Comparison of TP53 Mutations Identified by Oligonucleotide Microarray and Conventional DNA Sequence Analysis


ABSTRACT

As the rate of gene discovery accelerates, more efficient methods are needed to analyze genes in human tissues. To assess the efficiency, sensitivity, and specificity of different methods, alterations of TP53 were independently evaluated in 108 ovarian tumors by conventional DNA sequence analysis and oligonucleotide microarray (p53 GeneChip). All mutations identified by oligonucleotide microarray and all disagreements with conventional gel-based DNA sequence analysis were confirmed by re-analysis with manual and automatedideoxy DNA sequencing. A total of 77 ovarian cancers were identified as having TP53 mutations by one of the two approaches, 71 by microarray and 63 by gel-based DNA sequence analysis. The same mutation was identified in 57 ovarian cancers, and the same wild type TP53 sequence was observed in 31 ovarian cancers by both methods, for a concordance rate of 81%. Among the mutation analyses discordant by these methods for TP53 sequence were 14 cases identified as mutated by microarray but not by conventional DNA sequence analysis and 6 cases identified as mutated by conventional DNA sequence analysis but not by microarray. Overall, the oligonucleotide microarray demonstrated a 94% accuracy rate, a 92% sensitivity, and an 100% specificity. Conventional DNA sequence analysis demonstrated an 87% accuracy rate, 82% sensitivity, and a 100% specificity. Patients with TP53 mutations had significantly shorter overall survival than those with no mutation (P = 0.02). Women with mutations in loop2, loop3, or the loop-sheet-helix domain had shorter survival than women with other mutations or women with no mutations (P = 0.01). Although further refinement would be helpful to improve the detection of certain types of TP53 alterations, oligonucleotide microarrays were shown to be a powerful and effective tool for TP53 mutation detection.

INTRODUCTION

TP53 mutations are the most common genetic alterations in human malignancies. About 570 different TP53 mutations have been identified by analysis of more than 8000 human cancers since 1989 (1). Most mutations were identified by conventional methods such as SSCP3 and DNA sequencing. Other methods, such as denaturing gradient gel electrophoresis, heteroduplex analysis, and cleavage methods (2), have also been used. In general, these traditional gel-based sequencing methods are relatively time-consuming, labor-intensive, sequential processes. Relatively few studies have analyzed the entire coding sequence from exons 2 through 11. Therefore, current estimates of TP53 alterations and its mutational spectrum may be incomplete. A more accurate and rapid method would provide more complete information in future studies of TP53. Methods based on hybridization of test DNA or RNA with multiple, defined oligonucleotides or cDNA probes attached to a solid glass or nylon matrix have been developed and are referred to as “oligonucleotide microarrays” or “DNA microarrays” or “gene chips.” By analyzing different hybridization patterns or levels between control and test DNA or RNA, oligonucleotide microarrays have been used for the analysis of known genes (such as TP53, BRCA1, the ataxia-telangiectasia gene, the cystic fibrosis transmembrane conductance regulator gene, HIV reverse transcriptase and protease genes, and the cystochrome P450 gene), de novo DNA sequencing, comparative sequence analysis, and gene expression studies (3–5). However, relatively little is known about the sensitivity and specificity of microarray methods compared with gel-based DNA sequence analysis. In this study, we compared TP53 mutations detected by conventional gel-based DNA sequence analysis with those identified by oligonucleotide microarray (p53 GeneChip) in 108 ovarian cancers. The relationship between TP53 mutations and patient survival was also evaluated.

MATERIALS AND METHODS

Tumor Specimens. Clinical information and TP53 alterations identified by SSCP and conventional sequence analysis from most of these cases has been reported separately (6). The 108 ovarian carcinomas studied here for TP53 alterations included 77 serous carcinomas, 5 mucinous carcinomas, 12 endometrioid carcinomas, 5 clear cell carcinomas, 7 mixed epithelial carcinomas, and 2 undifferentiated ovarian adenocarcinomas.

DNA Extraction. Genomic DNA from 108 frozen ovarian epithelial carcinomas, available through the University of Southern California Tumor and Tissue Bank, was analyzed for TP53 mutations. Frozen tissue sections stained with H&E were used to confirm that the tumor tissue selected for analysis was composed predominantly of tumor cells. DNA was extracted from 10 to 20 serial frozen tissue sections (10 μm thick) of the tumor collected in Eppendorf tubes. The extraction solution consisted of 300 μl of 10 mM Tris-HCl, 25 mM EDTA, 100 mM NaCl, 0.5% SDS, and Proteinate K (0.1 mg/ml) incubated overnight at 50°C. After complete digestion, DNA was purified by centrifugation after deproteinization with phenol/chloroform/isoamyl alcohol (50:49:1) treatment and precipitation with ethanol and sodium acetate (3 M; pH 5.2) overnight at −20°C. The DNA yield was determined by spectrophotometry and analyzed by SSCP, DNA sequence analysis (6), automated DNA sequence analysis (7, 8), and p53 GeneChip assay as described below.

SSCP. The PCR was used to amplify each of the exons contributing to the open reading frame of the TP53 gene. Each of the oligonucleotide primer pairs was designed to span not only the exon of interest but also sufficient flanking intron sequence so that splice junction mutations would be included for analysis. The sequence for each primer pair is described elsewhere (6). Each exon of TP53 was amplified by the PCR technique through 35 reaction cycles in a thermal cycler using 100 ng of genomic DNA, 4 mM deoxynucleotide triphosphates, 6 μCi of [32P]dATP, 6 μCi [32P]dCTP, and 25 pmol of the appropriate oligonucleotide primer pair. Conformational differences in the PCR products were resolved on nondenaturing mutation detection enhancement polyacrylamide gels with the addition of 5% glycerol at room temperature. All samples identified by SSCP as having altered mobility were further characterized by DNA sequencing for the exon putatively identified as mutated. In previous studies from our laboratory, SSCP has had an 85% sensitivity and a 98% specificity for TP53 mutations (6).

Manual DNA Sequencing. DNA segments identified as having altered mobility by SSCP were evaluated by manual DNA sequence analysis. Ovarian

1 Received 6/23/99; accepted 3/17/00.

2 Supported in part by Grants CA48780 and CA50589 from the National Cancer Research Institute, Grant DAMD17-94-4234 from the United States Army Medical Research and Materiel Command and a California SEER Registry Special Study grant.

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4 The abbreviations used are: SSCP, single-strand conformational polymorphism.
carcinoma template DNA was reamplified with the appropriate PCR primer pair, and amplified PCR product was purified (PCR Purification kit; Qiagen, Inc.). Both the sense and antisense strands were analyzed by the dyeoxonucleotide chain termination technique with PCR sense and antisense primers, which were end-labeled using polynucleotide kinase. The products of these sequencing reactions were then separated by electrophoresis on 6% denaturing polyacrylamide gels (National Diagnostics, Atlanta, GA).

**Automated DNA Sequence Analysis of All SSCP-negative Samples.** Ovarian carcinoma cases with no mobility shift identified by SSCP screening were subjected to complete DNA sequence analysis of each exon contributing to the TP53 open reading frame (exons 2–11) by automated DNA sequence analysis, as described elsewhere (7), to identify those mutations that SSCP failed to identify.

**p53 GeneChip Assay.** Oligonucleotide microarrays are manufactured using light-directed combinatorial chemistry. In the context of the p53 GeneChip, the synthesis cycles were repeated until oligonucleotides of ~18 bases in length were constructed. Approximately 65,000 different oligonucleotide probes were synthesized in a 1.2 × 1.2-cm area (grid) consisting of 256 cells in each dimension. Each probe cell (50 μm × 50 μm) was arranged and constructed to accommodate 10² copies of each oligonucleotide. These probes were designed to interrogate each base of exons 2–11 of the human TP53 coding sequence (~1262 bases) and +2/-2 splice sites in a standard tiling format as well as a redundant tiling format for both sense and antisense strands (Fig. 1A). The first format, standard tiling, was the compilation of complementary probes designed to interrogate the normal sequence and every possible single-base mismatch, single-base deletion, and +2/-2 splice site junctions along the coding region of the TP53 gene. More specifically, probes in the standard tiles had a common substitution position located at the twelfth base from the 3’ end. Each probe set represents A, C, G, T, a 1-bp deletion, and an empty cell for background subtraction (Fig. 1B). Five probes per sense and antisense directions were arranged for each nucleotide position (Fig. 1C). The second format, redundant tiling, was designed to interrogate over 300 common mutations reported more than once in the TP53 database (9), with the exception of deletions or insertions ≥1 bp. Each redundantly tiled mutation had 12 probes (6 sense and 6 antisense) designed to interrogate the mismatch. The substitution position was placed at different locations on the probe for maximum hybridization and discrimination of the mutant target. The hybridization pattern and intensity was then determined by laser scanner and analyzed by software based on the mixture detection algorithm. When a mutation occurred, the software called the mutation according to the codon in which the mutation existed. Probes from sense and antisense strands in both tiling formats were used to generate a confidence score for mutations. Mutations that occurred in codons with redundantly tiled nucleotides, in addition to the standard tiling, had the highest confidence score. These scores were higher because of the increased number of probes available to calculate the average intensity from each probe cell. Confidence scores (GeneChip score) ranged from 1 to 36. A score of 36 was the highest indicator that a mutation was present. Cases with scores <10 were regarded as wild-type TP53. Through experience prior to initiating this study, we found that samples that showed alternative tiling and on initial processing scored <10 were associated with only standard tiling when processed a second time. These samples proved to be false-positive samples in our pilot investigations with non-study samples. No false-positives produced an alternative tiling pattern when processed on an array a second time.

Tumor DNA from all 108 specimens was analyzed by p53 GeneChip Assay (Affymetrix). Each sample DNA was PCR amplified, fragmented with DNase, labeled with Fluorescein-N6-ddATP (DuPont NEN, Boston, MA) by way of a terminal transferase reaction and hybridized to a p53 GeneChip Array. Fluorescently labeled fragmented DNA samples were washed over the chip and allowed to bind to complementary oligonucleotide probes. Hybridized probe arrays were then read using the GeneArray Scanner (Hewlett-Packard, HP G2500A). As a quality assurance step, a control oligonucleotide was also added to each sample during hybridization to examine the signal intensity and proper alignment of the probe array after the scan. Prior to the collection of image data, the scanner confirmed the correct position and alignment of the chip by focusing on a series of defined positions.

To account for any variations that occurred during the assay, each sample batch was processed with human placental DNA as a wild-type control (Sigma Chemical Co., St. Louis, MO). Any sequence mismatch present in sample DNA was identified by comparison to the control placental DNA (Fig. 1B).

**Repeat DNA Sequencing.** Automated and/or manual DNA sequencing was used to confirm each mutation identified by the p53 GeneChip assay. In addition, six samples that were wild type by p53 GeneChip but mutated by conventional gel-based assays were also re-analyzed as mutated with automated sequence analysis. Fourteen samples, initially wild type by the above “conventional” gel-based DNA sequence analyses but mutated by p53 GeneChip analysis, were also re-analyzed to confirm the presence of these mutations in either the manual sequence analysis or automated sequence analysis method or in both. Samples were sequenced on an ABI 377 sequencer using the ABI Prism DRhodamine Dye Terminator Cycle Sequencing kit. Each reaction was performed under the conditions outlined by the manufacturer (Perkin-Elmer-Applied Biosystems, Inc., Foster City, CA). Sequencing primers spanned exons 2–11 of the TP53 gene. Samples were analyzed using the ABI Sequence Navigator Software (version 1.0.1). It was not considered necessary to use a fourth method to confirm the presence of mutations in these samples because re-analysis with either of the “conventional” methods, manual sequencing or automated sequencing, did eventually demonstrate the mutations.

**Statistics.** Statistical analyses were conducted using the SAS and Epilog software packages. The differences between TP53 mutations identified by conventional sequence analysis and TP53 mutations identified by p53 GeneChip were evaluated using Fisher’s exact test. Differences in overall survival according to TP53 status were assessed by log-rank test. Cox proportional hazards analyses were conducted to assess the joint effects of TP53 alterations, age, grade, stage, and histological type of carcinoma on risk of ovarian cancer death.

**RESULTS**

**TP53 Mutations Identified by Conventional SSCP and DNA Sequence Analysis.** Among the 108 DNA samples of ovarian carcinoma analyzed, 54 cases were identified by gel shift with SSCP screening of PCR products, and 53 of these SSCP alterations were confirmed by manual gel-based DNA sequence analysis. All ovarian tumor DNAs that lacked SSCP alterations were subjected to complete automated DNA sequence analysis of exons 2–11 (7), and an additional 10 mutations were identified. The 63 cases with mutations, identified by these “conventional” DNA sequence methods, included 57 cases with single-bp substitution mutations, 3 with deletion mutations, and 3 with combined deletion and insertion mutations (Table 1). Among the 57 substitution mutations were 49 missense mutations, 4 nonsense mutations, and 4 splice junction mutations. In addition, 4 cases contained DNA polymorphisms. DNA from 41 cases showed no sequence alteration. Those cases with DNA polymorphisms and those cases with no sequence alterations were both considered to have wild-type TP53 sequences (45 cases; 42%).

**TP53 Mutations Identified by Oligonucleotide Microarray.** Among the 108 ovarian carcinoma DNA samples, 71 (66%) had GeneChip scores for at least one position that was between 10 and 36 and were, therefore, identified as mutated. Thirty-seven (34%) cases were identified as having wild-type TP53 gene with either a GeneChip score of <10 or a DNA polymorphism identified. The identified 71 ovarian tumors with mutations included 69 with single-bp substitution mutations and 2 with single-bp deletion mutations. Among the 69 ovarian tumors with substitution mutations were 56 with missense mutations, 7 with nonsense mutations, and 6 with splice junction mutations. Three ovarian tumors had two TP53 mutations. All three cases had two single-bp substitution mutations for a total of 72 single-bp substitution mutations identified among cases in this cohort. One case with two mutations had both a missense and a splice junction mutation, and the other two cases each had two missense mutations identified. Because no effort was made to characterize more than one mutation using the “conventional” DNA sequence analysis

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Fig. 1. Arrangement of p53 GeneChip (A) and examples of sequence analysis at a particular nucleotide (B and C). A, p53 GeneChip architecture. Approximately 65,000 different oligonucleotide probes were synthesized in a 1.2 × 1.2-cm area. These probes interrogated each base and flanking intron sequences of exons 2–11 of the human TP53 gene in a standard tiling format as well as a redundant tiling format for both sense and antisense strands. B, examples of oligonucleotide probe hybridization in wild-type target (left) and mixture target (right). Hybridization signal intensity for each individual tile of each nucleotide position can be ordered from high signal intensity to low signal intensity as follows: white > green > blue > black. The highest fluorescent tile indicates hybridization with the complementary nucleotide sequence. In the first illustration (wild type, left picture), the fluorescent green signal identified within the indicated area represents the strongest hybridization of the normal A nucleotide to a “T” nucleotide at the queried position. In the second illustration (wild type/mutant mixture, right picture), the fluorescent green signal identified within the indicated area represents hybridization of mutated G nucleotide in test DNA to a “C” nucleotide at the queried position. The weaker blue hybridization signal of wild-type A to the “T” nucleotide in the oligonucleotide probe is also shown, demonstrating lower signal than observed with wild-type sample (left), which shows a green signal. The white signal at multiple tiles in that nucleotide position for both wild-type and mutant target represents the hybridization by control oligonucleotide, which is shown as a white straight dotted line on the grid in A. C, hybridization signal intensities for test DNA at three queried positions in sense and antisense directions. Each probe in a tile set is perfectly complementary to the reference sequence, except for a mismatch at the 12th nucleotide position. At this position, each of the four possible nucleotide substitutions A, C, G, and T and a single-bp deletion are represented in the probe set. Sense and antisense probe hybridization data and probe set relative hybridization intensities are shown in individual columns using five different colors for each nucleotide position analyzed. The relative hybridization intensities for each nucleotide at a queried position permit a nucleotide identification call to be made. The wild-type base A (left) and mixture (mutant/wild type) A/G (right) correspond to the images of B (left) and B (right).
methods described above, only one of the two mutations identified in these three ovarian tumors by p53 GeneChip was included in the comparative evaluation of the methods (Table 1). In addition, among seven cases with a TP53 polymorphism, five also had a missense mutation. These cases were considered in the analysis as showing a single missense substitution.

Comparison of TP53 Mutations Identified by Oligonucleotide Microarray and Conventional DNA Sequence Analysis. A total of 77 ovarian cancers had TP53 mutations identified by at least one of the two approaches. The 77 tumors with identified mutations included 71 with single-bp substitution mutations, 3 with deletion mutations, and 3 with combined deletion/insertion mutations (Table 1). Among the 71 ovarian tumors with substitution mutations were 58 with missense mutations, 7 with nonsense mutations, and 6 with splice junction mutations.

Both methods identified a mutation in 57 ovarian cancers and no mutation in 31 ovarian cancers for a concordance rate of 81% (Table 2). As described previously, 71 ovarian tumors with mutations (71 of 108; 66%) were identified by DNA microarray and 63 (63 of 108; 58%) by conventional gel-based DNA sequence analysis. There were 6 cases identified by conventional DNA sequence analysis but not by DNA oligonucleotide microarray. There were 6 cases identified by DNA oligonucleotide microarray but not by conventional DNA sequence analysis (Table 4). These cases were all resequenced and confirmed as mutated by manual or automated DNA sequence analysis or both.

In this cohort of ovarian tumors, one case had an exon 4 (GTG→TTG) Val73Leu missense mutation, which was identified by conventional DNA sequence analysis but not by p53 GeneChip analysis (Table 3). The cohort contained two identical Lys132Arg missense mutations (AAG→AGG). One of these mutations was identified by both approaches; the other was detected initially only by the oligonucleotide microarray approach (Table 4). A Cys141Trp (TGC→TGG) missense mutation, missed by conventional DNA sequence analysis, was identified by p53 GeneChip analysis (Table 4). A nonsense Gln167Stop (CAG→TAG) mutation was identified by p53 GeneChip analysis but not by conventional DNA sequence analysis (Table 4).

One of two mutations involving codon 195 was detected by conventional DNA sequence analysis but not by p53 GeneChip. The other mutation at codon 195 was detected by p53 GeneChip but not by conventional DNA sequence analysis. The codon 195 mutation detected by conventional DNA sequence analysis but not by p53 GeneChip analysis was a deletion/insertion mutation involving codons 193–195 (9-bp deletion of CATCTTATC with a 6-bp insertion of GCCCTC, which encoded a deletion of His, Leu, and Ile and an insertion of Ala, Pro; Table 3). The codon 195 mutation detected by p53 GeneChip but not by conventional DNA sequence analysis was an Ile195Asn (ATC→AAC) missense mutation (Table 4). Neither of two Arg196stop (CGA→TGA) mutations were detected by conventional DNA sequence analysis, but both were detected by p53 GeneChip. Missense substitutions at codons 220 and 237 were identified with oligonucleotide microarray analysis but not with conventional DNA sequence analysis (Table 3). On the other hand, a missense mutation at codon 267 (CGG→CCG; Arg267Pro) was detected by conventional DNA sequence analysis but not by p53 GeneChip analysis (Table 4).

Table 1 Classification of TP53 mutations identified by conventional DNA sequence analysis and DNA oligonucleotide microarray*.

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>Sequencing</th>
<th>Microarray</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-bp substitutions</td>
<td>57</td>
<td>69</td>
<td>71</td>
</tr>
<tr>
<td>Missense</td>
<td>49</td>
<td>56</td>
<td>58</td>
</tr>
<tr>
<td>Nonsense</td>
<td>4</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Splice junction</td>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Deletion and/or insertion</td>
<td>6</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Single-bp deletion</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Multiple bp deletion</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Deletion and insertion</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>71</td>
<td>77</td>
</tr>
</tbody>
</table>

* Cases containing more than one TP53 mutation had the second mutation excluded from this comparison because no attempt was made to characterize more than one TP53 mutation by conventional DNA sequence analyses.

Table 2 Comparison of TP53 mutations identified by conventional DNA sequence analysis and DNA oligonucleotide microarray analysis.

<table>
<thead>
<tr>
<th>Mutations detected by sequencing</th>
<th>Wild-type by sequencing</th>
<th>Total no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutations detected by microarray</td>
<td>57</td>
<td>14</td>
</tr>
<tr>
<td>Wild-type by microarray</td>
<td>6</td>
<td>31</td>
</tr>
<tr>
<td>Total no.</td>
<td>63</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 3 TP53 mutations detected by conventional DNA sequence analysis but not identified by DNA microarray analysis.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Genomic location</th>
<th>Codon</th>
<th>DNA sequence change</th>
<th>Predicted amino acid changes</th>
<th>Mutation type</th>
</tr>
</thead>
<tbody>
<tr>
<td>715</td>
<td>Exon 4</td>
<td>73</td>
<td>GTG→TTG</td>
<td>Val→Leu</td>
<td>Missense</td>
</tr>
<tr>
<td>805</td>
<td>Exon 6</td>
<td>219</td>
<td>Deletion of CCC, Insertion of GTGTTC</td>
<td>Loss of Pro, Insertion of Val, Phe</td>
<td>Deletion/Insertion</td>
</tr>
<tr>
<td>2719</td>
<td>Exon 6</td>
<td>193–195</td>
<td>Deletion of CATCTTATC, Insertion of GCCCTC</td>
<td>Loss of His, Leu, Ile, Insertion of Ala, Pro</td>
<td>Deletion/Insertion</td>
</tr>
<tr>
<td>2001</td>
<td>Exon 7</td>
<td>247–251</td>
<td>Deletion of AACCGGAGGCCCATC, Insertion of GGCC</td>
<td>Truncated protein</td>
<td>Frameshift/Nonsense</td>
</tr>
<tr>
<td>2332</td>
<td>Exon 8</td>
<td>267</td>
<td>CGG→CCG</td>
<td>Arg→Pro</td>
<td>Missense</td>
</tr>
<tr>
<td>696</td>
<td>Exon 8</td>
<td>264–265</td>
<td>Loss of ACT</td>
<td>Loss of Leu</td>
<td>Deletion</td>
</tr>
</tbody>
</table>
sequence analyses ($P = 0.0024$, Fisher exact test). However, TP53 oligonucleotide microarray detected only two of the six deletion and/or insertion mutations, whereas conventional DNA sequence analyses detected all six. This difference was marginally statistically significant ($P = 0.06$, Fisher exact test).

Of the 49 missense mutations identified by conventional DNA sequencing, DNA microarray analysis detected 47 (96%). Of the four splice junction mutations identified by conventional DNA sequence analysis, microarray technology detected all of them (100%). Of the seven cancers with a premature stop codon identified by conventional DNA sequencing, oligonucleotide microarray analysis detected six, including four nonsense mutations caused by single-bp substitutions and two frameshift mutations caused by single-bp deletions. Among the four cases that had multiple-bp deletions and/or insertions, microarray analysis missed all of them, whether or not the mutation led to a frameshift. In conclusion, microarray achieved a 94% accuracy (102 of 108), a 92% sensitivity (71 of 77), and a 100% (31 of 31) specificity in mutation detection. Conventional DNA sequence analysis demonstrated an 87% (94 of 108) accuracy rate, an 82% (63 of 77) sensitivity, and a 100% (31 of 31) specificity.

**Relationship between TP53 Alterations and Survival.** One hundred and four of the 108 patients with survival data available were analyzed for the relationship between TP53 mutation and overall survival. The presence of TP53 mutations was associated with shorter survival ($P = 0.02$; Fig. 2A). Women with mutations in loop2, loop3, or the loop-sheet-helix domain had a significantly shorter survival than women who had other mutations in their ovarian cancers and women who had no mutations in their ovarian cancers ($P = 0.01$; Fig. 2B). Age (Trend test, $P = 0.03$), grade ($P = 0.02$), and stage ($P = 0.002$) were also associated with shortened overall survival. Mucinous histopathology was associated with a lack of TP53 mutations ($P = 0.023$) but not with survival. Serous (versus all others) tumors were not associated with overall survival ($P = 0.94$). Cox proportional hazards analysis, adjusting for the other factors such as size, stage, and grade, suggested that TP53 was an independent predictor of shortened overall survival after including these factors in the analysis. However, formal statistical significance was not demonstrated ($P = 0.09$), probably because of the limited sample size.

**DISCUSSION**

This study was designed to assess the sensitivity, specificity, and accuracy of DNA microarrays (p53 GeneChip) in identifying TP53 mutations relative to conventional gel-based DNA sequence analysis. Overall, the two methods showed a high level of concordance, with 88 of the 108 tumors (81%) showing the same result. With the inclusion

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Table 4  
**TP53 mutations identified by DNA microarray but not by conventional DNA sequence analysis**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Genomic location</th>
<th>Codon</th>
<th>DNA sequence change</th>
<th>Amino acid change</th>
<th>Mutation type</th>
<th>TP53 geneChip score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1748</td>
<td>Exon 5</td>
<td>132</td>
<td>AAG→AGG</td>
<td>Lys→Arg</td>
<td>Missense</td>
<td>26</td>
</tr>
<tr>
<td>2348</td>
<td>Exon 5</td>
<td>141</td>
<td>TGC→TGG</td>
<td>Cys→Trp</td>
<td>Missense</td>
<td>16</td>
</tr>
<tr>
<td>1566</td>
<td>Exon 5</td>
<td>167</td>
<td>CAG→TAG</td>
<td>Gln→stop</td>
<td>Nonsense</td>
<td>11</td>
</tr>
<tr>
<td>2741</td>
<td>Exon 6</td>
<td>195</td>
<td>ATC→AAG</td>
<td>Ile→Asn</td>
<td>Missense</td>
<td>24</td>
</tr>
<tr>
<td>2729</td>
<td>Exon 6</td>
<td>196</td>
<td>CGA→TCA</td>
<td>Arg→stop</td>
<td>Nonsense</td>
<td>11</td>
</tr>
<tr>
<td>2733</td>
<td>Exon 6</td>
<td>196</td>
<td>CGA→TGA</td>
<td>Arg→stop</td>
<td>Nonsense</td>
<td>12</td>
</tr>
<tr>
<td>712</td>
<td>Exon 6</td>
<td>220</td>
<td>TAT→TGT</td>
<td>Tyr→Cys</td>
<td>Missense</td>
<td>31</td>
</tr>
<tr>
<td>690</td>
<td>Intron 6</td>
<td></td>
<td>Splice acceptor</td>
<td>–Z: a→g</td>
<td>NA*</td>
<td>Splice junction</td>
</tr>
<tr>
<td>2347</td>
<td>Exon 7</td>
<td>237</td>
<td>ATG→ATG</td>
<td>Met→Ile</td>
<td>Missense</td>
<td>11</td>
</tr>
<tr>
<td>835</td>
<td>Intron 7</td>
<td>273</td>
<td>CGT→TGT</td>
<td>Arg→Cys</td>
<td>Missense</td>
<td>12</td>
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<tr>
<td>2735</td>
<td>Exon 8</td>
<td>273</td>
<td>CGT→TGT</td>
<td>Arg→Cys</td>
<td>Missense</td>
<td>12</td>
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<td>993</td>
<td>Exon 8</td>
<td>273</td>
<td>CGT→TGT</td>
<td>Arg→Cys</td>
<td>Missense</td>
<td>11</td>
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<td>2311</td>
<td>Exon 8</td>
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<td>CGT→TGT</td>
<td>Arg→Cys</td>
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<td>11</td>
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<tr>
<td>2327</td>
<td>Exon 8</td>
<td>273</td>
<td>CGT→TGT</td>
<td>Arg→Cys</td>
<td>Missense</td>
<td>11</td>
</tr>
</tbody>
</table>

*NA, not applicable.

Fig. 2. Cumulative probability of survival for women with ovarian carcinomas showing differences in TP53 tumor suppressor gene status. A, women whose ovarian cancer had TP53 mutation (—) showed a significantly shorter overall survival ($P = 0.02$) than women whose ovarian cancer had wild-type TP53 (——). B, a significantly shorter survival ($P = 0.01$) was observed for women whose ovarian cancer had mutations located in the highly conserved loop-sheet-helix, loop2, or loop3 domains of TP53 (—), compared with women whose ovarian cancer had mutations located in any other site in TP53 (-----) or in women whose ovarian cancer had wild-type TP53 (-----).
of 14 mutations initially not identified with conventional sequence analysis, the DNA microarray achieved an accuracy of 94% (102 of 108), whereas conventional DNA sequence analysis, including the six mutations not identified with p53 GeneChip analysis, achieved an accuracy of 87% (94 of 108) relative to the final TP53 status determined from assessment of both approaches together. In addition, three cases were identified with double mutations, two with double missense mutations, and one with a missense mutation and a splice junction mutation. Microarray analysis also detected polymorphisms in seven cases, five of which had coexistent missense mutations.

These observations provided an estimate of the frequency of cases in seven cases, five of which had coexistent missense mutations. The prevalence of deletions and insertions in these ovarian cancers occurred predominantly in exons 5–8. Nineteen (25%) mutations were in exon 5, 11 (14%) mutations were in exon 6, 15 (20%) mutations were in exon 7, 24 (31%) mutations were in exon 8. Six (8%) mutations were located in introns at splice junctions, and only one mutation was identified in exon 4 (1%) and exon 9 (1%). Most of the studies analyzing the entire open reading frame of TP53 (14–18) in ovarian cancer had a limited number of cases analyzed. Two of the five studies (19, 20) that analyzed more than 60 cases identified 15 and 18% of mutations outside exon 5–8, with a predominance of deletion/insertion mutations in these regions. However, we did not find a similar distribution in our study. Because we confirmed all SSCP-positive cases by manual sequencing and rescreened SSCP-negative cases by automated DNA sequencing, as well as reanalyzed all DNAs by a p53 GeneChip assay, it is unlikely that a significant number of mutations were missed. Therefore, our estimate of TP53 mutations in ovarian cancer is considered to be representative. The difference in mutation spectrum among studies might be attributed to environmental mutagenic exposure and/or endogenous factors, such as genetic differences, that contribute to carcinogenesis.

The most frequently mutated codons in our study were codon 273 (seven mutations), codon 179 (four mutations), and codon 234 (four mutations). Two of these (codon 179 and codon 273) are also “hot-spots” of tobacco-associated lung cancers, but most of the mutations (10 of 12; 83%) in these two codons in ovarian cancer were transition mutations rather than transversion mutations as observed in lung cancer (19). On the other hand, mutations frequently related to benzo(a)pyrene exposure in lung cancer, such as mutations in codon 157 (GTC→TTC), codon 248 (CGG→CTG), or related to aflatoxin exposure in liver cancer, such as mutations in codon 249 (AGG→AGT), were not identified. The higher overall percentage of transition (60%) compared with transversion mutations (32%) in our study suggest that most ovarian cancers arise spontaneously rather than because of exogenous carcinogen exposure.

The significance of TP53 alteration as a prognostic factor in ovarian cancers varies among different studies (reviewed in Ref. 6). The factors that cause variable results include insufficient samples, differ-
ence in screening methods, and incomplete analysis of the open reading frame. In our study, we tried to minimize these factors by using both conventional gel-based DNA sequencing and microarray analysis to analyze mutations in the entire open reading frame of TP53 in 108 ovarian carcinomas: (a) the high percentage (77 of 108; 71%) of TP53 mutations indicates that TP53 function is abrogated mostly by mutation of the gene itself in ovarian cancer, although other nonmutational mechanisms, such as defects in TP53 pathway that mediate its function or loss of mechanisms that activate TP53 (20), could be involved in the pathogenesis of those cancers that have wild type TP53; (b) the DNA-binding structural motifs (loop-sheet-helix, loop2, and loop3) of TP53 overlap almost exclusively with the conserved regions of the protein sequence, where the majority of mutations are found and affect its sequence-specific DNA binding activity (21). These “hot-spot” amino acids are highly conserved and are believed to represent regions of structural or functional importance (22). In our analysis, TP53 mutation was shown to be a predictor of overall survival in ovarian cancer. Patients with mutations in loop2, loop3, or the loop-sheet-helix domain had shorter survival than patients with mutations in other locations or no mutation. This observation supports the current concept that these sequence-specific DNA binding domains are functionally important, and mutations in these areas could have more deleterious effects as opposed to other mutant and/or wild-type TP53. Further in vivo functional characterization, such as the biological activity of these mutants, would provide more information.

A few possible mechanisms of false-negative and false-positive mutations detected by DNA microarray have been described. These include microarray design, imperfect hybridization conditions, and molecular interactions on microarrays (23, 24). The addition of more probes, such as redundant tiling based on common mutations related to deletion/insertion mutations, as well as other mutations, is expected to improve the sensitivity of the microarray in overall detection. In addition, enzyme approaches, such as polymerase and ligation, have also been adapted for use with microarray (24). It has been suggested that deletion and insertion would lead to the formation of energetically favored duplexes containing bulged nucleotides with wild-type probes over duplexes containing single-bp mismatches (23). All of these possibilities suggest that microarray detection can be improved. Although sequencing the entire coding region is a sensitive technique for overall detection of mutations, it is more time consuming and labor intensive than analysis by DNA microarray technology. For example, intensive analysis requires the amplification of 11 PCR products (exon 4 is split in two amplicons), 22 sequencing lanes, and the manual examination of over 1262 bases. The high costs and increased turnaround times impedes the usage of this technology on a regular basis. On the other hand, the time from purified DNA to data analysis was ~4.5 h for the p53 GeneChip. The average batch size/day was 12 samples, including a reference sample and negative control (blank). In conclusion, although there was a high concordance rate between the two methods, the p53 GeneChip assay detected more mutations, had greater overall accuracy for detection of sequence alterations, and was more effective in terms of time and cost.

ACKNOWLEDGMENTS

We thank Ivonne Villalobos, Norris Cancer Center, University of Southern California School of Medicine, for preparing the manuscript and Dr. Timothy Triche for the assistance of the Children’s Cancer Group Microarray Core Facility at Children’s Hospital Los Angeles, USC/ Norris Comprehensive Cancer Center.

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