Prognostic Significance of p53 Expression in Advanced-Stage Ovarian Serous Borderline Tumors

David M. Gershenson, Michael Deavers, Sheri Diaz, Guillermo Tortolero-Luna, Brigitte E. Miller, Robert C. Bast, Jr., Gordon B. Mills, and Elvio G. Silva

Departments of Gynecologic Oncology [D. M. G., G. T-L., B. E. M.] and Pathology [M. D., E. G. S.] and Division of Medicine [S. D., R. C. B., G. B. M.], The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

The purpose of this study was to investigate the frequency of p53 overexpression in the primary ovarian tumors of patients with stages II and III serous borderline tumors (SBTs) and to determine the relationship between p53 overexpression and risk of progression/recurrence and survival. Of 112 patients with stages II–IV SBTs, paraffin-embedded tissue from the primary ovarian tumor was available in 68 cases. Immunohistochemical staining for p53 was performed. Clinical information was abstracted from the medical records. The major endpoints selected for analysis were time to progression/relapse, disease-free survival, overall survival, and cause-specific survival. Univariate and multivariate regression analyses were also performed. The median patient age was 37 years (range, 17–67 years). Twenty-two patients had stage II disease, and 46 had stage III disease. The mean follow-up time was 105 months. Nineteen patients (28%) had either disease progression (1 patient) or relapse (18 patients). Eleven patients died: 10 patients died of their tumor, and 1 patient died of other causes. Thirteen cases (19%) had positive immunostaining for p53. Overexpression of p53 was significantly associated with an increased probability of progression/recurrence (P = 0.005) and a decreased overall survival (P = 0.012). After adjusting for age, International Federation of Gynecology and Obstetrics (FIGO) stage, the presence of residual tumor, and the presence of invasive implants, patients whose tumors overexpressed p53 had a 4-fold increased risk of progression/recurrence. Similarly, women whose tumor overexpressed p53 had an approximately 6-fold increased risk of death. p53 overexpression in the ovarian tumors of patients with stage II and III SBTs is significantly associated with increased probability of relapse and decreased overall survival. This information should provide better prognostic data to patients and their families and allow us to select patients who might benefit from postoperative treatment.

INTRODUCTION

First described in 1929 (1), ovarian SBTs now account for approximately 15% of all ovarian malignancies. Standard treatment for women with stage I SBT consists of surgery alone, and the prognosis of these women is excellent. For women with stages II, III, and IV SBT, however, at least 25% suffer relapse, and at least 10% die of tumor progression (2–11). A few studies have found an influence of residual tumor diameter on outcome (7, 10, 11). However, we do not currently have a reliable clinicopathological feature or a molecular marker with which to predict relapse or survival. In addition, no studies have demonstrated a beneficial influence of postoperative treatment.

The tumor suppressor gene p53 has been studied extensively as a prognostic indicator in ovarian carcinomas. In several studies, p53 overexpression has been observed in at least 50% of advanced-stage ovarian cancers (12–14). Although some investigators have found that p53 overexpression or mutation has a significant influence on survival (13, 15–17), others have not (12, 18, 19). Mutation or overexpression of p53 has also been studied in borderline ovarian tumors (15, 20–29). Although p53 mutations were not observed in any SBTs in two studies (20, 21), p53 overexpression has been reported in 0–50% of SBTs (15, 22–29). However, none of these studies have demonstrated a correlation between p53 overexpression and outcome.

The purpose of this study was to investigate the frequency of p53 overexpression in the primary ovarian tumors of patients with stage II and III SBTs and to determine the relationship between p53 overexpression and risk of progression/recurrence and survival.

MATERIALS AND METHODS

Patients. The medical records of all patients with a diagnosis of SBT of the ovary seen at The University of Texas M. D. Anderson Cancer Center from 1956 through 1996 were reviewed retrospectively, and 112 patients with stages II, III, and IV SBTs were identified. Although all cases were reviewed histologically at the time of initial referral, histopathological review of the primary tumor and the peritoneal implants was performed in all 112 cases by one of the authors (E. G. S.). Peritoneal implants were classified as either noninvasive or

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1 To whom requests for reprints should be addressed, at Department of Gynecologic Oncology, Box 67, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 745-2565; Fax: (713) 745-3510; E-mail: DGERSHEN@mdanderson.org.

2 The abbreviations used are: SBT, serous borderline tumor; FIGO, International Federation of Gynecology and Obstetrics; HR, hazard ratio; CI, confidence interval; SSCP, single-strand conformational polymorphism.
invasive. In addition, histological sections of recurrent tumor were reviewed, when available, and classified as SBT or serous carcinoma.

All patients underwent initial surgery. Surgical staging of these tumors was determined retrospectively according to criteria of the 1985 classification of the FIGO, based on careful review of surgical notes and pathology review (30). For purposes of staging, only documented sites of resection or biopsy were used. As in every study of this type, surgical staging was not comprehensive in every case. The extent of residual disease was determined on the basis of descriptions in written and dictated surgical reports and on clinical evaluation of patients in the early postoperative period. Detailed information concerning postoperative therapy and second-look surgery was also abstracted. During the study period, recommendations regarding postoperative treatment, including type of chemotherapy, were made at the discretion of the attending physician.

No patient in this study was lost to follow-up. The clinicopathological information for these patients has been reported previously (10, 11).

This study focuses on a subgroup of patients for whom paraffin-embedded tissue of the primary SBT specimens was available (68 of 112 cases). These patients did not differ statistically in clinicopathological characteristics (age at diagnosis, residual disease, FIGO stage, and type of peritoneal implants) or survival experience from those for whom tissue was not available for analysis.

**Immunohistochemistry.** Each tissue was sectioned on 4 μm thickness, deparaffinized, and rehydrated. The sections were then incubated with 0.3% hydrogen peroxide to block endogenous peroxidase activity, after which they were incubated with pepsin for 15 min. The sections were then washed four times with buffer. Next, the sections were immunostained with the mouse monoclonal anti-p53 antibody PAb 1801 (Oncogene Science, Manhasset, NY), which was applied at a 1:80 dilution for 2.5 h at room temperature. Then, following the manufacturer’s protocol (Lab Vision), a biotinylated secondary antibody was applied and incubated for 10 min at room temperature. After washing four times with PBS (plus Tween 20 on alternate washings), streptavidin-peroxidase complex was applied for 10 min at room temperature. The slides were again washed with PBS and then stained with diaminobenzidine. A hematoxylin counterstain was applied, and coverslips were applied. Sections from a human breast cancer known to have p53 overexpression served as positive controls, and sections of an overfixed human ovarian cell line served as negative controls.

Slides were scored by two of the authors (D. M. G. and M. D.), who were blinded to the clinical data at the time of interpretation. The interpretation of the p53 staining was based on the percentage of tumor cell nuclei staining and the staining intensity. The percentage was used to score a slide semi-quantitatively in one of four categories: (a) 1+, 5–25% staining; (b) 2+, 26–50% staining; (c) 3+, 51–75% staining; and (d) 4+, 76–100% staining. Sections with less than 5% tumor nuclei staining were considered negative. Intensity was graded from weak (1+) to strong (3+).

**Statistical Analysis.** Survival was measured in months from the date of diagnosis to the date of death or date of last follow-up. Progression-free survival was defined as the time from the date of diagnosis to the date of first evidence of tumor progression or recurrence. Both overall survival (deaths from all causes) and cause-specific survival (deaths from disease) were estimated.

The association between p53 overexpression and survival (overall and cause-specific) and disease-free survival was analyzed by means of the life-table methods of Kaplan and Meier. The statistical significance of the various factors was tested by the log-rank test. For the purpose of statistical analysis, p53 results were categorized as either positive (weak to strong) or negative. The independent influence of p53 overexpression as a prognostic factor for survival (overall and cause-specific) and disease-free survival was evaluated using Cox’s proportional hazards model. HRs and 95% CIs were used to calculate the relative risk of death or progression/recurrence after adjusting for other clinicopathological covariates. Variables included in the analysis were age (<30, 30–49, and 50+ years), FIGO stage (stage II versus stage III), the presence of residual tumor (none versus any), the presence of invasive peritoneal implants (no versus yes), and postoperative treatment (no versus yes). Both bivariate models (including p53 overexpression and each covariate separately) and multivariate models (including all clinicopathological variables of interest) were used to assess the prognostic value of p53 overexpression. Statistical significance was set at $P < 0.05$, and all reported Ps were two-sided.

**RESULTS**

The median age of the 68 patients at diagnosis was 37 years (range, 17–67 years). Table 1 summarizes other patient characteristics at diagnosis. All patients underwent primary surgery, and 48 patients (71%) received postoperative treatment.

The mean follow-up time was 105 months. Nineteen patients (28%) had either disease progression (1 patient) or relapse...
Thirteen cases (19%) had positive immunostaining for p53 (Fig. 1). The immunostaining distribution as determined by the percentage of tumor nuclei stained was almost always focal: 1+ in eight cases; 2+ in three cases; and 3+ and 4+ in one case each. The staining intensity was 1+ in four cases, 2+ in seven cases, and 3+ in two cases. The clinicopathological features of the 13 patients whose primary SBT had p53 immunostaining are detailed in Table 2.

Overexpression of p53 was significantly associated with an increased probability of progression/recurrence (P = 0.005) and a decreased overall survival (P = 0.012; Figs. 2 and 3). The median progression-free survival among patients whose tumors overexpressed p53 was 101 months (95% CI, 56–146 months) compared with 208 months (95% CI, 173–243 months) among patients whose tumors did not overexpress p53. Similarly, the median survival time among patients whose tumors overexpressed p53 was 154 months (95% CI, 106–201 months), compared with 252 months (95% CI, 225–279 months) among those whose tumors did not overexpress p53. The findings did not change when the end point was cancer-specific survival.

Crude HR and adjusted HR for both progression/recurrence and overall survival are presented in Table 3. After adjusting for age, FIGO stage, the presence of residual tumor, and the presence of invasive implants, patients whose tumors overexpressed p53 had a 4-fold increased risk of progression/recurrence (HR = 4.26; 95% CI, 1.53–11.82) compared with patients whose tumors did not. In addition, women 30–49 years old were almost four times more likely to have progressive/recurrent disease (HR = 3.64; 95% CI, 1.07–12.34) than women less than 30 years old.

Similarly, women whose tumors overexpressed p53 had an approximately 6-fold increased risk of death (HR = 5.75; 95% CI, 1.32–25.02) compared with those whose tumors did not after adjustment of other covariates. Age ≥50 years and the presence of residual tumor were also found to be independent adverse predictive factors for risk of death (HR = 9.25 and 95% CI = 1.29–66.12 for age ≥50 years; HR = 6.10 and 95% CI = 1.26–29.50 for the presence of residual tumor).

DISCUSSION

Although women with stage I ovarian SBT have excellent prognoses, with a disease-free survival rate approaching 100%, at least 25% of patients with stages II, III, and IV ovarian SBT suffer relapse, and at least 10% die of disease (2–11). These patients with stages II-IV SBT have peritoneal implants that are classified as either noninvasive or invasive. Noninvasive implants have an appearance similar to primary SBT. They are characterized by glandular or papillary proliferations with cell detachments; cellular atypia, psammoma bodies, and desmoplastic fibrosis may be present in some cases (2–9, 11). Invasive peritoneal implants are lesions similar to noninvasive implants but with epithelial cells infiltrating the surrounding stroma, singly or in clusters. They are characterized by irregular penetration of the underlying tissue (2–10). In our series (10, 11), invasive peritoneal implants were much less common than noninvasive implants, being observed in 35% of cases; in other reported series, invasive implants have been noted in 12–57% of cases, although several of these series are small (2–9). In addition, the majority of patients with invasive implants also have coexistent noninvasive implants [77% in our series and 100% in the study of Bell et al. (7, 11)].

For patients with noninvasive implants, relapse rates range from 8–33%, with a mean of 20%; death rates range from 0–17%, with a mean of 7% in the reported series (2–9, 11). For patients with invasive peritoneal implants, relapse rates range from 0–83%, with a mean of 39%; death rates range from 0–67%, with a mean of 28% in the reported series (2–10). The time from diagnosis to relapse varies considerably, with a range of 0.25–19.4 years in women seen at our institution (10, 11). However, the median time to relapse appears to be significantly shorter for patients with invasive peritoneal implants compared with those with noninvasive implants (2 years versus 7.1 years, respectively). The long interval to so-called ‘relapse’ in some patients raises the question of whether the appearance of a tumor represents a true recurrence or a new primary cancer.

Because a substantial proportion of patients with SBT and
peritoneal implants experience relapse, several groups, including our own, have historically recommended postoperative treatment, principally with platinum-based chemotherapy. However, in multivariate analyses, we have found no impact of postoperative therapy on the risk of recurrence or death from tumor (10, 11). Several other investigators have also been unable to demonstrate any benefit from postoperative therapy (31–34). Nevertheless, it still is possible that there is a beneficial influence of postoperative treatment that remains undetected because of the relatively small numbers of patients and variable follow-up times in reported series.

The observation that contemporary postoperative treatment may not be effective in reducing the risk of relapse in women with stage II-IV SBT does not negate the possible advantage of being able to identify those who are at higher risk for such an event. In addition, more effective therapies may be discovered in the future. Furthermore, an understanding of the molecular alterations associated with relapse may actually facilitate the discovery of more effective therapies for such patients. Therefore, we set out to investigate the relationship between p53 overexpression in the primary ovarian SBT and risk of relapse and survival.

Other investigators have studied p53 mutation or overexpression in ovarian SBT. Using the technique of PCR-SSCP, Wertheim et al. (20) found no mutations in 32 ovarian SBTs. Sixteen of these tumors were stage II-IV. Similarly, Kupryjanczyk et al. (21) studied 12 ovarian borderline tumors, 8 of which were serous. On immunostaining, p53 overexpression was observed in the ovaries of four patients, was not seen in the ovaries of three patients, and was not available in the ovaries of one patient. However, p53 mutation was not found in any of the ovarian borderline tumors using the PCR-SSCP and sequencing technique. In a previous report, the same group found p53 immunoreactivity in 6 of 43 (14%) SBTs in either ovarian or extraovarian tumor or both (23). Apparently, 18 of the 43 SBTs were associated with peritoneal implants.

The discordance between the findings of p53 mutations by PCR-SSCP and sequencing and p53 overexpression in ovarian SBTs suggests that p53 immunoreactivity is not always a reflection of a genetic alteration. Alternative explanations for p53 immunostaining include accumulation of wild-type p53 (35), formation of complexes between p53 and endogenous proteins (36), or mutations outside the exons examined. Hopefully, subsequent studies will elucidate the underlying mechanisms and differences between p53 mutation and p53 overexpression by immunostaining in ovarian SBTs.

Several other investigators have also found p53 immunoreactivity in ovarian SBTs, ranging from 7-50% of cases (Table 4; Refs. 15, 22, 24, 26, and 29). In three studies, no p53...
immunoreactivity was observed in a total of 18 ovarian SBT (25, 27, 28). However, it is interesting to note that in one of the these studies, a p53 mutation in exon 7 was observed in a case in which p53 immunostaining was negative (25).

Our study appears to be unique in that this is the largest series of patients with stage II and III ovarian SBTs reported to date. Furthermore, the follow-up time is quite long. Among 68 patients, our rate of 19% p53 immunoreactivity is consistent with other reports (15, 22–24, 29). In addition, there is a suggestion from other studies that p53 immunoreactivity may be more common in stage III ovarian SBTs than in stage I ovarian SBTs (22, 29). Other investigators have also observed the focal nature of p53 immunostaining (22, 23).

Among our 68 patients, 19 (28%) had either disease progression (1 patient) or relapse (18 patients). In multivariate analyses, we were able to demonstrate a significant association between p53 overexpression and an increased risk of recurrence and death from tumor. In other studies, a similar finding may not have been evident for a variety of reasons, including a relatively small number of patients, lack of information regarding treatment and outcome, or a brief follow-up time.

Another emerging concept regarding SBT deserves some discussion. Burks et al. (37) and Seidman and Kurman (38) have recently introduced the term ‘micropapillary serous carcinoma’ to describe a subset of patients with SBT whose primary tumor contains areas of a filiform or cribiform pattern or both patterns. These authors suggest that, compared with the typical SBT pattern, micropapillary serous carcinomas may or may not contain focal invasion, are more often bilateral, and are more likely to be associated with invasive implants. Most importantly, they contend that this histological pattern is associated with a significantly worse prognosis than typical SBT. They further recommend that these micropapillary serous carcinomas should be classified as malignant and that typical SBT be reclassified as a benign entity.

Subsequently, Eichhorn et al. (39) reported their study of 40 ovarian SBTs that contained a micropapillary or cribiform pattern and compared them with 44 SBTs that lacked these patterns. They concluded that the micropapillary and cribiform patterns are ‘associated with a higher frequency of exophytic ovarian growth, bilaterality, advanced stage, invasive implants, and unfavorable outcome than typical SBT.’ However, because the data suggest that tumors with the micropapillary or cribiform pattern have a better prognosis than frankly invasive serous carcinomas in both the stage I and stage II-III categories, they recommended that ‘micropapillary’ tumors should be retained within the borderline category.

In a recent report, Katabuchi et al. (40) studied p53 immunostaining and mutational analysis in 18 cases of ‘micropapillary serous carcinomas’ and compared their findings with those in 17 cases of typical SBT and six cases of frankly invasive serous carcinomas. Although none of the micropapillary serous carcinomas or typical SBTs contained p53 mutations, the micropapillary serous carcinomas had moderately intense p53 immunostaining, compared with only weak immunostaining in the typical SBTs and quite intense immunostaining in the invasive serous carcinomas. In this study, there were too few cases to attempt to correlate p53 status with the risk of relapse or death.

We are currently reviewing our series of patients with stage II–IV SBT for the presence of the micropapillary or cribiform pattern and the presence of microinvasion to correlate these findings with p53 immunostaining data, risk of relapse, and prognosis. Currently, we agree with the conclusions of Eichhorn et al. (39) that unless further study suggests otherwise, tumors with a micropapillary or cribiform pattern should be retained within the borderline category. In future studies, we plan to focus on expression of other molecular biomarkers in both the primary tumor and the peritoneal implants in stage II and III ovarian SBTs, and we plan to study p53 mutation by PCR-SSCP and sequencing in the cases with p53 overexpression. We eagerly await confirmation of our findings by other groups who have access to large patient numbers with adequate follow-up. The major implication of our findings is that the determination of molecular biomarkers that predict outcome will allow us to provide a risk assessment for future patients with stage II–IV ovarian SBT and to select patients who may benefit from postoperative therapy.

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