Detection of *p53* Gene in Breast Cancer by Denaturing Gradient Gel Electrophoresis and the DCode™ System

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Introduction

Denaturing gradient gel electrophoresis (DGGE) is one of the most consistently used mutation scanning methods. It has evolved enormously over the last decade to be widely used, particularly in gene diagnostic laboratories. A practical method on this topic has been reported recently (Fodde and Losekoot 1994). The more frequently used mutation screening methods are DGGE and single-strand conformational polymorphism (SSCP). However, the sensitivity of DGGE is far higher (~95%) than that of SSCP, making it an attractive technique for screening unknown mutations.

The principle of DGGE is based on the fact that DNA duplexes melt at high temperature in the presence of a gradient of chemical denaturant. DNA melting is sequence specific and occurs in discrete segments called melting domains. Since melting involves breaking the hydrogen bonds that hold the base pair (bp) together, a G-C rich region melts at a higher temperature than an A-T rich region.

A polymerase chain reaction (PCR) product begins to melt from the region which has the lowest melting point (lowest T_m) first when it is exposed to chemical denaturant and high temperature. If there are point mutations in the PCR product, the melting point will change, and thus its mobility on a polyacrylamide gel will be different from the mobility of the wild-type DNA. In other words, the mobility of the DNA fragment-containing mutation in the gel will be different from that of the wild type. In this paper we describe the use of DGGE to screen *p53* mutations in breast cancer.

Methods

One hundred and two genomic DNA samples obtained from breast cancers were examined from exon 5, 6, 7, and 8 of the *p53* gene. They were amplified by PCR with a pair of primers which contains a 40 bp GC clamp in one of them (Andersen and Borresen 1995). All products were analyzed both in perpendicular gradient gels and parallel gradient gels.

Perpendicular Gradient Gel Analysis

A 7.5 x 10 cm, 1 mm thick 25–65% denatured gradient gel was made using 6% aclylamide/bis (37.5:1) in 1x TAE buffer (50 mM Tris, 25 mM acetic acid, 1.25 mM EDTA). Four different PCR products each from exon 5, 6, 7, and 8 were mixed in a 25 µl volume, and the total sample volume made up to 100 µl. We added 100 µl 2x gel loading dye (70% glycerol, 0.05% Bromophenol Blue, 0.05% Xylene Cyanole, 2 mM EDTA) to these samples, and electrophoresed them on the DCode universal mutation detection system at 130 V for 2.5 hr at 56°C. After electrophoresis, the gels were stained in a 1:10,000 dilution of SYBR Green I (Molecular Probes, Inc.) in 1x TAE buffer for 45 min and destained in 1x TAE buffer for 45 min. The gels were imaged under ultraviolet (UV) transillumination.

Parallel Gradient Gel Analysis

A 16 x 16 cm, 1 mm thick 45–60% denatured gradient gel was made using 6% aclylamide/bis (37.5:1) in 1x TAE buffer. Five µl of PCR products (~200–300 ng) was mixed with 5 µl 2x gel loading dye and electrophoresed on the DCode system at 150 V for 3.5 hr at 60°C. The post-run analysis was the same as perpendicular gradient gel analysis.

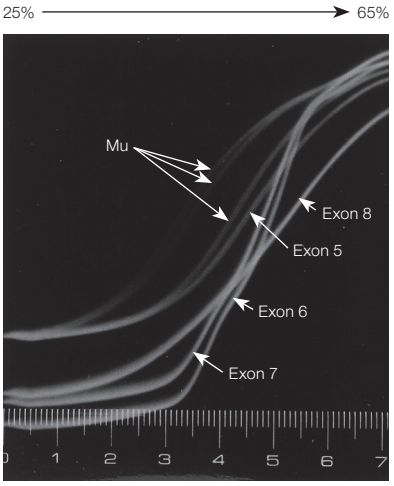

Fig. 1. The typical case of perpendicular analysis using the DCode system. Each wild-type exon is shown by an arrow, and mu of exon 5 is a mutant DNA. At this point in our investigations, the mutant DNA can be detected, but exon cannot be specified.

Results and Discussion

The optimum concentration of denaturant to be used for parallel gradient gels was determined from the perpendicular gradient gel analysis. All exons were separated and had a melting transition using a 25–65% gradient (Figure 1). Therefore a gradient of 45–60% was used for parallel gel analysis (Figure 2).

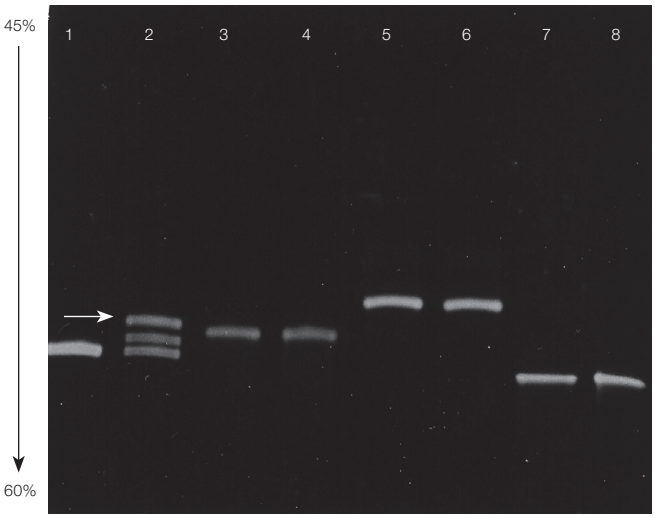

Fig. 2. Parallel gradient gel analysis using the DCode system. Lane 1, wild-type DNA of exon 5; lane 2, sample DNA of exon 5; lane 3, wild-type DNA of exon 6; lane 4, sample DNA of exon 6; lane 5, wild-type DNA of exon 7; lane 6, sample DNA of exon 7; lane 7, wild-type DNA of exon 8; lane 8, sample DNA of exon 8. The arrow in lane 2 shows a mutant of exon 5, which is the same as the one showed in Figure 1 (mu).

DNA from 34 cases was found to have a mutation on perpendicular gels and in 33 cases on parallel gels. The sample missed showed a mutation of exon 7 on perpendicular gels but not on parallel gels. A possible explanation for this could be that the parallel gel gradient of 45–60% was too wide for that particular sample. A narrow gradient may be required to identify the mutation of exon 7 on parallel gels.

We chose to use parallel gradient method because it provides high-throughput analysis. The detection of mutation was unclear in some cases where the rate of mutated allele was low or the genomic DNA was extracted from paraffin-embedded materials. In such cases, we used SYBR Green I instead of ethidium bromide to stain the gels. This improved sensitivity and enabled us to see the bands clearly.

These experiments indicate that DGGE is a powerful technique to screen mutations. The DCode system is easy to use and reliable, two important factors when scanning numerous mutations.

References

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