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Homoyzgous Deletion and Frequent Allelic Loss of Chromosome 8p22 Loci in Human Prostate Cancer


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Abstract

Allelic loss studies have been instrumental in identifying tumor suppressor gene loci in a variety of cancers. In this study we analyzed prostate cancer specimens from 52 patients for allelic loss using 8 polymorphic probes for the short arm of chromosome 8. Overall, 32 of 51 (63%) informative tumors showed loss of at least one locus on chromosome 8p. The most frequently deleted region is observed at chromosome 8p22-8p21.2. Loss of one allele is identified in 14 of 23 (61%) tumors at D8S163, in 15 of 32 (47%) tumors at lipoprotein lipase, and in 20 of 29 (69%) tumors at MSR, all on 8p22. Loss of one allele is identified in 16 of 27 (59%) tumors at D8S220 at 8p21.3-8p21.2. In addition to frequent loss of one allele at the MSR locus, one metastatic prostate cancer sample demonstrated homozygous deletion of MSR sequences. Loci telomeric and centromeric to this region are largely retained. A chromosome 8p deletion map is constructed and defines the smallest region of overlap to a 14-cM interval at 8p22 between D8S163 and lipoprotein lipase, flanking the MSR locus. Evidence of chromosome 8q multiplication at locus D8S39 was identified in the genome of a human prostate cancer specimen. A deletion map has been demonstrated homozygous deletion of MSR sequences. Loci telomeric and centromeric to this region are largely retained. A chromosome 8p deletion map is constructed and defines the smallest region of overlap to a 14-cM interval at 8p22 between D8S163 and lipoprotein lipase, flanking the MSR locus. Evidence of chromosome 8q multiplication at locus D8S39 was identified in the genome of a human prostate cancer specimen. A deletion map has been constructed which narrows the common region of deletion to a 14-cM interval on chromosome 8p22. The finding of a homozygous deletion in this region further suggests that it harbors a tumor suppressor gene which is frequently inactivated in prostate cancer.

Introduction

Prostate cancer is the most common cancer in United States men, with approximately 165,000 new clinically diagnosed cases and 35,000 deaths due to prostate cancer projected in 1993 (1). At the same time, histological evidence of subclinical prostate cancer is frequently found at autopsy (2), and based on these studies it is estimated that over 11 million United States men currently harbor “histological” prostate cancer. Prostate cancer thus has the unusual distinction of being clinically silent in a large number of men who harbor it (histologically) while it is also a major contributor to cancer mortality (it is the second most common cause of cancer death among United States men). Distinguishing prostate cancers destined to progress to lethal metastatic disease from those with little likelihood of causing morbidity is a major goal of prostate cancer research.

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In spite of the high prevalence of prostate cancer, little is known regarding the molecular pathogenesis of this disease. An understanding of the genetic events underlying prostate cancer progression may make accurate prediction of patient prognosis and better treatment selection possible. Inactivation of the p53 and Rb tumor suppressor genes has been observed in prostate cancer, but the frequency of these events appears to be rather low, at least in primary prostate cancers (3–5, 34). Evidence for the existence of other, more frequently inactivated tumor suppressor genes comes from allelic loss studies, which suggest that unidentified tumor suppressor genes located on chromosomes 8, 10, and 16 may be critical in prostate tumorigenesis (6, 7).

Materials and Methods

Tissue Samples. Prostate cancer tissue was obtained from patients undergoing radical prostatectomy for clinically localized prostate cancer between August 1988 and November 1992. None of the patients included in the study had been treated previously with chemotherapy or hormonal therapy. Prostate and seminal vesicle tissue was harvested and frozen at −80°C as described previously (8). Briefly, only clinically palpable tumors were eligible for the study, and only tumors palpable after surgical removal were harvested. The Gleason score (9) for the 42 primary tumors included in the study was 7.4 ± 1.1 (SD) with a range of 5–9. Focal or established capsular penetration was seen in all 42 primary tumors studied, and thus all tumors studied fell into the T3 category utilized in the recent tumor–nodes–metastasis classification of prostate cancer (10). Histological evidence of seminal vesicle invasion was seen in association with 16 of 42 (38%) of the primary tumors included in the study. Microscopic lymph node metastases were seen in 11 of 42 (26%) of the cases included in the study.

Harvested primary tumors were mounted and 6-μm sections were stained with hematoxylin and eosin. Forty-two primary prostate adenocarcinomas which could be trimmed to yield tissue containing greater than 70% tumor nuclei were selected for DNA analysis. Metastatic prostate adenocarcinoma tissue was available in ten cases from patients found to have palpable enlarged pelvic lymph nodes at the time of intended radical prostatectomy. A frozen section taken at the time of surgery revealed metastatic adenocarcinoma and radical prostatectomy was not performed. Nodal tissue not needed for histological diagnosis was snap frozen at −80°C and used for this study.

Paired noncancerous tissue (seminal vesicle, prostate, or blood lymphocytes) was obtained from each patient. Seminal vesicle or prostate tissue
serving as source material for noncancerous DNA was examined every 300 μm by frozen section, and all tissue containing dysplastic or cancerous epithelia was rejected.

Preoperative serum prostate specific antigen levels were measured by monoclonal immunoradiometric assay (Hybritech).

**DNA Preparation.** PSA containing greater than 70% prostate cancer nuclei was isolated from surrounding tissue (containing benign prostate epithelia, stroma, lymphocytes, etc.) as much as possible using a cryostat sectioning technique described previously (11). All prostate carcinomas studied were of the usual acinar type and were <2 cm in diameter. DNA isolation and quantification were performed as described previously (6, 12).

**Southern Analysis.** Samples were cleaved with restriction endonucleases (BRL and New England Biolabs) with the buffers recommended by the supplier, using 10 units of enzyme/μg of DNA for MspI digests and 7.5 units/μg for TaqI digests. Samples were electrophoresed in 0.8% agarose gels and transferred to Nytran nylon membranes (Schleicher & Schuell) in 0.4 μg/ml sodium hydroxide/0.6 μg sodium chloride after depurination in 0.25 M HCl for 10 min. After covariant linking of the DNA to the membrane using UV irradiation (Stratagene), membranes were prehybridized in 10 ml 1 mM NaCl/1% sodium dodecyl sulfate/10% Dextran sulfate at 65°C for 1 h. DNA probes KSR2, NF 5,1, and MCT 128.2 were obtained from the American Type Culture Collection. Probes C8-1, MSR-32, C8-319, and C8-277 are cosmid probes that have been described previously (13). Probes were labeled using random hexamer priming and incorporation of [α-32P]dCTP (Amersham) with the Klenow fragment of DNA polymerase I (Amersham). Probes C8-1, MSR-32, C8-319, and C8-277 were boiled with sheared human placental DNA (Sigma), (0.2 mg/ml), cooled briefly on ice, and hybridized at 65°C overnight. Probes KSR2, NEFL, and MCT 128.2 were boiled with 0.5 ml of 2 mg/ml denatured sonicated salmon sperm DNA, briefly cooled on ice, and hybridized at 65°C overnight. After hybridization, membranes were washed in 0.1X standard saline-phosphate-EDTA 0.1% sodium dodecyl sulfate for 15 min and were subsequently exposed to Kodak XAR-5 film at ~80°C in cassettes with amplifying screens.

Allelic loss was defined as the absence of one allele in prostatic tumor DNA compared to the noncancerous paired control DNA. 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.

Allelic multiplication using probe MCT 128.2 was defined as an increase in intensity of greater than 100% of one of two alleles present in tumor samples, or intensity differences of greater than 100% between tumor and normal alleles in homozygous cases when prior probing of the same blots demonstrated equal loading of DNA in tumor and normal lanes.

**Microsatellite Analysis.** Sequences for LPL (GZ 14,15) and Mfd 199 primer sets were as previously published (14). One of each pair of primers (LPL GZ 14 and Mfd 199R) was end-labeled with [γ-32P]dCTP (ICN Biochemicals) using polymerase kinase (Boehringer-Mannheim) and 5X kinase buffer [0.25 mM Tris, (pH 9.0), 50 mM MgCl2, 50 mM dithiothreitol, and 0.25 mg/ml bovine serum albumin]. Six μl primer (10 μg), 2.8 μl 5X kinase buffer, 0.7 μl kinase (9 units/μl), 1.5 μl sterile deionized water, and 3.0 μl [γ-32P]-dATP were combined and incubated at 37°C for 1 h. Products were purified using G-25 spin columns (Boehringer-Mannheim). One μl labeled primer was added to 1 μl unleaded primer (10 μg), 0.5 ml dodecylbenzene triphosphate mix (equal volumes of dATP, dCTP, dGTP, and dTTP each at 10 μg), 5.5 μl sterile deionized water, and 10X Taq DNA polymerase buffer (Perkin-Elmer), 10 μg genomic DNA were added (2.5 ng/μl), and the mixture was heated to 94°C. After addition of Taq DNA polymerase solution (5 units), thermocycling was then performed with 30 cycles of denaturation at 94°C for 30 s, annealing at 62°C (LPL) or 58°C (Mfd 199) for 30 s, and extension at 72°C for 30 s. This was followed by 72°C for 7 min. Products were then mixed with an equal volume of stop buffer containing 95% formamide, 0.05% xylene cyanol, 0.05% bromphenol blue, and 20 μl EDTA. Samples were heat denatured at 94°C and 3-μl aliquots of each sample were loaded on 6% acrylamide gels containing 8.0 μm urea. Gels were dried and exposed to Kodak XAR film. In this study, allelic loss using microsatellite analysis was determined according to criteria similar to those used in Southern analysis described above.

**Immunohistochemistry for MSR Protein.** Sections of primary prostate cancer and adjacent noncancerous prostate (including areas of benign prostatic hyperplasty and normal prostate) were examined in five patients. Liver tissue from a single patient obtained at autopsy served as positive control for MSR staining. Well preserved central and peripheral zone prostate tissue was obtained from the same patient at autopsy and stained for MSR protein. This patient had no evidence of malignancy at autopsy and prostate tissue was normal on gross examination and histologically. Unfixed air-dried 6-μm frozen sections on glass slides were warmed to room temperature and fixed in 2% formaldehyde/10 mM Tris, pH 7.4/150 mM NaCl/2 mM CaCl2 solution for 10 min and then incubated for 20 min at room temperature in 0.3% H2O2/absolute methanol solution. Slides were subsequently rinsed twice with 10 mM Tris, pH 7.4/150 mM NaCl/2 mM CaCl2 and then incubated at 37°C for 10 min in serum blocking solution (Zymed). Rabbit anti-human synthetic scavenger receptor peptide IgG (kindly provided by Dr. Tatsuhiko Kodama, University of Tokyo) (15) was then added (1:50) to each slide and incubated at 37°C for 30 min. The primary antibodies were detected with a biotinylated secondary antibody-streptavidin-peroxidase conjugate (Zymed).

**Results**

We analyzed 52 prostate cancer specimens for allelic loss using 8 polymorphic probes for the short arm of chromosome 8. Overall, 32 of 51 (63%) informative tumor specimens showed loss of at least one locus on chromosome 8p. The most frequently deleted region is observed at chromosome 8p22–21. Loss of one allele is identified in 14 of 23 (61%) tumors at D8S163 (12 of 19 primary tumors and 2 of 4 lymph node metastases) (Fig. 1), in 15 of 32 (47%) tumors at LPL (15 of 30 primary tumors and 0 of 2 metastases), and in 20 of 29 (69%) tumors at MSR (17 of 26 primary tumors and 3 of 3 metastases), all on 8p22. Loss of one allele is identified in 16 of 27 (59%) tumors at D8S220 (12 of 22 primary tumors and 4 of 5 metastases) on 8p21.3–21.2. (Fig. 2; Table 1).

In addition to loss of one allele at the MSR locus in a majority of tumors, one metastatic prostate cancer sample (N2) demonstrated remarkable chromosomal loss.

![Fig. 1. KSR2 (8p22) Southern analysis in human prostate cancer. Pairs purified prostate cancer DNA (T) and noncancerous DNA (N) from the same patients. The 1.9-kilobase allele is lost in the tumor tissue of patient 4. the 3.3-kilobase allele is lost in the tumor tissue of patient 6. Patient 7 is not informative at this locus.](image-url)
homologous deletion of MSR sequences. Hybridization of the same blot with the DCC probe 15-65 establishes the presence of intact DNA of equivalent or larger size in the N2 tumor lane (Fig. 3). Repeat digestion of N2 DNA with MspI, TaqI, and EcoRI and probing for MSR has confirmed this finding (data not shown). Fig. 3 illustrates the presence of one allele in N2 DNA at D8S163, while demonstrating complete loss of sequences at MSR. Both D8S39 alleles are present in tumor N2 and the intensity of the lower allele is multiplied 3-fold, bp, base pairs. For definition of T and N, see legend to Fig. 1.

In contrast to 8p22–21.2, loci telomeric and centromeric to this region are largely retained, with loss of one or more loci in only 9 of 48 (19%) of informative cases. Distal loci studied on 8p23 are largely retained, with loss in only 4 of 38 (11%) of informative cases at D8S201 and in only 3 of 22 (14%) of cases at D8S201 (Table 1). Loci studied on 8p11.2 and 8q24 are also infrequently deleted, with loss identified in 3 of 26 (12%) of informative cases at D8S194 and in 2 of 17 (12%) at D8S39.

The observation of homozygous deletion at the MSR locus prompted us to perform a preliminary assessment of the macrophage scavenger receptor gene as a possible tumor suppressor gene. Prostate tissue was analyzed for expression of MSR protein using a highly specific polyclonal antibody as described by Kodama et al. (15). Macrophage scavenger receptor protein was not detected among prostate cancer cells or noncancerous prostate epithelia. Scattered cells contained within the stroma of each of the prostate sections stained positively, consistent with staining in macrophages only.

To determine whether allelic loss on chromosome 8p correlates significantly with clinical parameters, we reviewed preoperative serum PSA levels, Gleason score, and final pathological staging for each patient included in the study. Mean Gleason score did not differ significantly with clinical parameters, we reviewed preoperative serum PSA levels, Gleason score, and final pathological staging for each patient included in the study. Mean Gleason score did not differ between the two groups, with a mean of 7.3 in patients with 8p loss, and a mean of 7.6 in those with no 8p loss demonstrated. Preoperative PSA levels were available for 34 of 42 patients whose primary prostate cancer tissue was studied. Mean PSA level for the entire group of patients was 11.2 ng/ml (range, 1.6–23.6). The mean preoperative PSA level for patients with 8p loss was 12.6 ng/ml, and for patients with no loss on chromosome 8p it was 9.3 ng/ml (analysis of variance, P = 0.105). Seminal vesicle invasion was observed in 11 of 27 (41%) patients with 8p loss and in 5 of 15 (33%) patients with no seminal vesicle invasion (χ², P = 0.055). Microscopic lymph node metastases were found in 9 of 27 (33%) of patients with 8p loss, and in 3 of 15 (20%) patients without 8p loss (χ², P = 0.35). In summary, there is a trend toward higher preoperative PSA levels, more frequent lymph node involvement, and more
frequent seminal vesicle involvement in patients with 8p loss demonstrated within their prostate cancers, but these trends are not statistically significant.

Discussion

This study confirms previous findings of frequent loss of 8p loci in human prostate cancer (7). Furthermore, we significantly extend this observation by defining a 14-cM common region of deletion at 8p22 and by demonstrating homozygous deletion of the MSR locus within this region. This is the first homozygous deletion identified in the genome of a human prostate cancer and the highest rate of loss yet reported on chromosome 8p in cancer.

Homozygous deletion has been associated with tumor suppressor gene inactivation in a number of instances (16, 17). Taken together, frequent allelic loss and homozygous deletion of the MSR locus is strong evidence of the presence of a tumor suppressor gene near this locus which is inactivated in the majority of prostate cancers. The extent of the homozygous deletion in this tumor is unknown at present. Further study of its boundaries should allow more precise definition of the critical region containing this putative tumor suppressor gene.

The MSR gene itself encodes the macrophage scavenger receptor, a membrane glycoprotein that mediates the endocytosis of a diverse group of macromolecules (18). MSR protein has been detected immunohistochemically in foam cells within atherosclerotic lesions consistent in appearance with macrophages, and MSR mRNA has been detected in liver, spleen and at low levels in brain (18). Similarly, expression of MSR in the liver is restricted to cells lining the sinusoids, and is not found in hepatocytes (15). Based on the lack of expression of MSR in noncancerous adult prostate epithelium, as well as the lack of a plausible role for MSR in prostate carcinogenesis, it is unlikely that the MSR gene itself is involved in the process of prostate cancer development. However, a recent report of induction of macrophage scavenger receptor mRNA in endothelial cells by phorbol ester (19) underscores the possibility that MSR could play a role in prostate physiology in certain metabolic states, e.g., growth. Further work will be necessary to fully address this question.

Alterations in chromosome 8 have been implicated in the pathogenesis of colorectal (20–22), prostate (7), hepatocellular (20), lung (20), and bladder carcinoma (23). Cytogenetic studies of prostate cancer revealed chromosome 8 abnormalities in primary cultures of prostate cancers (24–26) but these involved relatively few tumors and did not localize the changes to a specific region. Vogelstein et al. (21) found allelic loss of chromosome 8p in 50% of 22 colorectal carcinomas studied at NEFL (8p21.3–21.1) and SW50 (8p23.3–23.1) using RFLP analysis, localizing the area of interest to 8p. Bergerheim et al. (7) found loss at NEFL (8p21.3–21.1) in 5 of 5 metastases and 2 of 3 primary prostate cancers and based on deletion mapping localized the smallest region of overlap to between 8pter–8q11.2. Emi et al. (20) recently reported frequent loss of 8p loci in 22 of 46 (48%) informative primary hepatocellular carcinomas, 12 of 26 (46%) informative colorectal carcinomas, and 14 of 35 (40%) of informative non-small cell lung cancers but found relatively infrequent 8p loss in breast cancer (5 of 56, 9%) and in renal cell carcinoma (2 of 27, 7%). They found the smallest region of overlap in this study to include 8p21.3–8p23.1 and in a subsequent report (27) describe the finding of two commonly deleted regions in colorectal carcinoma, localized to 8p22.2–8p22 and 8p21.3–8p11.22, suggesting that at least two tumor suppressor genes important in colorectal cancer are located on chromosome 8p. Interestingly, they also found that only the more distal region (8p22.2–8p22) is commonly deleted in hepatocellular carcinoma.

Emi et al. (20) report relatively common interstitial deletion of 8p loci in colorectal and hepatocellular cancer. We observe interstitial deletion in 15 of 32 (46%) of the prostate cancers with 8p allelic loss. The reason for this high frequency of interstitial deletion on chromosome 8p in multiple cancers, and in particular in prostate cancer is unknown. One possibility is that retention of heterozygosity of alleles proximal and distal to the putative suppressor gene at 8p22 may result in a selective growth advantage for these cells.

The multiplication of chromosome 8q observed in a small fraction of prostate cancers may similarly confer a selective growth advantage to cells undergoing this change, particularly when occurring in concert with loss on 8p. Fujiwara et al. (28) observed 2–6 fold multiplication of chromosome 8q in 32 of 78 (41%) of informative hepatocellular carcinomas and at a lower frequency in colorectal cancers. Characterization of the putative tumor suppressor gene(s) on chromosome 8p and further characterization of the multiplied segment of chromosome 8q will be necessary to fully address this question.
8q will allow better understanding of this process. Furthermore, whether a second gene located at 8p21.3–8p11.22 is important in prostate cancer, as suggested in colorectal cancer (27), cannot be resolved with the data reported here. Further analysis of allelic loss at loci proximal to 8p22 in human prostate cancers should help resolve this question.

Most likely due to the selection criteria used in this study to obtain relatively pure tumors, the group of primary tumors studied is relatively high stage (100% T3, 26% N). Whether chromosome 8p22 loss is an early or late change in the development of prostate cancer thus cannot be determined in the present study.

Our findings narrow the most likely site of an important tumor suppressor gene in prostate cancer to chromosome 8p22. The finding of inactivation of the tumor suppressor genes p53 in colon cancer and RB in retinoblastoma led to the discovery of their frequent inactivation in a wide variety of other tumor types. Likewise, the finding of loss in the region of 8p22 in a variety of epithelial tumors suggests that inactivation of gene(s) at this site may be associated with cancer development in several different tissues. Identified genes known to reside in this region include clusterin (8p21) (29), glutathione reductase (8p21.1) (30), cathepsin B (8p22) (31), lipoprotein lipase (8p22) (32), and macrophage scavenger receptor (8p22) (33). The deletion mapping data presented in this study should prove useful for positional cloning efforts aimed at the identification of candidate prostate cancer tumor suppressor gene(s) located in the 8p22 region.

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