Association of $p53$ Mutations with Metastatic Prostate Cancer\textsuperscript{1}

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ABSTRACT

In prostate cancer, mutation of the $p53$ tumor suppressor gene has been associated with locally advanced disease and hormone-resistant disease that is predominantly localized to bone. However, little is known regarding the status of the $p53$ gene in metastatic prostate cancer that has not been treated with hormonal manipulation. We evaluated formalin-fixed, paraffin-embedded malignant tissues from 86 patients with various stages of prostate cancer, including pathologically confined, locally advanced, and metastatic disease, to detect abnormal $p53$ nuclear protein accumulation using immunohistochemistry. No abnormal $p53$ immunostaining was detected in 18 patients with prostate cancer confined to the gland. Two tumors from 21 patients with locally advanced disease (extracapsular extension and/or seminal vesicle invasion) had abnormal nuclear $p53$ accumulation, and a mutation in exon 7 of the $p53$ gene was detected in tumor DNA from one patient using single-strand conformation polymorphism–direct sequencing analysis. Of the remaining 47 patients studied in whom tissues from the prostate gland and a metastatic site (44 lymph node, 2 bone, and 1 lung) were available, only 3 had received hormonal therapy prior to obtaining metastatic tissue. In four patients both primary and metastatic tumors demonstrated accumulation of $p53$ protein, whereas seven additional patients exhibited $p53$ accumulation only at the metastatic site. In three patients the metastatic tumors harbored missense single-base substitutions in exon 5, as detected using single-strand conformation polymorphism–direct sequencing. These results indicate that $p53$ abnormalities are associated with lymph node metastases derived from prostate cancer patients that had not undergone hormonal therapy.

INTRODUCTION

The tumor suppressor gene $p53$ is centrally involved in inhibiting progression of the cell cycle at the $G_1\rightarrow S$-phase interface, giving the cell time to repair DNA damage before dividing, thus preventing replication of mutated DNA (1–3). This activity is accomplished in part via the transcriptional activation of p21, a cyclin-dependent kinase inhibitor (4–6). Mutations in the $p53$ gene could therefore permit DNA damage present in $G_1$ to be carried into the $S$-phase, resulting in genetically unstable cells more prone to the development of a malignant phenotype (3, 4, 7). A number of human cancers including colon, lung, and breast have been shown to have $p53$ mutations (8–10). The wild-type $p53$ protein is undetectable using immunohistochemistry because of its short half-life. However, certain mutant $p53$ proteins have a longer half-life, resulting in nuclear accumulation. The finding of nuclear staining with anti-$p53$ antibodies is strongly suggestive of mutations within the $p53$ gene (11).

Several investigators have suggested a role for $p53$ gene mutation in prostate cancer. Isaacs et al. (12) evaluated five prostate cancer cell lines and two specimens of primary human prostate cancer for $p53$ mutations by sequence analysis of exons 5–8. Three cell lines, all derived from metastatic sites, and one of the two clinical specimens demonstrated $p53$ mutations and transfection of these cell lines with a wild-type $p53$ gene markedly reduced colony formation. These studies suggested that the $p53$ gene plays a significant role in the development and/or progression of prostate cancer. Effert et al. (13) analyzed 10 primary and 2 metastatic human prostate carcinomas using SSCP\textsuperscript{3} and direct sequencing of PCR products. $p53$ mutations were detected in 1 of 10 primary prostate cancers and 1 of 2 metastatic tumors. Bookstein et al. (14) analyzed archival tumor material from 150 prostate cancer patients using immunohistochemistry with anti-$p53$ antibodies. Abnormal nuclear $p53$ accumulation was detected in 19 tumors and was strongly correlated with higher stage disease. Recently, Navone et al. (15) reported a relatively high frequency of $p53$ mutations in advanced hormone-refractory prostate cancer that was predominantly localized to bone. These authors concluded that $p53$ mutations were associated, in general, with progression of disease, and specifically with hormone-independent prostate cancer. An immunohistochemical evaluation of 137 primary untreated prostate cancers from Finland found an association between high levels of $p53$ accumulation and high-grade proliferating cell nuclear antigen-reactive, aneuploid tumors (16). Although only 6% of the tumors had high $p53$ accumulation, this subset had poor progression-free survival. A study by Chi et al. (17) reported a relatively high frequency of $p53$ mutations in

\textsuperscript{3}The abbreviations used are: SSCP, single-strand conformation polymorphism; dNTP, deoxynucleotide triphosphate; H & E, hematoxylin and eosin; TURP, transurethral resection of the prostate.
prostate cancer. Overall, the frequency of p53 gene abnormalities in all prostate cancer specimens examined was 42%.

In the present study, we further assessed the status of the p53 gene in human prostate cancer. Specifically, 86 patients with prostate cancer, for whom complete clinical and pathological information was available, were evaluated for p53 mutations using immunohistochemistry. This group included 47 patients for whom both primary and metastatic tissue samples were available. Prior to surgery, this subset of patients was considered to have clinically localized disease. To verify and further characterize mutations, all sections demonstrating nuclear accumulation of the p53 protein were analyzed by PCR amplification of DNA isolated from microdissected, formalin-fixed, paraffin-embedded sections followed by SSCP analysis of exons 5, 7, and 8 and direct sequencing of the PCR products.

PATIENTS AND METHODS

Patient Population/Tissue Samples. A total of 86 patients with adenocarcinoma of the prostate were evaluated. Medical records of the patients were reviewed to obtain age at the time of surgery, surgical methods used to obtain the sample(s), and pathological features of the tumor. Histopathological grading was performed by one author (T. M. W.) according to the method of Gleason (18). All patients were pathologically staged using the TNM classification (19).

Archival formalin-fixed, paraffin-embedded tissue was obtained from the pathology files of The Methodist Hospital or Veterans Affairs Medical Center (Houston, TX). Specimens were available from patients who underwent radical prostatectomy and pelvic lymph node dissection for carcinoma confined to the prostate gland (n = 18) or with locally advanced disease (n = 21). In addition, tissue from both the primary and metastatic sites was available from 40 patients who had either radical prostatectomy (n = 22) or TURP (n = 18). An additional seven patients had needle biopsies of the prostate available as well as pelvic lymph nodes. Serial 5-μm-thick sections were cut from each tissue block. One section was stained with hematoxylin and eosin, and an adjacent section was used for immunohistochemistry. Additional serial sections were used for microdissection to isolate DNA for PCR-SSCP and sequencing, which were followed by another section stained with hematoxylin and eosin to confirm the pathological features of the microdissected tissue.

Immunohistochemistry. Nuclear accumulation of the p53 protein was demonstrated by the use of the mAb DO7 (DAKO, Carpinteria, CA) and the polyclonal antibody CM-1 (kindly provided by Dr. David P. Lane, Dundee, Scotland, United Kingdom), which have been shown to recognize p53 in formalin-fixed, paraffin-embedded tissue specimens (20). Sections of a colon carcinoma, with a known p53 gene mutation, were used as a positive control with each experiment. Pelvic lymph nodes without histological evidence of tumor were used as a negative control. Sections (5 μm) from formalin-fixed, paraffin-embedded specimens were immersed in xylene to remove paraffin and then rehydrated in graded ethanol. Specimens to be stained with DO-7 were next immersed in 5 mM citrate buffer (pH 6.0) and boiled in a microwave oven for a total of 10 min (21). Endogenous peroxidase activity was blocked with a solution of 0.3% hydrogen peroxide in methanol for 30 min, after which sections were washed in PBS. Normal horse serum (3%) or normal goat serum (3%) was applied to the sections for 20 min to bind nonspecific antigens. Incubation with a primary antibody (DO-7 at 1:50 or CM-1 at 1:1500) was for 90 min at room temperature. Sections were washed in PBS. Corresponding to each primary antibody, biotin-conjugated antimonouse or antirabbit IgG diluted (1:200) in PBS (Vector Laboratories, Burlingame, CA) was applied for 30 min, after which the sections were incubated with avidin-biotin complex (Vectorstain ABC kit; Vector Laboratories) for 30 min at room temperature. After additional washing, sections were developed for 4 to 7 min using 3,3′-diaminobenzidine in PBS and 0.03% hydrogen peroxide. Slides were counterstained with 3% aqueous methyl green, dehydrated in alcohol, cleared in xylene, and permanently mounted. The accumulation of nuclear p53 protein was assessed by light microscopy using the following criteria: − (negative) if <5% of the tumor cell nuclei were p53 positive; + (positive) if 5–20% of the tumor cell nuclei were p53 positive; ++ if >20–50% of the tumor cells were p53 positive; and +++ if >50% of the tumor cell nuclei were p53 positive.

DNA Extraction. Areas of prostatic carcinoma in either primary or metastatic tissue, identified histologically on an adjacent hematoxylin and eosin-stained tissue section, were microdissected with sterile scalpels into 1.5-ml plastic Eppendorf tubes from two to three 5-μm-thick unstained sections. A fresh scalpel blade was used for each sample. Paraffin was removed by two extractions with xylene followed by two extractions with cold 100% ethanol. Specimens were lyophilized to dryness then resuspended in 100–200 μl tissue buffer containing proteinase K (0.2 mg/ml; Boehringer Mannheim Corp., Indianapolis, IN) and incubated at 65°C for 2 h. Proteinase K activity was destroyed by boiling the solution for 10 min. Specimens were centrifuged at 15,000 × g for 5 min and stored at −20°C. Approximately 10 μl of the supernatant were used directly in the initial PCR reactions.

PCR. Exons 5, 7, and 8 of the p53 gene were independently amplified using a two-step (heminested) PCR protocol to improve the efficiency and specificity of DNA amplification. Oligonucleotide primer sets were: exon 5, 5′-ACC CTG GCC AAC CAG CCC TGT-3′ plus 5′-GTT TGT TTC TTT GCT GCC GTG TT-3′ in the first “unnested” round followed by 5′-ACC CTG GCC AAC CAG CCC TGT-3′ plus 5′-TTG TCT TAT CTG TTC ACT TGT-3′ in the “nested” second round; and exon 7, 5′-ACA GGT CTC CCC AAG GCC GC AAC TC-3′ plus 5′-GTC AGC GGC AAG CAG AGG CT-3′ in the first unnested round and 5′-GTC AGC GGC AAG CAG AGG CT-3′ plus 5′-CCT CAT CTG GCG CCT GTG TT-3′ in the nested second round; and exon 8, 5′-TGG TAG TAC ATG TAG CTT GCC CCG TCC-3′ plus 5′-GCC TCC TTC ATG TGT GCT TCC GTC-3′ in the first round and 5′-GCC TCT TGC TTT GCC AT-3′ plus 5′-CGC TCC TTC TCC TGC GTG TT-3′ in the second round. All oligonucleotides were synthesized by the Nucleic Acid Core in the Institute for Molecular Genetics at Baylor College of Medicine. The initial PCR reaction was performed in a volume of 50 μl containing 1× Taq buffer (Promega Corp., Madison, WI), 1.25 mM MgCl2, 50 μM dNTP, 0.5 μM of each primer, and 0.25 units Taq polymerase (Promega). Samples were covered with 50 μl mineral oil. PCR conditions were 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C.
for 30 cycles followed by a final 5-min extension at 72°C in a DNA thermal cycler model 480 (Perkin Elmer/Cetus, Norwalk, CT).

A second round nested PCR reaction was carried out in a final volume of 50 µL containing 1 µL of the first round PCR reaction as a template. The buffer was as described above substituted with the nested primer set. After 20 cycles of amplification, adequate p53 gene amplification was verified by agarose gel electrophoresis on 3% NuSieve GTG:SeaKem GTG (2:1; FMC Corp., Rockland, ME) in 40 mM Tris-acetate-1 mM EDTA buffer (pH 7.8).

SSCP. SSCP analysis was performed in a modification of the procedure described by Orita et al. (22). Radioactive PCR product for SSCP analysis was generated with 1 µL of the nested amplified PCR product in a reaction volume of 25 µL with 1X Taq buffer, 1.25 mM MgCl₂, 50 µM dNTP, 0.5 µM of each corresponding second round nested primer, 25 µCi [α-32P]dCTP (3000 Ci/mmol; ICN Biomedicabs, Inc., Costa Mesa, CA), and 0.125 units Taq polymerase. The reactions were overlaid with an equal volume of mineral oil and cycled at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min for five cycles, with a final 5-min extension at 72°C. A 2.5-µL aliquot was added to 3.5 µL denaturation mix (0.01 M NaOH, 95% formamide, 0.005% bromophenol blue, and 0.005% xylene cyanol) and heated to 94°C for 3 min, then immediately placed on ice. After a brief centrifugation, the samples were loaded on a 5% polyacrylamide (29:1 acrylamide/bis) gel containing 7.8 M urea gel in Tris-borate-EDTA buffer at a constant power of 8 W for 15 h. The gels were dried and autoradiography performed.

DNA Sequencing. Sequencing templates were prepared by PCR amplification of the microdissected DNA as described above, except that one of the second round nested primers was replaced with a primer of the same sequence but containing a 5' biotin group. Reactions were performed in 30-µL volumes containing 1X Taq buffer, 1.25 mM MgCl₂, 200 µM dNTP, 1 µM of each primer, and 0.25 units Taq polymerase. The second round nested reactions were cycled according to the following parameters: 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, with a final 5-min extension at 72°C for a total of 35 cycles. An aliquot of 40 µL of each PCR reaction was added to 20 µL streptavidin-coated magnetic beads (Dynabeads M280 streptavidin; Dynal, Inc., Great Neck, NY) that had been prewashed with binding and washing buffer (5 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 1.0 M NaCl). This solute was allowed to stand at room temperature for 15 mins. The beads were immobilized to the side of an Eppendorf tube in a Dynal MPC magnetic apparatus, and the supernatant removed. The beads were washed twice with binding and washing buffer, then resuspended in 20 µL 0.1 M NaOH. After a 10-min incubation, the supernatant containing the denatured nonimmobilized DNA strand was removed. The immobilized single-stranded DNA was sequenced as per the manufacturer's instructions using Sequenase 2.0 (United States Biochemical Corp., Cleveland, OH) using the nonbiotinylated nested primer as the sequencing primer with [α-33P]dATP (DuPont New England Nuclear, Wilmington, DE). Sequencing reactions were separated on a 6% polyacrylamide/7 M urea gel in Tris-borate-EDTA buffer at 100 W for 2 to 3 h, the gels dried, and autoradiography performed. In some cases the PCR products were cloned using a TA cloning kit (Invitrogen, San Diego, CA) to confirm the mutant p53 allele. All p53 mutations were confirmed by direct sequencing from another independent PCR reaction or by cloning and sequencing multiple TA clones.

RESULTS

Clinical Features. A total of 86 patients with adenocarcinoma of the prostate were evaluated (age, 43–79 years; mean, 63 years). Eighteen patients underwent radical prostatectomy and pelvic lymph node dissection and were found to have disease pathologically confined to the prostate (T₁/N₁). None of these patients received hormonal therapy prior to surgery. Twenty-one patients were found to have locally advanced disease at the time of radical prostatectomy and pelvic lymph node dissection (T₂/N₁). Of these 21 patients, 12 had extracapsular extension while 9 had seminal vesicle invasion. None of these patients received hormonal therapy prior to surgery. An additional 47 patients had metastatic prostate cancer, all of whom had both primary and metastatic tissue available for study. In this group, 22 had undergone radical prostatectomy and pelvic lymph node dissection and 21 had undergone TURP or prostate biopsy followed by pelvic lymph node dissection at the time of radical prostatectomy and pelvic lymph node dissection (T₃/N₁). Of these 21 patients, 12 had extracapsular extension while 9 had seminal vesicle invasion. None of these patients underwent hormonal manipulation prior to surgery. Four other patients had undergone TURP or prostate biopsy followed by biopsy of a metastatic site: one lymph node, two bone, and one lung. Three of these four patients received androgen ablation treatment prior to biopsy of the metastatic site. Fourteen patients with metastatic disease had tumor samples available from multiple positive pelvic lymph nodes. Clinicopathological features of these 86 patients with 151 tumor samples are summarized in Table 1.

<table>
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<tr>
<th>Pathology</th>
<th>n</th>
<th>Average age (Range)</th>
<th>Gleason score</th>
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<tr>
<td>Confined</td>
<td>18</td>
<td>62 (50–70)</td>
<td>5 6 7 8 9</td>
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<td>Locally advanced</td>
<td>21</td>
<td>65 (51–78)</td>
<td>0 0 16 3 2</td>
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<td>Metastatic</td>
<td>47</td>
<td>67 (43–78)</td>
<td>2 13 15 8 9</td>
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<tr>
<td>Lymph node</td>
<td>44</td>
<td>63 (43–78)</td>
<td>2 12 15 7 8</td>
</tr>
<tr>
<td>Bone</td>
<td>2</td>
<td>66 (61–71)</td>
<td>0 0 0 1 1</td>
</tr>
<tr>
<td>Lung</td>
<td>1</td>
<td>70</td>
<td>0 1 0 0 0</td>
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Table 2  

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<thead>
<tr>
<th>Patient</th>
<th>Tissue</th>
<th>Gleason score</th>
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<th>SSCP*</th>
<th>Mutation</th>
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<tr>
<td>38⁴</td>
<td>Prostate</td>
<td>8</td>
<td>++</td>
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<tr>
<td>39⁵</td>
<td>Prostate</td>
<td>9</td>
<td>+++</td>
<td>Exon 7</td>
<td>Codon 245 GGC→AGC (gly→ser)</td>
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<td>46</td>
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<td>9</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>Prostate (radical)</td>
<td>6</td>
<td>-</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>Prostate (TURP)</td>
<td>8</td>
<td>-</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>Prostate (TURP)</td>
<td>9</td>
<td>-</td>
<td>ND</td>
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<tr>
<td>72</td>
<td>Prostate (TURP)</td>
<td>8</td>
<td>+++, Normal</td>
<td>None (exon 5)</td>
<td></td>
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<tr>
<td></td>
<td>LN 1</td>
<td>+++, Normal</td>
<td>Exon 5</td>
<td>Codon 163 TAC→TGC (tyr→cys)</td>
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<tr>
<td></td>
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<td>+++, Normal</td>
<td>ND</td>
<td></td>
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<tr>
<td>73</td>
<td>Prostate (biopsy)</td>
<td>7</td>
<td>-</td>
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<td>76</td>
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<td>+</td>
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<td></td>
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<td>Exon 5</td>
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<tr>
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<tr>
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<td>+++, Normal</td>
<td>None (exon 5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LN</td>
<td>+++, Normal</td>
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<td>57</td>
<td>LN</td>
<td>7</td>
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<td>68</td>
<td>LN</td>
<td>6</td>
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* Analyses included exons 5, 7, and 8 for all samples except ones from patients 38 and 39, for which only exons 5 and 7 were analyzed.
* ⁴ Patients 38 and 39 had locally advanced disease; all other patients had metastatic disease.
* ⁵ ++, >20–50% of tumor cells stained for p53; +, 5–20% of tumor cells stained for p53; -, <5% of tumor cells stained for p53; +++, >50% of tumor cells stained for p53.
* ⁶ LN, pelvic lymph node; ND, not done.

Table 2 summarizes the results of nuclear accumulation of the p53 protein. The number of tumor cells demonstrating accumulation of p53 protein was estimated for each section on a three-point scale and is presented in Table 2. None of the 18 patients with prostate cancer confined to the prostate showed accumulation of p53 protein within tumor cell nuclei. Twenty-one patients had locally advanced prostate cancer with no evidence of metastasis to either the pelvic lymph nodes or to distant sites, and two (9.5%) of these had intense staining for p53 protein within tumor cell nuclei (patients 38 and 39). Both tumors had high Gleason scores, 8 or 9, and extracapsular extension with seminal vesicle invasion. In the 47 patients with metastatic prostate cancer with tissue available from the primary as well as the metastatic site(s), 4 (8.5%) of the primary tumors and 11 (23%) metastatic deposits demonstrated nuclear staining for p53. Gleason grade and the percentage of cells positive for p53 protein accumulation did not completely concur. Only one of three patients (patient 59) that had undergone androgen-deprivation therapy prior to obtaining tissue from a metastatic site demonstrated nuclear accumulation of p53 protein. Overall, positive staining was observed in 8 of 44 pelvic lymph node metastases, 1 of 2 bone metastases, and 1 lung metastasis. In all four samples that demonstrated positive p53 staining in the primary tumor (patients 76, 77, 81, and 86), positive staining at a metastatic site was also observed. Tissue samples were available from multiple metastatic foci in 14 patients. In 12 patients all metastatic sites were negative, and in 2 (patients 72 and 76) all of the metastases were positive.

Whole-mount specimens of the prostate gland containing multiple foci of cancer (an average of three per gland) were available from 22 patients with metastatic disease who had undergone a radical prostatectomy. In 12 patients immunohistochemistry was performed directly on whole-mount slides, whereas in the remaining 10 patients punch biopsies from the
paraffin blocks were sectioned and stained. Of the 22 patients, 3 (patients 46, 56, and 86) had positively staining pelvic lymph node metastases, but only 1 patient from this group demonstrated positive staining in the primary tumor (patient 86).

**Mutational Analysis of p53 Exons 5, 7, and 8.** To detect mutations within the DNA sequence of the p53 gene, we microdissected samples from paraffin-embedded sections on glass slides, isolated the DNA, and amplified exons 5, 7, and 8 with intron-specific primers. The PCR products were analyzed by SSCP (22), and the results are summarized in Table 2. We evaluated 20 samples from 11 of 13 patients in which nuclear accumulation of the p53 protein was demonstrated in at least one tissue specimen. Two patients (patients 46 and 69) with microscopic metastases within a single pelvic lymph node had no tumor remaining in their tissue blocks and did not undergo subsequent PCR-SSCP evaluation. The remaining 11 patients included the 2 patients with locally advanced prostate cancer and 9 patients with metastatic disease. One of the two patients with locally advanced prostate cancer and accumulation of p53 protein had an abnormal band pattern suggestive of a mutation in exon 7. No other samples had an abnormal pattern for exon 7. A single primary tumor specimen from each of the nine patients with metastatic disease was also evaluated by PCR-SSCP and included four that had p53 protein accumulation. However, there were no aberrantly migrating bands apparent in any of these specimens. One lymph node from each of these nine patients that stained positive for p53 accumulation was also evaluated. Abnormal band patterns of PCR products for exon 5 were detected in DNA from three of the nine metastatic samples that demonstrated p53 immunostaining. No abnormal band patterns were detected on analysis of exons 7 and 8.

Representative PCR-SSCP analysis of exon 5 in metastatic samples is presented in Fig. 2A. Two of the samples (Fig. 2A, Lanes 1 and 8) have an abnormal migration pattern apparent as well as the normal pattern as evidenced in the control DNA (Fig. 2A, Lane 14). We also analyzed negatively staining lymph node samples from two patients (patients 57 and 68) and a DNA sample isolated from that prostate of a 42-year-old organ donor with no evidence of prostate cancer and negative p53 staining (data not shown). These samples had normal SSCP patterns and direct sequencing revealed a normal sequence. Overall, in a total of 20 specimens that were evaluated, 5 with negative staining also demonstrated normal SSCP patterns (Table 2). In 15 positively staining specimens, we detected abnormal SSCP patterns in 4 (27%). Seven of the 11 specimens with no SSCP abnormality but positive staining had only ++ staining and the remaining 4 had +++ staining.

DNA sequence analysis was performed directly on the PCR product from the four patients with an abnormal SSCP pattern and from several samples with a normal SSCP pattern. Analysis of sequencing reactions loaded as depicted in Fig. 2B facilitates mutation detection in multiple samples. A single-point mutation, which would result in an amino acid change of tyrosine to cysteine. The CCC to TCC transition in the DNA from the metastatic lymph node of patient 76 would result in a proline to serine alteration of amino acid 151. The only transcription we detected was an A to C in codon 132, which would result in a change of the amino acid lysine to glutamine, in the DNA microdissected from tumor cells in the lymph node of patient 77. Direct sequencing of the primary tumor DNA for patients 72, 76, and 77 was also performed for exon 5, and no mutations were detected. Fig. 2B shows a representative portion of the sequencing gel which detected the alteration in the DNA from the metastatic lymph node of patient 76 (Fig. 2B, Lane 1). There is a new band in Fig. 2B, Lane C, but Lane T reaction also has a band present at reduced intensity. Since the sequencing reactions were done on PCR amplified DNA, the presence of the band in Lane T indicates that some normal sequence PCR product was present. Although the microdissected tissue was composed predominantly of tumor cells that stained positive for p53, some p53-negative cells, including stromal cells, likely contributed normal p53 alleles to the initial DNA isolate. Alternatively, some tumor cells may have contained one mutant p53 allele and one allele with the normal wild-type sequence. To confirm the mutation we present three lanes with cloned PCR product (Fig. 2B, Lanes 4–6) with the codon 151 C to T transition.

**DISCUSSION**

Mutation of the tumor suppressor gene p53 has been implicated in the initiation and progression of several human cancers, and its role in prostate cancer is under increasing investigation (12–17). Some studies of human prostate cancer have shown an association between advanced stage and high grade of the primary tumor with p53 mutations. There is evidence for the involvement of mutated p53 in stepwise disease progression from our recent experimental data which indicate that loss of normal p53 function can lead to widespread metastasis in ras + myc-induced mouse prostate cancer (23). Thus, it is conceivable that loss of normal p53 function and/or the acquisition of additional downstream functional abnormalities acquired through p53 mutation may be directly related to metastasis.

In this study, we have focused on paired clinical specimens of primary and metastatic prostate cancer. We evaluated 86 patients with prostate cancer to determine the frequency of p53 abnormalities. Within this group, 18 patients had cancer that was pathologically confined to the prostate gland, 21 patients had locally advanced disease, and 47 had metastatic disease for which specimens of both the primary and metastatic lesions were available. None of 18 patients with cancer pathologically localized to the prostate showed positive p53 immunostaining using the criterion of <5% of cells being positive, which represents a lower cutoff value than in some previous studies. Two (9.5%) of 21 patients with locally advanced prostate cancer showed immunohistochemical nuclear accumulation of the p53 protein. In contrast, a greater number of patients, 11 (23.4%) of 47, demonstrated positive p53 nuclear immunostaining in metastatic sites; yet, in this subset only four patients also had p53 accumulation detectable in their respective primary tumors.
Fig. 2. PCR-SSCP and sequencing of exon 5 of p53. A, PCR followed by SSCP analysis of exon 5 was performed on microdissected DNA from metastatic samples (Lanes 1–12) and control DNA (Lane 14), and the PCR reaction was performed without added DNA (Lane 13). The un-denatured double-stranded PCR product migrates faster than the single-stranded products. Two samples (Lanes 1 and 8) have abnormal migration patterns (arrowheads). B, DNA sequencing gel of a region of exon 5 encoding the sequence indicated in the right margin. Sequencing reactions were performed on: Lane 1, PCR samples from the metastatic lymph node of patient 76; Lane 2, PCR product on microdissected DNA from the metastatic lymph node from patient 72 (wild type for codon 151); Lane 3, PCR product using control DNA with wild-type sequence at codon 151; and Lanes 4–6, PCR product from patient 76 cloned into a TA vector to confirm the C→T transition mutation.

Microdissected DNA isolated from tissue sections from patients with positive p53 immunostaining were further assessed with PCR-SSCP. Eleven of 15 samples with positive staining had normal SSCP patterns for exons 5, 7, and 8 (Table 2). The apparent discord between p53 staining and SSCP analysis may be the result of sampling bias, assay sensitivity, or a reflection of mutations in exons that were not screened in this study. The sampling bias is a genuine concern when microdissecting tissue samples from paraffin-embedded sections with potentially considerable variability in the extent and penetrance of tumor samples from slide to slide. It is likely that sections analyzed by immunohistochemistry may have staining patterns that do not adequately reflect the extent of cancer in nearby sections used for microdissection. We have observed that if <20% of the cells in a sample contain a mutant p53 allele, it is below the level of detection by SSCP [data not shown and supported by other studies (24)]. Based on our empirical and quantitative assessment of the combined immunohistochemistry-SSCP screening approach, as well as our results, we are of the opinion that significant levels of p53 mutations in prostate cancer could escape detection using this screening strategy. In all cases in which abnormal SSCP patterns were demonstrated, point mutations were detected in the p53 gene by direct sequencing. Because of the limited amount of malignant tissue available, our molecular analysis was limited to exons 5, 7, and 8. However, exons 5, 7, and 8 together account for approximately 80% of all reported p53 mutations in prostate cancer with more than 50% occurring in exon 5 (17).

In general, recent studies have found a consistent correlation of p53 mutations with end stage disease. The early studies of Effert et al. (13) demonstrate a trend toward disease with metastatic potential, and Bookstein et al. (14) clearly demon-
strated that p53 mutations were associated with high stage disease in a large, more comprehensive series of patient material. More recently, Navone et al. (15) demonstrated a relatively high frequency of p53 mutations in hormone refractory disease predominantly localized in bone. Our study adds a new dimension to the previous studies in that a large series of matched primary tumor and metastatic tissue that was predominantly composed of stage D1, i.e., lymph node disease was used for analysis. In this study we found a higher frequency of p53 mutations in metastasis relative to primary tumor specimens. Furthermore, in our study most patients had not received hormone therapy.

Overall, these results suggest that mutation of the p53 gene is associated with the development of metastatic disease in prostate cancer. A possible mechanism for the role of p53 in progression is related to genetic instability related to loss of G1→S-phase checkpoint functions (reviewed in Ref. 7). Alterations in p53 function may create a genetically unstable cell which can then acquire additional changes necessary for it to become metastatic. Recent data indicate that p53 may also act more directly in regard to progression via the transcriptional activation or repression of specific genes, e.g., those involved in the control of angiogenesis (25–27). Future studies will hopefully reveal additional information regarding the biological potential of specific mutations as well as a greater understanding of the broader role of p53 in prostate cancer metastasis.

REFERENCES

Association of p53 mutations with metastatic prostate cancer.


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