ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Invasive papillary carcinoma of the mammary gland: histopathologic and immunohistochemical aspects

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Abstract

Papillary lesions of the breast, both being and malignant, can prove to be a very challenging diagnosis in histological preparations. This study emphasizes on the importance of immunohistochemistry and in particular, the identification of myoepithelial cells for the correct evaluation of these lesions.

Keywords: papillary breast lesions, myoepithelial cells, immunohistochemistry.

Introduction

Breast cancer represents a very heterogeneous group of lesions with about 20 distinct subtypes. The evolution of mammary carcinomas depends on several factors such as histological subtype, tumor size, degree of invasion, the presence of metastasis. Also, the immunohistochemical profile has a role in the evolution, prognosis and treatment of breast cancer. Papillary carcinoma represents about 1–2% of all breast tumors. It is usually found in post-menopausal women and has a good prognosis. Clinically it is often asymptomatic and discovered at a routine examination or sometimes the patient may have bloody nipple discharge.

Papillary lesions of the mammary gland are characterized by the presence of a fibrovascular stalk and an epithelial proliferation. Lesions can be benign or malignant and the differential diagnosis can be difficult on HE staining. The staining of myoepithelial cells by immunohistochemistry using actin, p63 and CK5/6 can prove very useful.

Materials and Methods

The material consisted of nine cases of invasive papillary breast carcinoma, which were operated and treated at Emergency County Hospital of Craiova between 2005 and 2012.

Specimens were fixed in formalin and included in paraffin, and then serial paraffin sections were cut from tumor tissue at 5 µm and stained with Hematoxylin and Eosin (HE). These slides were examined in optical microscopy and the following parameters were studied: histological appearance of the lesion, size of nuclei and number of mitosis.

In the histological appearance, we identified the following features: papillary proliferation (single layered), florid hyperplasia, carcinoma *in situ*, myopithelial cell layer (classified into uniform, patchy – focal in 60% of cells, focal in 30%, partially absent, completely absent), metaplasia, fibrovascular core, the presence or absence of the epithelium lining the capsule, perilesional sclerosis, adjacent breast tissue and pre-existing lesions around the tumor (normal breast parenchyma, sclerosing adenosis, atypical ductal hyperplasia, carcinoma *in situ*, other injuries).

Immunohistochemical study

The paraffin sections were dewaxed and rehydrated, incubated, and the endogenous peroxidase was blocked with 3% hydrogen peroxide solution. The slides were stained using standard immunohistochemical methodology. For the study of myoepithelial cells, we used α-SMA, cytokeratins (CK5/CK6) and p63, and slides were interpreted as positive or negative. We also studied the hormonal profile of tumors with ER, PR as well as the HER2 status. The used antibodies, source and dilutions are presented in Table 1.

The most cellular areas of the tumor were identified

in each immunohistochemical staining and for hormone receptors the number of immunoreactive nuclei (%) of 100 malignant cells was calculated with a ×40 objective. Her-2/neu expression was evaluated as membrane staining of invasive tumor cells and scored to four classes (0/1+/2+/3+). For the other antibodies, just the presence or absence of antibodies was assessed. For all the tests, we had positive and negative controls and if required, they were repeated.

Table 1 – ***Antibodies used, source and dilutions***

Antibody	Source	Dilution
α-SMA	DAKO	1:100
P63	Santa Cruz	1:200
CK5/6	DAKO	1:50
ER	DAKO	1:50
PR	DAKO	1:50
HER2	DAKO	1:800

Results

We studied nine cases of invasive papillary carcinomas in women aged between 56 and 78 years, with a mean age of 64.11 years. There was no familial history of breast carcinoma in any of the cases.

Examination revealed tumors between 1.2 and 3.5 cm, firm, well-circumscribed. They were located in the subareolar region with concomitant retraction of the nipple, and in one case, there was clinical involvement of the axillary lymph nodes. Three cases presented ulceration. Surgical excision was the elected method of treatment for all our patients. Lymph node dissection was performed in one patient because of preoperatively known metastasis in axillary lymph node.

On cut sections, they showed a well-circumscribed, gray tumor, with pushing borders and solid features with an intracystic papillary component, which in three specimens occupied almost the whole breast. Areas of hemorrhage were detected in seven cases. The peritumoral area was fibrous in all cases.

Of the nine cases studied, three cases were intracystic breast carcinomas also known as encapsulated papillary carcinoma, with a malignant papillary proliferation involving a dilated duct, four cases were invasive papillary carcinoma and two cases were mixed invasive breast carcinoma (papillary and ductal). Surgical margins were free of tumor in all cases. Lymph node metastases were reported in one case.

Lesions that accompanied the invasive carcinomas were in two cases ductal carcinoma in situ carcinoma with comedocarcinoma pattern and in three cases atypical ductal hyperplasia and intraductal papilloma.

The benign papillomas were characterized by the presence of a continuous layer of myoepiothelial cells generally arranged in a single row and the presence of areas of epithelial hyperplasia. The fibrovascular stalk showed sclerosis in a case of sclerosing papilloma and in three cases (one of atypical hyperplasia and two cases of papillary carcinoma) we identified the presence of branching, giving a pseudocribriform appearance.

In cases of atypical hyperplasia, myoepithelial cells were present discontinuously in one case, and the fibrovascular stalks were thin, branched giving sometimes a solid pattern.

Microscopically, intracystic papillary carcinoma presented as a well-defined tumor, with edges with a fibrocollagen structure, partially lined by a single/several layers of epithelium with large nuclei and mitoses and intracystic papillary proliferation (Figure 1).

In the cases of invasive papillary carcinoma of solid type, the diagnosis was based on the invasion of the pseudocapsule and the presence of nests of tumor cells in the stroma and absence of the myoepithelial cells. (Figures 2 and 3). Papillary carcinoma was associated with atypical ductal hyperplasia and *in situ* and invasive ductal carcinoma.

Immunohistochemical study

The distinction between carcinoma and a benign papillary lesion was made by the absence of a myoepithelial layer.

The presence of myoepithelial cells was defined by the immunoreactivity for alpha SMA, p63 and CK5/6. In the cases of benign papillary lesions, the myoepithelial layer was continuous with only small gaps in some cases, as well as around normal ductal-lobular structures and around foci of conventional DCIS. α-Actin was strongly positive in the myoepithelial layer of benign injuries that accompanied the carcinomas. In all three cases α-SMA was intensely positive in myoepithelial cells, moderately positive in the stroma and negative in epithelial cells (Figures 4 and 5).

P63 was intensely positive in myoepithelial cells and absent in stroma and epithelial cells of benign papillary lesions (atypical hyperplasia, intraductal papilloma) (Figure 6).

Immunostaining for CK5/6 was scored as follows: present, focal, partially absent and absent. It was present in the basement membrane and cytoplasm in a continuous layer in papillomas, focally positive in ductal hyperplasia, partially absent in cases of atypical hyperplasia and negative in carcinomas (Figure 7). CK7 was positive in the area of invasive carcinoma (ductal and papillary) (Figure 8).

There was no positivity for any of the markers in myoepithelial cells at the periphery of the intracystic papillary breast carcinomas.

Hormonal receptors for estrogen and progesterone showed nuclear immunostaining for estrogen receptors in varying percentages in eight out of the nine cases studied and nuclear immunostaining for progesterone receptors in seven cases (Figures 9 and 10). Her2 showed 3+ immunostaining in the majority of cases (six cases), 2+ in two cases, and was negative in one case.

Discussion

Papillary lesions of the breast are a heterogeneous group, difficult to diagnose as benign or malignant. These lesions have a very varied morphological profile and a different prognosis. Papillary breast lesions include papillomas and malignant papillary lesions (micropapillary ductal carcinoma *in situ* – DCIS), non-invasive papillary carcinoma, invasive papillary carcinoma, and invasive micropapillary carcinoma). Invasive papillary

carcinoma is a rare form of invasive breast cancer. It was called papillary from the word "nipple-like". It generally affects older women in the post-menopausal period, the average age being around 60 years.

Papillary carcinoma is discovered on physical examination, when the tumor is around 2–3 cm or when bloody nipple discharge occurs, accompanied by redness, ulceration or nipple retraction. It can also be asymptomatic and only identified during screening ultrasound or mammographic examination [1]. In our study, all patients had an abnormal mammogram and/or ultrasound.

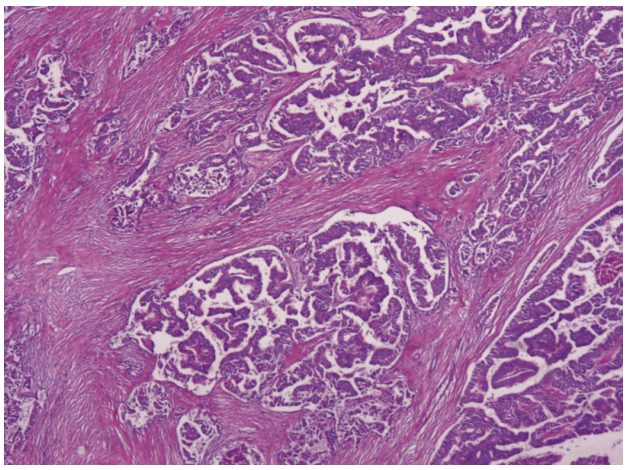

Figure 1 – ***Papillary invasive carcinoma with a well-circumscribed border and surrounding sclerosis (HE stain, ×100).***

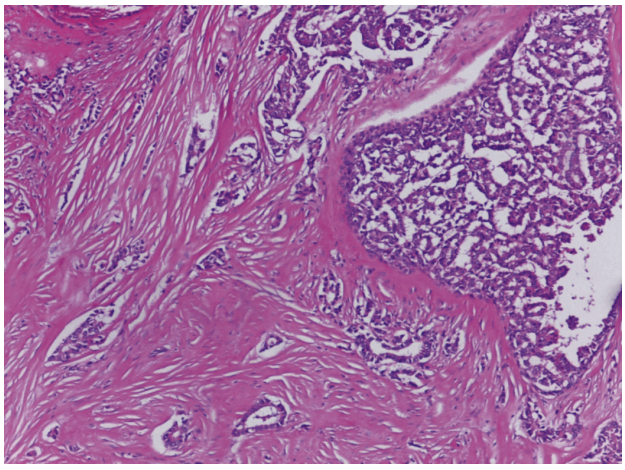

Figure 2 – ***Papillary invasive carcinoma and invasive ductal carcinoma (HE stain, ×200).***

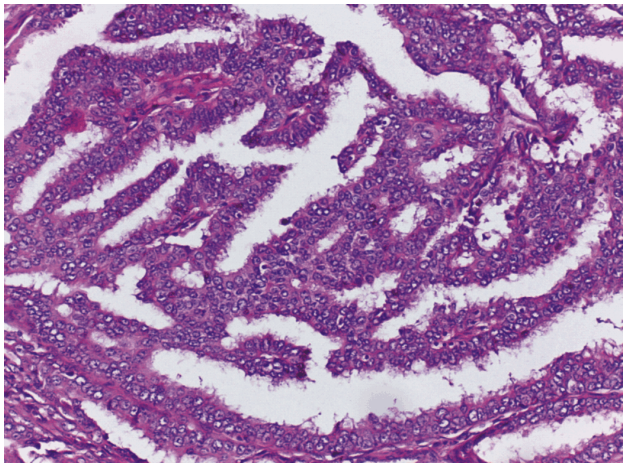

Figure 3 – ***High-power view of papillary invasive carcinoma that demonstrates classic papillary morphologic features and cytologic atypia (HE stain, ×200).***

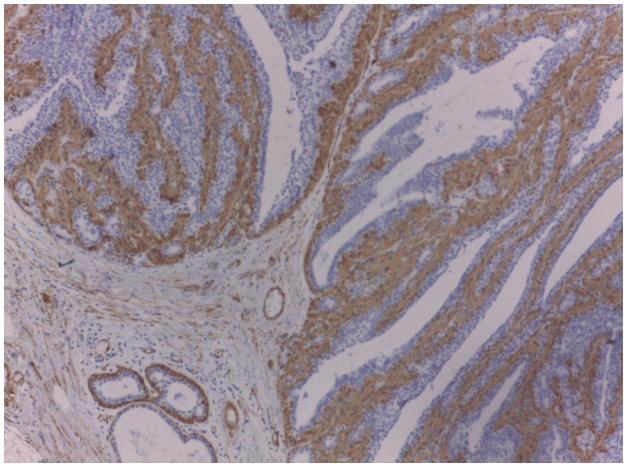

Figure 4 – ***An intracystic, benign papilloma: α-actin completely surrounds the perimeter of the papilloma and additionally highlights its fibrovascular cores, highlighting that smooth-muscle actin staining of the stromal cells (SMA immunostaining, ×100).***

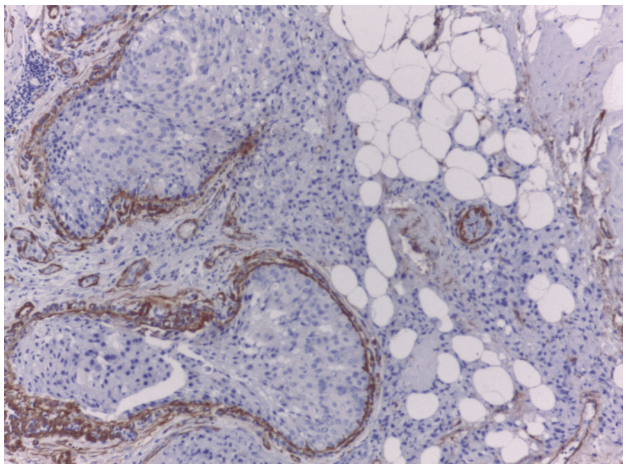

Figure 5 – ***SMA immunostaining, ×100.***

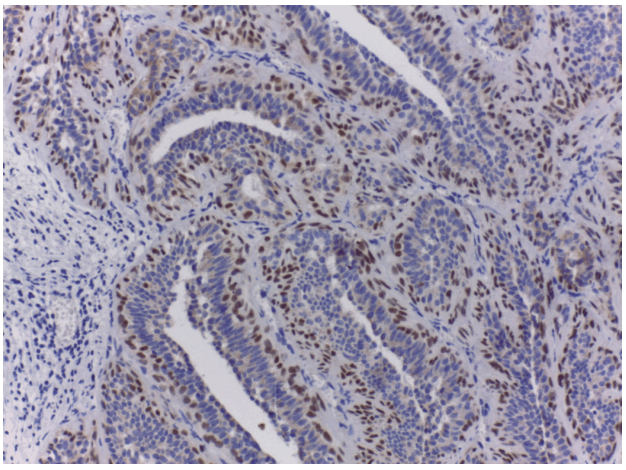

Figure 6 – ***P63 staining highlighting abundant myo-epithelial cells (immunostaining, ×200).***

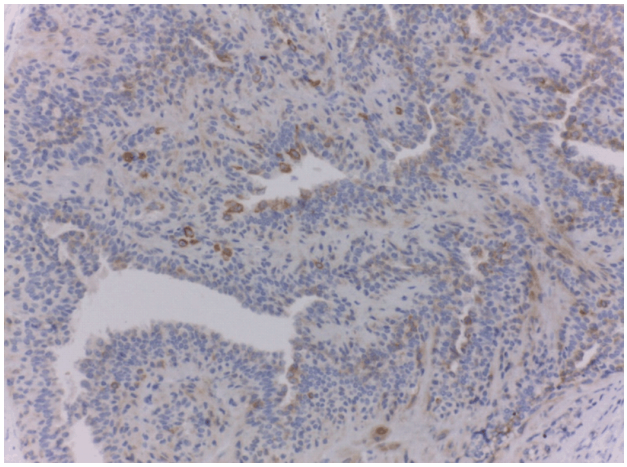

Figure 7 – ***The area with atypical histological features shows loss of epithelial CK5/6 staining compared with the non-atypical (immunostaining, ×200).***

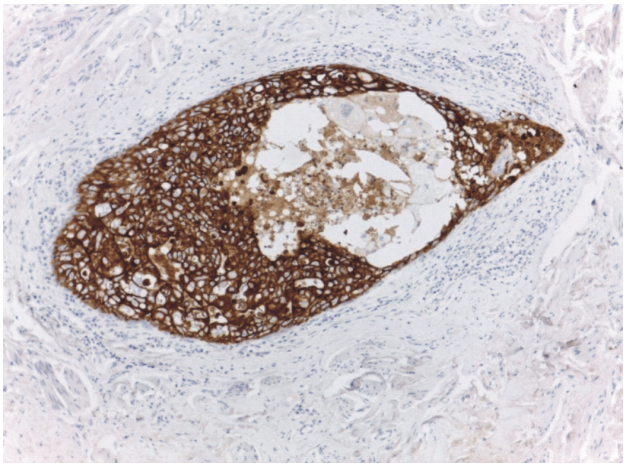

Figure 8 – ***Invasive ductal carcinoma (CK7 immunostaining, ×100).***

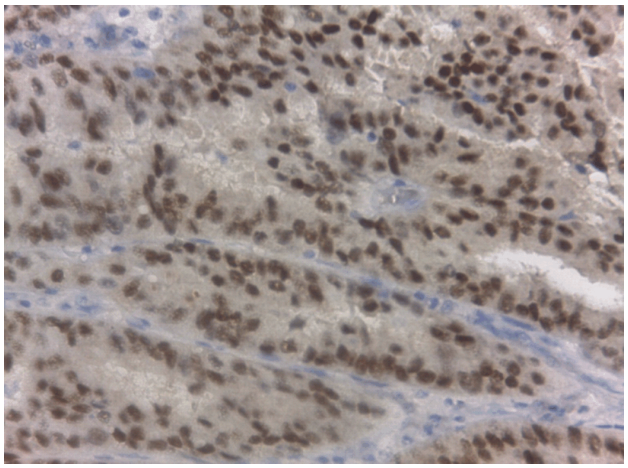

Figure 9 – ***Papillary invasive carcinomas were positive for estrogen receptor (immunostaining, ×400).***

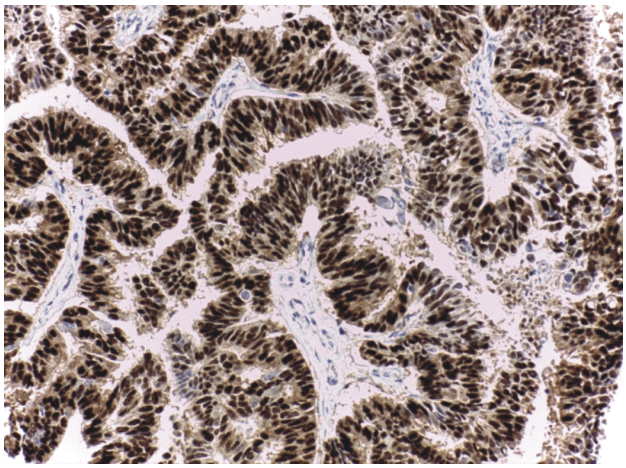

Figure 10 – ***Papillary invasive carcinomas were positive for progesterone receptor (immunostaining, ×200).***

The tumor is located beyond the nipple in over 50% of cases, but in our study, all cases had this location. On cut section, it is often cystic with solid areas, as well as areas with a mixture of blood and cancer cells.

Microscopically the tumor has well-defined borders and thin, finger-like papillary projections, which can sometimes form solid areas. Tumor cells have eosinophilic cytoplasm, roundish nucleus with moderate pleomorphism and frequent mitosis. Myoepithelial cells are absent. The rate of metastasis is low and the prognosis is good [2].

Intracystic papillary carcinomas of the breast have traditionally been, and still are according to some authors, considered as *in situ* lesions (DCIS) characterized by the presence of a duct with fibrovascular stalks lined by epithelial proliferation, despite the absence of myoepithelial cells [3].

We did not identify the presence of myoepithelial cells at the periphery of these lesions, data consistent with the study of Hill CB and Yeh IT in which more than half of the lesions classified histologically as intracystic/intraductal papillary carcinoma showed no positivity to calponin, SMMHC, and p63 in the periphery [4].

The differential diagnosis between benign and *in situ* or invasive malignant lesions can be challenging on standard HE examination. Thus, it is very important to evaluate the myoepithelial markers. The distinction of invasive papillary carcinoma from non-invasive forms is critical, but there is still confusion in what concerns distinguishing between *in situ* or invasive intracystic papillary carcinoma. Grabowski J *et al.*, after reviewing a large number of cases over several years, concluded that the differentiation is of no clinical significance because, regardless of the classification, the prognosis of situ or invasive intracystic papillary carcinoma is excellent [5].

The assessment of invasion was studied with the use of immunohistochemistry. The antibody panel we used in our study to determine the presence or absence of the myoepithelial cells was SMA, p63 and CK5/6. According to prior studies, malignant papillary proliferations of the breast lack the myoepithelial cell layer within the papillae, unlike benign intraductal papillomas [4].

Alpha-actin was present in myoepithelial cells in all benign lesions and in three cases there was a moderate positivity in myofibroblasts from the stroma. Some authors believe that actin does not prove to be a marker of choice, because of these cross-reactions with myofibroblasts from the stroma and pericytes [6].

P63 is a nuclear marker that stains not only myoepithelial cells but also epithelial cells of the skin,

cervix, basal cells of the mammary gland and prostate. Unlike actin, there is no cross reaction [7]. The positivity for p63 of myoepithelial cells was strong, but some authors recommend using p63 only together with another marker, because the nuclear staining pattern is too discontinuous to be reliable [4].

From the immunohistochemical point of view, papillary lesions are characterized by discontinuity of the rows of myoepithelial cells. SMA immunostaining is patchy and there is only focal staining for CK5/6. These immunostainings differentiate a benign lesion from a malignant one, but cannot differentiate an atypical from a malignant lesion [8].

The study of Grin A *et al.* showed that typical and atypical lesions could be differentiated by studying the expression of CK5 and ER: typical lesions show an ER low and CK5 rich profile, while atypical ones showed an ER rich and CK5 rich profile. Strong expression of ER is defined as a strong diffuse stain in a high percentage of cells and CK5 expression is defined as rich in the presence a mosaic pattern of staining in over 20% of cells and weak when staining was absent or below 20% of cells [9]. The genomic profiles of papillary carcinomas positioned them as part of the spectrum of ER-positive breast cancers, rather than as a distinct entity [10].

The excellent prognosis of papillary carcinoma guides the treatment, generally mastectomy or segmental resection, followed by axillary lymph node dissection or sentinel node biopsy in patients with invasive cancers. Despite the fact that one of the cases presented underwent mastectomy with axillary node dissection and the presence of nodal metastasis was identified, axillary nodal metastases are infrequent [11].

Conclusions

Correct diagnosis of papillary lesions remains a challenge when using standard HE staining. Identification of myoepithelial cells by immunohistochemistry and differentiation between benign and malignant lesions is a useful diagnostic tool with implication on management.

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Received: July 20th, 2012

Accepted: November 17th, 2012