Genetic Alterations in the PI3K Pathway in Prostate Cancer

XIUJU SUN1, JIAN HUANG1, TAKU HOMMA1, DAISUKE KITA1, HELMUT KLOCKER2, GEORG SCHAER3, PETER BOYLE1 and HIROKO OHGAKI1

1International Agency for Research on Cancer, Lyon, France; 2Urology and 3Pathology Departments, Innsbruck Medical University, Austria

Abstract. Alterations in the PIK3CA and PTEN genes were assessed in 40 prostate tumors (radical prostatectomy samples). Genetic analyses in glands of the highest Gleason pattern within each tumor revealed PIK3CA amplification in 13%, PIK3CA mutations in 3%, PTEN homozygous deletion in 13% and PTEN hemizygous deletion in 8% of the cases analyzed. Supporting the view that PTEN and PIK3CA act in the same PI3K signaling pathway, genetic alterations in the PIK3CA and PTEN genes were mutually exclusive, except in one tumor. Overall, 13 of the 40 (33%) prostate tumors had alterations in the PI3K pathway. For cases with genetic alterations, other tumor areas with lower Gleason patterns as well as non-tumorous prostate glands were also analyzed. Of nine tumors with Gleason score 7, five cases contained the same genetic alterations in tumor areas of Gleason patterns 3 and 4, whereas in another four cases, genetic alterations were detected only in tumor areas of Gleason 4 but not Gleason 3 patterns. There were no alterations in non-tumorous glands. These results suggest that genetic alterations in the PI3K pathway are common in prostate cancer, and occur mainly through PIK3CA amplification and PTEN hemizygous or homozygous deletion. Glands of Gleason pattern 3 are genetically heterogeneous, some containing the same genetic alterations observed in glands of Gleason pattern 4.

Prostate cancer is one of the most common malignances among men in developed countries, and the second highest cause of cancer death in males (1, 2). However, the molecular changes underlying its development have not been fully elucidated.

The signaling pathway involving PTEN, PI3K and AKT plays a significant role in the regulation of cell growth and death. Activation of growth factor receptors such as EGFR results in recruitment to the cell membrane of PI3K (phosphatidylinositol 3-kinase), which phosphorylates PIP2 (phosphatidylinositol-4,5-bisphosphate) to PIP3 (phosphatidylinositol-3,4,5-triphosphate). PIP3 activates downstream effector molecules such as AKT, leading to cell proliferation and blocking apoptosis (3, 4). PTEN inhibits PIP3 signaling, inhibiting cell proliferation (5). A variety of human neoplasms show gain of function of the PIK3CA gene, that encodes the p110α catalytic subunit of PI3K, and loss of PTEN function (3, 6), and therefore potential strategies for developing therapies targeted to this signalling pathway have emerged (3, 4).

Many studies have shown alterations in the PTEN gene in prostate cancer, but frequencies vary significantly across different studies. PTEN mutations have been reported in 0-15% of locally confined prostate cancers and 20-30% of their metastases (7-12). LOH 10q, in particular LOH at the PTEN locus (10q23), has been observed in 22-60% of prostate cancer (13-19). Homozygous or hemizygous PTEN deletion has been reported in 0-26% of locally confined cancers and metastases (8, 9, 15, 20-23). Both homozygous and hemizygous PTEN deletions were associated with significantly shorter survival of prostate cancer patients (24).

In contrast, there is less information on PIK3CA alterations in prostate cancer. One study reported absence of PIK3CA mutations in 12 cases of prostate cancer (25), while another, using array CGH, found that 39% of hormone-sensitive tumors and 50% hormone-independent tumors showed PIK3CA gene amplification (26). There have been no studies in which both PTEN and PIK3CA genes were analyzed in the same prostate cancer.

In the present study, prostate tumors were screened for PIK3CA alterations (mutations and amplification) and PTEN alterations (mutations, hemizygous and homozygous deletion), to assess alterations in the PI3K pathway in prostate cancer.

Patients and Methods

Prostate tumor samples. Forty samples from patients who underwent radical prostatectomy were obtained from the Pathology Department, Innsbruck Medical University, Austria. Whenever possible, cases in
which tumor areas of different Gleason patterns, in particular Gleason patterns 3, 4 and 5 were clearly separately recognizable were chosen. Tumor areas with glands of different Gleason patterns were marked on formalin-fixed paraffin-embedded sections and were manually microdissected, and DNA was extracted as previously described (27). The mean age of patients with prostate cancer was 62.1 years (range, 49-74 years). Genetic analyses were first carried out on DNA samples extracted from the tumor areas containing glands of the highest Gleason grade within the tumor, and for cases with positive results, further genetic analyses were performed in tumor areas with lower Gleason grades and as well as non-tumorous prostate glands.

PTEN homozygous deletion. PTEN homozygous deletion was assessed by differential PCR using primers for PTEN exon 2 (sense, 5'-TTT CAG ATA TTT TTT TTC TTA-3'; antisense, 5'-TGA AAT AGA AAA TCA AAG CAT-3'), together with primers for the GAPDH sequence (sense, 5'-AAC GTG TCA GTG GTG GAC CTG-3'; antisense, 5'-AGT GGG TGT CCG TGA AGT-3'). Differential PCR was performed in a total volume of 10 μL, consisting of 6 μL of DNA solution (75 ng/μL), 1 U of Taq DNA polymerase (Invitrogen), 1.5 mM MgCl₂, 0.25 mM of each dNTP, 1 μM of each primer, 0.1 μM of each GAPDH primer, 1 μL 10× buffer in the T3 thermocycler (Biometra), with an initial denaturing step at 95°C for 3 min, followed by 32 cycles of denaturation at 95°C for 1 min, annealing at 51°C for 1 min, polymerization at 72°C for 1 min and a final extension at 72°C for 5 min. PCR products were separated on an 8% acrylamide gel and ethidium bromide-stained bands were recorded by Kodak Digital Science ID Image software. Quantitative analysis of the bands for the PTEN gene and reference gene (GAPDH) was performed using image quantification software. The target gene dosage was calculated relative to normal DNA. A PTEN/GAPDH ratio of <0.3, relative to that of the average calculated in normal controls (formalin-fixed, paraffin-embedded sections from normal tissues) were regarded as evidence for homozygous deletion, as previously described (30).

Results

Genetic analyses in glands of the highest Gleason pattern within the tumor revealed PIK3CA amplification (copy numbers between 3 and 4.95) in 13% of cases, PIK3CA
mutations in one (3%) case (GAG->GCG at codon 545; Glu->Ala), *PTEN* homozygous deletion in 13% of cases, and *PTEN* hemizygous deletion in 8% of cases analyzed (Figures 1 and 2). These alterations were largely mutually exclusive except for one case. No tumor contained a *PTEN* mutation. Overall, 13 out of 40 (33%) prostate tumors showed at least one alteration in the PI3K pathway.

For cases with genetic alterations, other tumor areas with lower Gleason patterns as well as non-tumorous prostate glands were further analyzed. Of nine Gleason score 7 cases with genetic alterations, five cases contained the same genetic alterations in both tumor areas with glands of Gleason 3 and 4 patterns, while four cases contained genetic alterations in only tumor areas with glands of Gleason 4 pattern (Table I). Of three Gleason score 9 cases with genetic alterations, two cases contained the same genetic alterations in both tumor areas with glands of Gleason 4 and 5 patterns, while one case contained genetic alterations in only tumor areas with glands of Gleason 5 pattern (Table I). None of the non-tumorous prostate glands contained genetic alterations in the PI3K pathway.

**Discussion**

The present study shows that alterations in the PI3K signalling pathway are common in prostate cancer, and occur mainly through *PIK3CA* amplification and *PTEN* deletion. Supporting the view that *PTEN* and *PIK3CA* genes act in the same signaling pathway, genetic alterations of these genes in prostate cancer were largely mutually exclusive. A similar reciprocal association between *PTEN* hemizygous deletion and *PIK3CA* amplification in gastric cancer has been reported (32).

Table I. Genetic alterations in the PI3K pathway in prostate cancer.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gleason score</th>
<th>PIK3CA amplification</th>
<th>PIK3CA mutation</th>
<th>PTEN homozygous deletion</th>
<th>PTEN hemizygous deletion</th>
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<td>-</td>
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<td>G5, G4, N</td>
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</table>

G3, Gleason pattern 3; G4, Gleason pattern 4; G5, Gleason pattern 5. N, non-tumorous prostate glands. Numbers in bold letters indicate that alterations were present in tumor areas with the respective Gleason pattern. *Missense mutation at codon 545 of the *PIK3CA* gene (GAG->GCG; Glu->Ala).

It was noted in the present study that in all the prostate tumor samples with *PIK3CA* amplification, the level of amplification was relatively low (gene copy numbers of 3-4.95). Low-level amplification of *PIK3CA* (copy numbers 3-4) has also been reported to be common in other neoplasms. The majority (15/16) of primary cervical tumors had *PIK3CA* amplification with copy numbers >2.5 (33). Another study showed that 18 of 28 cervical cancers with *PIK3CA* amplification had copy numbers <4 (34). Even a low level of *PIK3CA* amplification may have significant functional consequences: cervical cancer cells with *PIK3CA* copy numbers 2.5-3.7 had increased p110α expression and kinase activity of PI3K, subsequently affecting aberrant cell proliferation and apoptosis (33); gastric cancer cells showing *PIK3CA* amplification (<5-fold) were associated with elevated levels of phospho-AKT (32).

Consistently with previous reports (8, 9, 15, 19-23), the present study showed that hemizygous or homozygous *PTEN*...
deletions are common in prostate cancer. Both hemizygous and homozygous PTEN deletions appear to have significant biological consequences. PTEN haploinsufficiency (hemizygous deletion) significantly promoted progression of prostate cancer in TRAP mice (35), and accelerated the formation of high-grade astrocytomas in mice lacking Nf1 and p53 (36). Yoshimoto et al. (24) reported that both homozygous and hemizygous PTEN deletions were significant prognostic markers of poor clinical outcome in prostate cancer patients.

The Gleason scoring system based on glandular differentiation is widely used for prostate cancer diagnosis, with Gleason patterns 3 and 4 being most common. Gleason pattern 3 is characterized by glands which are infiltrative between adjacent non-neoplastic glands, but each gland has an open lumen and is circumscribed by stroma. In contrast, the glands of Gleason pattern 4 appear to be fused or cribriform, and are composed of a group of glands that are no longer completely separated by stroma. Within the same tumor, separate areas with glands of Gleason 3 and 4 patterns may be observed, or glands of Gleason 3 and 4 patterns may be co-present in the same tumor area. Glands of Gleason 4 pattern may have evolved from neoplastic cells of Gleason pattern 3, or glands of Gleason 3 and 4 patterns may develop from independent cancer clones.

It is currently not clear whether differentiation status represented by different Gleason patterns reflects different genetic alterations. Using array CGH, Postma et al. (37) assessed tumor areas of Gleason patterns 3 and 4, and showed that there were no significant differences in genome-wide chromosomal imbalance between Gleason patterns 3 and 4, or between Gleason grades within one cancer. In the present study, carefully selected prostate cancer samples in which separated tumor areas of different Gleason patterns were recognized were used to show that glands of Gleason 3 pattern are genetically heterogeneous, with some but not all showing the same genetic alterations observed in those of Gleason 4 pattern within the same tumor.

Acknowledgements

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References


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