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ORIGINAL ARTICLE

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Infrequent genetic alterations of the *PTEN** gene in Japanese patients with sporadic prostate cancer

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Abstract Prostate cancer is a major cause of cancer death among elderly men in America, Europe, and Japan. However, the molecular mechanism of carcinogenesis is not yet well characterized. Frequent loss of heterozygosity (LOH) on chromosome 10q was reported in prostate cancer, and a candidate tumor suppressor gene, *PTEN*, was isolated on chromosome band 10q23.3. To investigate the genetic alterations of *PTEN*, we examined 45 primary prostate cancer specimens. LOH at the *PTEN* locus was observed in two (11.1%) of 18 tumors. However, no mutations were observed in any of the primary prostate cancers. These data suggest that mutation of the *PTEN* gene does not play a major role in prostate carcinogenesis of Japanese patients.

Key words *PTEN* gene · Prostate cancer · Loss of heterozygosity (LOH) · Tumor suppressor gene · Chromosome 10q

Introduction

Prostate cancer is the second leading cause of cancer deaths in males in the United States and the eighth in Japan. Despite the high incidence and mortality, molecular mechanisms underlying tumorigenesis and progression of prostate cancer are still open questions. Cytogenetic and loss of heterozygosity (LOH) studies have pointed out that a number of chromosomal regions, including 10q, are frequently lost in prostate cancer (Carter et al. 1990). One candidate gene, *MXI1*, which encodes a negative regulator of the Myc oncoprotein, was mapped to chromosome bands 10q24–q25. However, the incidence of mutation of *MXI1* in prostate cancer was not high (Eagle et al. 1995). More recently, *PTEN*, a candidate tumor suppressor gene, was isolated on chromosome 10q23.3 (Li et al. 1997; Steck et al. 1997; Li and Sun 1997), a region of frequent allelic loss in prostate cancer. Mutational analysis of this gene revealed that it was mutated in prostate cancers (Li et al. 1997; Steck et al. 1997). However, the incidence of mutation was much higher in endometrial cancers (Kong et al. 1997; Tashiro et al. 1997; Risinger et al. 1997) and it is not clear whether or not mutations of *PTEN* are deeply involved in prostate carcinogenesis. Hence, we analyzed this gene in primary prostate cancer.

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Materials and methods

A total of 45 paired specimens of primary prostate cancers and corresponding normal tissues from Japanese patients at Tohoku University Hospital (Sendai, Japan) and Yokohama City University Hospital (Yokohama, Japan) were analyzed. Twenty-seven specimens were snap-frozen in liquid nitrogen immediately after surgical resection and stored at −80°C until DNA extraction. Eighteen samples, fixed in formalin and embedded in paraffin, were retrieved from the surgical pathology file of the Division of Pathology, Tohoku University Hospital. Tumors were classified according to the Whitmore-Jewette system in clinical stages (Prout 1973). In terms of histologic subtypes or stages, there was no bias between samples of frozen tissue and those from paraffin blocks. Details of patients are summarized in Table 1.

Results

First, we analyzed LOHs in 19 primary prostate cancers in which relatively large amounts of DNA were available. LOHs were studied utilizing the 5-bp insertion/deletion

polymorphism in intron 4 of the *PTEN* gene (Sakurada et al. 1997) as well as microsatellite markers D10S2491 and D10S2492 at the *PTEN* locus (Cairns et al. 1997). Examples of the LOH studies are shown in Figure 1A. Two (11.1%) of 18 informative cases showed LOHs at one or more loci; similar to those by Cairns et al. (1997) in primary prostate cancers (11/60, 18.3%). We further studied allelic loss by fluorescence *in situ* hybridization (FISH) in 12 tumors in which fresh materials were available. Two (16.7%) of 12 tumors showed allelic loss; none of the tumors exhibited homozygous deletion. A typical example is shown in Figure 1B. Two centromeric signals were clearly seen, but only one signal for the *PTEN* gene (bac554d23) was observed in Case 20. There were no discrepancies between microsatellite and FISH analyses.

Next we analyzed mutations of *PTEN* in DNA from 27 primary prostate cancers in which the frozen tissues were available. Sequencing analyses were performed by Sequenase PCR Product Sequencing Kit (Amersham, Little Chalfont, UK) according to the manufacturer's protocol. Nucleotide sequences of the primers were described previously (Sakurada et al. 1997). We sequenced the entire coding regions as well as their surrounding introns and 194-bp of the 5′-noncoding region and 36-bp of the 3′-noncoding region, but no mutations were observed. In 18 tumors from the paraffin blocks, the amounts of DNA were not large, and PCR amplifications of 120-bp or more were not easy. In these tumors, we could only analyze mutational hot spots in exons 7 and 8 (Kong et al. 1997); no mutations were observed.

Table 1 Summary of tumors examined

Samples	
Formalin-fixed	18
Frozen	27
Average age (range)	67 (48–82) years
Grade	
Well-differentiated	11
Moderately differentiated	19
Poorly differentiated	15
Clinical stage	
A	0
B	23
C	9
D	13
Metastasis[a]	
Lymph node	14
Bone	4
Liver	1

[a] Four patients had metastases in multiple organs

Discussion

Cairns et al. (1997) reported that 18% (11/60) of primary prostate tumors and 60% (12/20) of pelvic nodal metastases had allelic losses at the *PTEN* locus. Suzuki et al. (1998) also reported mutations of *PTEN* in 21% (4/19) of prostate cancer patients, all of them with metastases. Based on these observations, mutations of *PTEN* are probably a rather late event, as Steck et al. (1997) initially suggested. In this study, however, no genetic alterations were found in the *PTEN* gene in Japanese patients, although the incidence of allelic loss was almost equivalent to that in other reports of

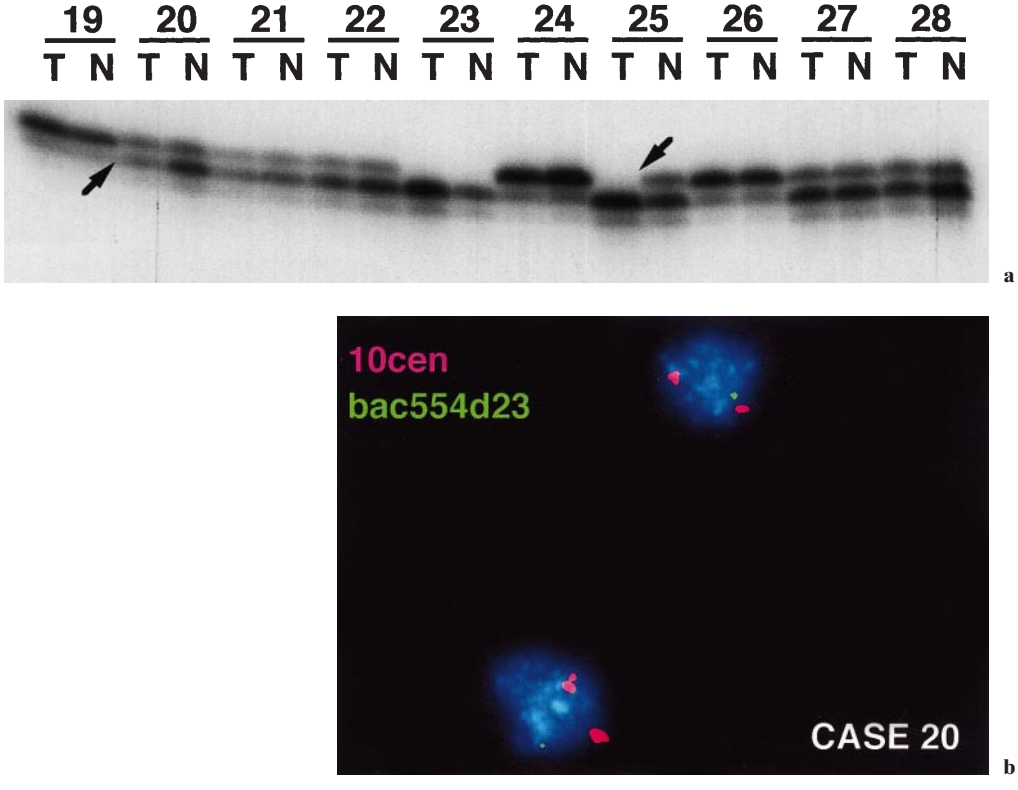

Fig. 1 **a** Microsatellite analysis at D10S2492. LOH at D10S2492 is clearly seen in tumor Cases 20 and 25 as indicated by *arrows*. *T* and *N* denote DNA samples from tumor and corresponding normal tissues. **b** FISH analysis of the *PTEN* gene in case 20. Tumor sample from Case 20 was hybridized with bac554d23, a *PTEN* BAC probe (*green*), and a 10cen probe pA10RR8 (*red*). Nuclei were counterstained with DAPI (*blue*). Two centromeric signals are clearly seen, but only one *PTEN* signal

American patients. There are several possible explanations: (a) the molecular mechanism underlying tumorigenesis and progression of prostate cancers differs between Japanese and American patients, (b) mutations of the *PTEN* gene play a major role in the late stage of prostate carcinogenesis, especially in the metastatic disease, and (c) inactivation of *PTEN* is important, and another mechanism such as methylation or imprinting may be involved in inactivation of this gene. Further studies are necessary to understand human prostate cancer.

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* Nomenclature comittee decided the gene symbol as *PTEN*. We would like to follow the decision in this article.

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