Identification and Characterization of Proximal 6q Deletions in Prostate Cancer

Kathleen A. Cooney, Jon C. Wetzel, Christina M. Consolino, and Kirk J. Wojno

Abstract

Allelic loss at 6q14—21 occurs in a significant percentage of sporadic prostate cancers and could be implicated in prostate carcinogenesis based on the identification of common regions of deletion.

Materials and Methods

Patient Material. Paired normal and tumor samples from radical prostatectomies were analyzed. Microdissection techniques were used to isolate tissue containing at least 70% normal or tumor nuclei as described previously. All cases were staged according to the American Joint Commission criteria. (16) For LOH determination, tumors obtained from radical prostatectomy samples were analyzed for loss of heterozygosity (LOH) using 10 polymorphic markers spanning chromosome 6 including one marker on 6p and nine markers on 6q. Markers were selected from databases, and a comprehensive linkage map was constructed. By this analysis, LOH for one or more polymorphic markers was detected in 17 of 52 sporadic prostate cancer cases (33%).

Results and Discussion

LOH of Proximal 6q Occurs in a Significant Percentage of Prostate Cancers. LOH was detected with one or more polymorphic markers on 6q in 21 of 31 primary prostate cancers studied. Two experienced observers (K.A.C. and J.C.W.) reviewed the slides, and all cases were staged according to the American Joint Commission criteria. (16) The prevalence of LOH in sporadic prostate cancer was significantly higher than that in benign prostatic hyperplasia (BPH) (P < 0.05).

LOH analysis DNA was amplified by PCR using a panel of nine microsatellite markers spanning the length of 6q and one marker on proximal 6p (Table 1). For PCR, one primer of each primer pair was 5'-end-labeled with [32P]dCTP using T4 polynucleotide kinase. PCR products were electrophoresed on 4.9% denaturing polyacrylamide gels at 65 W. Gels were exposed to film at —80°C for 2—20 h. Each PCR reaction was performed twice and scored visually for LOH

1 The abbreviations used are: LOH, loss of heterozygosity; PSA, prostate-specific antigen.

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distinct patterns of allelic loss of chromosome 6q markers are shown in Fig. 2.

Chromosome 6q allelic losses have not previously been well studied in prostate cancer. Kunimi et al. (20) examined 18 prostate cancers by Southern analysis using a panel of polymorphisms throughout the genome. They observed no evidence of allelic loss using a polymorphism in the MYB gene at 6q22—q23 in any of 6 informative cases. By comparative genomic hybridization, however, Visakorpi (6) described allelic loss in 7 of 31 primary prostate cancers (22%) and 4 of 9 recurrent cancers (44%). Review of the pattern of loss detected in their study reveals deletions involving proximal 6q, with the minimal overlapping region extending from 6cen—q21 in both primary and recurrent cancers. Our analysis has confirmed their initial observation of proximal 6q deletions occurring in approximately one-third of prostate cancers and has narrowed the interval for the location of a potential tumor suppressor gene to 6q14—q21 with evidence for a second potential locus at 6q16.3—q23.2.

Allelic loss of various regions of 6q has been observed in several other types of cancer. A region of deletion involving proximal 6q similar to that identified in prostate cancer has been described in ovarian (12, 13) and breast (9, 10) cancer. A translocation breakpoint in a human melanoma has been localized to 6q14—21 (21), which has narrowed the candidate region for a putative tumor suppressor gene important in melanoma from a much larger region identified by LOH (7). Biological evidence for a tumor suppressor gene in this region can be derived from microcell-mediated transfer studies demonstrating alterations in cell growth and/or metastatic potential of melanoma cell lines with introduction of all or part of chromosome 6 (22—24).

Interestingly, linkage of hereditary mixed polyposis syndrome has recently been reported to 6q with the highest LOD score attained using D6S283 (25), which flanks the major region of LOH in prostate cancer as determined in this current study. Patients affected with this autosomal dominant disorder have a large number of characteristic colonic polyps, colonic adenomas, and colorectal carcinomas. An increased risk of prostate cancer has not, however, been recognized in this syndrome. Whether this gene plays a role in sporadic forms of colorectal cancer is also unknown.

**Distal 6q Deletions Appear to Be Uncommon in Prostate Cancer.** LOH analysis has identified a common region of deletion involving 6q25—27 in ovarian cancer (11, 14). Additionally, the M6P/IGF2R gene at 6q26—27 has been proposed to function as a tumor suppressor gene in the development of hepatocellular cancer from the observations that (a) 60—70% of hepatocellular cancers have LOH at this locus (26) and (b) 25% of the tumors with LOH have a mutation in the remaining allele (27). Only 2 of 52 prostate tumors (4%) in our study had evidence of allelic loss with markers on distal 6q (Fig. 1). Tumor 372 demonstrated LOH with D6S264 and allelic retention at IGF2R. Similarly, tumor 380 had LOH at IGF2R (Fig. 2). Given the low frequency of allelic loss in prostate cancers at IGF2R and D6S264, it is unlikely that IGF2R or other tumor suppressor genes on distal 6q play a role in prostate carcinogenesis.

**Correlations of 6q Deletions with Clinical Features of Prostate Cancer.** The 52 prostate cancer samples used in this study were obtained from patients undergoing radical prostatectomy. Consequently, the average age of these patients was slightly younger than the average age of at diagnosis for all prostate cancer patients (72 years; Ref. 28), and complete pathology information was available for all cases. There was no difference in the average age of patients with or without 6q LOH (64.2 versus 62.3 years; Student’s t test; P = 0.41). Similarly, the presence of 6q deletion did not correlate with tumor stage (T2 versus T3 and T4 tumors) or grade (combined Gleason scores =6 versus ≥7; χ² analysis; P > 0.05). Finally, there was also no relationship between 6q loss and seminal vesicle involvement (Fisher’s exact test; P = 0.71).

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**Table 1. Linkage order for chromosome 6 markers**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Source</th>
<th>Location</th>
<th>Heterozygosity</th>
<th>cM</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6S430</td>
<td>Généthon</td>
<td>6p21.2—cen</td>
<td>0.88</td>
<td>7.4</td>
</tr>
<tr>
<td>D6S286</td>
<td>Généthon</td>
<td>6q14.3—q15</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>D6S251</td>
<td>Marshfield</td>
<td>6q14—16.2</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>D6S1082</td>
<td>CHLC</td>
<td>6q16.3—21</td>
<td>0.85</td>
<td>11.2</td>
</tr>
<tr>
<td>D6S501</td>
<td>CHLC</td>
<td>6q16.3—23.2</td>
<td>0.74</td>
<td>4</td>
</tr>
<tr>
<td>D6S283</td>
<td>Généthon</td>
<td>6q16.3—27</td>
<td>0.81</td>
<td>3.8</td>
</tr>
<tr>
<td>D6S404</td>
<td>Généthon</td>
<td>6q16.3—q27</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>D6S314</td>
<td>Généthon</td>
<td>6q25.2—q27</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>D6S264</td>
<td>Généthon</td>
<td>6q25.2—q27</td>
<td>0.71</td>
<td></td>
</tr>
</tbody>
</table>

* The relative distances between the first eight markers were calculated using the software program MultiMap (17) and are provided in Kosambi cM. The order of D6S286 and D6S251 cannot be determined by this analysis (see "Materials and Methods.").

The distance between D6S14 and D6S264 was estimated from mapping information provided in the Généthon database (18).
Follow-up PSA values are available on 49 of 52 patients with an average observation time of 8 months since the date of the radical prostatectomy. Ten of 48 patients (21%) have evidence of residual and/or recurrent disease defined as a PSA elevation of ≥0.1 ng/ml or PSA detectable above the assay limit. Four of these 10 patients (patients 372, 396, 532, and 544) also have 6q allelic loss. Visakorpi (6) noted an increase in the percentage of cases with 6q loss between primary prostate cancers and recurrent tumors (22 versus 44%), suggesting that 6q loss may be a late event in the transition from normal prostatic epithelium to cancer. More recently, Cher et al. (29) reported 6q loss in 39% of prostate cancer metastases, including both untreated and androgen-unresponsive cases. Continued follow-up of our cohort of patients may clarify whether deletion of chromosomal regions on 6q predicts poor outcome following definitive surgical therapy.

In conclusion, we have demonstrated evidence of 6q loss in 17 of 52 primary prostate cancers (33%). Thirteen tumors had evidence of a common overlapping region of deletion containing markers D6S1082 and D6S501, with evidence for a potential second locus at D6S404. Finaly, tumor 380 shows allelic loss at IGF2R, with allelic retention proximally at D6S314.

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**References**


28. Mettlin, C. J., and Murphy, G. The National Cancer Data Base report on prostate
29. Cher, M. L., Bova, G. S., Moore, D. H., Small, E. J., Carroll, P. R., Pin, S. S., Epstein,
J. I., Isaacs, W. B., and Jensen, R. H. Genetic alterations in untreated metastases and
androgen-independent prostate cancer detected by comparative genomic hybridiza-
30. Lew, D. J., Dulic, V., and Reed, S. I. Isolation of three novel human cyclins by rescue
31. Leopold, P., and O’Farrell, P. H. An evolutionarily conserved cyclin homolog from
32. Demetrick, D. J., Matsumoto, S., Hannon, G. J., Okamoto, K., Xiong, Y., Zhang, H.,
and Beach, D. H. Chromosomal mapping of the genes for the human cell cycle
proteins cyclin C (CCNC), cyclin E (CCNE), p21 (CDKN1) and KAP (CDKN3).
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, 32:
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