Distinct Regions of Allelic Loss on Chromosome 4 in Human Primary Bladder Carcinoma

Thomas J. Polascik, Paul Cairns, Wayne Y. H. Chang, Mark P. Schoenberg, and David Sidransky

ABSTRACT

Accumulating evidence implicates the presence of putative tumor suppressor genes on human chromosome 4 that are potentially inactivated in the genesis of several different neoplasms. To accurately determine the frequency of allelic loss on both arms of human chromosome 4, we screened 282 fresh-frozen human bladder carcinomas for allelic loss. Loss of heterozygosity of at least one marker for chromosome 4 was identified in 129 tumors (45.7%). Fine mapping was accomplished using up to 15 polymorphic markers on the p arm and 19 markers on the q arm. We identified a 3-cM minimal area of loss on the p arm between microsatellite markers D4S1608 and D4S404 deleted in 82 tumors (29.0%). A total of 68 tumors (24%) targeted a 14-cM critical region identified on the distal q arm between markers D4S426 and D4S408. Loss of these two regions correlated with advanced stage and grade of the lesions. These data identify distinct regions of loss on chromosome 4 potentially involved in the late progression of bladder carcinoma.

INTRODUCTION

Although the molecular events associated with the initiation and progression of TCC1 have not been fully elucidated, it has become increasingly clear that bladder carcinogenesis involves an accumulation of multiple genetic alterations including inactivation of tumor suppressor genes (1–5). Much attention has been devoted to studying the frequency of inactivation of established or putative tumor suppressor genes on chromosomes 9, lip, 13q, and l7p in human bladder cancer. A recent allelotype of human bladder cancer indicated that allelic loss on the short arm of chromosome 4 was a common molecular event (6). In this study, primarily using restriction fragment length polymorphism analysis, 21.7% of 83 informative bladder tumors displayed LOH on 4p. However, allelic loss on 4q was observed in only a minority of tumors. In contrast, we have identified a high frequency of allelic loss within a defined region on 4q in primary head and neck squamous cell carcinoma.4 Thus, chromosome 4 likely contains several tumor suppressor genes that may be frequently inactivated in TCC. Several reports confirm LOH on chromosome 4 involving other neoplasms including hepatocellular, colorectal, ovarian, cervical, head and neck squamous cell carcinoma, and Hodgkin’s disease (7–13).

We analyzed 282 primary bladder tumors for LOH with fine mapping to determine the frequency of allelic loss on both the p and q arms, to correlate allelic loss with tumor stage and grade, and to construct deletion maps for studies aimed at the isolation of putative tumor suppressor genes within these regions.

MATERIALS AND METHODS

Tissue Specimens and DNA Extraction. Primary bladder tumors were transurethrally resected using diathermy or cold-cup biopsy. A representative section of the tumor was fixed in formalin, stained with hematoxylin and eosin, graded, and staged according to WHO criteria (14) and the TNM classification (15), respectively. The remaining bladder tumor specimens were frozen fresh at −20°C, and macroscopically pure tumor was dissected before extraction. Venous blood from each patient was collected in EDTA and served as control DNA. Paired tumor and leukocyte DNA was extracted as described previously (16).

PCR Amplification and Microsatellite Analysis. The polymorphic dinucleotide primers used for microsatellite analysis were obtained from Research Genetics (Huntsville, AL). Primers were end-labeled with [γ-32P]ATP (DuPont New England Nuclear, Boston, MA) using T4-polynucleotide kinase (New England Biolabs). Fifteen ng of genomic DNA in a total reaction volume of 7.5 µl were subjected to 35 cycles of PCR (12). PCR products were separated by electrophoresis in denaturing 7% polyacrylamide-formamide gels (17), followed by autoradiography. LOH in informative cases was determined if at least a 30% change in ratio was observed between the intensity of one of the tumor alleles and the corresponding control allele.

In the initial screening of 282 bladder tumors, 2 widely spaced highly informative polymorphic markers on both the p (D4S1546, D4S174) and the q (D4S427, D4S171) arms were selected to assess for allelic loss. Additional microsatellite markers mapping close to these four markers were used as necessary to obtain an informative result for every tumor at each locus screened. Physical mapping data was based upon the Genethon map of chromosome 4 (18).

Statistical Analysis. All 282 bladder tumors were analyzed by the \( \chi^2 \) method to elucidate the relationship between LOH and tumor grade and stage.

RESULTS

The initial screening of 282 bladder tumors provided nearly 100% informativity at two widely spaced loci on both the p and q arms. Detailed mapping was accomplished using up to 15 polymorphic markers on the p arm and 19 markers on the q arm. A total of 47 tumors (16.7%) displayed allelic loss of all informative markers, indicating monosomy of chromosome 4. Fig. 1 denotes those tumors defining the minimal area of deletion on the p arm. We identified 2 tumors (T43 and T74) designating the proximal breakpoint at D4S404 and 2 tumors (T74 and T212) defining the distal breakpoint at D4S1608 (Fig. 2a). This minimal area of deletion between D4S1608 and D4S404 encompasses a 3-cM region on the p arm. The critical area may be further defined within a 1-cM region by a single tumor (T43) delineating a southern breakpoint at D4S1593 and another tumor (T212) defining a northern breakpoint at D4S1546. Although this would narrow the region of minimal deletion, one must be cautious in defining a breakpoint based on data derived from one tumor. A total of 35 tumors contained partial allelic loss extending into the minimal area of deletion on the p arm. Because monosomic loss can target either chromosomal arm, the total number of tumors with allelic loss targeting the critical area of deletion on the p arm was 82 (29.0%). Tumor 284 had intervening areas of allelic loss and retention, which is consistent with a complex chromosomal rearrangement (data not shown).

We identified a minimal area of deletion on the q arm between
Fig. 1. Deletion map of chromosome 4p. Minimal area of allelic loss is indicated by the solid vertical bar at the right. Note that markers D4S1608, D4S1546, D4S404, and D4S1551 are closely linked and are each separated by a distance of not more than 1 cM.

**DISCUSSION**

Several investigators have reported LOH on chromosome 4 in a variety of solid tumors, including hepatocellular, colorectal, ovarian, cervical, head and neck squamous cell carcinoma, and TCC, as well as Hodgkin's lymphoma (6-13). Although this implies that a tumor suppressor gene(s) is present on chromosome 4 and is involved in a variety of neoplasms, further conclusions from these studies are limited because of the small number of tumors studied and the lack of any definitive deletion mapping. Additional evidence was provided by

![Deletion map of chromosome 4p](image-url)
Ning et al. (19), who suggested that a gene involved with normal senescence may be located on chromosome 4 because introduction of a wild-type human chromosome 4 into immortal HeLa cells by microcell fusion induced senescence.

The only study to date that mapped the short arm of chromosome 4 identified a 750-kb region at 4p16.3 that was deleted in 14 of 178 (7.8%) bladder tumors and a second common area of loss centromeric to D4S174 involving 7 (3.9%) tumors (20). In that study, Elder et al. (20) identified the most frequent area of deletion at 4p16.3 between markers D4S127 proximally and D4S43 distally. Although their linkage order and relative map positions were based on Locke et al. (21), it appears that this area is very close to the telomere. In our study, we mapped the p telomere with microsatellite markers D4S412 and D4S394. The best available mapping data would place the 750-kb region flanked by D4S127 and D4S43 between markers D4S412 and D4S394. Although a 10-cM distance separates markers D4S127 and D4S394, it is unlikely that we would have missed small deletions located between but not extending beyond these two markers in 282 tumors screened. In contrast, we determined the most common region of deletion to lie near 4p15.1, which is clearly located between and exclusive of both areas of loss reported by Elder et al. (20). It is surprising that 82 of 282 tumors (29%) targeted the critical area in the present study, whereas only 25 of 178 tumors (14%) targeted the 750-kb region between D4S127 and D4S43 in the Elder et al. (20) study.

This study represents the first attempt to deletion map the q arm of chromosome 4 in TCC. We identified a commonly deleted 14-cM region between markers D4S426 and D4S408. In all, 68 tumors (24.1%) targeted this region, including 21 tumors with a selective deletion of the critical area. The high frequency of allelic loss within this area suggests the location of a tumor suppressor gene commonly altered in bladder cancer. Other investigators have identified a significant proportion of LOH on the q arm in cervical, head and neck squamous cell carcinoma, and Hodgkin’s disease, although fine deletion mapping was not undertaken (11–13). In a separate study involving primary head and neck squamous cell carcinomas, we mapped at least one other common area of allelic loss on 4q that was proximal to and exclusive of the critical area identified in TCC. This area was targeted in nearly one-half of the head and neck tumors analyzed.

We identified a potential third area of deletion around the centromere in 11 tumors (3.9%) that did not extend to a critical area on distal p or distal q. This finding is supported by Elder et al. (20), who identified a common region of LOH centromeric to D4S174 in a small number of tumors. We did not further attempt to define this region because of the lower frequency of occurrence and the large area this spans on the chromosome. Because there were more cases of proximal q than proximal p deletion in the present study, the critical region likely maps to proximal q. Perhaps the tumors with centromeric deletions are targeting a tumor suppressor gene implicated in other neoplasms (e.g. head and neck squamous cell carcinoma) but less commonly altered in bladder cancer.

The role of chromosome 4 in the initiation or progression of TCC has not been well studied, despite having a high frequency of allelic loss. We believe that LOH on chromosome 4 is a late genetic event involved with the progression rather than the initiation of bladder carcinogenesis, based on two observations: (a) allelic deletions on chromosome 4 occur with greater frequency in lesions of advanced stage and grade (Table 1); and (b) allelic imbalances were often more difficult to interpret when compared to microsatellite analysis of the same tumors on chromosomes 9 and 14 (22–23). This suggests that several tumors potentially demonstrating LOH are heterogeneous and have not yet become clonal for loss on chromosome 4. It should be noted, however, that a number of tumors of early stage and grade displayed LOH. This supports the contention that the biological properties of the tumor are determined by the progressive accumulation of genetic alterations rather than the specific order in which the alterations occur (24).

Deletion mapping is a reliable means to identify the most likely location of a critical tumor suppressor gene. To be most effective, deletion mapping should include a large number of tumors to maximize the probability of detecting tumors that accurately delineate the breakpoints. We used a PCR-based microsatellite analysis because it is a powerful technique to obtain useful information about the presence or absence of specific chromosomal loci in tumors. Although it is clear from this investigation that the breakpoints lie within a 3- (p arm) and a 14- (q arm) cM region, we were not able to obtain a more exact location due to a lack of available, well-mapped microsatellite markers for loci within these areas. Thus, positional cloning of candidate suppressor genes on chromosome 4 will require more precise mapping after isolation of additional microsatellite markers in these regions.

### ACKNOWLEDGMENTS

We thank Dr. Alan Partin for help with statistical analysis.

---

**Table 1**  **LOH and correlation with tumor grade and pathological stage**

<table>
<thead>
<tr>
<th>Grade (no.)</th>
<th>I (99)</th>
<th>II (99)</th>
<th>III (84)</th>
<th>T1 (94)</th>
<th>T2 (84)</th>
<th>≥T2 (104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOH on p⁶</td>
<td>13 (13%)</td>
<td>40 (40%)</td>
<td>29 (35%)</td>
<td>14 (15%)</td>
<td>31 (37%)</td>
<td>37 (36%)</td>
</tr>
<tr>
<td>LOH on q⁹</td>
<td>9 (9%)</td>
<td>29 (29%)</td>
<td>30 (36%)</td>
<td>11 (12%)</td>
<td>21 (25%)</td>
<td>36 (35%)</td>
</tr>
</tbody>
</table>

⁶ P < 0.005 between grades I and II and I and III for the p and q arms; P < 0.005 between stages T2 and ≥T2 for the p and q arms; P < 0.005 between stages T4 and T1 (p arm); P < 0.02 between stages T4 and T1 (q arm) by χ² analysis.

⁹ Includes only those tumors with LOH within the critical regions between markers D4S1508 and D4S404 on the p arm or between markers D4S426 and D4S408 on the q arm. Monosomic loss was included in both p and q LOH because this molecular event can target either or both nonacentric chromosomal arms.

---

**Fig. 3.** Deletion map of chromosome 4q. Markers D4S1552, D4S415, D4S1607, D4S1357, and D4S1584 are located within a 3-cM region; thus, not all tumors were evaluated using these markers. Minimal area of allelic loss is indicated by the solid vertical bar at the right.
REFERENCES


Distinct Regions of Allelic Loss on Chromosome 4 in Human Primary Bladder Carcinoma


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/55/22/5396

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.