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Mutations in p53 as potential molecular markers for human breast cancer

(protein expression/multiplex PCR/gene mutation)

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ABSTRACT **Based on the high incidence of loss of heterozygosity for loci on chromosome 17p in the vicinity of the p53 locus in human breast tumors, we investigated the frequency and effects of mutations in the p53 tumor suppressor gene in mammary neoplasia. We examined the p53 gene in 20 breast cancer cell lines and 59 primary breast tumors. Northern blot analysis, immunoprecipitation, and nucleotide sequencing analysis revealed aberrant mRNA expression, overexpression of protein, and point mutations in the p53 gene in 50% of the cell lines tested. A multiplex PCR assay was developed to search for deletions in the p53 genomic locus. Multiplex PCR of genomic DNA showed that up to 36% of primary tumors contained aberrations in the p53 locus. Mutations in exons 5–9 of the p53 gene were found in 10 out of 59 (17%) of the primary tumors studied by single-stranded conformation polymorphism analysis. We conclude that, compared to amplification of *HER2/NEU, MYC,* or *INT2* oncogene loci, p53 gene mutations and deletions are the most frequently observed genetic change in breast cancer related to a single gene. Correlated to disease status, p53 gene mutations could prove to be a valuable marker for diagnosis and/or prognosis of breast neoplasia.**

One in nine women in the U. S. will develop breast cancer in her lifetime. Nationally, the American Cancer Society estimates that 175,000 new cases of breast cancer will be diagnosed in 1991, and 44,500 women will die from the disease (1). Although major advances have been made in the early detection and treatment of breast cancer, little is understood regarding its etiology, biology, or the molecular events underlying its development.

The etiology of breast cancer is not well defined, although age at menarche, age at first childbirth, fat in the diet, alcohol consumption, family history, and, more recently, cigarette smoking have been identified as risk factors (2). To gain a better understanding of the genetic components involved in the initiation, promotion, and progression of breast cancer, several laboratories have undertaken molecular studies on tumor-specific alterations. To date, at least nine genetic alterations have been identified in breast malignancies, including amplification of c-*MYC, HER2/NEU,* and *INT2* and loss of heterozygosity in six chromosomal arms, 1q, 3p, 11p, 13q, 17p, and 18q (3, 4). The latter mechanism is believed to unmask recessive mutations in tumor suppressor genes (5). Studies on the overexpression or amplification of c-*MYC, INT2,* and *HER2/NEU* pointed to some utility of these protooncogenes as markers for predicting poor prognosis in a subset of breast cancer patients. The results are, however, controversial (3, 4). Deletions in the retinoblastoma tumor suppressor gene, *RB1,* were observed in a small percentage (7–10%) of breast tumors (3, 4). Allelic loss or duplication of 18q, which included the region of the chromosome where the *DCC* gene (ref. 6; deleted in colorectal carcinomas) is located, was also observed (7). The most promising lead, however, is offered by data showing that as many as 70% of breast tumors have deletions in the short arm of chromosome 17, in the vicinity of the tumor suppressor gene p53 (3, 4).

Studies in the last two years have clearly shown the p53 gene to be a target of molecular alterations in 40–75% of common human cancers (5, 8–10). Recent evidence from several laboratories suggests that such changes in the p53 gene may be important in breast tumorigenesis (11–16). In an effort to examine in depth the nature and frequency of p53 gene alterations in breast cancer, we examined 20 human breast cancer cell lines, a panel that included 9 previously studied. Point mutations in the p53 cDNA and the effect of point mutations on the expression of p53 mRNA and protein were studied. In addition, we examined the p53 locus in 59 primary breast cancer specimens by a multiplex PCR and by single-stranded conformation polymorphism (SSCP) analysis for deletions and mutations in the p53 genomic locus.

MATERIALS AND METHODS

Breast Cancer Tissue and Cell Lines. Resected specimens of breast cancer tissue and adjacent normal breast tissue were obtained frozen from hospitals in the San Diego area. The cell lines used in this study, with the exception of the cell lines MW and EP (17), were obtained from the American Type Culture Collection. Cells were grown under conditions recommended by the American Type Culture Collection and as described in ref. 17. In this study we used a soft agar-selected clone of the cell line MW called MW1C6.3. For controls, the promyelocytic leukemia cell line HL-60 was a source of DNA in the multiplex PCR assays, and the colon carcinoma cell line SW480 was a source of DNA in the SSCP assays.

Immunoprecipitations. Monoclonal antibodies PAb122 (18), an IgG2b, and PAb1801 (p53 Ab-2, Oncogene Sciences, Manhasset, NY), an IgG1 (19), were utilized. The supernatant of hybridoma cell lines MOPC21, secreting nonspecific IgG1, and MOPC11, secreting nonspecific IgG2b, were used as controls. Immunoprecipitation of p53 was accomplished by methods described in ref. 20.

Reverse Transcription Followed by PCR and DNA Sequence Analysis. Total cellular RNA of breast cancer cell lines was prepared by the guanidinium thiocyanate method (21). mRNA was reverse transcribed (8), and the following primer pairs were used to amplify the cDNA: no. 4026 (codons 34–40, 5′-CCCTTGCCGTCCCAAGCAATG-3′) and no. 4027 (codons 223–217, 5′-AGGCGGCTCATAGGGCACCAC-3′); no. 4025 (codons 157–163, 5′-GTCCGCGCCATG-

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Abbreviation: SSCP, single-stranded conformation polymorphism.
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GCCATCTAC-3′) and no. 4023 (codons 316–310, 5′-GGGAGAGGACCTGGTGTTGTT-3′). A 1-μl aliquot of a 1000-fold dilution of the PCR product was used in an asymmetric PCR for generating predominantly single-stranded products. Primers were used in the concentrations of 1 pmol (limiting primer) and 50 pmol each, in a 100-μl reaction. PCR was done on at least two independent cDNA preparations from each cell line. Sequencing by the dideoxy chain termination method was carried out using the limiting primer with the Sequenase 2.0 kit (United States Biochemical).

p53 Multiplex PCR. Four regions of the p53 gene including the entire protein coding sequence were chosen to be amplified with four sets of oligonucleotide primers in a single reaction. Additionally, primers for a housekeeping gene located on the long arm of chromosome 11 (*COX* gene; S. Maurer and G. A. Evans, personal communication) were included in the same PCR mix as an internal control. All the primers contain flanking intron sequences to restrict amplification to the p53 gene. The following p53 primers were used. Set I: Primer no. 3376 (5′-AGAATTCGATCCTCTTGCAGCAGCCAGAC-3′) and no. 3377 (5′-ACCTAGGCTCAGGGCAACTGACCGTGCAA-3′) were used to amplify exons 2–4 (codons 1–125) inclusive of intron 2 and 3 sequences. Set II: Primer no. 3378 (5′-AGAATTCCTCTTCCTGCAGTACTCCCCTG-3′) and no. 2379 (5′-CGGAATTCAGGCGGCTCATAGGGC-3′) were used to amplify exons 5 and 6 (codons 126–224), including intron 5. Set III: Primer no. 3380 (5′-AGAATTCCTCCTAGGTTGGCTCTGACTGT-3′) and no. 3381 (5′-ACCTAGGCCAAGACTTAGTACCTGAAGGG-3′) were used to amplify exons 7–9 (codons 225–331), inclusive of introns 7 and 8. Set IV: Primer no. 3451 (5′-GGGAATTCAGATCCGTGGGCGTGAG-3′) and no. 3383 (5′-ACCTAGGGCTGTCAGTGGGGAACAAGAAG-3′), beginning 30 bases downstream of the stop codon, amplified exons 10 and 11 (codons 332–393), including intron 10. The primers have different restriction enzyme sites included at their 5′ end.

The 100-μl PCR reaction mix contained 500 ng of genomic DNA, 150 nmol of each dNTP (Pharmacia), 5% (vol/vol) dimethyl sulfoxide, 50 pmol of each of the 10 primers, 5 units of *Taq* polymerase (Cetus), 10 μl of 10× buffer (170 μg of bovine serum albumin/700 mM Tris·HCl, pH 8.8/70 mM $MgCl_2$/150 mM ammonium sulfate/100 mM 2-mercaptoethanol/70 μM EDTA) overlaid with 50 μl of mineral oil. The amplification reaction was carried out in a thermocycler (Perkin–Elmer/Cetus or Ericomp, San Diego) with an initial denaturation step of 2 min at 94°C, followed by 30 cycles consisting of the following three steps: 94°C for 30 sec, 53°C for 30 sec, and 65°C for 2 min. The last cycle was followed by an extension step of 5 min at 65°C. Five to 10 μl of reaction mix were loaded either on 1.5% agarose (type II, medium electroendosmosis; Sigma) gels or on 8% polyacrylamide (Bio-Rad) gels.

Southern and Northern Blot Analysis of DNA and RNA. DNA analysis was carried out by using p53 cDNA pR4-2 (kindly provided by E. Harlow, Massachusetts General Hospital Cancer Center, Charlestown, MA) as a probe. RNA analysis was carried out as described elsewhere (21) by using pSLVHp53c-62 (22) as a probe.

SSCP. The procedure used was essentially according to those published (23, 24). Each 10-μl PCR reaction contained 10 pmol of each primer, each dNTP at 2.5 μM, 1 μCi of [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq), 100 ng of genomic DNA, and 0.02 units of thermostable *Taq* polymerase (Amplitaq, Perkin–Elmer/Cetus) under specified buffer conditions.

RESULTS

Southern Blot Analysis of the p53 Gene. Genomic DNAs of 20 human breast cell lines (listed in Fig. 1 legend) were analyzed for p53 gene rearrangements following digestion with *Eco*RI, *Bam*HI, or *Bgl* II by Southern hybridization with the p53 cDNA. Only 2 of the 20 cell lines tested showed restriction fragment size abnormalities. DNA from MDA-MB-453 digested with *Bgl* II yielded a fragment of 15.0 kilobases (kb) instead of the expected fragments of 12.0 kb and 3.0 kb, indicating the loss of a *Bgl* II site in the hemizygous p53 allele. The *Eco*RI fragment was ≈1 kb larger and of half the intensity of the wild-type p53 fragment (data not shown). Further, instead of the expected 7.5-kb *Bam*HI wild-type fragment seen in leukocyte and HBL-100 DNA, a single fragment of ≈12.0 kb was seen in MDA-MB-453. In MDA-MB-435, a 9.0-kb *Bam*HI fragment was present in addition to the expected 7.5-kb band.

Multiplex PCR Amplification of the p53 Gene. Small deletions and insertions in the p53 gene were revealed by fine structure analysis of genomic DNA based on multiplex PCR. This analysis was carried out on DNA from 20 breast cancer cell lines, 59 primary tumors, and 20 normal breast tissue samples.

For the region of exons 2–4, including introns 2 and 3 (primer set I), a smaller PCR product was generated from genomic DNA of MDA-MB-157 (Fig. 1, lane 6), whereas slightly larger PCR products, indicative of insertions in this region, were observed in EP, BT-549, and T-47D (Fig. 1, lanes 7, 13, and 15, respectively).

The region of exons 7–9, inclusive of introns 7 and 8, was not PCR-amplifiable in EP (Fig. 1, lane 7). In HS 578T and MDA-MB-453, no amplification of the fragment extending from exon 10 to the termination codon in exon 11 was seen (Fig. 1, lanes 4 and 14, respectively). By using a combination of primers complementary to sequences in the 11th exon and the 3′ untranslated region, situated at varying distance from each other, the extent of deletion in MDA-MB-453 was estimated to be about 30 base pairs (bp) in the 3′ end of the 11th exon.

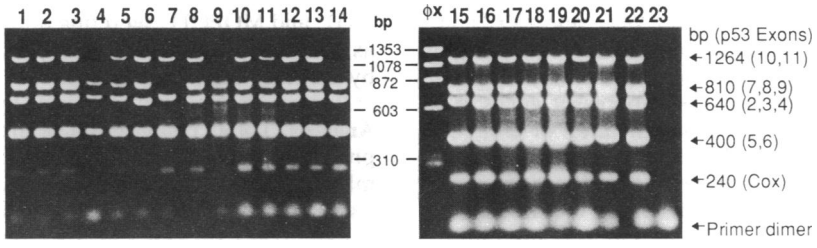

FIG. 1. p53 multiplex PCR analysis of genomic DNA from breast cancer cell lines. Lanes: 1, MDA-MB-231 DNA; 2, MCF7; 3, HBL-100; 4, HS 578T; 5, SK-BR-3; 6, MDA-MB-157; 7, EP; 8, HSAD10; 9, HS574BM; 10, MW; 11, MW1C6.3; 12, MDA-MB-435; 13, BT-549; 14, MDA-MB-453; 15, T-47D; 16, BT-474; 17, MDA-MB-361; 18, ZR-75-30; 19, MDA-MB-468; 20, ZR-75-1; 21, MDA-MB-436; 22, peripheral blood cells (control); 23, no DNA. The size (in bp) of the PCR product of the region of the p53 gene encompassed by each primer set and that of the *COX* gene is shown on the right. The migration of the molecular weight marker (φX174 digested with *Hae* III) is given in the center.

DNA from 38 out of 59 primary tumors of the breast showed a pattern of amplification similar to that seen in the 20 normal breast tissue samples (data not shown). The changes seen in the amplification products of the p53 gene in the remaining 21 (36%) tumor samples could be categorized as follows: partial (heterozygous) or total (homozygous) loss of bands in 6 out of 21, doublets of PCR products, and/or novel bands of aberrant size (13 out of 21), or larger sized bands (2 out of 21). The results of a representative experiment showing examples of loss of heterozygosity (lane 5) and also doublets and additional bands (lanes 1 and 3) is presented in Fig. 2. Multiplex PCR of DNA from HL-60 cells, known to have major deletions in the p53 locus (25), results in amplification of only 1 of 4 DNA fragments (Fig. 2, lane 7).

Expression of p53 Studied by Northern Blot and Immunoprecipitation. p53 mRNA of 17 breast cancer cell lines was analyzed on Northern blots. A 2.9-kb transcript was seen in the majority of the cell lines after 1 day of film exposure. In EP and MW1C6.3, p53 mRNA was detectable only after 2 weeks of exposure. In T-47D, MDA-MB-436, and MDA-MB-468, a 1.7-kb message was seen in addition to the 2.9-kb p53 transcript. MDA-MB-453, on the other hand, showed a single p53 mRNA species of 1.7 kb, possibly due to the preferential utilization of the promoter for the smaller species of mRNA (data not shown).

The level of p53 protein in the breast cancer cell lines was determined by immunoprecipitation with PAb122 and PAb1801. Representative results for 10 of the 15 cell lines tested are presented in Fig. 3. The 53-kDa protein was observed in 10 of 15 cell lines. In immunoprecipitations with PAb122 and PAb1801, no p53 protein was detectable in cell lines EP, MDA-MB-157, MDA-MB-453 (Fig. 3, lane 4), and MW1C6.3 (Fig. 3, lane 10). Low but detectable levels of p53 were seen in BT-549, HS 578T, MDA-MB-435, SK-BR-3 (Fig. 3, lane 11), and ZR-75-1 (Fig. 3, lane 8), whereas high levels of the protein were observed in MCF7, MDA-MB-361, MDA-MB-468, T-47D, MDA-MB-231 (Fig. 3, lanes 1, 2, 5, 6, and 7, respectively), and in the cell line HBL-100 (Fig. 3, lane 9), derived from normal breast epithelial cells with known integration of simian virus 40. The level of p53 in HBL-100 is high, perhaps due to the presence of the simian virus 40 large tumor antigen, which stabilizes p53.

Nucleotide Sequence Analysis of p53. Point mutations in the p53 cDNA were found in 8 of 15 breast cancer cell lines analyzed. In 7 out of these 8 cell lines missense mutations leading to a change of the amino acid sequence were present

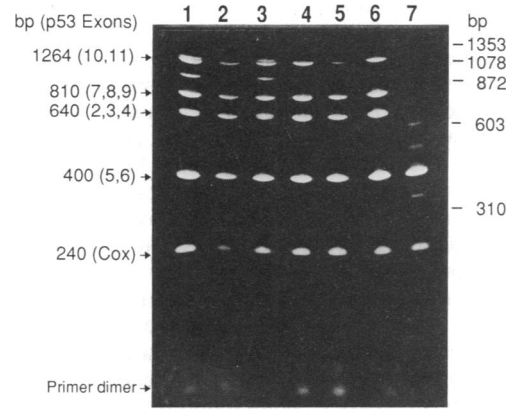

FIG. 2. p53 multiplex PCR analysis of genomic DNA from primary breast tumors. Lanes 1–5, DNA from primary breast carcinomas; lane 6, normal breast tissue; lane 7, HL-60 promyelocytic leukemia cell line. The size (in bp) of the PCR product in the region of the p53 gene encompassed by each primer set as well as that of the *COX* gene is shown on the left. The migration of the molecular weight marker (ϕX174 digested with *Hae* III) is given on the right.

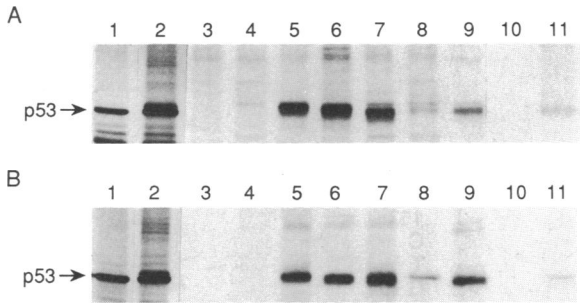

FIG. 3. Immunoprecipitation of equal amounts of trichloroacetic acid-precipitable labeled p53 protein in breast cancer cell lines. (*A*) Immunoprecipitation with monoclonal antibody PAb122. (*B*) Immunoprecipitation with monoclonal antibody PAb1801. Lanes: 1, MCF7; 2, MDA-MB-361; 3, HBL-100 with nonspecific IgG (control); 4, MDA-MB-453; 5, MDA-MB-468; 6, T-47D; 7, MDA-MB-231; 8, ZR-75-1; 9, HBL-100; 10, MW1C6.3; 11, SK-BR-3.

(Table 1). In the eighth cell line, MW1C6.3 (Fig. 4), the single point mutation (TAC → TAA) resulted in a stop codon at amino acid position 234. No background band of wild-type sequence was seen in repeated runs in any of the eight cell lines, indicating the absence of the wild-type allele.

SSCP Analysis of p53. Since nucleotide sequencing analysis of reverse-transcribed mRNA is laborious and time consuming, we screened exons 5–9 of the p53 gene in primary breast tumor DNA for mutations by SSCP (23, 24). We first confirmed that each of the mutations detected in the p53 gene of breast cancer cell lines by nucleotide sequencing leads to altered migration patterns. Next, DNA from the primary breast tumors were subjected to SSCP analysis. Results of a representative experiment are shown in Fig. 5. Ten of 59

Table 1. Summary of p53 nucleotide sequence, SSCP, and multiplex PCR analysis of the p53 gene in breast cancer

Cell line/primary tumor no.	Sequence analysis: Point mutation or SSCP	Sequence analysis: Change in codon/exon	Multiplex PCR: Exons	Multiplex PCR: Alteration
BT-474	GAG → AAG	285/exon 8*	2–11	None
BT-549	AGG → AGC	249/exon 7*	2–4	>
EP			7–9	∅
EP			2–4	>
HS 578T	GTC → TTC	157/exon 5	10, 11	∅
MDA-MB-157			2–4	<
MDA-MB-231	AGA → AAA	280/exon 8*	2–11	None
MDA-MB-436			5, 6	>
MDA-MB-453	Δ 30 bp	Exon 11	10, 11	∅
MDA-MB-468	CGT → CAT	273/exon 8†	2–11	None
MW1C 6.3	TAC → TAA	234/exon 7	2–11	None
SK-BR-3	CGC → CAC	175/exon 5	2–11	None
T-47D	CTT → TTT	194/exon 6†	2–4	>
4805	SSCP	Exon 6	2–11	None
4811	SSCP	Exon 8	2–11	None
4813	SSCP	Exon 6	2–4	Doublet
5293	SSCP	Exon 5	2–4	Doublet
5296	SSCP	Exon 5	2–11	None
5588	SSCP	Exon 6	2–11	None
5594	SSCP	Exon 9	2–11	None
5600	SSCP	Exon 6	2–11	None
5604	SSCP	Exon 6	2–4	Doublet
5633	SSCP	Exon 6	2–4	Doublet
			10, 11	Doublet

∅, Band not detectable; <, smaller than the expected size; >, larger than the expected size.
*Ref. 9.
†Ref. 10.

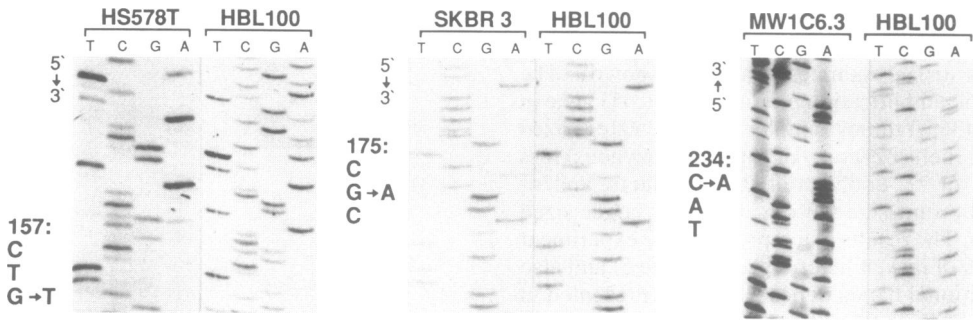

FIG. 4. Nucleotide sequence analysis of p53 cDNA of the cell lines HS 578T, SK-BR-3 and MW1C6.3. The wild-type sequence in HBL-100 in the same region is shown alongside.

(17%) primary tumors showed changes in SSCP profiles compared to fragments of wild-type p53 genes (Table 1) derived from breast tissue of six normal individuals.

Thus, 9 out of 18 (50%) breast cancer cell lines show abnormalities in the p53 locus. In primary breast tumors examined by multiplex PCR analysis, 21 out of 59 (36%) breast tumors showed alterations at the genomic locus of p53, whereas 10 out of 59 (17%) contained mutations as revealed by SSCP analysis. Four of the 10 tumors showed abnormal multiplex profiles in the area encompassing exons 2–11. Taken together, 24 out of 59 primary tumors (44%) showed mutationally altered p53 genes.

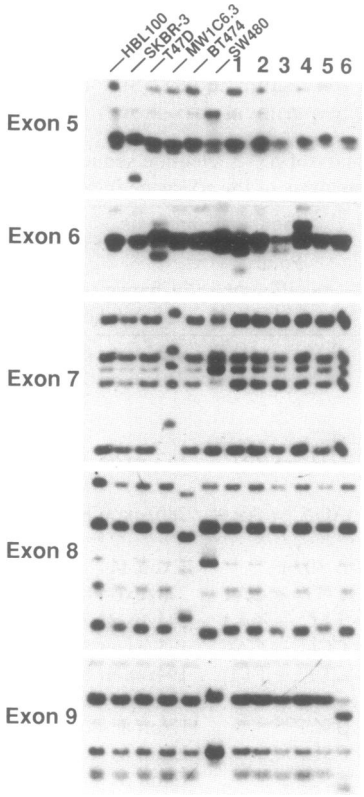

FIG. 5. SSCP analysis of exons 5–9 of the p53 gene in primary breast tumors. Three microliters of [α-^{32}P]dCTP-labeled, denatured, PCR-amplified DNA, mixed with an equal volume of sequencing stop solution, was loaded in the following order: HBL-100 representative of wild-type p53 in exons 5–9; breast cancer cell lines SK-BR-3, T-47D, MW1C6.3, and BT-574, with point mutations in exon 5, 6, 7, and 8, respectively; SW480 colon carcinoma cell line with known point mutation in exon 9 (8); primary breast tumor nos. 4813 (lane 1), 4805 (lane 3), 5633 (lane 4), 4811 (lane 5), and 5594 (lane 6); breast tissue from normal individual no. 1202 (lane 2). The polyacrylamide gel was dried and exposed to Kodak XAR for 5 hr at room temperature.

DISCUSSION

The results of this study indicate that alterations in the p53 locus are a common occurrence in human breast cancer. In agreement with several reports that failed to see any rearrangements in the p53 gene in carcinomas of different tissues including primary tumors of the breast (4, 26, 27), restriction fragment length changes were observed in only 2 of 20 breast cancer cell lines, which are equally likely to represent somatic mutations or uncommon variants. Gross alterations, therefore, do not appear to be a common mechanism for inactivation of the p53 gene. This analysis, however, does not rule out the possibility that small deletions or rearrangements in the p53 gene contribute to inactivation of the wild-type p53 function.

In genes, such as p53, that do not yield several DNA fragments with common restriction enzymes, it is often difficult to detect small deletions or insertions, unless they disrupt sequences at the recognition site (26, 27). In addition, for primary tissues, the availability of tissue material sufficient to carry out extensive Southern blot analysis often poses a problem. To circumvent these problems, we developed the p53 multiplex PCR, an approach similar to the one used for the detection of deletions in the large Duchenne muscular dystrophy gene (28). Smaller, larger, or no PCR products for the various regions of the p53 gene were seen in 7 of 20 breast cancer cell lines in multiplex PCR. DNA from the leukemia cell line HL-60, in which most of the p53 gene is deleted (25), yields only one of four products in multiplex PCR (Fig. 2, lane 7), suggesting that this assay can serve as a quick screening tool for such deletion mutants. Among the 59 primary breast tumors examined, 6 showed loss of fragments in the p53 multiplex PCR assay. Slower or faster migrating p53 gene fragments, as well as additional fragments absent in the normal breast tissue, were also detected in 15 other breast tumors. It is thus possible to visualize, in a single reaction, the coding sequences of the entire p53 gene, to determine which regions of the gene are rearranged or missing and the extent of allelic loss. Since the assay is performed on genomic DNA and includes large stretches of intron sequences, such changes could possibly contribute to altered regulation of p53 gene expression or may be inconsequential and irrelevant to p53 function.

A common mechanism of p53 gene alteration in human tumors is by point mutations. Nucleotide sequence analysis of conserved regions of the p53 gene revealed the presence of missense mutations in 8 of 15 breast cancer cell lines. While this study was in progress, point mutations in the p53 gene of 5 of the 15 breast cancer cell lines were reported (summarized in Table 1) (8, 11). In addition to the five point mutations at codons 194, 214, 249, 280, and 285, we found mutations in codons 175 (SK-BR-3), 157 (HS 578T), and 234 (MW1C6.3). A deletion mutation ($\approx$30 bp) in the 11th exon of the p53 gene in MDA-MB-453 was deduced by PCR analysis. The mutations in the p53 gene in breast cancer cell lines were therefore

situated close to, or at, codons previously reported to be mutated in other types of cancer (10). Methylated cytosines in the dinucleotide CpG in the p53 gene have been implicated as endogenous mutagens, since deamination of these residues can cause transitions to thymine (29). Unlike colon carcinomas, where more than half of the transition mutations were present at CpG dinucleotides, only two of eight breast cancer cell lines had G to A transitions at these residues. In agreement with other studies, point mutational alterations in p53 are common, whereas deletions occur less frequently. The breast cancer cell lines that show point mutations in the p53 gene usually possess high levels of p53 protein. Point mutations are believed to lead to changes in the conformation of the protein, resulting in prolongation of its very short half-life (9). While the potential of these mutations for creating oncogenic p53 proteins is under investigation, the transforming capabilities of at least two of these mutants (175H and 273H) have already been demonstrated (30). It is notable that in each of the breast cancer cell lines that displayed mutations or deletions in the p53 gene the normal allele had also been lost.

Although nucleotide sequencing is the most reliable method of detecting mutations in p53, SSCP analysis offers a quicker alternative for screening a large number of tissue samples. By using SSCP analysis of PCR-amplified DNA, band shifts were detected in 10 out of 59 (17%) primary tumors distributed through exons 5–9 of the p53 gene. Since such band shifts were seen in all (8 out of 8) breast cancer cell lines that were shown to contain mutations by nucleotide sequencing, we believe that band shifts represent mutations in the p53 gene in primary breast tumor DNA. However, the possibility that some represent nonsense mutations cannot be ruled out. There is no obvious correlation between abnormal multiplex PCR patterns and the presence of point mutations in p53 in the primary breast tumors tested by SSCP analysis (Table 1). Since the multiplex assay scans large stretches of DNA and the SSCP analysis reveals only mutations in exons 5–9 that result in band shifts, it is quite likely that our numbers reflect a lower than actual incidence of p53 abnormalities in primary tumors.

In summary, p53 alterations can occur in human breast cancer cells at the level of the gene structure, mRNA, or protein, by mechanisms involving point mutation. Additionally, as shown in this study, the p53 gene is susceptible to small rearrangements, deletions, or insertions. It is, however, clear that compared to the breast cancer cell lines the incidence of point mutations in the coding regions of the p53 gene in primary tumors is lower. It is likely that p53 mutations occur late in breast cancer and are confined to a subset of breast neoplasms. Further screening of the p53 gene in breast tumors at different stages of the disease and with respect to histopathology will allow us to determine the correlation between the occurrence of p53 gene mutations and disease status.

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