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Reduced Kv1.3 Potassium Channel Expression in Human Prostate Cancer

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Abstract. The presence of Kv1.3 voltage-gated potassium channels in rat and human prostate epithelial cells has been previously reported. We examined, by immunohistochemistry, Kv1.3 levels in 10 normal human prostate, 18 benign prostatic hyperplasia (BPH) and 147 primary human prostate cancer (Pca) specimens. We found high epithelial expression of Kv1.3 in all normal prostate, 16 BPH and 77 (52%) Pca specimens. Compared to normal, Kv1.3 levels were reduced in 1 (6%) BPH specimen and in 70 (48%) Pca specimens. We found a significant inverse correlation between Kv1.3 levels and tumor grade ($r = -0.25$, $P = 0.003$) as well as tumor stage ($r = -0.27$, $P = 0.001$). Study of an additional 30 primary Pca specimens showed that 15 (50%) had reduced Kv1.3 immunostaining compared to matched normal prostate tissue. Our data suggest that in Pca reduced Kv1.3 expression occurs frequently and may be associated with a poor outcome.

Key words: Prostate — Cancer — Potassium channel — Kv1.3 — Grade — Stage

Introduction

Voltage-gated potassium channels of the Kv1.3 subtype have been implicated in the regulation of many cellular functions, including membrane potential, solute and water transport, cell-volume, adhesion, motility, apoptosis and proliferation (Xu et al., 2003; Defarias, Stevens & Leonard, 1995; Kunzelmann, 2005; Fraser et al., 2003; Storey et al., 2003). Kv1.3 channels are known to play an essential role in the activation of T lymphocytes (Panyi, 2005). We and others have previously described the presence of Kv1.3 channels in human (Abdul & Hoosein, 2002a) and rat (Fraser et al., 2003; Ouadid-Ahidouch et al., 1999) prostate epithelial cells. Kv1.3 expression has also been reported in colon (Abdul & Hoosein, 2002b), kidney and breast (Grunnet et al., 2003; Abdul, Santo & Hoosein, 2003) epithelia. Kv1.3 channels contribute to the proliferation of normal glia and are present in human gliomas (Preussat et al., 2003).

We have previously reported strong immunostaining for Kv1.3 in normal human prostate epithelium and reduced expression in human prostate cancer (Pca) specimens compared to normal (Abdul & Hoosein, 2002a). In this study, we further examined Kv1.3 levels in normal, benign and neoplastic human prostate specimens. In addition, we examined the relationship between Kv1.3 immunostaining and patient clinicopathologic features. Our results show that reduced Kv1.3 protein levels in Pca correlate with high tumor grade and stage and may therefore indicate a poor prognosis.

Methods

Immunohistochemistry was done essentially as reported previously (Abdul & Hoosein, 2002a). The anti-Kv1.3 rabbit polyclonal antibody was purchased from Sigma-Aldrich (St. Louis, MO) and used at a dilution of 1:50. Immunostaining levels were visually scored (scale 0–5 points: 0, absent; 1, very low; 2, low; 3, moderate; 4, high; 5, very high) by evaluating the intensity and number of cells stained in the entire specimen. A tissue microarray (TMA) slide (TMA1), containing 294 Pca (147 patients in duplicate), 18 benign prostatic hyperplasia (BPH) and 10 normal prostate specimens, was obtained from the Cooperative Prostate Cancer Tissue Resource (CPCTR) and the Cooperative Human Tissue Network (CHTN) under the tissue array program (TARP) of the National Cancer Institute (NCI; Bethesda, MD). For each patient, the average of staining scores from two specimens was used. Statistical analysis was done using the software Statistica (Statsoft, Tulsa, OK).

Tumor grade (Table 1), tumor stage (Table 1), race and age were available for the 147 patients whose specimens were on the CPCTR TMA slide. Mean patient age was 64 years (range 44–79); 122 (83%) patients were Caucasian, 20 (14%) were African

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Table 1. Kv1.3 immunostaining scores in 10 normal human prostate, 18 human BPH and 147 human Pca specimens on a single slide (TMA1) provided by the CPCTR

Kv1.3 score	n[a]	0[b]	1	2	3	4	5
Normal	10	–	–	–	–	4 (40%)	6 (60%)
BPH	18	–	–	–	1 (6%)	5 (28%)	12 (67%)
Pca							
Gleason score							
5	7	–	–	–	1 (14%)	2 (29%)	4 (57%)
6	47	–	2 (4%)	9 (19%)	11 (23%)	13 (28%)	12 (26%)
7	77	1 (1%)	4 (5%)	11 (14%)	18 (23%)	31 (40%)	12 (16%)
8	11	1 (9%)	2 (18%)	3 (27%)	2 (18%)	2 (18%)	1 (9%)
9	5	–	1 (20%)	2 (40%)	2 (40%)	–	–
TNM stage							
2a	20	–	–	3 (15%)	2 (10%)	7 (35%)	8 (40%)
2b	78	–	5 (6%)	13 (17%)	16 (21%)	28 (36%)	16 (21%)
3a	25	1 (4%)	1 (4%)	6 (24%)	5 (20%)	11 (44%)	1 (4%)
3b	24	1 (4%)	3 (13%)	3 (13%)	11 (46%)	2 (8%)	4 (17%)

[a]Number of patients.
[b]Kv1.3 immunostaining level: 0, absent; 1, very low; 2, low; 3, moderate; 4, high; 5, very high.

American and 5 (3%) were designated "other" race. An additional TMA slide (TARP5), containing 49 human Pca and 39 normal human prostate specimens, was obtained from the CHTN.

Results

Kv1.3 immunostaining was observed in the epithelium of human prostate tissue (Fig. 1). Strong Kv1.3 expression (staining score 4 or 5) was seen in all 10 normal prostate (Fig. 1A–D) and 17 of 18 BPH (Fig. 1E–H) specimens in the CPCTR TMA slide (Table 1). Also, all 39 normal human prostate specimens in the TARP5 TMA (Fig. 2, bottom right) displayed strong Kv1.3 immunostaining (Fig. 1R, T).

Kv1.3 immunostaining levels in Pca specimens (Fig. 1I–P, Q, S) varied from very high to absent (Fig. 2, top). In the 147 Pca specimens on CPCTR TMA1 (Table 1), there was a significant inverse correlation between Kv1.3 levels and tumor grade (Spearman $r = -0.25$, $P = 0.003$) as well as tumor stage (Spearman $r = -0.27$, $P = 0.001$). In this series, there was a positive correlation between tumor grade and stage (Spearman $r = 0.40$, $P < 0.0001$). There was no correlation between Kv1.3 levels and patient race or age (Fig. 2, bottom left).

The frequency of decrease in Kv1.3 immunostaining (less than level 4) was 47% in CPCTR and 46% in the TARP5 TMA specimens (Fig. 2, top). In the TARP5 TMA, for 30 Pca specimens matched normal prostate tissue was provided. In 15 of the 30 Pca specimens (50%), Kv1.3 staining was lower compared to matched normal tissue (Fig. 1Q Pca *vs.* R normal prostate and S Pca *vs.* T normal prostate).

Discussion

Ion-channel expression and activity has been studied extensively in cancer-derived cell lines (Kunzelmann, 2005). However, in order to establish clinical relevance, there is a need for investigations of ion-channel expression in cancer tissue from patients (Kunzelmann, 2005). Our data show that expression of Kv1.3 channels is high in normal prostate as well as BPH specimens and reduced with a frequency of about 50% in Pca.

New biomarkers of Pca progression are needed to predict the course of the disease, to avoid unnecessary treatment and to develop more effective treatment strategies (Tricoli, Schoenfeldt & Conley, 2004). For patients with localized Pca, using markers currently available, it is often difficult to differentiate those who need aggressive treatment from those who do not (Tricoli et al., 2004). A significant inverse correlation was found between the level of Kv1.3 immunostaining and tumor grade as well as tumor stage. Therefore, reduced levels of Kv1.3 protein in patient prostate biopsies or resected tissue, compared to adjacent normal tissue, may be useful in both disease detection and outcome prediction. Further clinical studies are needed to determine if the Kv1.3 protein level in prostate tissue is a useful diagnostic or prognostic indicator.

A decrease in Kv1.3 expression in Pca suggests that Kv1.3 may act as a tumor suppressor. To explore this notion, protein and mRNA levels of Kv1.3 need to be determined in immortalized normal prostate and Pca cell lines. A possible tumor-suppressor role of Kv1.3 could then be examined by silencing the Kv1.3 gene in prostate cell lines that express Kv1.3. This could also be investigated by transfection-

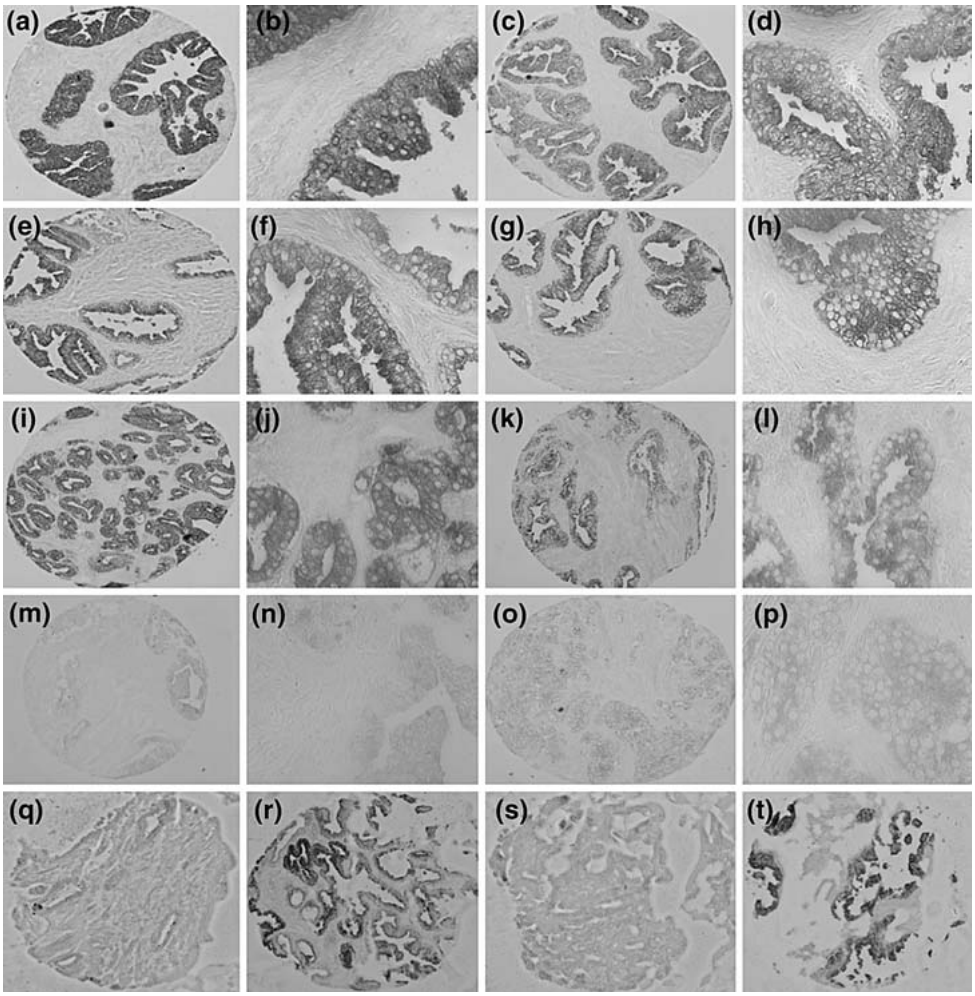

Fig. 1. Kv1.3 immunostaining in human prostate specimens: two normal (*A*/*B* and *C*/*D*), two BPH (*E*/*F* and *G*/*H*), Pca with Gleason grade 5 (*I*/*J*), Pca with Gleason grade 6 (*K*/*L*), Pca with Gleason grade 8 (*M*/*N*) and Pca with Gleason grade 9 (*O*/*P*). Pca (*Q, S*) with matched normal prostate (*R, T*) specimens from two patients (patient 1, *Q*/*R;* patient 2 *S*/*T*). Brownish black product of the horseradish peroxidase substrate 3,3′-diaminobenzidine was seen (no counterstain). Magnification x10 in *A, C, E, G, I, K, M, O, Q-T;* x40 in *B, D, F, H, J, L, N, P.*

mediated overexpression of Kv1.3 in prostate cell lines with low Kv1.3 levels.

Interestingly, hypoxia is known to downregulate Kv1.3 activity in freshly isolated human T lymphocytes as well as in leukemic Jurkat T cells, and this is accompanied by a decrease in Kv1.3 protein levels in Jurkat T cells (Conforti et al., 2003). Hypoxia occurs commonly in locally advanced, solid tumors and has been associated with malignant progression (Vaupel, 2004). Our results warrant further investigation of Kv1.3 downregulation in Pca.

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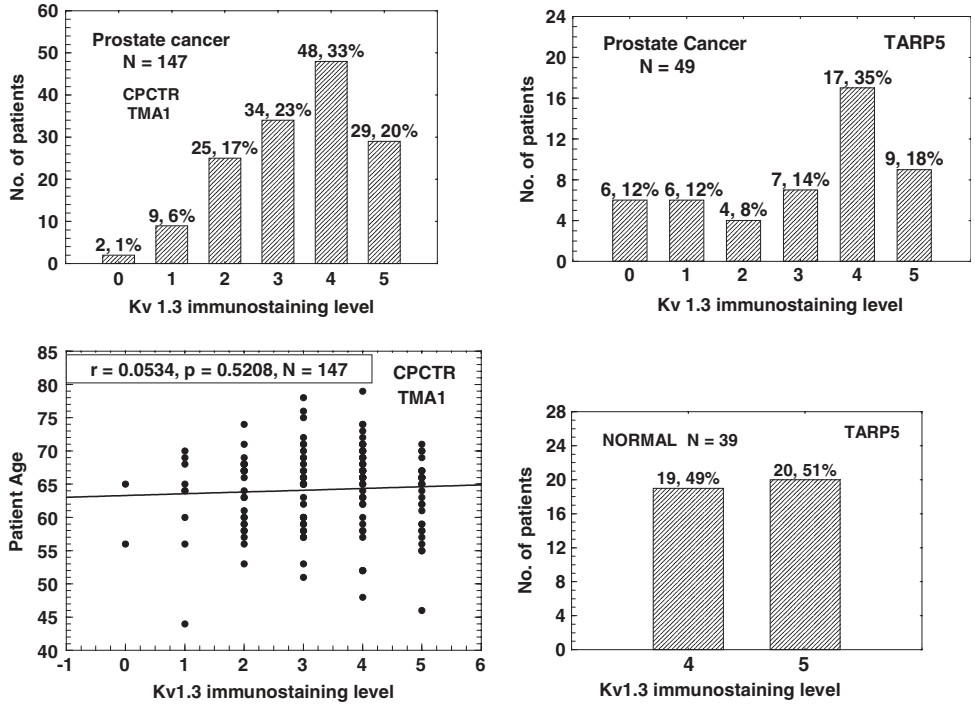

Fig. 2. Kv1.3 immunostaining levels in human Pca specimens (*top two figures*) and 39 normal human prostate specimens on the TARP5 TMA slide (*bottom right*). Relationship between Kv1.3 immunostaining and patient age (*bottom left*).

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