Expression of p53 and 17p Allelic Loss in Colorectal Carcinoma


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ABSTRACT

Mutations in the p53 gene are the most common genetic changes in cancer thus far. Many p53 mutations result in a protein product having a prolonged half-life compared to wild-type p53. The mutant protein is frequently detectable immunohistochemically, whereas the wild-type p53 present in normal cells is not. We examined 90 colorectal carcinomas for increased expression of p53 using 3 p53 specific monoclonal antibodies, PAb1801, PAb421, and PAb240. Overall, 70% of the colorectal carcinomas stained for p53. Each tumor's DNA was also assessed for loss of heterozygosity on chromosome 17p, the location of the p53 gene. Of those tumors that reacted with the anti-p53 antibodies, 76% showed loss on chromosome 17p. Tumors with loss of heterozygosity on 17p generally stained with all 3 antibodies, whereas those without loss tended to stain with just one antibody, typically PAb240. Fifteen tumors were examined for the presence of specific p53 mutations. A total of 10 mutations were found, 6 were missense and 2 were deletions, and all but one of the tumors with missense mutations stained for p53.

INTRODUCTION

Loss of heterozygosity at various chromosomal loci has been observed in a wide diversity of human tumors (1–3). Such gross chromosomal changes have been utilized to implicate the involvement of tumor suppressor genes (4) and as an aid to their initial localization. Chromosome 17p is one of the most common regions demonstrating allelic loss (5–10) and recently, one such tumor suppressor gene, p53, has been mapped to 17p13.1 (11–13).

The p53 gene encodes a nuclear phosphoprotein that is thought to play a critical role in controlling the cell cycle. This is based in part on its ability to bind DNA (14–16) and, under certain in vitro conditions, regulate transcription (17–19).Mutations in the p53 gene are generally thought to alter the functional capabilities of the molecule, rendering the cell devoid of the restraint engendered by normal p53. Although gross structural abnormalities of the gene have been observed (20–24), the vast majority of p53 mutations described to date are missense mutations occurring in 4 of 5 highly conserved regions of the p53 genome (25–33). Most of these missense mutations result in an abnormal protein that accumulates in cells by virtue of an increased half-life. In normal cells, however, wild-type p53 has a short half-life (34) and is generally not detectable. In cells that contain both wild-type and mutant p53, it has been proposed that the mutant form stabilizes the otherwise rapidly degraded wild-type p53 through the formation of oligomers (35–37) and alters its normal suppressive functions in a dominant-negative fashion. This could be achieved by altering the conformation of the wild-type protein (38).

Mutations are likely to alter the conformation of p53 and, perhaps its ability to associate with other cell products. Sequences required for binding of SV40 T antigen, for example, are frequently mutated (25), whereas those required for DNA binding and transcriptional activation rarely are. In addition, mutations in p53, while interfering with the suppressive function of the molecule, may also endow it with additional properties that promote an oncogenic state. Just how these mutations stimulate or promote a malignant state, however, is still not clear. More detailed analyses of mutant p53, by a variety of techniques, are needed if we are to fully understand the molecular and functional aspects of the p53 protein.

Immunohistochemical analysis is one method of assessing the frequency of abnormal p53 in human cancer. Studies that have taken advantage of this approach have utilized anti-p53 monoclonals with defined epitope recognition (39–41). In breast (42, 43), lung (44), ovarian (45), and colorectal carcinomas (46–48), about half of the tumors show p53 staining with p53 staining correlating with the presence of p53 mutations (44, 47, 49). Although a number of antibodies have been used in these studies, not all antibodies have comparable reactivity (42). In particular, PAb240 recognizes mutant p53 molecules that lack suppressor function (41, 50). In the present study, therefore, we used a panel of antibodies to explore the extent of p53 involvement in colorectal cancer. We studied the relationship of the expression of p53 to allelic loss on 17p and determined the specific mutation present in a group of these carcinomas by DNA sequencing.

MATERIALS AND METHODS

Patient Population. A total of 90 specimens from 87 patients with colorectal carcinoma were studied, none of which was considered to have a history of familial adenomatous polyposis. One of these had 2 tumors while another had 3. There were 48 males and 39 females. The mean age at surgery was 67 years (range, 25–91 years), and 7 were less than 45 years of age. There were 5 patients with Duke's A, 36 patients with Duke's B, 25 patients with Duke's C, and 21 patients with Duke's D. The anatomical distribution for the tumors was 19 caecal, 12 ascending, 9 transverse/descending, 20 sigmoid, and 30 rectal. A family history was extracted from the patients' medical records. With the exception of a few cases, information was available only for the immediate family members. Of the 87 patients studied, 40 had no significant family history of cancer, 10 had multiple tumors (i.e., colon cancer plus one other including cancer of the skin, prostate, breast, or leukemia), 12 had 1 other immediate family member with colon cancer, 7 had 2–3 family members affected with various cancers (including stomach, uterine, pancreatic, cervical, bladder, esophageal, and breast cancer) with at least 1 having colorectal cancer, 9 had 2–3 family members with cancer other than colon cancer (same spectrum as previously noted), and 9 had an insufficient amount of information.

Monoclonal Antibodies. PAb1801 (Cambridge Research Biochemical) is a p53 specific antibody that recognizes an epitope on human p53 between amino acids 72 and 79. PAb421 (Oncogene Sciences) recognizes an epitope between amino acids 370 and 378. PAb240 (supernatant; Dr. Lane, Imperial Cancer Research Fund) recognizes a denaturation resistant epitope on mutant p53 between 156 and 335. Normal mouse IgG (The Binding Site, Inc.) was used as a control. All antibodies were used at a dilution of 1 μg/ml in 20 mM phosphate-buffered saline, pH 7.2/1% bovine serum albumin/0.05% Tween 20 except for PAb240, which was used as undiluted supernatant.

Immunoperoxidase Staining. Samples were embedded in Tissue-Tek II ornithine carbamyl transferase compound and sectioned on a cryo-
stat. Five-μm sections were collected onto poly-L-lysine coated slides, briefly fixed in acetone, and stored at −70°C. Further fixation (10 min in acetone at 4°C) was carried out on thawed, dried sections just prior to use. Sections of colorectal carcinomas were incubated with anti-p53 monoclonal or control IgG overnight at 4°C. Bound IgG was detected by the avidin-biotin and horseradish peroxidase method as described by Van den Berg et al. (46) using a Vectastain Elite avidin-biotin complex kit. Initial experiments sought to identify specimens that could serve as positive controls for subsequent screening of the colorectal carcinomas. Also identified were specimens that expressed p53 at varying levels of intensity. Expression of p53 was primarily nuclear and was scored for: (a) distribution, an estimate of the percentage of stained cancer cells; and (b) intensity (equivocal, +, ++, or ++++), and inasmuch as this was frequently heterogeneous, upper and lower limits were recorded. For the various analyses, specimens were considered positive for p53 expression if >10% of the tumor cells stained with a level of intensity from 1+ to 3+. Cytoplasmic staining was observed occasionally and recorded as yes or no.

DNA Extraction. At the time of tumor processing, a hematoxylin and eosin stained frozen section of each tumor was examined and used as a template to dissect away as much non-tumor tissue as possible in order to enrich for tumor cells. The remaining tumor fragments were remounted for serial frozen sections in Tissue-Tek ornmithine carbamyl transferase compound. One hundred 10–20-μm-thick cryostat sections were then made. The 1st, 50th, and 100th sections were stained with hematoxylin and eosin, and the fraction of tumor nuclei present at these levels was assessed by a pathologist. Only those tumors containing ≥70% tumor nuclei were used for subsequent steps. DNA was extracted from tumor and “normal” control tissue (either normal mucosa or peripheral blood leukocytes) using an Applied Biosystem 340A Nucleic Acid Extractor (ABI, Inc.) in accordance with the manufacturer’s instructions and stored at 4°C until used for DNA sequence and Southern blot analysis.

Sequence Analysis. Sequence analysis was performed by the Sanger dideoxy chain termination method with the use of 32P-end labeled primers and PCR amplification fragments. All PCR reactions were performed on a Perkin-Elmer Cetus Thermal Cycler according to the manufacturer’s instructions. Typical reactions included 0.25 μg of genomic DNA, 20 pmol of each primer, buffer, dNTPs, and Taq polymerase in a 50-μl reaction volume. Sequence information needed to synthesize the oligonucleotides was obtained from Dr. B. Seizinger, Harvard University. Specific reaction conditions for the PCR included 30 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 2 min, and primer extension at 72°C for 2 min. Upon completion of the PCR, the amplified products were concentrated by ethanol precipitation, fractionated on agarose gels, purified by electroelution, and ethanol-precipitated again. Purified DNA was annealed with an equimolar concentration of sequencing oligonucleotide. The reaction mixture was aliquoted into 4 tubes containing deoxy- and dideoxy nucleotides, 0.5 μl of 0.1 M dithiothreitol, and 4 units of Sequenase. The reaction was terminated after 10 min at 37°C, heated to 95°C for 3 min, chilled, and electrophoresed on a 6% polyacrylamide/7 M urea gel at 50°C for 2–3 h. After drying, the gels were exposed to Kodak X-AR5 film at room temperature with the use of intensifying screens.

Statistical Methods. χ2 statistics were used to compare proportions of tumors demonstrating staining with each of the 3 antibodies. For small frequencies, Fisher’s exact statistic was used. Since allelic loss on 17p has been shown to be associated with tumor site, we performed stratified χ2 analyses by stratifying on the presence or absence of 17p loss in order to assess the association of antibody staining with tumor site, after removing the confounding effect of 17p loss.

**RESULTS**

**Immunohistochemical Analysis of p53 in Colorectal Cancer.** Three anti-p53 monoclonal antibodies (PAb240, PAb421, and PAb1801) were used to determine the extent of protein expression in a population of 90 colorectal carcinomas. For the purposes of this study, tumors were considered positive for a particular antibody when there was evidence of nuclear staining in greater than 10% of the cells (Table 1). Eleven tumors had ≤10% of cells staining with one or more antibodies, and these are also noted in Table 1. Of the 3 antibodies utilized, PAb240 and PAb1801 revealed the highest frequency of antibody reactivity, 64% (58 of 90) and 49% (44 of 90), respectively. Antibody PAb421 detected p53 in 36% (32 of 90) of the tumors examined. Detection of p53 by any one of the 3 monoclonals was achieved in 70% of the cases, a frequency higher than that reported in 70% of the cases, a frequency higher than that reported.

**Table 1. Summary of tumors that stained for p53**

<table>
<thead>
<tr>
<th>17p allelic loss</th>
<th>PAb240</th>
<th>PAb1801</th>
<th>PAb421</th>
<th>Any one of the three</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>No loss (n = 58)</td>
<td>43</td>
<td>43</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>Nl (n = 26)</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Nl (n = 6)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total (n = 12)</td>
<td>58</td>
<td>58</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Loss*PAb240</td>
<td>43(43)</td>
<td>39(55)</td>
<td>31(55)</td>
<td>48(53)</td>
</tr>
<tr>
<td>No loss*PAb240</td>
<td>35(55)</td>
<td>39(55)</td>
<td>31(55)</td>
<td>48(53)</td>
</tr>
<tr>
<td>Nl*PAb240</td>
<td>3(5)</td>
<td>2(3)</td>
<td>1(2)</td>
<td>4</td>
</tr>
<tr>
<td>Total*PAb240</td>
<td>51(57)</td>
<td>44(49)</td>
<td>32(36)</td>
<td>63(70)</td>
</tr>
<tr>
<td>P value*</td>
<td>0.006</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

* P value is for the comparison of the percentage of tumors that stained for p53 between the 17p allelic loss versus no loss groups.

**Table 1 Summary of tumors that stained for p53**

**Fig. 1. Immunohistochemical detection of p53 by monoclonal antibodies. A, a colorectal carcinoma with nuclear staining by PAb1801. B, normal mucosa demonstrating absence of staining by PAb421 (counterstained with hematoxylin).**

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1 The abbreviation used is: PCR, polymerase chain reaction.
2 B. Seizinger, personal communication.
EXPRESSION OF p53 IN COLORECTAL CANCER

Fig. 2. Distribution of stained cells (i.e., percentage of cells staining for p53 within a tumor) in colorectal carcinomas for each of the p53 monoclonal antibodies.

Fig. 3. Southern blot analysis of DNA isolated from tumor (T) and corresponding normal tissue (N) with the use of DNA probe YNZ22 and the restriction endonuclease BamHI.

previously (46–48). The distribution of staining for all 3 antibodies was predominantly nuclear, although some cells showed traces of cytoplasmic reactivity. There was no staining of normal mucosa, and tissue did not react with control mouse immunoglobulin. Examples of negatively and positively stained tissues are shown in Fig. 1.

Distribution of p53 Positive Cells. The number of cells exhibiting antibody staining and the overall intensity of staining varied broadly among the 3 different antibodies. For a given antibody, not all of the tumor cells stained for p53. In some specimens, nearly 100% of the tumor cells stained, while in the others, only a small percentage of the cells showed p53 reactivity. Fig. 2 shows this variability (ranging from 5 to 100% of the cells staining) for the 3 different antibodies. Furthermore, for any given tumor, the percentage of cells staining for p53 frequently differed among the 3 antibodies, with PAb240 generally staining the greatest number (Fig. 2). Thus, it would appear that p53 does not necessarily react equally with all 3 antibodies. This was particularly true for the “no loss” group, in which none of the positively stained tumors had an equal number of cells reacting with the 3 antibodies. For all tumors examined, however, patterns of reactivity with each antibody were reproducible.

Association of p53 Expression with 17p Allelic Loss. The frequency of allelic loss on chromosome 17p was assessed with the use of 2 DNA probes: YNZ22 and YNH37.3 (both of which map to 17p13.3). Loss of heterozygosity for 17p was found in 58 of the 90 tumors examined. Allelic loss was not detected in 26 cases, and could not be assessed in 6 cases because the DNA probes were uninformative (i.e., homozygous for the polymorphic alleles at each locus examined). Fig. 3 shows the results of a Southern blot analysis of representative tumor specimens.

Immunohistochemical detection of p53 by any one of the 3 antibodies was significantly higher in colorectal cancers with 17p allelic loss compared to those without loss (Table 1). A more detailed examination of the data, (Fig. 4), however, revealed interesting patterns of reactivity. (a) A majority of tumors in the “loss” group (48%) reacted with all 3 antibodies, whereas none of those in the “no loss” group did so. (b) An absence of staining by any antibody was noted for most of the tumors without allelic loss (58%), whereas only 17% of tumors with allelic loss did not stain. (c) A number of tumors [10 (17.2%) in the “loss” and 8 (31%) in the “no loss” group] reacted with PAb240 only.

Overall, tumors with allelic loss tended to react with all 3 monoclonals, the staining intensity was mild to strong, and the localization tended to be nuclear. Positively staining tumors in the “no loss” group showed reactivity to fewer antibodies (typically to just one, PAb240), and the intensity of staining was less for PAb240 but not for PAb1801.

In addition to the nuclear staining typically observed, mild cytoplasmic staining was detected occasionally. A small number of tumors (n = 11) demonstrated trace staining only in the cytoplasm, without a nuclear component. This was observed most frequently with PAb1801 and in tumors not demonstrating allelic loss (data not shown). An additional 4 tumors demonstrated trace cytoplasmic reactivity with one of the antibodies and nuclear staining by another monoclonal. As the significance

expression, and included 11 with 17p allelic loss and those selected at random from the 90 tumors initially tested for p53 correlated with specific mutations within the gene for p53, 15 without loss on 17p.

When broken down into the “loss” and “no loss” groups, this result should be viewed with caution inasmuch as due to small number), grade, or sex (data not shown). A significant correlation was obtained with site for PAb 1801 and PAb 240, and PAb 421 (P = 0.27). This significant correlation was obtained with site for PAb 1801 and PAb 421 (P = 0.01, 0.001), with a similar, although not statistically significant, correlation with PAb 240 (P = 0.27). This association is not surprising since our data and those of others have shown an association between site and loss of heterozygosity on chromosome 17p. When our data were adjusted for allelous loss on 17p, indicating correlation of allelic loss with presence of mutant forms of p53. In contrast to the “loss” group, a mutation was identified in only one of the 4 tumors in the “no loss” group. Overall, there was good correlation between the 3 types of analyses (i.e., immunohistochemistry, loss of heterozygosity, and sequence analysis). Tumors with allelic loss were more likely to contain mutant forms of p53 as detected by both sequence analysis and immunohistochemical analysis (results are summarized in Table 2).

Association of p53 Expression with Site, Stage, Grade, Sex, or Age. There was no association of p53 staining across Duke’s stages B-D (the 5 stage A tumors were excluded from analysis due to small number), grade, or sex (data not shown). A significant correlation was obtained with site for PAb 1801 and PAb 421 (P = 0.01, 0.001), with a similar, although not statistically significant, correlation with PAb 240 (P = 0.27). This association is not surprising since our data and those of others (51) have shown an association between site and loss of heterozygosity on chromosome 17. When our data were adjusted for 17p loss, the significance of the correlation with tumor site was lost. Interestingly, all tumors from patients less than 45 years of age (n = 7) expressed p53, regardless of their allelic loss status on 17p (3 in the “loss” group and 4 in the “no loss” group). This result should be viewed with caution inasmuch as the number included was small. The significance of this finding is not clear.

Association of p53 Expression with Specific Gene Mutations. To determine whether the altered expression of p53 protein correlated with specific mutations within the gene for p53, 15 tumors were selected for DNA sequence analysis. These were selected at random from the 90 tumors initially tested for p53 expression, and included 11 with 17p allelic loss and those showing both positive and negative p53 expression. Fig. 5 outlines our sequencing strategy of exons 5–9, the most highly conserved regions of the p53 gene in which the majority of previously defined mutations have been found (26; 33). 

Ten mutations were identified (Table 2). These consisted of a 2-base pair deletion (presumably a frame shift mutation), a 46-base pair deletion (spanning the boundary between intron 6 and exon 7), 6 missense mutations (5 of 6 resulting in a transversion), one non-sense mutation, and one base substitution that had no effect (no amino acid substitution). In this latter case, the putative causative mutation may lie outside of the region that we are monitoring. An example of a sequencing gel for a group of tumors is shown in Fig. 6. In all cases, an analysis from a second PCR was performed to verify the mutation. Analysis of DNA from normal tissue revealed wild-type p53 sequence in all cases. Thus, the mutations detected are specific for the tumors.

Nine of the 10 mutations were in tumors with loss of heterozygosity for 17p, indicating correlation of allelic loss with presence of mutant forms of p53. In contrast to the “loss” group, a mutation was identified in only one of the 4 tumors in the “no loss” group. Overall, there was good correlation between the 3 types of analyses (i.e., immunohistochemistry, loss of heterozygosity, and sequence analysis). Tumors with allelic loss were more likely to contain mutant forms of p53 as detected by both sequence analysis and immunohistochemical analysis (results are summarized in Table 2).

**DISCUSSION**

We have shown that the expression of p53 is present in a majority of colorectal carcinomas (70%), particularly tumors with allelic loss on chromosome 17p (83%). The overall frequency of p53 staining in this study is considerably higher than that reported previously (46–48). Several reasons may explain this finding. (a) We have examined a larger number of tumors (n = 90) compared to other studies (n = 29 and n = 26), reflecting possible population differences. (b) A second more likely explanation is that a panel of antibodies that recognizes different epitopes on p53 was utilized, allowing detection of a greater number of positive tumors (43). Had we used PAb 421 alone, for example, the frequency of positive tumors would have

**Fig. 4. Pattern of p53 staining in colorectal carcinomas, positive (+) or negative (−), by the 3 p53 antibodies. A, tumors demonstrating loss on 17p; B, tumors without loss on 17p.**

**Fig. 5. Strategy for amplification and sequencing of the p53 gene in DNA extracted from colorectal carcinomas. Exons 5–9 were examined using the indicated primers.**
**Table 2 p53 sequence and immunohistochemistry analysis**

<table>
<thead>
<tr>
<th>Tumor no.</th>
<th>Exon</th>
<th>No. of alleles 17p</th>
<th>Codon</th>
<th>Nucleotide mutation</th>
<th>Amino acid</th>
<th>p53 expression*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2705*</td>
<td>5</td>
<td>1</td>
<td>134</td>
<td>TTT→CTT</td>
<td>Phe→Leu</td>
<td>+ (20)</td>
</tr>
<tr>
<td>2528*</td>
<td>5</td>
<td>1</td>
<td>138*</td>
<td>GCC→GCT</td>
<td>Ala→Ala</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>2929</td>
<td>6</td>
<td>1</td>
<td>193</td>
<td>CAT→TAT</td>
<td>His→Tyr</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>1216*</td>
<td>6</td>
<td>1</td>
<td>209</td>
<td>2bp del</td>
<td>Frameshift</td>
<td>– – + (10)</td>
</tr>
<tr>
<td>2472</td>
<td>6</td>
<td>1</td>
<td>213</td>
<td>CGA→TGA</td>
<td>Arg→stop</td>
<td>± ± (5)</td>
</tr>
<tr>
<td>2347</td>
<td>7</td>
<td>1</td>
<td>225</td>
<td>46 bp del</td>
<td>***†</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>1810‡</td>
<td>7</td>
<td>2</td>
<td>245</td>
<td>GGC→AGC</td>
<td>Gly→Ser</td>
<td>++ (5)</td>
</tr>
<tr>
<td>2034‡</td>
<td>7</td>
<td>2</td>
<td>245</td>
<td>GGC→AGC</td>
<td>Gly→Ser</td>
<td>++ (5)</td>
</tr>
<tr>
<td>2683</td>
<td>7</td>
<td>1</td>
<td>248</td>
<td>CGG→CAG</td>
<td>Arg→Gln</td>
<td>++ + (50)</td>
</tr>
<tr>
<td>2296</td>
<td>8</td>
<td>1</td>
<td>270</td>
<td>TTT→ATT</td>
<td>Phe→ile</td>
<td>++ + + (50)</td>
</tr>
<tr>
<td>1556*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>+ (25)</td>
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<tr>
<td>2888*</td>
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<td></td>
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<td>+ + (40)</td>
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<tr>
<td>986</td>
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<tr>
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<tr>
<td>1219*</td>
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<td></td>
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<td></td>
<td>+ + + (10)</td>
</tr>
</tbody>
</table>

* Data represent relative intensities with most cells staining unless noted (percent stained).
† This mutation may result in a p53 molecule with short half-life, present in quantities insufficient to be detected immunohistochemically.
‡ p53 reactivity in absence of detectable mutation suggests that the mutations must be outside the region sequenced.
§ Deletion includes splice acceptor site; thus entire exon 6 would be lost.
∥ Two tumors with identical mutations but the 2 allele tumor displays reduced reactivity with PAbs 1801 and 421, whereas the hemizygous tumor reacts equally with all 3 antibodies.
* Minor subclones of positive cells whose mutations may not be detected by the DNA based assays.
† NF, none found.

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*Fig. 6. An example of a p53 sequencing reaction revealing a 2-base pair deletion in one tumor sample (Lane 2). Each of the other lanes is from a separate tumor.*

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been 32%. This frequency is comparable to that reported by others using PAb421 (46–48).

One of the major observations of this investigation is the lack of concordance in staining among the 3 different antibodies for some of the tumors. In contrast to a report by Rodrigues et al. (47), the reactivity of PAb421 did not parallel that of PAb240. Perhaps the most striking finding is that a number of tumors reacted only with PAb240. This antibody clearly recognizes p53 molecules that are not recognized by the other 2 antibodies. Reactivity with PAb240 only was seen in 31% of tumors without allelic loss and in 17% with 17p loss. It is likely that the former contain wild-type and mutant p53 alleles. The data suggest that the restrictive antibody reactivity to mutant p53 may be the result of its interaction with wild-type p53.

Data from 2 tumors, 1810 and 2034, further support this concept. These tumors were shown to have the same missense mutation in the p53 gene but displayed markedly different staining patterns with the 3 antibodies. Mutant p53 in tumor 1810 was detected by all 3 antibodies, whereas p53 in tumor 2034 reacted with only PAb240. One of these tumors (tumor 1810), however, showed allelic loss at 17p, whereas the other (tumor 2034) did not. Although not directly demonstrated, wild-type p53 is presumably present in tumor 2034 but not in tumor 1810. Thus, the presence of wild-type p53 may alter the availability of epitopes recognized by PAb1801 and PAb421, presumably through the formation of mutant wild-type heterooligomers. The existence of such heterooligomers has been demonstrated previously (35, 37, 52, 53).

Several tumors with loss of heterozygosity at 17p reacted with PAb1801 or PAb1801 and PAb421, but not with PAb240. Recently, Milner and Medcalf (38) showed that a truncated mutant p53 lacking a carboxyl terminus was unable to form oligomers or to adopt a mutant conformation detectable by PAb240. Thus, some tumors described in this study may contain mutations that similarly alter their immunoreactivity.

There was good correlation between the immunohistochemical, DNA sequencing, and loss of heterozygosity studies. In general, those tumors that demonstrated allelic loss also contained mutant forms of p53 that were detected by both sequence analysis and immunohistochemical analyses (Tables 1 and 2). In some cases, however, results from one type of analysis did not correlate with results from another (Table 2). One possible explanation for such discrepant results is that different portions of the tumor were used for these studies (DNA based assays...
versus immunohistochemical). These results, therefore, may reflect heterogeneity within the specimen. Other possible explanations are noted in Table 2 footnotes. It is also possible that allelic loss in some cases may be nonspecific and have no relationship to p53. Such nonspecific loss may occur as frequently as 10–20% (54). The DNA based assays for loss of heterozygosity and p53 sequence analysis depend upon sufficient concentration of affected cells, thus limiting their sensitivity. In this study, the immunohistochemical detection of p53 is clearly more sensitive than the DNA based assays inasmuch as the method allows detection of small numbers of cells bearing putative p53 mutations. This small population might be important clinically, however, since they may enjoy a selective advantage. Further experimentation is required to resolve these discrepancies.

Allowing for 17p loss, there was no association with other clinical variables considered. However, of particular interest is the finding that tumors from patients less than 45 years of age show p53 staining regardless of the allelic loss status on chromosome 17p (i.e., loss or no loss). Since we have analyzed so few of these tumors (n = 7), the clinical implications of these findings, as well as those previously described for the pattern of antibody reactivity, are as yet unknown.

The data from these analyses support and extend the model advanced by Baker et al. (27). In this model, the first event leading to the involvement of the proto-oncogene p53 in the process of tumorigenesis is the acquisition of a specific mutation within the gene. The mutant p53 protein may at this point exert a dominant-negative effect on the cell, thus conferring a selective advantage to that particular cell population (25, 55, 56).

Additionally, our data suggest that the p53 molecules in these cells, which are likely to be complexed with other intracellular proteins (such as wild-type p53), have restrictive-antibody reactivity, in this case to PAb240. Examples of this stage are those tumors that are negative for allelic loss and positive for p53 expression, detectable predominantly with PAb240 (e.g., tumor 2034). Following the acquisition of a mutation, a gross chromosomal change occurs leading to loss of the second wild-type p53 allele and subsequent clonal expansion. The pattern of antibody reactivity in this cell population is now less restrictive and all 3 antibodies are capable of reacting with mutant p53. One possible explanation for this change in antibody reactivity is an altered p53 interaction with other proteins.

This process of clonal evolution is further supported by examining the overall distribution of antibody staining within each of the tumors (Fig. 2). In some tumors, nearly 100% of the tumor cells stain with all 3 antibodies, while in others some of the tumor cells stain with just one antibody (typically PAb240) and other cells stain with all 3. In almost all cases where staining heterogeneity exists, the percentage of cells staining for p53 is highest for PAb240. Thus, the different staining patterns within a tumor may represent the different clonal populations as they evolve from one to another. Additional studies utilizing more sensitive techniques for DNA analyses are needed to assess this model in more detail.

ACKNOWLEDGMENTS

We would like to thank Dr. D. Lane for the PAb240 and Karen Erwin for her excellent secretarial assistance.

ADDITIONAL SOURCES

REFERENCES


Expression of $p53$ and 17p Allelic Loss in Colorectal Carcinoma

Julie Cunningham, John A. Lust, Daniel J. Schaid, et al.


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