Distinct Areas of Allelic Loss on Chromosomal Regions 10p and 10q in Human Prostate Cancer

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Abstract

Utilizing tissue microdissection and PCR techniques, we have examined 35 prostate tumors paired with normal tissues from the same patients for allelic loss at 24 polymorphic loci spanning chromosome 10. Twenty-five tumors (71%) were deleted for at least one chromosome 10 locus. Of the total 35 tumors, 6 (17%) were deleted for 10p loci only, 5 (14%) for 10q loci only, and 14 (40%) were deleted for both 10p and 10q loci. The common region of deletion on 10p included loci D10S211-D10S89-D10S111. Fluorescence in situ hybridization of yeast artificial chromosome probes encompassing these loci demonstrated that the 10p region of deletion maps to 10p11.2. Losses involving 10p loci alone were most common in localized (5/14, 36%) and least common in metastatic (0/8) tumors. The common region of deletion on 10q included loci D10S219-D10S215, consistent with the major region of deletion recently defined for prostate tumors on 10q. Losses involving 10q loci alone were lowest in localized and locally invasive tumors (1/14 and 2/12, respectively) and highest in tumors metastatic to regional lymph nodes (2/8). These results suggest that 10p losses may define less invasive tumors, whereas 10q losses may play a role in the progression to more advanced tumor states in the prostate. Furthermore, this is the first report of allelic loss of a defined region on 10p potentially harboring tumor suppressor gene loci in human prostate cancer.

Introduction

Deletion of chromosome 10 sequences, especially of those localized to 10q, occurs frequently in prostate tumors. Deletions of 10q22-q24 in short-term cultures of primary prostate carcinomas have been described cytogenetically (1-3). Molecular biological data has confirmed and expanded these cytogenetic findings. Several studies described relatively high frequencies (20-50% of informative cases) of 10q sequence losses in prostate tumors (4-8). Recently, Gray et al. (9) narrowed the region of deletion on 10q to the 10q23-q24 boundary. Loss of 10p sequences has been examined to a more limited extent. Although one study failed to find loss of 10p sequences in prostate tumors, other studies have reported loss of 10p sequences, often in conjunction with deletion of 10q sequences (4, 5, 8).

Taken together, these studies suggest that a tumor suppressor gene localized to 10q23-q24 and perhaps another gene localized to 10p are frequently inactivated in prostate tumors. The goal of the present study was to determine whether a discrete region on 10p, in addition to the major region on 10q, was specifically deleted in prostate tumors. To accomplish this aim, retention or loss of highly polymorphic microsatellite sequences at 24 loci (13 mapped to 10p and 11 to 10q) was examined in 35 prostate tumors to physically map potential regions of deletion. These studies confirmed the location of a major region of deletion on 10q, and identified another major region of deletion on 10p at 10p11.2. Loss of the region at 10p11.2 appeared to occur independently of loss at 10q and was not associated with monosomy for chromosome 10. This is the first study to show frequent, independent deletion of 10 p sequences in prostate tumors and to physically map this domain within a 4-7 cM region of band 10p11.2 inclusive of loci D10S211, D10S89, and D10S111.

Materials and Methods

Tissue Characterization. Prostate tissue was obtained after radical prostatectomy from 35 patients diagnosed with prostate cancer. Tumor pathological stage, degree of differentiation (combined Gleason score), and the ethnic composition of the patient population are detailed in Fig. 1. After an initial pathological evaluation of radical prostatectomy tissue, presumed malignant and normal tissue were snap frozen in liquid nitrogen and stored at −70°C. Tumor specimens comprising areas of at least 70% malignant cells and normal specimens comprising normal or hyperplastic epithelium were physically sectioned. One section was stained with H&E to define areas of discrete tumor and normal tissue were snap frozen in liquid nitrogen and stored at −70°C.

Analysis of DNA for Allelic Loss. PCR amplification assays targeted 24 sequences containing highly polymorphic microsatellite repeats at loci of interest on chromosome 10: 13 loci that map to 10p, and 11 loci that map to 10q. The linkage order of these markers has been reported as: pter-D10S189-D10S570-D10S226-D10S191-D10S674-D10S211-D10S89-D10S111-D10S611-D10S213-D10S208-D10S675-D10S176-centromere-D10S469-D10S141/D10S196 - D10S202 -D10S219 -D10S215-D10S91-D10S198-D10S608/D10S670-D10S187 (where / indicates markers of uncertain relative order; Fig. 1). In addition, polymorphic microsatellite sequences at the F8VWF locus (12pter-p12) were amplified for use as unlinked dosage controls. Primer sequences, additional linkage and contig information, and genetic mapping information were obtained from public databases maintained by the Human Genome Data Base (http://www.gdb.org/), Center for Genome Research at the Whitehead Institute for Biomedical Research (http://www-genome.wi.mit.edu/), and Cooperative Human Linkage Center (http://www.chlc.org/) as accessed through the Internet.

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Mapping of Chromosomal Sequences Using FISH Techniques. FISH was performed essentially as described previously (12, 13). Briefly, human metaphase chromosomes prepared from normal peripheral lymphocytes were G-banded and examined. Suitable metaphase chromosomes were localized to specific x,y microscopic coordinates and photographed. Three hundred ng each of Genehong YAC probe DNAs (746-G-7, 746-D-9, and 965-D-10) were labeled with biotin, combined with 1 µg Co, DNA in 50% formamide, 2X SSC (20X SSC is 3 M sodium chloride and 0.3 M sodium citrate), and 10% dextran sulfate, and hybridized separately to denatured chromosomes at 37°C overnight. Probe signals were detected with fluorescein-conjugated avidin D and biotinylated avidin D (sometimes with amplification) after sequential washes at 42°C in 50% formamide/2X SSC, 48°C in 2X SSC, and 48°C in PN buffer (0.1 M sodium phosphate and 0.1% NP40, pH 8.0). After counterstaining with propidium iodide and visualization on a fluorescence microscope using a triple band pass filter, metaphases localized to the same x,y coordinates as previously photographed G-banded chromosomes were identified and photographed. Probe localizations were then assigned based on comparison of probed and G-banded chromosomes from the same metaphases for 10 cells/probe. In addition, dual FISH was performed with YACs 746-D-9 and 965-D-10 (rhodamine and fluorescein antidigoxygenin detection, respectively, with DAPI counterstaining) to double check localizations.

Results

Frequency and Distribution of Allelic Losses on Chromosome 10

Of the 35 tumors examined, 25 (71%) demonstrated loss of at least one locus on chromosome 10. Six tumors (6/35, 17%) demonstrated loss of 10p loci only, 14 (40%) of 35 tumors were characterized by loss of both 10p and 10q loci, and 5 tumors (5/35, 14%) displayed loss of 10q loci only. These data are shown schematically in Fig. 1 and summarized in Table 1. None of the tumors demonstrated allelic loss at the chromosomal region 12p locus F8VWF, used as an unlinked dosage control (Fig. 1).

Physical Mapping of 10p Allelic Losses

Of the 20 tumors with loss on 10p (6 with loss of 10p loci only and 14 with loss of 10p and 10q sequences), 12 sustained losses involving adjacent loci D10S211, D10S89, and/or D10S111. The remaining nine tumors demonstrated losses at one or more of nine other 10p loci, the most frequent comprising loss at the locus D10S570 (4/19 tumors, or 21%). Allelic loss was not observed in any tumors at two 10p loci, D10S213 and D10S208. Thus, the highest concentration of allelic losses on 10p involved adjacent loci D10S211-D10S89-D10S111,
Table 1 Summary of 10p and 10q sequence losses

<table>
<thead>
<tr>
<th>Allelic loss</th>
<th>Combined Gleason score</th>
<th>Pathological stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.9</td>
<td>7</td>
</tr>
<tr>
<td>10p only</td>
<td>0/10</td>
<td>5/19 (28)</td>
</tr>
<tr>
<td>10p + 10q</td>
<td>6/10 (60)</td>
<td>5/18 (28)</td>
</tr>
<tr>
<td>10q only</td>
<td>2/10 (20)</td>
<td>2/18 (11)</td>
</tr>
<tr>
<td>None</td>
<td>2/10 (20)</td>
<td>7/18 (39)</td>
</tr>
</tbody>
</table>

- *Stage information not available for one tumor with 10p + 10q loss.
- Allelic loss refers to deletion of sequences within the 10p or 10q chromosomal regions, and is represented as the number of tumors with loss/total number of tumors per category.
- LN: tumors metastatic to regional lymph nodes; SV: tumors invasive to seminal vesicles; EPE: tumors that have extended through the prostatic capsule; LOC: tumors localized to the prostate.
- Numbers in parentheses, percentage of frequencies of loss.

Chromosomal Localization of Sequences Deleted on 10p

To determine the chromosomal localization of the majority of 10p allelic losses, YACs were chosen that contained cloned human DNA specific to the D10S211 (Genethon YACs 746-D-9 and 965-D-10) and D10S89 (Genethon YAC 746-G-7) loci. The specificity of the YACs was verified by PCR using primers for microsatellite sequences at the D10S211 locus with YACs 746-D-9 and 965-D-10 and for the D10S89 locus with YAC 746-G-7 (data not shown). The YACs were fluorescently labeled and hybridized to human metaphase chromosomes. These experiments localized all three YACs to chromosome band 10p11.2, indicating that both the D10S211 and D10S89 loci mapped to 10p11.2. Localization of YAC 746-G-7 to 10p11.2 is shown in Fig. 3. Since the D10S211, D10S89, and D10S111 loci map within the same YAC contig (contig WC-10.2, as defined by the Whitehead Institute/MIT Center for Genome Research), it may be assumed that all three loci, which account for 63% of all 10p loss, map to 10p11.2. Current genetic mapping information suggests that the D10S211-D10S89-D10S111 loci span a 4–7 cM region within 10p11.2. Therefore, the major region of 10p loss lies within a 4–7 cM domain of band region 10p11.2.
Physical Mapping of 10q Allelic Losses

Ten of the 19 tumors (53%) with loss on 10q (5 with loss on 10q alone and 14 with loss of 10q and 10p sequences) sustained losses involving locus D10S219 (Figs. 1 and 2B). Four of the 10 tumors deleted at D10S219 were also deleted at D10S215, and 4 additional tumors were deleted at D10S215 but not D10S219. Therefore, a total of 14 (78%) of 18 tumors deleted for sequences on 10q were deleted at adjacent loci D10S219-D10S215. The remaining four tumors demonstrated losses at one or more of nine other loci, the most frequent comprising six losses at nonadjacent locus D10S196 (6/17, or 35%). No allelic loss was observed at locus D10S608. D10S219 lies approximately 8 cM proximal to D10S215 on the Cooperative Human Linkage Center framework map, and D10S215 lies within the most frequently deleted region on 10q in prostate tumors as recently mapped by Gray et al. (9). Therefore, the observed high frequency of allelic losses involving loci D10S219-D10S215 is consistent with recent physical mapping of the most frequently deleted region on 10q in human prostate cancers.

Correlation of 10p and 10q Sequence Losses with Tumor Grade, Stage, and Patient Ethnic Origin

Tumor Grade and Stage. No positive statistical correlation between 10p loss or 10p + 10q loss was observed with the combined Gleason score (tumor grade). A trend toward decreasing 10p loss (as a sole event) with tumor stage was observed that approached statistical significance (P = 0.068). Localized tumors demonstrated the highest frequencies of 10p loss (5/14, 36%) compared to tumors invasive to the seminal vesicles or prostatic capsule (1/12, 8%) or tumors metastatic to regional lymph nodes (0/8) (Table 1). Conversely, a trend toward increasing 10q loss (as a sole event) with tumor stage was observed. Tumors metastatic to regional lymph nodes demonstrated the highest frequencies of 10q loss (2/8 tumors, or 25%) compared to tumors invasive to seminal vesicles or the prostatic capsule (2/12, or 17%) or tumors localized to the prostate (1/14, or 7%; Table 1).

Patient Ethnic Origin. Of the 35 tumors examined, 17 were derived from African American and 18 from Caucasian prostate cancer patients (Fig. 1). No correlation was observed between the ethnic origin of the prostate cancer patient and the status of 10p or 10q retention or loss in the tumors.

Discussion

In this study, 71% of 35 tumors examined demonstrated loss of at least one locus on chromosome 10. The observed high frequency of deletion of chromosome 10 loci suggests that these events contribute to prostate tumorigenesis. Both 10p-specific and 10q-specific deletions were observed.

Twenty of the 35 tumors examined exhibited deletion of 10p-specific sequences. Few cytogenetic or molecular genetic studies have reported rearrangements or deletions involving 10p sequences, and no study has precisely defined a common region of deletion on 10p in prostate or other cancers (4, 5, 8, 14–16). Our study localized a common region of deletion on 10p to loci D10S211-D10S89-D10S111, lost in 12 (60%) of 20 tumors with deletions on 10p. All three loci map within the same YAC contig (WC-10.2, as reported by the Center for Genome Research at the Whitehead Institute for Biomedical Research), and FISH analysis of YACs encompassing D10S211 and D10S89 sequences localized them to 10p11.2. Therefore, the common region of deletion on 10p maps to band 10p11.2 and includes loci D10S211-D10S89-D10S111. Current genetic mapping information suggests that the D10S211-D10S89-D10S111 loci span a 4–7 cM region within 10p11.2. Interestingly, our study also suggests that this region may comprise one or more deletional domains, since some tumors appeared to be deleted exclusively at D10S211 or D10S89-D10S111. Finer physical mapping should reveal the exact number and extent of deletional domains mapping to 10p11.2 in prostate tumors and permit isolation and characterization of putative tumor suppressor genes that map to this region.

Of the 20 tumors demonstrating loss of 10p sequences, 6 exhibited loss of 10p sequences as sole events. Most of the losses involving 10p sequences as sole events (5/6 tumors) occurred in tumors confined to the prostate, suggesting that these events contribute toward a tumorigenic but noninvasive malignant pathway.

Fourteen of the 19 tumors exhibiting 10q losses demonstrated allelic loss of adjacent D10S219-D10S215 loci, suggesting that these loci comprise the major region of 10q deletion. These observations are consistent with those reported by Gray et al. (9), which mapped D10S215 within the most frequently deleted region on 10q in prostate tumors near the 10q23–24 boundary. These observations are also consistent with studies reporting karyotypic abnormalities involving chromosomal band 10q24 in short-term cultures of prostatic tumors (1–3, 17), and molecular studies localizing allelic deletions to band region 10q23–25 in prostate and other cancers (4–9, 15, 16, 18, 19).

Of the 19 tumors demonstrating loss of 10q-specific sequences, 5 exhibited loss of 10q sequences as sole events. Of these five tumors, two were metastatic to regional lymph nodes, two were invasive to seminal vesicles, and one was localized to the prostate. Moreover, the majority of all tumors demonstrating 10q losses, either as sole events or in conjunction with 10p sequence losses, were metastatic or invasive (13/20, or 65%) rather than localized (5/14, or 36%). This suggests that 10q sequence losses may occur later in prostate tumorigenesis and may contribute to an invasive, perhaps metastatic, phenotype. This is consistent with recent work mapping a putative prostate metastasis-suppressor gene within the same major region of 10q deletion reported here (20).

None of the 35 tumors appeared to exhibit monosomy for chromosome 10 as evinced by reduction to homozygosity for all informative loci of the 25 examined. Reports in the literature vary regarding the frequency of monosomy for chromosome 10. Baretton et al. (21) reported monosomy 10 in 40% of the prostate tumors examined, whereas studies by Visakorpi et al. (22), Brown et al. (23), and Macoska et al. (6) reported much lower frequencies, from 0 to 9%. If monosomy for chromosome 10 is a relatively infrequent event, a larger population of tumors than those examined here may be required to observe reduction to homozygosity at all informative chromosome 10 loci. Alternatively, other techniques, such as FISH, might be better suited to address the issue of monosomy 10 in prostate tumors than PCR or Southern blotting.

The current study localizes one or more putative tumor suppressor gene regions within a 4–7 cM region mapping to 10p11.2 and confirms earlier studies identifying another region at 10q23–25. Moreover, loss of 10p sequences is associated with localized disease, whereas loss of 10q sequences appears to correlate with invasive and/or metastatic prostate cancers. Additional studies should allow isolation of these putative tumors suppressor genes, and determine whether assessment of 10p or 10q losses may have prognostic value in the diagnosis and treatment of prostate cancers.

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TUMOR SUPPRESSOR GENE LOCI ON 10p AND 10q IN PROSTATE CANCER

References

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