Allelic Imbalance and Loss of Heterozygosity at 5q11 in Human Prostate Cancer: A Novel Region for a Tumor Suppressor Gene

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KEY WORDS Prostate cancer genetics; LOH; allelic imbalance; tumor suppressor gene; microsatellite instability.

ABSTRACT Loss of heterozygosity (LOH) and allelic imbalance (AI) are widely recognized ways of localizing tumor suppressor genes. We have demonstrated structural alterations of chromosome 5 in 12 human prostate cancer cell lines. After mapping the breakpoints involved in translocations and deletions in chromosome 5, the 5q11 region was found to be the most consistent breakpoint. In this study, we further analyzed the 5q11 region in several prostate cancer cell lines as well as tumor samples from prostate cancer patients. Five different polymorphic sequence tagged sites (STSs) (D5S430, D5S822, D5S664, D5S645, and D5S2068) from this region were evaluated for LOH in order to discern the specific breakpoint in chromosome 5. A breakpoint was shown between the markers D5S2068 and D5S407 in the PC-3M human prostate cancer cell line. Allelotyping of prostate tumor samples revealed LOH in 6 (65%) of 9 tumor samples in at least one of the markers tested. In addition, in three cell lines (DU-145, MDAPCa2b and C4-2) microsatellite instability (MSI), shown as gain of alleles, was observed, suggesting RER+ phenotype. These results implicated 5q11 and specifically the region surrounding markers D5S2068 as a region of interest for locating a tumor suppressor gene in human prostate cancer.

INTRODUCTION

Prostate cancer is considered a disease of older men. The identification of genetic catastrophe in the germline or in somatic cells would identify family members at increased risk and could provide insight into the pathogenesis of the disease. Loss of heterozygosity (LOH) is a widely recognized way of localizing tumor suppressor genes. LOH or allelic imbalance (AI) on chromosomes 8p, 10q, 12q, 13q, 16q, 17p and 18q has already been reported in prostate cancer by other investigators (Ozen et al. 1996, 1998). Mapping of the breakpoints involved in translocations and deletions in chromosome 5 showed that the 5q11 region was the most consistent breakpoint. Furthermore, the tumorigenicity of a human prostate cancer cell line was inhibited by microcell-mediated transfer of a normal human chromosome 5 (Ewing et al. 1995).

In this study, we further analyzed the 5q11 region in several prostate cancer cell lines as well as tumor samples from prostate cancer patients. Several polymorphic sequence tagged sites (STSs) from this region were evaluated for LOH in order to discern the specific breakpoint in chromosome 5.

MATERIALS AND METHODS

Prostate Cancer Cell Lines and Prostate Tissue Samples

The following five prostate cancer cell lines were used in the study: PC-3M, DU-145 (Stone et al. 1978), ARCaP (Zhau et al. 1996), MDA PCa 2a (Navone et al. 1997) and C4-2 (Thalmann et al. 1994). All were grown in culture conditions described previously (Ozen, et al. 1998). Paired prostate tumor and adjacent normal prostate tissues were obtained from patients undergoing radical prostatectomy at the Mayo Clinic in Rochester, MN, and at The University of
DNA Isolation

Each cell line was grown to 70-80% confluence. The cells were harvested by trypsinization and pelleted for DNA isolation. For patient samples, normal prostate tissue and tumor biopsy samples from paraffin-embedded sections were trimmed by microdissection using H&E-stained sections as reference, and then the DNA was isolated. When available, DNA isolated from peripheral blood was used as a normal control. Tissue processing and DNA isolation were carried out following the procedures of Feddersen and Van Ness (1989) and Cunningham et al. (1996). Briefly, cells were lysed in 0.5 M NaCl/0.1% sodium dodecyl sulfate (SDS)/10 mM EDTA, pH 7.5. Pre-digested proteinase K was added to 1-2 mg/ml, and digestion was allowed to proceed at 50 °C for from 4 h to overnight. DNA was then extracted once with phenol, once with phenol:chloroform (1:1, vol:vol), and once with chloroform:isoamyl alcohol (24:1, vol:vol), as described elsewhere (Nagarajan et al. 1994).

Sequence Tagged Sites (STSs)

Table 1 summarizes the STS markers by heterozygosity index, primers, and mapping position from the top of the human chromosome 5 linkage group.

Polymerase Chain Reaction (PCR)

A sensitive PCR technique was used to detect allelic losses in all our samples (Fairman et al. 1994; Fairman and Nagarajan 1997). Briefly, each PCR amplification consisted of ~ 50 ng of template DNA, 0.5 µg of each primer, 13 nM 32P-end labeled primer, 250 µM concentrations of each deoxynucleoside triphosphate, 10 nM Tris-HCl (pH 8.4), 40 mM NaCl, and 2.0 mM MgCl2, and 5 units of Taq DNA polymerase in a volume of 25 µl. The following amplification protocol was used: 1) 1 cycle at 94 °C for 5 min; 2) 30 cycles, each at 94 °C for 1/2 min, 55°C for 1/2 min, and 72 °C for 1/2 min; and 3) 1 cycle at 72 °C for 10 min. After PCR, 25 µl of stop solution was added to each sample. Two microliters of the sample was then loaded onto a 6% polyacrylamide gel containing 7 M urea, 450 mM Tris-borate (pH 7.5) and 1 mM EDTA (pH 7.0) running buffer. Loaded gels were electrophoresed for 3-4 h. The gels were dried and exposed to X-ray films for 2 h to overnight at -70 °C, and the autoradiograms were analyzed for genotyping. The markers were selected because of their high heterozygosity index. A continuous region with one allele for a cell line would indicate a region of loss.

Quantitation of Amplification

Samples were analyzed on a Molecular Dynamics Phosphor Imager to determine the incidence of the two alleles. LOH was considered to happen when the intensity of one of the two alleles in tumor DNA was less than 50% of that in corresponding normal tissue DNA for a given amount of DNA. Selected bands were marked on the gel. Samples were cut out from the dried polyacrylamide gels, and the radioactivity measured on a Beckman LS1800 scintillation counter (Beckman Scientific Instruments, Irvine, CA).

Table 1: Descriptions of STS markers used in prostate tumor samples

<table>
<thead>
<tr>
<th>Heterozygosity Index</th>
<th>Primers Location (cM)</th>
<th>Location (cM)</th>
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<tbody>
<tr>
<td>D5S430</td>
<td>0.78</td>
<td>Left = TCTGCCAGCAATTCATAG</td>
</tr>
<tr>
<td>D5S822</td>
<td>0.76</td>
<td>Left = GCTCAATGGCTCAATTTCC</td>
</tr>
<tr>
<td>D5S664</td>
<td>0.84</td>
<td>Left = AATTGTTCCAGGACCCATCCCC</td>
</tr>
<tr>
<td>D5S645</td>
<td>0.76</td>
<td>Left = CTGGTCTTGAACTCCCAAC</td>
</tr>
<tr>
<td>D5S2068</td>
<td>0.81</td>
<td>Left = ACAATTGATGAGGAGGACAAAG</td>
</tr>
<tr>
<td>D5S407</td>
<td>0.86</td>
<td>Left = TTTGATTTGATTTTTCATGAGG</td>
</tr>
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</table>
RESULTS

Allelic Imbalance at 5q11 in Prostate Cancer Cell Lines

As seen in table 2, the PC-3M cells showed continuous loss of one allele for the markers D5S430, D5S822, D5S664, D5S645, and D5S2068. However, both alleles were intact for the marker D5S407. These observations indicate that the breakpoint was between the markers D5S2068 and D5S407 in this cell line. These two markers are 1.1 cM apart.

The ARCaP and MDAPCa2b cell lines showed losses of one allele for the markers D5S430 and D5S822, respectively. The other two cell lines retained both alleles in all markers studied. Figure 1 demonstrates losses and intact copies of alleles on LOH studies in selected prostate cancer samples.

Replication Errors

In three cell lines (DU-145, MDAPCa2b and C4-2) microsatellite instability (MSI), shown as gain of alleles, was observed, suggesting RER+ phenotype (Table 2). Most authors state that the tumor is described as RER+ (suggesting replication error) if the tumor has MSI in at least one marker (Cunningham et al. 1996; Risinger et al. 1993; Rhyu et al. 1994; Terrell et al. 1995; Watanabe et al. 1996; Dahiya et al. 1997; Bapat et al. 1999).

LOH at 5q11 in Tumors from Prostate Cancer Patients

In prostate tumor samples, allelotyping analysis revealed LOH in 6 (65%) of 9 tumors in at least one of the markers tested (Table 2). Patients 24, 33 and 11 retained both alleles at D5S407. However, patients 24 and 11 exhibited LOH at D5S2068 and D5S645. In addition, patient 33 showed LOH at only D5S2068. Two additional patients were non-informative for this marker. Only two (16%) of 9 patients retained both alleles at D5S2068. These results implicated the region surrounding marker D5S2068 as the locus of interest for further experimentation and locating a tumor suppressor gene in human prostate cancer.

Of 9 patients studied, four (45%) were not

<table>
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<th>Table 2: Results of LOH studies on chromosome 5q11 in prostate cancer cell lines and tumor samples</th>
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<tr>
<td><strong>Cell Lines</strong></td>
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<tr>
<td>PC-3M</td>
</tr>
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</table>

•: LOH, ![ ]: Retention of both alleles, NI: Not informative, Rer: Replication errors

<table>
<thead>
<tr>
<th>Table 3: Clinical characteristics of prostate cancer patients used in LOH studies</th>
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<tr>
<td><strong>Patient</strong></td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>24</td>
</tr>
<tr>
<td>33</td>
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<td>26</td>
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* 1: diploid, 2:tetraploid, 3: aneuploid
$ NED= No evidence of disease
& Radiation was started after PSA increase was detected (4.26.94)
Fig. 1. LOH at 5q11 in PC-3M prostate cancer cell line (a) and in selected prostate tumor samples from cancer patients (b). N: Normal DNA; T: Tumor DNA; STS markers used are given next to the appropriate bands. Prostate tumors from patients 26 and 7 showed LOH in markers D5S407 and D5S2068, respectively.
informative at the marker D5S430, and 3 (33%), at the marker D5S822. The heterozygosity index for these markers was 0.78 and 0.76, respectively. The MDAPCa 2b cell line also showed only one allele at D5S822. These results suggest a second region of interest between these two markers. In addition, patient 22 showed constitutionally non-informative results for the markers D5S822, D5S664, D5S645 and D5S2068. Taken together, these results suggest that mitotic recombination might have been occurring in the adjacent, so-called normal prostate tissue. Clinical features of all patients whose normal tissues and prostate tumors were allelotyped are summarized in Table 3.

DISCUSSION

In this study, we used six different microsatellite markers spanning the 5q11 region to narrow down a region of loss in prostate cancer cell lines and LOH in paired prostate tumor and normal samples. The PC-3M cell line showed loss of one allele within the region studied except at D5S407, where both alleles were retained. Introduction of a normal chromosome 5 into the PC-3 cell line suppressed tumorigenicity in nude mice (Ewing et al. 1995). Since then, no group has reported a loss of chromosome 5 region in PC-3M cells. The present data confirm our previous report in which we studied breakpoints at chromosome 5 in the PC-3M cell line. It also narrows down the break point to the ~1 cM region flanking markers D5S2068 and D5S407.

To further substantiate these findings, we examined additional paired prostate tumors and normal samples from cancer patients, some of whom were known to have an AI at 5q13. Interestingly enough, 65% of the patients with known AI at 5q13 showed LOH at marker D5S2068. We chose markers with a high heterozygosity index (0.76-0.86) to obtain more informative results. However, samples from 68% of the patients were non-informative, at least for one marker. Moreover, one prostate cancer patient gave constitutionally non-informative results. These observations taken together suggest that some genetic changes might have occurred in “normal” prostate tissue adjacent to a tumor, leading to malignant changes. Another possibility is that genomic instability might have occurred in the germ cells of these few patients. Ability to detect these very early changes might not only help early diagnosis of prostate tumor but also contribute to our understanding of prostate tumor biology and novel treatment strategies.

The physical loss or functional inactivation of tumor suppressor genes is known to be involved in the development of most solid tumors. Looking for deletions and AI and determining LOH within a chromosomal region in tumor samples are commonly used methods of searching for tumor suppressor genes. The most commonly lost chromosomal regions in primary prostate cancer are 6q, 8p, 9p, 13q and 18q (Carter et al. 1990; Kunimi et al. 1991; Bova et al. 1993). Several reports have shown LOH at 8p, 10q, 12q, 13q, 16q, 17p and 18q in prostate cancer (Macoska et al. 1992; Berube, et al. 1994; Isaacs et al. 1994; Trapman et al. 1994; Fan et al. 1994; Gray et al. 1995; Ittmann 1996; Elso et al. 1997; Isaacs 1997; Latil et al. 1997; Hugel and Wernert 1999; Mocera et al. 1999; Phillips et al. 1994). However, losses at chromosome 5 have rarely been reported in prostate cancer (Ozen et al. 1996, 1998; Cunningham et al. 1996), owing to their cryptic nature and/or the requirement for a molecular approach.

Cher et al. (1996), detected 5q losses in 39% of their androgen-independent prostate cancer cases by comparative genomic hybridization (CGH) studies. They also suggested that losses at 5q might be associated with reduced levels or absence of alpha catenin or E cadherin. Another study showed 5q losses in 44% of recurrent prostate cancer cases, in which the APC gene was localized to 5q21; LOH of this region has been found in 20-30% of advanced prostate carcinomas (Visakorpi et al. 1995). We have reported earlier a consistent breakpoint at the 5q11 region in 12 human prostate cancer cell lines (Ozen et al. 1998). A previous study reported AI on chromosome 5 (Cunningham et al. 1996). AI on 5q is seen more often in tumors of high pathological stage than in those of low pathological stage.

MSI observed in prostate cancer cell lines may suggest defective DNA mismatch-repair
(MMR) mechanisms. MSI may not affect the phenotype of the cell, but they are a sensitive measure of defective DNA MMR (Risinger et al. 1993; Rhyu et al. 1994; Terrell et al. 1995; Watanabe et al. 1996; Dahiya et al. 1997; Wada et al. 1994; Honchel et al. 1995). Mutations in at least one of the four MMR genes (hMSH2, GTBP, hMLH1, or hPMS2) in MMR-defective cell lines have been reported by different investigators (Bapat et al. 1999; Boyer et al. 1995; Thibodeau et al. 1998).

Just as we finished writing and were about to submit our paper for publication, Lin et al., (2000) reported a novel human prostate-specific, androgen-regulated gene, PART-1. This gene was mapped to chromosome 5q12 between the markers D5S2376 and D5S427, which are telomeric to the markers we have studied. Their findings, together with ours, suggest that chromosome 5q might contain one or possibly more tumor suppressor genes possibly associated with human prostate cancer development.

In conclusion, our data suggest a potential specific region in chromosome 5q for the presence of tumor suppressor gene(s) for human prostate cancer: between markers D5S2068 and D5S407. We further suggest that “normal” prostate tissue surrounding the prostate tumor should be examined more carefully for possible genetic alterations. Any constitutionally non-informative case should also be noted for future investigations because prostate tumor biology appears to be more complex than previously thought by most investigators.

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