Progressive Deregulation of the Cell Cycle With Higher Tumor Grade in the Stroma of Breast Phyllodes Tumors

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Key Words: Breast; Phyllodes tumors; Cell cycle; Prognosis; p53

Abstract

We studied cell cycle–regulating proteins in phyllodes tumor pathogenesis by immunohistochemical analysis for Ki-67, cyclin A, cyclin D1, retinoblastoma protein (pRb), p53, p16INK4A, bcl-2, and p21waf1 in the epithelium and stroma of 40 primary (benign, 21; borderline, 8; malignant, 11) and 7 recurrent tumors of different grades.

In most cases, the epithelium showed no altered expression of cell cycle regulators. Stromal overexpression of p16INK4A, p53, cyclin A, pRb, and p21waf1 correlated significantly with tumor grade. The number of altered proteins in stroma increased with higher grade and was accompanied by increased proliferation. Stromal cyclin A expression was the best separating marker between tumor grades. Correlations existed between stromal overexpression of p16INK4A and p21waf1, p16INK4A and p53, and p53 and pRb. No immunostaining differences were detected between primary tumors and recurrences. Four or more altered proteins and p53 expression in the stromal component were independent negative prognosticators for disease-free survival.

The stromal component of mammary phyllodes tumors displays an increasing level of cell cycle deregulation with higher tumor grade; the epithelial compartment mostly remains inconspicuous. Several combinations of aberrantly expressed cell cycle proteins seem important in the stromal progression of phyllodes tumors. The number of stromal cell cycle aberrations and stromal p53 expression might predict clinical behavior.

Phyllodes tumors are fibroepithelial breast tumors, composed of epithelial and stromal components. Kuijper et al1 previously demonstrated that, mostly, the stroma of phyllodes tumors is monoclonal and the epithelium polyclonal. Morphologically, phyllodes tumor is characterized by overgrowth of stroma in a pericanalicular or leaf-like intracanalicular pattern, increased stromal cellularity, stromal cellular atypia, and increased numbers of stromal mitoses.2 Phyllodes tumors are graded as benign, borderline, or malignant.2,3 Recurrence occurs in 8% to 65% of cases, depending on the grade of the primary tumor.4 Metastases are encountered in up to 22% of malignant tumors.2 Several studies suggested that a positive surgical margin is a major prognosticator for recurrence,2,5 but others failed to confirm this.6,7 Similar contradictory reports exist with regard to stromal overgrowth, mitotic activity, and flow cytometric features.8-15 Therefore, there is a need for better prognostic criteria. Few studies have addressed the role of tumor suppressor genes in phyllodes tumors. Immunohistochemical accumulation of p53 correlated with tumor grade but not with clinical outcome in some studies,16-18 while another larger study found a relation with disease-free survival (DFS).13 Accumulation of p53 was detected in several phyllodes tumors, but p21waf1 and mdm2 expression were absent.19 To our knowledge, no comprehensive studies exist on the role and prognostic value of cell cycle regulators in phyllodes tumors.

Distortion of the cell cycle machinery is a major phenomenon in carcinogenesis. The cell cycle is regulated by cyclins, cyclin-dependent kinases (CDK) and 2 groups of CDK inhibitors (CDKIs).20,21 The CIP/KIP family members, including p21waf1, p27, and p57, are universal CDKIs. The INK4 family members p15, p16INK4A, p18, and p19 exert a negative regulatory function on CDK4/CDK6. The cyclin-CDK
complexes phosphorylate the retinoblastoma protein, pRb, which allows cells to enter the S phase. Distortion within the proliferation controlling cyclin D1/CDK4/p16INK4A/Rb-pathway leads to loss of control at the G1-S checkpoint. The role of p53 as a major regulatory protein in the cell cycle is well established. Through promotion of expression of p21waf1, p53 can halt cellular growth and induce apoptosis in response to DNA damage or other types of cellular stress.

By studying primary tumors of different grades and their recurrences, we attempted to gain insight into the role of cell cycle regulators in the development, progression, and prognosis of breast phyllodes tumors.

Materials and Methods

Tissue Samples

We retrieved 40 primary formaldehyde-fixed, paraffin-embedded phyllodes tumors from our archives, as well as 7 recurrences of these tumors. Tumors were graded as benign, borderline, or malignant based on the degree of stromal cellularity, stromal overgrowth, cellular atypia, invasiveness of the tumor margin, and the mitotic activity index (MAI), as described previously in detail by Moffat et al. Mitoses were counted using established criteria in an area of 1.6 mm² at x400 magnification. Tumors were graded in the most unfavorable areas, provided they comprised at least 10% of the total tumor area. Clinical data were obtained from medical charts.

Immunohistochemical Analysis

We cut 4-µm sections and mounted them on coated slides. To stop endogenous peroxidase activity, deparaffinized and rehydrated sections were submersed in 0.3% hydrogen peroxide. Subsequently, antigen retrieval was performed by autoclaving the slides in a pressure cooker (120°C for 20 minutes) in a 0.01-mol/L concentration of citrate buffer (pH = 6.0) or EDTA (pH = 8.0) when staining for p16INK4A. Normal rabbit serum was used to block nonspecific staining. Slides were incubated overnight at 4°C with the following mouse monoclonal antibodies: cyclin D1 (DCS-6, dilution 1:400; Neomarkers, Fremont, CA), p16INK4A (G175-405, dilution 1:500; Pharmingen, San Diego, CA), pRb (G3-245, dilution 1:1,000; Pharmingen), p21waf1 (Ab-1, dilution 1:50; Oncogene Science, Cambridge, MA), p53 (DO-7, dilution 1:500; DAKO, Carpinteria, CA), cyclin A (6E6, dilution 1:100; Novocastra, Newcastle upon Tyne, England), Ki-67 (MIB-1, dilution 1:40; DAKO), and bcl-2 (124, dilution 1:50; DAKO). A secondary biotinylated rabbit antimouse antibody diluted 1:500 was applied for 30 minutes at room temperature. Thereafter, slides were incubated with avidin-biotin-peroxidase complex (DAKO) at 1:200 dilution for 1 hour at room temperature.

We used 3,3′-diaminobenzidine-tetrahydrochloride as the chromogen and hematoxylin as the counterstain. Between steps, slides were rinsed in phosphate-buffered saline. Negative (primary antibody omitted) and appropriate positive control samples were included throughout.

Immunoreactivity was scored in the epithelial and stromal components. The percentage of positive cells was estimated on a semicontinuous scale (by A.K. and P.J.vD.) in the area displaying the highest (Ki-67, cyclin A, cyclin D1, p53, p21waf1) or lowest (bcl-2) density of positive cells, believed to represent the biologically most relevant areas. These areas had to involve at least 10% of the total tumor area (see “Tissue Samples”). Because accumulation and loss of pRb reactivity might be important, staining for pRb was estimated for the whole slide. p16INK4A was assessed similarly because scoring of p16INK4A immunoreactivity varied between different studies. For statistical analysis, high expression was defined as 10% or more positively staining cells in accordance with previous studies. Loss of expression of pRb was indicated by less than 1% nuclear immunostaining.

Statistical Analysis

P values (SPSS software, SPSS, Chicago, IL) less than .05 were considered significant. The χ² test was used to test for differences in expression of cell cycle proteins between tumor grades and between primary and recurrent tumors. Stepwise discriminant analysis was applied to detect the best separating features between tumor grades. Immunohistochemical expression of cell cycle regulators and proliferation markers were correlated among themselves by using the Fisher exact test. Differences in the number of altered proteins between grades were analyzed by using the Kruskal-Wallis test. The clinical endpoint for survival analysis was local or distant recurrence (DFS). Patients treated by mastectomy were excluded from survival analysis. For univariate survival analyses, Kaplan-Meier curves were plotted and evaluated with the log-rank test. Cox regression was performed to identify independent prognostic variables.

Results

Patient Characteristics

We identified 21 benign, 8 borderline, and 11 malignant primary phyllodes tumors. Epithelial hyperplasia, mostly focal, was found in 15 cases (38%) and was not related to grade. Mean ± SD ages of patients with benign, borderline, and malignant phyllodes tumors were 45.5 ± 16.8 years, 57.9 ± 12.8 years, and 54.3 ± 12.9 years, respectively (P = .080). Mean ± SD tumor sizes were 4.5 ± 2.5 cm, 7.1 ± 7.0 cm, and 4.8 ± 2.5 cm for benign, borderline, and malignant grades,
respectively ($P = .855$). Five patients were treated by mastectomy and the remainder by local excision. Excision was incomplete for 13 primary tumors; for 6 tumors this information could not be retrieved.

**Differences Between Tumor Grade and Cell Cycle Expression**

Altered expression of cell cycle proteins seldom was found in the epithelial component. Only 3 cases of p21$^{waf1}$ overexpression, 3 of altered pRb expression, 1 of cyclin A overexpression, and 3 of cyclin D1 overexpression were found in the epithelium, all without relation to tumor grade or epithelial hyperplasia. In contrast, aberrant expression was frequent in the stromal component. Differences in stromal overexpression of cell cycle regulators for the different grades are summarized in **Table 1**. All malignant phyllodes tumors displayed high Ki-67 expression in the stromal component. Stromal p53 overexpression correlated significantly and positively with tumor grade ($P = .015$) and was found in nearly half of malignant-grade tumors. Stromal overexpression of cyclin A showed a striking increase from borderline to malignant tumors, from no tumors to nearly 75% of tumors (8/11 [73%]). In contrast with negative staining for pRb, high expression correlated positively with grade ($P = .003$) and was seen exclusively in malignant tumors. Immunostaining for p16$^{INK4A}$ displayed nuclear and cytoplasmic staining. Cyclin D1 expression seemed to parallel nuclear staining; both were correlated positively with tumor grade ($P = .005$ and $P < .001$, respectively). Stromal cyclin A expression was the single best discriminating factor between tumor grades ($P < .001$; 77.5% accuracy) **Figure 1**.

**Concerted Expression of Cell Cycle Regulators**

No interrelations existed between studied markers in the epithelial component. **Table 2** gives correlations among cell cycle regulators and proliferation markers in stroma. In the stroma, several combinations of aberrantly expressed cell cycle regulators were found, making both pathways of cell cycle control ineffective (Table 2). Stromal overexpression of p16$^{INK4A}$ correlated with stromal overexpression of p53 ($P < .001$ and $P .031$), thereby affecting both pathways. Likewise, stromal pRb overexpression correlated positively with stromal overexpression of p53 ($P = .013$).

**Number of Events**

The number of proteins with altered expression (further denoted as “events”) in the stroma displayed a steady increase with grade. Excluding Ki-67 expression, benign, borderline, and malignant phyllodes tumors displayed a mean of 0.7, 1.6, and 3.5 events in the stromal compartment,
respectively \((P < .001)\). The distribution of the number of stromal events in the 40 tumors was as follows: 14 (35%) displayed no aberrations, 8 (20%) showed 1 event, 2 altered proteins were found in 6 (15%), 3 and 4 events were found in 5 (13%), and 5 altered proteins were seen in 2 tumors (5%). The number of events was correlated with proliferation as measured by Ki-67 expression \((P < .001)\) and MAI \((P < .001)\). In the majority of phyllodes tumors \((30/40 [75\%])\) no epithelial events were found; 9 (23%) showed altered expression of 1 protein, and only 1 tumor (3%) had 2 abnormalities. No differences in the number of epithelial events were detected between tumor grades \((P = .542)\).

### Recurrent Disease and Prognosis

Three patients were lost to follow-up. The median follow-up time for the remaining 37 patients was 93 months \(\text{range, 4-215 months}\). In 10 (27%) of 37 patients, tumors recurred; of these, 5 (50%) were malignant, 1 (10%) was borderline, and 4 (40%) were benign. The median time to recurrence was 38 months \(\text{range, 4-162 months}\). Most were local recurrences, but 1 malignant tumor metastasized to the lung.

The corresponding local recurrences of 3 benign and 3 malignant tumors were recovered. One malignant tumor recurred twice, resulting in 7 available recurrent tumors. Two tumors had progressed to a higher grade, and 1 malignant tumor recurred with a borderline grade. No statistically significant differences in the expression of cell cycle regulators could be detected between primary and recurrent tumors.

Univariate survival analysis \(\text{Table 3}\) showed an inverse relation between DFS and stromal overexpression of p53 \(\text{Figure 3}\), cyclin A, Ki-67, and pRb; 4 or more stromal events; tumor diameter of 5 cm or more; and tumor grade. Margin status had no prognostic significance. However, recurrent tumors from incompletely excised tumors recurred earlier than those from completely excised tumors \(\text{Figure 4}\). When entering all variables that showed a univariate correlation with DFS in Cox regression, stromal p53 overexpression and 4 or more stromal events were independent prognosticators for recurrent disease.

### Discussion

This is the first study evaluating 2 major cell cycle control pathways in phyllodes tumors of different grades. With higher tumor grade, the stromal compartment of phyllodes tumors showed progressive cell cycle derailment. Although recent studies have suggested more than a passive role for

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**Table 2**

<table>
<thead>
<tr>
<th>Interrelations Between Altered Expression of Cell Cycle Regulators and Proliferation Markers in Stroma of Breast Phyllodes Tumors¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
</tr>
<tr>
<td>p16(^{INK4A})</td>
</tr>
<tr>
<td>Cyclin A</td>
</tr>
<tr>
<td>Cyclin D1</td>
</tr>
<tr>
<td>p21(^{waft})</td>
</tr>
<tr>
<td>p53</td>
</tr>
<tr>
<td>Retinoblastoma protein</td>
</tr>
<tr>
<td>&lt;1%</td>
</tr>
<tr>
<td>≥1%</td>
</tr>
<tr>
<td>bcl-2</td>
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</table>

MAI, mitotic activity index; NS, not significant.

¹ \(P\) values by the Fisher exact test.

² Cases were divided into high and low MAI groups using a cutoff of 10 mitoses.
the epithelium, the epithelial component did not display conspicuous cell cycle changes.

\(\text{p16INK4A is a tumor suppressor that blocks cell cycle progression at the G}_1\ \text{restriction point by inhibition of cyclin-CDK4 and cyclin-CDK6 activity.}\)

Surprisingly, high stromal expression of p16\(^{\text{INK4A}}\) correlated positively with stromal p53 overexpression, mitotic count, tumor grade, and stromal Ki-67 overexpression. Although initial reports in breast cancer showed that loss of p16\(^{\text{INK4A}}\) expression indicated an adverse prognosis, more recent studies related high p16\(^{\text{INK4A}}\) protein and messenger RNA levels to a more malignant phenotype and a poor outcome. Furthermore, a relation between cytoplasmic overexpression of p16\(^{\text{INK4A}}\) and aggressive features has been described. In the present study, cytoplasmic p16\(^{\text{INK4A}}\) overexpression seemed to parallel nuclear overexpression (data not shown). The mechanisms behind up-regulation of p16\(^{\text{INK4A}}\)

### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>With Disease</th>
<th>Disease-Free</th>
<th>(P)</th>
<th>Log-Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>p53 Stroma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10% (n = 27)</td>
<td>5 (19)</td>
<td>22 (81)</td>
<td>(&lt;.001)</td>
<td>12.54</td>
</tr>
<tr>
<td>≥10% (n = 6)</td>
<td>5 (83)</td>
<td>1 (17)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Cyclin A stroma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10% (n = 26)</td>
<td>6 (23)</td>
<td>20 (77)</td>
<td>(.002)</td>
<td>9.70</td>
</tr>
<tr>
<td>≥10% (n = 7)</td>
<td>4 (57)</td>
<td>3 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ki-67 stroma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10% (n = 19)</td>
<td>4 (21)</td>
<td>15 (79)</td>
<td>(.015)</td>
<td>5.91</td>
</tr>
<tr>
<td>≥10% (n = 14)</td>
<td>6 (43)</td>
<td>8 (57)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Rhabdomyosarcoma protein stroma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10% (n = 30)</td>
<td>8 (27)</td>
<td>22 (73)</td>
<td>(.030)</td>
<td>4.74</td>
</tr>
<tr>
<td>≥10% (n = 3)</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td></td>
<td></td>
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<tr>
<td><strong>No. of stromal events</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;4 (n = 28)</td>
<td>6 (21)</td>
<td>22 (79)</td>
<td>(&lt;.001)</td>
<td>14.27</td>
</tr>
<tr>
<td>≥4 (n = 5)</td>
<td>4 (80)</td>
<td>1 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grade</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign (n = 18)</td>
<td>4 (22)</td>
<td>14 (78)</td>
<td>(.023)</td>
<td>7.57</td>
</tr>
<tr>
<td>Borderline (n = 5)</td>
<td>1 (20)</td>
<td>4 (80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant (n = 10)</td>
<td>5 (50)</td>
<td>5 (50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Size (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 (n = 19)</td>
<td>2 (11)</td>
<td>17 (89)</td>
<td>(.042)</td>
<td>4.12</td>
</tr>
<tr>
<td>≥5 (n = 12)</td>
<td>6 (50)</td>
<td>6 (50)</td>
<td></td>
<td></td>
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</table>

\* Data are given as number (percentage).
are poorly understood, but possible explanations include loss of pRb,36 accumulation owing to high proliferation and long half-life of the protein,33 and cellular stress such as replicative senescence, ultraviolet radiation, or hyperthermia.36,37 No inverse relation was present between pRb and p16INK4A in our group, but p16INK4A was related to proliferation.

Stromal cyclin D1 overexpression was not correlated with established prognostic markers, and its role in phyllodes tumors, therefore, seems limited. A recent study detected overexpression of cyclin D1 in epithelium in 46% and in stroma of 19% of phyllodes tumors.31 We detected stromal cyclin D1 overexpression in 6 tumors (15%) but in epithelium in only 3 cases (8%). Cyclin D1 might be overexpressed in ductal hyperplasia,38,39 but like Sawyer et al31 found, epithelial cyclin D1 overexpression was not related to the presence of hyperplasia. Although comparisons are hard to make because the cutoff point for cyclin D1 overexpression used in the study by Sawyer et al31 was not specified, the reason for this difference in the frequency of epithelial cyclin D1 expression is unknown.

The p53 protein is involved in DNA damage control. In keeping with previous reports,13,16-19,40-44 stromal p53 overexpression was correlated with grade and was found in nearly 50% of malignant cases (5/11 [45%]). Although not all immunohistochemically detectable p53 accumulation is caused by mutation of the gene, p53 mutations have been detected in phyllodes tumors.19,42,45,46 A defective p53 protein might lead to uncontrolled progression through the cell cycle and associated uncontrolled growth and tumor progression. Indeed, stromal p53 accumulation was correlated with increased proliferation. Furthermore, we found that stromal p53 accumulation was an independent prognostic factor, confirming the results of a large study by Niezabitowski et al.13 p53 seems to have an important role in the progression of phyllodes tumors.

Recently, cycdin A is involved in the late S, G2, and M phases of the cell cycle, shortly before mitosis, and is required to initiate DNA replication.48 Not surprisingly, therefore, stromal cycdin A expression was correlated with MAI and stromal Ki-67 overexpression. Benign and borderline tumors on the one hand did not display stromal cycdin A overexpression, whereas 8 (73%) of 11 malignant tumors showed overexpression. Stromal cycdin A overexpression might, therefore, reflect a final step toward uncontrolled proliferation in many cells. By stepwise discriminant analysis, stromal cycdin A expression was the best discriminating marker between tumor grades (Figure 1) and, therefore, seems a useful aid in accurately grading phyllodes tumors. Furthermore, stromal cycdin A expression had prognostic power in univariate DFS analysis, but this was not an independent effect.

p21waf1 is a transcriptional target of p53 and blocks cell cycle progression by interaction with CDKs,49 but p53-independent p21waf1 activation pathways exist.50,51 In breast cancer, increased p21waf1 expression has been associated with decreased DFS,23 but others failed to detect this.52,53 In our study, p21waf1 stromal overexpression correlated positively with the adverse prognosticators grade and MAI but did not have prognostic value. Overexpression of p21waf1 was not found in a previous study,19 but malignant tumors were strongly underestimated in the study. Indeed, stromal p21waf1 overexpression was seen preeminently in malignant phyllodes tumors (4/11 [36%]). Of 5 p21waf1 overexpressing phyllodes tumors, 2 also were p53+, indicating that p21waf1 induction might occur independent of p53, eg, due to specific growth factors.50 Although p21waf1 overexpression was found in the epithelium of 3 tumors, no relation with other variables was found.

Because mitotic counts are part of almost all grading systems, one can expect a positive correlation between tumor grade and Ki-67 expression. Indeed, like the present study, all previous articles could confirm this.13,18,42,43,54-55 In our study, all malignant tumors expressed Ki-67 in more than 10% of stromal cells. Therefore, malignant grade might be denied by a finding of less than 10% positive stromal Ki-67 staining. Furthermore, stromal Ki-67 overexpression was predictive of DFS, in part agreeing with a previous study that found it to be predictive of overall survival but not of DFS.13 Another study detected no prognostic power, though.17

The intratumor heterogeneity of phyllodes tumors is reflected in pRb staining; areas with complete loss and with moderate or overexpression coexisted within the same tumor. Because both loss and overexpression of pRb might be important in carcinogenesis,23-25 immunohistologic quantification was problematic and pRb results should therefore be interpreted with caution. Stromal pRb overexpression was correlated with grade and a high proliferation rate. Although most studies have focused on loss of pRb expression, the relation between proliferation and accumulation of pRb has been described previously in breast cancer.56 In fibrosarcomas, phenotypically related to stroma of malignant phyllodes tumors, overexpression of pRb was associated with decreased DFS.24 These observations were attributable to dominance of hyperphosphorylated forms of the protein. In our study, pRb overexpression was associated with decreased DFS, but pRb was not an independent prognosticator. Possible explanations for accumulation of this tumor suppressor gene product include abnormalities of upstream regulators of pRb expression or stabilization of pRb by binding to other proteins or viral oncoproteins.25 Furthermore, pRb overexpression might provide protection against apoptosis and growth inhibition.59 Loss of stromal pRb was not correlated with grade or other variables.

Like Kuenen-Boumeester et al19 found, we found loss of the antiapoptotic protein bcl-2 in stroma in only 1 primary
phyllodes tumor, indicating seemingly minor importance. Conflicting bcl-2 data are found in the literature. Loss of bcl-2 in a recurrent tumor and metastasis in contrast with the primary tumor indicates a potential role for loss of bcl-2. We did not observe loss of bcl-2 in recurrent tumors. Another study found progressive loss of bcl-2 expression in both compartments with higher grade. Moore and Lee found loss of bcl-2 in the stromal component of malignant tumors but not in the epithelial compartment. The epithelial component in the present study invariably expressed bcl-2, as does normal breast epithelium.

We evaluated 6 pairs of primary and recurrent tumors. Two tumors progressed to a more malignant phenotype, whereas 1 malignant primary tumor recurred as a tumor of borderline grade. Comparison with the initial lesion revealed that this recurrence had a strong histologic and immunophenotypic resemblance to a small peripheral area of the primary tumor that was located outside the high-grade area. Therefore, the apparent “regression” was most likely due to the incomplete excision of the peripheral lower grade area. In general, no large differences in immunophenotype were found between primary and recurrent tumors. This suggests that the recurrent tumors were the result of residual disease and that wide local excision is the treatment of choice in phyllodes tumors. However, in survival analysis, a positive surgical margin was not predictive of recurrence. This is at variance with some previous reports. As shown in Figure 4, a short follow-up time might result in an inverse correlation between positive margins and DFS. Therefore, differences in length of follow-up might partially explain these discrepancies.

Like most previous studies, we found that tumor grade was predictive of recurrent disease. In agreement with a recent study, tumor size was related inversely to DFS in our series. Others found size to be predictive of death, not of DFS. Besides being accompanied by an increase in proliferation, the number of events in the stromal component was of prognostic value as well. Four or more stromal events in a tumor turned out to be an independent prognosticator for DFS.

Although a paracrine role has been suggested for the epithelium, the epithelial compartment did not show cell cycle alterations in the vast majority of the tumors. Previously a model was proposed in which growth of stroma in early stages of tumorigenesis is under the control of the epithelium. Uncoupling of this interaction in later stages would lead to autonomous growth of stroma. It was unclear, however, which mutations would initiate this process and in which compartment they would occur. Our findings suggest that cell cycle abrogation in the stromal component might be responsible for this loss of stromal-epithelial communication and progression toward a malignant phenotype.

The transition from borderline to malignant grade seems to be of particular importance. The number of tumors with stromal overexpression of p53, p16, p21, pRb, and cyclin A is sharply increased during this final step toward a malignant phenotype. It is thought that multiple derailments in one pathway, ie, the proliferation controlling Rb pathway or the apoptotic p53 pathway, do not add to a greater growth advantage. Indeed, in the stromal component, overexpression of p53 was not correlated with overexpression of p21, and overexpression of pRb was not correlated with overexpression of p16. Furthermore, overexpression of p16 was correlated with overexpression of p53 and p21, whereas overexpression of pRb was related to p53, assigning these 3 combinations of normally expressed G1-S checkpoint regulators a role in the progression of phyllodes tumors.

Taken together, our results suggest that the stromal component of mammary phyllodes tumors is characterized by an increasing level of cell cycle deregulation with higher tumor grade. The epithelial compartment does not display aberrant expression of cell cycle regulators. Several defined combinations of defective cell cycle regulators seem responsible for malignant progression of the stromal component of breast phyllodes tumors. Furthermore, the number of stromal events and stromal p53 expression might predict clinical behavior.

References


