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Genetics

Allelic loss of chromosomes 16q and 10q in human prostate cancer

(tumor suppressor genes/loss of heterozygosity/DNA polymorphism)

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ABSTRACT **Recent advances in understanding the molecular genetics of common adult tumors have indicated that multiple genetic alterations including the activation of oncogenes and the inactivation of tumor suppressor genes are important in the pathogenesis of these tumors. Loss of heterozygosity is a hallmark of tumor suppressor gene inactivation and has been used to identify chromosomal regions that contain these genes. We have examined allelic loss in the most common tumor in men, prostate cancer. Twenty-eight prostate cancer specimens have been examined for loss of heterozygosity at 11 different chromosomal arms including 3p, 7q, 9q, 10p, 10q, 11p, 13q, 16p, 16q, 17p, and 18q. Fifty-four percent (13/24) of clinically localized tumors and 4 of 4 metastatic tumors showed loss of heterozygosity on at least one chromosome. Chromosomes 16q and 10q exhibited the highest frequency of loss of heterozygosity with 30% of tumors showing loss at these chromosomes. These data demonstrate that allelic loss is a common event in prostate cancer and suggest that chromosomes 16q and 10q may contain the sites of tumor suppressor genes important in the pathogenesis of human prostate cancer.**

Prostate cancer is the most common cancer in men. Over 100,000 cases of this disease will occur in the United States in 1990 (1). Thirty thousand men die of prostate cancer each year in the United States, making this disease the second leading cause of male cancer deaths (1). Despite the magnitude of the morbidity and mortality associated with this disease, very little is known regarding the molecular mechanisms involved in prostate tumorigenesis. Recent advances in understanding the molecular genetics of other common adult tumors, including colorectal (2), bladder (3), lung (4), and breast cancers (5), have indicated that multiple genetic alterations including the activation of oncogenes and the inactivation of tumor suppressor genes are important in the pathogenesis of these tumors. Tumor suppressor genes are normal cellular genes whose products regulate cellular growth and differentiation and through this action have an important role as inhibitors of the uncontrolled cellular proliferation characteristic of cancer. Inactivation of such genes can occur via a variety of mechanisms, including deletion of one copy of the gene (allelic loss) and mutational inactivation of the other copy. Frequent detection of allelic loss at specific chromosomal regions implicates these regions as sites of tumor suppressor genes that become inactivated in tumor development. The localization of allelic loss to specific chromosomal regions has permitted the identification of tumor suppressor genes that are important in the pathogenesis of a variety of tumors. Established and candidate tumor suppressor genes include the retinoblastoma gene on chromosome 13q14 (6–8), the p53 gene on chromosome 17p13 (9), the Wilm's tumor gene on chromosome 11p13 (10, 11), the DCC gene on chromosome 18q21 (12), and the neurofibromatosis gene on chromosome 17q (13, 14). In addition, chromosomes frequently exhibiting allelic loss in various cancers (e.g., 3p in renal and small cell lung cancer and 9q in bladder cancer) are thought to harbor as yet unidentified tumor suppressor genes. Some tumor suppressor genes such as p53 on chromosome 17p appear to be inactivated in multiple common tumor types (colon, lung, breast cancer), whereas others may be inactivated only in a specific tumor type. For example, chromosome 9q, frequent loss of which has been observed only in bladder cancer (3), may harbor a tumor suppressor gene whose function is particularly important in suppressing the neoplastic phenotype of bladder epithelial cells.

To date, there have been no reports describing allelic loss in prostate cancer. Furthermore, cytogenetic analyses of prostate cancer have not revealed consistent chromosomal deletions. One study by Atkin and Baker (15), however, showed that 4 of 4 patients with late-stage prostate carcinomas exhibited 10q deletions and 3 of 4 exhibited 7q deletions. In addition, deletions of 10q have been observed in several prostate cancer cell lines (16–19). To pursue these observations at the molecular level, we studied allelic loss in prostate cancer by using polymorphic DNA probes for chromosomes 7q and 10q as well as probes for chromosomes containing documented and putative tumor suppressor genes (3p, 9q, 11p, 13q, 17p, 18q). We find that the regions most frequently exhibiting allelic loss are found on the long arms of chromosomes 10 and, surprisingly, 16. These data implicate these regions as the sites of tumor suppressor genes whose inactivation is important in the development of prostate cancer.

MATERIALS AND METHODS

Human Tissue Samples. Prostate cancer tissue was obtained from two sources. The first source was a bank of 40 frozen prostate glands obtained from patients undergoing radical prostatectomy for clinically localized early stage prostate cancer. In general, these men were asymptomatic and had lesions detected through routine screening via digital rectal examination. None of the men in the first group had been previously treated with chemo-, hormonal, or radiation therapy. Prostate tumors obtained in these early stages are frequently an admixture of normal and tumor tissue. Because of this, an assessment of the percentage of cells that were cancerous in each specimen was made of all samples in the tumor bank. Twenty-four of these specimens contained areas of at least 75% tumor cells; DNA was isolated from these areas and normal adjacent peripheral zones by a cryostat sectioning technique previously described (20). Final pathologic staging revealed that there was a mixture of both moderately (Gleason grades 5–7) and poorly differentiated (Gleason grades 8 and 9) tumors in our samples. One case of prostatic ductal carcinoma in which the gland was exten-

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sively involved with tumor was in the group of samples. All of the other prostate carcinomas were of the usual acinar type and were <2 cm in diameter.

A second source of prostate cancer tissue was from four men undergoing transurethral resection for local obstruction after failing androgen ablation therapy (tumors 74–77 in Table 2). The prostate cancer in these men was late stage metastatic disease, thus offering the opportunity to examine genetic alterations in a group of men with more advanced cancer than the radical prostatectomy group described above. Peripheral blood lymphocytes served as the source of normal DNA for these men. Due to limited quantities of DNA, only a portion of the chromosomes were studied for allelic loss in the tumors of these patients.

Hybridization Analysis. The DNA from the paired tumor and normal samples from each patient was extracted as described (21). After restriction endonuclease digestion, samples underwent electrophoresis on 0.8% agarose gels. DNA was transferred to Nytran nylon membranes (Schleicher & Schuell) in 0.4 M sodium hydroxide/0.6 M sodium chloride. Filters were prehybridized in 1 M sodium chloride/1% SDS/10% dextran sulfate at 65°C. DNA probes were labeled via random priming (22). Filters were hybridized overnight under the same conditions with 2–3 × 10^6 dpm of denatured DNA probe and 100 μg of denatured sonicated salmon sperm DNA added per ml of hybridization solution. Two probes, g3 and MHZ47 (see Table 1), were preannealed with an excess of alkali-sheared human genomic DNA for 10 min at 68°C before being added to the hybridization solution. Filters were washed sequentially in 6× saline/sodium phosphate/EDTA (SSPE; 1× SSPE = 0.18 M sodium chloride/10 mM sodium phosphate, pH 7.7/1 mM EDTA)/0.5% SDS for 10 min at room temperature, 1× SSPE/0.1% SDS for 20 min at 37°C, and 0.1× SSPE/0.1% SDS for 30 min at 65°C. Blots were exposed to Kodak XAR-5 film at −70°C.

Table 1. Chromosomal locations of polymorphic DNA probes used to study loss of heterozygosity and restriction endonucleases revealing polymorphism

Chromosome	Probe (ref.)	Restriction endonuclease
3p	EFD 145.1 (23)	*Taq*
7q	G3 (24)	*Taq*
9q34	EFD 126.3 (25)	*Taq*
10p13–pter	MHZ15 (26)	*Taq*
10p13–pter	TBQ7 (27)	*Taq*
10q22–q23	1-101 (28)	*Taq*
10q22–qter	EFD75 (29)	*Taq*
10p24–qter	HUK-8 (30)	*Bam*HI
10q26	HOAT1 (31)	*Msp*
11p15	EJ988 (32)	*Taq*
13q	MHZ47 (33)	*Taq*
13q14	68RS20 (34)	*Rsa* I
16p12–p13.3	16/32 (35)	*Taq*
16q22–q24	79-2-23 (36)	*Taq*
16q22	HP2α (37)	*Bam*HI
17p13	144D6 (38)	*Msp*
18q21	15-65 (10)	*Msp*

Allelic loss was detected as the absence of one allele in prostatic tumor DNA compared to the normal prostatic tissue DNA from informative patients (i.e., patient's normal tissue contained two different alleles). In some cases, when there was residual signal from contaminating normal tissue, densitometry was used for analysis. A sample was scored as having allelic loss if a 60% reduction was present in the diminished allele compared to its normalized retained counterpart.

The chromosomal locations of the polymorphic DNA probes used and the restriction endonucleases used to reveal the polymorphisms are shown in Table 1. DNA probes were

Table 2. Allelic loss in 28 human prostate tumors

Tumor	Chromosome arms on which allelic markers were lost	Chromosome arms on which allelic markers were retained
4		3p, 9q, 10p, 10q, 13q, 16p, 16q, 17p
5	13q	3p, 10p, 10q, 11p, 16p, 16q, 17p, 18q
10	10q, 16q	9q, 10p, 11p, 13q, 16p
11		3p, 7q, 10p, 10q, 13q, 16p, 16q, 18q
13	16q, 18q	3p, 10q, 13q, 16p, 17p
14	10q	13q, 17p
18		3p, 7q, 9q, 10p, 10q, 11p, 13q, 16p, 17p
20	10q	7q, 11p
23	17p	7q, 9q, 10p, 10q, 11p, 16q, 18q
24	3p	10p, 17p, 16q, 18q
25	18q	7q, 10p, 10q, 17p
26		3p, 7q, 10p, 11p, 17p, 18q
29	7q, 10q, 13q, 16q, 17p	10p, 16p
30		3p, 7q, 9q, 10p, 10q, 11p, 18q
35	10q	7q, 9q, 10p, 11p, 13q, 16p, 16q, 17p, 18q
38		7q, 10p, 10q, 16q, 17p, 18q
42	16q	3p, 7q, 10p, 10q, 11p, 18q
46		3p, 7q, 9q, 10p, 10q, 11p, 16q, 18q
71	10q	7q, 17p
73	7q	10q, 17p
78		9q, 10p, 10q, 13q, 16q, 17p
79		7q, 9q, 10p, 10q, 17p
80		9q, 10q, 13q, 16q
81		7q, 9q, 10p, 10q, 16q, 17p
74*	10q	7q
75*	17p	7q
76*	9q, 16q	7q, 10q, 13q
77*	9q, 13q	10q

*Patients failing androgen ablation therapy.

obtained from the American Tissue Type Collection and as gifts from T. Dryja (68RS2.0), V. Ramesh (HOAT), and B. Vogelstein (g3, EJ988, 15-65).

RESULTS

Prostate cancer and normal tissue was obtained from 28 men undergoing either radical prostatectomy for clinically localized early stage prostate cancer or transurethral resection for local obstruction after failing hormonal therapy. Seventeen different polymorphic DNA probes on 11 different chromosomal arms (3p, 7q, 9q, 10p, 10q, 11p, 13q, 16p, 16q, 17p, 18q) were used for this analysis. These polymorphic DNA probes detect sequences within or near several tumor suppressor genes as well as genomic regions thought to be involved in prostate cancer by karyotypic analyses. To examine a control chromosome, not known to harbor any tumor suppressor genes or to frequently exhibit allelic deletions in human tumors, DNA probes for chromosome 16 were also selected for the analysis. The results of these analyses are shown in Tables 2 and 3 with representative Southern blots shown in Fig. 1. Of the 24 clinically localized tumors examined, 13 (54%) showed allelic loss on at least one chromosome. Allelic loss was detected in at least two samples on chromosomes 10q, 13q, 16q, 17p, and 18q, with chromosomes 3p and 7q showing loss in one tumor each. The highest frequencies of allelic loss were seen on chromosomes 10q and 16q, where 30% of the informative patients exhibited loss. No allelic loss was seen on chromosomes 10p, 11p, or 16p. Only one of the clinically localized tumors analyzed demonstrated loss of alleles at more than two loci. This tumor (number 29), a prostatic duct adenocarcinoma, exhibited loss on chromosomes 7q, 10q, 13q, 16q, and 17p.

We also examined the frequency of allelic loss in later stage prostate adenocarcinomas by analyzing tissue from patients failing androgen ablation therapy. Due to the small amount of DNA obtained from these specimens, only a limited number of loci were examined. In spite of this limited analysis, all four tumors from patients failing androgen ablation therapy showed allelic loss. Losses were observed on chromosomes 9q, 10q, 13q, 16q, and 17q. Interestingly, whereas none (0/11) of the clinically localized tumors exhibited loss on chromosome 9q, 2 of 2 metastatic tumors from patients that could be evaluated showed allelic loss in this region. As a group, these tumors exhibited allelic loss at 50% (6/12) of the informative loci examined. This compares to 14% (19/135) of the informative loci deleted in the clinically localized tumors.

As shown in Fig. 2, in tumors from three patients who were informative for multiple loci on chromosome 10, the 10q allelic deletions were distal to 10q23 with retention of proximal loci, thus implicating the distal region of chromosome 10q as a common region of deletion for these tumors. No allelic deletions were detected on chromosome 10p in any tumors analyzed, including the three tumors with 10q deletions that were also informative for loci on chromosome 10p. This suggests that allelic loss on chromosome 10 is specific for the long arm of the chromosome. Similarly, the allelic deletions on chromosome 16q also appear to be specific for the long arm of the chromosome as all three patients who exhibited 16q loss and were also informative for chromosome 16p showed retention of chromosome 16p.

Table 3. Summary of allelic loss by chromosomes

Chromosome	3p	7q	9q	10p	10q	11p	13q	16p	16q	17p	18q
Deleted	1	2	2	0	7	0	3	0	5	3	2
Informative	10	19	13	18	24	11	13	8	16	18	12
Percent	10	10	15	0	29	0	23	0	31	17	17

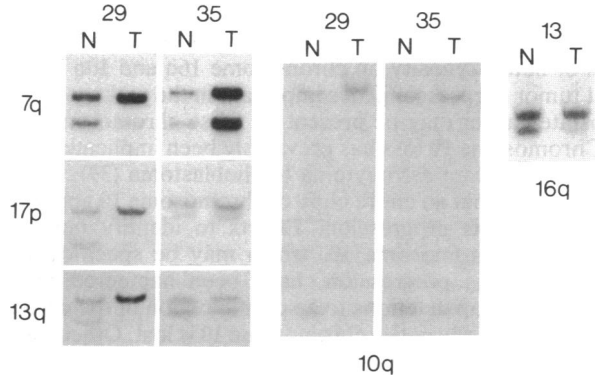

FIG. 1. Southern blot analysis of allelic loss in human prostate cancer. Paired DNA samples from normal (lanes N) and tumor (lanes T) tissue of patients 29, 35, and 13 are shown hybridized to probes detecting polymorphism on chromosomes 7q (g3), 17p (144-D6), 13q (68RS2.0), 10q (HUK-8), and 16q (HP2α). The absent or diminished signal present in lane T compared to lane N of patient 29 indicates allelic loss on chromosomes 7q, 17p, 10q, and 13q. Patient 35, in contrast, shows allelic loss only at 10q and not at 7q, 17p, and 13q. Patient 13 exhibits allelic loss on chromosome 16q.

DISCUSSION

The present study examines allelic loss in human prostate cancer. The majority (61%) of the tumors in the study exhibited allelic loss on at least one of the chromosomes examined. These losses have been detected on several chro-

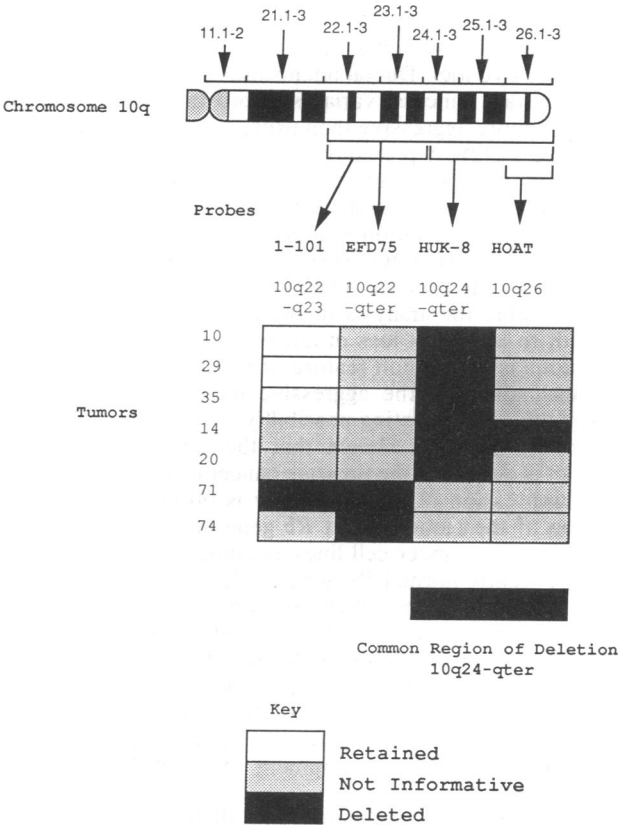

FIG. 2. Common region of deletion on chromosome 10q in prostate cancer. Allelic loss on chromosome 10q at informative loci for each of seven tumors. Four probes on chromosome 10q used for analysis are localized as follows: 1-101 (10q22–q23), EFD75 (10q22–qter), HUK-8 (10q24–q26), and HOAT (10q26). Three tumors have retained alleles at 1-101 but lost the more distal alleles detected by HUK-8, thus implicating a common region of deletion at the distal tip of the chromosome, 10q24–qter.

mosomes known to harbor tumor suppressor genes important in the development of other tumor types. Elevated rates of loss of heterozygosity on chromosome 16q and 10q suggest that tumor suppressor genes important in the pathogenesis of prostate cancer may be present on these chromosomes.

Chromosome 10 loss has previously been implicated in the progression from astrocytoma to glioblastoma (39). Studies have shown that an entire copy of chromosome 10 appears to be lost in this progression. Efforts to identify important regions of chromosome 10, which may be specifically involved in this progression, have been hampered by the inability to map deletions to a specific region of the chromosome because the entire chromosome 10 is lost. Other studies have suggested chromosome regions 10q24–26 as being involved in the early stages of melanocytic neoplasia (40) and 10q23–24 as being involved in T-cell acute lymphocytic leukemia (41). We find that the distal region of chromosome 10q is the common region of deletion in this study. The relationship between the alterations of chromosome 10 in these cancers and the 10q allelic loss seen in prostate cancer remains to be established.

The detection of allelic loss on chromosome 16q is an unusual finding. Chromosome 16 has not previously been implicated as a common site of allelic deletions in other tumors examined, although only in a few tumor systems such as colorectal cancer has a comprehensive search for allelic loss on all chromosomes been performed. A common region of deletion has not been identified for chromosome 16q, although deletions appear to be specific for the long arm of chromosome 16 as three tumors that had lost 16q did retain 16p.

Of 24 clinically localized tumors examined, only one, a prostatic ductal carcinoma (tumor 29), exhibited allelic loss at more than two loci. Ductal adenocarcinomas are rare (<1% of all prostate cancers) variants of prostate cancer that are generally more aggressive than usual acinar prostatic adenocarcinomas (42). A previous study that examined *ras* gene mutations in the same group of clinically localized prostate cancers as the present study demonstrated that tumor 29 also contains a point mutation at codon 61 of the *HRAS* gene and was the only primary tumor specimen in this group in which a *RAS* gene mutation was detected (43). Only one such tumor was available for analysis in the present study, making it difficult to infer that loss of alleles at multiple loci or *RAS* mutations is a common feature of prostatic duct adenocarcinomas, although the aggressive nature of these tumors makes this an interesting possibility.

Recent work has shown that the retinoblastoma gene product is altered in the prostate cancer cell line DU-145 and that suppression of tumorigenicity is observed upon introduction of the cloned intact *Rb* gene into these cells. Two other prostate cancer cell lines examined in the same study had apparently normal *Rb* gene products (44). In our study, we detected allelic loss of chromosome 13q in tumors from 3 of 13 informative patients. Interestingly, tumor five showed allelic loss of chromosome 13q but maintained heterozygosity at all other informative loci examined in this study including those on chromosomes 16q and 10q. Taken together, these data suggest that inactivation of the *Rb* gene may play a primary role in the development of a subset of prostate carcinomas.

Even the highest frequencies of allelic loss, which were seen at chromosomes 16q and 10q in this study, are lower than the rates of allelic loss that have been seen in some studies of other cancers. For example, >70% of colon tumors exhibit allelic loss on chromosomes 17p and 18q (1). One reason for this difference may be the early stage at which the majority of prostate cancers in the present study were analyzed. Previous studies have shown that clinically localized prostate cancers are predominantly diploid tumors (45, 46) and, therefore, might not be expected to have large numbers of genetic alterations associated with the gross chromosomal abnormalities of an aneuploid tumor. In addition, in a recent karyotypic study of 30 prostate cancers obtained from patients undergoing radical prostatectomy, the most common karyotype seen was a normal diploid karyotype (19). Thus, tumors obtained in these early stages may have less overall numbers of detectable genetic alterations at both the microscopic and molecular levels. Changes observed in these early lesions, however, may be those that are the most important to the initiation of the tumorigenic process.

In relation to tumor progression, we observed that, whereas just over half of the clinically localized tumors demonstrated allelic loss, four of four metastatic tumors, even when analyzed at a limited number of chromosomal loci, showed allelic loss. While the number of metastatic tumors analyzed is too low to be conclusive, these results suggest that increased frequency of allelic loss may be correlated with tumor aggressiveness. In the case of colon cancer, a strong correlation between allelic loss and tumor progression has been demonstrated (47, 48). Obviously, analysis of greater numbers of prostatic tumors is required to clarify this question in this tumor type.

In summary, we have detected allelic loss in a majority of prostate tumors analyzed. The most common sites of allelic loss are chromosomes 16q and 10q, suggesting that these chromosomes may contain tumor suppressor genes whose inactivation is involved in prostatic tumorigenesis.

Note Added in Proof. Tsuda *et al.* (49) recently reported that allelic loss on chromosome 16q is frequently observed in hepatocellular carcinoma.

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