Malignancy Grade–Dependent Expression of $K^+$-Channel Subtypes in Human Prostate Cancer

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Abstract. $K^+$ channels are key molecules in the progression of several cancer types and considered to be potential targets for cancer therapy. We examined the gene expressions of voltage-gated ($K_v$), $Ca^{2+}$-activated ($K_{Ca}$), and two-pore domain ($K_{2P}$) $K^+$-channel subtypes in needle-biopsy samples of human prostate cancer (PCa) by real-time PCR and compared them with those in PCa epithelial cell lines. The expression of $K_{v1.3}$, $K_{Ca1.1}$, $K_{Ca3.1}$, and $K_{2P1}$ markedly increased in the PCa group with Gleason score of 5–6 (GS5–6) but significantly decreased in the GS8–9 group. This malignancy grade–dependent $K^+$-channel expression pattern may provide a convenient marker to understand PCa progression level.

Keywords: prostate cancer, $K^+$ channel, tumor marker

Clinical diagnosis of prostate cancer (PCa) is usually confirmed by histopathological examination of prostate needle-biopsy among positive cases of blood tests such as PSA (prostate-specific antigen) test. The Gleason score (GS) is the most widely available system for discrimination of malignancy grade in PCa, and patients with GS over 7 have significant risks of death (1, 2).

In non-excitable cells including cancer cells, membrane hyperpolarization by increase in $K^+$-channel activities causes an increase in intracellular $Ca^{2+}$ by providing the driving force for $Ca^{2+}$ entry (3, 4). $K^+$ channels are classified into five superfamilies: voltage-gated, $K_v$; $Ca^{2+}$-activated, $K_{Ca}$; inward-rectifying, $K_{ir}$; ATP-sensitive, $K_{ATP}$; two-pore domain, $K_{2P}$. During tumor development, a series of genetic alternations affects $K^+$-channel expression and the activation of these channels controls cell proliferation and apoptosis in tumor cells via different signaling pathways (5–7). Therefore, $K^+$ channels have emerged as potential molecular markers and putative therapeutic targets in several types of cancers (8).

Due to unrestricted accessibility and convenience of experimentation, most studies on $K^+$ channels in PCa have been conducted on PCa epithelial cell lines such as LNCaP and PC-3. The data from native human PCa tissues are therefore much sparser and supply valuable information. In the present study, we found a novel malignant grade-dependent expression pattern of three distinct subfamilies of $K^+$-channel genes using needle-biopsy samples of PCa by real-time PCR and may provide a potential tool to quickly detect the malignant grade of PCa.

All tissue specimens were acquired in conformity with the protocols approved by ethics committee at Nagoya City University. Informed consent was obtained from all patients before the study with explanations of the purpose and methods. The age of the patients was $71.5 \pm 2.3$ years (age range: 66 – 82 years, n = 30). Most needle-biopsy samples are obtained from patients in grade GS5–7 to determine whether there was malignancy or not.

Total RNAs from human prostate biopsy samples and PCa epithelial cell lines (Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University, Sendai) were reverse-transcribed, and real-time PCR analysis was performed...
with the use of Syber Green chemistry on an ABI 7000 sequence detector (Applied Biosystems, Foster City, CA, USA) (9).

The following PCR primers were used: Kv1.3 (GenBank accession No. XM_084080, 1411–1517), amplicon = 107 bp; Kv4.3 (NM_004980, 1707–1807), amplicon = 101 bp; Kv3.1/BKα (XM_039587, 1116–1225), amplicon = 110 bp; Kv2.3/SK4 (NM_002250, 172–293), amplicon = 122 bp; Kv3.1/TWIK1 (NM_002245, 948–1048), amplicon = 101 bp; Kv3.3/TASK1 (NM_002246, 1739–1864), amplicon = 126 bp; β-actin (NM_001101, 411–511), amplicon = 101 bp.

Statistical significance between two groups or among multiple groups was evaluated using Student’s t-test, Welch’s t-test, or Tukey’s test after the F test or ANOVA. Data were presented as means ± S.E.M. Chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Wako Pure Chemical Industries (Osaka).

A total of 30 prostate biopsies were contributed to real-time PCR analysis: 12 unique samples of benign prostate glands (‘nonmalignant’) and 18 unique samples of primary PCa. The mean serum PSA was 5.9 ± 0.2 and 12.8 ± 1.6 ng/ml in nonmalignant and PCa samples, respectively (P<0.01).

We first examined the expression levels of three distinct K+ channel superfamilies (29 subtypes of Kv, 5 subtypes of KCa, 18 subtypes of Kv) in PCa needle-biopsy samples with GS7 using non-quantitative RT-PCR analysis (35 cycles). Six subtypes (Kv1.3, Kv4.3, KCa1.1, KCa3.1, Kv1.1, and Kv3.3) were abundantly expressed (not shown).

We then examined that the expression levels of Kv1.3, Kv4.3, KCa1.1, KCa3.1, Kv1.1, and Kv3.3 transcripts using quantitative, real-time PCR analysis (Fig. 1). In the nonmalignant group, the relative expression levels of Kv1.3, Kv4.3, KCa1.1, KCa3.1, Kv1.1, and Kv3.3 to β-actin were 0.020 ± 0.003, 0.006 ± 0.001, 0.007 ± 0.001, 0.004 ± 0.001, 0.006 ± 0.002, and 0.030 ± 0.010, respectively (Fig. 1). On the other hand, in PCa samples with GS5–9, the relative expression levels were 0.054 ± 0.007, 0.010 ± 0.002, 0.010 ± 0.002, 0.015 ± 0.003, 0.040 ± 0.004, and 0.078 ± 0.018, respectively (Fig. 1). The threshold cycle (Ct) values at 0.25 were 18.2 ± 0.2 and 18.3 ± 0.1 in nonmalignant and PCa groups, respectively, and no significant difference in β-actin expression was detected. These results showed that expressions of Kv1.3, KCa3.1, Kv1.1, and Kv3.3 transcripts in the PCa group were significantly increased compared with those in the nonmalignant one.

We further analyzed the grade-dependency of their expressions in the PCa groups with GS 5–6, GS 7, and GS8–9, using the data shown in Fig. 1. Of interest, expressions of both KCa1.1 and KCa3.1 in the PCa groups with GS7 and GS8–9 were markedly decreased compared with those in the GS5–6 group. The expression levels of KCa1.1 were 0.021 ± 0.004 (n = 6), 0.004 ± 0.001 (n = 8), and 0.003 ± 0.001 (n = 4) in the PCa groups with GS5–6, GS7, and GS8–9, respectively (Fig. 2C). The expression levels of KCa3.1 were 0.030 ± 0.004, 0.009 ± 0.002, and 0.004 ± 0.001, respectively (Fig. 2D). On the other hand, the expression levels of Kv1.3 and Kv1.1 transcripts showed no significant difference between the PCa
groups with GS5–6 and GS7, respectively. However, significant decreases were detected in the GS8–9 group (Kv1.3: 0.063 ± 0.006, 0.071 ± 0.004, and 0.006 ± 0.001, Kv4.3: 0.051 ± 0.007, 0.044 ± 0.004, and 0.017 ± 0.003, respectively) (Fig. 2: A and E). The changes in Kv4.3 and Kv3.1 were not significant among the three PCa groups, respectively (Fig. 2: B and F).

We showed here the systematic analyses of malignancy grade–dependent K+ channel expression in human PCa and first revealed novel biphasic changes in expression of key K+ channel transcripts (Kv1.3, Kv4.3, Kca1.1, and Kca3.1); there was a marked increase in the lower GS group (GS5–6) and a significant decrease in the higher GS group (GS8–9). The decreases in Kca1.1 and Kca3.1 were obvious in middle GS (GS7), whereas those of Kv1.3 and Kv3.1 were observed not in GS7 but in GS8–9. Abdul and Hosein have reported the ‘inverse’ correlation between K1.3 levels and tumor grade in human PCAs by immunohistochemistry (10). In non-excitatory cells such as T-lymphocytes and cancer cells, hyperpolarization of the membrane potential induced by activation of K+ channel increases the driving force for the Ca2+ entry (5, 7). K1.3 channel blockers have been developed as potential drugs for autoimmune diseases (11). In analogy, selective K1.3-channel blockers could be useful for chemotherapy of malignant PCa in a lower grade.

Kv2p1 channels contribute to maintaining the resting membrane potentials and are responsible for both cell apoptosis and tumorigenesis (12). Similar to the case of Kv1.3, the expression of Kv2p1 transcripts also changed in a biphasic manner with the progression grade. Recent reports have shown that Kv1.1 is expressed in a stage-specific manner in ovarian cancer with lowest expression in advanced tumor stages and is down-regulated with loss of the TA73 activity when TA73 is switched to oncogenic ΔN73, which is positively correlated with the GS in human PCa (13, 14).

Bloch et al. reported that gene amplification of Kca1.1, which is observed in a part of ‘late-stage’ PCa, is associated with overexpression of Kca1.1 transcripts and proteins (15). We first showed here the significant up-regulation of both Kca1.1 and Kca3.1 transcripts in PCa groups with GS5–6 and marked decrease in both in PCa groups with over GS7. These observations suggest that the detection of ‘inverse’ correlation of the expression between a set of Kca1.1 and Kca3.1 and another set of Kv1.3 and Kv3.1 may provide rapid and proper decision of GS7 with significant risks of death. The high expressions of all key K+ channels strongly suggest that the sample includes lower stage (GS5–6) PCa. All these findings are surely important in clinical settings because most needle-biopsy samples are obtained from patients in grade GS5–7 to determine whether there is malignancy or not.

Additional but notable information obtained in this study is that the expression profiles of key K+ channel transcripts in androgen-dependent and -independent PCa epithelial cell lines (LNCaP and PC-3) were not completely consistent with the patterns in native PCa samples with GS5–6 and GS7. Although the screening of chemicals for anti-PCa therapy is often carried out by use of these cell lines, the efficacy of modulators targeting these K+ channels to prevent cancer cell proliferation may not be adequately assayed by use of these cell lines. It is likely that the expression profiles in
these PCa cell lines correspond to that of PCa samples with GS8–9 since the malignancy is highly conserved in these cell lines. An alternative explanation with much lower possibility may be that the needle-biopsy sample included many cell types, among which the high expression of key K⁺ channels occurred in non-epithelial cells. This possibility, however, remains to be determined.

In conclusion, our findings suggest that quantification of specific K⁺-channel genes by real-time PCR is substantially useful for rapid and simple detection of malignancy grade during PCa progression, providing additional values to the established indicators of Gleason grade. In addition, the present study gives new insight into the roles of K⁺ channels during PCa progression and target molecules that may assist in the discovery of novel drugs for PCa treatment.

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