An Uncertain Role for p53 Gene Alterations in Human Prostate Cancers


Departments of Urology [J. D. B., G. S. B., C. M. E., B. S. C., J. C. R., W. B. I.], Pathology [G. S. B., J. I. E.], and Oncology [S. P., W. B. I.]. The Johns Hopkins Medical Institutions, Baltimore, Maryland 21287-2101

ABSTRACT

Inactivation of the p53 gene has been implicated in prostate cancer progression. To determine the role of p53 inactivation in the progression of clinical prostatic carcinomas, we assessed 67 tumors derived from patients with clinically localized disease for chromosome 17p and p53 gene allelic loss, p53 gene mutations using single-strand conformational polymorphism and direct sequencing, and p53 protein expression using immunohistochemical staining. Of 55 informative tumors, 10 demonstrated loss of 17p or the p53 gene; however, only a single tumor had a mutation in its remaining p53 allele. Significant p53 overexpression was observed in 2 of 38 tumors, and 9 others had faint staining of a few nuclei (<1%). p53 overexpression occurred in no informative tumor with allelic loss or mutation. In a 1-7-year follow-up, positive immunohistochemical staining did not confer an increased risk of recurrence (risk of recurrence, 0.86, P = 0.78), whereas allelic loss of chromosome 17p appeared to be highly correlated with recurrence (risk of recurrence, 3.7, P = 0.003). In an unrelated group of 42 patients with metastatic prostate cancer, p53 overexpression was found in 26 tumors (62%), and 15 (36%) had high grade staining. Neither the presence nor the degree of expression correlated with time to progression or time to death. This series suggests that p53 gene inactivation is rare in primary prostatic tumors, not essential to the development of prostatic cancer metastases, and of limited use as a prognostic marker in patients with primary or metastatic disease. Another gene or genes on chromosome 17p may be involved in prostate cancer progression.

INTRODUCTION

Alterations of the tumor suppressor gene p53 have been thought to play a role in prostate carcinogenesis since Carter et al. (1) observed loss of genetic material from the distal portion of chromosome 17p in 3 of 18 human prostate cancers, and Isaacs et al. (2) identified p53 gene mutations in three of five prostate cancer cell lines. Since these initial observations, a number of investigators have focused on defining the role of p53 alterations in clinical prostate cancer. Because mutations of the p53 gene commonly prolong the half-life of its protein product, IHC staining has been used as a measure of gene inactivation (3). In organ-confined prostate tumors (T1-T2), immunoreactive p53 polypeptide has been reported in 0-79% of the cases, although in most series less that 10% of the low-stage tumors stain positively (4-15). Cancers that invade the prostatic capsule (T3) have IHC* rates of 10-20%, whereas 40-95% of the bone metastases stain positively (9-13). SSCP analysis and sequencing of the p53 gene has confirmed that tumors which are IHC* positive harbor mutations, and that mutations appear to be more common with increasing tumor stage (10-19).

Because p53 alterations are more common in high-stage tumors, it has been inferred that inactivation of this gene is important in the progression of human prostate cancer. In vitro and in vivo studies offer some support to this hypothesis. Expression of wild-type p53 in prostate cancer cell lines with mutant alleles will suppress their growth (2). In a mouse prostate reconstitution model system, Thompson and colleagues (20, 21) have shown that loss of p53 function in the urogenital sinus cooperates with ras oncogene overexpression to produce metastatic hyperplasia and with ras and myc oncogene overexpression to produce metastatic prostate cancer. This group has suggested recently that p53 may be a suitable target for prostate cancer gene therapy (22).

However, several uncertainties remain regarding the role of p53 alterations in human prostate cancer. First, it has not been clearly demonstrated that p53 function is absent from clinical prostate cancers with p53 mutations. In a startling variety of human tumors, p53 is inactivated by a common mechanism; i.e., mutation of one copy of the gene and loss of the second (23). Of the many studies relating p53 to prostatic carcinoma, only three have interrogated both copies of the gene. Isaacs et al. (2) found a missense mutation in one of two primary tumors with loss of one copy of chromosome 17p. Bookstein et al. (10) found allelic loss of chromosome 17p in three of six prostatic tumors with documented p53 gene mutations. In a metastatic prostate cancer, Effert et al. (16) found chromosome 17p allelic loss coupled with a p53 gene mutation; however, further analysis revealed that the deletion did not include the p53 gene. Thus, of the nine clinical prostatic tumors in which both copies of the p53 gene have been analyzed, only four show conclusive evidence of inactivation of both alleles. From this limited data, it remains uncertain whether alteration of one copy of the p53 gene, either by mutation (4-19) or loss (1, 24-26), necessarily implies that the gene is functionally inactivated.

Equally uncertain is whether detection of p53 alterations offers prognostic information or aids in the selection of appropriate therapy in patients with prostate cancer. Since loss of p53 function appears to be associated with prostate cancer progression, it has been hypothesized that p53 inactivation confers biological aggressiveness to low-stage tumors and makes them more prone to metastasize (11). Indeed, Visakorpi et al. (4) found IHC* p53 staining in 17% of clinically localized prostate cancers, and observed that high-grade positive staining correlated with poor clinical outcome. Other investigators, however, have not found a relationship between p53 alterations and prostate cancer progression in low-stage tumors (8). In patients with metastatic prostate cancers, it is unknown whether the detection of altered p53 will predict clinical outcome.

In an effort to address these uncertainties, we have undertaken a comprehensive analysis of clinically localized and metastatic prostatic tumors for p53 gene inactivation and correlated our findings with patient outcome. Tumors from patients with clinically localized prostate cancer were assessed for allelic loss of chromosome 17p and the p53 gene. IHC staining for p53 protein was performed on informative samples and correlated with allele status. In all tumors with allelic loss, the remaining p53 allele was sequenced directly. IHC staining and allele status were correlated with tumor grade, stage, and recurrence at 1-7 years. IHC staining was also performed on an unrelated...
set of tumors derived from 42 patients with metastatic disease, and IHC status was correlated with patient survival.

**MATERIALS AND METHODS**

**Tissue Samples.** For genetic studies, fresh tissue was harvested from the primary tumors of 62 patients undergoing radical retropubic prostatectomy for clinically localized prostate cancer between 1988 and 1991. Only specimens with palpable tumors were used in this study. An additional five samples were obtained from lymph node metastases (samples 50, 133, 142, 170, and 207) at staging pelvic lymphadenectomy. None of the patients had received hormonal or radiation therapy prior to surgery. A portion of each tumor was snap frozen in liquid nitrogen and stored at −70°C, and the remainder was submitted for formalin fixation and paraffin embedding for pathological evaluation. Tumors were classified by pathological stage according to the TNM system and graded according to the method of Gleason (27, 28). After surgery, patients were evaluated every 3 months for 2 years, and then every 6 months in subsequent years. Prostate cancer recurrence was defined as elevation of the serum PSA above 0.2 ng/ml, palpable mass on rectal examination confirmed on biopsy to represent prostate cancer, or positive bone scan. Characteristics of these patients are given in Table 1. For IHC studies, sections of paraffin-embedded, formalin-fixed tissues were obtained from 38 of the 67 tumors.

We selected 42 patients with advanced prostate cancer seen at our institution between 1978 and 1992. None of these patients corresponded to those in the primary tumor group discussed above. Clinical data were obtained from a review of the medical charts. Site of biopsy, hormonal status at the time of biopsy, time from diagnosis to the development of metastases, time from metastases to death, and total survival time were recorded for each patient (Table 2). Paraffin-embedded specimens were obtained from the metastatic sites for IHC studies, and the presence of metastatic prostate cancer was confirmed by IHC staining for PSA and prostatic acid phosphatase expression (data not shown). H&E-stained sections were reviewed and graded by a single pathologist (J. I. E.). In all patients who had received hormonal therapy, biopsy specimens were taken after documented progression of their cancer and presumably represent hormone refractory disease.

**DNA Preparation.** Fresh prostate tumors were separated from surrounding tissue, areas of necrosis and fibrosis, benign prostatic epithelia, and inflammatory tissue using a microdissection technique described previously (1, 29). Only samples containing at least 70% prostate cancer nuclei were used in genetic studies. All prostate carcinomas studied were of the usual acinar histology and were less than 2 cm in diameter. Normal tissues comprised either the seminal vesicles or peripheral blood leukocytes. DNA isolation and quantitation were performed in accordance with established techniques (30).

**Southern Blot Analysis.** Purified genomic DNA was digested with either TaqI (7.5 units/μg DNA) or MspI (10 units/μg DNA), electrophoresed in 0.8% agarose, and transferred to nylon membranes. Membranes were hybridized overnight with 32P-labeled D17S34 or D17S73 probes (American Type Culture Collection) as described previously (1). Allelic loss was detected by the absence of one allele in prostate tumor DNA compared with paired noncancerous control DNA. In many cases, residual signal could be seen in the location of the lost allele due to contamination by normal tissue or heterogeneity in the tumor. When densitometry was used, samples with a 60% reduction in the signal of the lost allele compared to the retained allele were scored as having undergone allelic loss.

**p53 PCR Analyses.** Polymorphic regions of p53 exon 4 and intron 6 were amplified with primers and conditions described previously (31, 32). Products of exon 4 amplification were digested with 4 units BsrUI and those of intron 6 amplification with NciI. All PCR digestion products were resolved on 8% polyacrylamide gels and stained with ethidium bromide. Allelic loss in these PCR techniques was determined by the same criteria used in Southern blot analysis.

**Immunohistochemistry.** Prior to paraffin embedding, all bone specimens were decalcified in citric acid (2.5%/formic acid (8.75%). Sections (5 μm) of paraffin-embedded, formalin-fixed tissues were placed on poly-L-lysine slides. Sections were deparaffinized through graded alcohol and rehydrated in PBS. Endogenous peroxidases were quenched with a solution of 0.3% hydrogen peroxide in methanol for 30 min, and the slides were washed in PBS. Antigen retrieval was facilitated by incubation at 90°C for 10 min in preheated Target Unmasking Fluid (Kreatech Biotechnology B.V., Amsterdam, the Netherlands) or by boiling the samples in PBS for 10 min. Slides were allowed to cool to room temperature for 15 min, rinsed twice in distilled water and twice in PBS containing 0.1% BSA, and blocked with 2% goat serum. CM-1 (Signet Laboratories Inc., Dedham, MA), DO-1, and DO-7 (Novacasta Laboratories, Newcastle, United Kingdom) antibodies were used for detection of p53 antigen and were compared for staining efficacy. CM-1 (1:30 dilution), DO-1 (1:100 dilution), or DO-7 (1:100 dilution) primary antibodies were aliquoted onto each section, and the slides were incubated in a humid chamber for 30 min at 37°C. Slides were washed, and primary antibodies were detected using streptavidin-conjugated secondary antibodies and biotinylated peroxidase in accordance with the manufacturer’s instructions (Zymed). Slides were lightly counterstained with 1% aqueous hematoxylin. Sections of the human prostate cancer cell line DU145, grown as a xenograft in nude mice and a human colon cancer metastatic to the liver with documented stabilizing p53 mutations (kindly provided by Dr. Stan Hamilton, Johns Hopkins Hospital, Baltimore, MD), served as positive controls. Only samples demonstrating nuclear staining were scored as positive. Scoring for p53 immunoreactive protein was as follows: 0, no staining detected; 1+, <20% of the nuclei stain positive and are distributed diffusely; 2+, 20–50% of the nuclei stain positively; 3+, >50% of the nuclei stain intensely positive; and 4+, >50% of the nuclei stain intensely positive.

**PCR-SSCP.** PCR-SSCP was performed as described by Orita et al. (33) as modified by Bookstein et al. (10). Four segments (exon 4, exon 5/6, exon 7, and exon 8/9) of the p53 gene were amplified from genomic DNA using described primers and conditions (10). PCR products from exons 5/6 and 8/9 were digested with AaI or Styl, respectively. Samples were denatured and resolved on MDE gels prepared according to the manufacturer’s instructions (FMC BioProducts, Rockland, ME). Each sample was run twice at 4°C and at room temperature. After drying, gels were exposed to Kodak X-ray film with an intensifying screen at −70°C for 1 to 2 days.

**DNA Sequencing of p53.** A 1.8-kb fragment was PCR amplified from exons 5 to 9 of the p53 gene using primers 5'-biotin-TTCACTTGTGCCCT-GACCT-3' and 5'-CATGGAATTCGAGAACTTTCCCATGTAT-3'. Products were purified by centrifugation through Co-Star Spin-X columns, precipitated with absolute ethanol, resuspended in distilled water, and combined with avidin-coated Dynabeads (Dynal A.S., Oslo, Norway). Products were denatured by treatment with 0.1 M NaOH, and the strands were separated by magnetic precipitation of the biotinylated strand complexed to the avidin Dynabeads. Single-stranded products were sequenced directly using the described primers in accordance with the manufacturer’s instructions (Sequenase Version 2.0; United States Biochemical; Refs. 2 and 34). The prostate cancer cell lines TSU-Pr1 and DU145 served as positive controls (2).

**Statistical Analyses.** The primary statistical end points for this study were time to recurrence in the patients with clinically localized disease and time to death in patients with metastatic disease. Event time distributions were estimated using the product limit method (35). For categorical prognostic factors, overall event rates were estimated by dividing the number of events by the total follow-up time. Risk ratios for staining and loss were estimated using the proportional hazards model with and without stratification for stage and grade (36). Associations between categorical factors were assessed for statistical significance using the χ2 test with exact P values. All P values reported are two sided.

**RESULTS**

**Clinically Localized Tumors.** Follow-up was available in 65 of 67 patients and ranged from 2 months to 7 years, with a mean of 40.4 ± 21.6 months. During observation, 35 of 65 patients had disease progression defined as a PSA > 0.2 ng/ml (n = 34 patients) or the development of a positive bone scan (n = 1 patient). Mean time to progression for all patients was 19 ± 17.1 months. As expected, the Gleason grade and pathological stage of the tumors were strong prognostic factors for recurrence (Table 3).

**Allelic Loss of Chromosome 17p and the p53 Gene in Clinically Localized Prostate Cancers.** To screen for inactivation of the p53 gene, 67 tumors were analyzed for chromosome 17p allelic loss using polymorphic markers distal to the gene and for p53 allelic loss by...
Table 1  p53 alterations in clinically localized prostate cancers

<table>
<thead>
<tr>
<th>Case</th>
<th>Grade*</th>
<th>Stageb</th>
<th>Status</th>
<th>Time to progression (mo)</th>
<th>Follow-up (mo)</th>
<th>Chromosome 17p</th>
<th>p53 Gene</th>
<th>IHC gradingc</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>7</td>
<td>T2a</td>
<td>NEDb</td>
<td>26</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>1+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>T2a</td>
<td>NEDb</td>
<td>72</td>
<td>R</td>
<td>R</td>
<td>ND</td>
<td>1+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>T2b</td>
<td>PSA+</td>
<td>48</td>
<td>67</td>
<td>R</td>
<td>N</td>
<td>1+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>T2c</td>
<td>PSA+</td>
<td>60</td>
<td>84</td>
<td>N</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>T3a</td>
<td>NEDb</td>
<td>72</td>
<td>R</td>
<td>R</td>
<td>N</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>T3a</td>
<td>NEDb</td>
<td>84</td>
<td>R</td>
<td>N</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>6</td>
<td>T3a</td>
<td>PSA+</td>
<td>72</td>
<td>84</td>
<td>R</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>T3a</td>
<td>PSA+</td>
<td>33</td>
<td>60</td>
<td>N</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>9</td>
<td>T3a</td>
<td>PSA+</td>
<td>11</td>
<td>40</td>
<td>L</td>
<td>ND</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>7</td>
<td>T3a</td>
<td>NEDb</td>
<td>72</td>
<td>R</td>
<td>ND</td>
<td>0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>9</td>
<td>T3aN2</td>
<td>Unkn</td>
<td>48</td>
<td>R</td>
<td>ND</td>
<td>ND</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

Table cont. (68).

Case 50: 10 T2aN2 Unkn N N ND
Case 51: 8 T2aN2 Unkn N N ND
Case 61: 8 T2aN1 PSA+ 3 12 R R 1+
Case 71: 7 T2a PSA+ 9 15 R ND ND
Case 73: 7 T2c PSA+ 42 R ND ND
Case 74: 6 T2c PSA+ 48 R ND ND
Case 79: 5 T1a PSA+ 26 R ND ND
Case 80: 6 T2a PSA+ 60 R ND ND
Case 81: 6 T2a PSA+ 60 R ND ND
Case 82: 7 T2a PSA+ 12 24 R ND ND
Case 83: 8 T2a PSA+ 24 R N 0
Case 86: 7 T2a PSA+ 12 36 R R ND
Case 96: 9 T2aN2 PSA+ 10 29 L L 0 None
Case 100: 7 T2aN1 PSA+ 12 60 R N ND
Case 102: 6 T2a PSA+ 48 R R 0
Case 116: 6 T2a PSA+ 48 R ND ND
Case 123: 7 T2a PSA+ 32 ND ND ND
Case 125: 7 T2aN1 PSA+ 12 48 R N 2+
Case 125: 7 T2aN1 PSA+ 12 48 R N 2+
Case 125: 7 T2aN1 PSA+ 6 6 N ND
Case 133: 10 T2aN2 PSA+ 3 30 N L 0 None
Case 134: 6 T2c PSA+ 18 R N 4+
Case 138: 7 T2aN2 PSA+ 30 47 R R ND
Case 142: 10 T2aN2 PSA+ 3 24 L L 0 None
Case 144: 7 T2a PSA+ 15 R R ND
Case 146: 7 T2a PSA+ 12 48 N N ND
Case 152: 7 T2a PSA+ 24 R N 0
Case 155: 9 T2a BS+ 24 48 L N 0 None
Case 158: 8 T2a PSA+ 18 R N 0
Case 170: 10 T2aN1 PSA+ 12 12 R N 1+
Case 172: 8 T2c PSA+ 12 R N 1+
Case 177: 8 T2c PSA+ 24 24 N R 1+
Case 178: 7 T2a PSA+ 36 R R ND
Case 179: 9 T2aN1 PSA+ 12 12 R R 0
Case 185: 8 T2a PSA+ 48 60 R R 0
Case 192: 8 T2a PSA+ 12 R R 1+
Case 199: 7 T2aN1 PSA+ 3 48 R R 0
Case 198: 8 T2a PSA+ 11 25 L N 0
Case 199: 7 T2aN1 PSA+ 46 48 R R ND
Case 202: 6 T2aN1 PSA+ 48 R N 0
Case 204: 9 T2a PSA+ 12 R R 0
Case 207: 10 T2aN2 PSA+ 12 12 R N ND
Case 214: 7 T2a PSA+ 24 24 R N 0
Case 217: 7 T2aN1 PSA+ 10 48 N N ND
Case 219: 8 T2a PSA+ 12 36 R R 0

* Grade according to the Gleason system (28).
* Staging by TNM classification (27): T1 is used in cases where the pathological stage of the primary tumor is unknown.
* None, no staining detected; 1+, <2% of the nuclei stain positive and are distributed diffusely; 2+ , 2-20% of the nuclei stain positively; 3+ , 20-50% of the nuclei stain intensely positive; 4+ , >50% of the nuclei stain intensely positive.
* ND, not determined.

PCR amplification of polymorphic regions within the gene. Southern blot analysis, using D17S5 and D17S34 (which map distal to the p53 gene on chromosome 17p), identified 50 patients that were heterozygous in this region, and loss of one allele was detected in seven tumors (14%) from these informative patients (Fig. 1 and Table 1). There was complete concordance in the assessment of allele status in the 19 tumors informative for both probes. Naturally occurring polymorphisms within exon 4 and intron 6 of the p53 gene were exploited to...
p53 AND CLINICAL PROSTATE CANCER

Table 2. p53 expression in prostate cancer metastases

<table>
<thead>
<tr>
<th>Case</th>
<th>Grade</th>
<th>Site</th>
<th>Hormonal therapy</th>
<th>Time from Dx* to Mets (mo)</th>
<th>Time from Mets to death (mo)</th>
<th>Total survival (mo)</th>
<th>p53 IHC grading</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>10</td>
<td>Bone</td>
<td>N</td>
<td>24</td>
<td>8</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>M2</td>
<td>8</td>
<td>Bone</td>
<td>Y</td>
<td>0</td>
<td>18</td>
<td>18</td>
<td>1+</td>
</tr>
<tr>
<td>M3</td>
<td>8</td>
<td>Bone</td>
<td>Y</td>
<td>1</td>
<td>63</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>M4</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>85</td>
<td>13</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>M5</td>
<td>10</td>
<td>LN</td>
<td>N</td>
<td>107</td>
<td>26</td>
<td>133</td>
<td>0</td>
</tr>
<tr>
<td>M6</td>
<td>10</td>
<td>Testis</td>
<td>Y</td>
<td>91</td>
<td>53</td>
<td>144</td>
<td>1+</td>
</tr>
<tr>
<td>M7</td>
<td>10</td>
<td>Bone</td>
<td>Unkn</td>
<td>Unkn</td>
<td>Unkn</td>
<td>Unkn</td>
<td>2+</td>
</tr>
<tr>
<td>M8</td>
<td>10</td>
<td>Epidural</td>
<td>Y</td>
<td>25</td>
<td>50</td>
<td>75</td>
<td>2+</td>
</tr>
<tr>
<td>M9</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>5</td>
<td>13</td>
<td>18</td>
<td>2+</td>
</tr>
<tr>
<td>M10</td>
<td>10</td>
<td>Epidural</td>
<td>N</td>
<td>0</td>
<td>52</td>
<td>52</td>
<td>1+</td>
</tr>
<tr>
<td>M11</td>
<td>9</td>
<td>Bone</td>
<td>Y</td>
<td>3</td>
<td>31</td>
<td>34</td>
<td>3+</td>
</tr>
<tr>
<td>M12</td>
<td>10</td>
<td>Pleura</td>
<td>Y</td>
<td>39</td>
<td>16</td>
<td>55</td>
<td>0</td>
</tr>
<tr>
<td>M13</td>
<td>8</td>
<td>Bone</td>
<td>Y</td>
<td>0</td>
<td>80</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>M14</td>
<td>10</td>
<td>Bone</td>
<td>N</td>
<td>25</td>
<td>2</td>
<td>27</td>
<td>3+</td>
</tr>
<tr>
<td>M15</td>
<td>8</td>
<td>Bone</td>
<td>Y</td>
<td>42</td>
<td>31</td>
<td>73</td>
<td>3+</td>
</tr>
<tr>
<td>M16</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>0</td>
<td>44</td>
<td>44</td>
<td>1+</td>
</tr>
<tr>
<td>M17</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>11</td>
<td>2</td>
<td>13</td>
<td>3+</td>
</tr>
<tr>
<td>M18</td>
<td>8</td>
<td>Bone</td>
<td>N</td>
<td>0</td>
<td>31</td>
<td>31</td>
<td>1+</td>
</tr>
<tr>
<td>M19</td>
<td>10</td>
<td>Bronchus</td>
<td>Y</td>
<td>6</td>
<td>37</td>
<td>43</td>
<td>1+</td>
</tr>
<tr>
<td>M20</td>
<td>10</td>
<td>Bone</td>
<td>Unkn</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>M21</td>
<td>7</td>
<td>Bone</td>
<td>Unkn</td>
<td>0</td>
<td>13</td>
<td>13</td>
<td>1+</td>
</tr>
<tr>
<td>M22</td>
<td>7</td>
<td>Bone</td>
<td>Unkn</td>
<td>0</td>
<td>58</td>
<td>58</td>
<td>1+</td>
</tr>
<tr>
<td>M23</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>18</td>
<td>19</td>
<td>19</td>
<td>1+</td>
</tr>
<tr>
<td>M24</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>96</td>
<td>48</td>
<td>144</td>
<td>4+</td>
</tr>
<tr>
<td>M25</td>
<td>8</td>
<td>Bone</td>
<td>Y</td>
<td>45</td>
<td>5</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>M26</td>
<td>9</td>
<td>Bone</td>
<td>N</td>
<td>65</td>
<td>33</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>M27</td>
<td>9</td>
<td>Adipose</td>
<td>Y</td>
<td>141</td>
<td>124</td>
<td>243</td>
<td>3+</td>
</tr>
<tr>
<td>M28</td>
<td>9</td>
<td>Bone</td>
<td>N</td>
<td>74</td>
<td>59</td>
<td>133</td>
<td>4+</td>
</tr>
<tr>
<td>M29</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>9</td>
<td>2</td>
<td>11</td>
<td>2+</td>
</tr>
<tr>
<td>M30</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>12</td>
<td>29</td>
<td>29</td>
<td>1+</td>
</tr>
<tr>
<td>M31</td>
<td>6</td>
<td>Bone</td>
<td>Unkn</td>
<td>Unkn</td>
<td>Unkn</td>
<td>Unkn</td>
<td>0</td>
</tr>
<tr>
<td>M32</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>0</td>
<td>20</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>M33</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>52</td>
<td>9</td>
<td>61</td>
<td>3+</td>
</tr>
<tr>
<td>M34</td>
<td>9</td>
<td>Bone</td>
<td>N</td>
<td>58</td>
<td>5</td>
<td>63</td>
<td>1+</td>
</tr>
<tr>
<td>M35</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>19</td>
<td>24</td>
<td>43</td>
<td>3+</td>
</tr>
<tr>
<td>M36</td>
<td>9</td>
<td>Bone</td>
<td>N</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2+</td>
</tr>
<tr>
<td>M37</td>
<td>9</td>
<td>Bone</td>
<td>Y</td>
<td>18</td>
<td>5</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>M38</td>
<td>8</td>
<td>Bone</td>
<td>N</td>
<td>0</td>
<td>18</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>M39</td>
<td>9</td>
<td>Epidural</td>
<td>N</td>
<td>60</td>
<td>24</td>
<td>84</td>
<td>0</td>
</tr>
<tr>
<td>M40</td>
<td>9</td>
<td>Bone</td>
<td>N</td>
<td>19</td>
<td>27</td>
<td>46</td>
<td>2+</td>
</tr>
<tr>
<td>M41</td>
<td>10</td>
<td>Bone</td>
<td>Y</td>
<td>11</td>
<td>2</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>M42</td>
<td>10</td>
<td>Bronchus</td>
<td>Y</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Grade according to the Gleason system (28).
* Androgen deprivation therapy.
* Unkn, unknown; Dx, initial prostate cancer diagnosis; Mets, development of metastases; Y, androgen deprivation therapy instituted prior to biopsy (presumed hormone refractory disease); N, no hormonal therapy prior to biopsy (presumed androgen-sensitive disease).
* 0, no staining detected; 1+, <2% of the nuclei stain positive and are distributed diffusely; 2+, 2-20% of the nuclei stain positively; 3+, 20-50% of the nuclei stain intensely positive; 4+, >50% of the nuclei stain intensely positive.

Directly assess tumors for allelic loss of p53 gene sequences. PCR amplification of exon 4 and intron 6 followed by restriction digestion of the products by BstUI or VauI, respectively, yielded 25 patients which were informative for p53 allelic status. Six tumors (24%) demonstrated allelic loss of the p53 gene (Fig. 2 and Table 1). There was complete concordance between PCR-based assessments of allele status between the two polymorphic regions of the p53 gene. All tumors that had lost distal loci on chromosome 17p as determined by Southern blot analysis also demonstrated loss of the p53 gene. In contrast to the observation of Effert et al. (16), no tumor lost distal elements of chromosome 17p while retaining the p53 gene. Interestingly, two tumors (tumors 30 and 82) had an apparent interstitial deletion in the region of the p53 gene, since they demonstrated allelic loss at p53 using PCR analysis but retained distal elements of chromosome 17p using Southern blot analysis. Combining the data from the PCR and Southern blot analyses, 55 of 67 (82%) patients were informative within the p53 gene or at loci distal to the gene on chromosome 17p, and 10 tumors from this group (18%) demonstrated allelic loss.

Although the number of tumors with allelic loss of 17p or the p53 gene was low, loss appeared to correlate with the increasing Gleason grade of the tumor (P = 0.05). However, loss was not significantly related to final pathological stage in this set of clinically localized tumors (P = 0.93). Most important, loss appeared to offer some prognostic value in determining the risk of recurrence after prostatectomy (Table 3 and Fig. 3). When loss was considered as a single...
Fig. 1. Examples of allelic loss of chromosome 17p in prostate tumors. A, MspI digests of genomic DNA from paired tumor (T) and normal (N) samples from patients 29 and 96. Hybridization with D17S35 reveals loss of alleles in the tumor samples. B, TaqI digests of DNA from tumor (T) and normal (N) tissues from patients 142 and 198 hybridized with D17S34.

Fig. 2. Examples of allelic loss of the p53 gene in primary prostate cancers. A, amplification of a 259-bp fragment of the p53 gene (top band) which spans a polymorphic region of exon 4. Digestion of this product with BstUI distinguishes noncutting (top band) from cutting (lower two bands, 160 bp and 99 bp, respectively) alleles. In both tumor (T) 29 and 82, the cut alleles have been lost, evidenced by a relative diminution of signal relative to normal (N). B, a 359-bp segment of intron 6 of the p53 gene amplified by PCR followed by NcoI digestion to distinguish cutting (bottom two bands) from noncutting alleles (top band). Tumor 133 has lost the cutting allele, whereas tumor 142 has lost the noncutting allele.

p53 Gene SSCP and Direct Sequencing. In 7 of the 10 tumors with chromosome 17p/p53 gene allelic loss, exons 4–9 of the p53 gene were assessed for mutations using SSCP (tumors 50, 82, 96, 133, 142, 155, and 198). No mutations were detected because none of the tumors demonstrated aberrant bands (data not shown). The p53 gene was sequenced directly in all 10 tumors with allelic loss of chromosome 17p and/or the p53 gene. A missense mutation of codon 197 (GTG -> GGG) in tumor 23, previously reported by Isaacs et al. (2), was confirmed in the present analysis. In the remaining nine tumors, no mutations were detected by direct sequencing of exons 5–8 of the p53 gene. Given the infrequency of p53 gene mutations detected, no correlations could be drawn.

IHC Detection of p53 Expression in Clinically Localized Tumors. Formalin-fixed, paraffin-embedded sections of prostatic tissues were obtained from 38 of the 55 tumors with informative loci on chromosome 17p. These tissues were immediately adjacent to, and encompassed, the tumors used in the genetic studies. Conditions were optimized such that reliable staining of positive controls was obtained with each of the antibodies (data not shown). There was complete agreement in IHC detection of p53 protein between each of the three antibodies and between the two antigen retrieval systems (Target Unmasking Fluid versus boiling in PBS). In no case was p53 expression observed in normal prostate, prostatic stromal cells, or benign prostatic hyperplasia. Cytoplasmic staining was noted in two tumors with the CM-1 antibody; however, this staining was not confirmed with the DO-7 antibody. In the primary tumors, three predominant nuclear staining patterns were observed. In the majority of cases (71%), no expression of p53 was noted using IHC staining. In 9 of 38 tumors, rare (<1%) isolated nuclei were found with faint to moderate staining (Fig. 4B). These single, weakly IHC+ nuclei were widely dispersed throughout each specimen and did not occur in clusters. A third pattern of staining was recognized in two tumors and entailed dense staining of nuclei, which occurred in clusters of cells (case 125), or was diffusely distributed throughout the sample (case 134) (Fig. 4, C and E).

IHC detection of p53 expression did not correlate with genetic events in our set of primary prostatic tumors. None of the tumors with...
allelic loss of loci on chromosome 17p had overexpression of p53 as assessed using IHC staining, including the single tumor with allelic loss and a mutation of the remaining copy of the p53 gene (Fig. 4A). Interestingly, in these data, tumors with allelic loss showed significantly less staining than those without loss (P = 0.04). Furthermore, IHC+ status failed to correlate with pathological features of the tumors, including the Gleason grade (P = 0.83) and stage (P = 0.96). IHC+ expression of p53 protein was not associated with an increased risk of recurrence (Table 3 and Fig. 5). The estimated relative risk for IHC+ expression after adjustment for pathological grade and stage was 0.86 (P = 0.78).

**p53 Expression in Advanced Tumors.** p53 expression was assessed using IHC staining in the bone and visceral metastases of a separate group of 42 patients with advanced prostate cancer. Follow-up was available for 39 of 42 patients and averaged 54.2 ± 41.5 (range, 1–144) months. The mean time from diagnosis of primary...
Inactivation of both copies of the p53 gene, by mutation and/or loss, was a rare event in the tumors from our 67 patients with clinically localized prostate cancer. Mutation coupled with allelic loss was observed in only a single tumor. Two clinically localized tumors had significant expression of p53 protein using IHC staining and likely harbored p53 gene mutations. Nine additional primary tumors demonstrated minimal staining in a few scattered nuclei. Whether these tumors have a mutation in their p53 gene is uncertain (10, 12, 13, 37, 38). Taken together, 11 of 38 tumors (29%) showed IHC+ expression of p53, which agrees with staining rates reported previously (4–15). Somewhat surprisingly, staining was a poor indicator of allelic inactivation of the p53 gene, because all IHC+ primary tumors retained both copies of chromosome 17p. Although it is possible that tumors which were IHC+ had a mutation of both copies of their p53 genes, or had a single mutated p53 gene which behaved in a dominant negative manner to abolish p53 function, these means of p53 inactivation appear to be distinctly unusual in clinical specimens (23). Similarly, detection of allelic loss was a poor indicator of mutation of the remaining copy of the p53 gene. In our 55 informative prostate tumors, 10 demonstrated loss (18%). To our surprise, only one of these tumors had a mutation of its remaining allele documented using SSCP and sequencing, and none of the tumors with allelic loss were IHC-. This lack of concordance between chromosome 17p allelic loss and p53 gene mutation has been occasionally observed in bladder and head and neck tumors and more commonly in breast cancers and astrocytomas (36–44). Ours is the first series to document this pattern of discord in prostate cancer, and is the most striking example of this genetic pattern thus far reported.

It is unlikely that mutations of p53 were missed in tumors with chromosome 17p/p53 gene allelic loss. In this set of primary prostate tumors, we have been able to detect high rates of allelic deletion at several chromosomal loci (1, 29, 45). Our ability to detect allelic loss reflects the clonality of the tumors and a lack of contamination by DNA from normal tissues. Because of the paucity of contaminating
demonstrated minimal staining in a few scattered nuclei. Whether these tumors have a mutation in their p53 gene is uncertain (10, 12, 13, 37, 38). Taken together, 11 of 38 tumors (29%) showed IHC+ expression of p53, which agrees with staining rates reported previously (4–15). Somewhat surprisingly, staining was a poor indicator of allelic inactivation of the p53 gene, because all IHC+ primary tumors retained both copies of chromosome 17p. Although it is possible that tumors which were IHC+ had a mutation of both copies of their p53 genes, or had a single mutated p53 gene which behaved in a dominant negative manner to abolish p53 function, these means of p53 inactivation appear to be distinctly unusual in clinical specimens (23). Similarly, detection of allelic loss was a poor indicator of mutation of the remaining copy of the p53 gene. In our 55 informative prostate tumors, 10 demonstrated loss (18%). To our surprise, only one of these tumors had a mutation of its remaining allele documented using SSCP and sequencing, and none of the tumors with allelic loss were IHC-. This lack of concordance between chromosome 17p allelic loss and p53 gene mutation has been occasionally observed in bladder and head and neck tumors and more commonly in breast cancers and astrocytomas (36–44). Ours is the first series to document this pattern of discord in prostate cancer, and is the most striking example of this genetic pattern thus far reported.

It is unlikely that mutations of p53 were missed in tumors with chromosome 17p/p53 gene allelic loss. In this set of primary prostate tumors, we have been able to detect high rates of allelic deletion at several chromosomal loci (1, 29, 45). Our ability to detect allelic loss reflects the clonality of the tumors and a lack of contamination by DNA from normal tissues. Because of the paucity of contaminating

demonstrated minimal staining in a few scattered nuclei. Whether these tumors have a mutation in their p53 gene is uncertain (10, 12, 13, 37, 38). Taken together, 11 of 38 tumors (29%) showed IHC+ expression of p53, which agrees with staining rates reported previously (4–15). Somewhat surprisingly, staining was a poor indicator of allelic inactivation of the p53 gene, because all IHC+ primary tumors retained both copies of chromosome 17p. Although it is possible that tumors which were IHC+ had a mutation of both copies of their p53 genes, or had a single mutated p53 gene which behaved in a dominant negative manner to abolish p53 function, these means of p53 inactivation appear to be distinctly unusual in clinical specimens (23). Similarly, detection of allelic loss was a poor indicator of mutation of the remaining copy of the p53 gene. In our 55 informative prostate tumors, 10 demonstrated loss (18%). To our surprise, only one of these tumors had a mutation of its remaining allele documented using SSCP and sequencing, and none of the tumors with allelic loss were IHC-. This lack of concordance between chromosome 17p allelic loss and p53 gene mutation has been occasionally observed in bladder and head and neck tumors and more commonly in breast cancers and astrocytomas (36–44). Ours is the first series to document this pattern of discord in prostate cancer, and is the most striking example of this genetic pattern thus far reported.

It is unlikely that mutations of p53 were missed in tumors with chromosome 17p/p53 gene allelic loss. In this set of primary prostate tumors, we have been able to detect high rates of allelic deletion at several chromosomal loci (1, 29, 45). Our ability to detect allelic loss reflects the clonality of the tumors and a lack of contamination by DNA from normal tissues. Because of the paucity of contaminating
wild-type DNA, our samples with p53 allelic loss should be ideal for identifying mutations in the remaining copy of the gene. Furthermore, SSCP can detect 10% mutant sequence admixed with wild type (38). Since all seven tumors analyzed had normal banding patterns, it is improbable that these tumors, or a subpopulation of cells within these tumors, harbored p53 gene mutations.

In agreement with several studies, nearly one half of our metastatic prostatic neoplasms were IHC+ for p53 expression and likely had a mutated copy of the p53 gene (9–14). Since mutation of p53 is more common in prostate cancer metastases, several authors have suggested that mutation of p53 contributes to the metastatic phenotype, and that the detection of p53 mutations in primary tumors may serve as a marker for poor prognosis (4, 5, 9–13, 21). This hypothesis has been born out in other tumor types. For instance, p53 mutations and IHC detection of p53 expression are common in high-grade, muscle invasive and metastatic bladder cancers (39, 40). Detection of p53 mutations in superficial and locally invasive bladder tumors is associated with high rates of development of metastatic disease and worse prognosis (46, 47). In patients with primary lung, breast, colon, ovarian, and stomach cancers, loss of p53 function has been associated with tumor progression and is an independent prognostic marker for shortened survival (40). p53 gene inactivation appears to play a central role in the progression of prostate cancer in a mouse model (21). However, uncertainty remains whether p53 inactivation is involved in human prostate cancer progression and whether detection of p53 mutations is a useful prognostic marker in patients diagnosed with prostate cancer (4, 5, 8).

IHC detection of p53 expression was not a useful prognosticator in our patients with primary and metastatic prostate cancer. Of the 38 patients whose primary tumors were stained for p53 expression, 20 suffered disease progression. Only one of the tumors which progressed had a moderate degree of p53 expression using IHC staining. The remaining primary tumor with dense p53 staining did not progress in limited follow-up. Inclusion of the nine patients with grade 1+ staining did not improve our prediction of those patients whose primary tumors were prone to progress. In patients with metastatic prostate cancer, positive staining of the metastatic lesion did not correlate with time from diagnosis of their primary tumor to the development of metastases, time from the development of metastases to death, nor overall survival time. Interestingly, all three patients who had positive staining of their primary tumor had long-term survival (61, 80, and 133 months, respectively), and two patients had a long interval between diagnosis of their primary tumor and the development of metastases (52 and 74 months, respectively). Given the lack of correlation between p53 mutation (as detected using IHC staining) and disease progression in primary and metastatic tumors and the infrequency of inactivation of both copies of the gene, it appears that p53 inactivation is not essential in the development of human prostate cancer metastasis. Consistent with this view is our finding of a prostatic neoplasm with significant staining in the primary tumor and absent staining in the metastasis which developed years later. It is possible that p53 inactivation may represent one of several pathways in the development of prostate cancer metastases.

It is unknown whether nuclear p53 expression seen on IHC staining in the occasional cell is an indicator of p53 dysfunction in prostatic carcinomas. It has been proposed that these cells represented subpopulations with mutated or inactivated p53, which gives rise to metastases (5, 48). Since these cells represent only a fraction of the tumor, screens for p53 inactivation using either gene sequencing or the determination of chromosome 17p/p53 allele status would be negative. If these cells give rise to metastases, patients with grade 1 staining in their primary tumors would be predicted to be at an increased risk for progression. In our series, this was not the case.

Recurrence after radical prostatectomy was not related to the presence or degree of IHC detectable expression of p53 in the primary tumor. This finding is in agreement with Visakorpi et al. (4), who found no correlation between low-grade staining and progression in a group of 137 clinical stage T1,2 primary prostate cancers. IHC detection of p53 expression in a small fraction of tumor cells is not associated with a significantly worse prognosis in several other tumor types as well (40, 46, 47). Thus, it is unlikely that these occasionally staining cells represent clones with inactivated p53 which will ultimately give rise to metastases.

We were curious whether mutation of the p53 gene, commonly observed in prostate cancer metastases, acts as a biological modifier in advanced disease. Several studies have related p53 mutation to the acquisition of hormonal resistance (9, 11, 12). In our group of patients with metastatic disease, however, p53 expression was not related to hormonal status. An intriguing possibility is that p53 inactivation affects the acquisition of radioresistance in prostate cancers (49). We are currently investigating this possibility in our patients with metastatic prostate cancer.

Unlike IHC detection of p53 expression, chromosome 17p allelic loss did correlate with the clinical course in our patients with localized disease. Patients whose tumors had allelic loss of chromosome 17p had a 3-fold risk for tumor recurrence after radical prostatectomy which remained to a lesser degree (2-fold) after adjusting for stage and Gleason grade. In addition, chromosome 17p allelic loss was related to increasing Gleason grade, a reliable indicator of clinically aggressive disease (28). Since all tumors retained a normal p53 gene, these clinical correlates argue that a second gene on chromosome 17p is involved in prostate cancer progression (37, 38, 41–44). In agreement with this hypothesis, three of five lymph node metastases lost 17p and retained a normal p53 allele. Of the 11 prostatic tumors with chromosome 17p loss reported in the literature, 7 are metastases, and the remaining 4 are of advanced stage (Ts; Refs. 1, 10, and 24–26). Alternatively, chromosome 17p allelic loss may be reflective of global genetic alterations associated with poor prognosis (50). Deletion mapping of prostate cancer metastases will likely provide additional insights into the region of loss associated with disease progression (44).

REFERENCES

20. Lu, X., Park, S. H., Thompson, T. C., and Lane, D. P. ras-Induced hyperplasia occurs
29. Bova, G. S., Carter, B. S., Buscumakers, M. J. G., Emi, M., Fujiwara, Y., Kyprianou,
28. Mellinger, G. T., Gleason, D., and Bailar, J. The histology and prognosis of prostatic
43. W. W., Von Eschenhach, A. C., and Conti, C. J. p53 AND CLINICAL PROSTATE CANCER
Sidransky, D., Von Eschenbach, A., Tsai, Y. C., Jones, P., Summerhayes, I., Mars-
19. Kubola, Y., Shuin, T., Uemura, H., Fujinami, K., Miyamoto, H., Torigoe, S., Dobashi,
17. Uchida, T., Wada, C., Shibata, T., Egawa, S., and Koshiba, K. Infrequent involvement
Sidransky, D., Von Eschenbach, A., Tsai, Y. C., Jones, P., Summerhayes, I., Mar-
9. Kubota, Y., Shuin, T., Umura, H., Fujinami, K., Miyamato, H., Torigoe, S., Dobashi,
29. Bova, G. S., Carter, B. S., Buscumakers, M. J. G., Emi, M., Fujiwara, Y., Kyprianou,
28. Mellinger, G. T., Gleason, D., and Bailar, J. The histology and prognosis of prostatic
9. Kubota, Y., Shuin, T., Umura, H., Fujinami, K., Miyamato, H., Torigoe, S., Dobashi,
Sidransky, D., Von Eschenbach, A., Tsai, Y. C., Jones, P., Summerhayes, I., Mar-
30. Burton. K. Determination of DNA concentration with diphenylamine. Methods En-
9. Kubota, Y., Shuin, T., Umura, H., Fujinami, K., Miyamato, H., Torigoe, S., Dobashi,

Downloaded from cancerres.aacrjournals.org on January 5, 2018. © 1996 American Association for Cancer Research.
An Uncertain Role for *p53* Gene Alterations in Human Prostate Cancers


**Updated version**

Access the most recent version of this article at:
[http://cancerres.aacrjournals.org/content/56/16/3814](http://cancerres.aacrjournals.org/content/56/16/3814)

**E-mail alerts**

Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**

To request permission to re-use all or part of this article, use this link [http://cancerres.aacrjournals.org/content/56/16/3814](http://cancerres.aacrjournals.org/content/56/16/3814). Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.