Loss of Heterozygosity in Human Primary Prostate Carcinomas: A Possible Tumor Suppressor Gene at 7q31.1

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Abstract

We studied loss of heterozygosity (LOH) on human chromosome 7q to determine the location of a putative tumor suppressor gene (TSG) in human primary prostate carcinomas. Samples were obtained from 16 primary prostate carcinomas surgically removed from patients at The University of Texas M. D. Anderson Cancer Center. Paired normal and tumor DNAs were used as template for PCR amplification of a set of 14 CA microsatellite repeats on 7q21-qter. Twelve of 16 cases studied had LOH at one or more loci on 7q. Eighty-three percent LOH (five of six informative cases) was detected with D7S26 at 7q31.1-7q31.2. Percentage of LOH was normally distributed around D7S22. The high incidence of LOH in primary prostate carcinomas suggests that there is a TSG relevant to the development of prostate cancers at 7q31.1-31.2, confirming our previous functional evidence for a TSG at this location. Further research needs to be conducted to establish the identity and function of this putative TSG.

Introduction

Alterations in oncogenes and TSGs are considered to be critical in the multistep process leading to the development of tumors (1, 2), and the succession of these events is very conserved in some types of cancer, such as colon carcinoma (3). Ever since the idea of recessive-acting TSG was formulated (4), cytogenetic techniques have been used to determine their locations. Although producing metaphase spreads of prostate carcinoma cells is technically difficult, there have been cytogenetic reports that many chromosomes (1, 3, 6, 7q, 8p, 10q, 17, 18, 21, Y) are frequently altered (5, 8). Consistent deletions or inversions of part of a chromosome in tumor cells are indicative of inactivation of a TSG in this region during neoplastic progression (9). For instance, deletions of chromosome 7 are common in many different cancers including ovarian cancer, prostate carcinomas, and malignant myeloid disorders (10-16). Recently, we reported that LOH in 7q31.1-31.2 is a very common event in human primary breast cancers (17). Also, our experiments with microcell fusion transfer of human chromosome 7 into a murine SCC cell line indicated that the inserted chromosome can delay the onset of tumors by 2-fold and in some cases even completely repress the tumorigenic potential of the SCC cell line. In situ hybridization revealed that the clones that reverted to the malignant phenotype had expelled the inserted chromosome (18). Moreover, a recent report demonstrated that insertion of an intact human chromosome 7 into tumor cells is indicative of inactivation of a TSG (19). Although cytogenetic techniques are useful, they do not detect the entire spectrum of inactivating events; for example, microdeletions and homologous recombination with a defective chromatin (20, 21) are beyond the range of detection by karyotyping procedures. More sensitive molecular methods should be used to screen for genetic alterations in tumors and to determine the smallest chromosomal region involved in those alterations. Therefore, LOH analysis of DNA extracted from solid tumors is the method of choice for mapping TSG (22). In the case of prostate carcinomas, LOH of chromosomes 8, 10, 16, and 18 has been found (23-25). In prostate carcinomas, all of these chromosomes, with the exception of chromosome 16, have been shown by cytogenetical analysis to have alterations.

To determine the extent and types of alterations in chromosome 7 in prostate carcinomas, we used an extensive set of highly polymorphic markers on q21-qter. By comparing the results obtained with tumor and normal DNA, we were able to narrow down the site of the TSG to a 1.2 cM region.

Materials and Methods

Tissue Samples. Prostate tumor specimens were obtained from the prostate glands and a pelvic lymph node of 16 patients who had undergone pelvic lymphadenectomy with or without radical prostatectomy for clinically localized prostate carcinoma. The surface of each prostate was inked and the gland was sectioned throughout the palpable tumor in a transverse plane perpendicular to the posterior surface. A thin slice of the tumor was made with a scalpel and immediately frozen at -80°C. An adjacent frozen section was examined histologically to confirm the tumor's presence and to determine the percentage of the sample that was tumor. We used only samples that had a minimum of 70% tumor cells. As normal controls, we used cells from peripheral blood or the tip of the seminal vesicle contralateral to the tumor if it had been histologically confirmed to be tumor free.

DNA Extraction. Specimens were processed as described previously (26). Briefly, frozen seminal vesicle or tumor tissue (0.1-0.5 g) was pulverized with a mortar and pestle in liquid nitrogen. The powdered material was thawed in 1 ml lysis buffer (4 M guanidine thiocyanate, 5 mM sodium citrate, pH 7.0, 0.1 M β-mercaptoethanol, and 0.5% sarcosyl). Next, 0.48 g/ml CsCl2 was added, and the lysate was layered on top of 1.2 ml of 5.7 M CsCl2 and 0.1 M EDTA (pH 7.5) in a Beckman SW50.1 polylamellar tube. The tubes were then centrifuged at 35,000 rpm for 20 h at 25°C. High-molecular-weight DNA was recovered by aspirating the viscous interface. The DNA in it was precipitated with ethanol, and the pellet was washed with 70% ethanol, dried, and resuspended in water. DNA extracted from blood leukocytes was done by standard procedures (27).

CA Microsatellite Repeat Amplification Analysis. Fourteen CA microsatellite repeats in the 7q21.3-qter region (28) were amplified in a Thermocycler 9600 (Perkin-Elmer Cetus, Norwalk, CT). The 25-μl reaction mixtures contained 2.5 μl 10X standard PCR buffer, 100 ng DNA, 1 unit Taq polymerase, 400 PM each primer, and 200 μM each deoxynucleotide triphosphate. The hot-start protocol was performed with a Taq polymerase-specific antibody that inactivates the enzyme and is released during the first denaturation cycle (Taq Start, Clontech, CA). The DNA was amplified for 27 cycles of 20 s of denaturation at 94°C, 30 s of annealing at 50°C, and 15 s of extension at 72°C. The number of cycles used was determined to be in the linear part of the
amplification process, i.e., before product saturation, permitting us to assume that equal amounts of both alleles would be amplified if no LOH had occurred.

The PCR products were separated in a 3.5% Metaphor agarose (FMC Bioproducts, Rockland, ME) gel at 5.5 V/cm for 3 h in Tris-borate EDTA buffer (89 mM Tris-borate, 89 mM boric acid, and 2 mM EDTA, pH 7.5) with 0.5 mg/ml ethidium bromide in Tris-borate EDTA buffer (89 mM Tris-borate, 89 mM boric acid, and 2 mM EDTA, pH 7.5) with a standard loading buffer (27). The gel was photographed with a Fotodyne 3–4400 UV transilluminator (Fotodyne Inc., New Berlin, WI) and Polaroid Positive-Negative 4 X 5 Instant Film (Polaroid Corp., Cambridge, MA).

**Determination of LOH.** We considered a sample to have LOH if an entire band was absent or if the band was less than 30% as intense as the normal band (29). The signal intensity of fragments was determined by densitometry, by visual examination by two reviewers, or both. Although PCR amplification cannot be considered to be quantitative, we optimized the PCR conditions so that equal amounts of template should have produced equal amounts of amplified product. We used 27 amplification cycles, which we demonstrated to be in the linear part of the amplification process, i.e., before product saturation (data not shown). We also conducted a series of titrations with different proportions of homozygous and heterozygous templates to assess the influence of stromal tissue contamination of our amplification reactions. We determined that equal amounts of both alleles would be amplified if no LOH had occurred.

**Table 1 Chromosome 7 LOH in 16 primary prostate carcinomas**

<table>
<thead>
<tr>
<th>Markers</th>
<th>No. of informative cases (% of total)</th>
<th>No. of cases with LOH</th>
<th>% LOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7S527</td>
<td>8 (50)</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>D7S518</td>
<td>11 (68.7)</td>
<td>3</td>
<td>18.2</td>
</tr>
<tr>
<td>D7S496</td>
<td>8 (50)</td>
<td>2</td>
<td>28.5</td>
</tr>
<tr>
<td>D7S523</td>
<td>7 (43.8)</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>D7S486</td>
<td>6 (37.5)</td>
<td>3</td>
<td>42.8</td>
</tr>
<tr>
<td>D7S633</td>
<td>7 (43.8)</td>
<td>3</td>
<td>42.8</td>
</tr>
<tr>
<td>D7S677</td>
<td>8 (50)</td>
<td>5</td>
<td>62.5</td>
</tr>
<tr>
<td>D7S522</td>
<td>6 (37.5)</td>
<td>5</td>
<td>83.3</td>
</tr>
<tr>
<td>D7S655</td>
<td>14 (87.5)</td>
<td>6</td>
<td>42.9</td>
</tr>
<tr>
<td>D7S480</td>
<td>11 (68.7)</td>
<td>3</td>
<td>27.7</td>
</tr>
<tr>
<td>D7S490</td>
<td>6 (37.5)</td>
<td>1</td>
<td>16.2</td>
</tr>
<tr>
<td>D7S487</td>
<td>8 (50)</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>D7S498</td>
<td>4 (25)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D7S550</td>
<td>8 (50)</td>
<td>1</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Fig. 1. Representative PCR amplifications of DNA of prostate carcinoma. The case numbers are shown at the top of the lanes; T and N, matched DNA samples isolated from tumor tissue and peripheral leukocytes, respectively. A, D7S527; B, D7S522; C, D7S487.

Our results indicated that the loss of part or all of 7q was a common event in prostate cancer. LOH occurred in at least one locus on 7q in 12 (75%) of 16 tumors. As can be seen in Fig. 2, the most frequent marker lost was D7S522 (7q31.1–31.2) (83.3%). Furthermore, the segment flanked by the CA repeats D7S633 and D7S655 (7q22-q31.2), which includes D7S522, was lost in 57.8% of the prostate carcinomas studied.

Analysis of a histogram of the LOH data from our tumor samples by the Kolmorogov-Smirnov test (30) indicated that they were normally distributed, as would be expected from a stochastic process such as the inactivation of a tumor suppressor gene.

Fig. 3 is a schematic representation of the deleted regions of human chromosome 7 in nine cases in which large deletions could be predicted by the molecular techniques used. The probability of three or more allelic losses in the same fragment being caused by independent events is very small, so such a series of LOH in contiguous markers is more likely due to deletion of the entire segment. In most of our samples, these deletions were interstitial. Comparison of the deletions revealed a 1.2 cM SCDR that is flanked by the CA repeats D7S677 and D7S522. Only one case with LOH (42636) had not lost this fragment. In that case, however, a deletion was detected proximal to the SCDR that seems to be unrelated to the inactivation of the TSG.

**Discussion**

Although prostate cancer is the most prevalent neoplasia among men (31), there is very little information on its etiology and mechanisms of progression. LOH of chromosomes 8, 10, 16, and 18 (23–25) has been consistently reported in prostate cancers, suggesting the presence of TSGs on those chromosomes, but none of these genes have been cloned to date. Many reports have indicated that the p53 gene is somehow involved in the progression of prostate tumor toward a metastatic, more malignant phenotype (32–34). A metastasis suppressor gene has been localized in the short arm of chromosome 11 by microcell fusion transfer of human chromosome 11 into a highly metastatic rat prostate cell line (35). Since deletions in the long arm of human chromosome 7 are also a frequent trait of prostate carcinnomas, we studied 16 primary prostate...
Our results not only confirmed and expanded previous reports in prostate cancer, they also supported our other LOH studies of other types of tumors (17). Because deletions of chromosome 7q are a commonly observed cytogenetic event in many different types of tumors (10—16) and because we had functional evidence of a TSG on 7q (18), we studied 7q LOH in breast cancer (17). That tumor type had a very similar pattern of allele loss, with a normal distribution around a peak at CA microsatellite repeat D7S522. The extent of allele loss was 81.8% in primary breast carcinomas. The differences in the percentages of LOH found may be due to different patterns of inactivation of the TSG in different tumor types, or to differences in the amount of stromal contamination in the tumor tissues used.

The high frequency of LOH occurrence at 7q31.1—31.2 in prostate carcinomas shown in this report, a similar pattern of LOH in other types of neoplasias (17), and our repression of tumorigenicity by microcell-mediated transfer of chromosome 7 to a murine SCC cell line (18) indicate that human chromosome 7 harbors a TSG distal to c-met in band 7q31.1—31.2. This TSG seems to be relevant to several types of human neoplasias, as can be inferred by our previous reports of LOH in human primary breast cancer (17) and by cytogenetic evidence of chromosome 7 deletions in other neoplasias (10—16). Extensive work on the identity and function of this putative TSG is presently being conducted in our laboratory.

Fig. 2. Representation of 7q21.3-qter and approximate position of the microsatellite repeats (29). Histogram shows the percentage LOH for each of these microsatellites in the informative prostate samples studied.

carcinomas to determine the extent of LOH on 7q. Our data indicate that 12 (75%) of 16 tumors studied presented LOH at one or more 7q loci. This incidence is higher than that found at other frequently deleted regions in these tumor types (36, 37).

Five (83.3%) of six informative cases had LOH in CA microsatellite repeat D7S522 (7q31.1—31.2). Previous allelotype studies of prostate cancer (23—25) did investigate the long arm of chromosome 7 for LOH and revealed some LOH, but the percentages ranged from 8 to 10%. However, these results do not conflict with our findings because the markers used (one in each study) are several cM away from our SCDR. Moreover, the percentages of LOH we obtained with the markers closest to the ones used by Kunimi et al. (23) and Carter et al. (24) are very similar (12.5% for D7S527 and 18.2% for D7S518) to the percentages obtained by those two groups. In fact, the importance of the 7q LOH is reinforced by the finding by Carter et al. (24), that in one of the cases studied the only allelic aberration found was the loss of the 7q marker.

Fig. 3. Deletions suggested by the allele losses. Shaded columns, region is retained; open columns, loss of the region. Length of any column is arbitrary since we possess no exact information of the precise limits of a determinate deletion. Closed circles over shaded columns, retention of both alleles; open circles over open columns, LOH. Non-informative cases for each marker are symbolized by the absence of circles at that position. SCDR obtained by this analysis is indicated at the right. Case numbers are displayed at the top of each column.
Acknowledgments

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References

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