using an anti-human AR monoclonal antibody AR441. The mean AR density in the hot spots of different histological areas within the same sections were compared and the correlation of malignant epithelial AR density with clinicopathological parameters such as Gleason score, tumor, nodes and metastases (TNM) stage and pre-treatment prostate-specific antigen (PSA) value was assessed. **Results:** AR immunoreactivity was almost exclusively nuclear and was observed in tumor cells, non-neoplastic glandular epithelial cells and a proportion of peritumoral and interglandular stromal cells. Mean percentage of AR-positive epithelial cells was significantly higher in cancer tissues than that in normal prostate tissues (mean ± SD, 90.0% ± 9.3% *vs.* 85.3% ± 9.7%, $P < 0.001$). The histological score yielded similar results. The percentage of AR immunoreactive prostatic cancer nuclei and histological score were not correlated with existing parameters such as Gleason score, tumor, nodes and metastases stage and pre-treatment PSA value in this surgically treated cohort. **Conclusion:** The results of the present study suggest that there may be limited clinical use for determining AR expression (if evaluated in hot spots) in men with localized PCa. *(Asian J Androl 2008 Nov; 10: 855–863)*

1 Introduction

Prostate cancer (PCa) is the most frequently diag-

Correspondence to: Dr Peter M. Braun, Department of Urology and Pediatric Urology, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Kiel 24105, Germany.
Tel: +49-431-597-3781   Fax: +49-431-597-3783
E-mail: pbraun@uksh-kiel.de

nosed malignancy and the second leading cause of death as a result of cancer in men in industrial countries. Notwithstanding the importance of this malignancy, little is understood about its cause.

Androgens, mainly testosterone and 5-alpha-dihydrotestosterone (DHT), play a fundamental role in the growth, differentiation and maintenance of prostate tissue. Their effects are mediated via a specific androgen receptor (AR) that belongs to the nuclear receptor family. PCa, like the gland from which it arises, is initially

androgen dependent, and since the pioneering studies of Huggins [1], which showed that castration induces prostate tumor regression, front line therapy for metastatic PCa has been based on methods designed to prevent androgenic stimulation of the tumor.

The AR molecule is a major part of the regulatory androgen-AR complex and is therefore critical in the androgen-AR pathway of PCa [2, 3]. AR expression may represent a potential marker of prognosis and hormonal responsiveness in PCa. However, there have been variable results regarding the number of cells expressing ARs in cancer and the ability to predict clinical progression and survival [4–19].

The uncertainty surrounding the relationship between AR expression and advancing histological grade in prostate tumors encouraged us to undertake this project. We studied AR expression in a large series of patients of localized PCa undergoing radical prostatectomy to determine the relationship with several well-known clinicopathologic features, such as tumor, node, metastases (TNM) stage, Gleason score and pre-treatment prostate-specific antigen (PSA) value.

2 Materials and methods

2.1 Patients

We reviewed 232 patients who underwent radical prostatectomy for clinically localized prostatic adenocarcinoma at the Department of Urology and Pediatric Urology, Universitätsklinikum Schleswig-Holstein, Campus Kiel (Germany) between November 2001 and June 2005. The diagnosis of PCa was made by transrectal-ultrasound-guided octant biopsies in all patients. The indication for prostate biopsy was a suspicious finding on digital rectal examination and/or elevated serum PSA. None of the patients had received neoadjuvant hormonal therapy or chemotherapy before the tumor samples were taken.

The radical prostatectomy specimens were processed by the whole-mount technique and the pathologic parameters were evaluated in a manner previously described [20]. All clinical and clinicopathologic data, such as age, PSA, Gleason score and TNM, were obtained from medical records. Staging was based on the modified Whitmore-Jewett system and 2002 TNM classification. Sufficient tissue was available for immunohistochemical analysis in all cases.

2.2 Immunohistochemistry

Paraffin-embedded formalin-fixed archival prostatic tissue specimens were obtained. Serial sections of each case were cut and slides were stained with hematoxylin and eosin (HE) for routine histological evaluation; suitable blocks for examination were selected by an experienced pathologist (I. Leuschner). Diagnosis of each block was confirmed (Figure 1) by examination of a routinely stained HE section juxtaposed to the section used for AR immunostaining.

Immunohistochemical staining for AR was performed on routinely processed, paraffin-embedded tissue specimens. Sections (3 µm) were de-waxed and rehydrated in xylol and alcohol. After heating the slides for 4 min in a steam cooker, the sections were immersed in methanol with 0.6% hydrogen peroxide for 15 min to block endogenous peroxidase activity. The slides were incubated with primary monoclonal anti-human AR antibody AR441 (Dako, Carpinteria, CA, USA), dilution 1:50, at room temperature for 40 min. Bound antibody was detected using the avidin-biotin complex peroxidase method using an ABC Elite Kit (Vector, Burlingame, CA, USA) with 3,3'-diaminobenzidine used as the chromogen. Tissues were counterstained with Mayer's hematoxylin solution. Negative control slides were prepared by omitting the primary antibody.

The number and intensity of immunoreactive nuclei was assessed without any knowledge of the clinical data by two observers (Y. Q. Qiu and I. Leuschner). Owing to the heterogeneous content of positive staining cells in the tumors, each of the slides was scanned at × 40 to find

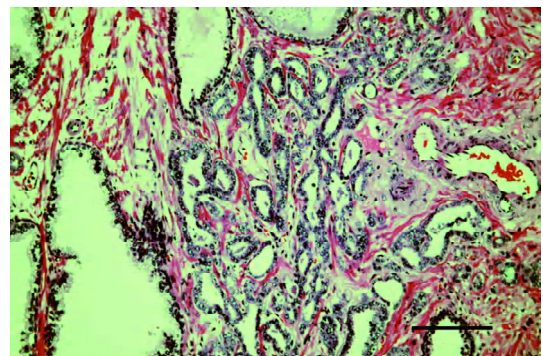Figure 1. Routinely stained hematoxylin and eosin (HE) section of prostate cancer (PCa) tissue (magnification × 400). Diagnosis of each block was confirmed by examination of a routinely stained HE section juxtaposed to the section used for androgen receptor immunostaining. Bar = 200 µm.

the areas of highest staining. For evaluating androgen receptors in malignant epithelium and adjacent non-tumorous prostate tissue, at least 1 000 epithelial cells within a hot spot were counted using an integration grid (magnification 400 ×). The number of positive nuclei is expressed as a percentage of the total number counted. Considering the nature of heterogeneous staining of PCa, we also used histological score (HSCORE), which is a measure of both the intensity and distribution of staining, to measure the immunohistochemistry staining of AR. The HSCORE was calculated using the equation: HSCORE = $\Sigma Pi(i + 1)$ [21]. The intensity of staining (i) was evaluated subjectively on a scale of 0–3, where 0 = no staining, 1 = weak equivocal staining, 2 = unequivocal moderate staining and 3 = strong staining. Pi is the percentage of stained epithelial cells for each intensity. This semiquantitative analysis has been shown to have a low intraobserver and interobserver error. The areas of focal staining with the highest percentage of nuclei for AR were used in each Gleason pattern observed in a particular tumor. If more areas from the same pathological category were identified within one prostate, the highest score was taken for that category.

2.3 Statistical analyses

Statistical calculations were performed using the Statistical Package for Social Sciences for Windows software (version 13.0; SPSS, Chicago, IL, USA). Comparison of the mean AR density in the different histological areas within the same sections was done using the paired *t*-test as appropriate. Correlation of malignant epithelial AR density with clinicopathological data was analysed as a Spearman rank coefficient. Nonparametric tests were used to study the relationships of AR protein density with other variables in univariate analysis. All tests were two-sided with significance set at 0.05.

3 Results

The levels of nuclear AR expression were evaluated by immunohistochemistry in 232 radical prostatectomy specimens from patients treated for clinically localized PCa. Mean patient age at prostatectomy was 64.9 years and mean pre-operative PSA was 10.6 ng/mL. Pathological stage was mostly T2c in 82 cases and T2b in 48 cases; ten patients confirmed lymph node metastasis. Average Gleason score was 6.54 ± 1.23 and the most frequent Gleason scores were 6 and 7, accounting for 70

and 72 cases, respectively.

The specificity and sensitivity of the anti-AR antibody used to recognize its antigen were confirmed by the absence of staining in negative controls (Figure 2) and positive reaction of all the prostate tissue sections (Figure 3). AR immunoreactivity was almost exclusively nuclear and was observed in the tumor cells, non-neoplastic glandular epithelial cells and a proportion of peritumoral and interglandular stromal cells (Figure 3). AR-positive cells were heterogeneously distributed in our study. Basal cells were only rarely positive. No cytoplasmic staining was noted in any case. Mean number of stained cells were significantly higher in cancer cells than in normal prostate tissues. The HSCORE yielded similar results (Table 1).

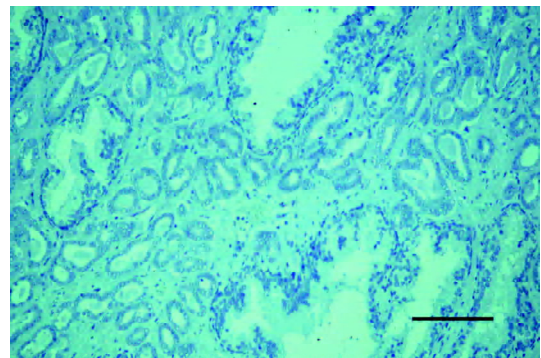Spearman rank correlations were used to explore potential associations between AR protein expression and

Figure 2. Negative controls (magnification × 400). No immunostaining in prostate tissue. Bar = 200 µm.
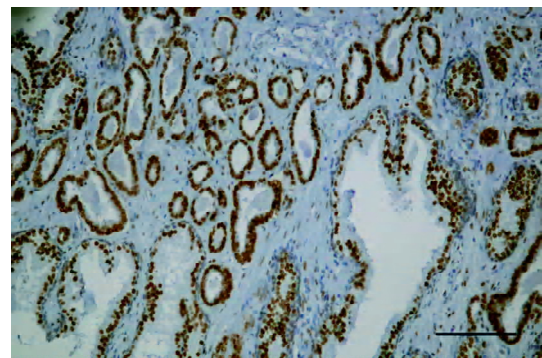

Figure 3. Androgen receptor (AR) immunoreactivity (magnification × 400). was almost exclusively nuclear and was observed in the tumor cells, non-neoplastic glandular epithelial cells and a proportion of peritumoral and interglandular stromal cells. Bar = 200 µm.

Table 1. Androgen receptor (AR) expression in prostate cancer (PCa) and adjacent benign tissue. HSCORE, histological score.

	Malignant (mean ± SD)	Benign (mean ± SD)	*P*-value
Percentage of positive cells (%)	90.0 ± 9.3	85.3 ± 9.7	0.001
HSCORE	3.2 ± 0.6	2.9 ± 0.6	0.001

Table 2. Spearman rank correlation coefficients of androgen receptor (AR) expression in malignant epithelium of prostate cancer (PCa) with clinicopathological variables. *$P < 0.01$, compared percentage with HSCORE. HSCORE, histological score; pre-PSA, pre-treatment prostate-specific antigen value.

	Percentage	HSCORE	Age	Pre-PSA
HSCORE	0.894*			
Age	−0.089	−0.069		
Pre-PSA	−0.004	0.049	0.14	
Gleason score	−0.009	0.071	0.041	0.301*

clinicopathological indicators of PCa severity, such as serum PSA levels and Gleason sum (Table 2). In this analysis, patient Gleason score and pre-prostatectomy PSA were significantly positively correlated with each other. When we compared two different staining measurement methods, we found they were strongly correlated.

Pathological stage was a dichotomous categorical variable with four levels (pT2a or less, pT2b, pT2c, pT3–T4 or lymph node positive). Pathological Gleason scores were categorical variables with three levels (6 or less, 7, 8–10). Pre-treatment PSA and age entered the model as continuous variables. Tables 3 and 4 show the mean, median and range of AR content (percentage and HSCORE) in a univariate analysis with each of the stratified covariate groups. AR protein expression density did not differ significantly compared with age, pre-treatment PSA, Gleason score or TNM stage.

4 Discussion

Although there are extensive studies of how androgen dependent PCa transits into androgen independence in advanced PCa [22], very little research attempts to unveil the actual mechanism of AR in early-stage PCa. An important issue that should be considered is that PCa is increasingly detected at earlier stages. Early detection may, in some cases, lead to over treatment because there are no molecular markers available that allow the detection of clinically indolent or potentially aggressive cancers. Thus, AR function should also be studied during the early stages of prostate carcinogenesis. A better understanding of the biologic mechanism and the role played by androgens and AR in patients with localized PCa would possibly allow improved clinical management and provide new targets for prevention and therapy in these patients.

PCa treated with radical prostatectomy may offer an ideal avenue for revealing the putative role and natural history of AR in PCa because the androgen-AR pathway is most likely undisturbed. With this in mind, the current study was designed with the following characteristics: 1) a large number of study cases for increased statistical power; 2) well-characterized patients, i.e. all received radical prostatectomy, no pre-operative hormonal therapy; and 3) two different measure methods for increasing objectivity in assessment of immunostaining.

We used a sensitive immunohistochemical method and a well characterized specific monoclonal antibody to determine the extent and intensity of AR expression in the benign and malignant prostate. We found that every case displayed intense nuclear immunoreactivity in benign epithelium and cancer. AR immunopositivity was significantly lower in these benign glands than in secretory cells in malignant epithelium within the same sections, which is consistent with previous reports [15].

In our study, there was no significant association between AR expression in PCa and clinical and pathological parameters such as Gleason score, TNM and pre-treatment PSA. This seems counterintuitive, since AR

level was initially thought to be higher in progressive local prostatic carcinoma. However, we found that this paradox might be explainable after reviewing similar studies published in recent years (Table 5). These studies investigated the potential clinical usefulness of AR levels in PCa and reported various conclusions.

Theodoropoulos *et al*. [6] studied 81 patients with Stage T1a PCa and revealed that well-differentiated tumors were associated with a high percentage of stained cells, as well as a high staining intensity, compared with moderately and poorly differentiated tumors, although this is not a prognostic factor of tumor progression. A greater AR content in patients with a low Gleason score compared with those with a high Gleason score has also been previously reported [4, 5, 7, 8]. Low AR expression has been considered a potential negative prognostic factor for the response to hormonal therapy and outcome in patients with metastatic PCa [4, 5].

However, some reports suggested that AR protein over-expression as a result of AR gene amplification may contribute to loss of growth control by enabling tumor cells to become hypersensitive to castrate levels of androgen in the prostate. Inoue *et al*. [9] studied 52 patients who underwent radical surgery and found high AR protein expression predicted shorter disease-free survival. Similarly, Li *et al*. [10] and Henshall *et al*.[11] demonstrated that high levels of AR in PCa denote a higher degree of malignancy, more advanced disease progression and worse biochemical recurrence-free survival. AR expression is positively correlated with standard clinical and pathologic parameters, including Gleason grade, clinical stage, lymph node status, extra-capsular extension and seminal vesicle invasion. It appears that increased AR activity is associated with enhanced tumor growth and accelerated disease progression; hormonally naive PCa cells may take advantage of higher AR status, which

Table 3. Nonparametric univariate analysis of relationship of histological score (HSCORE) in prostate cancer (PCa) cells with patient clinicopathologic parameters. PSA, prostate-specific antigen value. TNM, tumor, node, metastases.

Variable	*n*	HSCORE			*P* value
		Mean	Median	Range	
Age (years)					0.776
< 60	15	3.26	3.35	1.77–3.84	
60–64	16	3.25	3.28	1.75–3.94	
65–69	159	3.20	3.37	0.89–3.95	
≥ 70	42	3.15	3.13	0.40–3.94	
Total	232	3.21	3.29	0.40–3.95	
Pre-treatment PSA (ng/mL)					0.196
1–4	17	3.11	3.11	2.32–3.87	
4.1–10	112	3.19	3.32	0.40–3.94	
10.1–20	47	3.37	3.49	1.55–3.95	
Greater than 20.1	15	3.12	3.16	0.89–3.94	
Total	191	3.22	3.32	0.40–3.95	
Pathological Gleason score					0.830
6 or less	117	3.20	3.27	0.40–3.95	
7	72	3.24	3.37	1.75–3.94	
8–10	43	3.20	3.25	0.89–3.94	
Total	232	3.21	3.29	0.40–3.95	
Pathological TNM stage					0.208
T2a or less	31	3.29	3.22	2.26–3.95	
T2b	48	3.13	3.28	0.40–3.92	
T2c	82	3.16	3.19	1.55–3.94	
T3–T4 or N1	71	3.31	3.39	0.89–3.94	
Total	232	3.21	3.29	0.40–3.95	

Table 4. Nonparametric univariate analysis of relationship of percentage of positive cells in prostate cancer (PCa) tissues with patient clinicopathologic parameters. PSA, prostate-specific antigen.

Variable	Percentage			P value
	Mean	Median	Range	
Age (years)				0.723
< 60	90.7	94	66–97	
60–64	90.8	93	60–99	
65–69	89.7	92	40–99	
≥ 70	88.9	92	20–99	
Pre-treatment PSA (ng/mL)				0.177
1–4	89.1	88	76–98	
4.1–10	89.8	92	20–99	
10.1–20	92.2	93	60–99	
Greater than 20.1	86.2	91	40–99	
Pathological Gleason score				0.825
6 or less	90	93	20–99	
7	89.3	93	40–99	
8–10	90.4	91	60–99	
Pathological stage				0.459
T2a or less	90.7	92	73–99	
T2b	88.6	92	20–99	
T2c	89.5	92	60–99	
T3–T4 or N1	91.1	93	40–99	
Total	90	92.5	20–99	

Table 5. Selected studies of androgen receptor (AR) density in prostate cancer (PCa). RP, radical prostatectomy; TURP, transurethral resection of prostate; BPH, benign prostatic hyperplasia; TNM, tumor, node, metastases.

Authors	Specimens	Cohort size	TNM Stage	Effect on prostate cancer outcome
Takeda *et al.* [4]	Biopsies	62	Stage D2	Higher AR, better prognosis
Segawa *et al.* [5]	Biopsy, RP and TURP	42	Three metastases, others unclear	Higher AR, better prognosis
Theodoropoulos *et al.* [6]	TURP and RP	81	T1a	Not prognostic
Miyamoto *et al.* [7]	RP	10 BPH	10 primary	More heterogeneous in cancer sections
Ruizeveld de Winter *et al.* [8]	RP	26	Primary	Unavailable
Inoue *et al.* [9]	RP	52	Primary	Higher AR, worse prognosis
Li *et al.* [10]	RP	640	Primary	Higher AR, worse prognosis
Henshall *et al.* [11]	RP	96	Primary	Higher AR, worse prognosis
Schatzl *et al.* [12]	Biopsies	39	All stages	Unavailable
Pertschuk *et al.* [13]	Biopsies	90	unclear	Higher AR, better prognosis
Sweat *et al.* [14]	RP	197	Node positive	Higher AR, worse prognosis
Sweat *et al.* [16]	RP	172	T2	Not prognostic
Noordzij *et al.* [17]	TURP	68	All stages	Not prognostic
Gaston *et al.* [18]	RP	50	Primary	Unavailable
Ford *et al.* [19]	TURP	24	Recurrent	Unavailable
Sadi *et al.* [23]	Biopsies	17	Stage D	Not prognostic
Sadi *et al.* [24]	Biopsies	17	Stage D2	More heterogeneous in poor responders
Present study	RP	232	Primary	Unavailable

may lead to enhanced AR activity, resulting in more growth advantage.

In the present study, AR expression did not correlate with other well known clinicopathologic features, which is similar to the conclusions of some other reports [16–19]. Gaston *et al.* [18] reported that AR protein expression was 22% higher in the benign prostate and 81% higher in the PCa of black African men compared with white men who underwent radical prostatectomy for clinically localized PCa. However pathological evaluation revealed no differences in Gleason grade or stage. Visakorpi *et al.* [25] used immunohistochemistry to compare AR protein expression between tumors with and without AR amplification. They were unable to recognize any differences in the level of protein expression in primary versus recurrent tumors or in recurrent tumors that did or did not exhibit AR amplification. They attributed this finding to the "qualitative nature of the immunohistochemical reaction". Ford *et al.* [19] demonstrated that although AR amplification results in increased AR protein expression, it did not appear to impact survival after androgen deprivation for advanced PCa. Pertschuk *et al.* [13] found that men with AR-negative PCa had a worse prognosis than those with AR-positive PCa but failed to find any correlation between AR density with grade, stage or ethnicity. Similarly, Noordzij *et al.* [17] noticed a trend between AR expression and Gleason grade but it was not statistically significant.

It is important to pay attention to the study materials when reviewing reports of AR expression in PCa. We evaluated large tissue sections from radical prostatectomy specimens that contained the greatest amount of high grade cancer to minimize heterogeneity of AR expression, which might confound biopsy studies. In contrast to our study, some investigators evaluated AR immunoreactivity in biopsies [4, 5, 12, 13, 23, 24], the limited sample size in such specimens may in part account for the lack of predictive value for patient outcome. Several studies used tissue obtained from transurethral resection of the prostate [5, 6, 17, 19], despite the fact that AR are sensitive to thermal injury and may be damaged during this procedure.

Other factors that may contribute to the various conclusions of previous studies are antibodies used and evaluation methods for immunoreactivity. Many monoclonal and polyclonal antibodies were used to identify different antigenic epitopes with variable efficacy in paraffin-embedded tissues. Different thresholds of expression were chosen to stratify patients into prognostic group, usually with arbitrary cut points that were predefined by the investigators. Quantitation of AR immunoreactivity was performed using different methods, including manual evaluation or computer assisted digital image analysis.

The heterogeneity of AR immunostaining within tumors is a consistent finding [4, 8, 13, 23, 24, 26]. It may be a very important factor that resulted in different observations in the literature. In our study, we frequently found intense nuclear staining adjacent to unstained nuclei or areas of staining intermingled with areas that lacked staining. Some authors reported that not the percentage of positive cells but the degree of immunostaining heterogeneity [24] or mean immunostaining intensity [26] would determine the prognosis. Sadi and Barrack [24] analyzed AR staining patterns in 17 specimens of stage D2 PCa obtained before hormonal therapy and found that staining homogeneity was a significant predictor of subsequent response to hormone therapy. Good responders exhibited unimodal peaks for frequency of positive nuclei, whereas patients with disease progression, despite endocrine therapy, exhibited staining heterogeneity and flattened frequency distribution curves. Tilley *et al.* [26] analyzed AR staining with image analysis in 30 patients with stages A to D2 prostate tumors and found that averaged staining intensity per cell could predict the disease outcome. In the subgroup of stage D2 cases, the AR staining features were able to predict correctly response to hormone therapy in all 17 cases. Both studies made use of computerized image analysis systems and, in the latter study, significant data could only be obtained with the additional results of two antibodies against the C-terminal and N-terminal parts of the AR. However, since evaluation of clinically suitable markers should be fast and simple, we evaluated AR expression using two different, easily applicable measure methods in our study.

Owing to heterogeneous staining in the PCa, it is difficult to choose the right regions to evaluate. The methods of field selection varied in different published studies. We counted epithelial tumor cells within hot spots. Our method was similar to some other reports [6, 9, 12]. Theodoropoulos *et al.* [6] scanned each slide at × 40 magnification to find the areas with the most numerous positive cells first, then carefully examined each slide at × 400 magnification to count malignant epithelium cells. Some authors selected epithelial cell nuclei randomly from different areas [10, 18]. And some reports did not describe which areas they evaluated [11,

16, 17], ignoring the issue of heterogeneous staining in PCa. The discrepancy in methods may explain the conflicting results of AR immunostaining.

In our study, we constantly observed a higher AR immunoactivity in the malignant glands compared with their benign counterparts. Our result differs from the results of Sweat *et al.* [14, 16], who found reduced AR expression in PCa. This discrepancy could be due, in part, to AR expression heterogeneity in cancer tissue. We counted positive cells in the hot spots of cancer and Sweat *et al.* [14, 16] did not describe which areas they evaluated. We chose hot spots because we noticed that AR expression varied drastically in some cancer tissue and thus tried to avoid selection bias between cases. Also we noticed that AR immunoreactivity was more uniform in benign epithelium. The next logical step is to use different field selection methods to compare the results and analyze the discrepancies in published studies. A conclusion of up-regulation of AR expression in PCa might have been drawn if we counted the "coldest" spots of the cancer tissue in our series. Different evaluation methods should be attempted and compared, including using random areas, areas with lowest and highest expression levels, predominant AR expression pattern, *etc.* In addition, because PCa is frequently a multifocal disease, it will be necessary to stratify AR expression in different cancer foci within the same tumor and to correlate it with different Gleason patterns and PSA follow-up. To evaluate the usefulness of AR expression as an outcome predictor, we need to find an optimal evaluation method that shows the best correlation with disease prognosis parameters. Ultimately, we need to find a standard AR immunoreactivity counting system that is reliable, comparable and reproducible before AR immunostaining can become a valuable molecular marker of PCa.

In conclusion, AR nuclear expression was consistently present in benign and adenocarcinoma epithelium. The percentage of AR immunoreactive nuclei in hot spots of PCa was not correlated with clinicopathologic parameters such as Gleason score, TNM stage and pretreatment PSA. Our results indicate that there may be limited clinical use for determining AR expression in men with localized PCa before a standardized AR expression counting system is established.

Acknowledgment

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