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Immunofluorometric Assay of p53 Protein Versus Sequencing of p53 Exons 5 to 9 for the Detection of p53 Abnormalities in Ovarian Carcinoma

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Abstract. *p53 alteration, detected as mutation of the p53 gene or as accumulation of mutant p53 protein, is a common feature of most malignancies, including ovarian carcinoma, and may identify patients with unfavorable prognosis and resistance to chemotherapy. Tumor tissues from 55 patients with well or poorly differentiated (grades 1 or 3) primary epithelial ovarian carcinoma were assessed both for p53 protein overexpression by a sensitive time-resolved immunofluorometric assay employing DO-1 and CM-1 antibodies, and for genetic p53 abnormalities by direct sequencing of PCR-amplified exons 5 to 9. Sixteen p53 mutations (29%), including 3 deletions causing frameshifts as well as one nonsense and 12 missense point mutations were found in all exons except exon 9. Overexpression of p53 protein, defined as a concentration exceeding the 75th percentile, was found in 15 cases (27%), 10 of which had missense mutations ($P<0.01$). Tumors with nonsense and frameshift mutations were p53-negative by immunoassay. Both p53 mutation ($P=0.04$) and p53 protein accumulation ($P<0.01$) were associated with stage III-IV disease, while p53 mutation was more closely related to grade 3 lesions ($P=0.04$) and serous histotype ($P=0.01$). These results indicate that p53 protein accumulation correlates well with missense point mutation in carcinoma of the ovary and, together with other evidence that p53 abnormality may be prognostic of outcome in this disease, suggest that the immunoassay of p53 protein may have clinical value.*

Mutation of the chromosome 17p13-localized p53 gene has been reported with high prevalence in a wide variety of human neoplastic diseases [1], including epithelial ovarian carcinoma in which 30-70% of cases may harbor p53 mutational changes [2-8]. These alterations are typically missense mutations accompanied both by loss of heterozygosity [3-5,7] and mutant p53 protein accumulation [2,4,6], although deletions, insertions, and other genetic rearrangements affecting the p53 locus may also occur at greater frequencies in ovarian cancer than in many other malignancies [9]. Given the abundant evidence of the importance of p53 protein in the negative regulation of cellular growth in response to DNA damage, through mechanisms involving cell cycle arrest and apoptosis [10,11], mutational abrogation of p53 function would be expected to lead to dysregulated cell growth and tumor formation. This is supported by the relative paucity of p53 mutations or chromosome 17p losses in benign or borderline ovarian tumors compared to frankly malignant lesions [12-14], in which the p53 mutation rate may be increased in higher stage [8,15] and grade [6,8,16] tumors and associated with DNA aneuploidy [2], high proliferative activity [16], and serous histologic type [15-17]. Of great clinical interest are recent findings implicating p53 in the induction of apoptosis following exposure to a number of cancer chemotherapeutic agents; ovarian tumor cell lines and *in vivo* lesions with mutated p53 are frequently chemoresistant [18-20] and associated with reduced patient survival [16,20].

The close correlation between p53 genetic abnormalities and p53 protein overexpression has facilitated the use of simple and rapid immunohistochemical techniques to study the diagnostic and prognostic implications of p53 mutation in ovarian cancer. Complete DNA sequencing of the p53 gene, or as more commonly performed, of exons 5 to 9 within which up to 80% of the mutations occur, has been shown to offer greater sensitivity for the detection of mutations [21] than

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Table I. *Primers for PCR amplification and DNA sequencing of p53 exons 5 to 9.*

Exon	Strand	PCR primer pair	Sequencing primers
5	Sense	5′-CACTTGTGCCCTGACTTT-3′	5′-TCTTTGCTGCCGTGTTCC-3′
	Antisense	5′-CCTGGGGACCCTGGGCAA-3′	5′-CCTGGGACCCGTTGGTCG-3′
6	Sense	5′-TGTTCACTTGTGCCCTGACT-3′	5′-TGGTTGCCCAGGGTCCCC-3′
	Antisense	5′-GGAGGGCCACTGACAACCA-3′	5′-CCACCCTTAACCCCTCC-3′
7	Sense	5′-GGCGACAGAGCGAGATTCCA-3′	5′-CTCCCCTGCTTGCCACA-3′
	Antisense	5′-GGGTCAGCGGCAAGCAGAGG-3′	5′-TCAGCGGCAAGCAGAGG-3′
8	Sense	5′-GACAAGGGTGGTTGGGAGTAGATG-3′	5′-ATGGGACAGGTAGGACC-3′
	Antisense	5′-GCAAGGAAAGGTGATAAAAGTGAA-3′	5′-CATAACTGCACCCTTGG-3′
9	Sense	5′-GCGGTGGAGGAGACCAAGG-3′	5′-GGAGGAGACCAAGGGTGC-3′
	Antisense	5′-AACGGCATTTTGAGTGTTAGAC-3′	5′-GGAAACTTTCCACTTGA-3′

either indirect mutational analysis (such as single-strand conformation polymorphism analysis (SSCP), constant denaturant gel electrophoresis (CDGE) or other screening techniques) or immunohistochemical staining; however, the method remains laborious despite the widespread use of automated sequencing instruments. The majority of studies have therefore employed immunohistochemical methods. However, improved immunostaining in terms of sensitivity, specificity, and reproducibility for the detection of p53 protein in tumor tissue may be possible through quantitative immunoassays of p53, several of which have been developed and applied to extracts of various tumor types [22-24], including ovarian carcinoma [25]. Comparisons between the findings of such ELISA (enzyme-linked immunosorbent assay)-type assays of p53 protein performed in parallel with p53-immunostaining of breast [23], lung [26], gastrointestinal [27], and ovarian [25] tissues have demonstrated the general concordance between the two approaches. The evaluation of immunoassays of p53 protein have not yet included comparisons of tumoral p53 protein accumulation to the corresponding mutational status of the p53 gene ascertained by direct DNA sequence analysis. The purpose of this study was therefore to compare the findings of these two methods in a series of 55 well or poorly differentiated (grade 1 or 3) ovarian carcmomas.

Materials and Methods

Ovarian cancer patients. Fifty-five patients resected at the Department of Gynecology, Gynecologic Oncology Service of the University of Turin, Turin, Italy between November 1989 and February 1996 for treatment of primary epithelial ovarian carcinoma were included in this study. These patients constituted a subset of a larger patient population and were selected on the basis of having primary epithelial ovarian tumors of either low or high histologic grade (grade 1 or grade 3, respectively). Three patients for whom tumor specimens were available had been excluded since two had been diagnosed as having germinal ovarian neoplasms and one had had a primary colon cancer metastatic to the ovary. The age range of these patients was 20 to 79 years, with a median age of 57 years. Additional clinicopathologic variables for which the patients had been characterized at the time of surgery, including residual tumor size, stage according to the criteria of the International Federation of Gynecologists and Obstetricians (FIGO) [28] and histologic grade and type based on World Health Organization [29] criteria, were also assessed. The FIGO staging scheme assumes that an adequate staging operation, described elsewhere [25], has been performed. Twelve patients were found to have stage I disease, 3 patients were in stage II, 31 patients were in stage III, and 9 patients had stage IV ovarian cancer. Forty of the tumors were poorly-differentiated (grade 3), whereas 15 were well-differentiated (grade 1). With respect to histologic type, 3 tumors were clear cell, 12 were endometrioid, 7 were mucinous, 23 were serous, 9 were undifferentiated and one tumor had a mixed mullerian histologic type. Postsurgically, 20 patients were apparently free of residual tumor tissue, while 10 patients had residual tumor masses estimated in size to be between 1 and 4 cm in greatest diameter, 13 had masses between 5 and 8 cm in diameter, 11 had masses larger than 9 cm, and residual tumor size was unknown for 1 patient.

Tumor extraction. Immediately following surgery, a representative portion of each tumor was selected during quick-section procedures, snap-frozen in liquid nitrogen, and stored at -80°C until analysis (see below). Approximately 200 mg of each tumor tissue, which contained more than 70% tumor cells as determined by histologic examination, was pulverized to a fine powder at -80°C. DNA was extracted and purified from the tissue using a conventional phenol-chloroform-based procedure [30], quantified by absorbance measurements at 260 nm, and stored at 4°C until analysis. For p53 protein analysis, extracts were prepared as described previously [24] and assayed for total protein content by a commercially available method utilizing the bicinchoninic acid reagent (Pierce Chemical Co., Rockford, IL).

Immunofluorometric assay of p53 protein. p53 protein was quantitatively analyzed using an immunofluorometric procedure as previously described in detail elsewhere [24]. This method involves the capture of soluble p53 protein, both mutant and wild-type, onto microtiter wells

coated with monoclonal DO-1 antibody (gift of Dr. David Lane, University of Dundee, UK) and the detection of bound immunocomplexes by sequentially added polyclonal CM-1 antiserum (Novacastra Laboratories Inc., Newcastle upon Tyne, UK), alkaline phosphatase-conjugated anti-rabbit immunoglobulin (Jackson ImmunoResearch Inc., West Grove, PA), the enzyme substrate diflunisal phosphate, and finally a lanthanide metal chelate. Time-resolved fluorescence measurements were performed with a dedicated instrument (Cyberfluor Inc., Toronto, Ontario, Canada). Tumor extracts and assay calibrator solutions, ranging in concentration from 0.15 to 75 μg/L and prepared by dilutions of recombinantly expressed p53 protein, were assayed in parallel and as duplicates. Concentrations of p53 protein exceeding the detection limit of approximately 0.04 μg/L were divided by the total protein contents of the extracts to adjust for variable extraction efficiencies.

PCR amplification. The paired primer sequences flanking each of the exons 5 to 9 of the p53 gene are shown in Table I. All oligonucleotide primers were synthesized commercially (ACGT Corp., Toronto, Ontario, Canada). PCR amplification of each exon was performed in a final volume of 50 μL, containing approximately 500 ng of template DNA, 10 mmol/L tromethamine [Tris] (pH 8.3), 50 mmol/L KCl, 2 units AmpliTaq Polymerase (Hoffmann-La Roche, Basel, Switzerland), 200 μmol/L deoxynucleoside triphosphates, optimized $MgCl_2$ concentrations (1.5 mmol/L for exons 6 and 7, 2.0 mmol/L for exons 8 and 9, and 2.5 mmol/L for exon 5), and optimized concentrations of each primer (0.4 μmol/L for exons 5, 6, and 9, 0.6 μmol/L for exon 8, and 0.8 μmol/L for exon 7). The thermal cycling profile consisted of a 20 s denaturation step at 94°C, a 30 s annealing step at the optimal temperature determined for each exon (60°C for exons 5, 6, and 9, 62°C for exon 7, and 63°C for exon 8), and a 30 s extension step at 72°C, for a total of 30 cycles. Each PCR was initiated with a 3 minute denaturation at 94°C and terminated with a 3 minute extension at 72°C. Following determination of the approximate yield and purity in each case by agarose gel electrophoresis, the PCR products were incubated first at 37°C for 15 minutes with 10 units of Exonuclease I and 2 units of shrimp alkaline phosphatase (both from Amersham Life Science Inc., Arlington Heights, IL), and then incubated at 80°C for another 15 minutes to inactivate these enzymes. Dilution of the pretreated PCR products 1:5 to 1:10 preceded their sequencing.

DNA sequence analysis. The primers used for sequencing the PCR-amplified p53 exons 5 to 9 were designed using Oligo 5.0 software (National Biosciences Inc., Plymouth, MN) based on the genomic p53 sequence deposited into GenBank by Chumakov *et al* (accession # X54 156). The primers were synthesized, and labelled at the 5'-end with the fluorescent dye Cy5, at National Biosciences Inc., and their sequences are also given in Table I. The Thermo Sequenase cycle sequencing protocol (Amersham Life Science Inc.) was followed according to the manufacturer's instructions, after which the reaction products were resolved and sequenced using an ALFexpress DNA Sequencer (Pharmacia Biotech AB, Uppsala, Sweden).

Statistical analysis. The association of p53 gene mutation with other clinical or pathological variables, including patient age, stage, histologic grade and type, and residual tumor size, as well as with p53 protein concentrations dichotomized on the basis of a 75th percentile cutoff point, were examined using Chi-square or Fisher's Exact tests where appropriate. McNemar's test was also applied to the latter comparison of p53 status assigned by each of the two methods, in order to examine the distribution of discordant pairs. The medians of p53 protein concentrations in tissue extracts among different clinical or pathological groups were compared using Wilcoxon Rank Sum tests. Computer software SAS 6.12 (SAS Institute, Cary, NC) was used for these analyses, and 2-sided tests of significance were used throughout.

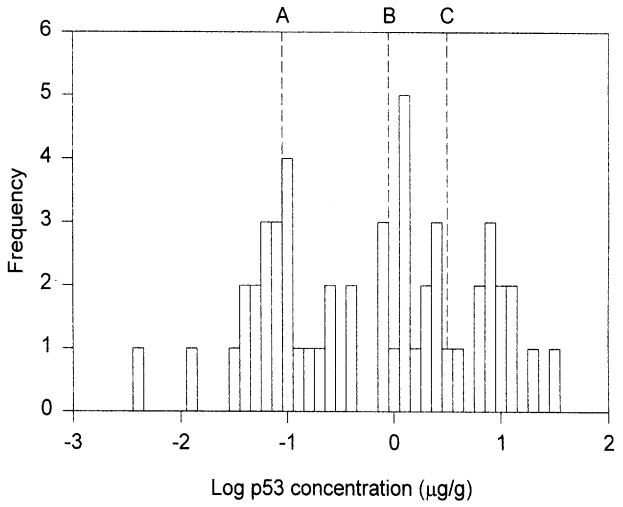

Figure 1. *Frequency distribution of logarithmically-transformed p53 protein concentrations in the 55 ovarian tumor extracts. The dashed lines A, B, and C indicate the 25th, 50th, and 75th percentiles of the distribution, respectively.*

Results

Quantitative p53 protein analysis. p53 protein concentrations above the detection limit of the immunoassay were found in all except four soluble protein extracts prepared from the 55 ovarian tumor specimens. These values ranged from 0.04 to 196.2 μg/L and had a median, standard deviation, and mean of 2.27 μg/L, 39.71 μg/L, and 19.80 μg/L, respectively. Adjustment of each p53 protein concentration to reflect the particular total protein content in each case yielded a highly skewed distribution (Figure 1) which ranged from 0.00 to 34.34 μg/g, had a mean of 3.38 μg/g and a standard deviation of 6.23 μg/g, and had 25th, 50th, and 75th percentiles of 0.08 μg/g, 0.94 μg/g, and 3.00 μg/g, respectively. The simple division of patients into two groups, p53-negative and p53-positive, was made on the basis of a cutoff point equal to the 75th percentile, at which and beyond, total protein-adjusted p53 concentrations were considered p53-positive.

Mutational analysis. Automated sequencing of the PCR-amplified exons 5 to 9 of the p53 gene in each of the ovarian carcinomas revealed mutations in 16 cases (29%) (Table II). These mutations were approximately evenly distributed across exons 5 to 8; exon 9 was found to have a wild-type sequence in every case. In addition to the three mutations detected at codon 248, missense point mutations at other p53 mutational hotspots (codons 175 and 273) were also found. One of the mutations at codon 248, as well as those detected at codons 196 and 282, represented GC→AT transitions at CpG dinucleotides. Five other mutations in specimens 5, 144, 62, 91, and 113, three of them transversions, also occurred at

Table II. *p53 mutations and corresponding p53 protein expression levels in ovarian carcinomas.*

Specimen	Exon	Codon	Nucleotide change[a]	Amino acid change	p53 protein[b]	Grade	Stage
189	5	172	GTT → TTT	Val → Phe	3.84	3	IV
5	5	175	CGC → CAC	Arg → His	3.07	3	III
8	5	175	CGC → CAC	Arg → His	0.00	3	III
205	5	183	Deletion of CAGATAGC	Truncated to 211 residues	0.11	3	II
112	6	193	CAT → GAT	His → Asp	10.76	3	III
88	6	194	CTT → CGT	Leu → Arg	7.86	3	IV
144	6	196	CGA → TGA	Arg → Stop codon	0.02	3	III
68	7	227	Deletion of TGAC	Truncated to 244 residues	0.06	3	IV
77	7	248	CGG → TGG	Arg → Trp	3.08	1	III
104	7	248	CGG → GGG	Arg → Gly	14.52	3	III
114	7	248	CGG → CTG	Arg → Leu	15.35	3	III
75	8	272	GTG → ATG	Val → Met	2.89	3	III
62	8	273	CGT → CAT	Arg → His	4.96	3	IV
91	8	280	AGA → GGA	Arg → Gly	20.31	3	III
113	8	282	CGG → TGG	Arg → Trp	8.70	3	III
175	8	292	Deletion of AAAG	Truncated to 303 residues	1.31	3	III

[a] Sequences flanking deletions given in text.
[b] p53 protein concentrations in units of μg/g; values equal to or exceeding 3 μg/g were considered p53-positive.

CpG sites encoding arginine residues. In total, substitutions affecting arginine occurred in 8 of the 12 missense cases. There was a slight predominance of transition (n=8) over transversion (n=5) mutations, and G to A or G to T substitutions accounted for 6 of the 16 mutations observed. Translational termination was encoded by a C to T transition within exon 6. Since additional noncancerous tissues were not collected from these patients, the possibility that these genetic changes may have represented germline p53 mutations could not be investigated. The same neutral genetic polymorphism, an A to G transition at the second position of codon 213 in exon 6 encoding an arginine residue, was detected in three tumor specimens. Sequence deletions, predicted to lead to premature translational termination, were also found to affect exon 5 (8 nucleotides in length), exon 7 (4 nucleotides), and exon 8 (4 nucleotides). Examination of sequences flanking the deletions revealed the presence of short repeats present also in the deleted segments: TGCTCAGATAGCGATG in exon 5; GGTTGGCTCTGACTGTAC in exon 7; and CAAGAAAGGGGAG in exon 8, where the deletion is underlined. Splice-site mutational changes in the p53 gene were not found.

Relationship between p53 gene mutation, p53 protein accumulation, and other variables. Missense p53 mutation and overexpresslon of p53 protein were closely associated, as was evident from the observation that of 12 ovarian tumors with such genetic changes, 10 were found to have p53 protein concentrations above 3 μg/g (Table II). The three deletion mutations, however, as well as the point mutation resulting in a stop codon, were all accompanied by much lower p53 protein concentrations - findings consistent with the expression of truncated p53 products either not subject to stabilization or unrecognizable by the immunoassay. The occurrence of any kind of p53 mutation was considered in relation to p53 protein status, as well as to the status of the other clinicopathologic features for which the patients had been characterized, as shown in Table III. The categorization of ovarian tumors into p53-negative and p53-positive groups by immunoassay agreed with the findings of mutational analysis in 10 of the 16 cases where mutations were demonstrated, and in 29 of the 34 cases where p53 mutations were not found. Missense p53 mutations were identified in 10 of the 15 cases in which p53 protein was overexpressed above the cutoff point. p53 protein accumulation above 3 μg/g and mutation of conserved p53 exons also agreed with respect to

Table III. *p53 gene mutation[a] in relation to clinicopathologic variables.*

Variable	Number of cases (%)		P value
	No p53 mutation	p53 mutation	
Age			
<57 years	20 (51.3)	7 (43.8)	
>57 years	19 (48.7)	9 (56.2)	0.612[b]
Stage			
I	12 (30.8)	0	
II	2 (5.1)	1 (6.2)	
III	20(51.3)	11 (68.8)	
IV	5 (12.8)	4 (25.0)	0.037[c]
Histologic type			
Serous papillary	12 (30.8)	11 (68.7)	
Endometrioid	12 (30.8)	0	
Undifferentiated	6 (15.4)	3 (18.8)	
All other histotypes	9 (23.0)	2 (12.5)	0.013[c]
Grade			
1	14 (35.9)	1 (6.2)	
3	25 (64.1)	15 (93.8)	0.043[b]
Residual tumor[d]			
<1 cm	18 (46.1)	2 (18.8)	
≥1 cm	21 (53.9)	13 (81.2)	0.057[b]
p53 protein[e]			
Negative	34 (87.2)	6 (37.5)	
Positive	5 (12.8)	10 (62.5)	<0.001[b]

[a] Mutation identified in exons 5 to 9 of the p53 gene.
[b] p value determined from Chi-square test.
[c] p value determined from Fisher' s Exact test.
[d] Residual tumor size unknown for one patient whose ovarian tumor had a p53 mutation.
[e] p53 protein concentrations equal to or exceeding 3 μg/g were considered p53-positive.

Table IV. *p53 protein concentrations[a] in relation to clinicopathologic variables.*

Variable	Number	Median	Range	P value[b]
Age				
<57 years	27	0.11	0.03 - 15.35	
≥57 years	28	0.38	0 - 34.34	0.395
Stage				
I - II	15	0.11	0 - 1.37	
III - IV	40	1.41	0- 34.34	0.004
Histologic type				
Serous papillary	23	1.49	0 - 20.31	
Endometrioid	12	0.35	0.04 - 34.34	
Undifferentiated	9	0.97	0- 15.35	
All other histotypes	11	0.26	0 - 7.86	0.378
Grade				
1	15	0.26	0 - 3.08	
3	40	1.14	0 - 34.34	0.054
Residual tumor[c]				
<1 cm	20	0.26	0-4.96	
≥1 cm	34	1.14	0 - 34.34	0.065
p53 mutation				
No	39	0.26	0 - 34.34	
Yes	16	3.46	0 - 20.31	0.015

[a] p53 concentrations expressed in μg/g.
[b] p values determined from Wilcoxon Rank Sum tests.
[c] Residual tumor size unknown for one patient whose tumor extract was p53-positive.

the similar number of cases positive by one method and negative by the other (p=0.763 by McNemar's test). Statistically significant associations were also found between p53 mutation and greater anatomic extent of disease, serous papillary histotype, and high grade. All except one of the 16 mutations identified were from poorly differentiated (grade 3) tumors, all except one were from patients with stage III or IV disease, and all except two were from patients who had been suboptimally debulked at surgery. There was no tendency, however, for the frequency of p53 mutation to increase or decrease with increasing patient age. Similarly, p53 protein levels did not differ significantly between patients of ages above or below the median cutoff or between ovarian tumors of different histologic types (Table IV). Although the statistical tests were borderline significant, there were tendencies for higher levels of p53 protein to associate with higher histological grade and residual tumor presence. On the other hand, p53 protein concentrations were shown to be elevated in tumor extracts from patients with stage III or IV compared to stage I or II assignments, and were strongly associated with p53 gene mutation. The distributions of p53 protein concentrations in tumors with and without mutation are shown in Figure 2.

Discussion

At least one in four patients with epithelial ovarian cancer, especially those presenting late at diagnosis, has a primary tumor in which the p53 tumor suppressor gene has been mutated. However, the impact of p53 mutation upon the

ovarian cancer patient in terms of the natural history of the disease and the likelihood of therapeutic success remains controversial. Relative to many of the more established markers (stage, grade, residual tumor), p53 alteration has been found to have minor prognostic value [2,5,15], although its importance may be greater for patients with particular clinical or pathologic features [25]. On the other hand, recent findings suggest that p53 functional status may be a critical determinant for the success of systemic chemotherapy with drugs which exert their antineoplastic effects by the induction of p53-dependent apoptosis [18,19]. Among these agents are platinum-containing compounds, and possibly taxol, which are components of regimes typically prescribed to patients whose malignancies exhibit extraovarian extension. In the present absence of accurate predictive factors for platinum-based chemotherapy, many patients with advanced disease are exposed to noxious but often ineffective treatments. A major focus of clinical research on ovarian cancer has therefore been the evaluation of novel prognostic and predictive factors, among which p53 mutational change has been the subject of intense interest.

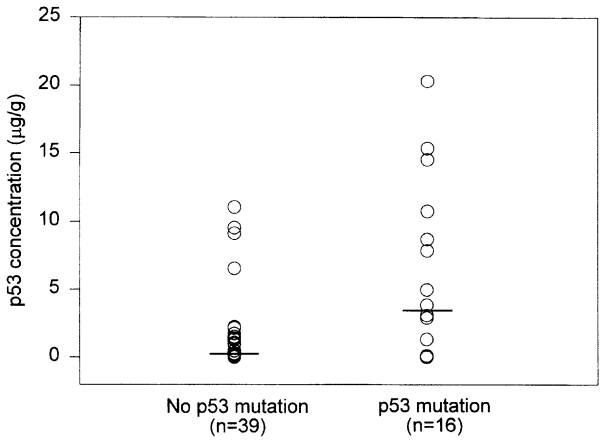

Figure 2. *p53 protein concentrations in ovarian tumors without and with mutation in exons 5 to 9 of the p53 gene. The horizontal bars indicate the median value in each of the two groups. Not shown in the figure is a specimen in which the absence of mutation was accompanied by p53 protein concentration of 34.34 µg/g.*

The primary purpose of this study was to compare two complementary methods of indirectly assessing the functional status of the p53 gene in ovarian carcinomas. These methods investigate p53 at the levels of genomic DNA sequence and protein expression. Structural alterations of the p53 gene, most commonly by missense point mutations within the evolutionarily-conserved DNA-binding domain [1], are associated with gene products impaired in their ability to function as transcription factors for genes mediating cell cycle arrest [10], programmed cell death [11], or DNA repair [31]. Failure of these processes following DNA damage is thought to contribute to the genomic instability predisposing to neoplastic transformation. Although DNA sequencing of the entire coding portion of the p53 gene is not usually necessary in order to identify the majority of mutations, which cluster within exons 5 to 8 of the 11 exon gene, it is possible that many of the 10-20% of mutations missed by sequencing only the central DNA-binding domain may also inactivate p53 function [21]. Nevertheless, most studies have restricted their analyses to p53 exons 5 to 8, alterations of which have usually been initially detected by screening techniques such as SSCP or CDGE prior to DNA sequence analysis of aberrant cases due to the considerable expenditure of resources associated with the latter procedure. The introduction of more rapid and affordable automated DNA sequencing technologies, targeting the clinical diagnostics setting, has made possible the processing and analysis of the large numbers of specimens required for meaningful epidemiologic studies [32]. Sequence analysis remains, however, technically far more demanding than simpler techniques taking advantage of another consequence of missense p53 mutation, namely, the conformational alteration of mutant p53 protein which confers resistance to normal degradative turnover. Cells harboring mutant p53 alleles usually display nuclear accumulation of the encoded protein, which can be detected by immunochemical methods employing a variety of monoclonal and polyclonal antibodies against epitopes exclusive to mutant p53 or shared by mutant and wild-type conformations of the protein. Most commonly, these antibodies have been applied as primary detection reagents for immunohistochemical staining of fixed or fresh tissue sections, although their use in ELISAs of soluble extracts of tissues has also been reported by a smaller number of groups [22-24]. While the advantages and disadvantages of immunoassays of p53 protein compared to conventional p53-immunostaining have been discussed elsewhere [26], use of an immunoassay of p53 protein has not yet been compared to DNA sequence analysis of the p53 core region.

The p53 mutation rate of 29% (16/55) in our series of ovarian carcinomas was somewhat low but within the range of those reported in other studies [2-8], even though specimens with grade 2 histology had been excluded from the analysis. Omission of moderately differentiated (grade 2) specimens served to enhance the partitioning of p53 mutations into high grade lesions but was unlikely a major source of bias since the 43% rate of p53 abnormality in our previous study [25], which included grade 2 tumors, was obtained from a similar number of low (n=16) and high (n=49) grade specimens. The rate of p53 protein overexpression in the present study (27%), however, is lower than that obtained in our previous work and may be explained largely by the selection of the cutoff point by which specimens were considered p53-positive - 3 µg/g compared to 0.23 µg/g used earlier. Although the immunoassays used in these two studies differed in their respective primary anti-p53 antibodies, they had been shown to yield highly correlated results when applied to extracts of various cell lines and resected breast tumors [24].

Comparison of the mutations identified in this study with those reported in a comprehensive database of p53 mutations [33] revealed several nucleotide changes detected at particular codons to be previously undescribed in ovarian carcinoma (G to T at codons 172 and 248; C to G at codon 193; A to G at codon 280). Our finding of a slight excess of transition over transversion mutations was consistent with the majority of other studies reporting a higher frequency of transitions [3,5,6,12] suggestive of endogenous mutagenic processes rather than exposure to carcinogens. The three deletions found in our series were novel for ovarian cancer and were found at a frequency (19% of identified mutations) similar to that observed in a recent study by our group [34]. In contrast to this earlier study, exon 8 was affected by deletions in addition to exons 5 and 7. Since the deletions were flanked by short direct repeats, misalignment of the template strands during DNA replication may have been responsible for their generation [35]. In spite of the fact that p53 coding sequences outside exons 5 to 9 were not surveyed for mutations, we feel that our analysis likely identified the majority of p53 genetic changes.

The strong relationship between p53 gene mutation, particularly in-frame substitutions, and p53 protein overexpression was confirmed in this study: 34 of the 39 cases without demonstrable p53 mutations in exons 5 to 9 were classified as p53-negative by immunoassay, while 10 of the 12 specimens in which missense mutations were detected were shown to have p53 protein concentrations above the cutoff point. To account for the eleven cases for which genetic analysis and immunoassay were not in agreement, several explanations might be invoked. Intragenic deletions and translational termination mutations comprised four of the six cases in which p53 mutation was accompanied by low p53 protein expression. The selection of the p53 protein concentration (3 μg/g) above which specimens were considered p53-positive may have resulted in the artifactual misclassification of another tumor in which a missense point mutation was found in exon 8 but in which the p53 protein concentration (2.89 μg/g) was only slightly below the cutoff. However, the other p53-negative tumor in which a missense mutation was found had no detectable p53 immunoreactivity. It is possible that the nonequivalence of tissues, with respect to the histologic distribution of p53 abnormalities, that were used for DNA sequence analysis and for immunoassay in each case might have led to discrepant findings, both for these two p53-negative cases and for the other five in which p53 mutations were not found and yet p53 protein accumulated. Nonmutational stabilization of p53 protein, such as by cytoplasmic sequestration, has been documented in many malignancies [36]. A less likely explanation would have been the presence of mutations outside exons 5 to 9 leading to the expression of a p53 gene product with an enhanced half-life. Since these explanations were not investigated, they remain speculative.

The results of this study provide evidence that the assessment of p53 abnormalities in epithelial ovarian carcinoma may be performed either by a simple immunochemical assay or by a more complex DNA sequence analysis. Although the results of p53 immunoassay were not in complete concordance with the sequence-based analysis of p53, the advantages of the former method, especially in terms of its ease of application, may make it more suitable for clinical laboratory use.

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