This study was supported by a Belgisch Werk Tegen Kanker Research Fellowship and The Frances and Augustus Newman Foundation and by a grant from The Cancer Research Campaign of Saint Bartholomew's Hospital.

References

Rapid Screening for \( p53 \) Mutations with a Sensitive Heteroduplex Detection Technique, Gregory J. Tsongalis, William K. Kaufmann, Sandra J. Wilson, Kenneth J. Friedman, and Lawrence M. Silverman\(^{2} \)

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Genetic changes such as point mutations, rearrangements, and amplification or deletion in a single cell may result in malignant transformation. It is widely accepted that mutations in the \( p53 \) tumor suppressor gene are among the most frequent alterations that occur during the malignant progression of many tumor types (I, 2). The product of \( p53 \) is a 393-amino acid nuclear phosphoprotein that was first described in 1979. Most mutations in \( p53 \) occur in exons 5 through 8 in 4 evolutionarily conserved domains (I). Mutations have been shown to cluster to these conserved regions of \( p53 \) in many human tumors, including breast, colon, lung, brain, and leukemias/lymphoma.

Li-Fraumeni syndrome (LFS) is a rare condition in which affected family members develop many different types of tumors similar to those sporadic tumor types that contain \( p53 \) mutations (3). Germline \( p53 \) mutations were first described in LFS patients who developed breast carcinomas, sarcomas, and brain tumors.

We examined the ability of a heteroduplex technique to detect single-base mutations in cells from two previously characterized LFS patients.

Two human fibroblast cell lines, MDAH041 and MDAH087, derived from two LFS patients were obtained from Michael Taieb (University of Texas–MD Anderson Cancer Center, Houston, TX) (4). MDAH041 contained a single-base deletion at codon 184 (exon 5), and MDAH087, a point mutation at codon 248 (exon 7). The cells were grown as previously described (5).

We resuspended a cell pellet containing \( 1 \times 10^{6} \) fibroblasts in 200 \( \mu \)L of extraction buffer (60 mmol/L Tris-HCl, pH 7.6; 100 mmol/L NaCl; 1 mmol/L EDTA; 5 g/L sodium dodecyl sulfate) and digested it overnight with 290 mg/L proteinase K at 37°C. The samples were then incubated for 10 min at 65°C to inactivate the proteinase before being exposed to RNase A for 1 h at 37°C. DNA was ethanol-precipitated after the addition of saturated NaCl and resuspended in 10 mmol/L Tris-\( \cdot \)1 mmol/L EDTA, pH 7.6.

We incubated genomic DNA (0.5-1.0 \( \mu \)g), isolated as described above, in a total reaction volume of 100 \( \mu \)L containing 300 ng of both the forward and reverse exon-specific primers, 2.5 \( \mu \)L of Taq polymerase, 200 mmol/L each deoxy-nucleotide triphosphate, 1.0 mmol/L MgCl\(_{2}\), 67 mmol/L Tris-HCl (pH 8.8), 10 mmol/L 2-mercaptoethanol, 16.6 mmol/L ammonium sulfate, and 6.7 \( \mu \)mol/L EDTA. The primers used were as follows: exon 5, 5\'GGTCACCCGTGTCCTATG3' and 5\'AGGAATACAGGCTCGGGGAG3'; exon 7, 5\'TGTCCTGCGACCTTCCG3' and 5\'AACACCCCTTTCTTGTG3'. DNA was initially denatured at 94°C for 6 min before amplification. Polymerase chain reaction (PCR) amplification was accomplished with 35 cycles consisting of 2 min annealing at 55°C, 3 min extension at 72°C, and 1 min denaturation at 94°C. The final cycle included a 2-min annealing step at 55°C and a 10-min extension step at 72°C.

The PCR-amplified product from each patient was heat-denatured at 100°C in a beaker of water for 3 min and then slowly cooled to 45°C by allowing the sample to remain in the water at room temperature on the benchtop. We mixed an aliquot of 40 \( \mu \)L of this product with 6 \( \mu \)L of gel loading buffer and electrophoresed the product on a 38-cm vertical Hydrolink-MDE gel (AT Biochem, Malvern, PA) that was 1.5 mm thick. We diluted the MDE gel to a 1x concentration (from 2x stock) in 0.6x Tris–boric acid–EDTA (TBE) buffer (1x = 133 mmol/L Tris, 81 mmol/L boric acid, and 3 mmol/L EDTA) and 150 g/L urea. After gel polymerization, electrophoresis was carried out for 16 h at 500 V. The gel

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was stained with ethidium bromide in TBE and then photographed under ultraviolet light.

In summary, two different single-base mutations were detected in two individuals with LFS (4). Exons 5 and 7 of the p53 gene were amplified from genomic DNA with PCR. An aliquot of the PCR products was electrophoresed in a 2% agarose gel to determine the quality of the amplification. Subsequently, amplified products were electrophoresed in a 38-cm Hydrolink-MDE gel that was stained with ethidium bromide. The detection of a heteroduplex band in exon 5 (Fig. 1A) and in exon 7 (Fig. 1B) is consistent with the presence of a single-base mutation in the amplified sequence, which was previously characterized for each individual patient.

The heteroduplex technique depends on PCR amplification of a target sequence, with subsequent heat denaturation and slow cooling to enhance annealing between the wild-type and mutant strands (6). In the case of homozygous mutations, wild-type DNA must be coamplified to allow heteroduplex formation. These products are then electrophoresed on a modified polyacrylamide-based vinyl polymer (Hydrolink-MDE) gel. Heteroduplexes migrate more slowly than homoduplexes, indicating the presence of a mutation. Heteroduplexes result from mismatches between complementary DNA strands, which retard electrophoretic mobility. This technique must be followed by DNA sequence analysis to determine the type of mutation or polymorphism present. This technology does not identify the mutation but can demonstrate the presence of a single base-pair change between the wild-type and mutant alleles. We believe that this rapid and simple heteroduplex technique may be a useful initial screening protocol to detect mutations in individuals at increased risk for developing cancer.

We thank Michael Tainsky (University of Texas MD Anderson Cancer Center, Houston, TX) for providing the cell lines and S. Adkins for technical assistance.

References

Fig. 1. Hydrolink-MDE gel electrophoresis showing heteroduplexes in exon 5 (A) and exon 7 (B) of the p53 gene in two different LFS patients. Arrowheads indicate the wild-type homoduplex. Lane 1, normal control; lanes 2 and 3, MDAH041 (A) or MDAH087 (B). Heteroduplex bands are visualized as higher molecular mass than the wild-type homoduplex band because of retarded migration.