Loss of Heterozygosity of Chromosome 8 Microsatellite Loci Implicates a Candidate Tumor Suppressor Gene between the Loci D8S87 and D8S133 in Human Prostate Cancer


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Abstract

To search for specific chromosome 8 aberrations in human prostate cancer, DNA was isolated from 44 human prostate tumor samples. Twenty-six tumor samples were obtained from locally progressive tumors by transurethral resection, 12 were from radical prostatectomy specimens, and 6 were from lymph node metastases. Tumor DNAs were screened for allelic losses using 16 highly polymorphic microsatellite loci (14 covering the p arm, 2 on the q arm). In general, the detected deletions were large. In 59% of the tumor DNAs, allelic loss of 3 or more 8p loci was observed. Loss of 8p loci occurred in between 36 and 69% of the informative cases; for the two 8q markers, the percentages of loss were 11 and 25%, respectively, indicating preferential loss of (part of) 8p. In one tumor, two separate 8p deletions were found. The percentage of loss of heterozygosity was considerably higher in transurethral resection (65%) and lymph node metastases (83%) than in radical prostatectomy specimens (33%), suggesting that 8p deletion is a relatively late step in tumor progression. The maximal overlapping deleted region in all tumor DNAs is between the distal locus D8S133 and the proximal locus D8S87, indicating the localization of a candidate tumor suppressor gene within this region.

Introduction

Prostate cancer is the most common cancer diagnosed and the second leading cause of male cancer death in North America, North and West Europe, and Australia (1). Substantial evidence indicates that a positive family history is a risk factor in prostate cancer (2). Thus far, our knowledge of genes which play an important role in the development and the progressive growth of prostate cancer is limited (see Refs. 3–5 for recent reviews). RAS oncogenes and the RB-1 tumor suppressor gene are mutated in a low percentage of prostate carcinomas. Data on p53 mutations are conflicting. On the one hand it has been shown that p53 mutations are infrequent in prostate cancer (6–8), but it has also been documented that a high percentage of (metastatic) tumors is mutated in the p53 gene (7, 9). Recently, more extensive data on chromosomal abnormalities in prostate tumors have become available. Most frequent losses have been found for chromosomes 8, 10, and 16 (10, 11), indicating the localization of candidate tumor suppressor genes on these chromosomes. In a recent study, a homozygous deletion of the MSR locus was found, suggesting a candidate tumor suppressor gene on chromosome 8p22 (12). In the present study we extend previous findings that chromosome 8p harbors a candidate tumor suppressor gene involved in prostate cancer. Our results indicate that deletion of 8p is a relatively late event in tumor progression. Interestingly, our data reveal chromosome 8p12–21 as the commonly deleted region, implicating the presence of at least two tumor suppressor genes on chromosome 8p.

Materials and Methods

Prostate Tumor Tissues. All analyzed tissues were from patients with a confirmed clinical history of prostate cancer. Tissue samples, which were obtained from locally confined tumors by RP, are from regional LM, and from locally progressive tumors by TUR were frozen directly after removal and stored in liquid nitrogen until use. RP and LM tissues were from patients without prior (endocrine) therapy. TUR samples were from patients with (17) or without (9) prior endocrine therapy.

Genomic DNA. Genomic DNA was isolated from 5 consecutive 5-μm cryostat sections according to standard procedures (overnight proteinase K incubation at 37°C, phenol extraction, and ethanol precipitation). DNA was dissolved in 200 μl of 10 mM Tris-HCl, pH 7.8-1 mM EDTA. Flanking tissue sections were stained routinely with hematoxylin and eosin and used to determine the tumor percentage in the DNA sample. Samples with a tumor percentage of 80 or higher were further processed; samples with less tumor were excluded. In the vast majority of cases, control DNA was from WBC. In some cases, control DNA was from nontumor regions in the resected prostate tissue.

Amplification of Microsatellite Loci. LOH was determined by amplification of highly polymorphic microsatellite loci on chromosome 8. The loci investigated are listed in Table 1. The positions of the different loci on the chromosome 8 genetic map are from references cited in Table 1 and from Refs. 19–21. The order of the loci D8S87, D8S283, and D8S339 is not precisely known. Most probably, they are very closely spaced on the genetic map. Sequences of primers are from references listed in Table 1. Standard (multiplex) amplification conditions were 30 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C in a 15-μl reaction volume, essentially as described (22).

Results

DNA was isolated from 44 prostate tumor samples. Twenty-six tumors were from locally progressive tumors (TUR), 6 specimens were from regional LMs, and 12 were from locally confined tumors (RP). DNA samples were analyzed for LOH with the 16 highly polymorphic chromosome 8 microsatellite loci listed in Table 1. Loci D8S255 to D8S262 are located on chromosome 8p; D8S260 and D8S167 are on the q arm. As summarized in Table 1, the highest
CHROMOSOME 8P IN PROSTATE CANCER

Table 1 Allelic loss of chromosome 8 microsatellite loci in prostate cancer

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chromosomal localization</th>
<th>Losses/inform. cases (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>D8S262</td>
<td></td>
<td>9/18 (50)</td>
<td>13</td>
</tr>
<tr>
<td>D8S277</td>
<td></td>
<td>11/26 (42)</td>
<td>13</td>
</tr>
<tr>
<td>D8S265</td>
<td></td>
<td>11/22 (50)</td>
<td>13</td>
</tr>
<tr>
<td>D8S261</td>
<td></td>
<td>17/53 (55)</td>
<td>13</td>
</tr>
<tr>
<td>LPL-GZ214/15</td>
<td>8q22</td>
<td>14/29 (48)</td>
<td>14</td>
</tr>
<tr>
<td>D8S282</td>
<td></td>
<td>13/27 (48)</td>
<td>13</td>
</tr>
<tr>
<td>D8S298</td>
<td></td>
<td>18/30 (60)</td>
<td>13</td>
</tr>
<tr>
<td>D8S133</td>
<td></td>
<td>16/29 (57)</td>
<td>15</td>
</tr>
<tr>
<td>D8S136</td>
<td></td>
<td>16/28 (58)</td>
<td>20</td>
</tr>
<tr>
<td>D8S137</td>
<td></td>
<td>16/23 (69)</td>
<td>16</td>
</tr>
<tr>
<td>D8S87</td>
<td></td>
<td>9/24 (37)</td>
<td>17</td>
</tr>
<tr>
<td>D8S265</td>
<td></td>
<td>11/28 (39)</td>
<td>13</td>
</tr>
<tr>
<td>D8S539</td>
<td></td>
<td>10/28 (36)</td>
<td>18</td>
</tr>
<tr>
<td>D8S255</td>
<td></td>
<td>10/28 (36)</td>
<td>13</td>
</tr>
<tr>
<td>D8S260</td>
<td></td>
<td>7/28 (25)</td>
<td>13</td>
</tr>
<tr>
<td>D8S167</td>
<td></td>
<td>4/35 (11)</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 2 Frequency of allelic loss on chromosome 8p in primary and metastatic prostate cancer

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of tissues</th>
<th>Allelic losses</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locally confined tumor (RP)</td>
<td>12</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td>Locally progressive tumor (TUR)</td>
<td>26</td>
<td>17</td>
<td>65</td>
</tr>
<tr>
<td>Regional LM</td>
<td>6</td>
<td>5</td>
<td>83</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>26</td>
<td>59</td>
</tr>
</tbody>
</table>

Discussion

In this study we describe the mapping of a commonly deleted region of chromosome 8p in prostate cancer, most frequently in advanced cases. Our data point to a candidate tumor suppressor gene located between the loci D8S87/D8S283 and D8S133 on chromosome 8p12-21. The length of the genetic interval between these two loci is not precisely known. In earlier genetic maps an estimated length of 19–36 cM has been reported (19, 20). However, data presented in the most recent Genethon linkage map indicate a size of 17 cM or less (13).

Our data extend the report of chromosome 8p deletions in prostate cancer by Bergerheim et al. (11), as determined with four chromosome 8p RFLP markers. Although only a small number of marker loci was tested, results more recently obtained by Bova et al. (12) and Chang et al. (23) indicated that the commonly deleted region was distal of the LPL locus, which is on chromosome 8p22. A homozygous deletion of the MSR locus suggested that a putative tumor
suppressor gene is located in the proximity of the MSR gene on band 8p22 (12). Importantly, our data (see Fig. 2) show that a candidate tumor suppressor gene is located at a different position, which is proximal to the LPL locus [four tumor samples exclude the MSR region, which is distal to LPL (see Ref. 12)]. Therefore, chromosome 8p most likely harbors two tumor suppressor genes implicated in prostate cancer, one at 8p12–21 as shown here, and one at 8p22 (12). Recently, MacGrogan et al. (24), using 16 chromosome 8p microsatellite markers, reported a commonly deleted region between loci D8S206 (which is distal to MSR) and D8S259 (which is closely spaced to D8S283; see Fig. 2 and Ref. 13). This large genetic interval encompasses the chromosome 8p12, 8p21, and 8p22 regions and could support our observation of a candidate tumor suppressor gene on chromosome 8p12–21.

Chromosome 8p deletions have not only been found in prostate cancer but also in colorectal, bladder, hepatocellular, and lung carcinomas (25–29). Similarly to what we postulate in this report for prostate cancer, at least two different tumor suppressor genes on chromosome 8p are supposed to be involved in colorectal carcinoma (26, 28). Although different marker loci have been used, which makes a direct comparison difficult, one of these regions most likely overlaps with the D8S87 to D8S133 interval as determined in our study. So, possibly one and the same tumor suppressor gene can be implicated in a high percentage of prostate and colorectal carcinomas.

The D8S87 to D8S133 interval is expected to contain many hundreds of genes. Only a few of these have been identified up till now. Genes of potential interest which are presumed to be located in this area are the CLU (clusterin) and BMP1 (bone morphogenetic protein 1) genes (30). Their structural analysis in prostate cancer DNA is currently under investigation. It is intriguing that in both colorectal and prostate carcinoma, mutations have been described in the POLB (DNA polymerase β) gene (31, 32), which has been assigned to chromosome 8p11–12 (20). However, the POLB locus did not show allelic loss in a small series of colorectal carcinomas investigated by RFLP analysis (28). Combined with the relatively small number of mutations found in prostate cancer (2 of 12), this might rule out a role of POLB mutations in the majority of cases with chromosome 8p deletions. On the other hand, these findings warrant a further investigation of the precise position of the POLB gene on the chromosome 8 linkage map, which is presumed to be at chromosome 8p11.2 (33), and its role in human prostate carcinoma.

Acknowledgments

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