Loss of Heterozygosity at Chromosome 16q in Prostate Adenocarcinoma: Identification of Three Independent Regions

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Abstract

Loss of heterozygosity (LOH) on chromosome arm 16q is one of the most consistent genetic alterations in sporadic prostate cancer and may be involved in cancer development through inactivation of tumor suppressor genes. A candidate tumor suppressor gene on this chromosome arm, CDHJ at 16q22.1, is dysregulated in prostate cancer. However, no specific deletion map has been constructed from prostate tumors to determine whether CDHJ is the potential target gene for the observed LOH on 16q. To narrow down the region of 16q loss, we constructed a detailed deletion map that incorporates CDHJ. We examined the pattern of allelic imbalance in prostate tissue from 22 patients with confined prostate tumors, 22 with local extracapsular extension, and 15 with metastatic forms, using 14 CA microsatellite repeats on 16q. Thirty-five of the 59 tumors tested showed LOH for at least one marker. We found evidence of 16q monosomy in 5 cases and partial allelic loss in 30. Our data provide evidence that three different target regions on 16q might be involved in the pathogenesis of prostate cancer. The first region is telomeric and lies at 16q24.3 between markers D16S520 and D16S413; the second, the most centromeric region in the 16q22.1 band, and limited by markers D16S347 and D16S318, is close to the CDHJ gene; the third, intermediate region, at 16q23.2, is bracketed by loci D16S318 and D16S507.

The rate of LOH at 16q24.3 was significantly higher in metastatic forms (80%; 12 of 15) than localized forms (32%; 7 of 22), pointing to a gene related to invasiveness in prostate cancer.

Introduction

Despite its high incidence and mortality rate, the exact molecular mechanisms underlying the tumorigenesis and progression of prostate cancer are still unclear. The most consistent genetic alterations in adenocarcinoma of the prostate are LOH involving chromosome arms 7q, 8p, 10q, 13q, 16q, 17q, and 18q, pointing to the presence of TSGs in these regions and their involvement in prostate carcinogenesis (1–7). One of the most recurrent aberrations observed in prostate tumors involves chromosome arm 16q, as shown by molecular analyses (1, 8–9). This suggests that one or more TSGs located on this chromosome arm might be involved in the initiation and/or progression of prostate carcinoma; however, their exact location and identity are unknown. Evidence for allele loss on 16q has also been found in other cancers, including breast cancer (10), hepatocellular carcinoma (11), ovarian cancer (12), and Wilms' tumors (13). The epithelial cell adhesion molecule E-cadherin, encoded by the CDHJ gene (14) located at 16q22.1, is of particular interest because it can function as an invasion suppressor gene (15). Indeed, decreased E-cadherin expression is associated with a poor prognosis in patients with prostate cancer (16). However, no specific deletion map has been constructed from prostate tumors to determine whether CDHJ is the potential target gene for the observed LOH on 16q. A recent report based on the fluorescence in situ hybridization technique with chromosome-specific probes has pointed to another novel site on 16q (17), suggesting that the CDHJ region is not the only site of deletion in this cancer.

To narrow down the deleted regions on chromosome 16q, we constructed a deletion map based on a large number of prostate tumors, using multiple polymorphic markers. We compared normal and tumor DNAs from 59 patients, using 14 microsatellite markers on chromosome arm 16q and tried to determine the fine location of commonly deleted regions. Furthermore, association of the LOH with clinical and histological parameters was examined to reveal the biological role of these regions bearing potential TSGs in prostate cancer development.

Materials and Methods

Patients and Samples. Fifty-nine prostate tumor specimens were obtained from patients undergoing surgery at St. Louis Hospital in Paris and La Cavale Blanche Hospital in Brest, France. The samples were obtained from locally confined and local extracapsular tumors by means of radical prostatectomy or needle biopsy, whereas those from patients with regional lymph node involvement or distant metastases were obtained by transurethral resection. The samples were examined histologically to confirm the presence of tumor cells. A sample was considered suitable for DNA analysis if the proportion of tumor cells was 60% or more. All suitable samples were stored in liquid nitrogen until high molecular weight DNA extraction. The histological diagnosis, Gleason score (18), and pathological tumor stage according to the tumor-node-metastasis classification of prostate cancer (19) were determined in each case during a routine clinical workup after surgery.

The Gleason score for the 59 primary tumors ranged from 4–9. We confirmed the well-established positive link between grade and stage, combining the Gleason score with the pathological stage.

The tumors were subdivided into three groups corresponding to the tumor-node-metastasis staging system: group A patients (n = 22; 37%) had disease limited to the prostate; group B patients (n = 22; 37%) had local extracapsular extension; and group C patients (n = 15; 26%) had regional lymph node involvement (5 cases) or distant metastases (10 cases).

We used 14 microsatellite markers on chromosome arm 16q to screen the 59 samples. Table 1 gives details of the loci investigated and their corresponding chromosomal location. The probable order of microsatellite loci was referred to integrated physical and genetic maps of human chromosome 16 (20–22).

Peripheral blood leukocytes were used as a source of normal DNA for each patient.

Detection of Microsatellite Markers by PCR. PCR was performed in a total volume of 50 μl containing 100 ng of genomic DNA, 20 μM each primer, 0.1 μM each deoxynucleotide triphosphate, 1 unit of Taq DNA polymerase, and 1.5–4 mM MgCl2. DNA amplification was performed conventionally (23) except that samples were subjected to 35 cycles of amplification consisting of 40 s of denaturation and 30 s of annealing. The final extension step at 72°C was extended to 10 min. The magnesium concentration and annealing temperature were optimized for each primer set. Products were diluted 1:3 in denaturing hybridization solution.
loading buffer and heat-denatured, then 1.5-μl aliquots of each sample were loaded on 6% acrylamide gels containing 7.5 M urea. DNA was then transferred to nylon membranes. CA repeat probes were labeled with [32P]dCTP by using terminal deoxynucleotidyl transferase. The membrane filters were hybridized overnight at 42°C with labeled probe, washed, and autoradiographed using film.

Detection of AI. Leukocyte and tumor DNA from each patient were analyzed in adjacent tracks. AI can only be identified in "informative" cases. Although simple PCR amplification cannot be considered informative, whereas homozygotes were "uninformative." The signal intensity considered to be present when the relative intensity of the two alleles in tumor samples containing >60% cancer cells (24). Evaluation based on the cutoff of 1.5 agreed well with the results of visual inspection. Each analysis was performed at least twice to ensure reproducible detection of AI (another independent PCR amplification, gel separation, and quantification). In an attempt to distinguish allelic gain from LOH, comparative multiplex PCR was performed (25) using microsatellite markers D4S244 or D21S222 (26), located in unaltered chromosomal regions (27), as internal controls.

Statistical Analysis. Differences in the distribution of AI between the different subgroups of patients were tested by the χ² test with Yates' correction for adjustment of the continuity of the χ² distribution.

Results

We analyzed normal DNA (peripheral blood lymphocytes) and autologous tumor DNA from 59 patients with prostate cancer, using 14 polymorphic probes for the long arm of chromosome 16 (Table 1). All patients were informative for four or more loci on 16q. AI on at least one locus was found in 35 of the 59 tumor DNAs (59%). Results obtained by means of comparative multiplex PCR supported the notion that all of the AIs observed in this study could be interpreted as LOH.

Of these 35 16q-altered tumors, 5 cases showed LOH at all of the informative loci tested on the long arm of chromosome 16 (data not shown), whereas the other 30 (Fig. 1) showed partial (interstitial and/or telomeric) alterations on 16q (Table 2).

The cumulative composite LOH pattern thus defined by the 30 tumors with partial deletions did not clearly point to a single common deleted region but suggested the existence of at least three different SCDRs (Fig. 1).

Mapping of the Noncontiguous Regions of Allelic Loss. Three nonoverlapping regions of allelic loss were pointed out by samples T56 and T63, which showed the loss of a common region at 16q24.3 between D16S520 and D16S413; T2, which defined an area bracketed by loci D16S347 (q22.1) and D16S515 (q23.1); and T51, which indicated the existence of a third region defined by loci D16S515 (q23.1) and D16S402 (16q24.2).

The most distal region of allelic loss has been mapped between D16S520 and D16S413 (T56 and T63). Cases T65 and T72 confirmed D16S520 as the proximal boundary, whereas case T45 confirmed D16S413 as the distal boundary for the most distal region (SCDR1).
Twenty-six (87%) of the 30 partially 16q-altered tumors showed LOH at loci D16S520 and/or D16S413 (Fig. 1).

The most centromeric region was characterized by deletion between loci D16S347 and D16S515 (T2). Two tumors (T48 and T73) had lost a single locus, D16S421, but showed allelic retention of adjacent, flanking loci (D16S318 distally and D16S347 proximally; Fig. 1). This region is within the region defined by T2, meaning that these results refine this LOH region and suggest that SCDR2 maps adjacent, flanking loci (D16S318 distally and D16S347 proximally; Fig. 1). This region is within the region defined by 12, meaning that these results refine this LOH region and suggest that SCDR2 maps between D16S318 and D16S347. Within this region lies the locus D16S421, which had disappeared from 7 (44%) of the 16 informative tumors with partial 16q alteration (Fig. 1). The high frequency of allelic loss involving this locus and the retention of adjacent, flanking loci suggest that D16S421 may be a central part of a deletion domain.

In addition to these two regions, SCDR1 and SCDR2, which correspond to regions observed at 16q24.3 and 16q22.1, respectively, in breast cancer (28), a third region of loss was defined by tumor 151, which showed allelic loss at D16S520 and/or D16S413 (Fig. 1). Interestingly, we observed two independently affected regions in 11 cases (T1, T10, T31, T39, T42, T48, T55, T57, T71, T72, and T73). Representative LOH results are shown in Fig. 2. Microsatellite instability was detected in two clinically localized tumors. One showed instability at the D16S318 locus and the other at the D16S413 locus.

**Table 2 Allelic losses on chromosome arm 16q in prostate cancer and corresponding pathological stages**

<table>
<thead>
<tr>
<th>Pathological stagea</th>
<th>16q status</th>
<th>Group A (22 cases)</th>
<th>Group B (22 cases)</th>
<th>Group C (15 cases)</th>
<th>Total (59 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No LOH on 16q</td>
<td>12 (54)%</td>
<td>9 (41)</td>
<td>3 (20)</td>
<td>24 (41)</td>
<td></td>
</tr>
<tr>
<td>16q</td>
<td>1 (5)</td>
<td>2 (9)</td>
<td>2 (13)</td>
<td>5 (8)</td>
<td></td>
</tr>
<tr>
<td>Partial 16q LOH</td>
<td>9 (41)</td>
<td>11 (50)</td>
<td>10 (67)</td>
<td>30 (51)</td>
<td></td>
</tr>
</tbody>
</table>

a Group A, patients with disease limited to the prostate; group B, patients with local extracapsular extension; group C, patients with regional lymph node involvement or distant metastases.

b No. of cases (%).

Although allelic losses involving 16q are known to occur in prostate cancer, precise deleted regions have not been clearly defined.

**Correlation of LOH on 16q with Grade and Stage.** Allelic loss at one or more loci on chromosome arm 16q was analyzed according to tumor stage and grade. A trend was found toward a higher frequency of LOH in metastatic cancer. Ten (45%) of 22 informative patients with locally confined prostate tumors (group A) showed LOH of one or several loci on 16q. Thirteen (59%) of the 22 patients with extracapsular tumor extension (group B) showed allelic loss on this chromosome arm, whereas LOH was observed in 12 (80%) of the 15 metastatic cancers (group C; Table 2).

The deletion frequency of each SCDR was also examined with respect to tumor stage. The only significant relationship (P = 0.009) was observed between LOH within the SCDR1 and metastatic forms (group C; Fig. 1). No essential difference in SCDR2 and SCDR3 frequencies was noted according to tumor stage. Similarly, there was no correlation between 16q loss, whether restricted to the three deleted domains or inclusive of all 16q loci, and the combined Gleason score (tumor grade).

**Discussion**

Although allelic losses involving 16q are known to occur in prostate cancer, precise deleted regions have not been clearly defined.
To determine the extent of allelic loss on 16q, we analyzed 44 clinically localized and 15 metastatic human prostate carcinomas, using 14 16q-specific markers. LOHs on the long arm of chromosome 16 were found in 35 (59%) of the 59 prostate tumors studied. We found evidence of potential loss of the entire long arm of chromosome 16 in only 5 (14%) of the 35 16q-altered tumors (not shown in Fig. 1). Rearrangements leading to monosomy were often associated with a breakpoint in the large constitutive heterochromatin. Our results suggest that breakpoints in the pericentromeric region of chromosome 16 are infrequent in prostate tumors (5 of 59; 8%), unlike breast cancer (29). Moreover, our data are in keeping with cytogenetic studies suggesting that monosomy 16q is not a frequent event in prostate cancer (30–32).

An interesting and fortuitous finding in this study was the high frequency of tumors showing partial (interstitial and/or telomeric) deletion on 16q. Indeed, most (30 of 35; 86%) 16q-deleted tumors showed partial alterations of 16q.

We did not observe a common breakpoint for all of these tumors, ruling out an important role of a 16q breakpoint in the development of prostate tumors through oncogene activation, contrary to hematologic malignancies (33). However, tumors displaying a switch from allelic loss to retention are of particular interest because they could provide information on the limits of deleted regions, in which TSGs should be located. With regard to these 30 tumor DNAs, we discriminated three distinct regions involved in allelic losses. Interestingly, the LOHs were observed in two separate defined LOH regions in 11 tumors. This further supports the presence of three defined locations of potential TSGs on 16q. However, we were unable to determine whether the LOH affecting two individual regions in the same tumor DNA sample involved the same parental copy of chromosome 16, or if one SCDR is from one chromosome and the other is from the other chromosome. It would be interesting to determine the parental haplotypes to obtain better definition in the LOH map.

Our results point to a SCDR at 16q24.3 (SCDR1), between D16S520 and D16S413. Current genetic mapping information suggests that the D16S520–D16S413 loci span a 4-cM region. Allelic loss in this region was seen in 31 (53%) of the 59 tumors studied. The existence of this LOH region on 16q, near the telomere, has been reported in breast cancer by many investigators (10, 28, 34, 35). Moreover, our data are in keeping with cytogenetic studies suggesting that monosomy 16q is not a frequent event in prostate cancer. Although several genes are located in this region, there are no obvious candidate TSGs.

In an attempt to regionally locate TSGs on chromosome arm 16q using fluorescence in situ hybridization analysis, Cher et al. (17) identified a region of common deletion with a proximal terminus somewhere on 16q23.1 and a distal end anywhere on 16q24.3. Our data are consistent with this report, and ours is the only study on prostate tumors reported thus far in this region. Moreover, these results provide a more accurate definition of the minimal area of LOH and identify, within the LOH region defined by Cher et al. (17), two minimal regions of loss (SCDR1 and SCDR2). Although the size of the three distinct commonly deleted regions is still too large to make it feasible to isolate the critical genes, a rapid refinement of the candidate regions should now be possible.

Allelic losses at one or more loci in 16q regions were analyzed according to tumor stage and grade. Although a trend toward higher frequencies was observed in metastatic cancers (80%; 12 of 15), the difference in the LOH rate for all 16q loci between group A and group C was not significant (Table 2). The only significant finding was the higher frequency of SCDR1 involvement in metastatic cancers (group C; F = 0.009). These results suggest that the TSG located at 16q24.3 might be related to invasiveness; the newly identified H-cadherin gene, expression of which is significantly reduced in human breast carcinoma (39), is a potential target gene.

There was no correlation between tumor stage and allelic loss at SCDR2 or SCDR3 (Fig. 1). Similarly, overall frequencies of allelic loss, whether restricted to the three deleted domains or inclusive of all 16q loci, were not associated with tumor grade.

Although in most cases allelic losses on 16q correlate with LOH on other chromosome arms (7q, 10q, 13q, 17q, or 18q), as indicated by our previous allelotype study (47), a large proportion of clinically localized prostate tumors showed LOH on 16q (52%; 23 of 44). This suggests that the allelic loss of 16q sequences, especially in SCDR2 and SCDR3, may be a relatively early event during prostate tumorigenesis.

In summary, the results presented here do not rule out the involvement of CDH1, but they do support the possibility that other TSGs on 16q are frequently inactivated in sporadic prostate cancer. Indeed, our findings suggest that in addition to CDH1, the TSGs involved in the genesis of prostate cancer are located in three distinct regions (16q22.1, 16q23.2, and 16q24.3) on chromosome arm 16q.

Likewise, LOHs on 16q are not restricted to prostate adenocarcinomas but have also been observed in breast cancer (10, 28), hepatocellular carcinoma (11) and ovarian cancer (12). Callen et al. (34) reported a consensus map of three similar LOH regions in breast tumors, suggesting the presence of TSGs at these locations that are involved in several tumor types.

The high SCDR1 LOH frequency in metastatic forms of prostate tumors suggests that inactivation of a putative TSG located in this region might be a relatively late step in prostate tumorigenesis. However, the precise role of this TSG, as well as those in the other SCDRs, remains to be determined in prostate carcinogenesis. Investigations...
will now focus on the different SCDRs to refine our knowledge of such genetic regions and to identify the putative TSGs affected by 16q LOH, together in their role in human prostate tumorigenesis.

References


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