Frequent Loss of Heterozygosity at 7q31.1 in Primary Prostate Cancer Is Associated with Tumor Aggressiveness and Progression


ABSTRACT

Cytogenetic analyses have demonstrated that chromosome region 7q22–32 is commonly altered in prostate adenocarcinomas. In addition, in recent fluorescence in situ hybridization studies, we have observed that aneusomy of chromosome 7 is frequent in prostate cancer and is associated with higher tumor grade, advanced pathological stage, and early prostate cancer death. These findings suggest that genetic alterations of chromosome 7 play a significant role in the development of prostate cancer. To better define the chromosome 7 alterations, PCR analysis of 21 microsatellite loci was performed on 54 paired prostate cancer and control DNAs. Overall, chromosome 7 allelic imbalance was identified in 16 of 54 cases (30%). Allelic imbalances of loci mapped to 7q were observed in 15 of the 16 cases. The allelic imbalances were classified as losses in 15 tumors (28%) and as gains in 1 (2%) by comparative multiplex PCR analysis. The most common site of allelic loss included loci D7S523 and D7S486 at 7q31.1. A comparison with clinicopathological features of the tested tumors revealed that the allelic loss of 7q31.1 correlated with higher tumor grade (P = 0.012) and lymph node metastasis (P = 0.017). These results indicate that 7q31 may be the site of a putative suppressor gene(s) important for the pathogenesis of prostate carcinoma, and that the genetic alterations at 7q31.1 may participate in tumor progression and metastasis.

INTRODUCTION

Prostate adenocarcinoma is the most common cancer and the second leading cause of cancer death in men in the United States (1). Prostate adenocarcinoma has extensive variability in clinical behavior (2). Accurate prediction of the probability of tumor progression and of patient survival is a major goal of current prostate cancer research. An understanding of the genetic events underlying prostate cancer progression may make possible accurate distinction of aggressive tumors from those with little likelihood of metastatic disease.

Substantial evidence suggests that inactivation of TSGs may play an important role in the pathogenesis of various human cancers (3). In an effort to identify genomic sites harboring TSG in prostate cancer, several laboratories have analyzed LOH on specific chromosome regions for this tumor type. Previous studies, mainly using Southern blot analysis of RFLP, have found frequent allelic deletions on chromosomes 8p, 10q, 16q, and 18q (4–7). Cytogenetic analysis has also offered an initial clue to putative TSG loci in human cancers. Alterations on chromosome region 7q22–32 have been reported to be one of the most common karyotypic anomalies in prostate adenocarcinomas (8, 9). In addition, in recent FISH studies, we observed that aneusomy of chromosome 7 is frequent in prostate cancer and is associated with higher tumor grade, advanced pathological stage, and early prostate cancer death (10–12). These findings suggest that genetic alterations of chromosome 7 play a significant role in the development of prostate cancer. However, previous Southern blot analyses of RFLP mapped to chromosome 7 did not show apparent LOH. Although the reason for this discrepancy is not clear, one possibility could be the limitations of conventional RFLP methods. This analysis has required relatively large quantities of DNA extracted from freshly frozen tissue. In addition, Southern blot analysis depends on the informativeness of RFLP markers (whose heterozygosity rates are usually less than 50%) and on their distribution (13). Indeed, in previous LOH studies of prostate cancer, relatively small numbers of cases were analyzed using only a few markers mapped to the long arm of chromosome 7 (4, 5). To overcome these limitations, PCR-based assays for the analysis of a number of microsatellite markers mapped to specific chromosomes have emerged (14–16). Recently, using this method, frequent loss of allele at 7q31.1 was identified in prostate carcinomas (17).

In this study, PCR analysis of 21 microsatellite loci on chromosome 7 was performed on 54 paired prostate cancer and control DNAs to better define the genetic alterations on this chromosome. We observed a significant incidence of allelic loss of markers mapped to 7q31.1. It is of interest that the results obtained from this study suggested that genetic alterations of this band play a significant role for tumor aggressiveness and progression.

MATERIALS AND METHODS

Tissue Samples. Primary prostate cancer tissue and paired benign tissue were obtained from patients who underwent radical prostatectomy for clinically localized prostate adenocarcinoma at the Mayo Clinic (Rochester, MN). Harvested tissue blocks were immediately frozen at −70°C and stored until the time of DNA extraction. Histological diagnosis, Gleason score (18), pathological staging, and flow cytometric DNA ploidy (19) were determined for each case during routine clinical workup after surgery. The mean Gleason score of the 54 primary tumors included in this study was 7.4 (range, 5–9). Twenty-six tumors (48%) were confined within the capsule and classified as pathological stage T1. Twenty-eight tumors (52%) penetrated through the capsule (pathological stage T2). Microscopic lymph node metastases were found in seven (13%) cases. Preoperative serum prostatic-specific antigen levels were measured with a monoclonal solid phase, two-site, immunoradiometric assay (Hybritech, Inc., San Diego, CA).

DNA Extraction. Tissues were mounted and examined microscopically on hematoxylin- and eosin-stained cryosections. Microdissection of the specimens was performed to remove normal tissue to assure a maximal percentage of tumor in each specimen. The primary prostate adenocarcinomas used in this study could be microdissected to yield tissue that contained >70% cancer cells. Tumor DNA was obtained from multiple 10-μm cryosections. Paired, noncancerous DNA was isolated from benign prostatic tissue, which was generally hyperplastic. All tissue containing atypical adenomatous hyperplasia or intraepithelial neoplasia were rejected for noncancerous tissue samples (20). To confirm the percentage of cancer cells in the trimmed tumor specimens and to determine that the control specimens were free of cancer cells, histological examinations were performed on two 5-μm hematoxylin- and eosin-stained sections adjacent to each side of the specimens used for DNA extraction. DNA extractions using phenol/chloroform purification were performed as described previously (21).

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2 The abbreviations used are: TSG; tumor suppressor gene; LOH; loss of heterozygosity; FISH; fluorescence in situ hybridization.

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PCR and Microsatellite Polymorphism Analysis. We used 21 pairs of primers for chromosome 7 (5 for the short arm and 16 for the long arm), which were obtained from Research Genetics (Huntsville, AL; 22). Markers used in this study were: D7S531, D7S513, D7S507, D7S484, D7S530, D7S542, D7S518, D7S515, D7S501, D7S496, D7S523, D7S486, D7S522, D7S490, D7S487, D7S514, D7S504, D7S530, D7S498, and D7S550 (Table 1). Most these markers were reported to have heterozygosity rates of >70% (mean 78%). To thoroughly survey potential genetic alterations of 7q22–32, the region in which frequent anomalies were observed in previous cyogenetic studies (8, 9), 13 markers targeted on this region were selected (22-24). As positive controls, four markers (D8S254, D8S261, LPL-GZ, and D8S258) for studies (8, 9) were included. As negative controls (23), because no significant allelic imbalance for these regions had been reported in prostate adenocarcinoma. Furthermore, in a previous FISH study from our institution using 12 different chromosome enumeration probes, no case with aneusomy of chromosome 4 was found among 40 randomly selected, clinically localized prostate cancers (11).

PCR was performed on genomic DNA samples using the following conditions: 25 ng of genomic DNA template; 4 pmol of each primer; 0.5 units of Taq DNA polymerase (Promega, Madison, WI); 1 mM [α-32P]dCTP (New England Nuclear, Boston MA); 200 mM dNTP (dATP, dCTP, dGTP, and dTTP); 50 mM KCl; 10 mM Tris-HCl; and 0.1% Triton X-100 in a 15-ml reaction volume. PCR cycling conditions were identical for all primers used: 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 2 min, and extension for 1 min at 72°C. PCR products were subjected to electrophoresis on a denaturing 6% polyacrylamide/15% formamide gel. The gel was dried and processed for autoradiography.

Assessment of Allelic Imbalance. Allelic imbalance was determined by comparing the intensity of heterozygous alleles of the matched tumor DNA and noncancerous DNA. Two reviewers (S. T., R. B. J.) evaluated the autoradiograms visually. Complete loss of one of the bands in tumor was rarely observed. When the pattern of the relative allelic dosage was not conclusively apparent by visual inspection, the intensities of the bands were quantified using densitometry and the image analysis software program NIH Image (Ver. 1.47). Quantitation of band intensity was performed by the area integration method (with subtraction of local background).

Table 1 Allelic imbalance on chromosomes 4, 7, and 8 in 54 clinically localized prostate carcinomas

<table>
<thead>
<tr>
<th>Locus</th>
<th>Location</th>
<th>Genetic distancea (cM)</th>
<th>Allelic imbalance/informative cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 7</td>
<td>D7S531</td>
<td>7q22</td>
<td>3/32 (9)</td>
</tr>
<tr>
<td></td>
<td>D7S513</td>
<td>7p21</td>
<td>6/42 (14)</td>
</tr>
<tr>
<td></td>
<td>D7S507</td>
<td>7p21–15</td>
<td>2/41 (5)</td>
</tr>
<tr>
<td></td>
<td>D7S484</td>
<td>7p15–14</td>
<td>5/38 (13)</td>
</tr>
<tr>
<td></td>
<td>D7S510</td>
<td>7p14–13</td>
<td>6/38 (16)</td>
</tr>
<tr>
<td></td>
<td>D7S482</td>
<td>7q11.2</td>
<td>4/32 (13)</td>
</tr>
<tr>
<td></td>
<td>D7S518</td>
<td>7q22</td>
<td>4/37 (11)</td>
</tr>
<tr>
<td></td>
<td>D7S515</td>
<td>7q22–31.1</td>
<td>7/45(18)</td>
</tr>
<tr>
<td></td>
<td>D7S501</td>
<td>7q31.1</td>
<td>8/37 (22)</td>
</tr>
<tr>
<td></td>
<td>D7S496</td>
<td>7q31.1</td>
<td>4/32 (13)</td>
</tr>
<tr>
<td></td>
<td>D7S523</td>
<td>7q31.1</td>
<td>4/10 (24)</td>
</tr>
<tr>
<td></td>
<td>D7S486</td>
<td>7q31.1</td>
<td>11/43 (26)</td>
</tr>
<tr>
<td></td>
<td>D7S522</td>
<td>7q31.1</td>
<td>1/23 (23)</td>
</tr>
<tr>
<td></td>
<td>D7S516</td>
<td>7q31.1</td>
<td>1/23 (23)</td>
</tr>
<tr>
<td></td>
<td>D7S490</td>
<td>7q31.2</td>
<td>6/43 (14)</td>
</tr>
<tr>
<td></td>
<td>D7S487</td>
<td>7q31.3</td>
<td>5/34 (15)</td>
</tr>
<tr>
<td></td>
<td>D7S514</td>
<td>7q31.3–32</td>
<td>2/5/29 (17)</td>
</tr>
<tr>
<td></td>
<td>D7S504</td>
<td>7q33.3</td>
<td>6/43 (14)</td>
</tr>
<tr>
<td></td>
<td>D7S530</td>
<td>7q33–35</td>
<td>4/31 (13)</td>
</tr>
<tr>
<td></td>
<td>D7S489</td>
<td>7q36</td>
<td>3/38 (8)</td>
</tr>
<tr>
<td>Chromosome 4</td>
<td>D4S174</td>
<td>4p15–13</td>
<td>2/45 (4)</td>
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<tr>
<td></td>
<td>D4S171</td>
<td>4q35–qter</td>
<td>2/45 (4)</td>
</tr>
<tr>
<td>Chromosome 8</td>
<td>D8S254</td>
<td>8p22</td>
<td>15/30 (50)</td>
</tr>
<tr>
<td></td>
<td>D8S261</td>
<td>8p22</td>
<td>15/30 (50)</td>
</tr>
<tr>
<td></td>
<td>LPL-GZ</td>
<td>8p22</td>
<td>19/35 (54)</td>
</tr>
<tr>
<td></td>
<td>D8S258</td>
<td>8q22</td>
<td>13/29 (44)</td>
</tr>
</tbody>
</table>

*The estimates of genetic distances between each marker and the one directly above it (loci on 7q22–32) are shown in centimorgans (cM; 21).

*One case without allelic imbalance showed microsatellite instability.

Fig. 1. Examples of allelic imbalance (loss) of microsatellite loci on chromosome 7 in representative prostate cancers. Analysis of tumor (T) and noncancerous tissue (N) DNAs obtained from cases 8, 11, 38, and 46. Arrows, alleles showing loss. Complete loss of one allelic band in tumor is rare, possibly because of contamination of noncancerous DNA or tumor heterogeneity. Case 11 shows a loss of alleles for loci D7S522 and D7S486 (band intensity ratios are 4.86 and 3.32, respectively) and retention of alleles for loci D7S496 and D7S522 (band intensity ratios are 1.08 and 1.22, respectively). Case 38 shows a loss of alleles for loci mapped to 7q31–32 (loci D7S496 and D7S504 are shown with band intensity ratios of 2.52 and 2.87, respectively). Retention of alleles for D7S523 at 7q22 and D7S530 at 7q32 are shown with band intensity ratios of 1.04 and 1.02, respectively. Case 46 shows a loss of alleles for loci mapped to the distal long arm (q22-qter; loci D7S501, D7S486, and D7S530 are shown). Case 8 shows allelic losses at all informative loci mapped to both arms of chromosome 7. See Table 1 and Fig. 3 for detailed information on loci locations.
Allelic imbalance was identified in 10 of 42 informative cases (24%) with allelic imbalance at each locus is shown in Table 1. Allelic imbalance was identified more frequently for the loci mapped to the chromosome 7p arm was likely deleted, did not lose either of these regions. Except for cases 26, 35, and 51) (31%) exhibited allelic imbalance at all of the informative loci on both the long and short arms of chromosome 7, thereby indicating probable allelic dosage alteration of the entire chromosome. For example, case 11 showed allelic deletions at D7S523 and D7S486 only; the closest flanking loci (D7S496 and D7S522) were retained (see Fig. 1). Cases 6 and 13 also had an interstitial allelic loss limited at 7q31.1 (commonly deleted loci were D7S486 and D7S522). The localization of the commonly deleted regions was inferred by the pattern of allelic loss in these tumors. The allelic loss pattern could be interpreted most simply by postulating that two commonly deleted regions were present on the chromosome 7q arm. Except for cases 26, 35, and 40, the most commonly deleted region seemed to include band 7q31.1. For this region, the minimal common site is defined by the LOH at loci D7S523 and D7S486 for cases 10, 11, and 13, by the retention of heterozygosity at locus D7S496 for cases 11 and 13, and by the retention of heterozygosity at locus D7S523 for cases 11 (Fig. 3). This region is also defined partially by the frequent loss of D7S523 and D7S486 in our group of tumors (Table 1). A second overlapped deletion region may also be present slightly more distally at 7q31.2. Although the frequency of allelic loss at this site was less than 20% (Table 1), this deleted region may be defined by the retention of heterozygosity at loci D7S496 and D7S487 for cases 40 and 35, respectively (Fig. 3). Only one tumor (case 26), in which the entire chromosome 7p arm was likely deleted, did not lose either of these regions.

**Correlation with Clinicopathological Characteristics.** To identify biological and clinical significance of allelic loss at 7q31.1, its relationship with clinicopathological characteristics was evaluated. Of 16 tumors with allelic imbalance on chromosome 7, 12 tumors had allelic loss for loci D7S496, D7S523, D7S486 and/or D7S522 at 7q31.1 (cases 19, 26, 35, and 40 were excluded). Table 2 shows a summary of the relationship between allelic loss of 7q31.1 and 8p22 and clinicopathological characteristics. For 10 of 12 tumors with allelic loss at 7q31.1 (83%), the Gleason score was ≥8, compared
with 31% in tumors without the allelic loss ($P = 0.001$). The existence of lymph node metastasis in tumors with allelic loss at 7q31.1 was approximately five times that in tumors without the allelic loss (33% and 7%, respectively; $P = 0.017$). Tumors with allelic loss at 7q31.1 also showed a trend of higher preoperative serum prostatic-specific antigen (≥10 ng/ml; $P = 0.056$). The addition of tumors with loss at 7q31.2 (cases 35 and 40) did not alter these correlations significantly (data not shown). In contrast, 28 (52%) tumors had allelic loss of at least one locus at 8p22 and did not show significant correlations with any clinicopathological parameters (Table 2).

### DISCUSSION

In this study using 21 microsatellite polymorphic markers, 30% of the 54 tumors investigated were found to have an allelic imbalance of loci mapped to chromosome 7. The frequently affected loci were D7S523, D7S486, and D7S522 at 7q31.1, where approximately 25% of the informative cases showed allelic imbalance. Because the frequency of allelic imbalance at control loci on chromosome 4 was quite infrequent (<4%), the allelic imbalance of loci mapped to 7q31.1 is likely to be a significant genetic alteration in prostate cancer. In addition, most of the allelic imbalances identified (except for case 19) were attributed to allelic loss. Therefore, the region of chromosome 7q31.1 may be a candidate locus for a putative TSG significant for prostate cancer. The deletion map obtained from this study narrowed down the common LOH region to 7q31.1 near D7S486. The critical region may possibly lie within the 1-centimorgan region between D7S523 and D7S486 (Table 1 and Fig. 3). Furthermore, another overlapping region of LOH may be located more distally at 7q31.2 within the 3-centimorgan region between D7S480 and D7S487 (Table 1 and Fig. 3). However, the frequency of allelic deletion at this region was less than 20%. Additional analysis, including an attempt to identify homozygous deletions, would be required to confirm the existence of this new putative TSG locus at 7q31.2.

Type 1 microsatellite instability (28) was clearly observed for only one locus (D7S490) in a single tumor (case 14). A low rate of microsatellite instability in prostate cancer was also observed for multiple markers spread throughout the genome. It is of interest that D7S490 has been mapped between D7S480 and D7S487 within the deleted region at 7q31.2.

Recently, Zenklusen et al. (17, 29) reported frequent loss of allele for locus D7S522 at 7q31.1 in prostate and breast cancers, suggesting that the genetic alteration at this locus may participate in the development of both of these common carcinomas. In their study of prostate carcinoma, five of six informative tumors (83%) showed LOH for locus D7S522 at 7q31.1 (17). In our study, the frequency of allelic loss for D7S522 was 23% (31 informative cases), apparently lower than that of Zenklusen et al. (17). The discrepancy between these findings may be explained by differences in tumor specimen...
selection. In their study of 16 clinically localized prostate carcinomas, neither Gleason score nor pathological T stage were indicated. One of the tumor specimens evaluated was obtained from a metastatic lymph node. The higher frequency of LOH observed by Zenklusen et al. (17) at 7q31 may result in the more aggressive characteristics of the tumors evaluated (see below). Our study of a larger number of informative, clinically localized prostate cancers confirms that allelic loss at 7q31.1 is frequent in prostate carcinoma, although allelic loss of this loci is a less common event than that at 8p22.

The loss of alleles mapped to 7q31.1 correlated significantly with a higher Gleason score and lymph node metastasis. Higher tumor grade and advanced stage (lymph node metastasis) are predictors of clinical aggressiveness and poor prognosis in prostate carcinoma (2). Several independent studies have implicated the presence of genes involved in invasion and metastasis on chromosome 7 (30, 31). A somatic cell fusion study has shown evidence for the existence of genes on chromosome 7 controlling invasion and metastasis of T-cell cancers (30). Of greater interest is that breast cancer patients with LOH at 7q31.1 (c-met oncogene locus) have been reported to show significantly shorter metastasis-free survival and overall survival compared with patients without this alteration (31). These findings suggest that the region of 7q31.1 is the site of a putative TSG significant for certain cancers, perhaps in association with tumor aggressiveness and metastasis. Because allelic loss at 8p22 did not correlate with any clinicopathological features, it is possible that the putative genetic alteration at 8p22 is important for cancer initiation and/or early development in the majority of prostate carcinomas. In contrast, the alteration at 7q31.1 may play a significant role for a subgroup of prostate cancers and is more likely to be important for later stages of tumor progression and metastasis.

Although microsatellite polymorphic markers have a number of advantages over RFLP markers (13–16), they also have limitations for LOH analysis. In general, complete loss of one allele in a tumor DNA lane is rare, which sometimes makes interpretation difficult. For example, in this study, complete deletion of alleles mapped to 8p22 or 7q31 was rarely observed. There are two likely reasons for these observations: (a) contamination by noncancerous DNA; and (b) tumor heterogeneity. The contamination by noncancerous DNA seems to be the most likely reason for partial allelic deletion. The complete elimination of noncancerous DNA from tumor specimens by microdissection is practically impossible because many prostate cancers are infiltrated by normal stromal elements. Therefore, it is possible that the frequency of allelic imbalance detected in this study is lower than is the true frequency of allelic imbalance in cancer cells. On the other hand, tumor heterogeneity cannot be excluded, because significant genetic heterogeneity has been reported in multistaged prostate cancers (32). In this study, based on an inspection of the densitometric results (see “Materials and Methods”), a paired tumor allele:control allele intensity ratio of $\geq 1.5$ was selected as the criterion for allelic imbalance. A similar value was also selected as the allelic imbalance criterion in a recent LOH study of colorectal cancer (25). Further, a recent detailed quantitative analysis of allelic imbalance at 8p22 in prostate cancer demonstrated that a cutoff value of $\geq 1.5$ is adequate to determine allelic loss of microsatellite loci in tumor samples containing $>60\%$ cancer cells (26).

Another limitation in PCR analysis of microsatellite polymorphic loci is the difficulty of characterizing allelic imbalance as loss or gain. It is possible to overestimate the frequency of LOH because of misinterpretation of allelic gain (33). In an attempt to overcome this limitation, we used comparative multiplex PCR analysis to control for the amount of tumor and noncancerous DNA amplified by the PCR and loaded on gel. As a result, it was possible to characterize the allelic imbalance. Recently, the utility of comparative multiplex PCR for detecting homozygous deletion of 9p21 (the p16 TSG locus) in bladder cancer was also reported (27). Comparative multiplex PCR analysis is more feasible than are other possible methods (e.g., confirmatory Southern blot analysis and FISH using region-specific probes) for characterizing allelic imbalance. In our study, comparative multiplex PCR could identify one case with likely allelic gain on chromosome 7, demonstrating that this method is useful to distinguish allelic gain from loss.

In summary, this study identified significantly deleted regions within relatively narrow portions of band 7q31. This observation will be useful for attempts to clone the putative TSGs within this region. In addition, the detection of allelic loss at 7q31.1 may help to identify patients with clinically localized prostate cancer who have a poor prognosis and a high risk of having or developing metastatic disease.

**REFERENCES**

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