[CANCER RESEARCH 51, 2979-2984, June 1, 1991]

Overexpression and Mutation of p53 in Epithelial Ovarian Cancer

J. R. Marks,[1] A. M. Davidoff, B. J. Kerns, P. A. Humphrey, J. C. Pence, R. K. Dodge, D. L. Clarke-Pearson, J. D. Iglehart, R. C. Bast, Jr., and A. Berchuck

Departments of Surgery [J. R. M., A. M. D., J. P., J. D. I.], Pathology [B. K., P. A. H.], Biostatistics [R. D.], Obstetrics and Gynecology/Gynecologic Oncology [D. L. C-P., A. B.], and Medicine, Microbiology/Immunology [R. C. B.], and the Duke University Comprehensive Cancer Center, Duke University Medical Center, Durham, North Carolina 27710

ABSTRACT

We examined p53 expression in 107 epithelial ovarian cancers with immunohistochemical techniques using monoclonal antibody PAb1801. High level expression of nuclear p53 protein was detected in the malignant epithelium in 54 (50%) of these cancers. Expression of p53 protein was undetectable in 13 benign gynecological tissues. p53 mRNA from three cancers that overexpressed the protein were sequenced and point mutations which altered the coding sequence of the highly conserved region of the gene were found in each case. Three cancers with undetectable protein levels also were sequenced and were found to be wild-type through the same region of the gene. As in other cancers, overexpression of the p53 protein in ovarian cancer appears to correlate closely with the presence of mutation in the *p53* gene. p53 overexpression did not correlate with stage, histological grade, or the ability to perform optimal cytoreductive surgery. A significant correlation ($P = 0.04$) was observed between p53 overexpression and aneuploidy in advanced stage (III/IV) disease. There was no significant relationship between overall survival and p53 expression. Since mutation and overexpression of p53 are common in epithelial ovarian cancers, further studies are warranted to clarify the role of p53 in ovarian tumorigenesis.

INTRODUCTION

p53 is a nuclear phosphoprotein that is expressed by many normal cells (1). Although p53 has been implicated as a protooncogene, the molecular mechanisms of p53 action in normal and malignant cells are not well understood. It has been shown, however, that the *p53* gene frequently acquires mutations during the development of many human malignancies including colon, breast, and lung cancers (2–4). The *p53* mutations which have been noted in these cancers invariably are located in a relatively large region of the gene that has been highly conserved through evolution (5). These mutations, which cause amino acid substitutions, appear to change the conformation of p53 resulting in increased stability and higher steady-state levels of this normally short-lived protein (6). Mutant p53 can immortalize cells *in vitro* and, in conjunction with an activated *ras* oncogene, can produce a fully transformed phenotype in primary fibroblasts (7–10).

It has been proposed that the loss of wild-type p53 function may play a role in malignant transformation. This loss may occur either by mutation of one copy of the *p53* gene followed by deletion of the remaining wild-type allele or through inactivation of the wild-type protein by oligomerization with the more stable mutant protein (11, 12). In addition, the findings of several groups that wild-type p53 can inhibit transformation *in vitro* have led to the hypothesis that p53 acts as a tumor suppressor gene (13–15).

Epithelial ovarian cancer is the fourth leading cause of cancer deaths in women in the United States. Relatively little is known of the molecular events which lead to the development of this cancer; however, the activation of a number of different oncogenes has been implicated. Several studies recently have suggested that in the progression of ovarian cancer, the alteration of genes on chromosome 17 may frequently be of significance. First, it has been shown that the *HER-2/neu* oncogene, which is located on 17q, is overexpressed in about 30% of ovarian cancers and that overexpression is associated with poor prognosis (16, 17). In addition, loss of heterozygosity for genes on chromosome 17, due to chromosomal deletion, is a common event that occurs in as many as three-fourths of ovarian cancers (18, 19). Since p53 is located on chromosome 17p and loss of heterozygosity is thought to play a role in its activation, we sought to determine whether p53 is aberrantly expressed in epithelial ovarian cancer.

MATERIALS AND METHODS

Patients. One hundred seven patients with ovarian cancer were included in this study. Each patient underwent exploratory laparotomy as part of treatment for epithelial ovarian cancer at Duke University Medical Center between 1985 and 1990. The stage of disease was assigned according to the International Federation of Gynecologists and Obstetricians staging system (20). In 69 patients, tumor samples were obtained at initial surgery while 38 samples were obtained at second-look or subsequent laparotomy. All of the patients with advanced stage disease in this study received cisplatin/cyclophosphamide chemotherapy following primary surgical exploration. Tissue was flash frozen in liquid N_2 and stored at −80°C. Histological material from each case was reviewed by a single pathologist for this study. The histological type and grade were assigned according to the WHO classification of ovarian cancer (21). In addition, samples of normal and benign reproductive tract tissues were collected from 13 patients undergoing surgery for benign gynecological diseases.

Immunohistochemistry. All tissue samples were snap frozen and stored at −80°C until analyzed. Tissue samples were frozen in Tissue Tek OCT compound (Ames Division, Miles Laboratory, Elkhart, IN) and 4-μm-thick cryostat sections were mounted on gelatin/potassium dichromate-coated slides. The slides were air dried overnight at room temperature to enhance cellular morphology. The next day, slides were fixed in acetone for 10 min at room temperature and then air dried again for 30 min before initiation of the staining assay.

Immunohistochemical staining was performed using the Elite Vectastain ABC kit (Vector Laboratories, Burlingame, CA). All incubations were performed in a humidified chamber at room temperature. Slides were first incubated with the appropriately diluted primary antibody for 60 min. Slides were then washed three times with PBS[2] for 5 min. Slides were blocked by incubation for 15 min in 5% normal horse serum diluted in PBS/2% bovine serum albumin. After a washing with PBS, biotinylated horse anti-mouse IgG antibody (1:100 in 2% bovine serum albumin) was added for 30 min followed by avidin-peroxidase complex, also for a 30-min incubation. Finally, the slides were developed for 4 min with the enzyme substrate diaminobenzidine (0.5% diaminobenzidine in 0.05 M Tris buffer, pH 7.6-0.6% hydrogen peroxide). The slides were then rinsed for 10 min in running tap water and counterstained with 1% methyl green in sodium acetate buffer, pH 5.2. Slides were then dehydrated, cleared in xylene, and mounted in permanent coverslipping medium.

Received 12/26/90; accepted 3/18/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at Box 3873, Duke University Medical Center, Durham, NC 27710.

[2] The abbreviations used are: PBS, phosphate-buffered saline; PCR, polymerase chain reaction.

Antibodies. Monoclonal antibody PAb1801 (Ab-2; Oncogene Science, Manhasset, NY) is affinity purified and recognizes a denaturation resistant epitope in human p53 located between amino acids 32 and 79 (22). This antibody was used at 0.5 μg/ml in all cases. The monoclonal antibody PAb421 was obtained from the supernatant of the hybridoma cell line (A. J. Levine, Princeton University, Princeton, NJ) and used at a 1:10 dilution. This antibody recognizes a denaturation resistant epitope located between amino acids 370 and 378 of the mouse p53 protein which corresponds to positions 372–380 of the human protein (23). Monoclonal PAb240 (Ab-3; Oncogene Science) is affinity purified and recognizes a denaturation resistant epitope on p53 located between amino acids 156 and 335 (24). PAb240 was used at 0.5 μg/ml. This antibody has been shown to preferentially recognize mutant forms of the protein, particularly in immunoprecipitations (25). In each case, serial sections were reacted with mouse IgG1 (0.5 μg/ml) to assess nonspecific staining and the nuclear proliferation antigen, Ki-67 (Dakopatts, Santa Barbara, CA), at 0.5 μg/ml to test for maintenance of nuclear reactivity (26).

Evaluation of immunohistochemistry was performed by P. A. H. Results of immunohistochemistry were reported categorically as either positive or negative. In order for the section to be scored as positive for p53, nuclear staining in the majority of the epithelial cells had to be evident.

DNA Quantitation. The DNA content was measured by using the Feulgen staining reaction on whole cell touch imprints from fresh frozen tissue. The intensity of the staining was measured by the CAS 200 Image Analysis System using the Quantitative DNA Analysis software (Cell Analysis Systems, Elmhurst, IL) according to the manufacturer's protocols. Briefly, frozen blocks were initially cut and stained with hematoxylin-eosin to be certain that representative tissue was present. The blocks were allowed to warm until the exposed surface of the block was sticky. Then, multiple touch preparations were made by firmly touching a cleaned glass slide to the thawed surface of the tissue. The first three imprints were discarded in an attempt to reduce the number of cut nuclei. The touch preparations were air dried overnight and hydrolyzed in 5N HCl. The slides were then incubated for 1 h in the freshly mixed dye (CAS DNA Stain Reagent) and then rinsed, dehydrated, cleared, and mounted. The amount of blue staining was quantitated from 100 cancer cells from each imprint by CAS 200 analysis. In each batch of experimental samples, a slide was included which contained cells with a known amount of DNA (rat tetraploid hepatocytes supplied by the manufacturer (Cell Analysis System). This was used as a reference which standardized the intensity of the stain from assay to assay. Histograms of the DNA content per cell were generated and determination of ploidy was made on the basis of these histograms by the location of the main and secondary peaks of DNA content [diploid DNA index, 1.0 ± 0.1 (SD)].

Sequence Analysis. Total RNA was extracted using the guanidinium isothiocyanate procedure (27). One 1 μg of total RNA was used as a template for p53 complementary DNA synthesis with murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Bethesda, MD). The reaction was performed at 37°C for 30 min in 1× Taq polymerase buffer (New England Biolabs, Beverly, MA) with an antisense oligonucleotide primer (25 pmol) from exon 10 (5′-CCTGGGCATCCTTGAGTT, all oligonucleotide primers were synthesized at the Duke Comprehensive Cancer Center), 2.5 mM $MgCl_2$, 125 μM deoxynucleotide triphosphates, and 10 units of placental RNase inhibitor (Stratagene, La Jolla, CA). Exons 4 through 10 were then amplified from this complementary DNA using the PCR (28) by adding an oligo primer from exon 4 (5′-GGGACAGCCAAGTCTGTGACT), more 1× Taq polymerase buffer, and 2.5 units of Taq DNA polymerase (New England Biolabs). This mix underwent thermocycling for 30 cycles as follows: 94°C for 1 min; 60°C for 1 min; and 72°C for 3 min. The 712-base pair product of this reaction was gel purified and reamplified using the same thermocycling conditions with 5 pmol of each primer, 200 μM deoxynucleotide triphosphates and 2.5 units Taq DNA polymerase in 1× Taq polymerase buffer. This product was purified away from primer and unincorporated nucleotides by gel filtration through a Sepharose CL-6B (Pharmacia, Piscataway, NJ) spin column, ethanol precipitated, and resolubilized in water. This material was the template for dideoxy sequencing using Sequenase 2.0 (United States Biochemical, Cleveland, OH). Oligonucleotides flanking each of the exons 5, 6, 7, and 8 (5′-TACTCCCCTGCCCTCAACAAG, 5′-CATC GCTATCTGAGCAGCGCT; 5′-GTCTGGCCCCTCCTCAG, 5′-CT CAGGCGGCTCATAGG; 5′-GTTGGCTCTGACTGTAC, 5′-CCGA ATTCAGTCTTCCAGTGTGATGATG; 5′-TTCCGTCCCAGTAGA TTACCA, 5′-TGGTAATCTACTGGGA) were used to prime the reactions which were performed by first boiling the primer-template mix, labeling on ice for 10 min with [^{32}P]dATP and then running the termination reactions at 45°C for 10 min. The reaction products were electrophoresed on a polyacrylamide gel which was then soaked in 10% acetic acid/12% methanol, dried, and set with Kodak (Rochester, NY) XAR-5 film overnight.

Statistics. Tables were analyzed using Fisher's two-tailed exact *t* test. Survival estimates were calculated using the Kaplan-Meier life table method (29). Differences in survival were tested using the log-rank statistic (30).

RESULTS

Expression of p53 Protein in Malignant and Benign Ovarian Tissue. Thirteen nonmalignant gynecological tissues and 107 epithelial ovarian cancers were stained using the human p53 specific monoclonal antibody, PAb1801. There was no detectable staining of any of the 13 benign tissues (2 normal ovaries, 7 benign ovarian serous cystadenomas, 3 normal endometria, and 1 fibroid). In contrast, 54 (50%) of the 107 cancers had strong nuclear immunoreactivity in the majority of malignant epithelial cells (Fig. 1). In two other cancers, staining was seen in a relatively small percentage of the malignant cells while the remaining 51 cancers had no detectable nuclear immunoreactivity with this antibody. To confirm that it was the p53 protein which was being detected, 10 cases were stained with 2 other p53 monoclonal antibodies, PAb421 and PAb240, which recognize distinct determinants (22–24). In each case, all three p53 antibodies yielded a similar pattern of nuclear staining with PAb240 giving the weakest immunoreactivity.

Sequence Determination of the *p53* Gene. To determine if mutations in the *p53* gene exist in ovarian cancers with high levels of p53 protein, sequence analysis was performed using whole cell RNA extracted from primary tissue samples. All mutations which are known to increase the stability and oncogenically activate the protein are located between exons 5 and 8 corresponding to amino acids 126 through 306. This region of the p53 message was sequenced from three cancers with high levels of the protein and three cancers with undetectable levels. Individual pieces of tumor tissue were assessed and selected by histological examination of thin sections for relative homogeneity of cancer cells. Material for dideoxy sequencing was obtained by first reverse transcribing mRNA using a p53 specific primer, followed by two rounds of PCR amplification of exons 4 through 9. The amplified product was then directly sequenced on both strands using a separate set of internal primers. This method effectively eliminates sequencing errors due to polymerase misincorporations. Random polymerase errors fade into the background by sequencing the entire population of PCR amplified molecules. Multiple independent sequence determinations from the same RNA sample have confirmed the fidelity of this method (31).

The three cancers that demonstrated high levels of p53 protein all contained single point mutations which altered the coding sequence (Figs. 2 and 3). Two of these mutations reside in a highly conserved region in exon 8 (codon 278, CCT to CGT, proline to arginine and codon 282 CGG to TGG, arginine

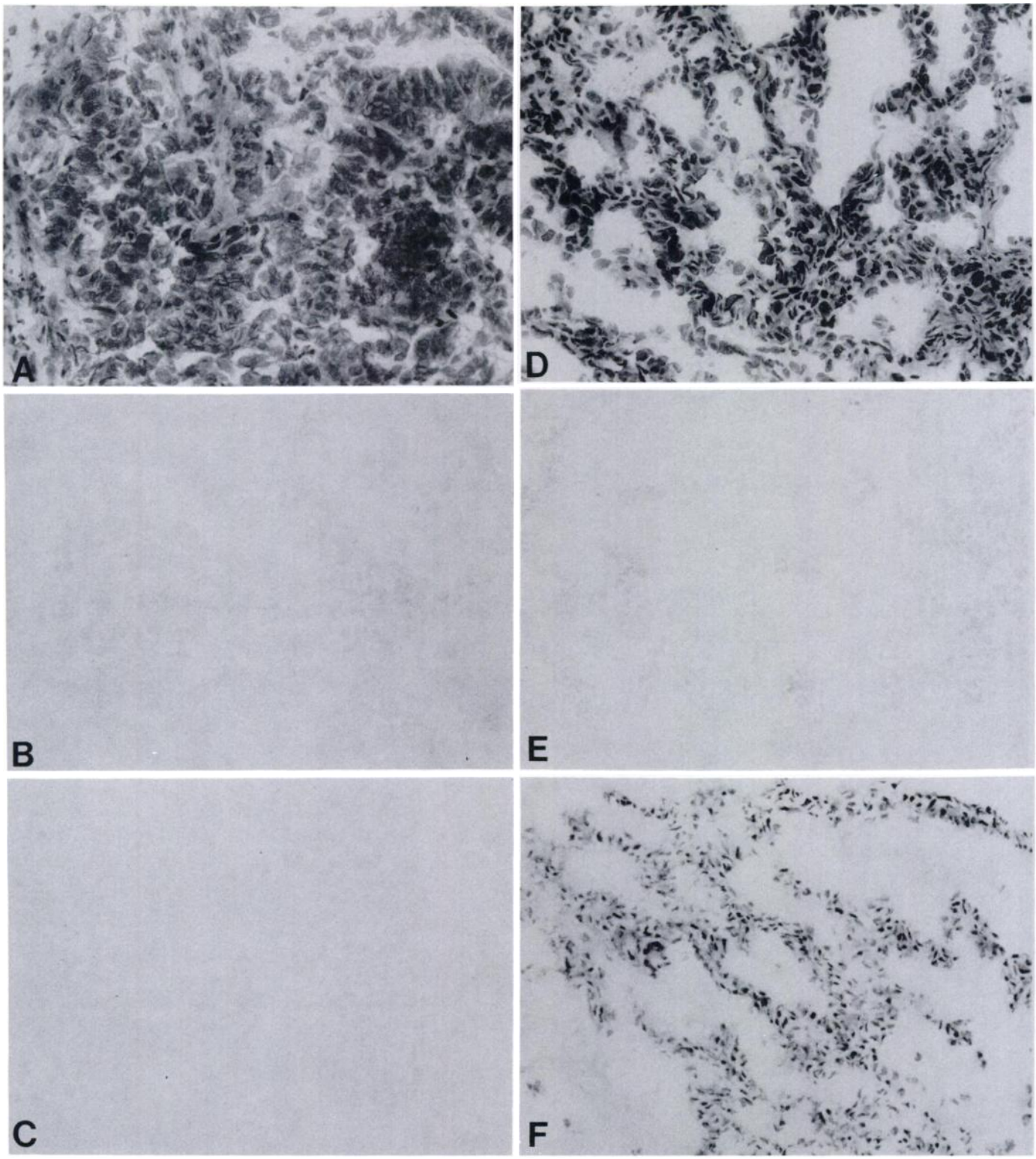

Fig. 1. Immunohistochemical staining for the p53 oncoprotein in two epithelial ovarian cancers. Normal p53 expression: *A*, H & E; *B*, negative control; *C*, p53. p53 overexpression: *D*, H & E; *E*, negative control; *F*, p53. × 250.

to tryptophan). The mutation in the third tumor was found at amino acid 216 (GTG to ATG, valine to methionine), a site that is positioned outside of the 5 stretches of evolutionary conservation but that is conserved in frogs, rodents, and humans (5). There are no known *p53* polymorphisms which reside at these locations. From the sequencing autoradiograms, it was apparent that two of the three tissues transcribed only the mutant allele, consistent with deletion of the wild-type allele. The third tumor contained a faint band corresponding to the wild-type nucleotide at amino acid 216 (Fig. 3); however, the relative intensities suggested that this was probably due to contamination with a small amount of normal cells rather than the presence of the wild-type allele in the cancer. Conversely, the three cancers with undetectable p53 protein all contained a

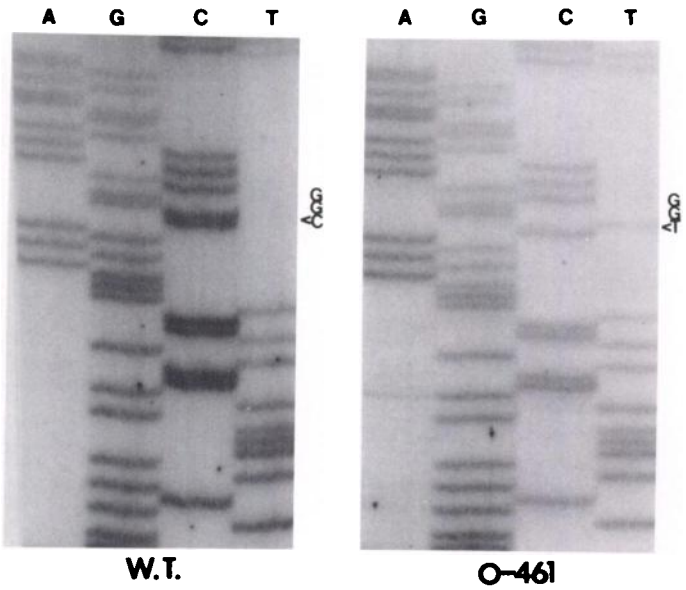

Fig. 2. Sequencing autoradiograms of the *p53* gene from an ovarian cancer with overexpression of the p53 protein. Point mutation at codon 282 changing a C to a T resulting in an amino acid change of arginine to tryptophan in exon 8. *W.T.*, wild type.

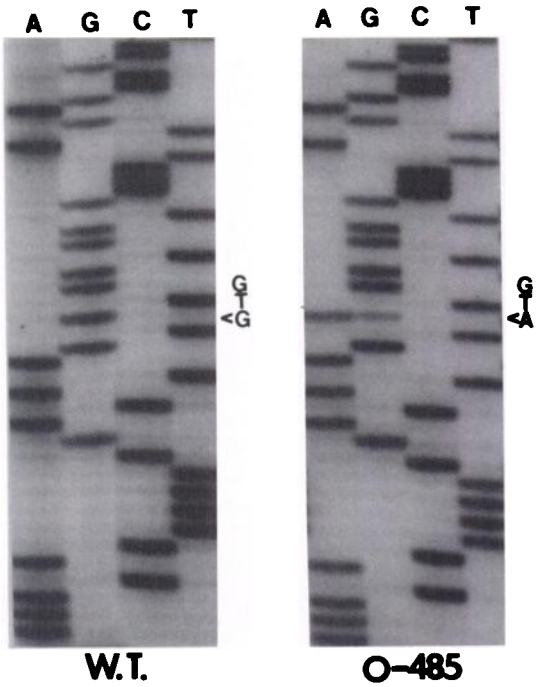

Fig. 3. Sequencing autoradiogram as in Fig. 2. Point mutation at codon 216 (exon 6) changing a G to an A and resulting in a valine to methionine alteration. The wild-type (*W.T.*) nucleotide is visible in this sequence; however, the relative intensities of the bands suggest that it may be due to normal ovarian cells in the tissue specimen that was used.

completely wild-type coding sequence throughout this same part of the gene (32). Therefore, a good correlation exists between high level expression of the p53 protein by immunohistochemistry and the presence of mutations in the highly conserved region of the gene.

Histopathological Criteria and p53 Expression. Staining for p53 (Table 1) was seen in 8 (53%) of 15 early stage cancers (stage I/II) and in 46 (50%) of 92 advanced stage cancers (stage III/IV). Among the 69 cancers (all stages) that were obtained

Table 1 *Relationship between p53 expression and clinical parameters in ovarian cancer*

	p53 negative	p53 positive
Av. age at diagnosis	57.3 yr	56.0 yr
Stage I/II	7 (47)[a]	8 (53)
Stage III/IV	46 (50)	46 (50)
Well differentiated[a]	4 (67)	2 (33)
Moderately differentiated	11 (41)	16 (59)
Poorly differentiated	19 (53)	17 (47)
Diploid[c]	14 (70)	6 (30)
Aneuploid	29 (42)	40 (58) $P = 0.04$
Optimal cytoreduction[d]	9 (60)	6 (40)
Sub-optimal cytoreduction	20 (44)	25 (56)
Median survival[c]	2.2 yr	1.85 yr

[a] Numbers in parentheses, percentage.
[b] ($n = 69$) patients in whom tumor sample was obtained at initial surgery.
[c] ($n = 92$) patients with advanced stage disease.
[d] ($n = 60$) patients with initial surgery on advanced stage disease.

at initial surgery, 52% were positive for p53 overexpression compared to 47% of the 38 cancers in which the sample was obtained subsequent to the administration of chemotherapy. There was no relationship between histological grade and p53 overexpression in the 69 cancers in which the tumor was obtained at initial surgery. In this group, p53 overexpression was seen in 2 of 6 (33%) well differentiated cancers, 16 of 27 (59%) moderately differentiated cancers, and 17 of 36 (47%) poorly differentiated cancers.

In the following statistical analyses, patients with advanced stage disease are presented separately for two reasons: (*a*) of the 107 cancers in this study, only 15 were early stage (I/II); (*b*) of the cancers that were early stage, in 6 cases tissue was obtained at the time of recurrence. Therefore, this sample of early stage cancers is relatively small and unrepresentative. We did not see a correlation between p53 overexpression and the ability to perform optimal cytoreductive surgery (maximum diameter of largest residual tumor nodule, <1 cm) in patients with advanced stage disease. Optimal cytoreduction was achieved in 19% of cancers with p53 overexpression compared to 31% of those with normal p53 expression ($P > 0.3$).

Ploidy is as an independent parameter that has been related to tumor aggressiveness and overall survival. The DNA content of 102 of the 107 cancers in this study was measured using static image analysis on Feulgen stained touch imprints. From the quantitative measurement of DNA content per cell, the cancers were classified as either diploid or aneuploid. Overall, 23% were diploid and 77% were aneuploid. A significant correlation existed between p53 expression and ploidy in advanced stage disease. Among cancers with p53 overexpression, only 13% were diploid compared to 33% of cancers that had normal levels of p53 protein ($P = 0.04$). This correlation was less significant ($P = 0.13$) if all cancers were considered.

Finally, we examined the relationship between p53 expression and overall survival in patients with advanced stage ovarian cancer. Although the median survival of patients with p53 overexpression (1.85 years) was somewhat worse than that of patients with normal p53 expression (2.2 years), the difference was not statistically significant (Fig. 4). Similarly, there was no significant difference in survival when the entire group of patients, including those with early stage disease, was analyzed.

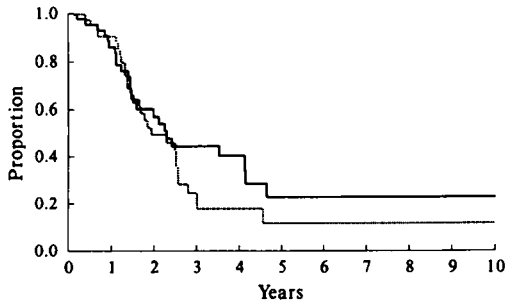

Fig. 4. Relationship between p53 overexpression and survival in ovarian cancer. Survival in 92 patients with advanced stage disease with p53 overexpression (. . . .) and normal expression (——). $P > 0.2$.

DISCUSSION

In this study, we have demonstrated that overexpression of the p53 protein occurs in approximately 50% of epithelial ovarian cancers. In general, virtually all malignant epithelial cells in a positive tumor homogeneously expressed the protein. Conversely, in those cancers which were scored negative for expression, no detectable staining was present. Additionally, in a variety of benign gynecological tissues, no staining for the p53 protein was detected. Therefore, the normal condition for ovarian epithelium is to have low or undetectable steady-state levels of the p53 protein while cancers containing high levels are aberrantly expressing this oncogene. Many of the tissue samples that were used in this study were obtained from recurrent cancers after alkylating agent chemotherapy (cisplatin and cyclophosphamide). Since the incidence of p53 overexpression was similar in samples obtained at initial surgery and in samples obtained after chemotherapy it does not appear that this gene is activated due to the mutagenic effect of chemotherapy.

Since mutations which render p53 oncogenic also increase the half-life of this normally unstable protein, we investigated whether high level expression correlated with the presence of mutations in the highly conserved region of the gene. RNAs from three ovarian cancers that overexpressed p53 and three with normal expression of the protein were sequenced. Point mutations, each creating an amino acid substitution, were identified in all three cancers that overexpressed p53. The same region of the gene in the three cancers expressing normal levels of p53 was identical to the reported wild-type sequence (32). We have previously made this same correlation in primary breast cancer (31) and Iggo *et al.* (33) have described a similar situation in lung cancers. In these three types of cancer, it appears that increased expression is tightly linked to the presence of mutations between exons 5 and 8 of the gene. The absence of mutations in ovarian and breast cancers with normal p53 expression supports the argument that mutation is causally related to increased expression of the protein.

The results of this study suggest that p53 is a frequently activated oncogene in ovarian cancer. For this reason, it was of interest to determine how aberrant p53 expression related to other prognostic factors in ovarian cancer. No correlation was seen between p53 overexpression and stage or histological grade of the cancer. Since stage is determined at the time of initial diagnosis and many of the relatively few early stage cancers in this study were obtained at the time of surgery for recurrent disease, this group of early stage tumors is disproportionately composed of aggressive cancers. It would require a larger and more representative sample of early stage ovarian cancers to demonstrate a correlation between p53 overexpression/mutation and stage if it exists. One parameter that did correlate with p53 overexpression in advanced cancers was ploidy. p53 positive cancers were more frequently aneuploid than those that were p53 negative. While p53 activation has been associated with allelic loss on chromosome 17p, it is unlikely that these relatively small deletions could account for the gross changes in DNA content that were measured. Deletions on one chromosome may, however, reflect more widespread chromosomal instability that would be detected in an overall assay of DNA content. It remains to be determined whether allelic loss on 17p corresponds to the mutational activation of the *p53* gene in ovarian cancer.

No relationship was seen between p53 overexpression and survival in either the entire cohort of patients or the subset of patients with advanced disease. Although there was a small difference in survival in patients with advanced disease, this was not statistically significant. Given the high percentage of patients with p53 overexpression (50%) and the extremely poor prognosis for patients with advanced disease (10–20% overall survival), a much larger sample size would be required to be certain that there was no relationship between p53 overexpression and survival.

It is widely held that most cancers are the result of multiple independent genetic lesions and that therefore it is likely that p53 acts in concert with other oncogenes in the ovary during tumorigenesis. In this regard, transgenic mice that carry a mutated *p53* gene, with resultant high level p53 expression in the ovaries, have not developed ovarian cancer (34). In addition, germ line *p53* mutations recently have been found in afflicted kindreds of the dominantly inherited Li-Fraumeni/SBLA cancer family syndrome (35), yet ovarian cancer is not one of the malignancies commonly associated with this syndrome. From these data, it appears that mutation of the *p53* gene is, in itself, probably insufficient to induce epithelial ovarian malignancies. p53 may well contribute to the development of these cancers, however, since overexpression (and presumably mutation) is common and must have been selected for and maintained during tumor progression.

ACKNOWLEDGMENTS

The authors would like to thank Nancy Glover, Regina Whitaker, and Mary Beth Moore for their excellent technical assistance and Dennis Pittman for assistance in the final preparation of the manuscript.

REFERENCES

1. Rogel, A., Popliker, M., Webb, C. G., *et al.* p53 cellular tumor antigen: analysis of mRNA levels in normal adult tissues, embryos, and tumors. Mol. Cell. Biol., *5:* 2851–2855, 1985.
2. Takahashi, T., Nau, M. M., Chiba, I., *et al.* p53: a frequent target for genetic abnormalities in lung cancer. Science (Washington DC), *246:* 491–494, 1989.
3. Nigro, J. M., Baker, S. J., Preisinger, A. C., *et al.* Mutations in the p53 gene occur in diverse human tumour types. Nature (Lond.), *342:* 705–708, 1989.
4. Prosser, J., Thompson, A. M., Cranston, G., *et al.* Evidence that p53 behaves as a tumour suppressor gene in sporadic breast tumours. Oncogene, *5:* 1573–1579, 1990.
5. Soussi, T., deFromentel, C. C., Mechali, M., *et al.* Cloning and characterization of a cDNA from *Xenopus laevis* coding for a protein homologous to human and murine p53. Oncogene, *1:* 71–78, 1987.
6. Finlay, C. A., Hinds, P. W., Tan, T-H., *et al.* Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-line. Mol. Cell. Biol., *8:* 531–539, 1988.
7. Parada, L. F., Land, H., Weinberg, R. A., *et al.* Cooperation between gene encoding p53 tumour antigen and *ras* in cellular transformation. Nature (Lond.), *312:* 649–651, 1984.
8. Eliyahu, D., Raz, A., Gruss, P., *et al.* Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. Nature (Lond.), *312:* 646–649, 1984.

9. Jenkins, J. R., Rudge, K., Chumakov, P., *et al.* The cellular oncogene p53 can be activated by mutagenesis. Nature (Lond.), *317:* 816–818, 1985.
10. Hinds, P., Finlay, C., and Levine, A. J. Mutation is required to activate the *p53* gene for cooperation with the *ras* oncogene and transformation. J. Virol., *63:* 739–746, 1989.
11. Kraiss, S., Quaiser, A., Oren, M., *et al.* Oligomerization of oncoprotein p53. J. Virol., *62:* 4737–4744, 1988.
12. Green, M. R. When the products of oncogenes and anti-oncogenes meet. Cell, *56:* 1–3, 1989.
13. Finlay, C. A., Hinds, P. W, and Levine, A. J. The p53 proto-oncogene can act as a suppressor of transformation. Cell, *57:* 1083–1093, 1989.
14. Eliyahu, D., Michalovitz, D., Eliyahu, S., *et al.* Wild-type p53 can inhibit oncogene-mediated focus formation. Proc. Natl. Acad. Sci., *86:* 8763–8767, 1989.
15. Baker, S. J., Markowitz, S., Fearon, E. R., *et al.* Suppression of human colorectal carcinoma cell growth by wild-type p53. Science (Washington DC), *249:* 912–915, 1990.
16. Berchuck, A., Kamel, A., Whitaker, R., *et al.* Overexpression of HER-2/*neu* is associated with poor survival in advanced epithelial ovarian cancer. Cancer Res., *50:* 4087–4091, 1990.
17. Slamon, D. J., Godolphin, W., Jones, L. A., *et al.* Studies of HER-2/*neu* proto-oncogene in human breast and ovarian cancer. Science (Washington DC) *244:* 707–712, 1989.
18. Eccles, D. M., Cranston, G., Steel, C. M., *et al.* Allele losses on chromosome 17 in human epithelial ovarian carcinoma. Oncogene, *5:* 1599–1601, 1990.
19. Russell, S. E. H., Hickey, G. I., Lowry, W. S., *et al.* Allele loss from chromosome 17 in ovarian cancer. Oncogene, *5:* 1581–1583, 1990.
20. Annual Report on the Treatment of Gynecologic Cancer. *In:* H. L. Kottmeier (ed.), International Federation of Gynecologists and Obstetricians Stockholm, Sweden, Vol. 17, 1979.
21. Serov, S. F., and Scully, R. E. Histological typing of ovarian tumors. *In:* International Histological Classification of Tumors, No. 9. Geneva: World Health Organization, 1973.
22. Banks, L., Matlashewski, G., and Crawford, L. Isolation of human-p53-specific monoclonal antibodies and their use in the studies of human p53 expression. Eur. J. Biochem., *159:* 529–534, 1986.
23. Wade-Evans, A., and Jenkins, J. R. Precise epitope mapping of the murine transformation-associated protein, p53. EMBO J., *4:* 699–706, 1985.
24. Gannon, J. V., Greaves, R., Iggo, R., *et al.* Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form. EMBO J., *9:* 1595–1602, 1990.
25. Bartek, J., Iggo, R., Gannon, J., *et al.* Genetic and immunochemical analysis of mutant p53 in human breast cancer cell lines. Oncogene, *5:* 893–899, 1990.
26. Gerdes, J., Lemke, H., Baisch, H., *et al.* Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by a monoclonal antibody. J. Immunol., *133:* 1710–1715, 1984.
27. Chomczynski, P., and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., *162:* 156–159, 1987.
28. Saiki, R. K., Gelfand, D. H., Stoffel, S., *et al.* Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science (Washington DC), *239:* 487–491, 1988.
29. Kaplan, E. L., and Meier, P. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc., *53:* 457–481, 1958.
30. Mantel, N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother. Rep., *50:* 163–170, 1966.
31. Davidoff, A. M., Humphrey, P. A., Iglehart, J. D., *et al.* Genetic basis p53 overexpression in human breast cancer. Proc. Natl. Acad. Sci., in press, 1991.
32. Lamb, P., and Crawford, L. Characterization of the human p53 gene. Mol. Cell. Biol., *6:* 1379–1385, 1986.
33. Iggo, R., Gatter, K., Bartek, J., *et al.* Increased expression of mutant forms of p53 oncogene in primary lung cancer. Lancet, *335:* 675–679, 1990.
34. Lavigueur, A., Maltby, V., Mock, D., *et al.* High incidence of lung, bone, and lymphoid tumors in transgenic mice overexpressing mutant alleles of the p53 oncogene. Mol. Cell. Biol., *9:* 3982–3991, 1989.
35. Malkin, D., Li, F. P., Strong, L. C., *et al.* Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science (Washington DC), *250:* 1233–1238, 1990.