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Constant denaturant gel electrophoresis as a rapid screening technique for p53 mutations

(tumor-suppressor gene/DNA screening/loss of heterozygosity/chromosome 17/breast cancer)

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ABSTRACT **At present, mutation of the p53 gene appears to be the most common genetic alteration found in human cancers. These mutations can occur within many different regions of the gene. We have developed a modification of denaturing gradient gel electrophoresis termed "constant denaturant gel electrophoresis" (CDGE), which provides a rapid and sensitive method to screen the four conserved regions within the p53 gene where the majority of p53 mutations have been reported. The sensitivity of CDGE was first tested with known p53 mutations in all four conserved regions. The CDGE technique was then used to screen 32 breast carcinomas that had been analyzed by immunohistochemical methods for altered p53 protein levels and whose DNA had already been shown to have loss of heterozygosity for a chromosome 17p marker. By immunostaining techniques, only 6 of the 32 tumors had elevated p53 expression. However, CDGE detected p53 mutations in 11 of the 32 tumors. DNA sequence analysis was performed to determine the nucleotide positions of these mutations in all 11 samples. Loss of heterozygosity for the pYNZ22 or p144D6 markers did not associate with either the loss of heterozygosity at the p53 locus or the mutations detected by CDGE. We conclude that CDGE is a rapid and effective technique to screen for p53 mutations.**

A wide variety of human tumors have been shown to be associated with changes in the p53 gene (1–8). As the list of cancers with p53 mutations increases, there is a need for an accurate and rapid screening technique for these mutations. Unlike some of the dominant oncogenes, p53 can be inactivated by a diverse set of mutations scattered through several important regions of the gene (7). Many recent surveys of p53 mutations have used loss of heterozygosity (LOH) at polymorphic markers closely linked to p53 as an indirect assay for p53 inactivation (9–11). While such a screen is rapid, it cannot detect point mutations, and its accuracy is limited, since some of the nearby polymorphic markers might undergo deletions that do not extend into the p53 locus. Alternatively, because mutations in the p53 gene frequently result in a mutant p53 protein that is significantly overexpressed compared with the low levels of the wild-type protein, other p53 surveys have used immunostaining techniques to screen for mutations (12–14). Unfortunately, the p53 mutations that result in either a lack of protein or the same low levels of p53 as are present in cells containing the wild-type p53 cannot be detected by this method. The most sensitive screening technique is to sequence the genomic region encoding p53. Although sequencing is quite sensitive, it is also labor-intensive. There are several new nucleic acid-based screening methods that can rapidly detect mutations within short fragments of DNA. These techniques include RNase protection assays (15), single-strand conformational polymorphisms (SSCP) (16, 17), denaturing gradient gel electrophoresis (DGGE) (18, 19), and detection of base-pair mismatches with hydroxylamine and osmium tetroxide (20).

To develop a screening method for p53 mutations, we have tested a modification of the DGGE system (18), termed constant denaturant gel electrophoresis (CDGE) (21). The DGGE separation technique relies on strand dissociation of DNA fragments in discrete sequence-dependent melting domains. This dissociation causes an abrupt decrease in mobility in a polyacrylamide gel containing a gradient of denaturant. The modification of DGGE employed in this study is to run constant denaturant gels that avoid the use of a gradient by selection of a specific denaturant concentration at which maximal separation between the wild-type and mutant fragments can be achieved. The CDGE technique has the advantage of enabling the fragments to migrate with a consistently different rate through the whole gel. This allows separation of several centimeters between mutant and wild-type fragments (21). In addition to the CDGE analyses, each potentially mutant p53 fragment was sequenced to confirm the position and nature of the mutations.

MATERIALS AND METHODS

Tumor Samples. Fresh tumor samples were obtained from 69 breast carcinomas from patients admitted to The Norwegian Radium Hospital. One part of the tumor was immediately frozen and stored in liquid nitrogen for DNA studies and immunohistochemistry. Formalin-fixed material from each case was processed for light microscopy. Blood was drawn from each patient into EDTA solution and stored at −40°C. These tumor samples have been analyzed for LOH and for gene amplification of several genetic markers (22). Thirty-two of the samples that had LOH for at least one of a panel of 17p markers were used for CDGE analysis.

DNA Analysis and Tissue Immunostaining. DNA was extracted from cell nuclei of tumor tissue and whole blood by standard procedures (phenol/chloroform extraction and ethanol precipitation). Allelic loss of chromosome 17p sequences in tumors was analyzed by Southern blot hybridization using a series of polymorphic probes (22). Frozen sections from the breast carcinomas were immunostained with a polyclonal antibody against p53 that recognizes both wild-type and mutant p53 (OM-11-918; Cambridge Research Biochemicals).

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Abbreviations: CDGE, constant denaturant gel electrophoresis; DGGE, denaturing gradient gel electrophoresis; LOH, loss of heterozygosity.

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Polymerase Chain Reaction (PCR). DNA from 32 tumor tissue samples, all with LOH of either of the previously tested 17p markers, and from 6 samples with known mutations was subjected to mutation analysis using PCR followed by CDGE. Three of these samples (LF1–LF3) were DNA extracted from patients with Li–Fraumeni syndrome (26). The other three samples were from sporadic tumors (33). The DNA fragments that were amplified are shown in Fig. 1 together with the position and type of the previously identified mutants. PCR was performed using 100–300 ng of template DNA in 50 mM Tris·HCl, (pH 8.6) with 10 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 25 or 100 pmol of each primer (25 of the purified and 100 of the unpurified), and 2.5 units of *Taq* polymerase (AmpliTaq, Cetus). The 100-μl mixture was incubated in a Perkin–Elmer/Cetus thermocycler for 35 cycles of 94°C (45 sec), 55°C (45 sec), and 72°C (45 sec). The reaction was initiated with one 7-min incubation at 94°C and ended with a 10-min incubation at 72°C. The primers were synthesized by Genosys (Houston), and only the 60-mer primers were ordered purified. The primers were designed using the OLIGO primer analysis program from National Biosciences. A "GC clamp" was attached to one of the primers in each set, creating a 60-mer primer (19). The following primer pairs were constructed: fragment A sense primer (5′-TTCCTCTTCCTGCAGTACTC-3′) and antisense primer (5′-CGGCACCCGCGTCCGCGCCACGGGCGGGGGCGGCGGGACGGGCGCGGGGCGCGGCGGGCG-3′); fragment B sense primer (5′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGTTCCACACCCCCGCCCGGCA-3′) and antisense primer (5′-GATGGTGAGCAGCTGGGGC-3′); fragment C sense primer (5′-CACCATCCACTACAACTACA-3′) and antisense primer (5′-CATCATCACACTGGAAGACTGCCCGCCCCCGCCGCCCTGCCCGCGCCCCGCGCCGCCCGC-3′); fragment D sense primer (5′-ATCCTGAGTAGTGGTAATCT-3′) and antisense primer (5′-AGGGAGCACTAAGCGAGGTAGCCCGCCCCCGCCGCCCTGCCCGCGCCCCGCGCCGCCCGC-3′). The primer sets amplify across the four conserved regions where >80% of p53 mutations have been identified. All the PCR products were analyzed for purity by 7.5% PAGE followed by staining with ethidium bromide.

Denaturing Gel Electrophoresis. The theoretical melting profiles of the amplified fragments were produced by the computer program of Lerman *et al.* (23), based on the statistical mechanical principles and algorithms developed by Poland (24) and the nearest-neighbor base-pair doublet parameters introduced by Gotoh and Tagashira (25).

Perpendicular denaturing gradient gels (10 × 8 × 0.1 cm) contained 12.5% acrylamide in TAE buffer (40 mM Tris acetate/1 mM EDTA, pH 8.0) with *N,N′*-diallyltartardiamide (0.93 g/100 ml), as crosslinker and varying denaturant concentrations consisting of urea and formamide (100% denaturant corresponds to 7 M urea and 40% formamide). The gels were polymerized with ammonium persulfate (5 mg per gel) and *N,N,N′,N′*-tetramethylethylenediamine (10 μl per gel). The gradient gels were cast with a gravitational gradient mixer. All reagents used were of electrophoretic grade. The PCR product was loaded into a long well along the top of the gel and run with the electrophoresis direction perpendicular to the denaturant gradient. Gels were run submerged in TAE buffer at 56.0°C at 80 V constant, in a self-constructed cell adapted to the Mini-Protean electrophoresis-cell system (Bio-Rad). The modification allowed the glass plates surrounding the gels to be in direct contact with the buffer on both sides. Extensive circulation of the buffer was provided during the runs. The running time was 1–2 hr. After electrophoresis, the gels were stained for a few minutes with ethidium bromide (2 mg/liter of TAE) and photographed using a UV transilluminator.

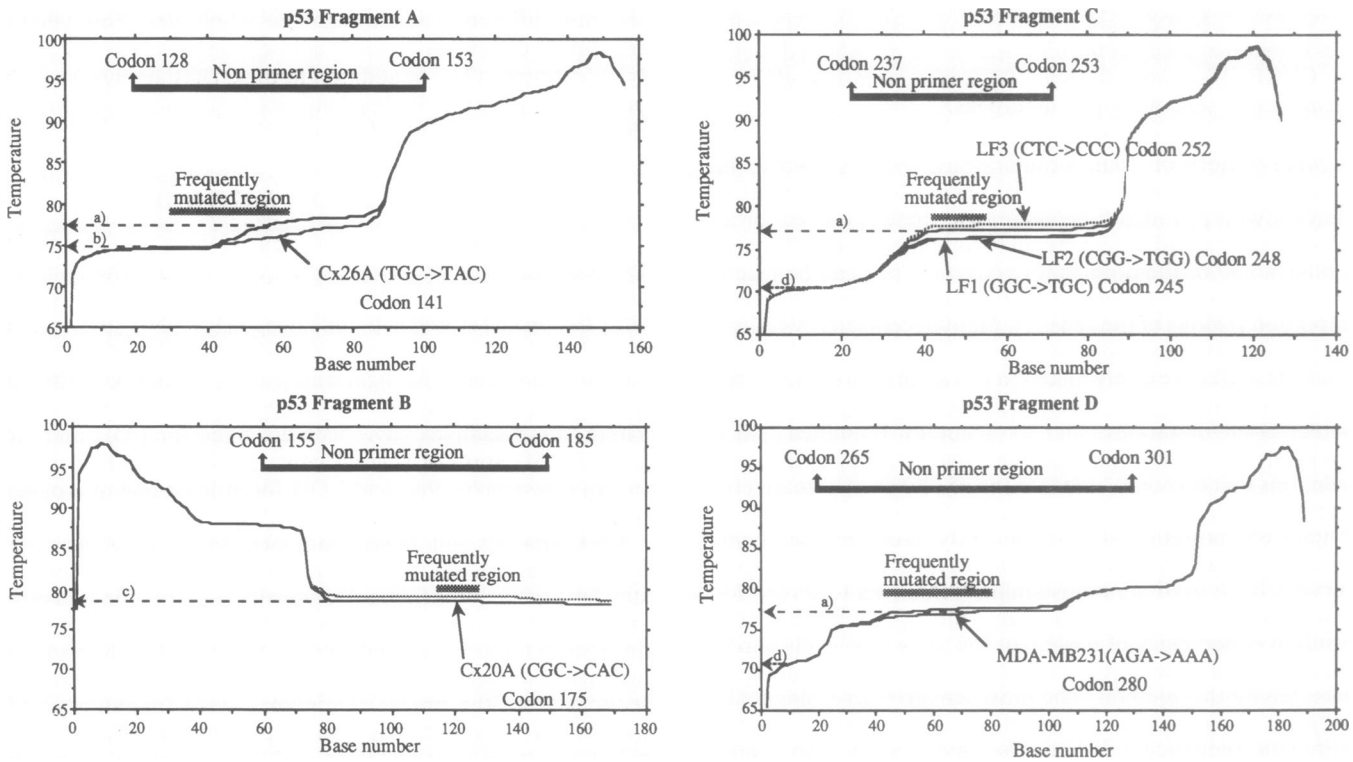

FIG. 1. Theoretical melting profiles of the four PCR-amplified fragments of the p53 gene at temperatures (°C) giving a 50% chance of the base pair being in either double- or single-stranded state. In fragment A, exon 5 begins at codon 126, which corresponds to base 16. In fragment B, exon 5's codon 149 begins at base 42. In fragment C, exon 7's codon 231 begins at base 2. In fragment D, exon 8's codon 262 begins at base 13. The broken lines show the profiles for the different control mutants with localization of the base mutations indicated by arrows. The theoretical melting temperatures a–d correspond to the following experimental denaturant concentrations at 56°C in our CDGE system: a (77.5°C), 50.7%; b (75°C), 42.0%; c (78°C), 51.7%; d (71°C), 32.0%.

The gels used in CDGE contained the same chemicals as the perpendicular denaturing gradient gels, but with a uniform denaturant concentration throughout the gel. Running conditions were the same.

Sequencing. A 1.7-kilobase fragment was generated from 0.5–2.0 μg of the genomic DNA template. This fragment was subcloned and sequenced with four pairs of internal primers as described (26).

RESULTS

LOH Studies of Chromosome 17 Markers and Immunostaining of p53. The LOH for the various chromosome 17 markers used in this study have been reported (22). LOH of either of the 17p markers tested was observed in 32 tumors. The most frequently lost marker was p144D6 (25/29, 86.2%) followed by pYNZ22 (21/26, 80.8%) and p53 (9/16, 56.3%).

Immunostaining of frozen sections showed that 6 of these 32 tumors (18.8%) had elevated p53 protein levels compared with normal tissues. All of the 6 tumors had LOH of pYNZ22 or p144D6 (Table 1), suggesting that these deletions were associated with elevated levels of p53. Surprisingly, for the 4/6 of these samples with elevated levels of p53 that were informative at the p53 locus itself, only 2 samples had LOH at the p53 locus. The 2 tumors with overexpression and with no LOH of p53 had LOH of pYNZ22, and one had LOH of p144D6 but the other did not. There is clearly a lack of correlation between these two tests. To determine which values are the false positive and negative ones, it appears to be important to actually screen for p53 mutations.

Development of a Screening Technique for p53 Mutation Analysis. Prior to CDGE analysis, theoretical melting maps were calculated for the four regions in p53 where >80% of the reported mutations cluster. Primers were designed for each region to give fragments with melting profiles with high- and low-melting domains, where most of the region to be screened resided in the low-melting area. A total of 321 base pairs encoding 107 amino acids resided in lower-melting domains between the primers used for amplification (Fig. 1). Previous studies (19, 21, 23) have shown that using a GC clamp increases the number of detectable mutations from 40% of all single base changes to close to 100%, if they reside in the lower-melting domains of the fragment. Most of the mutations in these codons (Fig. 1) should therefore be detected by our CDGE system.

The theoretical melting profiles of six previously identified mutants are shown in Fig. 1. These profiles predict a different melting pattern compared with the wild type; hence, it should be possible to observe a different migration of these mutants compared with the wild type by using the CDGE system. The theoretical calculations, however, are not always in agreement with what is actually seen (21), probably because possible conformational changes in the melted region are not taken into account. To test the theoretical prediction, perpendicular DGGE was performed on the control mutants.

In Fig. 2, the actual melting behaviors of four fragments containing previously identified mutations are compared with the melting behavior of wild-type DNA in perpendicular denaturing gradient gels. The observed strand separation indicates that mutant DNAs can be distinguished from the wild-type DNA. When both normal and mutant fragments were present in the same sample, heteroduplex formation was often observed (Fig. 2 *B* and *C*). An artifact that was seen at times is shown in Fig. 2*C*: a shadow curve that migrated faster than, but was parallel to, the normal band. The presence of this shadow band correlated with freezing and thawing of the long primer and was probably due to breakage within the G·C region.

We then tested the six mutants by CDGE. The denaturant concentration used for each region was determined from the theoretical melting curve and from the perpendicular gels (Figs. 1 and 2). For region A, the optimal concentrations were 42.5% and 50.7%. For region B only one denaturing concentration was used (51.7%); for regions C and D two denaturant concentrations were used (32% and 50.7%). With these conditions, CDGE was successful in separating all of the six previously identified mutants from the wild type (Fig. 3). The multiple bands in Fig. 3*C* represent heteroduplexes between the mutant and normal p53 amplified fragments.

CDGE Analyses of Tumors. Tumor DNA from 32 carcinomas, each with LOH of a 17p marker and previously analyzed by immunostaining for p53, was then analyzed for mutations in all four regions. Representative gels showing tumors with p53 mutations compared to tumors without such mutations are shown in Fig. 4. Each of 11 tumor samples yielded a PCR product with a mobility different from normal DNA, indicating a mutation residing in one of these fragments. The approximate position and the nature of the mutations could be predicted from these gels. For example, tumor T29 (Fig. 4*A*) migrated more slowly and hence would be predicted to have undergone a G·C → A·T mutation, because such a

Table 1. p53 mutations in human breast carcinomas

Tumor	LOH* D17S34	LOH* YNZ22	LOH* p53	p53 expression†	PCR fragment with mutation	Mutation CDGE	Mutation Sequencing	Codon	Amino acid change
Tumors with mutations detected by CDGE									
T29	Yes	ND	Yes	−	A	G·C → A·T	CCT → TCT	128	Pro → Ser
T59	Yes	Yes	U	−	A	A·T → G·C	TTT → CTT	134	Phe → Leu
T34	Yes	Yes	Yes	−	A	A·T → G·C	CCT → CCG	128	Pro → Pro
T22	U	No	Yes	−	B	A·T → G·C	GTTG → GTG	172	codon 173 → stop
T83	Yes	Yes	U	++	B	G·C → A·T	CGC → CAC	175	Arg → His
T50	Yes	No	U	−	C	G·C → A·T	ATG → AAG	237	Met → Lys
T104	Yes	Yes	Yes	−	C	G·C → A·T	GGC → GTC	245	Gly → Phe
T106	No	Yes	U	−	C	G·C → A·T	TGT → TTT	238	Cys → Phe
T112	Yes	No	Yes	++	C	G·C → A·T	CGG → TGG	248	Arg → Trp
T16	Yes	Yes	Yes	+++	D	G·C → A·T	CGT → CAT	273	Arg → His
T119	Yes	Yes	U	++	D	A·T → G·C	GAC → GGC	281	Asp → Gly
Tumors with no detected mutation but with increased expression of p53									
T65	Yes	Yes	No	+++	—	—	—	—	—
T115	No	Yes	No	++	—	—	—	—	—

Tumors T34 and T115 are metastases; the others are primary tumors.

*U, uninformative; ND, not determined, due to lack of material. For the p53 gene, LOH was detected by the probe pBHP53 on Southern analysis of *Bam*HI digests or by PCR and digestion with *Acc* II followed by PAGE.

†From immunostaining of frozen sections.

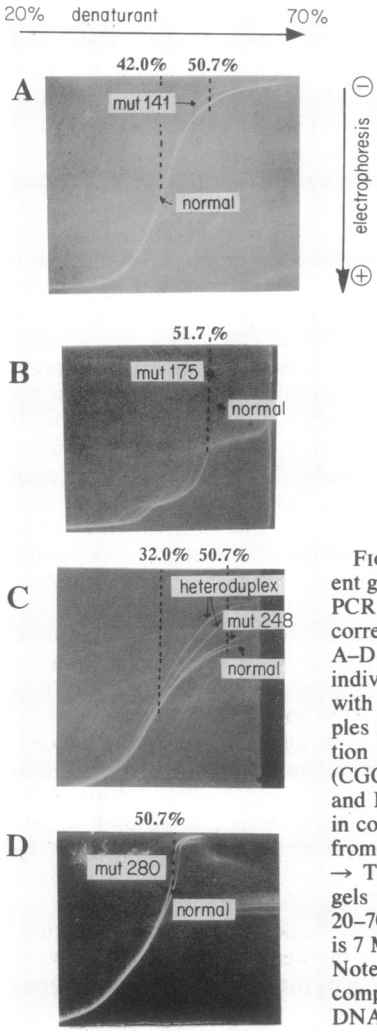

FIG. 2. Perpendicular gradient gel electrophoresis of the four PCR fragments analyzed. *A–D* correspond to amplified fragments A–D in Fig. 1. DNA from a normal individual was loaded together with DNA from mutant p53 samples Cx26A (TGC → TAC mutation in codon 141) (*A*), Cx20A (CGC → CAC in codon 175) (*B*), and MDA-MB231 (AGA → AAA in codon 280) (*D*), and with DNA from the blood sample LF2 (CCG → TGG in codon 248) (*C*). The gels were run in a gradient from 20–70% denaturant (where 100% is 7 M urea plus 40% formamide). Note the separation of mutants compared with the normal p53 DNA fragment.

mutation would result in the destabilizing loss of a hydrogen bond. All the tumors with mutations identified by CDGE were subjected to sequence analysis to confirm the mutations and to determine their exact nature (Table 1). In one tumor (T34) a silent mutation (Pro → Pro) was observed, indicating that tumors with aberrantly migrating bands on CDGE may not always have mutations that result in an amino acid change. In the two tumors with elevated p53 expression but without LOH of p53, no p53 mutation was detected by CDGE, suggesting that the overexpression of the wild-type p53 might be modulated by other factors. Sequencing could not be performed because there was not sufficient DNA. Mutations not detectable by our CDGE technique or mutations outside the screened regions could not be excluded.

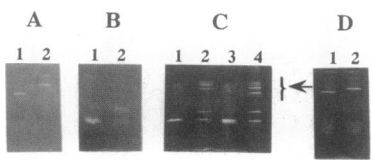

FIG. 3. Constant denaturant gels (stained with ethidium bromide) of normal DNA and DNA from six previously identified mutants. Differences in migration reflect differences in the conformation of the DNA fragments. *A–D* correspond to amplified fragments A–D in Fig. 1. The gels were run at 50.7% denaturant for *A*, *C*, and *D*, and at 51.7% for *B*. Lanes 1, normal DNA (*A–D*); lanes 2, DNA from Cx26A (*A*), DNA from Cx20A mixed with normal DNA (*B*), DNA from blood of LF2 (*C*), and DNA from MDA-MB231 (*D*); lane 3, DNA from blood of LF3; lane 4, DNA from blood of LF1. Heteroduplexes are indicated by an arrow.

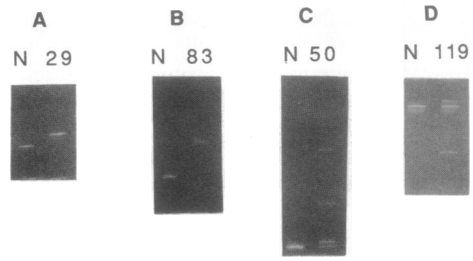

FIG. 4. Constant denaturant gels of normal DNA (N) and DNA from breast carcinomas with p53 mutations. (*A*) PCR-amplified fragments A of normal DNA and tumor T29 run at 50.7% denaturant. (*B*) PCR-amplified fragments B of normal DNA and tumor T83 run at 51.7% denaturant. (*C*) PCR-amplified fragments C of normal DNA and tumor T50 run at 32% denaturant. (*D*) PCR-amplified fragments D of normal DNA and tumor 119 (T119) run at 50.7% denaturant. See Table 1 for the nature of the mutations found in the tumors.

Four of the 11 tumors (36.4%) with p53 mutations detected by CDGE showed elevated levels of p53 protein compared with normal tissues, yet 2 of 21 tumors (9.5%) without detectable mutations had elevated levels of p53. All of the tumors with mutations had LOH of p53 when informative. Three of 10 tumors with no mutations and 1 with a silent mutation had LOH of p53. These four tumors seemed to have lost a whole chromosome 17 since all of the markers on both 17p and 17q showed LOH (22).

DISCUSSION

CDGE appears to be a rapid, efficient, and reliable method to screen for unknown mutations in the p53 gene in human breast carcinomas. Our results indicate that 34% of breast tumors with LOH of a 17p marker had p53 mutations detectable by CDGE using four fragments covering the regions where the majority of p53 mutations are found.

Several investigators have reported significant LOH of chromosome 17 sequences in human breast carcinomas (11, 22, 27–29). There appear to be two regions of overlap on the short arm that are frequently lost. One region, in band 17p13.3, contains probes pYNZ22 and p144D6, and the other region, containing the p53 gene, is located 20 centimorgans centromeric to the first region. Using the combined set of data for LOH of loci at 17p, we found a highly significant association between p53 mutations and LOH at the p53 locus ($P = 0.011$), whereas no significant association was found between p53 mutation and LOH of the other two 17p markers. This confirms and expands on previous data suggesting that the LOH of 17p markers involves more than deletions of the p53 gene (27, 30). The data also indicate that loss of the wild-type p53 allele might be necessary for tumorigenesis in certain organs.

Two of the six samples with increased p53 expression levels have retained heterozygosity for the p53 locus. No p53 mutations were found in these samples by CDGE screening with the four primer sets (Fig. 1). However, both tumors have LOH for pYNZ22. It is therefore tempting to speculate that one reason for the increased p53 expression is the loss of a controlling gene at pYNZ22, with a mechanism of tumorigenesis that does not involve mutation of the p53 gene.

Mutations found in the p53 gene in human carcinomas so far seem to cluster primarily in four "hot spots," all residing in the highly conserved region of the gene (encoding amino acids 118–280) (7, 31). To date, several methods have been available for rapid screening of point mutations in regions of interest in the human genome. Each of these previous screening methods has features that make it potentially less than optimal. The hydroxylamine/osmium tetroxide technique will not detect A → T or T → A changes (20). RNase

mismatch analysis requires the use of RNA (15). Direct sequencing is labor-intensive. These restrictions still leave several alternative DNA-based screening techniques. One might analyze single-strand conformational polymorphisms (SSCP), as this approach has been useful in identifying random mutations in small DNA fragments (16, 17). Instead, we developed a variant of the DGGE technique because theoretically it should be able to detect all mutants within low-melting domains. This modification greatly enhances the ability to screen for unknown mutations in a large series in any gene for which sequence information is available. One limitation of the CDGE approach is the number of melting domains that can easily be resolved in one fragment. We therefore concentrated on the evolutionarily conserved domains in the p53 gene where mutations have been frequently seen. Using four different amplification products, we were able to screen the portions of the p53 gene within which >80% of the mutations have been detected (31). There are several other potentially important domains outside of the regions covered by the four fragments. Thus, for example, the conserved domain in exon 2, the cdc2 kinase recognition motif (codons 311–318), and a conserved serine (codon 392) are not covered by these four fragments.

Using CDGE, we detected 11 aberrantly migrating fragments from the panel of 32 breast carcinomas. Fragments with an altered mobility were found in each of the amplified regions (A–D). The nature and the approximate position of the mutations predicted by CDGE were all confirmed by sequencing. In addition to the regions of the gene identified by CDGE as highly suspect for carrying a mutation, all conserved regions of the amplified fragment were sequenced to rule out the presence of mutations not detected by screening. No such mutations were identified. In this panel of breast carcinomas, 11 tumor DNA samples showed an aberrant migration pattern in CDGE. It is likely that a larger number of mutations might have been found if a larger screening area had been used for analysis. Thus far, there have been very few sequencing efforts for exons 1–2 and 9–11 and our view of mutation frequencies might be limited. Four of the 32 tumors had no mutations despite LOH of the region coding for p53. These might be examples of tumors with the mutations not detected by the CDGE primers described here. On the other hand, all 4 of these tumors appear to have lost the whole chromosome 17, and therefore the LOH may not indicate a specific inactivation of the p53 gene in these tumors.

In the present series of breast tumors, 3 of the 11 sequenced mutations (T6, T83, T112) were found where CpG sequences are located. These positions have been shown to be methylated in human tissues (31). The majority of reported mutations at these positions are consistent with a mechanism of induction of methylcytosine deamination leading to C → T or G → A transitions. The mutations analyzed by Prosser *et al.* (32), and the mutations presented here indicate that the clustering of mutations in breast carcinomas are less conspicuous than reported for colorectal tumors (7).

Screening for p53 gene mutations in the germ line, in premalignant lesions, in primary tumors, and in metastases may help answer the question of the importance of different mutations and the behavior of the tumor. CDGE seems to offer a rapid technique by which to screen for such mutations. These may help to elucidate the sequence of events that occurs in tumorigenesis and to determine the role of p53 in that process.

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1. Ahuja, H., Bar-eli, M., Advani, S., Benchimol, S. & Cline, M. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6783–6787.
2. Baker, S. J., Fearon, E. R., Nigro, J. M., Hamilton, S. R., Preisinger, A. C., Jessup, J. M., vanTuinen, P., Ledbetter, D. H., Barker, D. F., Nakamura, Y., White, R. & Vogelstein, B. (1989) *Science* **244**, 217–221.
3. Bartek, J., Iggo, R., Gannon, J. & Lane, D. P. (1990) *Oncogene* **5**, 893–899.
4. Bressac, B., Galvin, K. M., Liang, T. J., Isselbacher, K. J., Wands, J. R. & Ozturk, M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1973–1977.
5. Masuda, H., Miller, C., Koeffler, H. P., Battifora, H. & Cline, M. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7716–7719.
6. Mulligan, M., Matlashewski, G. J., Scrable, H. J. & Cavenee, W. K. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5863–5867.
7. Nigro, J. M., Baker, S. J., Preisinger, A. C., Jessup, J. M., Hostetter, R., Cleary, K., Bigner, S. H., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F. S., Weston, A., Modali, R., Harris, C. C. & Vogelstein, B. (1989) *Nature (London)* **342**, 705–708.
8. Takahashi, T., Nau, M. M., Chiba, I., Birrer, M. J., Rosenberg, R. K., Vinocour, M., Levitt, M., Pass, H., Gazdar, A. F. & Minna, J. D. (1989) *Science* **246**, 491–494.
9. Russel, S. E. H., Hickey, G. I., Lowry, W. S., White, P. & Atkinson, R. J. (1990) *Oncogene* **5**, 1581–1583.
10. Fults, D., Tippets, R. H., Thomas, G. A., Nakamura, Y. & White, R. (1989) *Cancer Res.* **49**, 6572–6577.
11. Sato, T., Tanigami, A., Yamaka, K., Akiyama, R., Kasumi, F., Sakamoto, G. & Nakamura, Y. (1990) *Cancer Res.* **50**, 7184–7189.
12. Cattoretti, G., Rilke, F., Andreola, S., Cohen, B. & McKenzie, I. M. (1988) *Int. J. Cancer* **41**, 178–183.
13. Iggo, R., Gatter, K., Bartek, J., Lane, D. & Harris, A. L. (1990) *Lancet* **335**, 675–679.
14. Scott, N., Sagar, P., Stewart, J., Blair, G. E., Dixon, M. F. & Quirke, P. (1991) *Br. J. Cancer* **63**, 317–319.
15. Gibbs, R. A. & Caskey, C. T. (1987) *Science* **236**, 303–305.
16. Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. & Sekiya, T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2766–2770.
17. Orita, M., Suzuki, Y., Sekiya, T. & Hayashi, K. (1989) *Genomics* **5**, 874–879.
18. Fischer, S. G. & Lerman, L. S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1579–1583.
19. Sheffield, V. C., Cox, D. R., Lerman, L. S. & Myers, R. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 232–236.
20. Cotton, R. G. H., Rodreques, N. R. & Campbell, R. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4397–4401.
21. Hovig, E., Smith-Sørensen, B., Brogger, A. & Børresen, A.-L. (1991) *Mutat. Res.* **267**, 63–67.
22. Andersen, T. I., Gaustad, A., Ottestad, L., Farrantz, G., Nesland, J. M. & Børresen, A.-L. (1991) *Genes Chromosomes Cancer*, in press.
23. Lerman, L. S., Fischer, S. G., Hurley, I., Silverstein, K. & Lumelsky, N. (1984) *Annu. Rev. Biophys. Bioeng.* **13**, 399–423.
24. Poland, D. (1974) *Biopolymers* **13**, 1859–1871.
25. Gotoh, O. & Tagashira, Y. (1981) *Biopolymers* **20**, 1033–1042.
26. Malkin, D., Li, F. P., Strong, L. C. S., Fraumeni, J. F., Jr., Nelson, C. E., Kim, D. S., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A. & Friend, S. H. (1990) *Science* **250**, 1223–1238.
27. Devilee, P., Cornelisse, C. J., Kuipers-Dijkshoorn, N., Jonker, C. & Pearson, P. L. (1990) *Cytogenet. Cell Genet.* **53**, 52–54.
28. MacKay, J., Steel, C. M., Elder, D. A., Forrest, A. P. M. & Evans, H. J. (1988) *Lancet* **ii**, 1384–1385.
29. Thompson, A. M., Steel, C. M., Chetty, U., Hawkins, R. A., Miller, W. R., Carter, D. C., Forrest, A. P. M. & Evans, H. J. (1990) *Br. J. Cancer* **61**, 74–78.
30. Coles, C., Thompson, A. M., Elder, P. A., Cohen, B. B., MacKenzie, I. M., Cranston, G., Chetty, U., MacKay, J., MacDonald, M., Nakamura, Y., Hoyheim, B. & Steel, C. M. (1990) *Lancet* **336**, 761–763.
31. Soussi, T., Fromentel, C. C. & May, P. (1990) *Oncogene* **5**, 945–952.
32. Prosser, J., Thompson, A. M., Cranston, G. & Evans, H. J. (1990) *Oncogene* **5**, 1073–1079.
33. Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C. C. (1991) *Science* **253**, 49–53.