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Expression of prostate specific membrane antigen (PSMA) in prostatic adenocarcinoma and prostatic intraepithelial neoplasia

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Summary. The prostatic membrane antigen (PSMA) is a protein that is expressed in the prostatic epithelium. We studied the expression of PSMA in a series of 55 patients with different stages of prostate cancer and we compared the PSMA staining in prostate cancer cells, in high-grade prostatic intraepithelial neoplasia (PIN) and in histologically benign prostatic epithelium for the same specimen. For this purpose archival paraffin-embedded specimens were studied by immunohistochemistry with a monoclonal antibody 7E11-C5.3 against PSMA using the streptavidin-biotin method. The mean percentage of PSMA immunoreactivity was 56.67% in prostate cancer (CaP) cells, and 48.6% in PIN cells, which was significantly higher than benign-appearing prostatic epithelium (5.72%) (for each pair, $p<0.001$). PSMA expression was greater in CaP with a higher Gleason score ($p=0.01$), but no relationship was found with serum PSA value. We conclude that PSMA overexpression is detected in high-grade PIN and is associated with a higher Gleason score of prostate cancer. It is a potential marker for studying carcinogenesis and progression of prostate cancer.

Key words: Prostatic carcinoma, High-grade prostatic intraepithelial neoplasia, Immunohistochemistry, Prostate-specific membrane antigen, PSMA

Introduction

Prostate cancer is the most common cancer diagnosed in men in the United States and the second most common cause of cancer death. Considerable progress has been made on defining the molecular events that contribute to the transformation of normal prostate epithelial cells into prostatic intraepithelial neoplasia, and to prostate cancer. Multiple oncogenes and tumor suppressor genes have been proposed to play important roles in prostate carcinogenesis (Walsh, 1998).

The antibody against prostate-specific membrane antigen (PSMA) (7E11-C5 clone) was obtained following immunization with LNCaP cells (Horoszewicz et al., 1987). PSMA is a type II membrane protein that is expressed in the prostatic epithelium (Chang et al., 2000). However, PSMA expression has also been observed in renal adenocarcinoma, urothelial carcinoma, adenocarcinoma of the colon and embryonic carcinoma of the testicle. One study reveals a significant association between PSMA expression in the neovascularisation of prostate cancer (Chang et al., 1999).

The PSMA gene located in the 11p chromosome (Israeli et al., 1994) codifies two types of fractions: the cytosolic fraction of lower molecular weight (Heston, 1997); and the membrane fraction, detected by fixing with the 7E11-C5 antibody.

PSMA presents NAALADase activity (demonstrated in mouse brains) and Folate hydrolase activity. N-acetylaspartilglutamate (NAAG) is converted into A-acetylaspartate and glutamate (Carter et al., 1996).

The technique of the reverse transcription polymerase chain reaction (RT-PCR) for PSMA has been employed to detect the presence of circulating tumor cells for identifying micro-metastatic disease. Horoszewicz et al. (1987) were able to detect circulating PSMA epitope in 20 out of 43 sera from CaP patients. Okegawa et al. (1998) have observed a significant correlation of serum PSMA value with the pathological state of the cancer, but Basso et al. (2000) observed no significant findings.

An immunoconjugate complex of 7E11-C5.3, termed CYT-356, can be marked and used to detect bone metastasis (Zukier et al., 1997) and lymph metastasis (larger than 5 mm), with a sensitivity of 44% and a specificity of 86% (Babaian et al., 1994). This immunocomplex could be of future use, both at the

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tumoral staging and in the treatment of an advanced stage of the illness (Bostwick et al., 1998).

PSMA expression in adenocarcinoma and prostatic intraepithelial neoplasia (PIN) has received little attention in the literature (Bostwick et al., 1998; Chang et al., 2000). We present a series of 55 cases of patients with different stages of prostate cancer, and compare PSMA expression in benign-appearing prostatic epithelium, PIN and prostate cancer. In addition, we evaluated the association of PSMA expression with the levels of serum PSA, the cancer clinical stage and the Gleason score for all these patients.

Materials and methods

Prostatic specimens obtained by transrectal ultrasound (TRUS) biopsy from 55 patients at the Costa del Sol Hospital, Marbella. Málaga. Spain, between 1996 and 1999 were chosen for study. In each case, pre-treatment serum PSA was evaluated by immunoassay (Roche Diagnostic, Mannheim, Germany) obtained from the patients record, and cancer Gleason scores were determined by two pathologists (JQ, DGB). The stage was established according to the UICC classification, and was always based on clinical stage, because the percentage of patients undergoing radical prostatectomy (following the Partin's nomogram) was low (32.7%). To obtain prognostic conclusions, the patients were divided into 2 groups: localised cancer (T1-2 N0M0) and advanced cancer (T3-4 N1-3M1). High-grade prostatic intraepithelial neoplasia was diagnosed using criteria described previously (Epstein et al., 1995). Moreover, we classified these lesions into adjacent (< 2mm) or not (≥ 2 mm) to carcinoma.

Formalin-fixed and paraffin-embedded tissue sections (5 μm) were used for the study. Mouse PSMA monoclonal antibody (7E11-C 5.3 clone) (Cytogen Corp. Princeton, NJ) was applied to tissue sections as previously described (Bostwick et al., 1998).

Briefly, tissue sections were deparaffinized, dehydrated, rehydrated and soaked in 3% H_2O_2 for 15 minutes, then steamed in EDTA buffer (pH 8.0) for 30 minutes. Tissue sections were incubated with primary antibody (PSMA; 20 mg/ml) for 60 minutes, biotinylated goat-anti mouse immunoglobulin Ig and goat anti-rabbit IgG (1:400, Dako Corp. Santa Barbara), for 30 minutes, and peroxidase-labeled streptavidin (1:500, Dako Corp. CA) for 30 minutes. Immunoreactivity was visualized by incubations of sections with 3-aminoethylcarbazole in the presence of the hydrogen peroxide. The sections were counterstained with light hematoxylin and mounted with a coverslip. Positive and negative controls were run in parallel with each batch and gave appropriate results (data not shown).

The percentage of cells exhibiting PSMA staining in each case was estimated in 10% increments. A numerical intensity score between 0 and 3 was assigned to each, using the following criteria: 0=no staining; 1=weak staining; 2=moderate staining; 3=strong staining. Only cells with an intensity of staining >1 were considered positive.

Statistical study

The relationship of PSMA immunoreactivity with the cancer Gleason score and serum PSA levels was evaluated by a linear correlation test. The association between the PSMA immunoreactivity and clinical cancer stage was studied by the ANOVA test. The T-test was used to measure the differences between the percentage of stained cells in CaP, PIN and benign-appearing prostate epithelium. Due to the significant correlation between the staining intensity and the percentage of positive cells, only the results for the percentage of positive cells are displayed in this paper (Table 1).

Results

The patient age ranged from 51 to 87 years (mean 71.1) and the pretreatment serum PSA ranged from 1 ng/ml to 137 ng/ml (mean 27.9 ng/ml). The cancer Gleason scores were: 4 to 6 (27 cases), 7 (12 cases), 8 to 10 (16 cases) and the clinical cancer stages were: localised stage (35 cases) and advanced stage (20 cases).

The 47% of patients were diagnosed with prostate cancer by the first biopsies and the 33% by second biopsies, with the remaining 20% being diagnosed by more than two randomized biopsies. In 85% of cases, ≥ 4 cores were involved, with a percentage of tumoral infiltrate superior to 75%, while in 15% of cases, cores were < 4, with 25% infiltration.

Cytoplasmic immunostaining with accentuation on the cell membrane was observed in 56.67% of CaP cells (Fig. 1), 48.6% of PIN cells (Fig. 2), and 5.7% of normal epithelium. PSMA immunoreactivity was significantly higher in CaP and high-grade PIN than histologically benig epitelium ($p<0.001$ for each pair); however, there were no differences between high grade PIN and CaP

Table 1. Association between PSMA Immunoreactivity in CaP and Clinical Stage, PSA, PIN, Gleason score and staining percentage in CaP.

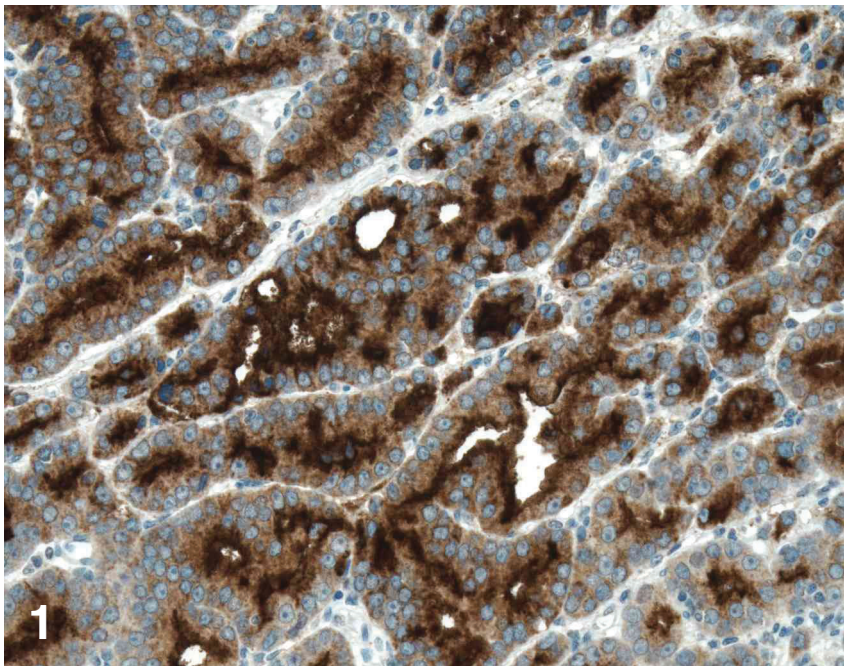
	CORRELATION COEFFICIENT Pearson coefficient (p value)	VARIANCE ANALYSIS F Snedecor (p)	STUDENT-T TEST t Student (p)
Clinical Stage	-	0.69 (p=0.19)	-
PSA levels	0.19 (p=0.15)	-	-
High grade PIN	-	-	1.41 (p=00.17)
Staining intensity	-	22.14 (p<0.001)	-
Gleason Score	0.31 (p=0.01)	-	-

(Table 1). PSMA immunoreactivity was strongly associated with a higher Gleason score (p=0.01), but not with serum PSA (p=0.15, Table 1). This higher PIN value was adjacent to the tumors in 75.6% of the cases versus 24.3% in non adjacent areas. However, there were no statistical differences between these zones in the percentage of staining from PSMA. Moreover, no statistical differences were observed between samples corresponding to prostatectomies and samples obtained by TRUS biopsies.

A trend of a higher percentage of stained cells in the advanced stages than in the localised ones was observed, although the difference was not statistically significant (p=0.19, Table 1).

Discussion

We examined a group of patients with prostate cancer and found that the PSMA (7E11-C5) antibody was strongly positive in prostate cancer as well as in high-grade PIN lesions. A previous work found a similarly strong expression in PIN lesions (Bostwick et



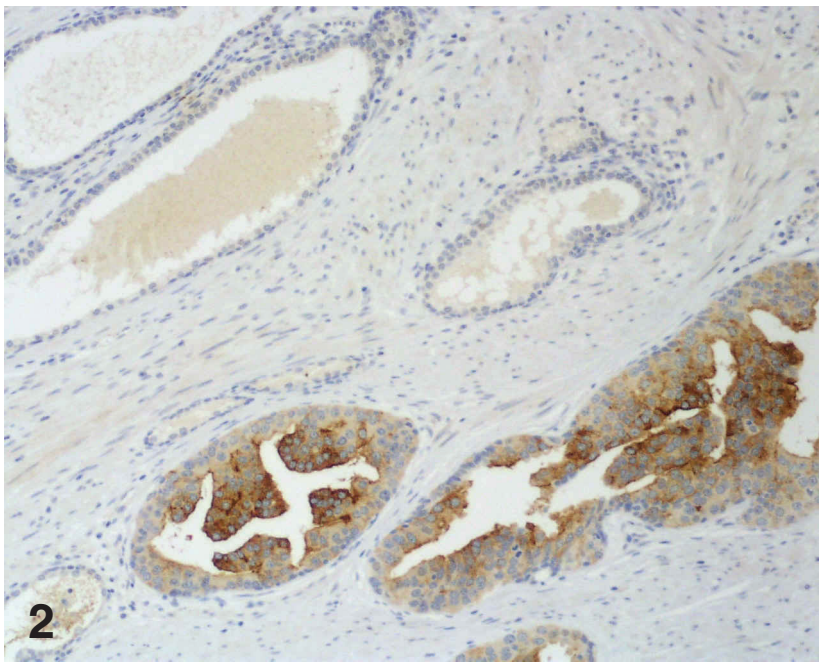


Fig. 1. Strong PSMA staining in a high grade prostate carcinoma. x 200

Fig. 2. Prostatic glands strongly positive for PSMA staining in a high grade intraepithelial neoplasia (PIN). x 200

al., 1998). The expression of the mRNA of this PSMA is greater in states of hormonal deprivation (Israeli et al., 1994; Chang et al., 2000). However, the expression of PSA is decreased in hormone-refractory tumors.

Earlier studies associated PSMA expression with the malignancy of prostate cancer (Israeli et al., 1994; Kawakami and Nakayama, 1997). In the present study, a positive correlation between PSMA staining and the Gleason score was observed, which support these observations.

Douglas et al. (1997) found an association of serum PSMA expression with an advanced pathological stage, although other authors have been unable to confirm these findings (Murphy et al., 1995; Beckett et al., 1999). We found a trend of higher PSMA immunoreactivity in advanced stages than in localised tumor stages, but the difference was not significant. However, it should be noted that we used clinical stage for this comparison. Therefore we cannot exclude a possible relationship with pathological stage.

Neither did we find any correlation of PSMA immunoreactivity with levels of serum PSA (Beckett et al., 1999). In fact, serum PSMA was detectable in cases of relapse or treatment failure, while PSA was not detectable (Murphy et al., 1995). These findings suggest that the regulation of the PSMA protein is independent of PSA and that PSMA is a potential sensitive marker for detection of recurrence of prostate cancer.

In this study, we found that PSMA immunoreactivity was similar between prostatic adenocarcinoma and high-grade PIN (56.7% vs. 48.6%). Similar results have been reported previously (Bostwick et al., 1998), which suggests that these two lesions share PSMA phenotypic features. The benign-appearing epithelium, on the other hand, presented lower PSMA reactivity than the PIN and adenocarcinoma lesions, in agreement with the findings of other studies (Chang et al., 2000).

In summary, PSMA immunoreactivity is associated with a higher Gleason score suggesting that it might be a potential marker for cancer progression. Prostate cancer and high-grade PIN have similar a PSMA immunophenotype, which supports the hypothesis that high-grade PIN is a precursor of prostatic adenocarcinoma.

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