A Case-Case Analysis of Factors Related to Overexpression of *p53* in Endometrial Cancer following Breast Cancer

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Abstract

We studied 54 patients diagnosed with endometrial cancer between 1981 and 1994 following a diagnosis of breast cancer. We used a case-case analysis, comparing tumors with and without overexpression of the *p53* gene product to evaluate the association of putative *p53* mutations with tamoxifen use and other risk factors for endometrial cancer. Twenty-four % of the tumors showed strong positive staining for the *p53* gene product. Tumors in a more advanced stage (stage 2, 3, or 4, compared to stage 1) were more likely to overexpress *p53* [odds ratio (OR) = 4.2; 95% confidence interval (CI), 1.1–16.2], as were tumors with serous or clear cell, compared to endometrioid, histology (OR = 5.8; 95% CI, 1.3–26.5). There was a small association between *p53* overexpression and treatment with tamoxifen for breast cancer (OR = 2.6; 95% CI, 0.69–9.8). There was a strong relationship between overexpression of *p53* and having a first-degree relative with breast cancer (OR = 12.3; 95% CI, 2.6–57.4) and between overexpression of *p53* and having an additional cancer, *i.e.*, at sites other than breast or endometrium (OR = 7.9; 95% CI, 1.6–40.1). In this group of women, genetic predisposition to cancer, as reflected in family history of breast cancer and personal history of an additional primary cancer, was strongly associated with overexpression of *p53* in endometrial tumors. The results suggest that use of tamoxifen may be associated with an increase in tumors that overexpress *p53*, although the results could be due to chance.

Introduction

Tamoxifen was introduced as treatment for advanced breast cancer in the early 1970s and came into general use as adjuvant therapy for estrogen receptor-positive, early-stage breast cancer in the early 1980s. In randomized clinical trials, tamoxifen has been found to be effective in reducing the progression of breast cancer and the occurrence of cancer in the contralateral breast. However, women in the clinical trials who received tamoxifen were at an increased risk of developing endometrial cancer (1, 2). There has been conflicting evidence as to whether endometrial cancer that develops after the use of tamoxifen is a more virulent disease with a poorer prognosis than endometrial cancer in general (3, 4).

Mutation of the *p53* tumor suppressor gene is an event in carcinogenesis common to many cancers. In endometrial cancer, recent investigations have found the prevalence of *p53* mutations to be between 10 and 48% (5–13). Overall, about 20% of endometrial tumors overexpress *p53*. Mutation in *p53* is a late event in endometrial cancer, usually associated with more advanced disease (5, 6, 9, 11) and with poor prognosis (8, 14–16). Studying associations between overexpression of *p53* in tumors and other subject characteristics can offer an indication of etiological heterogeneity; that is, whether there are different pathways associated with different tumor types (17). The purpose of this study was to investigate the relationships between treatment with tamoxifen, other risk factors for endometrial cancer, and other subject characteristics and overexpression of the *p53* gene product in women with endometrial cancer following breast cancer.

Materials and Methods

Study Subjects and Data Collection. The study group consisted of 54 women who were diagnosed with epithelial endometrial cancer at the Memorial Sloan-Kettering Cancer Center 6 months or more after a diagnosis of breast cancer. We reviewed the medical records of these patients to ascertain their exposure to risk factors for endometrial cancer and other subject characteristics.

Immunohistochemical Methods. We detected overexpression of the *p53* gene product by immunohistochemical staining of paraffin-embedded tumors. Tissue sections (6 μm in thickness) were deparaffinized in xylene and rehydrated in graded alcohols. Sections were then incubated in Target Unmasking Fluid (Signet Laboratories, Inc., Dedham, MA), warmed to 90°C for 10 min, and washed in water and then in PBS. The sections were treated with 0.3% hydrogen peroxide in PBS for 30 min to block endogenous peroxidase activity and then washed in PBS. Normal horse serum (10%) was added to sections to block nonspecific protein binding for 30 min. Excess serum was aspirated off, and the primary monoclonal antibody (Ab-6, DO-1 clone; Oncogene Science, Manhasset NY) was applied to each section for 90 min in a humid chamber. Sections were washed in PBS and then incubated with biotinylated antimouse immunoglobulin (1:100 dilution; Vector Laboratories, Burlingame, CA) for 45 min. Sections were washed in PBS and then incubated with avidin-biotin complex (Vectastain ABC Kit; Vector Laboratories) for 30 min. Sections were washed again in PBS and incubated with 3,3′-diaminobenzidine in PBS containing 0.03% hydrogen peroxide as the

Received 12/17/96; revised 5/2/97; accepted 5/2/97.
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substrate for 6–10 min. Sections were rinsed in water, counterstained with Harris' hematoxylin, rinsed in water again, air-dried, and coverslipped with Histoclad (Clay Adams-Becton Dickinson, Parsippany, NJ). All incubations were performed at room temperature. Specific immunostaining was visualized by light microscopy as a brown reaction product. Slides were evaluated without knowledge of tamoxifen use or other subject characteristics.

Statistical Methods. We calculated crude ORs[2] to describe the relationships between subject characteristics and *p53* overexpression in tumors. Because of the small sample size, we used an exact procedure (18) to calculate ORs adjusted for potential confounding variables.

Results

Subject Characteristics. The year of endometrial cancer diagnosis ranged from 1981 to 1994, and the year of breast cancer diagnosis ranged from 1946 to 1992. The mean age at diagnosis was 67.1 ± 11.1 years (range, 34–89 years) for endometrial cancer and 58.8 ± 12.1 years (range, 26–78 years) for breast cancer. Most of the endometrial tumors were classified as stage 1 (n = 41), and most were of the endometrioid type (n = 45). About half the subjects (28 of 54) had been treated with tamoxifen.

***p53* Overexpression According to Stage and Histological Type.** Of the 54 cases of endometrial cancer following breast cancer that were evaluated for presence of the *p53* gene product in tumor tissue, 13 (24%) were strongly positive (*i.e.*, more than 50% of the cells were positive), 9 (17%) were weakly positive (*i.e.*, usually <10% of the cells were positive), and 32 (59%) were negative for *p53*. We compared those patients whose tumors exhibited strong positive reactions to those whose tumors were weakly positive or negative. Strong positive results were more common in tumors classified as stage 2, 3, or 4 than in those classified as stage 1 (OR = 4.2; 95% CI, 1.1–16.2). Positive results were also more common in serous or clear cell tumors than in endometrioid tumors (OR = 5.8; 95% CI, 1.3–26.5).

Association of *p53* Overexpression with Tamoxifen Use. Table 1 shows the results for the association of tamoxifen and other risk factors with *p53* overexpression. There was a small association between *p53* overexpression and use of tamoxifen (OR = 2.6; 95% CI, 0.69–9.8). Use of tamoxifen was more common among women who were older, heavier, diagnosed with breast cancer more recently, or had a relative with breast cancer or a third primary cancer (in addition to breast and endometrial cancer). After adjusting for these possible confounding variables, we found that the relationship between tamoxifen and *p53* was independent of all these factors, except history of a third cancer (OR adjusted for a third cancer = 1.7; 95% CI, 0.38–7.7).

Association of *p53* Overexpression with Other Subject Characteristics. The major risk factors for endometrial cancer, older age and higher body mass index, were not strongly related to expression of *p53* in these endometrial tumors, with ORs of 1.1 (95% CI, 0.32–3.9) and 1.2 (95% CI, 0.35–4.3), respectively. A strong relationship between overexpression of *p53* and family history of breast cancer was noted, with an OR of 12.3 (95% CI, 2.6–57.4). Having a history of another primary cancer was also strongly associated with overexpression of *p53*, with an OR of 7.9 (95% CI, 1.6–40.1). There were smaller associations between *p53* overexpression and use of estrogen replacement therapy (OR = 2.9; 95% CI, 0.56–15.1), smoking (OR = 0.39; 95% CI, 0.09–1.6), and higher parity (OR = 2.1; 95% CI, 0.51–9.0; see Table 1).

Table 1 Overexpression of *p53* in tumors of women with endometrial cancer according to patient characteristics

	No.	% positive for *p53*	OR (95% CI)
Total	54	24	
Stage			
1	41	17	1.0
2, 3, or 4	13	46	4.2 (1.1–16.2)
Histology			
Endometrioid	45	18	1.0
Serous or clear cell	9	56	5.8 (1.3–26.5)
Treated with tamoxifen			
No	26	15	1.0
Yes	28	32	2.6 (0.69–9.8)
Age (y)			
≤67	26	23	1.0
≥68	28	25	1.1 (0.32–3.9)
Body mass index			
<25.5	27	22	1.0
≥25.5	27	26	1.2 (0.35–4.3)
Breast cancer in first-degree relative			
No	40	13	1.0
Yes	11	64	12.3 (2.6–57.4)
Additional primary cancer			
No	46	17	1.0
Yes	8	63	7.9 (1.6–40.1)
Ever smoked cigarettes			
No	31	29	1.0
Yes	22	14	0.39 (0.09–1.6)
Ever used estrogen replacement therapy			
No	41	17	1.0
Yes	8	38	2.9 (0.56–15.1)
Parity			
0–1	19	16	1.0
≥2	35	29	2.1 (0.51–9.0)

Because of the importance of stage and histological type, we examined the associations between family history of breast cancer and personal history of a third primary and *p53* overexpression adjusted for stage and type. Stage (1 *versus* 2–4) and histological type (endometrioid *versus* others) were dichotomized for these analyses. Family history remained strongly associated with *p53* overexpression after adjusting for stage (OR = 9.0) and for histology (OR = 18.8), as did personal history of a third cancer (OR = 9.1 after adjusting for stage and OR = 7.7 after adjusting for histology). Because of the small numbers, CIs were wide, but all excluded 1.

Women with first-degree relatives with breast cancer were somewhat younger at diagnosis of breast cancer (mean age, 52.2 ± 13.8, compared to 59.7 ± 11.1 in other women) and endometrial cancer (mean age, 59.3 ± 13.1, compared to 68.7 ± 9.8). Among the eight women who had other primary cancers in addition to breast and endometrial cancer, several other types of cancer were found, including thyroid, melanoma, colon, cervix, ovary, vulva, Hodgkin's disease, and chronic lymphocytic leukemia. The mean ages at diagnosis of breast cancer and endometrial cancer were similar to those for other subjects, 59.4 ± 17.8 and 68.5 ± 15.0, respectively.

[2] The abbreviations used are: OR, odds ratio; CI, confidence interval.

Discussion

Among the patients who had endometrial cancer after breast cancer, the proportion of tumors that expressed *p53* was 24%, similar to that reported in other studies (5–13). This study was consistent with earlier reports finding higher levels of overexpression in tumors at more advanced stages and those with serous or clear cell histology (6, 10, 11).

Only a small number of studies to date have reported on the association of risk factors for endometrial cancer and alterations in *p53*. Older age has been found to be associated with overexpression of *p53* in some studies (6, 16) but not others (12, 19). Inoue *et al.* (6) reported that having a previous history of cancer was associated with *p53* overexpression. Another study (16) has also found no association with body mass index.

The finding that family history of breast cancer is an important factor in development of endometrial tumors that express *p53* raises the question of whether overexpression of *p53* in endometrial tumors among these women is due to a germ-line mutation. If this were so, these cases would be different from other families with germ-line mutations in *p53* (Li-Fraumeni families), first, because endometrial cancer is not usually found in these families and, second, because breast cancer is usually diagnosed at earlier ages (20). Most of the families with Li-Fraumeni syndrome reported in the literature were identified through probands with childhood sarcomas. The present results suggest the possibility of another *p53* germ-line mutation with different characteristics of expression.

For those women with 3 or more cancers, the overexpression of *p53* would seem to reflect general genomic instability, perhaps with multiple mutations that eventually lead to a mutation in *p53*. Although they were selected for the present study because they had both breast and endometrial cancer, there was no pattern in the type of third cancers. A germ-line mutation in, for example, a DNA repair gene might explain their propensity to multiple cancers. However, they were no more likely than other women in this study to have first-degree relatives with cancer, and their mean ages at diagnosis of breast and endometrial cancer were similar to those of the other women. Thus, these women do not fit the usual pattern of inherited susceptibility to cancer, although the number of women in this study is too small to draw definitive conclusions.

In other studies, the relation between family history and overexpression of *p53* has not been consistent. In breast cancer, Thor *et al.* (21) found that family history of breast cancer was associated with higher level of expression of the *p53* protein. In endometrial cancer, Inoue *et al.* (6) found no relationship between *p53* overexpression and family history of cancer (not further defined). In colon cancer, two studies (22, 23) have reported conflicting results on the association between history of cancer in first-degree relatives and *p53* overexpression.

In conclusion, we found a small association between the use of tamoxifen and overexpression of *p53* in these endometrial tumors. The relationship between family history of breast cancer and between history of a third primary cancer in these patients and *p53* overexpression suggests that genetic predisposition is an important determinant of *p53* expression in these patients.

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