Allelic Loss on Chromosome 13q in Human Prostate Carcinoma

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ABSTRACT
To clarify the role in prostate tumorigenesis played by loss of the three known or putative tumor suppressor loci on the centromeric portion of chromosome 13q, we examined 80 clinically localized and 15 advanced prostate carcinomas for allelic loss at microsatellite markers mapped to this region, including markers tightly linked to the BRCA-2, retinoblastoma (Rb), and DBM (deleted in B-cell malignancy) loci. Among the 80 clinically localized cases, 24 showed allelic loss at one or more 13q loci. In all cases with loss, the Rb and/or DBM loci were lost. No cases were found with loss of Rb without loss of DBM or loss of DBM without loss of Rb, implying a role for both the Rb and DBM loci in clinically localized prostate cancer. Loss of the BRCA-2 locus was less common (4 of 55 informative cases) and was always associated with loss of Rb and/or DBM loci. Thus, the BRCA-2 locus does not appear to play as important a role in clinically localized prostate cancer as the Rb and/or DBM loci. Allelic loss on 13q was extremely common in the clinically advanced cases; it was present in 14 of the 15 cases. The rate of allelic loss at each of the three tumor suppressor loci was increased significantly in the advanced cases (P < 0.01, Fisher’s exact test). Thus, loss of heterozygosity on 13q is very common in prostate cancer and occurs at all three known or putative tumor suppressor loci on the centromeric portion of chromosome 13q.

INTRODUCTION
LOH has been observed repeatedly in a number of different chromosomal loci in human prostate cancer, including 8p (1), 18q (1, 2), 16q (1, 2), 10q (1–3), and 13q (1, 2, 4–7), using a variety of techniques including analysis of RFLPs and microsatellites and by comparative genomic hybridization. Such LOH is generally believed to indicate the presence of a tumor suppressor gene (or genes) for that neoplasm in the affected region.

At present, three known or putative tumor suppressors have been identified on chromosome 13q. The Rb gene, located on 13q14.2, is the prototype tumor suppressor gene, which was cloned as the gene altered in familial retinoblastoma. Alterations in the retinoblastoma gene and its encoded protein that lead to loss of its normal function have also been described in a variety of sporadic malignant neoplasms. There is evidence that alterations of the Rb gene may be involved in carcinomas of the prostate. LOH at the Rb locus has been observed in 20–60% of prostate cancers, depending on the clinical stage (4–9). Decreased or absent Rb protein, as determined by immunohistochemistry, has been observed in 10–100% of prostate cancers, including the prostate cancer (4, 6, 8, 9), again depending on clinical stage, and mutations of the Rb gene were found in 16% of prostate cancers examined by Kubota et al. (10).

Given the above, it is clear that in a subset of prostate cancers, there are alterations of the Rb gene. However, the rate of loss of Rb protein (4, 9) or Rb gene mutations (10) in clinically localized cancers (10–20%) is less than the approximately 30% rate of LOH on chromosome 13q found in such prostate cancers (4, 5, 9). Thus, there is the possibility that another tumor suppressor gene on 13q is also affected in prostate cancer. Similar observations of a discordance between the rate of LOH on 13q and the rate of Rb inactivation as assessed by immunohistochemistry has been observed in a number of different neoplasms (11). The existence of a second tumor suppressor gene on 13q, closely linked to Rb, has been postulated. Brown et al. (12) have found that the region near D13S25 (approximately 530 kb telomeric of Rb) is frequently homozygously deleted in low-grade B-cell malignancies, particularly CLLs, and postulated the presence of a tumor suppressor that they named DBM near this locus (12). A number of laboratories have confirmed this observation (13, 14). A detailed analysis by Devilder et al. (14) of LOH at microsatellites in this region has clearly delineated a region from D13S319 to D13S294, containing the D13S25 locus, which is lost in some cases of CLL without loss of the adjacent Rb locus. Thus, the question arises whether this locus might also be affected in human prostate cancer.

Finally, a familial breast cancer susceptibility locus, BRCA-2, located at 13q12, has been cloned recently (15). Given the known linkage between the occurrence of breast and prostate cancer in first-degree relatives (16), it is a possibility that BRCA-2 might also be a target for inactivation in sporadic prostate cancers.

To resolve these questions, we have examined in detail the pattern of loss on the proximal portion of chromosome 13q in 80 clinically localized (stage B) and 15 clinically advanced (stage C and D) prostate cancers using PCR analysis of microsatellites, followed by video densitometry, to detect LOH. We have found that loss of the Rb and DBM loci is tightly linked in human prostate cancer, consistent with a role for both genes in this
common malignancy. Loss of the BRCA-2 region is uncommon in clinically localized prostate cancers. However, all three loci are lost at significantly higher rates in advanced prostate cancers, raising the possibility of a role for all three genes in prostate cancer progression.

MATERIALS AND METHODS

Prostate Carcinoma Specimens. Prostates from patients undergoing radical prostatectomy for stage B prostate carcinoma were received fresh, and portions were frozen in liquid nitrogen. Frozen sections were prepared, and tissues containing a minimum of 50% carcinoma or benign tissue only were identified. Care was taken to avoid areas of prostatic intraepithelial neoplasia. DNA was extracted as described previously (17).

Prostate cancer and control benign DNAs from stage C or D carcinomas were extracted from microdissected, paraffin-embedded tissue from transurethral resections of prostate, perivesical neoplasia. DNA was extracted as described previously (17).

Determination of LOH on 13q. To determine if there was loss of heterozygosity on 13q, we carried out PCR on matched benign and tumor DNAs using primers specific for microsatellites in the region. Primers for microsatellite markers were obtained from Research Genetics (Huntsville, AL) with the exception of D13S319 (18), which was synthesized at the Kaplan Cancer Center Oligonucleotide Core Facility. Chromosomal location was determined from the Whitehead Genome Database as well as from published data (7, 11, 14, 15, 18, 19). The markers are located from approximately 13q12.1 to 13q21.1, with the following map positions from the top of chromosome 13: D13S120 (25 cM); D13S267 (30 cM); D13S155 (50 cM); D13S118 (51 cM); D13S153 (52 cM, within the Rb gene); D13S272/319 (52 cM, less than 1 megabase telomeric of the Rb gene); D13S176 (56 cM); D13S133 (59 cM); and D13S135 (64 cM). The D13S272 and D13S319 markers are extremely close and have not been mapped relative to each other. The positions of D13S120, D13S118, and D13S133 was estimated using the published map distances from markers in the Whitehead Genome Database (18, 19). PCR was performed using [32P]dCTP, followed by electrophoresis in 7% acrylamide/8 M urea gels and autoradiography as described previously (3).

Following autoradiography, the relative band intensity of the two alleles was quantitated by video densitometry of autoradiograms using MCID image analysis software (Image Research, Inc., Bedford, MA) as described previously (3). A given locus was scored as showing LOH if the relative band intensity of one allele was at least 50% decreased in the tumor sample compared to the control benign DNA analyzed simultaneously as determined by calculation of the AI index, defined by McGrogan et al. (20) as the ratio of the band intensities of the larger to the smaller bands in the tumor DNA sample divided by the same ratio in the benign DNA sample. An AI index of >1.5 (loss of the smaller allele) or <0.67 (loss of the larger allele) corresponds to at least a 50% deduction in relative band intensity. This general methodology has been validated previously in this laboratory using mixing experiments with known hemizygotes (17).

RESULTS

LOH on 13q in Clinically Localized Prostate Carcinomas. A total of 80 clinically localized prostate carcinomas were analyzed for LOH at the eight microsatellite markers indicated at the top of the figure (with the D13S prefix removed). The 24 cases with LOH are shown with the corresponding case number at the left. Filled ovals, LOH; open ovals, retention of heterozygosity; hatched ovals, noninformative loci. * two cases (nos. 17 and 51) that were noninformative at D13S153 and showed LOH at the Rb 1.2 marker. Three groups of cases are indicated: A, cases with LOH in the BRCA-2 and Rb/DBM regions; B, cases with LOH in the Rb/DBM region and indeterminate status at the BRCA-2 locus; C, cases with LOH at the Rb/DBM locus and retention of heterozygosity at the BRCA-2 locus.

Fig. 1. LOH on chromosome 13q in clinically localized prostate carcinomas. A total of 80 stage B prostate carcinomas was analyzed for LOH at the eight microsatellite markers indicated at the top of the figure (with the D13S prefix removed). The 24 cases with LOH are shown with the corresponding case number at the left. Filled ovals, LOH; open ovals, retention of heterozygosity; hatched ovals, noninformative loci. * two cases (nos. 17 and 51) that were noninformative at D13S153 but showed LOH at the Rb 1.2 marker. Three groups of cases are indicated: A, cases with LOH in the BRCA-2 and Rb/DBM regions; B, cases with LOH in the Rb/DBM region and indeterminate status at the BRCA-2 locus; C, cases with LOH at the Rb/DBM locus and retention of heterozygosity at the BRCA-2 locus.
was present in 4 of the 24 cases showing LOH. A second pattern was loss of the Rb/DBM region, with an indeterminate status for the BRCA-2-linked locus at D13S267 because of noninformative loci at D13S267, and often at D13S118 as well, which was found in 10 of the 24 cases with LOH on 13q (Fig. 1B). This category presumably includes cases with and without loss of the BRCA-2 locus. The third pattern was loss of the Rb and/or DBM loci with retention of the BRCA-2-linked locus D13S267 (Fig. 1C).

Several observations can be made regarding the pattern of LOH on 13q in clinically localized prostate cancers:

(a) In 23 of 24 cases with LOH on 13q, there was loss of the Rb and/or DBM loci. Two of these cases (nos. 17 and 51) were noninformative at D13S153, D13S319, and D13S272 but showed LOH at the Rb locus when analyzed at the Rb 1.2 locus, performed as described previously (4). In addition, one case (no. 120) was noninformative at all three loci in the Rb/DBM region but had losses at D13S118 and D13S135, which bracket this region; therefore, the entire Rb/DBM region was probably lost in this case. Thus, the Rb/DBM region is lost in all cases showing LOH in this region, in contrast to the BRCA-2 locus, which is lost in only a subset of such cases.

(b) No cases were identified in which there was loss of Rb with retention of the DBM loci or loss of DBM with retention of Rb. In all cases, there was either loss involving both loci or loss of one locus with an indeterminate status at the second locus.

(c) Loss at centromeric (D13S120) and telomeric (D13S135) loci was markedly less frequent than loss of the 13q14.3 region containing Rb and DBM loci, implying that this region is not being lost secondary to loss of centromeric or telomeric loci.

LOH on 13q in Clinically Advanced Prostate Carcinomas. A total of 15 clinical stages C, D1, and D2 prostate cancers were analyzed at six microsatellite markers on the centromeric portion of 13q, including three of the markers closely linked to known or putative tumor suppressor loci, specifically D13S267 (BRCA-2), D13S153 (Rb), and D13S272 (DBM). The results of this analysis are shown in Fig. 3. Overall, 14 of 15 cases (93%) showed LOH at one or more loci on 13q, with the exception being a single stage C prostate carcinoma (case 1). Several observations can be made regarding the pattern of loss on 13q in the clinically advanced prostate cancers: (a)
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This is in agreement both with our prior results (4) and those of clinically localized, disease is statistically significant expressed as a percentage. U, number of cases with LOH divided by the total number of informative cases, and advanced prostate carcinomas. The percentage of cases with LOH at each of the three tumor suppressor loci was much more common in clinically advanced disease when compared to clinically localized disease (Fig. 4). The increase in the rate of LOH at each of the three loci in clinically advanced, as compared to clinically localized, disease is statistically significant (P < 0.01, Fisher’s exact test); (b) loss of the BRCA-2 locus appears to be more common in stage D2 disease (four of four informative cases with loss) as compared to stage C (zero of one) and stage D1 (one of three); (c) as in the clinically localized cases, no instances of LOH at the Rb locus with retention of DBM or loss of DBM with retention of Rb were seen; and (d) as in the clinically localized cases, loss at centromeric (D13S120) and telomeric (D13S176) loci was markedly less frequent than loss at the BRCA-2/Rb/DBM loci, implying that this region is not being lost secondary to loss of centromeric or telomeric loci.

DISCUSSION

We have analyzed 80 clinically localized prostate carcinomas for LOH on 13q and detected loss in 30% of such cases. This is in agreement both with our prior results (4) and those of other investigators, who have found loss of 13q in 23–42% of clinically localized prostate cancers using RFLP, microsatellite analysis, and comparative genomic hybridization analysis (1, 2, 5–7, 9). For example, analysis of a group of primarily clinically localized carcinomas using RFLP analysis found a 23% incidence of LOH on 13q (2). Brooks et al. (5) have found LOH at the Rb locus at 13q14 in 27% of 41 informative, primarily clinically localized, cases of prostate cancer, whereas Phillips et al. (6) have found deletion of the Rb locus in 3 of 7 stage B carcinomas (6). Using comparative genomic hybridization, Visakorpi et al. (1) found loss of 13q in 32% of primary prostate carcinomas. We detected a significantly higher rate (93%) of LOH in clinically advanced disease (stages C and D) when compared to clinically localized disease. This is in agreement with the findings of Phillips et al. (6), who found a 65% rate of LOH at the Rb locus in their stages C and D cases using PCR analysis of microsatellites, RFLPs, and variable number of tandem repeats. Visakorpi et al. (1) found a 54% rate of loss on 13q in recurrent carcinomas analyzed by comparative genomic hybridization. Thus, a number of laboratories, using a variety of techniques, have found LOH on 13q in approximately 30% of clinically localized and 50–90% of clinically advanced prostate cancers.

A variety of evidence supports the notion that at least one reason for such losses on 13q is their role in the inactivation of the Rb tumor suppressor gene. Loss of Rb protein in prostate carcinomas by immunohistochemistry, consistent with inactivation of the retained Rb allele, has been observed by a number of laboratories. Bookstein et al. (8) found decreased or absent Rb protein expression in three of seven, primarily stage C or D tumors, whereas Phillips et al. (6) reported decreased or absent Rb protein in all nine clinically advanced cases examined by immunohistochemistry. Cooney et al. (9) have shown loss of Rb protein by immunohistochemistry in 8 of 27 clinically localized prostate cancers. We have shown that LOH at the Rb locus occurred in 35% of informative clinically localized cancers (4). Of the cases that showed LOH, 33% also had markedly decreased or absent Rb protein in tumor cells by quantitative immunohistochemistry. In contrast, none of the cases without LOH showed loss of Rb protein. Thus, LOH on 13q was correlated with loss of Rb protein. In addition, using single-stranded conformation polymorphism analysis and direct sequencing, mutations have been identified in 16% of prostate carcinomas examined by Kubota et al. (10). Finally, it has been shown that expression of normal Rb in DU145 prostate carcinoma cells, which lack a functional Rb gene, suppresses tumorigenicity in nude mice (22).

Given the above, it is clear that in a subset of prostate cancers, there is inactivation of the Rb gene based on a decreased Rb protein by immunohistochemistry or mutations documented by sequencing. However, the rate of such alterations in clinically localized cancers (10–20%) is less than the approximately 30% rate of LOH that has been observed consistently (4, 9). Indeed, both in this laboratory (4) and others (9), cases in which there is LOH at the Rb locus but retention of Rb protein expression (as determined by immunohistochemistry) have been identified. In part this may be due to the fact that immunohistochemistry may underestimate the loss of functional Rb protein, because some mutant Rb proteins are detected using this technique (23). Similarly, screening for mutations using PCR-single-stranded conformation polymorphism may miss a variable percentage of mutations. However, it is also possible that a closely linked gene may be the target for inactivation in a subset of tumors. A body of evidence has emerged that such a gene, designated DBM, may be the target for loss of 13q in chronic lymphocytic leukemia and other low-grade B-cell malignancies.
(12–14). Strongly supporting this idea is the observation by Devilder et al. (14) that there is a subset of B-CLLs showing loss of the putative DBM locus without loss of Rb. We did not identify any such cases among our 95 prostate carcinomas, including 38 with loss on 13q. However, we have not found the converse, i.e., loss of Rb without loss of DBM. Such a finding would have argued against a role for the DBM locus in prostate cancer. In our cases, loss of the Rb and DBM loci appeared to be tightly linked. The absence of cases showing loss of Rb without loss of loci linked to the putative DBM tumor suppressor locus raises the possibility that this locus plays a role in prostate cancer. Ultimately, to answer this question definitively, the putative tumor suppressor gene in this region will need to be cloned and analyzed for alterations in prostate cancers.

The BRCA-2 tumor suppressor gene at 13q12.2 could also be a target for inactivation in prostate carcinoma. We have found loss of the BRCA-2 region in only 4 of 55 informative clinically localized prostate cancers (7.3%). In all of these cases, this loss was associated with loss of the Rb and/or DBM loci. Recently, Latil et al. (7) have reported an analysis of LOH in the BRCA-2 and Rb regions in 39 prostate carcinomas of unspecified clinical stage. They found an overall rate of LOH on 13q in these cases of 41%. The BRCA-2 region was lost in 21% of cases, but such loss was always associated with loss of Rb, similar to our finding. They concluded that BRCA-2, therefore, probably was lost secondary to loss of Rb and did not have a role in sporadic prostate cancer. Essentially the same results were seen by Cooney et al. (9) in their analysis of 40 clinically localized prostate cancers. They found loss of the BRCA-2-linked loci in 5 of 40 clinically localized prostate cancers, and in all of these cases, the Rb locus was either lost or noninformative. This is in contrast to the finding in sporadic breast cancers that BRCA-2 and Rb were independently lost in some cases, implying a role for both genes in sporadic breast cancer (21). Our data in the clinically localized cases are thus consistent with the conclusion that loss of BRCA-2 plays a less important role in sporadic stage B prostate carcinomas than loss of the Rb and/or DBM loci. However, we have found that the BRCA-2 locus is lost in a substantially higher fraction of clinical stages C and D carcinomas, with loss of the BRCA-2 locus in over 60% of informative cases. This raises the possibility that the BRCA-2 gene, or a gene in the BRCA-2 region, may play a role in prostate cancer progression. Among the clinically advanced cases, loss of the BRCA-2 region was present at a higher rate in stage D2 cases (four of four informative cases) as compared to stage C (zero of one) and stage D1 cases (one of three). Supporting the idea that loss of the BRCA-2 region is associated with disease progression is the observation that the clinically localized cases with loss of the BRCA-2 locus (Fig. 1, group A) had a mean Gleason sum of 7.75 (n = 41), whereas those with retention of the BRCA-2 locus had a mean Gleason sum of 6.1 (n = 41). This difference is statistically significant (P < 0.05, t test). Thus, the clinically localized cases with LOH at the BRCA-2 locus were more poorly differentiated, which is correlated with eventual disease progression. At the present time, the clinical follow-up on our series of cases is too brief to be meaningful; therefore, direct correlation of eventual disease progression of the stage B cases with loss of the BRCA-2 region cannot be performed. It does not seem that loss of the BRCA-2 locus is simply secondary to an increased rate of loss at the Rb/DBM region. If the 13 clinically advanced cases with loss of Rb and/or DBM had the same proportion of loss at the BRCA-2 locus as the clinically localized cases (i.e., 16.6%), one would expect 2 cases with loss at BRCA-2 among the advanced cases.

We observed a total of eight clinically advanced cases with loss of the BRCA-2 region, which is clearly disproportionately increased.

We have found that LOH at all three known or putative tumor suppressor loci on chromosome 13q is increased in advanced prostate cancers, implying a role for these genes in prostate cancer progression. Alternatively, it is possible that the increased rates of loss seen in advanced cancers reflects a generalized increase in genetic instability in advanced prostate cancers. However, Visakorpi et al. (1) have found, using comparative genomic hybridization, that although advanced cancers do show increased rates of loss relative to primary cancers, such losses are in specific regions (including 13q), and some regions do not show losses, consistent with a targeting of specific regions for loss rather than a generalized loss of genetic stability at all loci.

In summary, we have presented evidence for LOH in human prostate cancer at three loci on chromosome 13q that are tightly linked to tumor suppressor genes and that such losses occur at increased rate in advanced cancers. However, to truly establish the role of these known or putative tumor suppressor loci in prostate cancer, the data regarding LOH will need to be linked to a comprehensive analysis of each gene for mutations and homozygous deletions and to quantitative studies of tumor suppressor protein expression. Such studies will of course require the cloning of the putative tumor suppressor DBM and will be difficult because of the size of the involved genes; but these studies ultimately the only approach, in the absence of unambiguous biological assays, to establishing the role of each gene in prostate cancer.

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