Allelic Loss on Chromosome 10 in Prostate Adenocarcinoma

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ABSTRACT

A total of 83 prostate adenocarcinomas was evaluated for allelic loss on chromosome 10 by analysis of loss of polymorphic microsatellite repeats. Initially, 64 stage B carcinomas were analyzed at 12 loci on chromosome 10. Nine cases showed loss of chromosome 10 sequences, with a fractional allelic loss of 20%. These nine cases were then analyzed at an additional 19 loci to define better the regions of loss. Four areas of loss were identified, including 10q (2 of 64 cases), 10q23.1 (7 of 64 cases), 10q23.3 (4 of 64 cases), and 10q26 (2 of 64 cases). Three loci in these regions, D10S111, D10S185, and D10S192, were then analyzed in 19 advanced (stage C and D) carcinomas. Seven (37%) of 19 advanced carcinomas showed allelic loss at one or more of these loci. A statistically significant increase in the fractional allelic loss at both D10S111 (10p) and D10S185 (10q23.1) was observed. Thus, a complex pattern of loss is seen on chromosome 10 in prostate carcinoma, with regions of loss on 10p and 10q, and such loss occurs at a higher rate in clinically advanced disease.

INTRODUCTION

Prostate cancer is the most common cancer in men in the United States and the second leading cause of cancer deaths. Recent studies have begun to clarify the molecular genetics of human prostate cancer. Alterations in proto-oncogenes have not been found in a high percentage of cases to date, although alterations in the expression of growth factors and/or growth factor receptors have been reported (1). In contrast, alterations in tumor suppressor genes or putative tumor suppressor loci (based on LOH at those loci) have been described in at least nine different chromosomes or chromosomal regions in prostate cancer, including 17p (2, 3), 13q (4, 5), 18q (5-7), 5q (5), 6p (7-9), 16q (5, 7, 10), 9p (5), and 10 (3, 5-7, 9, 10).

Alterations of chromosome 10 in prostate carcinoma are complex. Bergerheim et al. (9) examined 18 prostate carcinomas using 10 RFLP probes and found apparent monosomy of chromosome 10 in two cases, two cases with LOH of the distal region of 10q, two cases with LOH on 10p, and two cases with loss of both 10p and the proximal portion of 10q. Overall, 8 (44%) of 18 cases had LOH somewhere on chromosome 10. Carter et al. (7) examined 28 tumors by RFLP analysis with two 10p and four 10q markers. No LOH was found on 10p, whereas 7 (29%) of 24 informative cases showed LOH on 10q. On the basis of a subset of three patients with multiple informative loci, the 10q deletions appeared to be localized to the distal portion of 10q (10q24-qter). Phillips et al. (10) analyzed 21 primarily advanced-stage tumors using three RFLP probes, one on 10p and two on 10q. Overall, 9 (48%) of 19 informative cases showed LOH on one or more loci on chromosome 10. Of the nine cases with LOH, two showed apparent monosomy, one had loss of 10p only, one had loss on both 10p and 10q, and five had loss on 10q only. Latil et al. (6) examined 20 clinically localized prostate cancers using two RFLP probes from 10q and found a 20% rate of LOH. Previously, we have analyzed a series of 26 clinically localized adenocarcinomas at four microsatellite repeats on chromosome 10 and found loss of 10p, proximal 10q, or distal 10q in 29% of the tumors (3). In summary, a number of laboratories using RFLP or microsatellite analysis have found loss of chromosome 10 in 20-48% of informative cases with a complex pattern, including monosomy and loss of 10p, portions of 10p and 10q, and portions of 10q only. However, Visakorpi et al. (5), using CGH, found only 10% of 40 cases with deletions of chromosome 10. Thus, both the rate of loss and the exact location of such losses on chromosome 10 in human prostate cancer are still unclear.

We have analyzed a series of 83 human prostate carcinomas for LOH on chromosome 10 to determine: (a) the rate of LOH on chromosome 10; (b) the regions of chromosome 10 most frequently lost; and (c) whether LOH on chromosome 10 is associated with prostate cancer progression. Our results are consistent with the presence of at least four regions of LOH on chromosome 10 in human prostate carcinoma, with the most common region of LOH at 10q23.1. We have also found a statistically significant correlation of LOH on chromosome 10 with advanced stage prostate cancer.

MATERIALS AND METHODS

Tissue Samples and DNA Isolation. The DNAs used in this study were isolated from fresh or paraffin-embedded prostate adenocarcinomas. A total of 64 matched tumor and benign DNAs was isolated from fresh radical prostatectomy specimens after frozen section analysis to ensure at least 50% carcinoma in tissue used for isolation of tumor DNA as described previously (2). All of these cases were clinical stage B and pathological stage T2 or T3, N0. High-molecular-weight DNA was also extracted from matched benign and tumor tissue as determined by frozen section from four clinically advanced cases, two stage D1 (pelvic lymph nodes) and two stage D2 (transurethral resections). An additional 15 matched DNAs were isolated as described previously (2) using paraffin-embedded tissue from transurethral resections or pelvic lymph nodes corresponding to stage C, D1, and D2 cancers.

Allelic Imbalance Analysis. Allelic imbalance was determined by PCR of microsatellite repeats in the presence of [32P]deoxyctydine triphosphate using matched benign and tumor DNAs as described previously (2, 3). Primers were obtained from Research Genetics (Huntsville, AL). For all high-molecular weight DNAs, the annealing temperature was 58°C, and it was 63°C for DNAs from paraffin-embedded tissues. The labeled reaction products were separated on denaturing gels and autoradiography performed. After autoradiography, the relative band intensity was determined by video densitometry using Microcomputer Imaging Device image analysis software (Image Research Inc., Bedford, MA). Allelic imbalance was present if the relative band intensity was decreased by at least 50% in one allele of the tumor sample, as described previously (3). All cases showing allelic imbalance were confirmed at least in duplicate. The validity of this approach has been confirmed by MacGrogan et al. (8) as well as by mixing experiments using known hemizygotes in this laboratory (2). The map position for all microsatellites used was provided by Drs. Katherine Call and Jen-I Mao (Genome Therapeutics Corp., Waltham, MA). The corresponding chromosomal band localization was estimated from the published FISH data from the same group (11).

RFLP Analysis. Ten μg each of matched benign and tumor high-molecular weight DNAs from cases showing allelic imbalance by PCR were digested with TaqI restriction endonuclease, subjected to electrophoresis on 1% agarose gels, and blotted onto nylon membranes as described previously (2). Blots were hybridized with [32P]random primed probes, washed, and autoradiography
performed as described previously (2). The RFLP probes were obtained from the American Type Culture Collection (Rockville, MD) except for the MXII cDNA probe, which was obtained from Dr. T. Lee (New York University, New York, NY). The localization of D10S4 and D10S20 was based on correlation of the microsatellite and FISH data described above with the results of Kapetaki et al. (12) and Lichter et al. (13). The presence of the TaqI polymorphism in the MXII gene was described previously by Bova et al. (14), and the chromosomal localization was based on correlation of microsatellite and FISH data as described above.

RESULTS

Allelic Imbalance in Stage B Prostate Carcinomas. A total of 64 stage B prostate carcinomas was analyzed for allelic imbalance by PCR at 12 microsatellite repeats located throughout chromosome 10 (Fig. 1, underlines). Nine cases showed allelic imbalance at one or more of these loci, for an overall rate of allelic imbalance of 14%. Given that the percentage of informative loci for these 12 microsatellites was 69%, the rate of LOH (number with LOH/number informative) was 20%. These nine cases were then analyzed at an additional 19 microsatellites to better define the region of loss. As can be seen in Fig. 1, four of the cases (nos. 5, 6, 60, and 61) had a limited region of allelic imbalance centered around D10S608 and D10S185, corresponding approximately to 10q23.1. Representative microsatellite PCR reactions, showing the allelic imbalance in this region for these four cases, as well as adjacent informative loci without allelic loss, are shown in Fig. 2. Another case (no. 32) had a limited region of allelic imbalance more telomeric on 10q near D10S669 (10q26). Two other cases (nos. 35 and 46) had larger areas of allelic imbalance that, including adjacent noninformative loci, involved major portions of 10q (10q22-10q26). Two cases (nos. 31 and 45) showed allelic imbalance on 10p, with additional noncontiguous areas of allelic imbalance on 10q. These results can be explained by assuming the presence of four independent regions involved preferentially by allelic imbalance (indicated as 1-4 on Fig. 1), specifically regions on 10p (2 of 64 cases), 10q23.1 (7 of 64), 10q23.3 (4 of 64), and 10q26 (2 of 64) with involvement of multiple regions in some instances.

RFLP Analysis in Stage B Prostate Carcinomas. The presence of allelic imbalance as determined by PCR only shows that unequal numbers of the two alleles are present. This imbalance could arise by the loss of one allele (LOH) or the duplication or amplification of one allele. These two possibilities cannot be distinguished reliably by PCR, given the inherent variability in the efficiency of different PCR reactions. To determine whether the observed allelic imbalance was...
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PCR at three loci that showed the highest rates of LOH in the clinically localized cancers, i.e., D10S111, D10S185, and D10S192. The advanced cases included 3 clinical stage C, 5 stage D1, and 11 stage D2 cases. Seven (37%) of 19 showed LOH at one or more of these loci, as shown in Fig. 5, with loss of 10p, 10q, or both 10p and 10q markers. Fig. 6 shows a comparison of the rate of LOH at these three loci in clinically localized versus advanced cancers. In all cases, the rate of loss was greater in the advanced cancers. This difference was statistically significant for both the D10S111 and D10S185 markers (Fisher’s exact test; P < .05) and approached statistical significance for the D10S192 marker. Thus, loss of both 10p and 10q loci is more common in advanced prostate cancer.

Fig. 3. Autoradiograms of representative Southern blots with chromosome 10 RFLP probes. TaqI digested DNAs were hybridized with the indicated RFLP probes after electrophoresis and blotting to nylon membranes as described in “Materials and Methods.” Lanes with DNA from benign tissue are indicated as $N$ and $T$ if they are from tissue containing at least 50% carcinoma. The two alleles recognized by the RFLP probe are indicated. $A$. D10S4: $A_1 = 7.4$ kb, $A_2 = 4.9$ kb; $B$. MXI1: $A_1 = 3.3$ kb, $A_2 = 2.6$ kb. Three cases show LOH, whereas case 35 with MXI1 is noninformative.

Fig. 4. Summary of RFLP analysis of cases showing LOH by microsatellite analysis. Seven of nine cases with LOH on chromosome 10 based on microsatellite analysis were analyzed by Southern blotting with RFLP probes as described in “Materials and Methods.” Filled ovals, LOH; open ovals, retention of heterozygosity; hatched ovals, noninformative cases.

due to loss or gain of genetic material we analyzed seven of nine cases showing allelic imbalance by PCR using Southern blotting with RFLP probes. For informative cases, if gain of genetic material had occurred, then one band in the tumor sample should be more intense than the control benign DNA, while loss would be indicated by decreased band intensity. A total of four TaqI RFLPs was analyzed. Three instances of allelic loss were found, one in case 35 and two in case 45 (Fig. 3). No evidence of gain of genetic material was seen in any case. Comparison of benign and carcinoma DNAs hybridized with control probes confirmed that all matched lanes contained equal amounts of DNA (data not shown). Thus, the allelic imbalance observed by PCR of microsatellites is due to LOH, not gain of genetic material. A summary of the results of the RFLP analysis is shown in Fig. 4. As can be seen by comparison with Fig. 1, there is complete concordance between the areas lost as determined by the two different methods.

LOH in Advanced Prostate Cancers. To determine whether advanced-stage prostate cancers had a higher rate of LOH on chromosome 10, a total of 19 clinical stage C and D tumors was analyzed by

Fig. 5. Summary of LOH on chromosome 10 in advanced prostate cancers by microsatellite analysis. LOH at three chromosome 10 loci was determined by microsatellite analysis as described in “Materials and Methods” in 19 advanced-stage prostate carcinomas. The pattern of loss in the seven cases showing LOH is shown. Filled ovals, LOH; open ovals, retention of heterozygosity; hatched ovals, noninformative loci. The locus number is shown but is abbreviated by omission of the D10S prefix.

Fig. 6. Rate of LOH at three chromosome 10 loci in localized and advanced-stage prostate carcinomas. LOH at the three indicated chromosome 10 loci was determined for 64 clinically localized and 19 advanced stage prostate cancers. The locus number is shown but is abbreviated by omission of the D10S prefix. The rate of LOH, expressed as a percentage, is shown for the clinically localized (open bars) and advanced cases (hatched bars). The rate of LOH is defined as the number of cases with LOH at a given locus divided by the total number of informative cases at that locus.
DISCUSSION

We have found a complex pattern of loss on chromosome 10 in clinically localized prostate adenocarcinomas. The most common region of loss was that containing the microsatellite markers D10S608 and D10S185, near 10q23.1. This region has not been identified previously as an area showing LOH in human prostate cancer. Three other less frequently involved loci were also seen, namely, 10p, D10S192 (10q23.3), and D10S169 (10q26). These last three regions were lost individually in less than 10% of the stage B carcinomas analyzed, and one must consider that one or more of these loci might reflect "background" LOH, given that LOH at low rates can be found in malignant neoplasms in areas not containing tumor suppressor genes. However, the finding that there was a substantial rate of LOH (20-40%) for the two loci examined in clinically advanced disease argues that at least these two regions probably contain tumor suppressor genes. Additional study of the D10S169 region is needed to determine whether this region is also lost at higher rates in clinically advanced disease. Previously published analyses of LOH on chromosome 10 in prostate cancer have used primarily RFLP analysis. These studies have found loss on 10p as well as on 10q. The probes on 10q used in these studies have been localized centromeric and telomeric to our most common region of loss, near 10q23.1. However, loss of 10q23.3 (D10S4 and D10S20), with or without loss of more telomeric sequences near 10q26 (D10S25), has been observed (7, 9). This is in agreement with our localization of two areas of loss to D10S192 (10q23.3) and D10S169 (10q26). Visakorpi et al. (5), using CGH, found loss on 10q in 4 of 40 cases of human prostate carcinoma with the minimal deleted region encompassing 10q22-10q23, which would contain our most common region of LOH near 10q23.1. Thus, our results, based primarily on microsatellite analysis, are in agreement with prior studies using RFLP and CGH analysis.

The loss of multiple loci on chromosome 10 is similar to the pattern seen in glioblastoma multiforme. LOH on chromosome 10 is found in 50-100% of glioblastomas examined (15-20). Most investigators have found a complex pattern of LOH with monosity and deletions of 10p, the proximal portion of 10q, and the terminal portion of 10q (18-21). Karlbom et al. (18) have concluded that there are at least three separate loci on chromosome 10, i.e., telomeric on 10p and telomeric and centromeric on 10q, which may harbor tumor suppressor genes. Whether there are one or two tumor suppressors on 10p and the proximal portion of 10q is not completely agreed upon, but deletions of the distal half of 10q are observed consistently (15, 18, 19, 21). The finding of multiple areas of loss by RFLP analysis is supported by the high incidence of monosomy of chromosome 10 by CGH (22). Functional studies in hybrid cells have also indicated the presence of at least two tumor suppressor loci on chromosome 10, one on 10p, and the other on 10q between CYP2c and D10S169 (23). This is agreement with our results, because the most common region of loss in our study is near D10S185, which maps near CYP2c.

LOH on chromosome 10 has been observed in a number of other malignant neoplasms, particularly malignant meningiomas (24), melanomas (25, 26), endometrial carcinomas (27, 28), renal cell carcinomas (29), and hepatocellular carcinomas (30). In renal cell carcinoma, 41% of informative cases showed LOH on 10q centered on a region around 10q21-23 (29). Fujimori et al. (30) have shown LOH in the distal portion of 10q in 25% of hepatocellular carcinomas. In meningioma, a high frequency of monosomy and loss of 10q has been observed (25, 26). Peiffer et al. (27) have found loss of 10q23-10q26 in approximately 40% of endometrial carcinomas. Alterations in chromosome 10 have also been identified at lower frequencies in other cancers. In summary, analysis of a variety of malignant tumors supports the idea that there are multiple tumor suppressor genes on chromosome 10.

The second major observation in this study is a statistically significant correlation between loss of both 10p and 10q and advanced clinical stage in prostate cancer. In Table 1, the rates of loss observed on 10p and 10q in clinically localized or advanced (stage C and D) prostate cancer in earlier studies using RFLP analysis and this study are summarized. There is a very good correlation between the rates of loss observed in this study and earlier RFLP studies. In general, there is a low rate of loss of 10p in clinically localized disease (3.2-6.2%), whereas 10p is lost in a much higher percentage of advanced cases (27.3-36.3%). Loss of 10q in clinically localized disease is more common than loss of 10p, ranging from 14.1 to 21.7%, with a substantially increased rate of loss in advanced disease (42.8-50%). Thus, our conclusion that loss of both 10p and 10q is associated with advanced disease is in concordance with prior observations in smaller numbers of cases using RFLP analysis.

A similar phenomenon has been observed in other malignant neoplasms. Low-grade astrocytomas have very few alterations in chromosome 10 (16), in contrast to the high rate in glioblastomas, indicating that alterations in chromosome 10 are associated with a more malignant phenotype in astrocytic neoplasms. Similarly, no LOH on chromosome 10 was found in 20 benign meningiomas, but LOH was present in 4 of 13 malignant meningiomas (24). Herbst et al. (26) examined sequential tumor samples from seven patients with meningioma as their disease progressed and showed LOH on chromosome 10 in metastatic lesions that were not present in the initial tumor samples in four patients. Thus, LOH on chromosome 10 appears to be correlated with clinically aggressive disease and disease progression in a number of different neoplasms.

As can be seen in Table 1, RFLP and microsatellite analysis indicates that approximately 20% of clinically localized and 40 to 50% of advanced cases show loss of chromosome 10 sequences.

Table 1 Summary of rates of allelic loss on chromosome 10 in human prostate carcinoma

<table>
<thead>
<tr>
<th>Reference</th>
<th>Localized</th>
<th>Advanced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10p</td>
<td>10q</td>
</tr>
<tr>
<td>Latil et al. (6)</td>
<td>ND*</td>
<td>2/10 (2)</td>
</tr>
<tr>
<td>Carter et al. (7)</td>
<td>0/18 (2)</td>
<td>6/22 (4)</td>
</tr>
<tr>
<td>Bergerheim et al. (9)</td>
<td>2/10 (2)</td>
<td>1/10 (1)</td>
</tr>
<tr>
<td>Phillips et al. (10)</td>
<td>0/4 (1)</td>
<td>1/4 (2)</td>
</tr>
<tr>
<td>Combined RFLP (6, 7, 9, 10)</td>
<td>2/32 (6.2%)</td>
<td>10/46 (21.7%)</td>
</tr>
<tr>
<td>Current analysis</td>
<td>2/62 (3; 3.2%)</td>
<td>9/64 (9; 14.1%)</td>
</tr>
</tbody>
</table>

* ND, not determined.
However, Visakorpi et al. (5) examined 31 localized and 9 advanced cases using CGH and found only 4 of 40 had deletions of chromosome 10. There are two possible explanations for this discrepancy: (a) CGH would not detect loss of a chromosome followed by duplication of the remaining chromosome (5); and (b) CGH can only detect deletions of at least 10 Mbp (5). It should be noted that a number of our losses particularly near 10q23.1 are relatively small, consistent with the latter possibility. Additional analysis is needed to clarify the basis for this apparent discrepancy.

Finally, it has recently been reported that the MXII gene is mutated in several prostate cancers with karyotypically proven 10q24–10q25 deletions, although the percentage of mutant cells was small (31). The MXII gene is a negative regulator of Myc-Max transcriptional complexes and is thus a potential tumor suppressor gene. The MXII gene maps quite closely to DIOSI73, and as can be seen in Fig. 1, this region is lost in three of the tumors showing LOH on chromosome 10, and this loss is confirmed by RFLP analysis in one case (Figs. 3 and 4). However, this region was lost less frequently than the other loci on 10q discussed previously. Thus, whether the MXII gene is a tumor suppressor gene for prostate cancer is still unclear, and additional investigations are needed to clarify this point.

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ADDENDUM

After the submission of this manuscript, Gray et al. (32) published an analysis of LOH in the 10q23–25 region in 37 clinically advanced prostate carcinomas. Of these 37 cases, 22 showed LOH in the region between DIOS532 and DIOS185, which overlaps our most frequently deleted region between DIOS608 and DIOS185. The finding of frequent LOH in the same region by two independent groups supports the hypothesis that this region contains a tumor suppressor gene for prostate carcinoma.

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