Pathologic, Immunohistochemical, and Molecular Features of Benign and Malignant Phyllodes Tumors of the Breast

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The histologic distinction between benign and malignant Phyllodes tumors (PT) is often difficult and arbitrary. We analyzed a group of benign and malignant PT to determine whether specific histologic features and expression of Ki-67 and p53 could be useful in distinguishing benign PT from malignant tumors. We also determined whether deletions in Chromosome 3p at the FHIT and hMLH1 loci are common abnormalities in PT. Twenty PT were histologically classified as benign (7) or malignant (13). Seven of the malignant PT were low grade, and six were high grade. Ki-67 and p53 immunohistochemistry was performed on all tumors and analyzed for the stromal and for the epithelial component. PCR-based loss of heterozygosity analyses were performed with the following markers on Chromosome 3p: D3S1478 (3p21.2–21.3), D3S1289 (3p21.1–21.2), and D3S1295 (3p14.3–21.1). The distribution of immunoreactivity for Ki-67 was analyzed by quantifying the percentage of positive nuclei and expressed as the labeling index (LI). Patients' ages ranged from 13 to 71 years (median: 51 y). After a mean follow-up period of 8 years, none of the PT metastasized, whereas three recurred locally. Although malignant PT were larger than benign PT (means, 7.1 *versus* 4.3 cm), this difference was not statistically significant. Five tumors had infiltrating margins, and 14 were circumscribed. The Ki-67 LI in low-grade malignant PT (16 ± 25.5) was significantly higher than that in benign PT (3.6 ± 4.8), whereas the LI in the high-grade malignant PT group (50 ± 21.9) was significantly higher than that in low-grade malignant tumors (P = .012). The Ki-67 LI in the three tumors that recurred was less than 10%. Two of seven (29%) benign PT were focally positive for p53, whereas four of seven (57%) low-grade malignant and three of six (50%) high-grade malignant PT were diffusely positive for p53. The three tumors that recurred initially were histologically benign, as were two of the recurrences. One recurrent tumor evolved to a high-grade malignant PT. Margins were greater than 1 cm in all tumors except four, three of which recurred locally. No allelic loss of 3p was found. In summary, Ki-67 expression may assist in distinguishing benign from malignant PT in diagnostically difficult cases. 3p deletions do not play a significant role in the development of these tumors. Neither Ki-67 nor p53 can reliably predict recurrence. Histologically high-grade malignant PT have a favorable prognosis if widely excised. We emphasize the importance of adequate margins in the treatment of benign and malignant PT.

KEY WORDS: Phyllodes tumor, Breast neoplasm, Cystosarcoma phyllodes.

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Periductal stromal tumors of the breast, including fibroadenomas and Phyllodes tumors (PT), arise from the epithelial and stromal components of the terminal duct-lobular unit. Malignancy develops in the mesenchymal component of PT, whereas the ductal component rarely exhibits premalignant or malignant changes. On the basis of histologic criteria, PT may be classified as benign or malignant and further subdivided into borderline or low-grade malignant or high-grade malignant (1–4). PT are usually readily diagnosed by characteristic histopathologic features (1–3). However, the distinction between benign and malignant PT (especially low-grade malignant PT, also classified as borderline PT by some authors) can be difficult. Thus,

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additional diagnostic features would be clinically useful.

Prognostic assessment of cellular periductal stromal tumors based solely on the above histologic classification continues to be problematic. Histologically benign PT have been reported to have metastasized, whereas many histologically malignant tumors have neither recurred nor metastasized (5–8). Although local recurrences or metastases of seemingly benign tumors can be attributed to inadequate sampling for microscopical evaluation or to inadequate assessment of resection margins, these circumstances cannot account for all clinicopathologic inconsistencies in the literature. Attempts to correlate various gross and microscopical features of PT with clinical behavior have not been uniformly successful in predicting the clinical outcome of these tumors (5–12).

Recent studies have suggested that Ki-67, a proliferation marker, and p53, a tumor suppressor gene, may be implicated in predicting behavior of breast carcinomas in general and of PT in particular (13–15). In addition, cytogenetic investigation has been performed in a small number of PT (16, 17). The most common chromosomal abnormalities found were interstitial deletions of 3p. Therefore, we hypothesized that loss at 3p, possibly at the FHIT and/or hMLH1 loci; alterations in p53; and/or assessment of proliferation by Ki-67 expression might be useful in distinguishing tumors that pursue a benign course from those with an aggressive clinical outcome.

The goals of this study are to analyze the histologic features and expression patterns of Ki-67 and p53 in benign and malignant PT of the breast to assess the usefulness of these markers in distinguishing benign from malignant PT (especially from low-grade malignant PT) and to determine whether these markers are helpful clinical prognosticators.

MATERIALS AND METHODS

Cases and Tissues

Formalin-fixed, paraffin-embedded tissues from 20 PT of the breast diagnosed in our institution (13 cases) and from cases submitted for consultation (7 cases) were studied. These included seven benign PT, seven low-grade malignant PT, and six high-grade malignant PT. At least three hematoxylin and eosin sections of all tumors were available and were reviewed independently by two of the authors (CGK and HAO) for verification of diagnosis. PT were histologically classified as benign, low-grade malignant (or borderline), and high-grade malignant, taking into account stromal cellularity and overgrowth, mitotic activity, and the microscopic tumor border (circumscribed or infiltrating; 1–3). Benign PT were well circumscribed microscopically, with few or no mitoses (less than one per 10 HPF) and no stromal overgrowth. High-grade malignant PT, on the other hand, were characterized by marked stromal overgrowth with numerous mitoses (more than 10 per HPF) and by nuclear pleomorphism. These tumors were poorly circumscribed with invasion of adjacent breast parenchyma. The low-grade malignant PT (or borderline) had a microscopically infiltrating border and stromal overgrowth and hypercellularity but less nuclear pleomorphism and fewer mitoses than did the high-grade malignant tumors (one to five mitoses per 10 HPF).

In addition to histologic features, including the presence of stromal overgrowth, the presence of metaplasias, and mitotic activity, proximity to the resection margin was recorded as positive when there was tumor at the inked margin; as negative when the tumor was more than 1 cm from the margin; and as close when the tumor was within 1 cm of the margin.

Patient Characteristics

Patient history and follow-up information were obtained by chart review and by information provided by the referring pathologist or surgeon. Clinical information including age, mean follow-up, tumor size, and the presence of locally recurrent or metastatic disease are summarized in Table 1.

Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded tissue sections were cut at 5 μm and treated with 0.1 mol/L citrate, pH 6, in an 800-W microwave oven for 15 minutes for antigen retrieval before immunostaining. A monoclonal antibody to Ki-67 (1:100 dilution, DAKO, Carpenteria, California) and a monoclonal antibody to p53 (DO-7 monoclonal, Novocastra, Newcastle-upon-Tyne, UK) were used as previously reported (18). Immunostaining was done with the Elite avidin-biotin-peroxidase kit (Vector, Burlingame, CA), according to the manufacturer's specifications. Slides were counterstained with hematoxylin for 1 second. Internal positive controls such as lymphocytes were used for Ki-67.

Quantitation of Immunostaining

The distribution of immunoreactivitiy was analyzed in the epithelial and in the stromal components of PT by quantifying nuclear staining in each case without knowledge of the diagnosis or outcome. The percentages of cells expressing Ki-67 were determined by counting 1000 cells per slide. The percentage of positive nuclei was expressed as the labeling index (LI). Staining of the cells for p53

TABLE 1. Clinical and Pathologic Features of Phyllodes Tumors (PT)

Diagnosis	*n*	Mean Age (y)	Mean Follow-Up (y)	Mean Tumor Size (cm)	Recurrence	Metastases
Benign PT	7	56	8.8	4.3	3	0
Low-grade malignant PT	7	46	8.4	8.6	0	0
High-grade malignant PT	6	49	4.5	5	0	0

was assessed according to both the intensity and the proportion of cells staining. Tumors were considered focally positive when unequivocal nuclear staining was present in 10%– 50% of the tumor cells and as diffusely positive when more than 50% of the tumor cells were positive.

Molecular Studies

In eight tumors, allelic losses at 3p were evaluated using a panel of three microsatellite probes specific to Chromosome 3p: D3S1478 (3p21.2–21.3), D3S1289 (3p21.1–21.2), and D3S1295 (3p14.3–21.1). These probes are within the chromosomal region that has been previously shown to contain interstitial deletions (16, 17). DNA was extracted from paraffin-embedded tissue sections after selective microdissection of the epithelial component of PT. This was accomplished by using a 30-gauge needle to microdissect under direct light-microscope visualization, as previously described (19). DNA was amplified by polymerase chain reaction. A locus was considered informative for a particular patient when the constitutional DNA from that patient displayed two different alleles; that is, heterozygous at that locus, loss of heterozygosity was considered to be present when one of the two polymorphic alleles present in the corresponding normal-tissue DNA was absent or reduced in the tumoral DNA by at least 70%.

Statistics

Because of the small sample sizes, nonparametric tests were used to examine differences between the three groups of tumors. First, a Kruskal-Wallis test was used to test for differences between the three groups in tumor size as well as level of Ki-67 expression. Fisher's Exact test was used to test for differences in the proportion of p53 expression.

RESULTS

The clinical and pathologic features, including follow-up information, are presented in Table 1. Treatment information was available for 17 of the 20 patients. All tumors were treated with excision. Two tumors had positive margins (tumor at the inked margin), and two were close to the margin (within 0.1 cm of the margin). One patient with a very large low-grade PT underwent preoperative chemotherapy, followed by radical mastectomy. This patient had a positive margin, and postoperative radiotherapy was used. This patient had no evidence of disease at 6 years after treatment. The other patient with a positive margin after excision and the two patients with close margins experienced local tumor recurrences. The tumors were re-excised. After the follow-up period, two patients had no evidence of neoplasm, and one patient died of another, unrelated cause. Of the six high-grade malignant PT, two were treated with total mastectomy and three with lumpectomy. One patient also received adjuvant chemo- and radiotherapy. No treatment information was available for one patient with a high-grade malignant PT. After the follow-up period, none of the patients developed metastases. Three of the 20 tumors (15%) recurred locally. Grossly, all of the tumors were well circumscribed with a tan, lobulated, sometimes cystic, cut surface. One PT had areas of necrosis and punctate hemorrhage. Malignant PT were softer than benign tumors. The mean size of malignant PT (7.1 cm) was larger than that of benign PT (4.3 cm). Although all tumors were grossly circumscribed, 5 (25%) had microscopical infiltrating margins. The overall histologic pattern of PT was analogous to that emphasized in prior reports (1–3, 20, 21). Metaplastic foci were seen in four tumors (20%), three (15%) had lipocytic metaplasia of the stroma, and one (5%) had squamous metaplasia of the ductal component. Stromal multinucleated giant cells were present in one tumor (5%), and one tumor (5%) had extensive stromal myxoid change. Neither perineural nor vascular invasion was evident in any PT. Seven tumors were histologically benign (35%), and 13 (65%) were malignant, of which seven were low-grade PT and six were high-grade PT. As shown in Table 1, three of seven (43%) tumors with benign histologic features recurred locally. Two of these had close margins, and one had positive margins. Two of these three tumors recurred as histologically benign PT and one as a high-grade malignant PT.

Immunohistochemical staining revealed nuclear localization of Ki-67 protein in all groups of tumors in the epithelial and in the stromal cells (Fig. 1). Analysis of the three groups of PT showed a significant difference between benign, low-grade malignant, and high-grade malignant tumors ($P = .012$). Fig. 2 shows the Ki-67 LI for the three groups of tumors. Benign PT had a lower Ki-67 LI than ma-

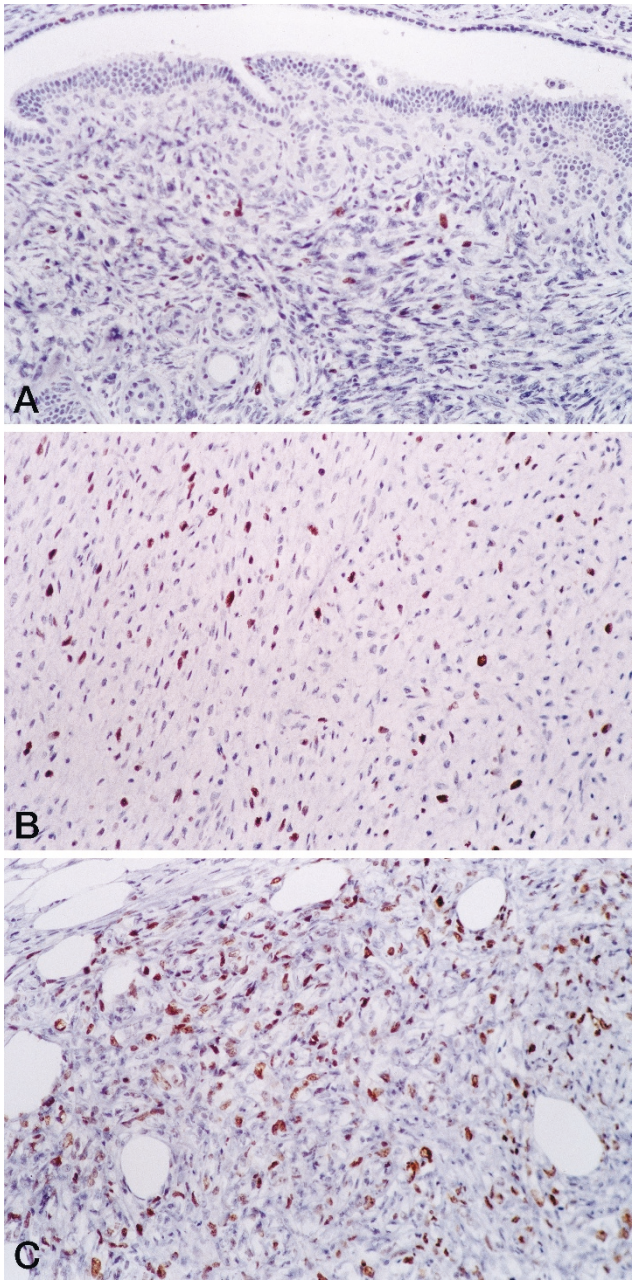

FIGURE 1. Ki-67 immunostaining in Phyllodes tumors. **A**, benign PT with few subepithelial stromal nuclei staining; **B**, low-grade malignant PT with increased Ki-67 LI; and **C**, high-grade malignant PT with numerous nuclei staining.

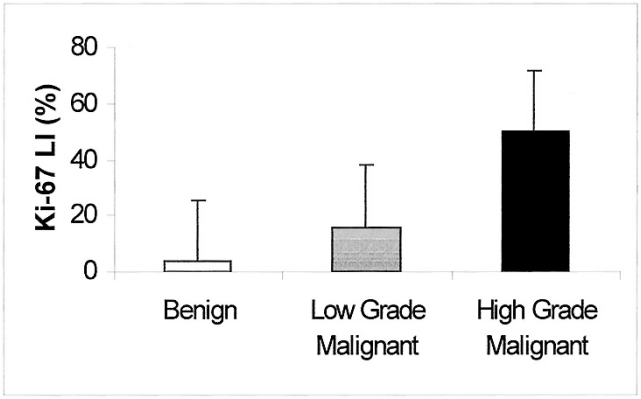

FIGURE 2. Analysis of Ki-67 in Phyllodes tumors. The distribution of Ki-67 labeling indices and the mean labeling index for each group of neoplasms is shown. High-grade malignant PT had the highest labeling index, 3-fold higher than that of benign PT ($P = .012$).

lignant PT. Benign PT had a lower Ki-67 LI than did low-grade malignant PT (3.6 ± 4.8 *versus* 16 ± 25.5, respectively). High-grade malignant PT had a greater than 3-fold higher Ki-67 LI than did benign tumors (50 ± 21.9 *versus* 3.6 ± 4.8, respectively).

Immunostaining for p53 protein was positive in two of seven benign PT (29%), in four of seven low-grade malignant PT (57%), and in three of six high-grade malignant PT (50%). No significant difference was found between p53 expression or tumor size for the three groups of tumors. The pattern of immunostaining for both p53 and Ki-67 was remarkable, as it was confined almost exclusively to the periductal stromal cells.

Of the eight tumors studied by PCR, six were informative at the D3S1478 locus, two were informative at the D3S1289 locus, and five were informative at the D3S1295 locus. No loss of heterozygosity at 3p was found in any of the eight tumors (four benign, two low-grade malignant, and two high-grade malignant).

DISCUSSION

The unpredictable clinical course of PT is demonstrated by several studies that attempted to predict clinical outcome based solely on histological classification, with little or no success (5–12). Some studies noted that several histologic features correlated with prognosis in patients with PT. Stromal overgrowth, cellular pleomorphism, and high mitotic rates were considered possible indicators of metastatic potential (5, 9, 10). Other investigators, however, questioned the usefulness of these histological features in predicting tumor behavior (22, 23).

In this study, analysis of the cell cycle proliferation marker Ki-67 showed significant differences between benign and malignant PT ($P = .012$). Although there was overlap at the lower and higher Ki-67 LIs, Ki-67 was effective in distinguishing between malignant and benign PT. As a group, malignant PT had a 2-fold higher Ki-67 LI than did benign PT. Our results support those of a previous study, which showed that Ki-67 expression correlates with the histologic classification of PT (13). Our study also is in agreement with others in that p53 protein expression correlates with the histologic classification of PT (14, 15). In our study, however, neither Ki-67 nor p53 expression correlated with clinical behavior. The finding that both p53 and Ki-67 proteins were predominantly expressed

by the stromal cells immediately beneath the epithelium indicates that this layer contains the proliferative pool of cells resulting in the stromal hypercellularity characteristic of PT and supports the hypothesis that PT arise from periductal rather than intralobular stromal cells (2).

Recently, Dietrich *et al.* (16, 17) studied PT by cytogenetics and found that interstitial deletions of the short arm of Chromosome 3, del (3)(p12p14) and del (3)(p21p23), were found in benign PT. Interestingly, 3p14 is the location of the FHIT (fragile histidine triad) gene, reported to be abnormally transcribed in primary carcinomas of the gastrointestinal tract, lung, and, more recently, breast (24). DNA mismatch repair gene homologue hMLH1 resides in 3p21–23. This gene has been found to be mutated in patients with hereditary nonpolyposis colon cancer (25). 3p12–14 and 3p21–23 have also been associated with bladder cancer progression (26). This is the first study that addresses whether there is loss of FHIT and/or hMLH1 alleles in PT. We did not find allelic loss at any of the 3p loci analyzed, and this may indicate that losses at 3p may not play a significant role in the development and progression of PT, as opposed to other neoplasms.

Although PT carries a risk of metastatic potential, this risk is low (reportedly less than 10%). Grimmes *et al.* (22) reported the clinical outcome of 100 patients with PT. Local recurrences developed in 14 benign tumors (27%), 7 borderline tumors (low-grade malignant; 32%), and 7 malignant tumors (high-grade malignant; 26%). In addition, 3 of the 22 borderline tumors (13.6%), 4 of the 27 malignant PT (15%), but none of the benign tumors metastasized. These results attest to the imperfect correlation of histologic findings with the subsequent clinical course. Salvadori *et al.* (27) found no differences in terms of local recurrence or distant metastases between borderline and malignant tumors. None of the PT in our study metastasized. Three of the tumors recurred locally. These three tumors were histologically benign. One of these tumors had a positive resection margin, and the margin was close (less than 1 cm) in the other two tumors. The recurrence of two of these tumors was histologically benign, and one was a high-grade malignant PT. All other tumors were farther than 1 cm from the resection margin.

In summary, analysis of the cell cycle protein Ki-67 in benign and malignant PT may be clinically useful in histologically classifying PT in diagnostically difficult cases. Unlike other forms of breast cancer, allelic losses at 3p do not occur in PT. Our study emphasizes that wide resection margins are pivotal in the treatment and subsequent prognosis of PT, regardless of the histologic classification.

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Book Review

Epstein JI, editor: The Johns Hopkins Atlas of Surgical Pathology, CD ROM, London, Churchill Livingstone, 1999 ($210.00).

Electronic publishing is ever expanding. *The Johns Hopkins Atlas of Surgical Pathology* is another entry into this nascent field, which is inextricably changing the way we assimilate the written word and captured image. The application program is Windows and Macintosh compatible, requiring a minimum of 12 MB of RAM, 8 MB of disk space, and a 16-bit color monitor. The clock speed only needs to be above 66 MHz. The program runs under most versions of Windows, including NT and Me.

The atlas consists of a single CD-ROM containing 4000 images stored as PDF files of approximately 156 KB. Adobe Acrobat Reader is used to open these files, but they can be accessed by Photoshop and converted to a form suitable for PowerPoint presentations. The Folio Corporation supplies the mechanics of the disk. Installation is tedious and requires loading Acrobat Reader 3.0, Folio Bound, and Apple Quick Time. All files can be loaded onto your hard disk (656 MB), but this process is exceeding slow. Once accomplished, however, one can run the *Atlas* without the CD in place. The program is easy to use, and navigation is intuitive. However, for the uninitiated, there is a distinct difference between single and double mouse clicks in the operation of the program. The *Atlas* has a so-called recognition mode and a quiz mode. Search capabilities and hyperlinks are straightforward. Image quality is good.

Ignoring all the bells and whistles, the true measure of any volume is content. In this instance, the *Atlas of Surgical Pathology* can best be described as inclusive but not necessarily comprehensive. Most topics contain something for everyone, but there is rarely any discussion of pathophysiology. On the other hand, this is an atlas and not a textbook. The section on medical kidney is restricted to light microscopic images. Cryoglobulinemia mentions only two types of cryoglobulins and fails to describe clonality. The section on malignant mesotheliomas makes reference to calretinin but ignores the use of CK7, CK5/6, and thyroid transcription factor. Conversely, the paragraph on synovial sarcoma does reference the characteristic X:18 translocation.

Overall, the restricted depth of the *Atlas* will limit its audience. The breadth of coverage, however, will meet the needs of most medical students and junior residents. Experienced pathologists will find the material most useful as an outline and image archive for teaching and presentations.

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