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Original Article

Clinicopathologic features of intraductal papillary neoplasm of breast: analyses of three cases

Yan Zhao[1*], Nan Li[1*], Xiaomeng Gong[1], Lan Yu[1], Xin Jin[2]

[1]Department of Pathology, The First Affiliated Hospital of Bengbu Medical College, Bengbu Medical College, Bengbu, Anhui, P. R. China; [2]Department of Surgical Oncology, The First Affiliated Hospital of Bengbu Medical College, Bengbu, Anhui, P. R. China. [*]Equal contributors.

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Abstract: Intraductal papillary neoplasm of breast (IDPN) belongs to a pathological heterogeneous group of diseases, which spans the spectrum of benign, atypical, and malignant. It constitutes less than 10% of benign breast lesions and less than 1% of malignant breast cancers. The majority of IDPNs begins within the ductolobular system of the breast and shows cystic structure with intracystic finger-like projection containing fibrovascular cores. The reasons that made the diagnosis of IDPNs difficult were the proliferation of breast epithelium and the emergence of some illusions. We systematically reviewed 47 cases of breast IDPNs, including 19 cases of intraductal papilloma (IDP), 2 cases of intraductal papilloma with atypical ductal hyperplasia (IDP with ADH), 4 cases of intraductal papilloma with ductual carcinoma in situ (IDP with DCIS), 22 cases of intraductal papillary carcinoma (IDPC), and underwent p63, CD10, SMA, calponin, CK5/6, ER immunohistochemistry Envision staining analysis. This study was focused on three cases which were easy to misdiagnosis and combined the WHO classification to sort out the pathological changes, arousing attention in daily pathological diagnosis.

Keywords: Breast, intraductal papillary neoplasms, diagnosis, immunohistochemistry

Introduction

In the most recent (4th) edition of the WHO classification of breast tumor in 2012, Breast IDPNs are classified as intraductal papillomas (intraductal papilloma with atypical hyperplasia, intraductal papilloma with ductual carcinoma in situ, intraductal papilloma with lobular carcinoma in situ), intraductal papillary carcinomas [1]. In clinical, Breast IDPNs may appear as palpable masses, bloody or bloodless nipple discharges or densities seen on mammography. They would cause bloody nipple discharge as a result of the rotation of its stalk or bloodless nipple discharge as a result of irritating papilloma duct [2]. Histologically, Breast IDPNs consist of fibrovascular center, myoepitelial layer and outer cuboidal or columnar epithelium. The analysis of papillary tumors begins with the evaluation of three fundamental features: the geometrical characteristics of the fronds, the amount and quality of the stroma, and the cellular characteristics of the epithelium [3]. Superimposed pathologic processes such as scarring and epithelial proliferation can obscure the appearance of these fundamental features and thereby complicate the analysis [4]. Sometimes it was challenging of distinction between benign, premalignant, and malignant components of IDPNs, and the architectural variety accompanying them attributes to this difficulty [5]. Among the histopathological criteria for differentiating benign from malignant IDPNs, the most emphasized histological hallmark of benignity is the preservation of myoepithelial cells along the epithelial-stromal interface of the papillary fronds [6]. But sometimes myoepithelial cells are not readily discernible on hematoxylin and eosin (HE) stained sections. At this time immunostains are very helpful in identifying a neoplastic process within a papillary lesion of the breast [7]. We performed histological observation on 47 cases of breast IDPNs and studied by immunohistochemical method, which focused on 3 cases of IDPNs that would misdiagnose easily. This study was

Table 1. Sources of the antibodies used in the immunohistochemistry analysis

Source	Antibody
p63	Monoclonal, clone 4A4
CD10	Monoclonal, clone 56C6
SMA	Monoclonal, clone 1A4
calponin	Monoclonal, clone CALP
CK5/6	Monoclonal, clone D5/16B4
ER	Monoclonal, clone SP1

All antibodies were obtained from Maixin Biotech, Inc. (Fuzhou, China), and were ready to use.

to investigate the morphological features and the significance of the application of immune markers of IDPNs.

Materials and methods

We collected 47 cases of breast IDPNs in the First Affiliated Hospital of Bengbu Medical College from January 2010 to July 2015 including 19 cases of IDP, 2 cases of IDP with ADH, 4 cases of IDP with DCIS, 22 cases of IDPC. HE-stained sections (4 um thickness) were re-examined to evaluate the tumor's histological features and immunohistochemistry was performed with Elivision technique. Antibody details are given in **Table 1**. Clinical demographics and follow-up data were obtained from medical records and referring physicians.

This study was approved by the Ethics Committees of the First Affiliated Hospital of Bengbu Medical College and was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Results

Case presentation

Case 1: A 49-year-old woman experienced bloody discharge from the right nipple. A small amount of bloody fluid appeared when pressure was exerted on the nipple. The sonographic examination revealed a solid mass measuring 2.5 cm (long axis) within a dilated duct inside the anterosuperior quadrant. Duct-lobular segmentectomy was performed to determine the exact diagnosis of the lesion and to resolve the symptoms.

Histologically, the lesion was multiple and basically intraductal with papillary structures. Epithelial cells lined the fibrovascular cores. The tissue around the duct was fibrosis and dense (**Figure 1A**). It can be seen that the irregular and even distorted glands were scattered. The stroma was sclerotic with elastofibrosis. There were eosinophilic, thick fibrous bundles surrounding each proliferating gland (**Figure 1B**). Immunohistochemically, myoepithelial markers (CD10, calponin, SMA, p63) were continuously positive along the fibrovascular stroma and at the outermost part of papillary lesion (**Figure 1C**). CK5/6 was widely positive for the epithelium of any area (**Figure 1D**). The lesion was diagnosed as intraductal papilloma with benign sclerosing stroma.

Case 2: The patient was a 54-year-old perimenopasual woman. One year ago, she had left breast mass resection in other hospital, postoperative pathology showed benign lesions. 7 days ago, the patient found a mass in the original incision; the tissue was 2.0 cm × 1.0 cm × 1.0 cm, slightly hard, no tenderness. Lumpectomy (wide excision) of the tumor was performed. Pathology findings revealed an intracystic papillary lesion. Epithelial cells lined the fibrovascular cores had two distinct morphological characteristics (**Figure 2A**). One kind cells located in the superficial layer of the papillary structure. The cytoplasm was opaque, the staining was deep, the nucleus was low or intermediate. The second kind of cells was round or polygonal, located in the inner layer of the papillary structure. Single cell like Paget spread or a small cluster or nest shaped. The cytoplasm was clear and nucleoplasm ratio increased. The clear cells had nuclei with irregular borders and differed from the superficial cells in their rounded borders and clear cytoplasm (**Figure 2B**). Using immunohistochemistry, the clear cytoplasm of basally situated cells were negative for P63, CD10, calponin and SMA. To prove that these cells were not myoepithelial cells (**Figure 2C**). CK5/6 was widely negative for the epithelium cells of any area (**Figure 2D**). The diagnosis was breast intraductal dimorphic papillary carcinoma.

Case 3: A 57-year-old woman experienced a breast lump on the outer upper quadrant of her right breast. Radiological examinations revealed a widely distributed intraductal lesion. FNAC revealed abundant epithelial cells with solid and sieve-like structure. Lumpectomy of the tumor was performed. Histologically, the

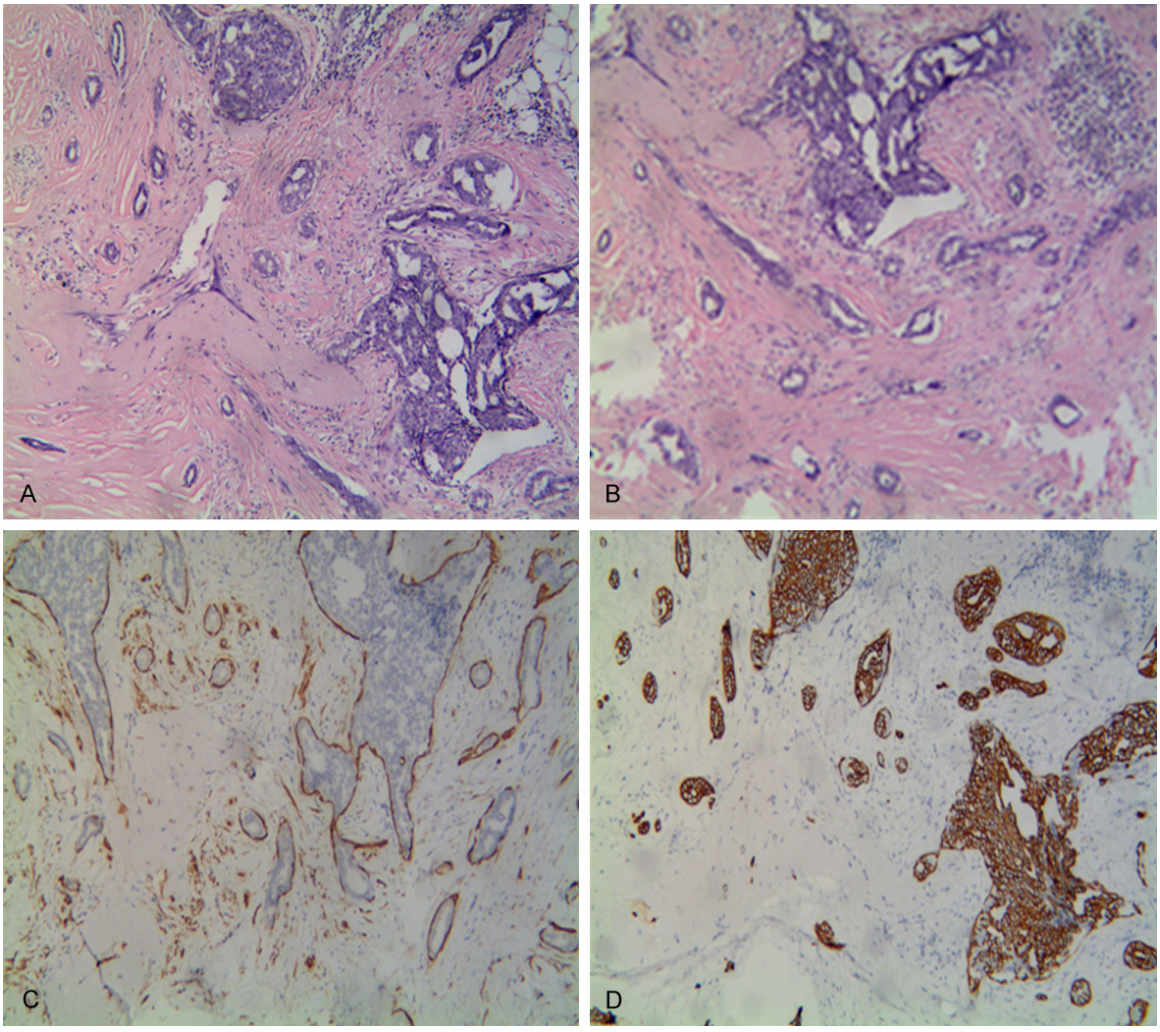

Figure 1. Histological and immunohistochemical features of the tumor from the patient described in case 1. A: Fibrosis at the edge of papillomas can entrap benign glands. (magnification, × 100). B: Entrapped glands often flow circumferentially and in parallel with the collagen bundles. (magnification, × 100). C: Myoepithelial cells positive for SMA. (magnification, × 100). D: Epithelial cells positive for CK5/6 protein. (magnification, × 100).

lesion was basically intraductal, with solid and papillary structures (**Figure 3A**). Proliferation cells were solid and most of the nuclei were low level, also can be seen high-level nuclear and small focal necrosis. The structure and cytological characteristics of proliferative epithelium in some regions reached the DCIS standard (**Figure 3B**). But we can also see the area of benign IDP which consisted of just a few broad blunt fronds and fitted together well. The solid epithelial population measured ≥3 mm within the IDP. Immunohistochemically, myoepithelial cells (detected by CD10, SMA, calponin and p63) were absent or missing at the area of solid and sieve-like structure (**Figure 3C**), CK5/6 was negative too (**Figure 3D**). In the region of benign IDP, myoepithelial cells markers were positive. The lesion was diagnosed as breast intraductal papilloma with ductual carcinoma in situ (IDP with DCIS).

To investigate the morphologic and immunophenotypic characteristics of breast IDPNs and explore the diagnosis criteria, we collected 47 cases of breast IDPNs and observed with HE stains and studied by immunohistochemical method. (1) 19 cases of IDP were confirmed as principal disease. They were characterized by a papillary structure composed of fibrovascular stalks covered by a layer of myoepithelial cells with overlying glandular epithelial cells within a distended duct or ductule. Among them, 11

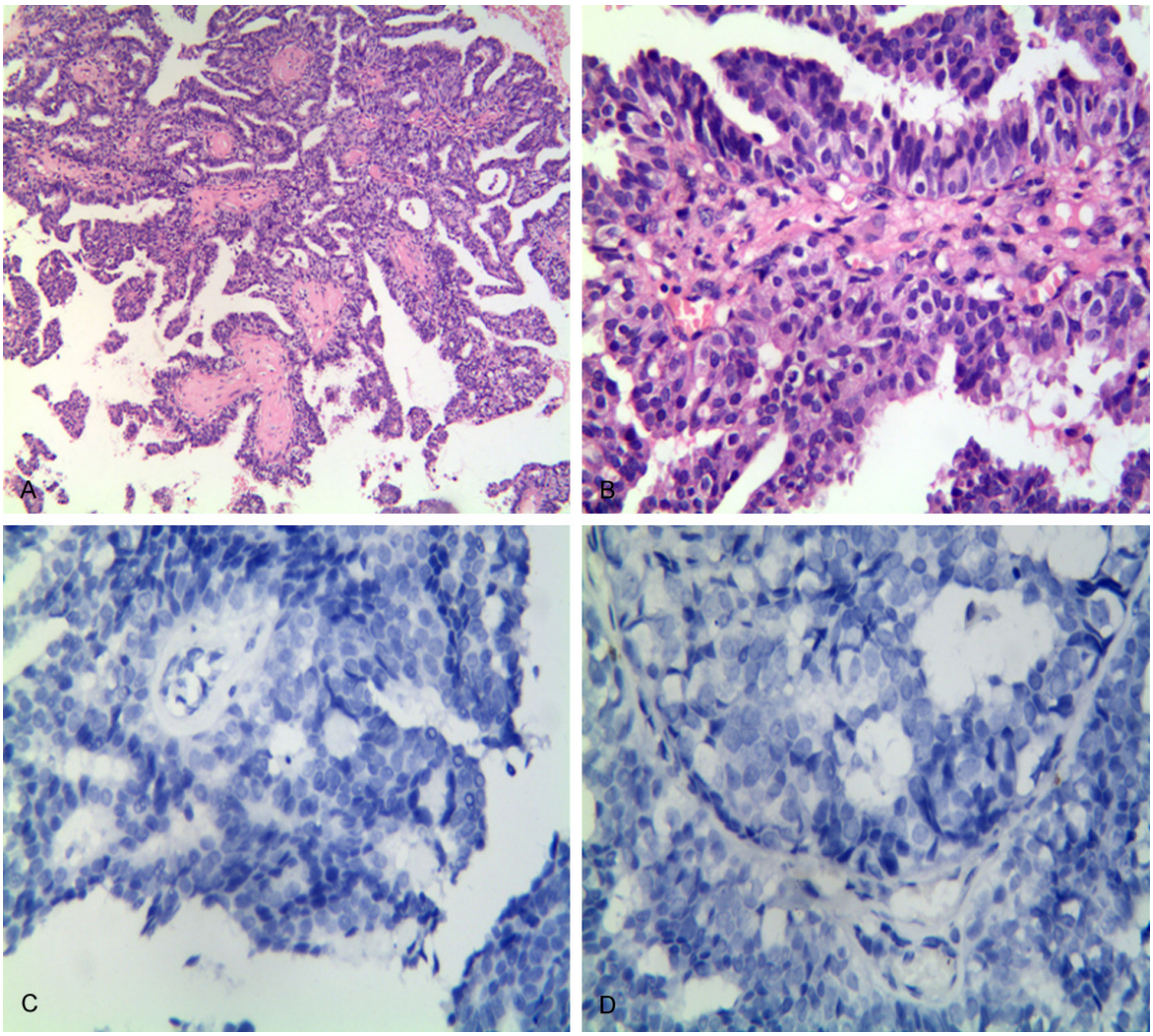

Figure 2. Histological and immunohistochemical features of the tumor from the patient described in case 2. A: We call the cells as "dimorphic cells" when the cytoplasm of basally situated carcinoma cells becomes unusually clear. (magnification, × 100). B: Dimorphic carcinoma cells with clear cytoplasm were similar to myoepithelial cells. (magnification, × 400). C: Dimorphic carcinoma cells negative for p63 protein. (magnification, × 400). D: Epithelial cells negative for CK5/6. (magnification, × 400).

cases were single lesions, 8 cases were multiple lesions. 3 of the 19 cases displayed usual epithelial hyperplasia, and 4 cases were associated with apocrine hyperplasia. 4 of the 19 cases were confirmed as concomitant disease (1 case with adenosis, 2 cases with fibrous adenoma, 1 case with UDH). Epithelial cells of all cases were moderate to strong positive of CK5/6 and myoepithelial cells were positive of p63, SMA, calponin and CD10. Epithelial cells were heterogeneous positive of ER. (2) There were 2 cases of IDP with ADH. They were characterized by a low nuclear grade atypical epithelial proliferation measuring <3 mm within an IDP. There were 4 cases of IDP with DCIS. They were characterized by similar cytoarchitecturally abnormal epithelial population measuring ≥3 mm within an IDP. The epithelial cells with atypical hyperplasia were negative of CK5/6 and myoepithelial cells markers with atypical hyperplasia were missing. (3) 22 cases of IDPC were confirmed. They had three kinds of microscopic morphology. 6 cases were characterized by clearly branched papillary structure with slender fibers axis, covered with high columnar cells, deep nuclear staining, atypia was mild or mild to moderate, no obvious myoepithelial cells around. 11 cases were characterized by sieve-like structure, forming a round and consistent sieve, the nucleus level was low, like

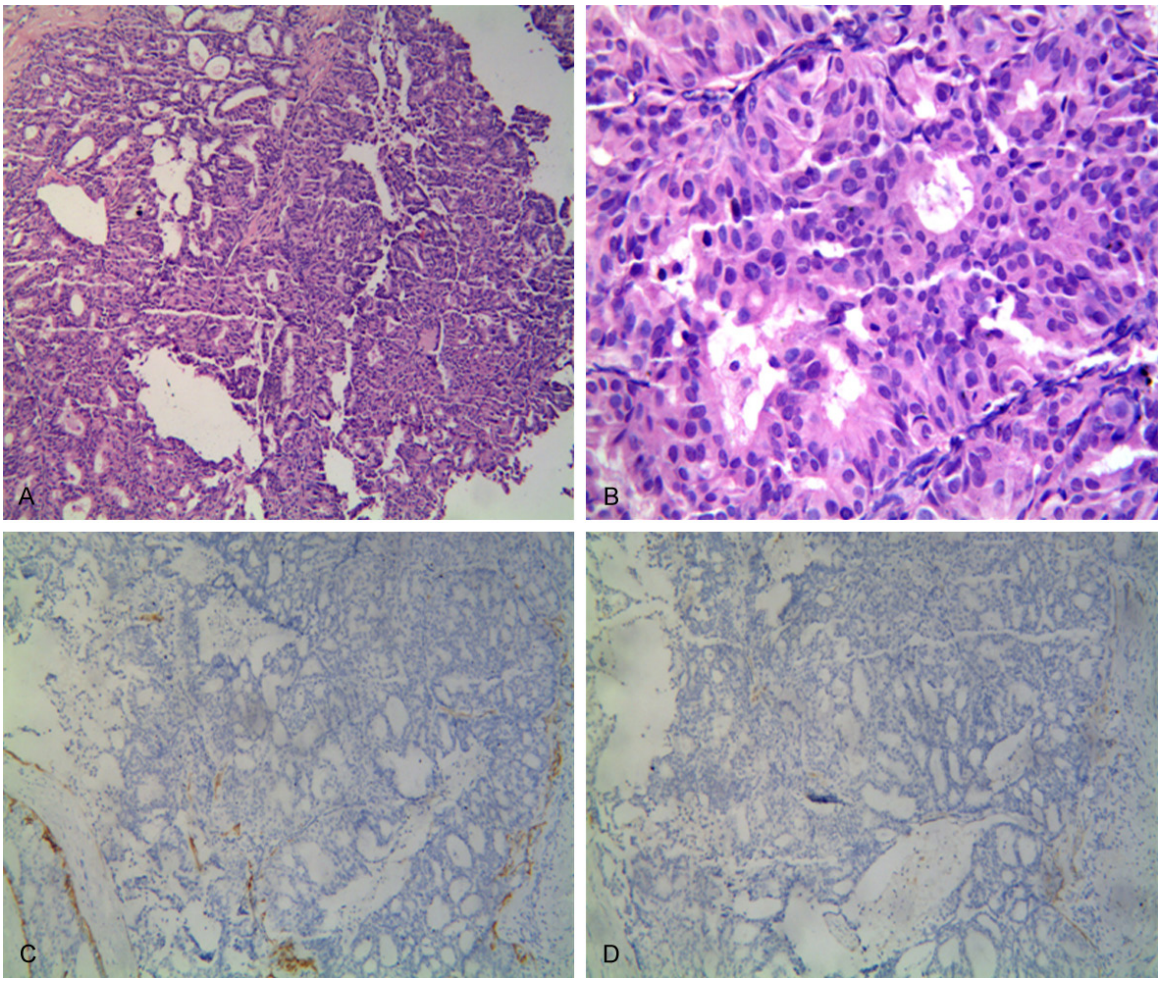

Figure 3. Histological and immunohistochemical features of the tumor from the patient described in case 3. A: IDP with an atypical epithelial proliferation measuring ≥3 mm in maximum dimension, fulfilling the criteria for low nuclear grade DCIS within an IDP. (magnification, × 100). B: Proliferation cells were solid and sieve-like structure, most of the nuclei were low level. (magnification, × 400). C: Myoepithelial cells negative for p63 protein. (magnification, × 100). D: Immunohistochemistry for CK5/6 showed negative staining among the atypical epithelial cell population. (magnification, × 100).

low-grade intraductal carcinoma, but the slender fiber axis still can be visible seen, myoepithelial cells significantly reduced or lacked. The microscopic morphology of the remaining 5 cases were the mixture of the above two features. In IDPCs, CK5/6 was negative in all and myoepithelial cells markers were negative too. ER was uniform and strong positive. It is noteworthy that the myoepithelial cells markers were typically absent in the fronds but retained at the duct periphery.

Discussion

Commonplace breast IDPNs do not cause diagnostic problems for experienced pathologists. But the superimposition of secondary processes can create confusing patterns. In the first case, the fibrosis around papillomas was obvious and the scarring can entrap and distort neighboring glands. Observers were unfamiliar with this phenomenon and misinterpret this entrapment as invasion, hence misdiagnose a papilloma as a papillary carcinoma [8]. To avoid this misinterpretation, the diagnosis of carcinoma must rest on the cytological and architectural characteristics of the papillary tumor itself. Only when the papillary tumor demonstrates the criteria for malignancy can we consider whether there are irregular epithelial clusters that reflect entrapment or invasion [9]. Several features help to differentiate entrap-

ment of epithelial clusters from invasive nests [10]. (1) The morphology of false infiltrating glandular was similar to IDP. Entrapped epithelium consists of benign cells arranged irregularly but smoothly contoured clusters. (2) The stroma of false infiltration area was almost collagen and hyaline degeneration, rare fibroblast proliferation would be seen. The glands maintain an orderly relationship with the bundles of collagen. (3) Entrapped benign glands consist of both glandular epithelium cells and myoepithelial cells. The immunohistochemistry detection of myoepithelial cells provides especially important evidence to establish the benign nature of glandular clusters.

In the second case, the presence of basal carcinoma cells with clear cytoplasm and the formation of short stubby fronds sometimes cause misdiagnose papillary carcinomas as papillomas. These clear cytoplasm cells usually form a layer just 1 or 2 cells thick, but sometimes they can become the dominant population. Lefkowitz et al referred to these cells as dimorphic tumor cells and tumors containing these cells as dimorphic papillary carcinomas [11]. To prevent these errors, we should observe the characteristics of the two kinds of epithelial cells carefully. The quality of the chromatin and the characteristics of the nucleoli of the two kinds of cells appear identical. Furthermore, the dimorphic cells look somewhat more cohesive. The negative result of immunohistochemical staining for myoepithelial proteins can help us in diagnosis [12].

Breast papillomas with areas of monotonous epithelial proliferation resembling DCIS or ADH remain a source of common diagnostic problems [13]. Reproducible classification of such borderline papillary lesions are important because their natural history and the optimum treatment have yet to be elucidated [14]. The term ‘atypical papilloma’ is avoided in the 4th edition. The 2012 WHO Working Group recommends rely on size as a criterion, with 3 mm being the cutoff. A low nuclear grade atypical epithelial proliferation measuring <3 mm within an IDP is diagnosed as ADH, whereas a similar cytoarchitecturally abnormal epithelial population measuring ≥3 mm is regarded as DCIS within an IDP. When the abnormal epithelial proliferation shows intermediate or high nuclear grade, DCIS should be diagnosed regardless of extent. The 4th edition also makes a distinction between papilloma with DCIS and papillary DCIS/intraductal carcinoma [1]. Papillary DCIS is considered to be a de-novo in-situ malignant papillary process without a morphologically recognizable benign papilloma in its background. In contrast, papilloma with DCIS shows an underlying, identifiable benign papilloma upon which the abnormal epithelial proliferation is engrafted. Although the paucity or absence of myoepithelial cells is a recognized criterion for IDPC, the presence of myoepithelial cells does not negate its diagnosis if other features are characteristic, such as a monotonous epithelial cell population, intermediate or high nuclear grade features, and slender fibrovascular cores within the malignant intraductal papillary process [15]. Myoepithelial cells, whether observed on light microscopy or detected with immunohistochemistry are very important in diagnosis of breast IDPNs.

The WHO Working Group recommends using a panel of two to three antibodies to demonstrate myoepithelial cells on immunohistochemistry, such as p63, SMA, CD10, calponin [16]. Among various myoepithelial markers, we selected p63 because it is a sensitive and relatively specific marker for myoepithelial cell nuclei. Importantly, it is not expressed in stromal cells including myofibroblasts and pericytes, circumventing the diagnostic pitfalls associated with smooth muscle-related myoepithelial markers such as SMA and calponin [17].

Although high-molecular weight cytokeratin (CK5/6) is also known as a myoepithelial marker, we used it to distinguish the UDH from low-grade DCIS [18]. The UDH is characterized by a heterogeneous and intense immunoreaction for CK5/6, whereas monotonous intraductal proliferations resembling DCIS or ADH in IDPC were typically negative for CK5/6. These results indicate that CK5/6 may provide the key information in the differential diagnosis of IDPNs with solid or quasi-solid epithelial proliferation. A combination of immunohistochemistry for CK5/6 and myoepithelial markers is useful in assessing difficult papillary lesions of the breast [19]. Nevertheless, the morphology is more important than the immunostaining pattern, and diagnosis of neoplastic proliferation should not be made on the immunostaining pattern alone. Furthermore, the immunostains must be used in the correct context; otherwise

the implications of the staining pattern can cause an incorrect classification of the lesion [20].

In conclusion, Intraductal papillary neoplasm of the breast is a heterogeneous group that can usually be distinguished via careful histologic evaluation. Immunohistochemical staining, clinical features and X-ray are useful in the diagnosis and differential diagnosis.

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Disclosure of conflict of interest

None.

Address correspondence to: Xin Jin, Department of Surgical Oncology, The First Affiliated Hospital of Bengbu Medical College, 287 Changhuai Road, Bengbu 233004, Anhui, P. R. China. Tel: +86-552-3086189; E-mail: 13865031688@163.com

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