p53 and Ki-ras Gene Mutations in Epithelial Ovarian Neoplasms

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ABSTRACT

In an effort to define the pathogenic relationship between ovarian neoplasms spanning the clinicopathological spectrum from benign to malignant, the incidence of Ki-ras and p53 mutations was determined in 20 ovarian cystadenomas, 20 low malignant potential (LMP) tumors of the ovary, and 23 ovarian carcinomas. Using DNA extracted from paraffin embedded tissue, polymerase chain reaction amplification, designed restriction fragment length polymorphism analysis, and DNA sequencing, 1 cystadenoma (5%), 6 LMP tumors (30%), and 1 ovarian carcinoma (4%) demonstrated an activated Ki-ras gene. All of the Ki-ras mutations identified except one were GGT to GAT transversions at codon 12. One LMP tumor demonstrated a CAA to CAC transversion at codon 61. Using polymerase chain reaction/single strand conformational polymorphism, DNA sequencing, and immunohistochemistry, 11 ovarian carcinomas (48%) demonstrated a p53 mutation. These mutations included 5 missense, 2 nonsense, and 1 frameshift mutation located within exons 6-8 and 3 mutations that were identified only by immunohistochemical staining. No p53 mutations could be identified in cystadenomas or LMP tumors. Clinically, the presence of either a Ki-ras or p53 mutation was associated with advanced stage disease. The pattern of Ki-ras and p53 mutations appears to distinguish LMP tumors from invasive carcinomas and suggests that they may be separate biological entities.

INTRODUCTION

Epithelial ovarian neoplasms span a clinicopathological spectrum ranging from ovarian cystadenoma to LMP3 tumor of the ovary to invasive ovarian carcinoma. Although these tumors are comprised of cells which may share a common origin, each exhibit unique clinical and histological characteristics (1-3). Ovarian cystadenomas follow a benign clinical course and lack the cytological and nuclear atypia seen in LMP tumors and invasive carcinomas. In turn, LMP tumors, which retain an overall cellular and nuclear architecture similar to invasive carcinomas and have the ability to metastasize, lack the invasive histological properties of their fully malignant counterparts (2). While these clinical and histological features define a spectrum of disease, the precise pathogenetic relationship between these tumors remains unclear. For instance, some investigators suspect that LMP tumors, as an intermediate form of malignancy, may be a precursor of invasive carcinomas (4). However, the observation that LMP tumors follow a more benign clinical course compared to invasive carcinomas suggests that LMP tumors may be separate biological entities. In addition, the relationship between ovarian cystadenomas and LMP tumors remains poorly understood. Therefore, a more detailed molecular and pathological analysis of these neoplasms is required to elucidate their relationship to one another. The characterization of dominant and recessive oncogenes, such as ras and p53, might help demonstrate important differences or similarities among these tumors.

The ras gene family consists of 3 closely related genes: Ha-ras, Ki-ras, and N-ras. These genes encode Mr 21,000 proteins (21) which are localized to the inner plasma cellular membrane, demonstrate GTPase activity, and appear to be involved in normal cell receptor signal transduction pathways (5). Mutant p21 produced by specific amino acid substitutions at codons 12, 13, or 61 results in the loss of GTPase activity and activation of the gene product (5, 6). ras mutations are a common occurrence in human cancers. Up to 90% of pancreatic adenocarcinomas have an activated Ki-ras gene, while rates of at least 30% have also been reported for non-small cell lung cancer (Ki-ras), colon cancer (Ki-ras), seminoma (Ki-, N-ras), melanoma (N-ras), and thyroid cancer (all ras genes) (5, 6). In ovarian neoplasms, only ovarian carcinoma has been systematically studied for ras gene abnormalities where they appear to be uncommon events (7).

The p53 gene encodes a Mr 53,000 DNA binding phosphoprotein which functions as a tumor suppressor gene (8, 9). The loss of wild type p53 function through single base pair mutations or deletions in exons 5-8, where several highly conserved domains are located, can lead to deregulated cellular proliferation and transformation (9, 10). p53 mutations have been identified in malignancies of the colon, lung, breast, esophagus, head and neck, and hematopoietic system as well as soft tissue sarcomas (10, 11). In ovarian neoplasms, mutation and/or overexpression of the p53 gene have been described previously in epithelial ovarian carcinomas (12, 13), but only rarely in other ovarian neoplasms (14).

We determined the frequency of Ki-ras and p53 gene mutations in a series of ovarian neoplasms in an attempt to define the molecular relationship between ovarian cystadenomas, LMP tumors of the ovary, and ovarian carcinoma. Using DNA isolated from paraffin embedded tissues and PCR amplification, we searched for activated Ki-ras genes by designed RFLP analysis and DNA sequencing. In addition, p53 mutations within exons 5-8 were identified by PCR amplification, SSCP analysis, and IHC staining, and characterized by DNA sequencing.

MATERIALS AND METHODS

Case Selection. Tumor specimens dating from 1985 to 1992 were selected from the pathology archives at the National Naval Medical Center, Bethesda, MD. For the LMP tumors and ovarian carcinomas, a surgical stage had previously been assigned based on the staging system of the International Federation of Obstetrics and Gynecology. In all of the ovarian cystadenomas, ovarian carcinomas, and 19 of 20 LMP tumors, the patients underwent surgery at the National Naval Medical Center and tissue obtained at the initial surgery was used for analysis. In one LMP tumor case, secondary cytoreductive surgery was performed for recurrent disease at the National Naval Medical Center and ovarian tissue from the second surgery was used for analysis. For cases where a Ki-ras mutation was identified, histologically normal tissue from the original surgery, if available, was also obtained for analysis.

Histological Analysis and DNA Extraction. All paraffin blocks were serially sectioned: first for H&E staining, second for DNA analysis, and third for H&E and IHC staining. In this manner, tissue for molecular analysis was "sandwiched" between two H&E sections to ensure the presence of pathological tissue. An unstained 8-μm section was then deparaffinized and digested as previously described (15).
PCR Amplification: Ki-ras Gene. PCR was performed by using either a Perkin Elmer Cetus Model 480 thermocycler (Perkin Elmer Cetus, Norwalk, CT) or Techne Model PHC-3 thermocycler (Techne, Ltd., Cambridge, United Kingdom). A 10-μl aliquot of the DNA solution was used for amplification in a 100-μl volume containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 200 μM concentrations of each deoxynucleoside triphosphate, 5 units of Taq DNA polymerase (Bethesda Research Laboratories, Bethesda, MD), and 0.5 to 1 μg of each oligonucleotide primer (16). Forty amplification cycles were performed under the following conditions: 94°C for 1 min, 50°C (codon 12) or 55°C (codon 61) for 2 min, and 68°C for 2 min. Oligonucleotide primers were synthesized by using a DNA synthesizer (Model 380B, Applied Biosystems, Foster City, CA).

Detection of Ki-ras Codons 12, 13, 61 Mutations. Activated Ki-ras genes were identified by designed RFLP analysis using mismatched primers (16).

Restriction enzyme digestion with the use of 20 μl of the PCR mixture was performed according to the manufacturer’s recommendations (Banl and Earl, New England Biolabs, Beverly, MA. BstI and MboI, Bethesda Research Laboratories, Bethesda, MD). DNA fragments were visualized by electrophoresis through a 3% Nusieve/1% Tris-acetate EDTA-agarose gel. The uncut DNA fragment was then digested by undigested and digested PCR products were paired side by side during electrophoresis to facilitate RFLP identification. For cases not demonstrating a mutation of codon 12 or 61 by designed RFLP analysis, DNA sequencing was used for detection of codon 13 mutations.

Detection of Ki-ras Codon 12 Mutations in Ovarian Cystadenomas. In one of the ovarian cystadenomas, RE digestion of amplified DNA with Banl yielded a faint, uncleaved fragment. In order to selectively amplify the uncleaved DNA fragment, a nested primer amplification was performed followed by two rounds of mismatched PCR amplification and RE digestion with Banl. An initial amplification in a 100-μl volume was performed by using nonmismatched primers (16). Two sequential PCR amplifications using mismatched primers were performed using 1 μl of the PCR product digested with Banl. After the final Banl digestion, the entire specimen was analyzed on a 3% Nusieve/1% Tris-acetate EDTA-agarose gel. The uncut DNA fragment was then removed from the gel, purified (GeneClean, San Diego, CA), and sequenced as described below.

PCR/SSCP Analysis for p53 Mutations in Exons 5-8. For PCR/SSCP analysis, amplified DNA fragments spanning either exons 5 and 6 or exons 7 and 8 were analyzed separately as previously described with slight modifications (17, 18). In brief, PCR amplification of exons 5 and 6 was performed by using 3 μl of the sample DNA solution in a 10-μl volume containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 200 μM concentrations of each deoxynucleoside triphosphate, 0.5 unit of Taq DNA polymerase, 0.05 to 0.1 μg of each primer (17), and 1 μl of [α-32P]dCTP (10 μCi/μl, 3000 Ci/mmol, Amersham Corp., Arlington, IL). For exons 7 and 8 PCR amplification, nested primers were used to improve the overall yield of detectable DNA. An initial outer nested amplification in a volume of 50 μl was performed by using the following primers:

3’ AAAGAGGCAAGGAGGAGTAGTAA

DNA Sequencing of Ki-ras and p53. For Ki-ras codon 12/13 and codon 61, direct sequencing of DNA fragments obtained by PCR was performed by using an automated DNA sequencer (Model 373A, Applied Biosystems). To obtain sufficient DNA for sequencing (0.5 to 1.0 μg), a DNA fragment including either codons 12 and 13 or codon 61 was amplified in a 100-μl volume using mismatched primers (15). The amplified DNA was purified by passage through a Qiagen-20 spin column (Qiagen Inc., Chatsworth, CA). Enzymatic sequencing of the PCR product using fluorescent dye labeled deoxyribonucleotides was performed by using the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems).

For p53 gene sequencing, a DNA fragment containing an individual exon was obtained by PCR amplification as described above, using the following primers:

exon 6: 5’ CGGCCCTGTATCTCAGTTGCT
3’ ATATTTGACACTTCAGCCCTTA
exon 7: 5’ CGGTCGACCGGAGTGAACA
3’ CATGCGGAGGCTGGCAAGT
exon 8: 5’ TCTTCTGTCCTCTCTTCTCTTCT
3’ AATAGTGAAAGGAACGGGAGAA

The fragment was then cloned by using the TA Cloning System, version 1.3 (Invitrogen Corp., La Jolla, CA). Plasmid isolation and purification was performed using Qiagen Mini columns (Qiagen Inc.). Bidirectional sequencing using Sp6 and T7 promoter primers (Bethesda Research Laboratories) was performed as described above.

Immunohistochemistry. A mouse monoclonal antibody, DO-7, directed against human p53 was used for IHC staining (Dako Corp., Carpenteria, CA). Primary incubation was performed overnight at 4°C with 9.5 μg/ml of the antibody. IHC staining was performed by the avidin-biotinylated peroxidase complex technique using a Vectastain kit (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions and modified as previously reported (19). Positive controls included a formalin fixed paraffin embedded cell pellet of a non-small cell lung cancer cell line (NCI-H1155) known to express high amounts of mutant p53 protein (20), and a positively staining primary non-small cell lung cancer. Controls for specificity consisted of the incubation of serial ovarian tumor sections with: (a) an unrelated mouse monoclonal antibody, and (b) phosphate buffered saline. Results of the IHC staining were reviewed without knowledge of the molecular data by four authors (M. G. T., M. E., R. I. L., M. J. B.) who scored both for the intensity of the staining (0, negative; 1, weak; 2, moderate; 3, strong reaction), and the percentage of positive cells within the tumor. In addition, each specimen was assessed for the percentage of tumor within each paraffin section.

RESULTS

Clinical Characteristics of Study Patients. A total of 20 cystadenomas, 20 LMP tumors, and 23 ovarian carcinomas were selected for analysis. Although the selection of cases was not random, the clinical characteristics of the study patients and the pathology of the tumors (Table 1) were generally consistent with the known behavior of these diseases in terms of stage distribution, histological findings, and disease status. All of the patients with an ovarian cystadenoma underwent at least oophorectomy, and the majority (14 of 20) underwent surgery because of a postmenopausal adnexal mass. Of the patients with LMP tumors, 16 of 20 (80%) had surgically defined stage I disease and the remaining 4 patients had stage III disease based on the presence of microscopic extrapelvic disease. In patients with ovarian carcinoma, 18 of 23 (78%) presented with stage III or IV disease. All patients with stage III or IV ovarian carcinoma underwent cytoreductive staging surgery, and following surgery, 10 (56%) had optimal residual disease (<1 cm residual disease), and 8 (44%) had suboptimal residual disease (>1 cm residual disease).
Frequency of Activated Ki-ras Genes in Ovarian Neoplasms.

Sixty-three ovarian neoplasms were screened for the presence of activated Ki-ras genes. Mismatched PCR and BanI digestion of DNA from 5 LMP tumors and 1 ovarian carcinoma revealed a codon 12 mutation (Fig. 1A). In each of these cases, the specific mutation was identified by a second mismatched PCR amplification and digestion with MboI (Fig. 1B). Digestion of amplified mismatched DNA with MboI only occurs with a GTT to GAT transversion at codon 12. The mutation in each of these specimens was confirmed by DNA sequencing (data not shown). One ovarian cystadenoma demonstrated a weak uncult DNA fragment (mutant DNA) after BanI digestion. Selective amplification of the uncult DNA was accomplished by repeated PCR amplifications and enzymatic digestions. Isolation and sequencing of the uncult DNA identified a GTT to GAT transversion at codon 12 (data not shown). The small quantity of mutant DNA most likely reflects the small fraction of tumor cells present in a tumor with a single cell layer epithelium. Evaluation of the ovarian neoplasms for codon 61 mutations by digestion of amplified mismatched DNA with EarI and BclI revealed only one mutation which occurred in an LMP tumor (Fig. 1C). The specific mutation was identified by DNA sequencing to be a CAA to CAC transversion (data not shown). One ovarian cystadenoma demonstrated a weak uncult DNA fragment (mutant DNA) after BanI digestion. Selective amplification of the uncult DNA was accomplished by repeated PCR amplifications and enzymatic digestions. Isolation and sequencing of the uncult DNA identified a CAA to CAC transversion at codon 61 (data not shown). The small quantity of mutant DNA most likely reflects the small fraction of tumor cells present in a tumor with a single cell layer epithelium. Evaluation of the ovarian neoplasms for codon 61 mutations by digestion of amplified mismatched DNA with EarI and BclI revealed only one mutation which occurred in an LMP tumor (Fig. 1C). The specific mutation was identified by DNA sequencing to be a CAA to CAC transversion (data not shown).

To ensure specificity and to exclude the possibility of a germ line mutation, normal tissue including cervix, myometrium, or the uninvolved contralateral ovary was also analyzed for the presence of the Ki-ras mutation identified in the corresponding tumor specimen. No Ki-ras mutations in histologically normal tissue were identified (data not shown).

Clinical Characteristics of Study Patients and Pathological Features of Ovarian Neoplasms with Activated Ki-ras Genes. LMP tumors with an activated Ki-ras gene were evenly divided between serous and mucinous histology (Table 2). A trend toward advanced stage disease was seen in patients with activated Ki-ras genes where 2 of 4 patients (50%) with stage III disease had a activated Ki-ras gene compared with 4 of 16 patients (25%) with localized disease. In addition, the only patient with recurrent disease had an activated Ki-ras gene. The one patient with ovarian carcinoma and an activated Ki-ras gene had stage Ic disease and died of recurrent disease 23 months following initial diagnosis and treatment.

Frequency of p53 Mutations in Ovarian Neoplasms by PCR/SSCP. PCR/SSCP analysis of the 63 ovarian neoplasms for p53 mutations revealed abnormal SSCP patterns in 9 ovarian carcinomas (39%), 1 ovarian cystadenoma (5%), and none of the LMP tumors. To confirm and determine the specific nature of these mutations, all tumors which demonstrated an abnormal SSCP pattern were sequenced (Table 3). Five ovarian carcinomas demonstrated mutations in exon 6 (Fig. 2), 2 in exon 8 (data not shown), and 1 in exon 7 (data not shown). Five missense mutations, 2 nonsense mutations, and 1 frameshift mutation (leading to a downstream termination codon) were localized between codons 189 and 273. Two of the 8 mutations

<table>
<thead>
<tr>
<th>Ovarian neoplasm</th>
<th>FIGO stage</th>
<th>Tumor histology</th>
<th>Codon</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystadenomas M59</td>
<td>III</td>
<td>Mucinous</td>
<td>12</td>
<td>GGT → GAT</td>
</tr>
<tr>
<td>LMP tumor M28</td>
<td>III</td>
<td>Serous</td>
<td>12</td>
<td>GGT → GAT</td>
</tr>
<tr>
<td>M32</td>
<td>I</td>
<td>Seromucinous</td>
<td>12</td>
<td>GGT → GAT</td>
</tr>
<tr>
<td>M40</td>
<td>I</td>
<td>Serous</td>
<td>12</td>
<td>GGT → GAT</td>
</tr>
<tr>
<td>M43</td>
<td>III</td>
<td>Serous</td>
<td>12</td>
<td>GGT → GAT</td>
</tr>
<tr>
<td>M55</td>
<td>I</td>
<td>Mucinous</td>
<td>12</td>
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</tr>
<tr>
<td>M45</td>
<td>I</td>
<td>Mucinous</td>
<td>61</td>
<td>CAA → CAC</td>
</tr>
<tr>
<td>Carcinoma M82</td>
<td>I</td>
<td>Clear cell</td>
<td>12</td>
<td>GGT → GAT</td>
</tr>
</tbody>
</table>

* FIGO, International Federation of Obstetrics and Gynecology.
p53 AND Ki-ras MUTATIONS IN OVARIAN NEOPLASMS

Table 3 Ovarian carcinomas with p53 mutations by PCR/SSCP and DNA sequencing or immunohistochemical staining

<table>
<thead>
<tr>
<th>Tumor code</th>
<th>FIGO* stage</th>
<th>Histology</th>
<th>Residual disease</th>
<th>SSCP Codon</th>
<th>DNA sequencing</th>
<th>% of tumor in section</th>
<th>% of Tumor cells staining positive</th>
</tr>
</thead>
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<tr>
<td>M19</td>
<td>III</td>
<td>Transitional</td>
<td>O</td>
<td>Exon 6</td>
<td>215</td>
<td>AGT → ATT; Ser to Ile</td>
<td>&gt;75</td>
</tr>
<tr>
<td>M78</td>
<td>III</td>
<td>Serous</td>
<td>O</td>
<td>Exon 7</td>
<td>237</td>
<td>ATG → ATA; Met to Ile</td>
<td>&gt;75</td>
</tr>
<tr>
<td>M37</td>
<td>IV</td>
<td>Serous</td>
<td>O</td>
<td>Exon 8</td>
<td>273</td>
<td>CGT → CAT; Arg to His</td>
<td>&gt;75</td>
</tr>
<tr>
<td>M52</td>
<td>IV</td>
<td>Serous</td>
<td>O</td>
<td>Exon 8</td>
<td>266</td>
<td>GGA → GTA; Gly to Val</td>
<td>20</td>
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<tr>
<td>M51</td>
<td>III</td>
<td>Clear cell</td>
<td>O</td>
<td>Exon 6</td>
<td>189</td>
<td>GCC → CCC; Ala to Pro</td>
<td>&gt;75</td>
</tr>
<tr>
<td>M22</td>
<td>IV</td>
<td>Serous</td>
<td>S</td>
<td>Exon 6</td>
<td>224</td>
<td>GAG → TAG; Glu to Stop</td>
<td>&gt;75</td>
</tr>
<tr>
<td>M23</td>
<td>IV</td>
<td>Serous</td>
<td>S</td>
<td>Exon 6</td>
<td>205</td>
<td>TAT → TAG; Tyr to Stop</td>
<td>&gt;75</td>
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<tr>
<td>M76</td>
<td>III</td>
<td>Serous</td>
<td>O</td>
<td>Exon 6</td>
<td>212</td>
<td>TTT → TT; 1 base pair deletion</td>
<td>&gt;75</td>
</tr>
</tbody>
</table>

* FIGO, International Federation of Obstetrics and Gynecology; NT, not tested.

Following cytoreductive surgery, O: optimal residual <1 cm, S: suboptimal >1 cm.

Percentage of each paraffin section demonstrating malignant tissue was estimated by light microscopy of H&E sections.

Percentage of tumor cells within a specimen with positive nuclear staining estimated by light microscopy. In the cases with positive staining, intensity ranged from moderate to strong.

This frameshift mutation leads to a termination codon at 215.

Minor abnormal SSCP bands were noted in exon 8, but were scored as normal.

(M37 and M78) were present in highly conserved domains within exons 5–8 (10). No particular type of mutation predominated, although G:C to A:T or T:A transversions were present in 5 cases. One case (M37) demonstrated a known “hotspot” mutation at a CpG dinucleotide at codon 273 (10). Identical exon 6 SSCP abnormalities were identified in an ovarian carcinoma (M79; Fig. 2) and an ovarian cystadenoma (M95, data not shown). Both of these tumors were sequenced and both demonstrated a CGA to CGG transversion at codon 213. This transversion has been identified by others as a neutral polymorphism of the p53 gene (21). To validate the reliability of SSCP in identifying wild type p53, specimen M18 (carcinoma) and M56 (LMP tumor), which demonstrated normal exon 6 and exon 7 patterns, respectively, on SSCP, were shown by DNA sequencing to have the normal sequence (data not shown). In comparing all cases with either a Ki-ras or p53 mutation, there were no tumors which demonstrated both a Ki-ras and p53 mutation.

Frequency of p53 Mutations in Ovarian Carcinomas by IHC Staining. Since p53 mutations were identified only in ovarian carcinomas, further study of this group of tumors by IHC staining was undertaken for confirmation of the molecular analysis. Positive staining for p53 was seen in 7 of 23 ovarian carcinomas (Table 3). Four of the 7 cases (M19, M52, M76, M78) had been identified by unequivocally abnormal SSCP patterns and were shown to have missense mutations by DNA sequencing. The staining in these 4 cases was very intense and comprised a substantial percentage of the tumor cells. Interestingly, the other three positive cases (M21, M74, M77) had intense but focal p53 staining. The SSCP results for two specimens (M74, M77) revealed only faint band shifts in exon 8 which could not be convincingly distinguished from background. The other case (M21) was normal by SSCP analysis. In addition, no staining was observed in 4 cases where SSCP and DNA sequencing had identified the presence of a mutation. These cases included 3 nonsense mutations (M22, M23, M76) and one missense mutation (M51). The lack of staining in the latter case may have been related to suboptimal tissue preservation seen on H&E staining.

With the molecular and IHC analysis combined, a total of 11 of 23 ovarian carcinomas (48%) were identified with a mutation of the p53 gene.

Clinical Characteristics of Study Patients and Pathological Features of Ovarian Neoplasms with p53 Mutations. In the 11 ovarian carcinomas with a p53 mutation (Table 3), several histological types were represented: 7 serous (64%), 1 clear cell (9%), 1 transitional cell (9%), 1 endometrioid (9%), and 1 mucinous (9%) tumors. As noted with Ki-ras and LMP tumors, a trend toward advanced stage disease was seen in patients with a p53 mutation where 11 of 18 patients (61%) with stage III/IV disease had a p53 mutation in contrast to 0 of 5 patients with stage I/II disease. Additionally, 6 of 11 stage III/IV patients (55%) with a p53 mutation had optimal residual disease following cytoreductive surgery, compared to 4 of 7 (57%) without a p53 mutation.

Clinical follow-up information was available for 22 of 23 patients with ovarian carcinoma. Although the number of cases was small and precluded a statistical analysis, median survival time was determined for patients according to stage and p53 status. With a mean follow-up time of 30.8 months (range, 1 to 73 months), the median survival for the 17 patients with stage III/IV disease was 37 months. For the 10 patients with a p53 mutation, clinical follow-up was available for 9 patients (all stage III/IV). The overall median survival for these 9 patients was 45 months (mean follow-up, 43 months), while the 8 stage III/IV patients without a p53 mutation had a median survival of 26 months.

DISCUSSION

In the present study, the incidence of Ki-ras and p53 gene mutations was determined in a series of ovarian neoplasms spanning the histological spectrum from benign to malignant. We found that activated
Ki-ras genes occur at a higher frequency in LMP tumors (30%) than in ovarian carcinoma (4%), and that p53 mutations only occur in ovarian carcinoma (48%) and not in ovarian cystadenomas or LMP tumors.

In ovarian carcinoma, previous work has indicated that ras gene amplification or mutation appears to be uncommon with a combined incidence of 5–10% (7). Although Zhou et al. (22) reported Ki-ras amplification in 3 of 7 (41%) ovarian carcinomas, subsequent studies have not confirmed this observation (23–26). Recently, Enomoto et al. (27) reported the presence of an activated Ki-ras gene in 10 of 31 epithelial ovarian carcinomas; however, the majority of these cases had mucinous histology which actually represents only 15% of ovarian carcinomas (2). In fact, only 3 nonmucinous tumors in their study had an activated Ki-ras gene. Therefore, our result demonstrating an activated Ki-ras gene in 1 of 23 ovarian carcinomas (4%) appears consistent with these published results and suggests that activated ras genes are not of major importance in the pathogenesis of this disease.

The specific role of the ras gene in other ovarian neoplasms has not been systematically explored. Yaginuma et al. (28) has described the overexpression of the ras p21 oncoprotein in several benign and LMP tumors of the ovary. Enomoto et al. (27) also reported a single case of a mucinous LMP tumor with an activated Ki-ras gene. However, the incidence of activated ras genes in ovarian cystadenomas or LMP tumors cannot be accurately determined from these studies. Our study suggests that 30% of LMP tumors have an activated Ki-ras gene. Thus, while the ras gene appears to play a minor role in the pathogenesis of invasive ovarian carcinoma, our data suggest that it might be more important in LMP tumors. Additionally, the identification of an activated Ki-ras gene in a benign ovarian tumor is of interest. Other benign tumors have been identified with activating ras mutations, including approximately 40% of colonic adenomas which are known precursors of colon carcinoma (29). Whether ovarian cystadenomas with activated ras genes behave differently from those with a normal ras gene will require further analysis of a larger number of specimens.

In addition, the finding of an activated Ki-ras gene in an ovarian cystadenoma emphasizes the sensitivity of our designed RFLP assay. In this particular case, the abnormal epithelium represented only a small fraction of the total amount of the paraffin embedded tissue. Through the use of repeated cycles of PCR amplification and enzyme digestion, the mutant DNA band was selectively amplified in order to obtain a sufficient quantity of DNA for sequencing. This technique allowed us to detect mutant DNA that comprised only 5% of the total DNA content of the sample. It is conceivable that conditions could be designed to further improve the efficiency of this technique such that even smaller quantities of mutated ras gene could be identified.

The PCR/SSCP technique for identifying p53 mutations relies on the conformational molecular changes resulting from base sequence alterations. Using this approach, we identified mutations in 8 of 23 ovarian carcinomas (35%), a finding consistent with the previous observations by Mazars et al. (13) who used a similar technique. However, the incidence of p53 mutations detected by PCR/SSCP can be underestimated due to electrophoretically silent mutations, mutations outside of exons 5–8, and the overall sensitivity of the assay. As a result, we were interested in confirming and comparing our PCR/SSCP findings in ovarian carcinoma with an independent assay such as IHC staining. This technique detects p53 mutations by virtue of the fact that mutation of the gene frequently results in prolongation of protein half-life (9, 12, 20). Of interest, three cases with equivocal or normal SSCP findings had focally intense staining. In all three cases, ≤10% of the paraffin embedded tissue stained positively for p53. Thus, the molecular analysis may not detect mutations in specimens with a small percentage of cells which contain the mutant p53 gene. Conversely, we found no p53 staining in 3 cases with unequivocally abnormal SSCP patterns which were identified as having nonsense mutations by DNA sequencing. This type of mutation, which would result in a truncated form of the protein, has been shown previously as not being detectable by IHC analysis (20). Thus, our findings suggest that these two techniques are complementary and, in our sampling of cases, identified an overall incidence of p53 mutations in ovarian carcinoma of 48%. This finding is similar to that reported by Marks et al. (12), who demonstrated in a much larger sampling of cases, overexpression of p53 protein in 50% of ovarian carcinomas.

Of interest, we were unable to detect any p53 mutations in cystadenomas and LMP tumors. This suggests that either LMP tumors are a discrete biological entity separate from ovarian carcinoma which follow a different molecular pathway leading to malignant transformation, or that LMP tumors are a precursor of ovarian carcinoma which acquire a p53 mutation at a later stage in malignant transformation. Our finding of a higher incidence of activated Ki-ras genes in LMP tumors strongly supports the former hypothesis. The molecular data are also in agreement with the clinical observation that LMP tumors rarely progress to invasive carcinoma and have a favorable prognosis compared to carcinoma (1, 3). Further, the finding of an activated Ki-ras gene and the lack of p53 mutations in benign tumors of the ovary suggest that LMP tumors and ovarian cystadenomas may be more closely related to each other than to ovarian carcinoma.

In LMP tumors, although the number of cases is small, the presence of an activated Ki-ras gene was more common in patients with advanced stage disease. In fact, the only patient with recurrent disease had stage III disease and an activated Ki-ras gene in contrast to no recurrences in the other 14 patients without a Ki-ras mutation. No further comment can be made regarding Ki-ras mutations and prognosis, since as a whole, patients with LMP tumors have a favorable long term prognosis, as was observed in our study group (1, 4). In patients with ovarian carcinoma, correlation of p53 status with known prognostic variables revealed that p53 mutations were more common in patients with advanced stage disease, but not in cases with suboptimal residual disease. Additionally, while median survival in patients with a p53 mutation appeared to be improved over those without a p53 mutation, the relatively small sample size and the nonrandom selection of cases limit the value of this correlation.

In conclusion, this study identifies different patterns of oncogene mutations which help distinguish LMP tumors from ovarian carcinoma and suggests that each may follow a different molecular developmental pathway. It would also appear that activated Ki-ras genes are more frequent in advanced stage LMP tumors of the ovary and that p53 mutations are more common in advanced stage ovarian carcinomas. From a practical standpoint, this pattern of oncogene mutations may be useful in distinguishing LMP tumors from ovarian carcinomas. An analysis of this type may have clinical utility in cases of low grade ovarian malignancies where the exact histopathological diagnosis is unclear. Further study of a wider sampling of cases will be necessary before a definitive statement can be made concerning the precise role and prognostic significance of either Ki-ras or p53 mutations in ovarian neoplasms.

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