Prognostic Significance of p53 Overexpression in Endometrial Cancer

Kiyoshi Ito, Keiko Watanabe, Suhail Nasim, Hironobu Sasano, Shinji Sato, Akira Yamada, Steven G. Silverberg, and Carleton T. Garrett

Department of Obstetrics and Gynecology [K. I., K. W., S. S., A. Y.] and Pathology [H. S.], Tohoku University School of Medicine, Sendai, Japan, and Department of Pathology, George Washington University Medical Center, Washington, DC 20032 [S. N., S. G. S., C. T. G.]

ABSTRACT

Abnormalities of p53, a tumor suppressor gene, have been considered to play an important role in tumorigenesis. Clinically, overexpression of p53 has been reported to correlate with poor prognosis in several types of tumors. In this study, we examined 221 cases of endometrioid endometrial carcinoma for overexpression of p53 using immunohistochemistry in patients with a median follow-up of 41 months. Immunohistochemical analysis was performed with monoclonal antibody pAb801. Overexpression of p53 was detected in 47 of 221 cases (21.3%). There was a statistically significant correlation between p53 overexpression and poor prognosis (P < .0001). In the early stages of disease (stages 1 and 2), p53 was overexpressed in 11 of 22 patients (50%) who died or had a recurrence during follow-up. In contrast, overexpression was detected in only 14.7% of the 156 disease-free patients during the same period (P < .0001). In advanced stages (stages 3 and 4), tumors from patients with recurrent disease had a higher frequency of overexpression (41.2%; 7 of 17) than those of disease-free patients (23.1%; 6 of 26). However, the difference between these frequencies was not statistically significant. In multivariate analysis using the Cox proportional hazard model, p53 overexpression was an independent risk factor when compared with clinical stage, nuclear grade, and patient age. Our results indicate that p53 immunohistochemical evaluation of the most common form of endometrial cancer may be useful in identifying cases of aggressive carcinoma, especially in the early stages.

INTRODUCTION

The p53 gene, which encodes a M, 53,000 nuclear phosphoprotein, is considered to be a tumor suppressor gene (1). p53 mutations have frequently been identified in a wide variety of human malignancies including colon, breast, and lung cancer (2, 3). Wild-type p53 protein is expressed at low levels in most normal fetal and adult tissues and has a half-life of only 5 to 45 min (4). Missense mutations of the p53 gene result in proteins with greatly extended half-lives (4 to 8 h), which form stable complexes with heat shock protein 70 and with normal p53 protein (5). Moreover, mutant p53 proteins generally have a much longer half-life than do the wild p53 proteins on the order of 4 to 8 h. The extended half life of mutant p53 protein leads to its accumulation within the nuclei of affected cells. Recent reports have shown that immunohistochemical detection of p53 protein is closely correlated with the presence of mutations in the gene (6–8). In a previous study, we demonstrated that overexpression of p53 observed by immunohistochemical staining is not due to an increase in the steady-state level of p53 mRNA in carcinoma cells (9).

Endometrial cancer is the most common female pelvic cancer in the United States with approximately 33,000 new cases diagnosed every year (10). However, compared to cancer of other sites, studies of the molecular genetic alterations involved in the development of endometrial cancer are limited. We previously reported that p53 overexpression was observed in endometrial carcinoma cells but was not observed in endometrial hyperplasia or normal endometrium (9). Recently, Kohler et al. (11) described the same observation. Two other studies have reported an association between p53 overexpression and several prognostic factors (12, 13). However, the role of p53 overexpression as an independent predictor of survival has not been thoroughly evaluated.

To investigate the possible relationship between p53 overexpression and prognosis, we studied 221 cases of endometrioid endometrial cancer with follow-up data. We observed a significant correlation between p53 overexpression and decreased survival, especially in stages I and II disease. Our results indicate that p53 immunohistochemical evaluation may be useful as a new prognostic indicator and could be especially useful in evaluating low-stage disease.

MATERIALS AND METHODS

Tissues. Two hundred twenty-one cases of endometrioid endometrial carcinoma were studied. Patients were Japanese with a median follow-up of 41 months. The cases were accessioned between 1979 and 1991 at Tohoku University Hospital and affiliated hospitals. The carcinoma samples were obtained at surgery (n = 217) and curettage (n = 4). None received preoperative pelvic radiation. The lesions were classified according to the Histological Typing of Female Genital Tract Tumors by WHO (14) and staged according to the International Federation of Gynecology and Obstetrics system (15). One hundred and fifty-six lesions were stage I, 22 were stage II, 33 were stage III, and 10 were stage IV.

Tissue Preparation. One hundred ninety-four cases were routinely formalin-fixed tissues. Twenty-seven cases were fixed in 4% parafomaldehyde in phosphate-buffered saline, pH 7.4. Fixed tissues were then embedded in paraffin.

Immunohistochemical Staining. A single 2.5-μm section from paraffin-embedded specimens was routinely deparaffinized and incubated in methanol with 0.3% H2O2 for 30 min at room temperature to block endogenous peroxidase activity. After treatment with 1% normal rabbit serum for 30 min at room temperature, the samples were incubated with the primary antibody for 18 h at 4°C in a moisture chamber. The primary antibody used in this study was the monoclonal antibody pAb1801 (Nobocora Laboratory, Newcastle, United Kingdom). This antibody can recognize a denaturation-resistant epitope in human p53 located between amino acids 32 and 79 (16). The optimal dilution for the antibody was 1:50. The Histofine avidin-biotin method (Nichirei, Inc., Tokyo, Japan) was then used for immunostaining. After treatment with biotinylated anti-mouse immunoglobulin for 45 min at room temperature, the sections were incubated with peroxidase-conjugated streptavidin for 30 min at room temperature. A final wash was followed by immersion of the reacted sections for 5–10 min in a solution containing 0.06 mM 3,3′-diaminobenzidine and 2 mM hydrogen peroxide in 0.05% Tris-HCl buffered at pH 7.6. Specific staining was identified by the presence of brown reaction products. The sections were counterstained with 1% methyl green, and coverslips were mounted with a glycerol-gelatin, water-soluble medium.

Interpretation of Immunohistochemical Staining. The sections were evaluated according to the intensity of staining: when nuclear immunoreactivity was intense and/or many positive cells (>80% of tumor) were observed, the tumor was considered to be strong/diffuse positive (Fig. 1A); when nuclear immunoreactivity was weak or rare positive cells were present, the tumor was regarded as weak/focal positive (Fig. 1B); and no reactivity was negative. For purposes of evaluation of the data in this report, a case was regarded as demonstrating p53 overexpression if it was either strong/diffuse or weak/focal positive.
RESULTS

Association of p53 Overexpression with Clinical Stage and Tumor Grade. p53 immunoreactivity was observed as nuclear staining (Fig. 1) and was present in 47 of 221 cases of endometrioid endometrial carcinoma (21.3%). As previously reported, only tumor cells demonstrated immunoreactivity (9).

The relationship between p53 overexpression and surgical stage or tumor grade was examined. As can be seen in Table 1, the incidence of p53 overexpression was greatest in high grade tumors and appeared to decrease with decreasing tumor grade. No statistically significant relationship could be discerned between clinical stage and frequency of cases overexpressing p53 (data not shown). Stage IV tumors did demonstrate an approximately 2-fold greater incidence of p53 overexpression as compared with other stages, but this may have been due in part to the relatively small sample of stage IV lesions.

Association of p53 Overexpression with Disease-free Survival. Next, p53 overexpression was evaluated as a prognostic variable in the 221 cases by univariate analysis (Table 2; $\chi^2$ test). In the total 221 cases, p53 was overexpressed in 18 of 39 patients who died or had recurrence during follow-up (46.2%), while overexpression was detected in only 29 (15.9%) of 182 disease-free patients for the same period. Patients whose tumors demonstrated overexpression were at much greater risk to develop recurrent disease ($P < .001$). As would be expected, classical indicators of prognosis including stages III and IV disease, grades 2 and 3 morphology, and older age were likewise significantly associated with poor outcome. The relationship between p53 overexpression and decreased survival was also highly significant ($P < .001$) using the Mantel-Haenszel, Wilcoxon-Gehan, and log-rank tests (17, 18), all of which are sensitive to the length of survival

In order to determine whether the prognostic value of p53 overexpression was independent of other risk factors, we examined the data by multivariate analysis according to the Cox proportional hazard analysis model (17, 19). The prognostic factors included p53 immunohistochemistry, clinical stage, tumor grade, and age of the patient. We did not include lymph node metastasis in the analysis, because lymph node dissection had not been done in 118 of the cases. The findings are summarized in Table 2 (multivariate analysis). p53 immunohistochemistry, stages III and IV, grade 3, and age of the patient were independently statistically significant as risk factors in our data.

Significance of p53 Overexpression in Patients with Low-Stage Disease. While the outlook for patients with high-stage disease (stages III and IV) is generally recognized to be poor, a small but significant percentage of patients with low-stage disease (stages I and II) will likewise develop recurrence and die. Therefore, it was of interest to examine the prognostic utility of p53 overexpression for patients with low- and high-stage disease separately. Fig. 2 shows the relation between p53 staining and survival for patients with early-stage disease. The relation between p53 overexpression and decreased survival for stages I and II patients was highly significant ($P < .0001$; Mantel-Haenszel, Wilcoxon-Gehan, and log-rank tests). Fig. 3 shows this relationship for patients in advanced stages. Although there was a trend for p53-positive cases to expire more quickly than p53-negative ones, the differences were not statistically significant.

The clinico-pathological variables analyzed previously for the total group of patients were examined for the 178 patients with stages I and II disease (Tables 3 and 4). The mean age of both groups of patients was 56 years. Some degree of covariance was observed between p53 immunostaining and tumor grade (Table 3) as had been the case with

---

Table 1 Comparison of p53 status and tumor grade of endometrioid endometrial carcinoma for patients of all stages

<table>
<thead>
<tr>
<th>Grade</th>
<th>No. of cases (%) positive for p53</th>
<th>Total no. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13 (12.0%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108</td>
</tr>
<tr>
<td>2</td>
<td>19 (24.7%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77</td>
</tr>
<tr>
<td>3</td>
<td>15 (21.7%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36</td>
</tr>
</tbody>
</table>

<sup>a</sup> The proportion of cases overexpressing p53 was statistically significantly different between the three grade categories ($P < 0.001$, df = 2; $\chi^2$ analysis).

<sup>b</sup> The proportion of grade 1 cases overexpressing p53 was statistically significantly different from the proportion in grade 2 ($P = 0.025$, df = 1) and grade 3 ($P < 0.010$, df = 1; $\chi^2$ analysis).

<sup>c</sup> The proportion of grade 2 cases overexpressing p53 differed from grade 3 by 0.05 < $P < 0.10$ ($\chi^2$ analysis).

---

Table 2 Comparison of survival to prognostic factors by univariate and multivariate analysis for total patients regardless of lymph node status

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>Univariate ($P^*$)</th>
<th>Hazard ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 immunohistochemistry</td>
<td>&lt;0.0001</td>
<td>2.75</td>
<td>1.4 to &gt;5.3</td>
</tr>
<tr>
<td>Stage II</td>
<td>0.0603</td>
<td>0.832</td>
<td>-</td>
</tr>
<tr>
<td>Stage III</td>
<td>0.0339</td>
<td>0.999</td>
<td>4.3</td>
</tr>
<tr>
<td>Stage IV</td>
<td>&lt;0.0001</td>
<td>0.899</td>
<td>6.6 to &gt;41</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0.023</td>
<td>0.979</td>
<td>-</td>
</tr>
<tr>
<td>Grade 3</td>
<td>0.001</td>
<td>0.994</td>
<td>1.5 to &gt;8.2</td>
</tr>
<tr>
<td>Age</td>
<td>0.254&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.029</td>
<td>1.04</td>
</tr>
</tbody>
</table>

<sup>a</sup> $\chi^2$ analysis of 221 patients.

<sup>b</sup> Cox proportional hazard analysis of 221 patients.

<sup>c</sup> Univariate comparison for age = a comparison of survival versus either greater or less than the mean age of 56 years for all patients.
The total group of patients (Table 1), but p53 remained a strong independent predictor of poor outcome when evaluated by multivariate analysis (Table 4).

FIG. 2. The relationship between p53 staining and survival for patients with early-stage disease (stages I and II).

FIG. 3. The relationship between p53 staining and survival for patients with advanced stage disease (stages III and IV).

DISCUSSION

Immunohistochemistry is a simple and useful method to detect p53 overexpression in surgical pathology specimens. Close correlation of p53 overexpression with the presence of mutations in the gene has been demonstrated in ovarian cancer, lung cancer, breast cancer, and others (6–8). Recently, Barnes et al. (20) analyzed 195 primary breast cancers and demonstrated that immunohistochemical detection of p53 protein was useful as a prognostic marker. This prompted our own interest in determining whether p53 overexpression in endometrial cancer might be useful as a prognostic indicator.

There have been a few reports demonstrating molecular genetic alterations of p53 in endometrial cancer. Okamoto et al. (21) examined 57 loci on 23 different chromosomes and demonstrated loss of heterozygosity in 24 cases of endometrial cancer. These findings also suggested that inactivation of the p53 gene is involved in the development of endometrial cancer (21). Enomoto et al. (22) detected mutations of the p53 gene in 23% of endometrial cancers and suggested that inactivation of p53 usually occurred as a late event in endometrial carcinogenesis. In a previous study, we analyzed whether p53 mRNA is overexpressed in endometrial cancer by combined in situ hybridization and simultaneous immunohistochemistry (9). We found that overexpression of p53 protein as measured by immunohistochemistry is not due to an increase in the steady-state level of p53 mRNA in carcinoma cells. In two other reports (12, 13), p53 overexpression was detected in 20–30% of endometrial cancer cases and correlated with several prognostic factors. At the same report, Kohler et al. (13) confirmed the close relationship between p53 overexpression and mutation, that DNA sequencing revealed point mutations in each of five cancers that overexpressed p53, while the wild-type of sequence was found in three cancers that did not overexpress the protein.

In our study, we used paraffin-embedded tissues and analyzed enough cases with follow-up data to demonstrate a clear correlation between p53 overexpression and adverse prognosis. Overexpression of p53 was detected in 47 of 221 cases (21.3%), and there was a statistically significant relationship between cases demonstrating overexpression and disease recurrence. In multivariate analysis using the Cox proportional hazard model, we found that p53 overexpression was an independent risk factor when compared with clinical stage, nuclear grade, and patient age.

Endometrial carcinoma is the most common pelvic gynecological carcinoma, and 80 to 90% of all cases are early stage (23). Five-year survival data reveal approximately 10 to 20% mortality in early-stage cancers. Several studies tried to identify prognostic factors for early-stage disease. Tornos et al. (24) demonstrated that myometrial invasion, vascular invasion, 8 or more mitoses per 10 high-power fields, and an absence of progesterone receptors were significant adverse prognostic factors in stage I/grade 1 endometrial carcinomas. Zaino et al. (25) reported that a new nuclear grading system did not provide prognostic information superior to architectural grading system in stage I endometrial carcinomas. In our study, p53 overexpression was observed in 11 of 22 carcinomas of patients with early-stage disease who subsequently expired. Only 12 of 152 disease-free patients with
stages I and II disease also demonstrated p53 overexpression. Thus, p53 overexpression has a statistically significant correlation with poor prognosis in early-stage disease.

In conclusion, these findings indicate that p53 overexpression as determined by immunohistochemistry may be an important tool to identify patients at high risk of recurrence who are otherwise not detected by current clinical and pathological evaluation, especially in the early stages. This interesting result may be helpful for planning postoperative follow-up and therapy.

REFERENCES
Prognostic Significance of *p53* Overexpression in Endometrial Cancer

Kiyoshi Ito, Keiko Watanabe, Suhail Nasim, et al.


Updated version

Access the most recent version of this article at:

http://cancerres.aacrjournals.org/content/54/17/4667

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.