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Blinded study determination of high sensitivity and specificity microchip electrophoresis–SSCP/HA to detect mutations in the p53 gene

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Abstract

Knowledge of the genetic changes that lead to disease has grown and continues to grow at a rapid pace. However, there is a need for clinical devices that can be used routinely to translate this knowledge into the treatment of patients. Use in a clinical setting requires high sensitivity and specificity (>97%) in order to prevent misdiagnoses. Single strand conformational polymorphism (SSCP) and heteroduplex analysis (HA) are two DNA-based, complementary methods for mutation detection that are inexpensive and relatively easy to implement. However, both methods are most commonly detected by slab gel electrophoresis, which can be labor-intensive, time-consuming, and often the methods are unable to produce high sensitivity and specificity without the use of multiple analysis conditions. Here we demonstrate the first blinded study using microchip electrophoresis-SSCP/HA. We demonstrate the ability of microchip electrophoresis-SSCP/HA to detect with 98% sensitivity and specificity >100 samples from the p53 gene exons 5–9 in a blinded study in an analysis time of less than 10 minutes.

Keywords

microchip electrophoresis; SSCP; HA; blinded

1 Introduction

Genetic screening has the potential to improve the diagnosis and treatment of diseases such as cancer. With completion of the sequencing of the human genome in 2001 and the upcoming sequencing of the human cancer genome, the potential for treating patients based

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on understanding the genetic changes associated with these diseases has greatly increased [1, 2]. However, in order for these methods to be commonly used in a routine clinical setting, they must be rapid, accurate, and inexpensive for detecting the genetic changes in each patient and correlating them with treatment and outcome.

The p53 gene is the most commonly altered gene in human cancers [3]. The p53 gene is a large gene composed of 11 exons and 10 introns that are made up of >20,000 bp [4, 5]. However, >90% of the known mutations exist in exons 4–9, with ~70% of research studies focusing on exons 5–8 [6]. This focus is because p53 exons 5–8 code for the DNA binding domain of the p53 protein, an area where the structure can be highly affected by sequence changes [6]. This area is also highly conserved among mammals, amphibians, birds, and fish [7]. The p53 exons 5–8 also include 3 “hotspot” residues (codons 175, 248, and 273), where the frequency of mutations is especially high [3]. For our study we used a set of samples generated at the National Institute of Standards and Technology (NIST) from cell lines similar to those described previously [8, 9]. These samples contain many of the most frequent mutations in both germline and somatic mutations as indicated by the IARC TP53 mutation database (<http://www-p53.iarc.fr>), therefore creating a highly useful sample set for testing the abilities of a mutation detection method [5].

There are several methods of genetic mutation detection or screening that have been demonstrated in literature, including automated DNA sequencing by capillary array electrophoresis, DNA hybridization chips (Affymetrix GeneChips™), denaturing HPLC (DHPLC), single strand conformation polymorphism (SSCP), and heteroduplex analysis (HA). The recent introduction of next generation sequencing provides great promise for the future of genetic sequencing, but is still a costly and time consuming method [10]. Table 1 compares many of the important properties of these different mutation detection methods that have been used to examine mutations in the p53 gene. In this table, sensitivity is defined as the number of mutations detected by the system divided by the total number of mutations actually present and specificity is defined as the number of wild-type samples detected by the system divided by the total number of wild-type samples actually present. These numbers represent the ability of a system to both detect all mutations present and to not call mutations when they are not present, respectively. Routine clinical use of these mutation detection methods would require a sensitivity and specificity of >97% at a reasonable cost. All of the methods except DHPLC demonstrate a high enough specificity (when determined) for use in a clinical setting. Only the SGE-SSCP and CE-SSCP/HA studies demonstrate a high enough sensitivity (>97%) for routine clinical use. However, the SGE-SSCP study required 4 different analysis conditions to achieve 100% sensitivity and neither CE-SSCP/HA study determined specificity, which would be required before clinical use. In the Kozlowski *et al.* study, they examined the BRCA1 and BRCA2 genes instead of p53 and 5 additional mutants were generated from one of the patient samples and used in determining sensitivity. However, none of these studies have yet demonstrated the clinically needed >97% sensitivity and specificity.

We felt that tandem SSCP/HA had the potential to be a clinically relevant technique if translated to a faster and potentially more clinically useable platform like microchip electrophoresis and tested with a clinically relevant sample size. Here we present a blinded study of 106 patient samples by microchip electrophoresis tandem SSCP/HA with the ability to detect mutations with >97% specificity and sensitivity in <10 minutes of analysis time. To our knowledge this is the first blinded study to be performed by microchip electrophoresis-SSCP/HA. In the following paper, we will explore the keys to sensitive and specific mutation detection by tandem SSCP/HA on a microfluidic chip, give the results of our blinded study in terms of which exons were the most detectible, and clearly state what work needs to be done to bring this technique into an actual clinical setting.

In our previous work with capillary electrophoresis (CE) tandem SSCP/HA, we studied a panel of 32 mutants derived from p53 exons 7 and 8, and showed that by optimizing the polymer matrix and sample preparation conditions, we could obtain a mutation detection sensitivity of 100%, compared to 93% for SSCP alone and 75% for HA alone [11, 12]. In another study by Kozłowski *et al.*, they were able to obtain 100% sensitivity for 31 different mutations in 24 samples of BRCA1 and BRCA2 using combined capillary electrophoresis SSCP and HA, compared to 90% for SSCP alone and 81% for HA alone [13]. Because the sensitivity of the SSCP and HA methods have been a limiting factor for clinical use of SSCP and HA, there was a need for an optimized microchip electrophoresis-based, tandem SSCP/HA method that would be rapid, inexpensive, and provide 97% or greater sensitivity and specificity of mutation detection.

Microchip electrophoresis HA has been demonstrated by Tian *et al.* [14, 15]. In a different study, Tian *et al.* demonstrated the ability to detect mutations by microchip electrophoresis SSCP [16]. Vahedi *et al.* demonstrated a unique method of microchip electrophoresis tandem SSCP/HA in which they added formamide to the microchip well containing the DNA sample of interest and used it to form the SSCP and HA conformers [17]. Sukas *et al.* constructed a parylene-c micro-electrophoresis system and were able to detect a 3 bp mutation using HA [18]. ME-SSCP has been recently demonstrated also for detection of point mutations in the human obesity gene and p53 gene [19, 20]. Although all of these previous studies demonstrated that SSCP and HA could be used to detect mutations by microchip electrophoresis, each study was limited to a small sample set (<6 samples) and they did not investigate the sensitivity and specificity of their systems, which is critical for use in a clinical setting. In a study of 21 mutations in the HFE, MYL2, MYL3, and MYL7 genes, Tian *et al.* found that they would detect mutations with 95% sensitivity using microchip electrophoresis-SSCP at one electrophoresis temperature (25 °C) and with 100% sensitivity using 2 temperatures (25 and 40 °C) [21].

In order to make a truly usable clinical mutation detection method, not only must sensitivity and specificity be determined, but also they should be determined in a blinded study to prevent any biasing. Of the thousands of articles about SSCP (>6500), there are only ~45 that tested their samples in a blinded study. There have been <10 blinded studies looking at capillary electrophoresis-SSCP and <5 studies for capillary electrophoresis-HA. To our knowledge, there have been no blinded studies conducted with either microchip electrophoresis HA or combined SSCP/HA. Therefore, if these commonly used research methods are to be translated to a clinical setting the sensitivity and specificity of SSCP and HA must be determined to be >97% in a blinded, unbiased study.

With our study we hoped to prove the clinical potential for rapid mutation detection of cancer related genes in a microchip electrophoresis system. SSCP and HA have shown great promise for fulfilling the screening of candidate genes if their sensitivity and specificity could be improved. We felt that a clinically relevant system would be one that was able to achieve both a sensitivity and specificity of >97%. Therefore, we performed a blinded study on >100 samples from exons 5–9 of the p53 gene to determine the sensitivity and specificity of microchip electrophoresis-SSCP/HA.

2 Materials and methods

2.1 Fluorescently labeled amplicons from cell line-derived samples

Mutant DNA specimens of the p53 gene were stored in stock solutions in the form of plasmids or PCR amplicons. The cell line-derived plasmids were created at the National Institute of Standards and Technology (NIST, Gaithersburg, MD) as previously described [8, 9]. The presence of a mutation in these samples was previously confirmed by sequencing

at NIST. Amplicons of p53 exons 6–9 were produced using a PCR protocol previously described by O'Connell *et al.* for generating SSCP fragments with all forward (sense) primers fluorescently labeled with FAM and all reverse primers fluorescently labeled with JOE [26]. Exon 5 was amplified using the same PCR protocol, but modified to use the following primers: forward primer TGCCCTGACTTTCAACTCTGT; reverse primer GCAACCAGCCCTGTCGTCTCT [27]. The primers were fluorescently labeled with FAM on the forward strand primers and with JOE on the reverse strand primers. This labeling scheme allowed for two-color laser induced fluorescent (LIF) detection, which we have previously found to allow the unambiguous assignment of DNA peaks in the electropherograms [11, 12].

2.2 DNA sample purification, quantitation, and storage

Crude PCR products were purified by the Qiaquick PCR purification kit (Qiagen, Inc., Valencia, CA) following the manufacturer's instructions. Quantitation of purified cell line DNA was determined either spectrophotometrically or with the use of the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Palo Alto, CA). Typically, the DNA samples were prepared in stock solution at a concentration of 10 ng/μL in 10 mM Tris-HCl. These stock solutions were stored at –20 °C.

2.3 SSCP and HA sample preparation and blinding

Fluorescently labeled amplicons of the 53 mutants and the 5 wild-types for exons 5–9 of the p53 gene were produced as described above and listed in Table 2. From these stock solutions, the 53 wild-type and 53 wild-type + mutant samples were generated for blinded analysis by microchip electrophoresis tandem SSCP/HA. The wild-type samples were prepared by adding 1–3 μL of 10 ng/μL wild-type from the appropriate p53 exons to 5–9 μL 10 mM Tris-HCl to make a final solution volume of 10 μL. A spreadsheet containing the labels for the set of 106 samples and their mutation status was sent to a statistician outside the analysis lab to be coded. Once the blinded code was generated, the samples and the blinded code were given to a researcher with no involvement in this project. This researcher coded the samples by labeling new tubes with the blinded code and pipetting the samples from the old uncoded tubes to the new coded tubes. The coded samples were then returned for analysis. Before analysis, 3.1 μL of the stock solution from the blinded tubes were pipetted into a thin-walled tube, denatured at 95 °C for 3 minutes, and snap cooled on ice for ~3 minutes. The samples were also stored on ice. After all the samples were analyzed, a spreadsheet containing the coded number for each sample and the mutational status determined from the blinded analysis were sent to a statistician outside the analysis lab for statistical analysis.

2.4 Tandem SSCP/HA sample preparation

We previously conducted a systematic study to determine the optimum preparation method for tandem SSCP/HA samples [11]. Briefly, the samples were diluted using 1× Tris-HCl (10 mM, pH 8.5), denatured at 95 °C for 3 minutes, and snap-cooled on ice. Using this protocol, we were able to generate both SSCP and HA conformers in the same sample tube. In this study, we examined the importance of sample concentration. If sample concentration was too high, we noticed the predominant formation of HA conformers and a significant loss of SSCP conformers. Conversely, at very low DNA concentrations we noticed a predominance of SSCP conformers and a loss of HA conformers. Therefore, for the blinded study we noted concentrations of 10–20 nM seemed to give similar and detectable amounts of both SSCP and HA conformers.

2.5 Polymer matrix selection

In our previous CAE-SSCP/HA studies we had determined 6% 600,000 g/mol linear polyacrylamide (LPA) to be the optimum sieving matrix for p53 exons 7 and 8 [12]. In a recent study, we looked at the influence of molecular weight and concentration of LPA for microchip electrophoresis-SSCP/HA for p53 exons 5–9 [28]. In that study, we found the optimum polymer matrix for high resolution, highly repeatable separations to be 8% 600,000 g/mol. In addition, we found that adding a small amount of the coating polymer (0.25% poly-*N*-hydroxyethylacrylamide) to the 8% 600,000 g/mol LPA improved the resolution of the polymer matrix and made the polymer solution easier to load into the narrow microchannels of the glass chip.

2.6 Polymer synthesis and characterization

LPA was synthesized by free radical polymerization under conditions previously determined to produce a molecular weight of around 600,000 g/mol [12]. Once the polymer was synthesized, the solution was purified by dialysis, lyophilized, and characterized by tandem gel permeation chromatography (Waters, Milford, MA) and multi-angle laser light scattering (Wyatt Technology, Santa Barbara, CA). After analysis, the polymer was dissolved to 8% (w/v) in 1× TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) containing 10% glycerol, which has previously been reported to improve resolution [22, 23].

2.7 Dynamic microchip coating

Before performing any analyses, the borosilicate glass microchips (T8050, Micronit, The Netherlands) used in this study were coated with adsorbed poly-*N*-hydroxyethylacrylamide (BioWhittaker Molecular Applications, Walkersville, MD), which was previously reported to be a favorable coating for resolution of mutation detection by SSCP [12]. We used the optimized coating procedure from Albarghouthi *et al.* [25] but modified it slightly for use on microchips. First the chip was rinsed with DI water. Next, 1M HCl was placed into the sample, buffer, and injection waste wells. The 1M HCl was then dynamically pulled through the separation channel by applying vacuum pressure at the separation waste well for 15 minutes, making sure to keep the other wells filled with 1M HCl. The 1M HCl was then removed from the wells and channels by vacuum pressure and replaced with 0.1% (w/v, aq) poly-*N*-hydroxyethylacrylamide. The poly-*N*-hydroxyethylacrylamide solution was pulled through the separation channel using vacuum for 15 minutes, then removed using vacuum, and the chip was rinsed with water.

2.8 Polymer matrix loading into microfluidic chip

Due to the relatively high viscosity of the polymer matrices and the small channel dimensions of the microfluidic chips, a high pressure loading device was designed for loading the microchips. This device consisted of a pneumatic press (Carver, Wabash, IN), two metal plates, and stacks of neoprene rubber gaskets (McMaster-Carr, Atlanta, GA) cut in a manner to hold the chip in place and has been described previously [24]. The microfluidic chips were loaded with the linear polyacrylamide (LPA) separation matrices under N₂ pressure (~100 psi).

2.9 Microchip electrophoresis

The samples prepared by tandem SSCP/HA were injected at 800–1000 V/cm for 20 seconds and electrophoresed at 350–450 V/cm at ambient temperature (17–20 °C) in a running buffer of 1× TBE with 10% glycerol. Each run was completed within 7.5–10 minutes. The microchip electrophoresis system has been described previously [24]. Borosilicate glass microfluidic chips (Micronit, The Netherlands) with the following properties were used in

the experiments: double T injector with an offset of 100 μm , separation length of 80 mm, channel width of 50 μm , and channel depth of 20 μm .

3 Results and discussion

3.1 Analysis of SSCP/HA electropherograms

Examples of electropherograms for an easily identified and a challenging mutation detection by tandem microchip electrophoresis-SSCP/HA obtained in this study are shown in Figure 1 for p53 exon 8. The double stranded DNA peaks are the first peaks to elute and can be identified in the electropherogram by the overlap of the two dyes (red dye FAM and blue dye JOE) that label the forward and reverse DNA strands respectively. The next peaks are the single stranded DNA peaks and can be identified by the dominance of a single dye. The presence of the less dominant and opposite dye in the single stranded DNA peaks is due to a slight spectral overlap in the emission region of the two dyes. By labeling the forward and reverse strand with different fluorescent dyes, the peaks can easily be identified, as we noted in our previous CE-SSCP/HA studies [11]. As Figure 1 also demonstrates, all the mutation detection analyses were completed within 7.5–10 minutes (450–600 s) by microchip electrophoresis-SSCP/HA. This is a decrease in analysis time of 4–5 times our previous capillary electrophoresis-SSCP/HA studies [12].

3.2 Temporal stability of SSCP and HA conformers

The stability of SSCP and HA conformers over time is important for developing a clinically useable and robust method of mutation detection. Ideally the conformers would be stable over the period of a normal workday (8–10 hours), allowing mutation detection analyses to be run at any time during that period. In a previous capillary electrophoresis study, Kozłowski et al. found that SSCP and HA samples were stable over the full analysis time of their instrument (ABI 310, ~20 hrs) [13]. In our study, samples were denatured at 95 °C, snap-cooled on ice, and then stored at 0–4 °C. As Figure 2 shows, the SSCP and HA conformers were very stable and allowed mutation detection for a 24-hour period and even for 16 days after being denatured and snap-cooled. Therefore, the tandem SSCP/HA conformers are highly stable, indicating their high robustness for mutation detection.

3.3 Mutant to wild-type ratios

Detecting mutations in tumor tissue can often be complicated by the presence of normal tissue. The amount of normal tissue in the tumor varies depending on the affected organ or the individual [29]. Therefore it is important to determine the limitations of any mutation detection method in terms of its ability to detect mutations when the mutant DNA is present in a smaller ratio than the normal DNA. We explored the ability of SSCP and HA to detect mutations in samples with different amounts of mixed wild-type and mutant DNA. We tested 2 mutant samples from each exon 5–9 (10 total samples) and found that we were able to detect mutations when the mutant DNA concentrations were as low as 18%. In several of the exons (exons 5, 7, and 9), we could detect mutations when the mutant DNA concentration was as low as 15%. Representative wild-type and wild-type + mutant electropherograms for the largest (exon 5, 263 bp) and smallest (exon 9, 102 bp) p53 exons are shown in Figure 3. We also found that the mutation detection in these samples was highly temporally stable (>1 week). Below these concentrations, there did not appear to be consistent mutation detection in any of the samples.

3.4 Blinded study mutant samples

The 53 mutant samples used for determining the sensitivity in the blinded study are listed in Table 2. Single nucleotide polymorphisms (SNPs) are the most frequent type of genetic

variation [30]. As this table indicates, most of the mutations (~74%) were single nucleotide substitutions, which can be challenging to detect. Of these mutations, 37.5% were transition mutations, while 62.5% were transversion mutations. In addition, some samples contained two mutations that were either located next to each other or a significant distance apart. We felt that having this mix of samples created an interesting challenge for testing the sensitivity of tandem SSCP/HA-microchip electrophoresis. In addition, we felt that it was important to include mutations from the codons that are frequently found in the population (codons 175, 248, and 273). Although mutant samples from codon 175 were not available during the time of the blinded study, we were later able to obtain, amplify, purify, and test these samples. The samples from codon 175 are included at the bottom of Table 2, although they are not included in any of the sensitivity or specificity calculations. As Table 2 shows, we were able to detect mutations in all the samples that we tested from these codons (3 codon 175, 6 codon 248, 11 codon 273), therefore demonstrating our ability to detect samples of high clinical relevance.

3.5 Sensitivity and selectivity determination in blinded study

Sensitivity reflects the ability of a mutation detection method to detect all the mutations that are present, while specificity reflects the ability of a mutation detection system not to call false mutations. These numbers need to be very high (>97%) in order to be used in a clinical setting where lives are affected by the results.

For the blinded study, we analyzed a total of 106 DNA samples (53 mutants and 53 wild-types) from p53 exons 5–9. The blinded sample set was comprised of 12 samples from p53 exon 5, 14 samples from exon 6, 40 samples from exon 7, 30 samples from exon 8, and 10 samples from exon 9. Each sample was run at least 3 times (consecutively) to ensure repeatability of the electropherograms. Table 2 provides information about the mutant samples analyzed, a detailed summary of how the mutation was detected (SSCP and/or HA), and the overall sensitivity and specificity from the μ CE-SSCP/HA analysis. For p53 exons 5, 7, 8, and 9 (four of the five p53 exons of primary interest for the detection of genetic mutations related to cancer), the tandem μ CE-SSCP/HA method yielded 100% sensitivity and 100% specificity. For p53 exon 6, the sensitivity and specificity were 86%, based on the miscalling of 1 mutant and 1 wild-type. This gives an overall sensitivity and specificity for p53 exons 5–9 of 98% (52/53). The miscalled mutation is a single base substitution of G to A. When the wild-type and mutant sequences for this mutation were input into MFOLD to predict the secondary structures, we found that the structures were very highly similar indicating that the conformation change was slight and therefore highly challenging to detect.

In order to understand the importance of using SSCP and HA in tandem, we examined the ability of each method alone to detect the mutations in the blinded study. HA alone was able to detect 51% of the mutations while SSCP alone was able to detect 94% of the mutations, as shown in Table 2. By combining the two complementary methods we were able to obtain the clinically significant sensitivity of 98%. Examining the mutation detection by SSCP in more detail, we see the importance of labeling both the forward (sense) and reverse (antisense) strands of DNA. As indicated by Table 2, the mutation detection by forward strand SSCP was ~72% and by reverse strand SSCP was ~68%, but by detecting both strands the total SSCP mutation detection is 94%.

4 Concluding remarks

There is a need for a mutation detection method that is inexpensive, rapid, sensitive, and specific so that it can be used for routine clinical testing. Here we demonstrate the ability of microchip electrophoresis-SSCP/HA to detect mutations with 98% sensitivity and specificity

in a blinded study of 106 cell-line derived samples. We demonstrate HA alone provides a sensitivity of 51% and SSCP alone provides a sensitivity of 94%, which confirms the need to use SSCP and HA in tandem to achieve a clinically relevant high sensitivity (>97%). In addition, we show that in order to obtain a high sensitivity in the SSCP conformer it is important to label both single strands of DNA because each strand alone did not provide a high enough sensitivity (72% forward SSCP, 68% reverse SSCP).

Another benefit of this method is the high temporal stability of the SSCP and HA conformers. We found that our samples were stable for at least two weeks when stored between 0–4 °C. We have shown the ability to detect mutations in samples where wild-type DNA is predominant over mutant DNA. We demonstrated the ability to detect mutations in samples from all exons at 18% mutant DNA and from 3 of the 5 exons at 15% mutant DNA. In addition, the mutations in these samples were highly stable over time when stored properly.

Although we have shown that microchip electrophoresis-SSCP/HA has great potential for routine use in a clinical setting, there are still a couple of challenges remaining. An integrated microchip that included DNA amplification, purification, and analysis that was also capable of analyzing multiple samples would make the system more desirable because it would increase the throughput and decrease the cost of the analysis. In addition, the system will need to be proven to work just as sensitively and specifically on a large number of patient samples. However, we feel that this study lays the foundation for these challenges to be addressed and overcome.

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Abbreviations

μCE	microchip electrophoresis electrophoresis
HA	heteroduplex analysis
LIF	laser-induced fluorescence
C(A)E	capillary (array) electrophoresis electrophoresis
LPA	linear polyacrylamide
SGE	slab gel electrophoresis
SSCP	single-strand conformation polymorphism

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Figure 1.

Representative electropherograms of p53 exon 8 wild-type (a) and wild-type + mutant samples for an easily identified mutation (b) and a more challenging mutation (c), with the detection of the mutations highlighted by green arrows

. Mutations were detected by tandem SSCP/HA on a microfluidic device using the following conditions: ambient temperature, 2-color laser induced fluorescence (LIF) detection (

FAM

for the forward strand,

JOE

for the reverse strand), 0.1% PHEA dynamically coated channel, 350 V/cm applied electric field strength. Baselines adjusted to value of zero to remove background noise.

Figure 2.

Electropherograms of p53 exon 8 demonstrating temporal stability of SSCP and HA conformers that allowed mutation detection for at least 16 days after initial heat denaturing and snap cooling when stored at 0–4 °C. Detection of the mutations by tandem SSCP/HA is highlighted by **green arrows**

. Mutations were detected on a microfluidic device using the following conditions: ambient temperature, 2-color LIF detection (

FAM

for the forward strand,

JOE

for the reverse strand), 0.1% PHEA dynamically coated channel, 350–450 V/cm applied electric field strength. Baselines adjusted to value of zero to remove background noise.

Figure 3.

Electropherograms of p53 exons 5–9 wild-type and wild-type + mutant samples demonstrating ability to detect mutations at mutant DNA concentrations as low as 15–18% mutant DNA with detection of the mutations by tandem SSCP/HA highlighted by green arrows

. Mutations were detected on a microfluidic device using the following conditions: ambient temperature, 2-color LIF detection (

FAM

for the forward strand,

JOE

for the reverse strand), 0.1% PHEA dynamically coated channel, 350–450 V/cm applied electric field strength. Baselines adjusted to value of zero to remove background noise.

Table 1

Comparison of DNA-based mutation detection methods for the p53 gene.

Mutation Detection Method	Samples Analyzed	Gene	Sensitivity	Specificity	Reference
DNA sequencing	100 lung cancer patients; 108 ovarian cancer patients	p53 exons 5-9; p53 exons 2-11	76%; 82%	100%; 100%	Alrendt <i>et al.</i> [31]; Wen <i>et al.</i> [32]
DNA hybridization chips	100 lung cancer patients; 108 ovarian cancer patients	p53 exons 5-9; p53 exons 2-11	80%; 92%	98%; 100%	Alrendt <i>et al.</i> [31]; Wen <i>et al.</i> [32]
DHPLC	7 breast, 2 urothelial, and 18 colorectal carcinomas, 1 sample from Li-Fraumeni patient	p53 exons 5-8	~95%	~95%	Keller <i>et al.</i> [33]
Slab gel SSCP	105 ovarian cancer patients; 112 colorectal, 28 esophageal, and 33 hepatocellular carcinomas	p53 exons 2-11; p53 exons 5-9	85%; 95-100%	98%; N/D	Wen <i>et al.</i> [34]; Makino <i>et al.</i> , [35]
CE-SSCP	20 colon tumor samples	p53 exon 7	86%	100%	Ru <i>et al.</i> [36]
CE-SSCP/HA	32 cell line-derived mutant; 24 samples from breast and/or ovarian cancer patients	p53 gene exons 7-8; BRCA1 and BRCA2 genes	100%; 100%	N/D N/D	Kourkine <i>et al.</i> [12]; Kozłowski <i>et al.</i> [13]

* N/D indicates that the study did not determine this information.

Table 2

Panel of 53 cell line–derived mutant samples used for blinded genetic screening of p53 gene by tandem ME-SSCP/HA.

Sample #	Exon	Codon	DNA Mutation	HA	SSCP FWD	SSCP REV	SSCP
1	5	144	CAG to TAG	–	+	+	+
2	5	149	TCC to CCC	–	+	–	+
3	5	163	TAC to TGC	–	+	–	+
4	5	164	AAG to AGG	+	+	–	+
5	5	173	GTG to CTG	–	+	–	+
6	5	173	GTG to TAG	+	–	–	–
7	6	196	CGA to CTA	–	+	+	+
8	6	197	GTG to GAG	–	+	–	+
9	6	197, 213	GTG to GAG, CGA to CTA	+	+	–	+
10	6	197, 213	GTG to GCG, CGA to CCA	–	+	–	+
11	6	197, 213	GTG to GGG, CGA to CCA	+	–	–	–
12	6	198	GAA to AAA	–	–	–	–
13	6	213	CGA to CCA	+	+	–	+
14	7	242	TGC to AGC	+	+	–	+
15	7	242	TGC to ACC	+	+	+	+
16	7	242	TGC to ATC	+	+	+	+
17	7	242	TGC to CTC	+	+	–	+
18	7	242	TGC to GGC	–	+	+	+
19	7	242	TGC to TTC	–	+	–	+
20	7	245	GGC to TGC	+	–	+	+
21	7	245	GGC to AGC	–	+	+	+
22	7	245	GGC to CGC	+	+	–	+
23	7	245	GGC to GCC	–	–	+	+
24	7	245	GGC to GTC	–	+	+	+
25	7	245	GGC to TGC	+	–	+	+
26	7	248	CGG to TGG	–	+	+	+
27	7	248	CGG to AGG	+	+	+	+

Sample #	Exon	Codon	DNA Mutation	HA	SSCP FWD	SSCP REV	SSCP
28	7	248	CGG to CAG	-	+	+	+
29	7	248	CGG to GGG	+	+	+	+
30	7	248	CGG to TGG	-	+	-	+
31	7	248	CGG to CTG	-	+	+	+
32	7	249	AGG to AGC	-	+	+	+
33	7	249	AGG to AGT	-	+	+	+
34	8	273	CGT to AGT	-	-	+	+
35	8	273	CGT to ATT	-	-	+	+
36	8	273	CGT to CAT	-	-	+	+
37	8	273	CGT to CTT	+	+	+	+
38	8	273	CGT to TAT	+	-	+	+
39	8	273	CGT to TCT	+	-	+	+
40	8	273	CGT to TGT	-	-	+	+
41	8	273	CGT to AAT	+	-	+	+
42	8	273	CGT to CAT	-	-	+	+
43	8	273	CGT to CCT	+	+	+	+
44	8	273	CGT to CTT	+	+	+	+
45	8	282	CGG to TGG	+	+	+	+
46	8	282	CGG to AGG	+	-	+	+
47	8	282	CGG to GGG	-	+	+	+
48	8	282	CGG to GGG	+	+	-	+
49	9	312	ACC to ACG	+	+	+	+
50	9	312, 321	ACC to ACA, AAA to CAA	-	+	+	+
51	9	312, 321	ACC to ACT, AAA to CAA	+	+	+	+
52	9	321	AAA to GAA	+	+	+	+
53	9	328	TTC to CTC	+	+	+	+
175 #1	5	175	CGC to CTC	-	+	+	+
175 #2	5	175	CGC to CCC	-	+	+	+
175 #3	5	175	CGC to CAC	-	+	+	+

Sample #	Exon	Codon	DNA Mutation	HA	SSCP FWD	SSCP REV	SSCP
Overall sensitivity				51%	72%	68%	94%

* The location of the mutations has been highlighted in **red**.
. A (+) indicates that the mutant was detected by the method (HA or SSCP) and a (-) indicates that mutant not detected by the method (HA or SSCP).