Identification and Characterization of Proximal 6q Deletions in Prostate Cancer

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

Advances in Brief

Abstract

Allelic loss of 8p, 10q, 13q, 16q, and 18q has been frequently demonstrated in prostate cancer, implying the existence of putative tumor suppressor genes in these regions. However, there are likely a number of additional genetic events that define the progression from normal prostatic epithelium to prostate cancer that have yet to be identified. To characterize a novel region of deletion in sporadic prostate cancers, 52 tumors obtained from radical prostatectomy cases 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 available 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%). Thirteen of 17 tumors were shown to have a common region of allelic loss extending from D6S286 to D6S283 or 6q14—21, with a minimum region of loss containing markers D6S1082 and D6S501. A second separate region of deletion centered around marker D6S404. LOH of one or more 6q markers did not correlate with Gleason grade or pathological stage of the disease.

Introduction

LOH studies have been used to identify chromosomal regions containing putative tumor suppressor genes that contribute to the development of malignancy. The regions most frequently studied by this type of approach in prostate cancer include 8p (1—3) and 10q (4, 5). However, there are likely a number of additional genetic events that contribute to the development of prostate cancer that have not yet been identified. Visakorpi et al. (6) used comparative genomic hybridization to analyze 31 primary prostate cancers. They reported that the most common regions of chromosomal deletion were located on 8p (32%), 13q (32%), 6q (22%), 16q (19%), 18q (19%), and 9p (16%). Allelic loss of 6q has been described in a number of common cancers including melanoma (7), breast (8—10), ovarian cancer (11—14). There are several candidate genes on 6q that may contribute to prostate carcinogenesis, including the receptor for the insulin-like growth factor II (IGF2R) at 6q26, implicated in the genesis of hepatocellular cancers, and cyclin C (CCNC), a cell-cycle regulatory protein, at 6q21. Taken together, these studies suggest that one or more tumor suppressor genes on 6q may play a role in several common forms of cancer. This current study was undertaken, therefore, to determine whether 6q deletions occur in a significant proportion of prostate cancers and whether any specific candidate genes 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 performed at the University of Michigan Medical Center are frozen in liquid nitrogen and stored at −70°C for future studies. Fifty-two cases were chosen for analysis based on the availability of adequate specimens. Microdissection techniques were used to isolate tissue containing at least 70% normal or tumor nuclei as described previously (15). All cases were staged without consideration of margin status using the standard TNM criteria according to the American Joint Commission on Cancer (16) on wholly embedded prostate specimens. Statistical calculations were performed using the software package Statistica for Windows, Version 4.5 (Statsoft, Inc., Tulsa, OK).

Linkage Map. Polymorphic microsatellite markers were selected from available databases with the following characteristics: (a) maximal heterozygosity value >0.70; and (b) favorable allele distribution for LOH interpretation using PCR-based techniques (Table 1). A comprehensive linkage map was subsequently constructed for the chosen markers using the computer program MultiMap (ftp://chimera.gene.cwru.edu/multimap/; Ref. 17; from centromere to telomere): D6S430—D6S286/D6S251—D6S1082/D6S501—D6S283—D6S404—D6S314. The order of markers D6S286 and D6S251 could not be determined by this analysis and are likely <1 cM apart. The recombination distance between markers D6S314 and D6S264 was estimated to be 36 cM based on information from Généthon (18). The IGF2R polymorphism contains a tetranucleotide deletion/insertion polymorphism and a dinucleotide polymorphic repeat in the 3′-untranslated region of the IGF2R gene (19).

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 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 using the standard TNM criteria according to the American Joint Commission on Cancer (16) on wholly embedded prostate specimens. Statistical calculations were performed using the software package Statistica for Windows, Version 4.5 (Statsoft, Inc., Tulsa, OK).

Results and Discussion

LOH of Proximal 6q Occurs in a Significant Percentage of Prostate Cancers. LOH was detected with one or more polymorphic 6q markers in 17 of 52 prostate cancer cases (33%; Fig. 1). Eleven of the 17 tumors had evidence of LOH with more than one adjacent polymorphic marker; however, none of the tumors examined had evidence of loss of the entire chromosome. Thirteen of 17 tumors demonstrated a common region of allelic loss extending from D6S286 to D6S283 or 6q14—21, with a minimal region of allelic loss containing markers D6S1082 and D6S501 (Fig. 1). Six tumors also showed evidence of allelic loss with marker D6S404. Three of these six tumors with LOH at D6S404 had evidence of retention proximally at D6S283 and distally at D6S314 (tumors 450, 540, and 554). This data suggests that there may be a second potential locus important in prostate cancer at 6q16.3—23.2. Representative tumors demonstrating...
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), which has narrowed the candidate region for a putative tumor suppressor gene in melanoma from a much larger region identified by LOH (21). Interestingly, linkage of hereditary mixed polyposis syndrome has recently been reported to 6q with the highest LOD score attained in Fig. 2.

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).

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>11.2</td>
</tr>
<tr>
<td>D6S1082</td>
<td>CHLC</td>
<td>6q16.3–21</td>
<td>0.85</td>
<td>4</td>
</tr>
<tr>
<td>D6S501</td>
<td>CHLC</td>
<td>6q16.3–23.2</td>
<td>0.74</td>
<td>3.8</td>
</tr>
<tr>
<td>D6S283</td>
<td>Généthon</td>
<td>6q16.3–q27</td>
<td>0.81</td>
<td>22.4</td>
</tr>
<tr>
<td>D6S314</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 D6S314 and D6S264 was estimated from mapping information provided in the Généthon database (18).

* 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.”).
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 6q16.3–q23.2. One potential candidate gene is this region is CCNC, which encodes the cell cycle regulatory protein cyclin C (30, 31). The CCNC gene, localized to 6q21 by fluorescent in situ hybridization (32), was recently shown to be deleted in 12 of 13 cases (92%) of childhood acute lymphoblastic leukemia with concurrent cytogenetic abnormalities of chromosome 6 (33). Analysis of prostate cancer specimens with additional polymorphic markers will better define the deleted region containing one or more potential tumor suppressor genes and clarify whether CCNC or other genes located on 6q are important in prostate carcinogenesis.

Acknowledgments

We thank William Nichols and David Siemieniak for assistance with the use of MultiMap Software. We also acknowledge Elizabeth Petty, Jill Macoska, and Kenneth Pienta for critical review of the manuscript.

References


4152
Identification and Characterization of Proximal 6q Deletions in Prostate Cancer

Kathleen A. Cooney, Jon C. Wetzel, Christina M. Consolino, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/18/4150

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.