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ARTICLE

Proliferating Activity in Differential Diagnosis of Benign Phyllodes Tumor and Cellular Fibroadenomas: Is It Helpful?

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Benign phyllodes tumors and fibroadenomas are two types of fibroepithelial tumors of breast that are usually difficult to differentiate. The purpose of this study is to evalute the proliferative activity of these tumors and to find out if it helps in differential diagnosis. Thirty-one benign phyllodes tumors and twelve cellular fibroadenomas were retrieved from the archives of Pathology Department of Akdeniz University, School of Medicine. Proliferating activity of epithelial and stromal cells were evaluated by using labeling index (LI) of proliferating cell nuclear antigen (PCNA) and Ki-67 antigen by immunohistochemistry. The results were compared with other clinicopathologic findings. There was not any significant difference between the proliferating activity of phyllodes tumor and cellular fibroadenomas. Mean LI of PCNA was 28.01 (±22.85) in stromal cells and 56.57 (±30.98) in epithelial cells of phyllodes tumor where it was 28.92 (±24.02) and 62.53 (±32.56) in fibroadenomas. Ki-67 indices were 0.05 (±0.19) in stromal cells, 2.65 (±12.53) in epithelial cells of phyllodes tumors and 0.0 (±0) in stromal cells, 0.43 (±0.63) in epithelial cells of fibroadenomas. There was no correlation between the diameter of tumors and proliferating activity in both groups. Proliferating activity, determined by immunohistochemistry with PCNA and Ki-67 antibodies did not reveal significant difference between phyllodes tumor and fibroadenoma. (Pathology Oncology Research Vol 7, No 3, 213–216, 2001)

Keywords: Phyllodes tumor, fibroadenoma, proliferating activity

Introduction

Phyllodes tumor and fibroadenoma are two types of biphasic tumors of the breast. They are composed of various combinations of proliferating epithelial and stromal elements.[1,4] Phyllodes tumor (PT) is a rare fibroepithelial neoplasm of the breast that account for 0.3% to 1.5% of breast tumors in females and approximately 2.5% of all fibroepithelial breast tumors.[5] Fibroadenoma (FA) is the most common benign tumor that causes breast masses in young women.[3] Generally PT occur in an older age group than fibroadenomas; most patients are middle aged or elderly.[2] Phyllodes tumors are classified as benign, borderline or malignant on the basis of stromal cellularity, nuclear atypia, mitotic activity, stromal overgowth and type of border (infiltrating or pushing).[12] Histopathologically, PT (PT) is composed of a benign epithelial component and a cellular spindle cell stroma; the stroma is characterised by formation of leaf-like processes protruding into cystic spaces.[5] Benign PT is generally used as a synonym of fibroadenoma phyllodes where the cellularity of the stroma is similar to fibroadenoma, but the leaf-like processes are readily identifiable.[1] There is a distinct difference in the clinical course between FA and borderline/malignant PT. Clinically most PTs tend to behave in a benign fashion, but, unlike FA, they can recur locally (in 16-30%)[5] and can undergo malignant progression to sarcoma.[11,12] In many instances, differential diagnosis of FA and BPT poses no difficulty but in complicated cases such as FA with a slightly higher cellular stroma or with focal phyllodes structure differential diagnosis is problematic.[13] However, in general, the stromal cell cellularity in PT is higher than in FA, and the nuclear atypia of the former is more prominent than the latter.[3] The histogenesis of PT and that of FA of the breast appears to be closely related

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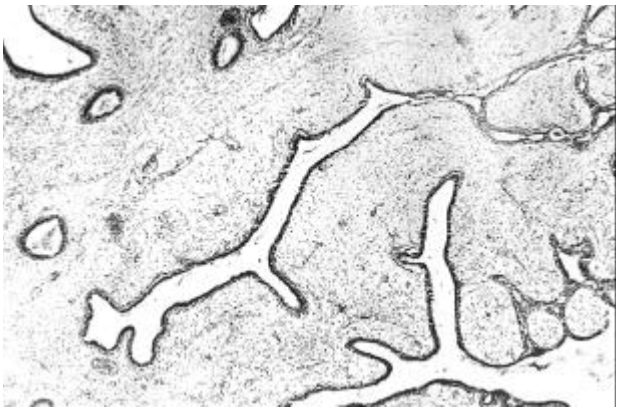

Figure 1. Benign phyllodes tumor, identifiable leaf-like structures, HE, x40

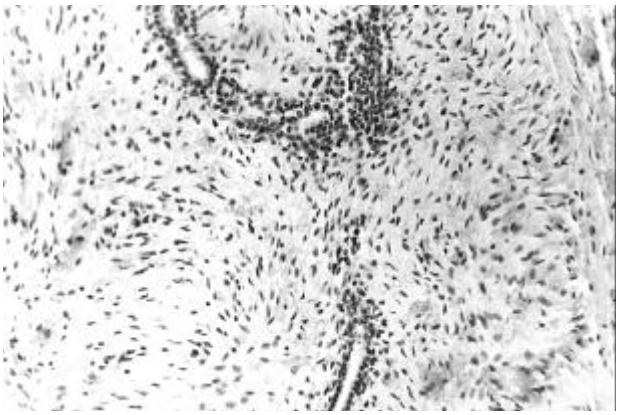

Figure 2. Cellular fibroadenoma, cellular stroma, HE, x200

and discrimination between them by histopathological analysis is sometimes problematic.[8,14] There is no histologic feature that will always provide an absolute and universally accepted distinction between the two lesions.[2] The aim of this study is to find out whether the immonohistochemical assessment of proliferative activity using Ki-67 antigen and proliferating cell nuclear antigen (PCNA) can help to differentiate BPT from cellular FA.

Materials and Methods

Patients and tissues

Thirtyone benign PT and 12 cellular FA were retrieved from the archives of Pathology Department of Akdeniz University, School of Medicine. According to mentioned criteria all of PT were benign that had similar stromal cellularity with fibroadenomas but identifiable leaf-like processes (*Figure 1*). The designation of cellular FA was used if the tumor lacked or had rare and poorly formed leaf-like processes and the stroma showed minimally increased cellularity (*Figure 2*).[1] All of FA cases were cellular. Mean age of PT patients and FA patients were 32.6 years (range:19-45) and 24.3 years (range: 14-36) respectively. Median tumor size of BPT patients and FA patients were 2.5 cm (range: 1-5) and 2.5 cm (range: 1.5-12). The tumor sizes of three FA patients were 6, 7 and 12 cm which were defined as giant fibroadenomas (4 cm and larger).[6]

Immunostaining

For immunohistochemical analysis, 4-5μm thick sections of paraffin blocks of FA and PT cases were rehydrated according to standard protocol. Sections were immunostained in automated immunohistochemistry system (Techmate 500, DAKO, Denmark) by streptavidin biotin peroxidase protocol. Primary antibodies were PCNA (diluted at 1:50, monoclonal, PC10, DAKO, Denmark) and Ki-67 antigen (diluted at 1:50, monoclonal Ki-S5, DAKO, Denmark). In each group proliferating activity of epithelial and stromal cells were evaluated. Positive stained percent of nuclei were assessed as labeling index (LI) by counting at least 500 cells at high power (x400). The fields with the highest cellular area within the tumor were selected for cell counting.

Statistical analysis

Statistics between FA and PT groups were performed by Mann-Whitney U test and parameters were compared by correlation tests in each group. P values ≤ 0.05 were regarded significant.

Results

We retrospectively examined 31 benign PT and 12 cellular FA. The average values of age, tumor size, LI of PCNA and Ki-67 antigen (%) are summarized in *Table 1*. FA patients were younger than BPT patients (p=0.008). PCNA LI in epithelial and stromal components demonstrated a positive correlation overall (*Figure 3*) (r=0.55, p=0.0001) and inside FA group (r=0.64, p=0.025). There was not statistically significant difference in tumor sizes between PT and FA. *Figure 4* shows the PCNA positivity in PT. In both groups, there were not any correlation with the diameter of tumors and proliferative activity. There was no significant difference between the proliferating activity of BPT and cellular fibroadenomas.

Discussion

Phyllodes tumor of the breast is well-known for its unpredictable behavior in terms of local recurrences and distant metastases. There is now a clear consensus that histopathological appearence and biological behavior in PT of the breast may poorly correlate, and histopathological features

Table 1. **The average values of tumor size, age, labeling index of PCNA and Ki-67 antigen**

Features	*Benign phyllodes tumor*	*Fibroadenoma*	*p value**
Tumor size (cm)	2,5 (median) range:1-5	2,5 (median) range:1,5-12	NS**
Age	33.81 (mean) range 19-45	24.3 (mean) range:14-36	0.0008
PCNA stromal (mean±SD)	28,01 ± 22,85	28,92 ± 24,02	NS
PCNA epithelial (mean±SD)	56,57 ± 30,98	63,53 ± 32,56	NS
Ki67 stromal (mean±SD)	0,05 ± 0,19	0,00 ± 0	NS
Ki67 epithelial (mean±SD)	2,65 ± 12,53	0,43 ± 0,63	NS

*p< 0.05 is significant
**NS: Nonsignificant

alone are of relatively limited value in discriminating benign and malignant PT.[5] Recently the determination of proliferative activity has given additional information on the biological behavior and clinical outcome of different neoplasms.[5,15] Proliferative activity can be determined by two different immunohistochemical marker, Ki-67 and PCNA. Ki-67 (MIB 1) is a non-histone nuclear protein that is present through the whole cell cycle.[10] However some investigators observe that Ki-67 reaches the peak value during the G2/M phase of the cell cycle.[7] On the other hand PCNA is also a non-histone nuclear protein and is present in the late G1 phase, with the peak in G1/S interphase of the cell cycle.[9] As the two markers indicate the different cell cycle phases of proliferating cell, we use both of them. In several studies the determination of proliferating activity and Ki-67 antigen proved to be a useful parameter to distinguish between benign and malignant tumors. Witte et al found a significant correlation between proliferation rate and dignity, tumors with a low proliferative activity mostly had a benign histology whereas a high Ki-67 index indicated a malignant PT.[15] This correlation was also demonstrated by Kocova et al[5] and Umekita et al.[8]

Fibroadenomas with hypercellular stroma are often difficult to distinguish from benign PT, and there are some PT with foci that are indistinguishable from FA. Fibroadenomas with hypercellular stroma may be considered to be cellular variant fibroadenoma (FACV), and some FACV cases have stromal cellularity values that are equivalent to PT.[3] There is a broad consensus that the discrimination between PT and FA is very important because the malignant potential of PT is higher than that of FA.[8] It has been well acknowledged that MIB1 antibody, which recognizes Ki-67 antigen, is a suitable and reliable marker for the assessment of cell proliferative activity and Umekita et al demonstrated that there were two different benign PT according to MIB 1 index. They found no significant difference between cellular FA and FA with focal phyllodes structures but observed a significant difference between the MIB indices of conventional FAs and aforementioned two types of Fas.[13] In our study, we defined FA with phyllodes structure as a benign PT and we also did not find any statistically significant difference in the proliferative activity of cellular FA anf benign phyllodes tumor. Kocova et al found that there was a statistically significant difference in MIB1 indices of benign PT and FA.[5] The histopathologic criteria used in this study differed from the criteria of current study. We used the histopathologic criteria designated by

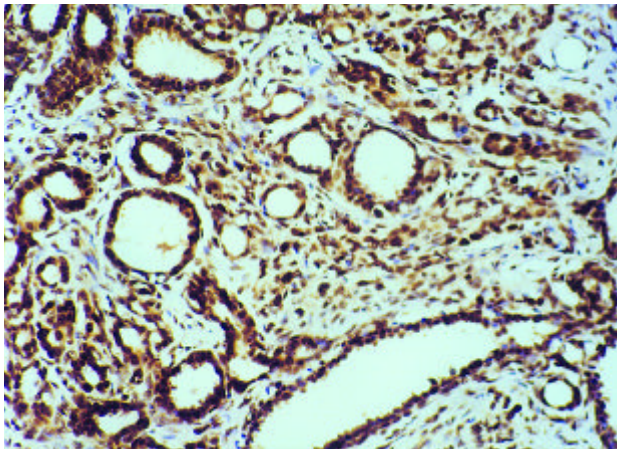

Figure 3. PCNA positivity in stromal and epithelial cells of FA, DAB, x200

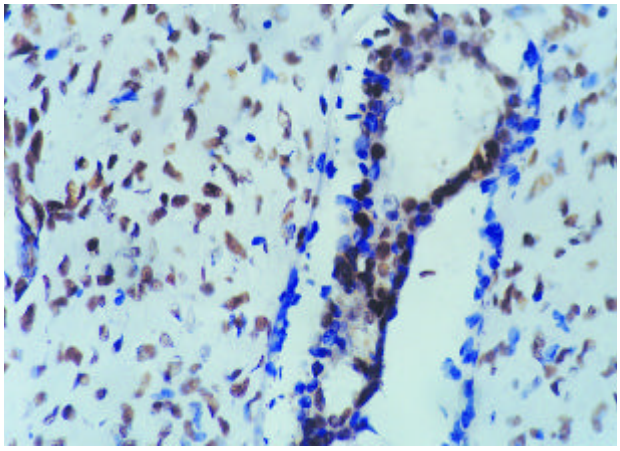

Figure 4. PCNA positivity in PT, DAB, x400

Tavassoli et al. in AFIP.[1] In the current study, though we used two different proliferative markers, we did not find any significant difference between benign PT and cellular FA. As we had no malignant or borderline PT, no comparison could be made between benign mnd malignant PTs.

We concluded that determination of the proliferating activity of benign PT and cellular FA did not help to differentiate these neoplasms.

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