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Distribution of p63, a Novel Myoepithelial Marker, in Fine-Needle Aspiration Biopsies of the Breast

An Analysis of 82 Samples

Jorge S. Reis-Filho, M.D.[1,2]
Fernanda Milanezi, M.D.[1]
Isabel Amendoeira, M.D.[3]
André Albergaria, B.Sc.[1]
Fernando C. Schmitt, M.D., Ph.D.[1,4]

[1] Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal.

[2] School of Health Sciences, University of Minho, Braga, Portugal.

[3] Hospital São João, Medical Faculty, University of Porto, Porto, Portugal.

[4] Medical Faculty, University of Porto, Porto, Portugal.

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Address for reprints: Fernando C. Schmitt, M.D., Ph.D., Institute of Molecular Pathology and Immunology, University of Porto, R. Roberto Frias, S/N, 4200 Porto, Portugal; Fax: 011 (351) 225570799; E-mail: fernando.schmitt@ipatimup.pt

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BACKGROUND. The presence of myoepithelial cells (MECs) in fine-needle aspiration biopsies (FNAB) of the breast constitute an important criterion used to diagnose benign breast lesions. However, MECs sometimes have a distorted cytomorphology, and most of the previously evaluated myoepithelial markers do not have satisfactory sensitivity and specificity. p63, a recently characterized p53 homolog, is a nuclear transcription factor that is expressed in basal cells of multilayered epithelia and myoepithelial cells of the breast. The authors analyzed the immunocytochemical distribution of p63 in a series of 82 breast FNABs (30 benign lesions and 52 malignant breast lesions).

METHODS. Eighty-two archival, Papanicolaou-stained smears of breast lesions were retrieved from the files of the authors' institutions. Immunocytochemistry was performed according to the streptavidin-biotin-peroxidase complex technique using the antibody 4A4 (against all p63 isoforms). Two pathologists evaluated the distribution of p63 positive cells. Only nuclear reactivity was considered specific.

RESULTS. In benign lesions, p63 decorated the nuclei of MECs in all samples. p63 also stained naked nuclei in fibroadenomas. In malignant lesions, p63 was positive in MECs overlying malignant cell clusters in all 8 samples of ductal carcinoma in situ (DCIS), in 9 of 16 samples of pure invasive carcinomas (IC), and in 16 of 20 samples that contained both DCIS and IC. In 18 samples (36%), a variable population of p63 positive, malignant cells was observed. p63 failed to decorate stromal, neural, adipocytic, and smooth muscle cells in all samples.

CONCLUSIONS. p63 is a reliable nuclear marker of MECs in breast aspirates. Regardless of the fact that variable proportions of p63 positive, malignant cells were observed in 36% of breast carcinoma aspirates, p63 may be a useful adjunct antibody to confirm the presence of MECs in FNABs of benign breast lesions. ***Cancer (Cancer Cytopathol)* 2003;99:172–9.** *© 2003 American Cancer Society.*

KEYWORDS: p63, myoepithelial cells, myoepithelium, breast, fine-needle aspiration, immunocytochemistry, naked nuclei.

Fine-needle aspiration biopsy (FNAB) of the breast is a widely accepted technique in the diagnosis and management of both palpable breast lesions and nonpalpable breast lesions due to its simplicity, accuracy, and utility for the avoidance of more invasive procedures.[1,2] Regardless of the growing popularity of core needle biopsies of the breast, FNAB remains one of the methods included in the *triple-test* approach to breast lesions, in which clinical, mammographic, and cytologic features are used to tailor the best management for a given patient.[1–4] Moreover, FNAB specimens may be used to assess prognostically significant pathologic parameters of breast carcinoma, including nuclear grade, histologic type, ploidy, prolifer-

ation index, immunocytochemical expression of hormone receptors, c-erb-B2, and p53.[3]

Breast aspirates usually are comprised of epithelial (secretory) and myoepithelial cells as well as mesenchymal cells, including stromal cells, endothelial cells, pericytes, adipocytes, and neural cells.[1,2,4] The architectural distribution of epithelial, myoepithelial, and mesenchymal cells as well as cytologic features should be assessed carefully. In some situations, the identification of myoepithelial cells is of utmost importance, because their presence has been used as a marker of benign breast lesions.[1,5] However, these cells sometimes have unusual cytomorphologic features and may be confused with apoptotic cells, macrophages/histiocytes, and stromal cells.[1,2]

Several antibodies have been employed as myoepithelial markers, including S-100 protein, α-smooth muscle actin, calponin, myosin heavy chain, and maspin.[1,2,4–12] Recently, p63, a p53 homologue, has been characterized as a reliable marker of myoepithelial cells of breast lobules and ducts.[13,14] Most importantly, p63 is the first nuclear marker for myoepithelial cells.[13,14] To the best of our knowledge, only a brief, preliminary assessment of p63 expression in FNAB of the breast has been published to date.[13] We evaluated the distribution of p63 in 82 FNABs of the breast as well as its putative applications in routine cytopathology practice.

MATERIALS AND METHODS

FNABs of the breast were retrieved retrospectively from the cytopathology archives of the Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal, and the Department of Pathology, Hospital São João, Porto, Portugal. Only Papanicolaou-stained slides were included in the current study. Eighty-two samples were collected and reviewed by two of the authors: 30 benign breast lesions and 52 carcinomas (in situ and invasive).

The cytologic samples were reviewed by two of the authors, and the most representative Papanicolaou-stained cytology slide was chosen for the immunocytochemical analysis, as described previously.[15] Briefly, the coverslips of Papanicolaou-stained slides were removed. Heat-induced antigen retrieval in a wet bath, using either the Dako antigen-retrieval solution or citrate buffer, pH 6.0, was performed for 20 minutes in all samples. Immunocytochemistry was performed according to the streptavidin-biotin-peroxidase technique using the antibody 4A4 raised against p63 (dilution 1:200; Neomarkers, Freemont, CA), as described elsewhere.[16] Only cells that showed strong and distinctive nuclear immunoreactivity for p63 were considered positive. Cytoplasmic and membranous staining was considered nonspecific. Positive controls (histologic sections of a sclerosing papilloma with myoepithelial hyperplasia) and negative controls (omission of the primary and secondary antibodies) were included in each slide run. All controls yielded appropriate results.

Two of the authors independently evaluated the distribution of p63 in epithelial cells, myoepithelial cells, stromal tissue fragments, adipocytes, blood vessels, and neoplastic cells. Special attention was paid to the immunoreactivity of p63 in the so-called *naked nuclei* (NN) observed in benign lesions.

RESULTS

Benign Lesions

The benign breast lesions assessed in this study included 11 fibroadenomas, 1 papillary lesion, and 6 lesions that were diagnosed *hypercellular for patient age*. The latter seven lesions were diagnosed histologically as one intraductal papilloma (Table 1, Patient 24) and six fibrocystic changes with ductal hyperplasia and adenosis (Table 1, Patients 25–30). Twelve patients were diagnosed with benign lesions due to the absence of clinical, radiologic, or cytologic criteria to diagnose malignancy (Table 1, Patients 1–12).

All FNABs of benign lesions showed a consistent distribution of p63 in the nuclei of myoepithelial cells, which were arranged in three distinctive patterns: 1) overlying epithelial sheets and clusters (Fig. 1A,B), 2) on the borders of epithelial sheets, and 3) isolated cells admixed with epithelial clusters. In all lesions that were diagnosed as fibroadenomas, bipolar NN consistently were decorated by p63 (Fig. 1C). In contrast, stromal cells and fat cells lacked p63 in all but one lesion. There was no immunoreactivity observed in stromal cells or endothelial cells in fibrovascular cores from papillary lesions (Fig. 1D). In 17 lesions, no background staining was observed; in 12 lesions, a faint background was found but by no means impaired the evaluation of the cytologic specimens. In one lesion, a strong background was found that partially impaired the evaluation of the sample. Table 1 summarizes the distribution of p63 in benign specimens.

Malignant Lesions

Forty-four histologically confirmed lesions were diagnosed either as ductal carcinoma in situ (DCIS) (8 lesions), invasive ductal carcinoma (IDC) of no special type (28 lesions), or special types of invasive carcinoma (8 lesions). Eight other lesions had a diagnosis of malignancy, and the patients were referred for adjuvant therapy at another hospital. Tables 1 and 2 summarize the clinical pathologic characteristics of the benign and malignant lesions evaluated herein.

TABLE 1
Summary of Clinical Pathologic Features of 30 Patients with Benign Results

Patient[a]	Gender	Age (yrs)	Cytologic diagnosis	P63+ overlying	P63+ naked nuclei	p63+ Bipolar cells
1	F	49	Benign	+	−	−
2	F	42	Benign	+	+	−
3	F	44	Benign	+	Rare	+
4	F	49	Benign	+	+	+
5	F	47	Benign	+	+	−
6	M	29	Benign	NA	NA	−
7	F	40	Benign	+	−	−
8	F	51	Benign	+	+	−
9	F	51	Benign	+	−	−
10	F	21	Benign	+	+	−
11	F	54	Benign	+	−	−
12	F	52	Benign	+	−	−
13	F	48	Fibroadenoma	+	+	+
14	F	26	Fibroadenoma	+	+	−
15	F	34	Fibroadenoma	+	+	+
16	F	44	Fibroadenoma	+	+	−
17	F	25	Fibroadenoma	+	+	−
18	F	53	Fibroadenoma	+	+	−
19	F	52	Fibroadenoma	+	+	−
20	F	29	Fibroadenoma	+	+	+
21	F	63	Fibroadenoma	+	+	−
22	F	35	Fibroadenoma	+	+	−
23	F	20	Fibroadenoma	+	+	−
24	F	18	Papillary lesion	+	Rare	−
25	F	52	Hypercellular for patient age	+	+	−
26	F	64	Hypercellular for patient age	+	+	−
27	F	53	Hypercellular for patient age	+	+	+
28	F	64	Hypercellular for patient age	+	+	−
29	F	36	Hypercellular for patient age	+	+	−
30	F	73	Hypercellular for patient age	+	+	−

−: negative; +: positive; P63+ overlying: p63 positive myoepithelial cells overlying malignant cell clusters; F: female; M: male; NA: not available.
[a] Patients 1–12 did not have histologic follow-up.

There were p63 positive myoepithelial cells overlying tridimensional, malignant cell clusters observed in all DCIS specimens (Fig. 2A). In 4 lesions, scattered, p63 positive, bipolar cells with ovoid-to-elongated nuclei and spindle-shaped cytoplasm also were observed.

Myoepithelial cells overlying malignant cell clusters were observed in 9 of 16 pure invasive carcinoma (IC) samples (Fig. 2B). However, in these samples, p63 positive myoepithelial cells were much less conspicuous compared with the cells observed in DCIS samples. In seven samples, scattered myoepithelial cells admixed with noncohesive, neoplastic cells were found. In six samples, no myoepithelial cells were observed.

In IC lesions that were associated with DCIS, p63 positive myoepithelial cells overlying malignant cell clusters were found in 16 specimens. In 10 specimens, scattered myoepithelial cells were observed in the background. It should be noted that, in these specimens, p63 positive cells were as abundant as they were in DCIS samples.

In no sample did p63 decorate cells with cytomorphologic features of stromal cells, adipocytes, endothelial cells, or nerve sheath cells. In 50 samples, no background staining was found. In two samples, a strong background that partially impaired evaluation was observed.

One unexpected finding was the presence of p63 positive malignant cells in 16 samples (3 of 8 samples of pure DCIS, 6 of 16 samples of pure IC, and 7 of 20 samples of IC associated with DCIS) (Fig. 2C,D). It should be emphasized, that in 9 samples, only a minority of neoplastic cells showed p63 staining (1–5% of neoplastic cells); in 6 samples, 5–15% of neoplastic cells showed p63 immunoreactivity; and, in 1 sample, the majority of the neoplastic cells were decorated by p63 (it is interesting to note that this patient had a metaplastic breast carcinoma comprised of high-

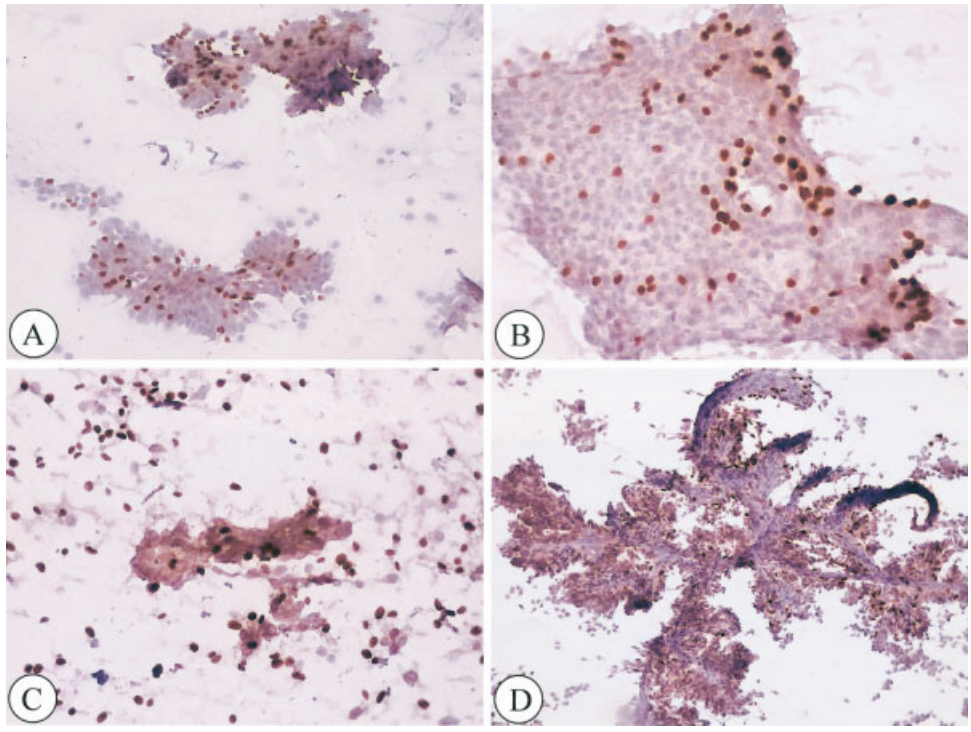

FIGURE 1. (A) Low-magnification photomicrograph showing p63 positive myoepithelial cells overlying epithelial clusters. (B) Medium magnification showing p63 positive myoepithelial cells overlying an epithelial cell sheet. (C) p63 Decorated myoepithelial cells and naked nuclei. (D) Papillary lesion showing p63 positive myoepithelial cells. Note the absence of p63 immunoreactivity in the nuclei of stromal cells of this papillary lesion (streptavidin-biotin-peroxidase/diaminobenzidine tetrahydrocloride; original magnification × 100 [A], × 200 [B, C], × 40 [D]).

grade spindle cells with foci of squamous differentiation) (Table 2, Patient 37).

Eight additional lesions that were diagnosed as carcinoma (Table 2, Patients 45–52) also were evaluated for p63 distribution. In these lesions, the cytologic criteria for malignancy were fulfilled completely. Two lesions showed p63 positive myoepithelial cells overlying malignant cell clusters, and two lesions showed scattered myoepithelial cells admixed with noncohesive, neoplastic cells. In three lesions, p63 immunoreactivity was observed in the nuclei of malignant cells (<5% of neoplastic cells).

DISCUSSION

In routine cytologic preparations, the precise identification of myoepithelial cells plays a major role in the diagnostic assessment of several types of breast lesions. These cells are a constituent of the normal basal layer of the breast lobules and ducts and usually are lost during malignant progression.[1,2,4–14,17–20] However, identification of myoepithelial cells in breast biopsies and FNAB specimens sometimes is difficult using Papanicolaou-stained or Giemsa-stained preparations.[1,4,12]

Based on their biphenotypic (epithelial and smooth muscle-like) properties,[21] several antibodies directed against myoepithelial cells have been raised. These target either smooth muscle-related antigens (α-smooth muscle actin, calponin, h-caldesmon, and smooth muscle myosin heavy chain)[4–6,8–12] or cytokeratins that are expressed specifically by basal/myoepithelial cells (cytokeratin 5/6, cytokeratin 14, and cytokeratins that are recognized by the antibody 34βE12).[17–20]

Currently, several investigations mitigate against using S-100 protein, because it has a high sensitivity but a very low specificity for myoepithelial cells.[4,5,11,14] Most of the smooth muscle-related antibodies, such as α-smooth muscle actin, calponin, h-caldesmon, and smooth muscle myosin heavy chain, lack specificity for myoepithelial cells, because they cross react with breast stromal cells and myofibroblasts[4,5,11,14] as well as with neoplastic cells.[11] Basal layer specific cytokeratins have a low sensitivity for myoepithelial cells,[17,19,20] mainly for those located in the lobules, and also stain a variable proportion of breast carcinomas.[17–21]

The recently characterized p53 homolog, p63, is expressed consistently in the basal cell population of several types of stratified epithelia.[13,14,22–24] The p63 gene is located on 3q27 and encodes at least six different isoforms, three with a transactivating (TA) N-terminal domain and three dominant negative (δN) isoforms that lack the N-terminal TA domain.[13,14,22–24] The δN-p63 isoforms may participate in an alternative mechanism to overcome p53-related cell cycle arrest and apoptosis, thus constituting an efficient mechanism to maintain a basal cell population.[13,14,22–24]

TABLE 2
Summary of the Clinical Pathologic Features of 52 Patients with Malignant Findings

			Cytologic findings			Histologic findings			
Patient	Gender	Age (yrs)	P63+ overlying	P63+ on the background	P63+ Neoplastic cells	Diagnosis	Nuclear grade (DCIS)	IDC type	IDC grade
1	F	49	+	−	−	DCIS	−	−	−
2	F	79	+	−	+	DCIS	H	−	−
3	F	47	+	+	−	DCIS	H	−	−
4	F	65	+	+	+	DCIS	H	−	−
5	F	80	+	−	−	DCIS	I	−	−
6	F	59	+	+	−	DCIS	I	−	−
7	F	63	+	−	+	DCIS	L	−	−
8	F	69	+	+	−	DCIS	L	−	−
9	F	53	+	−	−	DCIS + IDC	H	Ductal	I
10	F	64	+	−	−	DCIS + IDC	H	Ductal	III
11	F	64	+	−	+	DCIS + IDC	H	Ductal	I
12	F	51	+	+	−	DCIS + IDC	H	Ductal	II
13	F	68	+	+	−	DCIS + IDC	H	Ductal	III
14	F	53	+	+	−	DCIS + IDC	H	Ductal	II
15	F	48	+	+	+	DCIS + IDC	H	Ductal	III
16	F	41	+	+	+	DCIS + IDC	H	Ductal	I
17	F	68	+	+	++	DCIS + IDC	H	Ductal	III
18	F	50	+	+	++	DCIS + IDC	H	Ductal	II
19	F	76	−	−	−	DCIS + IDC	I	Ductal	II
20	F	60	−	−	−	DCIS + IDC	I	Ductal	II
21	F	62	+	−	−	DCIS + IDC	I	Ductal	I
22	M	67	−	+	−	DCIS + IDC	I	Ductal	II
23	F	71	+	+	−	DCIS + IDC	I	Ductal	I
24	F	63	+	+	+	DCIS + IDC	I	Ductal	I
25	F	74	−	−	−	IDC	−	Ductal	II
26	F	55	−	−	−	IDC	−	Ductal	III
27	M	59	−	−	−	IDC	−	Ductal	III
28	F	71	+	−	−	IDC	−	Ductal	I
29	F	67	+	−	−	IDC	−	Ductal	III
30	F	54	+	−	++	IDC	−	Ductal	III
31	F	44	−	+	++	IDC	−	Ductal	II
32	F	63	+	+	−	IDC	−	Ductal	I
33	F	49	+	+	−	IDC	−	Ductal	I
34	F	59	+	+	+	IDC	−	Ductal	I
35	F	51	+	+	+	IDC	−	Ductal	II
36	F	75	+	+	++	IDC	−	Ductal	III
37	F	82	−	−	++	IDC	−	MBC	III
38	F	48	−	−	−	IDC	−	MC	II
39	F	71	+	+	−	IDC	−	MC	II
40	F	68	−	−	−	DCIS + IDC	H	MC	II
41	F	54	+	−	++	DCIS + IDC	H	MC	II
42	F	40	+	+	−	DCIS + IDC	H	MC	III
43	F	80	−	−	−	IDC	−	Mucinous	I
44	F	62	−	−	−	DCIS + IDC	H	Mucinous	I
45	F	69	−	−	−	N.O.	N.O.	N.O.	N.O.
46	F	77	−	−	+	N.O.	N.O.	N.O.	N.O.
47	F	58	+	−	+	N.O.	N.O.	N.O.	N.O.
48	F	49	−	−	−	N.O.	N.O.	N.O.	N.O.
49	F	79	+	+	−	N.O.	N.O.	N.O.	N.O.
50	F	60	−	+	+	N.O.	N.O.	N.O.	N.O.
51	F	47	−	−	−	N.O.	N.O.	N.O.	N.O.
52	F	71	−	−	−	N.O.	N.O.	N.O.	N.O.

DCIS: ductal carcinoma in situ; H: high; I: intermediate; L: low; IDC: invasive ductal carcinoma; F: female; −: negative; +: positive; MBC: metaplastic breast carcinoma; MC: mixed carcinoma; N.O.: not observed; P63+ overlying: p63 positive myoepithelial cells overlying malignant cell clusters; P63+ on the background: p63 positive myoepithelial cells admixed with the neoplastic population; p63+ neoplastic cells: p63 positive neoplastic cells.

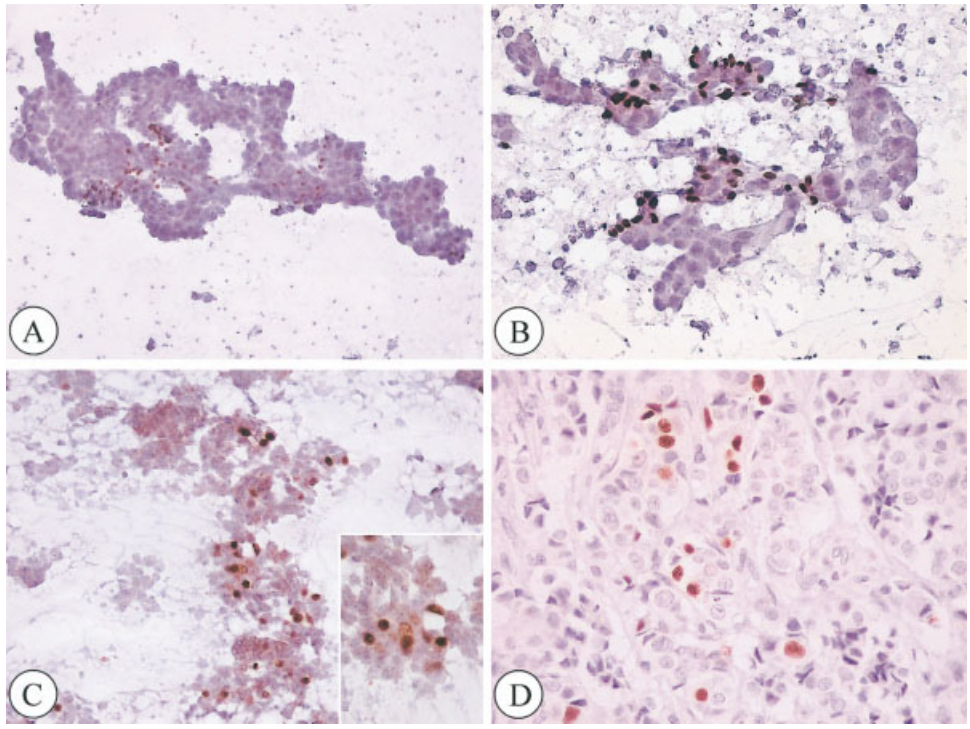

FIGURE 2. (A) Low-magnification view of a ductal carcinoma in situ with p63 positive myoepithelial cells overlying malignant cell clusters. (B) Medium magnification of an invasive ductal carcinoma with malignant cells arranged in duct-like structures and showing p63 positive overlying myoepithelial cells. (C) p63 Positive malignant cells. (Inset) Malignant cells forming lumen and showing p63 immunoreactivity. (D) Histologic section showing an invasive ductal carcinoma with scattered p63 positive nuclei (streptavidin-biotin-peroxidase/diaminobenzidine tetrahydrocloride; original magnification × 100 [A], × 200 [B, C], × 400 [inset, D]).

Along with 14 other authors,[13,25,26] we have demonstrated that p63 is expressed consistently in myoepithelial cells of the breast lobules and ducts as well as in the myoepithelial cells of sweat glands.[16]

In breast FNAB samples, p63 is expressed selectively by myoepithelial cells, for which it is a very reliable marker.[13] It is interesting to note that the consistent expression of p63 in NN of benign breast FNAB samples favors a myoepithelial origin for these cells.[13] NN have been described in fibroadenomas, Phyllodes tumor, fibrocystic change, pseudoangiomatous stromal hyperplasia, sclerosing lobular hyperplasia, benign papillary lesions, papillary adenoma of the nipple, gynecomastia, and other benign conditions.[4,13,27,28] In all of the fibroadenoma lesions analyzed herein, we observed p63 positive NN admixed with sheets of epithelial cells. Our results are in accordance with early reports[28–30] suggesting that NN have a myoepithelial origin and with the preliminary results of Barbareschi and colleagues.[13] Their designation suggests that NN do not have intact cytoplasm. Hence, we do not expect these nuclei to react with cytoplasmic markers of smooth muscle differentiation.[4,27] In the current study and in that of Barbareschi et al.,[13] most NN showed evidence of a myoepithelial origin.

The presence of myoepithelial cells overlying malignant cell clusters has been suggested as a very specific indicator of an in situ component.[1] Despite its high prevalence in DCIS samples and DCIS plus IC samples, p63 positive myoepithelial cells also were observed in 56.25% of pure IC samples. Thus, based on our findings, the presence of p63 positive myoepithelial cells should not be used as a specific criterion to rule out the presence of an invasive component.

Another important aspect of p63 immunocytochemistry is that, whereas some markers show strong background staining or even aberrant nuclear immunoreactivity in cytologic samples,[9] p63 showed strong immunoreactivity that was confined to the nuclei of myoepithelial cells. In only three lesions, the background was so high that it impaired evaluation of the cytologic preparation.

Most importantly, the major surprising and interesting finding of p63 immunoreactivity in cytologic samples is the presence of p63 positive malignant cells. In previous studies, Barbareschi et al.[13] and Kaufmann et al.[26] reported the presence of a variable proportion (≈5–15%) of p63 positive neoplastic cells in up to 4.6% and 11% of breast carcinomas, respectively. In the current study, we observed rare p63 positive malignant cells (1–5%) in 12 specimens (23%) and frequent positive cells (>5%) in 7 specimens (13%). It should be noted that, in all but one specimen, the majority of neoplastic cells were p63 negative, and a correct diagnosis of malignancy would obviously be achieved. The sample with diffuse p63 immunoreactivity was a metaplastic carcinoma. It is interesting to note that we have demonstrated,[31] along with oth-

ers,[13,32] that p63 is expressed consistently in up to 62.5% of metaplastic breast carcinoma samples, independent of their morphologic appearance. In addition, several lines of evidence support the finding that up to 18% of high-grade, invasive ductal carcinomas of the breast show myoepithelial-like or basal-like differentiation.[21,33,34] Thus, one possible explanation for the expression of p63 in malignant cells may reflect an aberrant or partial myoepithelial-like or basal-like differentiation.[13,14,21,33]

In conclusion, the current study demonstrated that 1) p63 is a reliable nuclear marker of myoepithelial cells in the breast and may be used to distinguish these cells from their mimics in FNABs; 2) benign lesions usually contained p63 positive myoepithelial cells, and we demonstrated it is a useful marker for highlighting these cells; and 3) malignant, p63 positive cells were observed frequently in samples of DCIS and IDC, although, based on careful cytomorphologic evaluation, they may have been classified correctly as malignant cells. Hence, based on previously published data and on our findings, we advocate that anti-p63 antibodies may be used to identify myoepithelial cells as well as to overcome the cytomorphologic distortion of myoepithelial cells in FNABs of the breast. However, by no means may the evaluation of p63 staining preclude a careful search for classic cytopathologic criteria to rule in or rule out a diagnosis of malignancy.

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