Genetic mapping of allelic loss on chromosome 6q within heterogeneous prostate carcinoma

Noboru Konishi,[1] Mitsutoshi Nakamura,[1] Munehiro Kishi,[1] Eiwa Ishida,[1] Keiji Shimada,[1] Syuichi Matsuyoshi,[1] Hisaki Nagai[2] and Mitsuru Emi[2]

[1]Department of Pathology, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8521; and [2]Department of Molecular Biology, Institute of Gerontology, Nippon Medical School, 1-396 Kosugi-cho, Nakahara-ku, Kawasaki, Kanagawa 211-8533

(Received May 29, 2003/Revised July 7, 2003/Accepted July 7, 2003)

A number of genetic events have been reported in prostate carcinogenesis, including frequent loss of heterozygosity (LOH) on chromosomes 8q, 10q, 16q and 18q. In samples of heterogeneous, multifocal prostate carcinomas, we focused on chromosome 6q using PCR-based techniques with 15 microsatellite markers to identify the specific 6q deletion within tumors. LOH of one or more polymorphic markers was detected in 10 of 21 tumors (48%). Two of these 10 tumors demonstrated LOH in all cancerous foci at specific loci and 4 tumors showed deletion in one focus. Different deletion patterns were found in 3 tumors when different polymorphic markers were used. In 90% of tumors showing LOH in one or more foci, however, two common regions of LOH were identified; one at 1.81 cM on 6q15-16.3 between markers D6S1631 and D6S1056, and the other at 5.11 cM on 6q16-21 between markers D6S424 and D6S283. By RT-PCR analysis, the *TAK1* gene located at these loci did not correlate with LOH status, indicating that *TAK1* is not a target gene in prostate carcinoma. The 6q deletion occurs heterogeneously and LOH was more frequent in tumors of higher pathological stages, implying that this alteration is a late event in prostate carcinogenesis. Because prostate carcinomas are genetically multicentric and of multifocal origin, it remains unclear whether the foci containing 6q deletions specifically expand within tumors or to what extent they contribute to the histological heterogeneity characteristic of the disease. (Cancer Sci 2003; 94: 764–768)

Cytogenetic studies of prostate carcinomas indicate that various chromosomal deletions containing tumor suppressor genes contribute to the development and progression of tumors. Recent intensive investigations using PCR of polymorphic microsatellite markers, fluorescent *in situ* hybridization (FISH), and comparative genomic hybridization (CGH) have shown consistent genetic alterations on chromosomes 2q, 3q, 5q, 6q, 7q, 8q, 9q, 10q, 13q, 16q and 18q.[1,2] Although malignant tumors are now believed to develop through a multi-step process involving both oncogene activation and tumor suppressor gene (TSG) inactivation,[3] putative TSG are postulated to be the primary targets of these carcinogenesis-associated events.

Allelic losses on 6q have been described in a number of cancers including melanoma and breast, ovarian, and renal cancers.[4–9] Genes associated with prostate carcinoma in the 6q region have also been reported and, while the incidences varied, 6q alterations have been found to occur in up to 48% of prostate cancers.[1,2,10,11] However, the role of these chromosomal aberrations remains unclear. At least two common regions of 6q deletion have been identified in prostate carcinoma. Using 13 polymorphic markers, Srikantan *et al*.[2] reported that 6q16, 6q3-21, and the distal region 6q23-24 were deleted. Hyytinen *et al*.[11] examined two regions, 6q16-21 and 6q22, and claimed that deletion of 6q16-22 is a frequent event in prostate cancer. They further showed that loss of 6q24 was associated with androgen-independence and tumorigenicity.[12] Several candidate genes, such as the *insulin-like growth factor II receptor* (*IGF2R*) at 6q26 or *cyclin C* (*CCNC*) at 6q21, may participate in prostate carcinogenesis.[1] A novel gene located on chromosome 6q23-24 and designated *UROC28* was recently identified as overexpressed in prostate, breast, and bladder cancers,[13] while in breast and cervical cancers, the 6q23-24 region was shown to be deleted.[14,15]

Carcinoma of the prostate is a histologically heterogeneous lesion containing a number of both abnormal and frankly cancerous foci. We have previously suggested that multicentric genetic events, comprised of both known and unknown genetic and epigenetic changes, lead to tumor progression within prostate lesions.[16–18] Those foci histologically identified as aggressive or invasive do not always show accumulation of genetic alterations in known oncogenes or TSGs. In addition, the predominant genetic changes observed in prostate carcinoma appear to be losses of chromosomal regions; however, previous loss of heterozygosity (LOH) studies in multifocal prostate cancer used only a limited number of polymorphic markers within only a few cancerous foci.[19–21] For an improved understanding of the mechanisms of tumor development, more data on cytogenetic changes in heterogeneous prostate cancer are needed. More extensive studies using more polymorphic markers may more fully delineate how pre-neoplastic and pre-malignant foci within tumors are affected by genetic events. To investigate the relationship between allelic losses and both the pathological grade and growth patterns of prostate tumors, we analyzed LOH on 6q within multiple histologically different cancerous foci using gene mapping with a wide array of microsatellite markers.

Materials and Methods

Tumor samples and histology. The 21 prostate carcinomas examined in this study were obtained from radical prostatectomies. No initial chemotherapy or hormonal treatments were instituted before tumor excision. The patients' ages ranged from 54 to 82 years (median, 67 years). All cases were staged without consideration of margin status using the standard TNM criteria on whole embedded prostatic specimens according to the American Joint Committee on Cancer.[22] A slice of whole prostate was fixed in 10% neutral buffered formalin and embedded in paraffin, while the remaining portions of the tumors were frozen at −80°C for later DNA extraction. Consecutive sections were cut at 4 μm and mounted for immunohistochemical analyses and histopathological evaluation using conventional hematoxylin and eosin (H&E) staining; the H&E-stained sections also served as a guide for the DNA analyses. Four to eight different foci from each tumor were selected based on representative morphology, size, and lack of contamination with normal prostatic tissues, and histologically graded according to the Gleason system for prostate carcinoma.[23] Control genomic DNA was derived from separate normal tissues not

E-mail: nkonishi@naramed-u.ac.jp

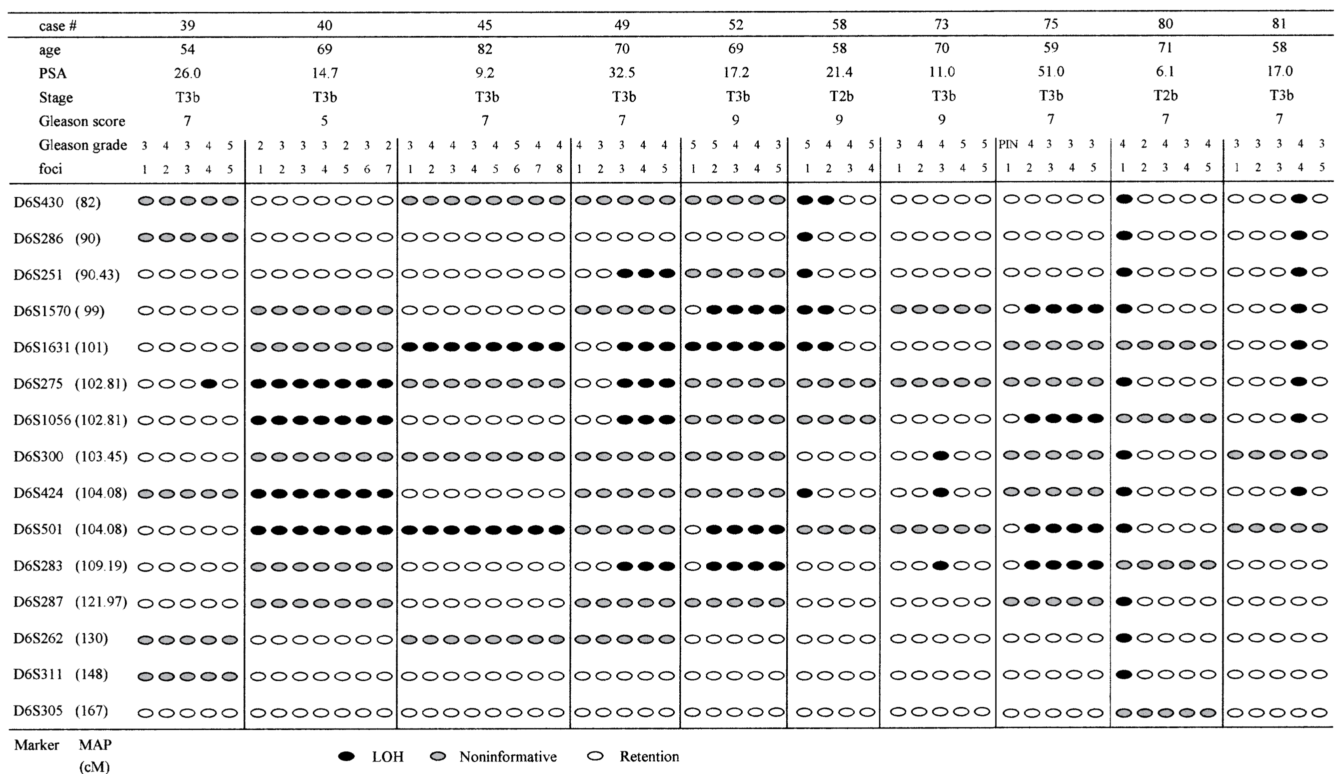

Fig. 1. LOH of microsatellite markers from chromosome 6q in 10 prostate carcinomas. Four to 8 foci were selected from each tumor into according to histologic heterogeneity and only those cases that revealed loss of at least one marker are shown. Case number, marker names and the genetic map in cM are listed on the left. Tumor grading and staging were done according to the Gleason scoring system and TNM criteria, respectively. PIN: prostatic intraepithelial neoplasia.

affected by the tumor. Informed consent was obtained from all patients before the collection of specimens as appropriate.

LOH assay. Fifteen polymorphic microsatellite markers were selected from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/genemap/) and the Genome Data Base (http://gdbwww.gdb.org/) databanks based on heterozygosity frequency, as well as coverage and flanking of the region of interest. Chromosomal maps and distances for each marker were obtained from the Whitehead Institute web site (http://www-genome.wi.mit.edu/). The names of the polymorphic microsatellites and their linear order are shown in Fig. 1.

For each marker, the sense primer was labeled with a fluorescent dye and paired normal and tumor DNA samples from each patient were amplified for 26 cycles with an annealing temperature of 58°C. PCR was performed on genomic DNA samples made up as follows: 25 ng of genomic DNA template, 10 pmol of each primer, 2.0 m*M* $MgCl_2$, dNTPs mix (200 µ*M* each), 1× PCR buffer, and 0.5 unit of "Platinum" *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), in a 10 µl final volume. The PCR program consisted of one cycle of 95°C for 5 min, followed by 26 cycles of denaturing at 95°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. One microliter aliquots of the PCR product were then mixed with 12 µl of deionized formamide and 0.5 µl of GeneScan Internal Lane Size Standard (ABI, Foster City, CA), denatured for 2 min at 94°C, and subjected to capillary electrophoresis on a 6% denaturing gel on a Genetic Analyzer 310 (ABI). The data were automatically collected and analyzed with GeneScan software as described in the manufacturer's protocol. Analyses of each marker were repeated independently at least twice and showed a variation of no more than 3% in the allelic ratio. Only samples heterozygous for a given locus were regarded as informative; homozygosity and/or microsatellite instability rendered a particular locus unsuitable for LOH or amplification.

RT-PCR analysis of the PTPRK transcript. Total RNAs were isolated from cancerous and normal loci of each case using RNA TRIzol Reagent (Gibco-BRL, Rockville, MD) following the manufacturer's instructions. cDNA was synthesized from 2 µg of total RNA, and RT-PCR was performed as described previously.[24] The sequences of the primers are: forward primer: 5′-TGGACGTTTAAGCTTGGGAGC-3′, the reverse primer: 5′-CCAGTTCTGCAACTAGTTCTTGC-3′. The PCR conditions were as follows: one cycle of 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min. After the last cycle, the reaction mixture was held at 72°C for 7 min. The PCR products were then analyzed by electrophoresis in a 2% agarose gel.

Statistical analysis. Statistical analyses were performed with Student's *t* test for age and PSA value, with the Mann-Whitney *U* test for Gleason score, and with Fisher's exact test for Gleason grade and pathological stage. A P value of <0.05 was considered significant.

Results

Using 15 polymorphic markers, LOH on chromosome 6q was detected in 10 of 21 (48%) prostate carcinomas. The results for the 10 tumors that showed LOH are summarized in Fig. 1. A total of 1605 foci were tested and 1123 foci (70%) were found to be informative. The number of informative foci in tumors with 6q LOH and without 6q LOH were 566/810 (69.9%) and 557/795 (70.1%), respectively, showing no significant difference. Nine of 10 tumors (90%) had evidence of LOH with more than one polymorphic marker, but none of the tumors had evidence of loss of the entire chromosome 6q. Nine of 10 tumors

Table 1. Relationship of 6q deletion and clinicopathological variables of 21 prostate carcinomas

Clinicopathological variables	LOH (n=10)	No LOH (n=11)	P
Age	66±8.5	68±4.3	0.517
PSA	20.6±15.8	13.2±11.6	0.156
Gleason score	7.40±1.26	7.55±1.44	0.805
Gleason grade	3.58±0.82 (3.58±0.81)[1)]	3.96±0.89	0.313
Stage I & II	2	9	0.009
Stage IIIa	0	2	
Stage IIIb	8	0	

1) Average Gleason grade in all foci examined of a tumor with 6q deletion.

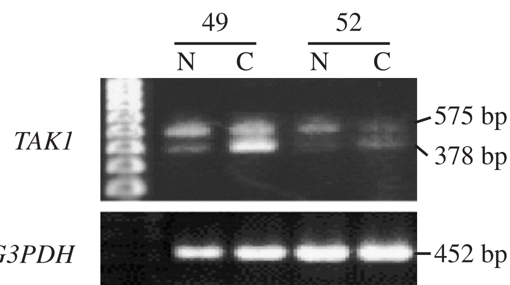

Fig. 3. Detection of *TAK1* transcripts by RT-PCR analysis. The 378 and 575 bp *TAK1* transcripts were ubiquitously expressed in the tumor samples (case nos. 49 and 52) positive for LOH on 6q.

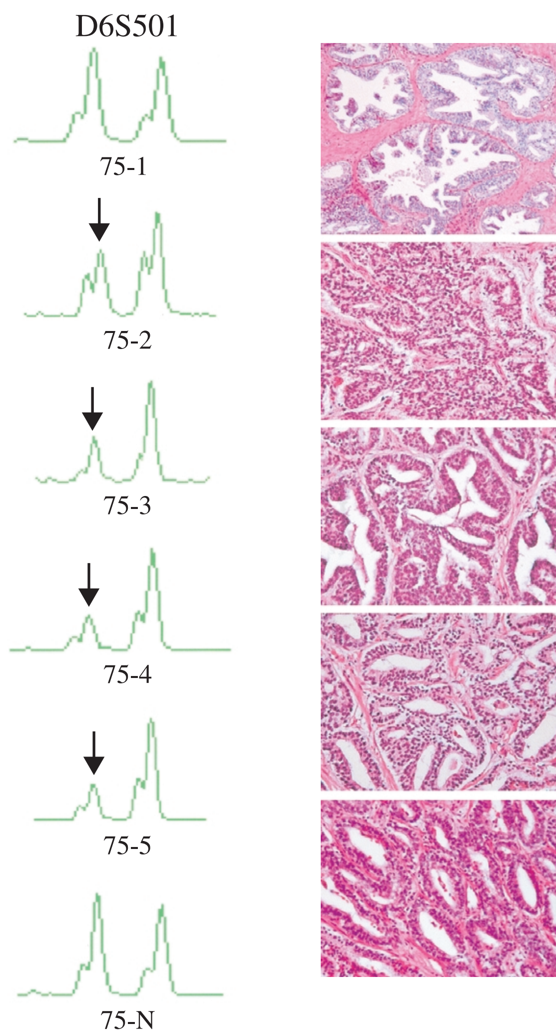

Fig. 2. Representative analysis of microsatellite loci on chromosome 6q in prostate carcinoma. Cancerous (C) and normal tissue (N) DNA were obtained from radical prostatectomy specimens as described in "Materials and Methods." High-grade PIN showing retention of heterozygosity, while 4 other adjacent foci with Gleason grades 4, 3, 3 and 3 show a deletion in 6q in case no. 75.

showing LOH exhibited common regions of allelic loss extending from D6S1631 to D6S1056, which encompasses 6q15-16.3. Another area with a high frequency of 6q losses was seen from D6S424 to D6S283 or the 6q16-21 region; this also was found in 9 of the 10 LOH-positive lesions.

Of the 10 LOH-positive tumors, 9 showed heterogeneous LOH patterns. In only one tumor (case no. 45) did all informative foci exhibit an identical pattern of allelic loss. Five other tumors (case nos. 40, 49, 52, 58 and 75) demonstrated LOH in two or more foci, while 4 tumors (cases 39, 73, 80 and 81) demonstrated LOH in only one focus of the tumor examined. In case no. 39, LOH was only found in one focus, and only at D6S275. However, 3 tumors showed LOH in one focus for at least 3 polymorphic markers. With regard to Gleason grading in 10 tumors showing 6q deletion, 6q LOH-positive foci averaged 3.58±0.82 while 6q LOH-negative foci averaged 3.60±0.82 within a tumor. Eleven LOH-negative tumors scored an average grade of 3.96±0.89, showing no significant differences as regards Gleason grade of foci and 6q deletion (Table 1).

A relatively large area of high-grade prostatic intraepithelial neoplasia (PIN) was found adjacent to the prostate carcinoma in one sample (case no. 75). The informative foci within this tumor exhibited obvious LOH at 4 polymorphic markers; however, LOH was never detected at any marker in the adjacent PIN (Fig. 2).

The relationship of LOH to clinicopathological variables in the 21 tumors is shown in Table 1. The frequency of deletion was independent of age, preoperative PSA values, and Gleason score. The average Gleason score for a whole tumor exhibiting LOH was 7.40±1.26, whereas the score for those without LOH was 7.55±1.44, which was not statistically significantly different. Although the average Gleason grades assigned to foci described above and the average Gleason score assigned to each whole tumor were higher in LOH-negative tumors than in those positive for LOH, the differences were very small and statistically insignificant. While 8 of 10 (80%) tumors diagnosed at pathologic stage T3 (non-organ confined) were LOH-positive, only 2 tumors at stage T2 (organ confined) exhibited the deletion. Of the 11 tumors without LOH, only 2 tumors were stage T3, whereas 7 lesions were diagnosed as T2, and 2 tumors were staged at T1. The only statistically significant difference emerged when examining the stage T3 tumors; all stage T3b tumors were LOH-positive while stage T3a tumors were negative for LOH (P=0.009).

Prostate carcinomas positive for 6q deletions were also analyzed for the presence of *TAK1* transcripts by RT-PCR. We found, however, that all tumors examined ubiquitously expressed *TAK1* (Fig. 3); we could not detect any correlation between LOH and *TAK1* expression.

Discussion

Recent intensive cytogenetic studies have achieved a more precise evaluation of the chromosome 6q region that is frequently lost in human prostate carcinomas.[1, 2, 11, 25] The incidence of LOH varied up to a high of 48% of tumors evaluated,[11] probably due to the polymorphic markers used in each study and the

heterogeneous/multifocal nature of prostate lesions.[16–18] Genetic heterogeneity has been investigated at specific regions on chromosomes 3p, 8p, 10q, and 17q[20, 21]; however, both the number of polymorphic markers for each chromosome and the number of individual tumor foci examined were too few to yield much information. For this reason, we have investigated and focused on 6q deletions in particular, using 15 polymorphic markers, multiple tumor samples, and no less than 4 histologically cancerous foci within each tumor.

We have previously reported on both known and unknown genetic and epigenetic changes within prostate carcinomas,[16–18] but have so far been unable to detect specific combinations of genetic or epigenetic changes in representative focal areas. In previous LOH studies of this disease, a random discordant pattern of allelic deletion emerged.[19–21] Hügel *et al*.[26] analyzed 25 chromosomal loci in 47 prostate carcinomas and demonstrated a significant correlation between frequency of focal LOH and malignant progression of affected foci. In our current study, only a single focus in 4 of 10 tumors positive for 6q LOH actually showed the deletion in 6q. Of these 4 single-positive-focus tumors, the deletion was revealed at only one polymorphic marker, while the remaining 3 tumors demonstrated single foci with deletions at multiple markers in chromosome 6q. This suggests that LOH on 6q is an additional genetic event in the stepwise progression of prostate carcinoma.

As was found in a previous investigation, more highly staged lesions appear to have more frequent LOH. Srikantan *et al*.[2] reported the risk for finding a 6q deletion was greater in stage T3 (non-organ confined) disease. A study employing CGH demonstrated LOH in 6q in only 22% of primary cancers, but in fully 44% of recurrent cancers.[27] Recent LOH studies have similarly shown that LOH at 6q16-22 is a frequent event at higher stages of this disease[11] and we also detected more 6q deletions in LOH-positive stage T3 tumors than T3 lesions negative for LOH.

Previous studies indicate that prostate cancer is closely linked to the presence of PIN, and, further, that multiple prostatic tumors may arise from PIN.[28, 29] The same chromosomal aberrations detected in full-blown carcinoma have often been observed in PIN as well.[30] In a survey of various prostatic lesions, LOH at 6q has been detected in 18% of PIN, in 13% of primary cancer, and in 41% of metastatic cancer.[31] Although only one PIN focus was examined in the current study, a 6q deletion was not detected, but it was apparent in adjacent cancerous foci. Therefore, while PIN remains the most likely precursor to prostate cancer, the evidence would point to LOH at 6q as likely to occur as a later event in the process of carcinogenesis.

In attempts to define the common region of deletion in prostate carcinoma, CGH and cytogenetic studies have shown that frequent genetic alterations occur in the 6q15-22 region and indicate that one or more TSGs are located here.[1, 2, 11, 26, 32, 33] An analysis of 9 polymorphic markers in 52 tumors uncovered a common region of deletion in 6q14-21 19 cM between markers D6S251 and D6S286 in 13 of 17 (76.5%) informative neoplasias.[1] More recent investigations have further identified 2 distinct regions around the locus; Srikantan *et al*. revealed a region of 15 cM between D6S1056 and D6S300 on 6q16.3-21 as a common region in which deletions occur at a relatively high frequency (in 6 of 11 tumors, or 54.5%). Another potential locus was at marker D6S314 at a more distal location on 6q23-24. However, this locus was found in only one case among 38 tumors examined for homozygous deletion.[1] Hyytinen *et al*. recently defined two regions of LOH, one at 7.5 cM on 6q16-21 between D6S1716 and D6S1580, and a second at 4.3 cM on 6q22 between D6S261 and D6S1702; however, the second region of LOH defined in their analysis was found in cell lines or xenografts, rather than in primary tumors.[11] Three potential candidate genes are present in the region implicated, and they encode human cyclin C (*CCNC*), thiol-specific antioxidant protein 1 (*TSA1*), and the glutamate receptor, ionotropic kainate 2 (*GRIK2*) (NCBI, http://www.ncbi.nlm.nih.gov/genemap/). The *CCNC* gene has been shown to be deleted in acute lymphoblastic leukemias[34] and thiol-containing proteins with antioxidant capacity have recently been characterized and shown to be overexpressed in a number of human malignancies.[35] There is no evidence, however, that these genes participate in prostate carcinogenesis. The glutamate receptor 6 (*GluR6, GRIK2*) gene, which has been linked to autism, is also located on 6q21.[36]

A second region spanning 1.81 cM between D6S1631 and D6S1056 on 6q15-16.3 was also identified at high frequency in 9 of 10 LOH-positive tumors (90%) in our study; this area is within the region examined in the investigation by Cooney *et al*.[1] and overlaps a short segment identified in both Srikantan's and Hyytinen's studies.[2, 11, 12] One candidate gene, transforming growth factor β (TGF-β)-activated kinase 1 (*TAK1*), is present in the region. In the mouse, *TAK1* was isolated as a MAPKKK downstream of the TGF-β receptor.[37] We found no indication of *TAK1* transcripts in our series of 6q LOH-positive tumors, but this does not preclude the possibility of a role for the downstream mediators of TGF-β-elicited signals in the pathogenesis of prostate carcinoma. It is also likely that one or more putative TSGs that are important in prostate carcinogenesis, but have yet to be identified, might be located on 6q15-16.3.

We thus found frequent LOH at 6q15-21 in our analysis of 21 primary tumors using 15 microsatellite markers. Two common regions were identified, which were within those described in previous studies; however, we were able to define narrower regions spanning 1.81 cM on 6q15-16.3 and 5.11 cM on 6q19-21 at higher frequencies in lesions showing LOH. The *TAK1* gene located at these loci seems to have no correlation to tumorigenesis since the transcripts were detected in our LOH-positive tumor samples, but definition of this region of chromosome 6q should be useful in identifying any putative tumor suppressor genes that might be located here. Further, we suggest that 6q deletion might be a late event in prostate carcinogenesis based on the clinicopathological data indicating no deletion in PIN, and correlating more frequent LOH on 6q with higher clinical/histological tumor stage.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

1. Cooney KA, Wetzel JC, Consolino CM, Wojno KJ. Identification and characterization of proximal 6q deletions in prostate cancer. *Cancer Res* 1996; **56**: 4150–3.
2. Srikantan V, Sesterhenn IA, Davis L, Hankins GR, Avallone FA, Livezey JR, Connelly R, Mostofi FK, Mcleod DG, Moul JW, Chandrasekharappa SC, Srivastava S. Allelic loss on chromosome 6q in primary prostate cancer. *Int J Cancer* 1999; **84**: 331–5.
3. Knudson AG. Antioncogenes and human cancer. *Proc Natl Acad Sci USA* 1993; **90**: 10914–21.
4. Milkin D, Meese E, Vogelstein B, Witkowski C, Trent J. Loss of heterozygosity for loci on the long arm of chromosome 6 in human malignant melanoma. *Cancer Res* 1991; **51**: 5449–53.
5. Morita R, Saito S, Ishikawa J, Ogawa O, Yoshida O, Yamakawa K, Nakamura Y. Common regions of deletion on chromosome 5q, 6q, and 10q in renal cell carcinoma. *Cancer Res* 1991; **51**: 5817–20.
6. Orphanos V, McGown G, Hey Y, Boyle JM, Santibanez-Koref M. Proximal 6q, a region showing allelic loss in primary breast cancer. *Br J Cancer* 1995; **71**: 290–3.

7. Orphanos V, McGown G, Hey Y, Thorncraft M, Santibenez-Koref M, Russell SE, Hickey I, Atkinson RJ, Boyle JM. Allelic imbalance of chromosome 6q in ovarian tumors. *Br J Cancer* 1995; **71**: 666–9.
8. Hankins GR, DeSouza AT, Bentley RC, Patel MR, Marks JR, Iglehart JD, Jirtle RL. *M6P/IGF* receptors: a candidate breast tumor suppressor gene. *Oncogene* 1996; **12**: 2003–9.
9. Theile M, Seitz S, Arnold W, Jandrig B, Frege R, Schlag PM, Haensch W, Guski H, Winzer KJ, Barrett JC, Scherneck SA. Defined chromosome 6q fragment (at D6S310) harbors a putative tumor suppressor gene for breast cancer. *Oncogene* 1996; **13**: 677–85.
10. Alers JC, Rochat J, Krijtenburg P-J, Hop WCJ, Kranse R, Rosenberg C, Tanke HJ, Schröder FH, Dakken H. Identification of genetic markers for prostatic cancer progression. *Lab Invest* 2000; **80**: 931–42.
11. Hyytinen ER, Saadut R, Chen C, Paul L, Koivisto PA, Vessella RL, Frierson HF, Dong J-T. Defining the region(s) of deletion at 6q16-q22 in human prostate cancer. *Genes Chromosom Cancer* 2002; **34**: 306–12.
12. Hyytinen ER, Thalmann GN, Zhau HE, Karhu R, Kallioniemi OP, Chung LW, Visakorpi T. Genetic changes associated with the acquisition of androgen-independent growth, tumorigenicity and metastatic potential in prostate cancer model. *Br J Cancer* 1997; **75**: 190–5.
13. An G, Ng AY, Meka CSR, Luo G, Bright SP, Cazares L, Wright GL, Veltri RW. Cloning and characterization of *UROC28*, a novel gene overexpressed in prostate, breast, and bladder cancers. *Cancer Res* 2000; **60**: 7014–20.
14. Bilanges B, Varrault A, Basyuk E, Rodrigues C, Mazumdar A, Pantaloni C, Bockaert J, Theillet C, Spengler D, Journot L. Loss of expression of the candidate tumor suppressor gene ZAC in breast cancer cell lines and primary tumors. *Oncogene* 1999; **18**: 3979–88.
15. Mazurenko N, Attaleb M, Gritsko T, Semjonova L, Pavlova L, Sakharova O, Kisseljov F. High resolution mapping of chromosome 6 deletions in cervical cancer. *Oncol Rep* 1999; **6**: 859–63.
16. Konishi N, Hiasa Y, Matsuda H, Tao M, Tsuzuki T, Hayashi I, Kitahori Y, Shiraishi T, Yatani R, Shimazaki J, Lin J-C. Intratumor cellular heterogeneity and alterations in *ras* oncogene and p53 tumor suppressor gene in human prostate carcinoma. *Am J Pathol* 1995; **147**: 1112–22.
17. Konishi N, Hiasa Y, Nakamura M, Kitahori Y, Matsubara K, Nagai H. Different patterns of DNA alterations detected by restriction landmark genomic scanning in heterogeneous prostate carcinomas. *Am J Pathol* 1997; **150**: 305–14.
18. Konishi N, Nakamura M, Kishi M, Nishimine M, Ishida E, Shimada K. Heterogeneous methylation and deletion patterns of the *INK4a/ARF* locus within prostate carcinoma. *Am J Pathol* 2002; **160**: 1207–14.
19. Cheng L, Song S-Y, Pretlow TG, Abdul-Karim FW, Kung H-J, Dawson DV, Park W-S, Moon Y-W, Tsai M-L, Linehan WM, Emmert-Buck MR, Liotta LA, Zhuang Z. Evidence of independent origin of multiple tumors from patients with prostate cancer. *J Natl Cancer Inst* 1998; **90**: 233–7.
20. Macintosh CA, Stower M, Reid N, Maitland NJ. Precise microdissection of human prostate cancer reveals genotypic heterogeneity. *Cancer Res* 1998; **58**: 23–8.
21. Takimoto Y, Shimazui T, Akaza H, Sato N, Noguchi M. Genetic heterogeneity of surgically resected prostate carcinomas and their biopsy specimens is related to their histologic differentiation. *Cancer* 2001; **91**: 362–70.
22. Fleming ID, Cooper JS, Henson DE, Hutter RVP, Kenndy BI, Murphy GP, editors. AJCC cancer staging manual. 5th ed. American Joint Committee on Cancer. Philadelphia: JB Lippincott-Raven; 1997.
23. Gleason DF. Histologic grading of prostate cancer. *Hum Pathol* 1992; **23**: 273–9.
24. Sakurai H, Shigemori N, Hasegawa K, Sugita T. TGF-β-activated kinase 1 stimulates NF-κB activation by an NF-κB-inducing kinase-independent mechanism. *Biochem Biophys Res Commun* 1998; **243**: 545–9.
25. Cunningham JM, Shan A, Wick MJ, McDonnel SK, Schaid PJ, Tester DJ, Qian J, Takahashi S, Jenkins RB, Bostwick DG, Thibodeau SN. Allelic imbalance and microsatellite instability in prostate adenocarcinoma. *Cancer Res* 1996; **56**: 4475–82.
26. Hügel A, Werrnert N. Loss of heterogeneity (LOH), malignancy grade and clonality in microdissected prostate cancer. *Br J Cancer* 1999; **79**: 551–7.
27. Visakorpi T, Kallioniemi AH, Syvanen AC, Hyytinen ER, Karhu R, Tammela T, Isola JJ, Kallioniemi OP. Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. *Cancer Res* 1995; **55**: 342–7.
28. Bostwick DG, Pacelli A, Lopez-Beltan A. Molecular biology of prostate intraepithelial neoplasia. *Prostate* 1996; **29**: 117–34.
29. Bostwick DG. High grade prostatic intraepithelial neoplasia: the most likely precursor of prostate cancer. *Cancer* 1995; **75**: 1823–36.
30. Qian J, Bostwick DG, Takahashi S, Borell TJ, Herath JF, Lieber MM, Jenkins RB. Chromosomal anomalies in prostatic intraepithelial neoplasia and carcinoma detected by fluorescence *in situ* hybridization. *Cancer Res* 1995; **55**: 5408–14 .
31. Saric T, Brkanac Z, Troyer DA, Padalecki SS, Sarosdy M, Williams K, Abadesco L, Leach RJ, O'Connell P. Genetic pattern of prostate cancer progression. *Int J Cancer* 1999; **81**: 219–24.
32. Cher ML, Bova GS, Moore DH, Small EJ, Carroll PR, Pin SS, Epstein JI, Isaacs WB, Jensen RH. Genetic alterations in untreated metastases and androgen-independent prostate cancer detected comparative genomic hybridization and allelotyping. *Cancer Res* 1996; **56**: 3091–102.
33. Nupponen NN, Kakkola L, Koivisto P, Visakorpi T. Genetic alterations in hormone-refractory recurrent prostate carcinomas. *Am J Pathol* 1998; **153**: 141–8.
34. Li H, Lahti JM, Valentine M, Saito M, Reed SI, Look AT, Kidd VJ. Molecular cloning and chromosomal localization of the human cyclin C (CCNC) and cyclin E (CCNE) genes: deletion of the CCNC gene in human tumors. *Genomics* 1996; **32**: 253–9.
35. Berggren MI, Husbeck B, Samulitis B, Baker AF, Gallegos A, Powis G. Thioredoxin peroxidase-1 (peroxiredoxin-1) is increased in thioredoxin-1 transfected cells and results in enhanced protection against apoptosis caused by hydrogen peroxide but not by other agents including dexamethasone, etoposide, and doxorubicin. *Arch Biochem Biophys* 2001; **392**: 103–9.
36. Jamain S, Betancur C, Quach H, Philippe A, Fellous M, Giros B, Gillberg C, Leboyer M, Bourgeron T. Linkage and association of the glutamate receptor 6 gene with autism. *Mol Psychiatr* 2002; **7**: 302–10.
37. Yamaguchi K, Shirakabe K, Shibuya H, Irie K, Oishi I, Ueno N, Taniguchi T, Nishida E, Matsumoto K. Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. *Science* 1995; **270**: 2008–11.