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Deletion at 13q21 Is Associated with Aggressive Prostate Cancers[1]

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ABSTRACT

Previous cytogenetic and molecular genetic analyses suggest that the q21 band of chromosome 13 harbors a tumor suppressor gene(s) involved in prostatic carcinogenesis. The precise genetic location, however, has not been defined. In this study, we examined prostate cancer specimens and cell lines/xenograft for genetic deletions at 13q21, using the methods of tissue microdissection and duplex PCR. Deletions at 13q21 were detected in 13 of 147 (9%) prostate cancer samples. Deletion of the same region was also detected in the LNCaP cell line and the PC-82 xenograft of prostate cancer. The overlapping region of deletion in LNCaP and PC-82 spans 3.1 cM or 2.9 cR, which is equivalent to 1–3 Mb. The endothelin receptor B gene, a possible tumor suppressor gene at 13q21, was not located in the region of deletion. Among the 13 prostate neoplasms with deletion at 13q21, 5 were metastases, and 7 were poorly differentiated primary tumors. The only primary tumor that was not poorly differentiated but had deletion occurred in one of the youngest patients (49 years) at diagnosis. These results provide evidence that 13q21 may harbor an unidentified gene(s) whose inactivation occurs in some aggressive carcinomas of the prostate. In addition, this study provides a framework for the cloning and identification of the 13q21 gene(s).

INTRODUCTION

Molecular determinants important in the development and progression of prostate cancer are poorly understood, despite the fact that this neoplasm has become a significant health problem (1). Cytogenetic and molecular genetic analyses have indicated that interstitial deletions on chromosomes 8p, 13q, 10q, 6q, 7q, 17q, and 18q occur frequently in human prostate cancer, suggesting the existence of tumor suppressor genes on these chromosomal arms (2, 3). At present, however, only 10q has been identified as having a tumor suppressor gene (*i.e., PTEN*) that has been implicated in prostate cancer (4, 5). The target genes from the remaining chromosomes have yet to be mapped and identified.

Deletion of portions of chromosome 13 has been detected by various genetic approaches in human prostate cancer. In a cytogenetic banding study, nonrandom loss of chromosome 13 was observed in a xenografted cell line (6). CGH[3] demonstrated that loss of 13q is the second most frequent chromosomal alteration, having occurred in 32% of primary tumors, 56–75% of recurrent and metastatic tumors, and each of the four commonly used prostate cancer cell lines derived from metastatic prostate cancer (7–11). One CGH study suggested a deletion region at the q21 band of chromosome 13 (10). In our LOH assay, we identified a distinct region of LOH in a 7-cM DNA segment involving markers D13S269 and D13S162 at 13q21 (12). These studies suggested that a tumor suppressor gene is located at 13q21; however, the DNA segment containing this gene was still too large for its identification.

To fine map the region of deletion and to evaluate the clinical significance of 13q21 deletion in prostate cancer, we analyzed a number of STS markers at 13q21 for genetic deletions in prostate cancer, using the approaches of tissue microdissection and duplex PCR. The minimal region of the deletion was confined to a DNA fragment of 3.1 cM or 2.9 cR (1–3 Mb), and the deletion at 13q21 appeared to be associated with tumor aggressiveness.

MATERIALS AND METHODS

Tumor Specimens, Cell Lines, and Xenograft. A total of 147 prostate cancer tissues from 125 patients were examined for deletion in this study. Of them, 103 were primary tumors, 6 were lymph node metastases obtained at surgery, and 38 were either primary tumors (12 specimens) or metastases (26 specimens) from various organ sites obtained at autopsy from 16 patients who died of prostate cancer. Among the 103 primary tumors from surgery, one was a well-differentiated tumor (Gleason score, 4), 19 were moderately differentiated cancers (Gleason score, 5 or 6), 40 were moderately poorly differentiated tumors (Gleason score, 7), and 43 were poorly differentiated neoplasms with Gleason scores of 8–10. Each of the primary tumors from autopsy was a high-grade cancer. Patient age ranged from 42–88 years. Tumor tissues were zinc formalin-fixed and paraffin-embedded, and the cells for DNA isolation were collected from 7-μm H&E-stained sections using a previously described protocol for preparation of histological sections on glass slides before microdissection (13), which ensured a minimum of 70% neoplastic cells. Nonneoplastic cells from lymph nodes or seminal vesicles in most of the cases or from normal prostate stroma or urothelium in the remainder of the cases were obtained from paraffin blocks that contained no neoplastic cells.

Prostate cancer cell lines LNCaP, PC-3, DU 145, and TSU-Pr1 were purchased from American Type Culture Collection (Manassas, VA) and propagated following the manufacturer's instructions. The PC-82 prostate cancer xenograft was described previously (14).

DNA Preparation. For most of the tumor specimens, DNA was isolated from microdissected cells by adding proteinase K solution and incubating at 55°C overnight, followed by boiling the solution for 10 min to inactivate proteinase K, as described previously (12). One μl of DNA sample was used in each PCR. For tumor specimens including all of the autopsy cases where greater amount of tissues were available, phenol/chloroform extraction and ethanol precipitation were performed after the proteinase K treatment. For these samples, 5–50 ng of DNA were used for each PCR. For the cell lines and the PC-82 xenograft, genomic DNA was isolated by using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI).

STS Markers. We initially analyzed the two mapped genetic markers, *i.e.*, D13S269 and D13S162, that are located in the 13q21 LOH region in our previous study (12). After deletion for either of these markers was detected, additional markers that flank the deleted ones were analyzed to further define the segment with deletion. Selection of additional markers was based on the latest version of the integrated human genomic map (15),[4] which is available on line from the Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research, Human Genetic Mapping Project and from the high-resolution yeast artificial chromosome-cosmid-STS map of human chromosome 13 (16). Primer sequences of these markers are available from the Genome Database.[5] The endothelin receptor B (*EDNRB*) gene, which is close to but telomeric to marker D13S162 at 13q21 (17), was also examined using a STS marker derived from its fourth exon (18). Primer sequences for exon 4 of the *EDNRB* gene are 5′-ATCCCTATAGTTTTACAAGACAGC-3′ (for-

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[3] The abbreviations used are: CGH, comparative genomic hybridization; LOH, loss of heterozygosity; STS, sequence tagged site.

[4] Supplementary data from the Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research, Human Genetic Mapping Project (http://www-genome.wi.mit.edu).

[5] http://gdbwww.gdb.org.

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ward) and 5′-ATTTTCTTACCTGCTTTAG GTG-3′ (reverse). PCR primers were either purchased from Research Genetics (Huntsville, AL) or synthesized by Life Technologies, Inc. (Gaithersburg, MD).

In addition to 13q21 markers, each PCR contained one internal control STS marker, *i.e.*, one of the exons from the KAI1 gene whose deletion has not been found in prostate cancer (19, 20). Internal controls were necessary for reliable detection of chromosomal deletions. Depending on the size of PCR products of a 13q21 marker, exon 5, 7, or 8 of the KAI1 gene was used. The primer sequences of these KAI1 exons have been described previously (19). Sizes of PCR products, annealing temperatures, KAI1 control exons, genetic and physical maps, and deletion status in LNCaP and PC-82 cells for the 13q21 markers are listed in Table 1.

Deletion Analysis. The duplex PCR approach was used for the deletion detection. Each PCR, which was in a volume of 10 μl, contained 1 μl of genomic DNA, 1× PCR buffer [20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100, and 0.1 mg/ml BSA], 0.4 μM of each primer, 20 μM of each deoxynucleotide triphosphate, 1 μCi of [α-^{33}P]dATP (3000 Ci/mmol; ICN, Irvine, CA), 0.6 unit of platinum Taq antibodies (Life Technologies, Inc.), and 0.6 unit of Taq DNA polymerase. After an initial denaturation at 95°C for 5 min, 30 cycles, each consisting of denaturation at 94°C for 45 s, annealing for 45 s, and extension at 72°C for 1 min, were performed.

PCR products were separated in 5% nondenaturing polyacrylamide gels (size, 20 × 20 cm) at 200 V for 1–2 h. The gels were dried and exposed to Kodak Biomax MR film at room temperature overnight. Absence of a PCR product for a 13q21 marker in a tumor defined a deletion. However, the microdissected tumor samples were sometimes contaminated with nonneoplastic cells sufficient to give rise to PCR products. When this occurred, signal intensities for PCR products were quantitatively measured by scanning and analyzing PCR bands from a film using ScanDNASIS software (Hitachi Software, San Bruno, CA); the ratio of signal intensity of a 13q21 marker to that of the internal control marker was calculated for each DNA sample, and a deletion was considered to be present when such a ratio in a tumor was less than half of that in its matched nonneoplastic cells.

All experiments were repeated one to three times, and the deletions were detected in each of the experiments.

RESULTS

Based on the multiplex PCR method used for the detection of homozygous deletion in our previous study (21), we first adjusted the experimental procedures to maximize the sensitivity and consistency in detecting genetic deletion. Compared with regular PCR and agarose gel electrophoresis, we found that the procedure of radioactive PCR with [α-^{33}P]dATP, use of the hot-start approach by adding Taq antibodies, separation of PCR products with nondenaturing PAGE, and exposure of gels to Kodak Biomax MR film was more consistent, quantitative, and sensitive in demonstrating genetic deletions in tumor samples.

We first analyzed the two markers (*i.e.*, D13S269 and D13S162) that were located in the LOH region at 13q21, based on our previous study (12), in each of the 147 tumor specimens using the improved method of duplex PCR assay (Table 1). Some tumors showed absent or reduced band intensities for D13S269 and/or D13S162 compared with that of the internal control marker and with that of a normal control. We then repeated the PCR for these tumors, along with their matched nonneoplastic cells. As shown in Fig. 1 for some specimens, deletions at D13S269 and D13S162, which could be either homozygous deletions or hemizygous deletions, were repeatedly detected in 13 tumor specimens. Whereas 11 of 13 tumors lost both D13S269 and D13S162, 2 tumors lost D13S269 only (Table 2).

In total, 13 of 147 (9%) prostate cancer samples showed deletion at 13q21. Patient age at diagnosis and Gleason score for these neoplasms are shown in Table 2. Among these tumors, five were metastases, and seven were poorly differentiated primary tumors. The only primary tumor that was not poorly differentiated but had a deletion (case 233) occurred in one of the youngest patients (49 years) at diagnosis.

To determine whether deletion at 13q21 also occurred in prostate cancer cell lines and xenograft, we analyzed D13S269 and D13S162 for deletion using the same duplex PCR method. As shown in Table 1 and Fig. 2, whereas three cell lines did not show any deletions, the LNCaP cell line and the PC-82 xenograft showed a significant signal reduction at D13S269 and D13S162. Measurement of signal intensities using the ScanDNASIS program indicated that the signal ratio of the deleted marker to the internal control in LNCaP cells was only about one-fourth of that in the normal placenta control DNA. Deletion analysis of cell lines and xenograft was also repeated using regular PCR and agarose gel electrophoresis, and deletion of D13S269 and D13S162 was demonstrated in each experiment (data not shown).

Table 1 *Genetic and radiation hybrid (RH) maps, sizes of PCR products, annealing temperatures, internal KAI1 control markers, and deletion status in LNCaP and PC-82 cells for the 13q21 markers*

Marker names[a]	Genetic map (cM)	RH map (cR)	PCR product (bp)	Annealing temperature (°C)	KAI1 exons	LNCaP	PC-82
D13S1273	49.4		115	55	7	+	+
D13S1260	50.7		141	57	7	+	+
D13S1317	51.9		223	57	8	+	+
D13S275	52.7		239	55	7	−	+
D13S1310	53.5		143	55	7	−	+
D13S1318	54.0		259	57	7	−	+
WI-5860		191.5	224	57	8	−	+
WI-3660			132	61	7	−	+
D13S1090E[b]	56.6		107	61	7	−	+
D13S152	56.6		138	55	7	−	−
D13S745			189	57	7	−	−
D13S791			294	61	7	−	−
D13S1249	57.3		220	60	8	−	−
D13S166	57.3		115–125	57	7	−	−
D13S156	57.3	193.2	272–286	60	7	−	−
D13S269	58.3		142	60	7	−	−
H65656	59.7	194.4	~150	61	7	−	−
D13S162	59.7	194.4	182–202	55	7	−	−
BS610/611[b]	59.7	194.4	95	57	7	+	−
D13S1562			148	60	7	+	−
WI-16413			132	60	7	+	−
D13S160	62.7	207.7	229–241	57	8	+	+
EDNRB			170	60	5	+	
D13S170	65.4		113	57	7	+	+

[a] The markers are listed in order from centromere to telomere according to the published genomic maps (15, 16).[4]

[b] Markers D13S1090E and D13S152 are located in one BAC clone and are thus considered to be at the same genetic location (data not shown). Similarly, markers BS610/611, H65656, and D13S162 are in another BAC clone. Primers sequences for marker BS610/611 are 5′-TATTCATCCAGCCCCCTCAATG-3′ and 5′-AGATGTGCAGGAGATGAATGGC-3′.

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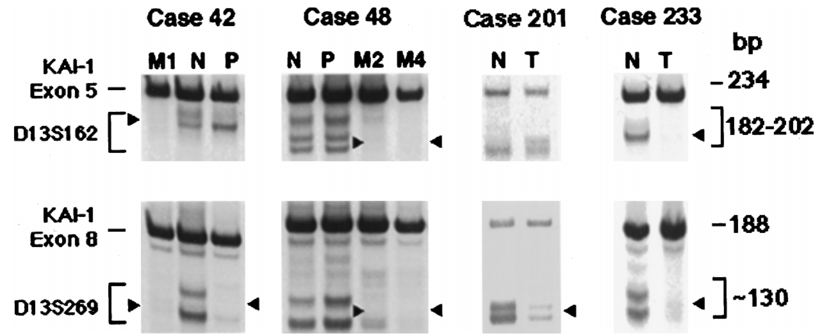

Fig. 1. Detection of deletion at 13q21 in prostate cancer by duplex PCR assay. Case number and tissue type are indicated at the *top*, STS markers are indicated at the *left*, and the size of the PCR products is indicated at the *right*. Each *arrow* denotes a deletion at a marker in a neoplasm. *Lanes P* and *M*, primary tumor and metastasis obtained from autopsy specimens; *Lane T*, primary tumor obtained at surgery; *Lane N*, matched nonneoplastic cells in each case. Due to the polymorphic feature of microsatellite markers, two bands that represent two alleles are seen for both markers in most cases. Case 42 (*Lane P*) appears to have LOH instead of deletion at D13S162.

To determine the size of the region with deletion, we selected more STS markers (Table 1) that flank D13S269 and/or D13S162 at 13q21 and examined them in LNCaP and PC-82 tumor cells that showed deletions at D13S269 and D13S162 and had a sufficient quantity of pure tumor DNA for analysis (Table 1; Fig. 2). The deletion region in LNCaP was different from but overlapped that in PC-82, and the common region of deletion was defined as a DNA segment of 3.1 cM within markers D13S1090E and BS610/611 or 2.9 cR within markers WI-5860 and BS610/611 (Table 1). According to the current genomic maps (15, 16), the size of this common region of deletion was estimated to be 1–3 Mb. The endothelin receptor B (*EDNRB*) gene, which is located at least 3 cM telomeric to the common region of deletion at 13q21 and has been suggested as a tumor suppressor gene, was also analyzed in five tumors and in all of the cell lines but showed no deletion (data not shown).

DISCUSSION

In this study, we first improved the multiplex PCR procedure used in our previous study (21) for the detection of genetic deletion in human tumors. Use of radioactive PCR with a hot-start approach, PAGE, and exposure to Kodak Biomax MR film made deletion detection more reliable when compared with regular PCR and agarose gel electrophoresis. As shown in Fig. 1, some tumor samples had no signal or a very faint signal at the deleted markers D13S269 and D13S162 compared with their matched nonneoplastic cells. Detection of deletion in the LNCaP cell line but not in any other cell lines further indicated the feasibility of our methods because a high-resolution cytogenetic banding study showed that each LNCaP cell has an interstitial deletion at 13q21 in some but not all of its copies of chromosome 13 [it has a near tetraploid karyotype (22)].

Genetic deletion at 13q21 in prostate cancer was first demonstrated by a cytogenetic banding study in the LNCaP cell line (22). In CGH analyses, deletion of 13q was detected in human prostate cancer tissues and in each of the four commonly used prostate cancer cell lines (7–11), and a common region of deletion was suggested to be located at 13q21 (10). Recently, our LOH study further showed that the deletion at 13q21 involved markers D13S313, D13S269, D13S162, and D13S1306 in a DNA interval of 7 cM (12). Using the methods of tissue microdissection and an improved duplex PCR assay, we found that genetic deletions at 13q21 occurred in 13 of 147 (9%) prostate cancer specimens and 2 of 5 (40%) prostate cancer cell lines and xenograft. Consistent with previous studies, our data provide additional evidence for the existence of a tumor suppressor gene(s) at 13q21.

Although previous studies identified a region of deletion at 13q21 in prostate cancer, the size of the region with deletion was still too large for gene identification, and the precise location of the target gene remained to be defined. Using densely mapped genetic markers and the LNCaP cell line and PC-82 xenograft of prostate cancer, we defined the common region of deletion to be in a 3.1-cM segment within markers D13S1090E

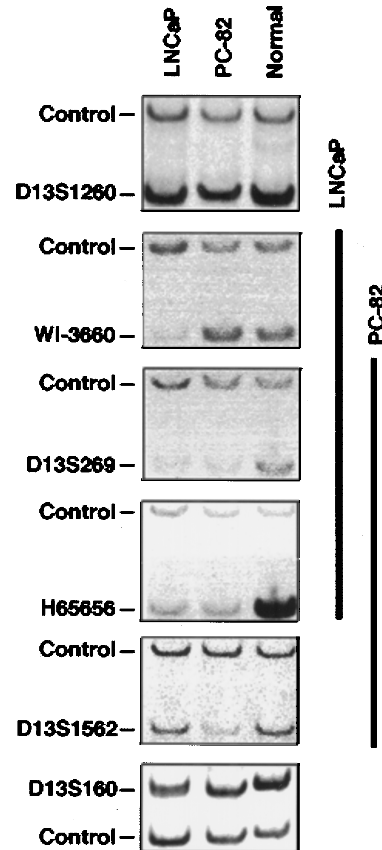

Fig. 2. Deletions of 13q21 markers in LNCaP cell line and PC-82 xenograft of prostate cancer. Sample names are indicated at the *top*, and marker names are indicated at the *left*. Markers deleted are indicated by *vertical bars* at the *right*. Normal DNA was from a normal human placenta.

Table 2 *Prostate cancers showing deletions at D13S269 and D13S162 and patient age at diagnosis and Gleason score (G.S.). "M" indicates a metastasis*

Presence or absence of a marker in a tumor is indicated by + or −, respectively.

Case no.	Age (yrs)	G.S.	D13S269	D13S162
42-P	68	9	−	+
42-M1	68	M	−	−
48-M2	71	M	−	−
48-M4	71	M	−	−
53-P	67	9	−	−
53-M3	67	M	−	−
104-T	75	9	−	−
110-T	65	9	−	−
122-M	68	M	−	−
200-T	71	8	−	−
201-T	80	10	−	+
228-T	69	9	−	−
233-T	49	7	−	−

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and BS610/611 and a 2.9-cR segment between WI-5860 and BS610/611 (Table 1). Considering that, on average, 1 cM is equivalent to 1 Mb and 1 Mb is equivalent to 3.7 cR for the Genebridge4 Radiation Hybrid Panel,[6] our findings indicate that the minimal region of deletion containing the target gene should be in the size range of 1–3 Mb.

Twelve of the 13 prostate cancer samples with homozygous deletion at 13q21 were either poorly differentiated primary tumors or metastases. The only primary tumor that was not poorly differentiated but had a deletion occurred in one of the youngest patients (49 years) at diagnosis (Table 2). The LNCaP cell line and the PC-82 xenograft, which were noted above to have a deletion at the same region of 13q21, were also derived from metastases of prostate cancer (23). These data indicate that deletion at 13q21 occurs in biologically aggressive prostate cancers. Consistently, a previous study found that patients whose prostate cancers showed LOH at 13q were diagnosed at a significantly younger age than those whose tumors lacked LOH at 13q (12).

The endothelin receptor B (*EDNRB*) gene is located at 13q21, telomeric to marker D13S162 but centromeric to marker D13S160 (17). Considering that promoter methylation of the *EDNRB* gene occurs frequently in prostate cancer and that this gene has been suggested to act as a tumor suppressor (24), we analyzed its deletion status in five tumors that showed deletion at D13S269 and D13S162 and in all of the prostate cancer cell lines. No deletion at the *EDNRB* gene was detected. Based on the current genomic map, *EDNRB* is at least 3 cM telomeric to the common region of deletion. Therefore, *EDNRB* is not the target gene for the 13q21 deletion region in prostate cancer. Currently, there is no gene that is located in the region of deletion that has been identified as a tumor suppressor. This study provides a framework for the identification of this gene.

Genetic deletion involving 13q21 has also been detected by CGH in malignant fibrous histiocytomas (25, 26) and other sarcomas (27, 28), and gliomas (29, 30). These studies suggest that different types of tumors may share the same genetic alteration at 13q21 during carcinogenesis or progression.

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