Use of Immunohistochemical Stains in Epithelial Lesions of the Breast

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Background: During the last few decades, immunohistochemistry (IHC) has become an integral part of pathology. Although hematoxylin and eosin (H & E) stain remains the fundamental basis for diagnostic pathology of the breast, IHC stains provide useful and sometimes vital information. Moreover, considering the role of hormonal therapy in hormone receptor–positive breast tumors, as well as the availability of targeted chemotherapeutic agents for HER2-positive cases, IHC studies represent a major part of workups.

Methods: A literature search was performed to explore the uses of IHC stains related to the diagnoses of breast lesions and prognostic/predictive information.

Results: Selective use of IHC stains in conjunction with H & E examination helps resolve most diagnostic issues encountered by surgical pathologists during their day-to-day practice. Pathologists should be familiar with the use of each immunostain and its limitations to avoid interpretative errors.

Conclusions: IHC stains help guide the differential diagnosis of challenging epithelial lesions of the breast. They should be selectively and judiciously used and their findings must be interpreted with the differential diagnoses in mind and with an understanding of possible pitfalls.

Introduction

In the majority of cases, diagnoses of benign and malignant epithelial lesions of the breast are achievable using hematoxylin and eosin (H & E) microscopic sections alone. However, in some circumstances, such as in atypical and borderline breast lesions, as well as in core needle biopsy (CNB), this morphological distinction can be problematic. In addition, interobserver variability exists among pathologists when interpreting difficult and borderline lesions of the breast.\textsuperscript{1,2}

Therefore, using immunohistochemical (IHC) stains can be of help when dealing with these challenging lesions, particularly in cases for which the diagnosis carries a significant impact on management and prognosis. Pathologists involved in the diagnosis of breast cases should be familiar with diagnostic challenges they may encounter during their daily practice.

The purpose of this article is to describe the ancillary studies used for the diagnosis of benign, borderline, and malignant epithelial lesions of the breast as well as to describe studies used for the prognostic assessment of cancer. For the purpose of discussion, IHC stains used in breast pathology are divided into...
2 groups: those used for diagnostic purposes and those used to obtain prognostic/predictive information.

**Diagnostic Immunohistochemical Stains**

**Benign and Atypical Ductal Proliferative Lesions**

Diagnosing benign (usual and florid) ductal hyperplasia from atypical ductal hyperplasia (ADH) can be difficult on H & E examination. In addition to providing information regarding long-term cancer risk stratification, distinguishing the 2 groups in CNB specimens helps the clinical team plan the next step in the workup process.

The likelihood of finding a more severe lesion (ie, in situ, invasive carcinoma) on excision after the diagnosis of ADH ranges from 12% to 36%.

Therefore, in most centers, the diagnosis of ADH is followed by excisional biopsy; consequently, making the correct diagnosis in borderline cases has great clinical implications. For several years, IHC has been used by pathologists for this diagnostic purpose. High-molecular-weight keratins (cytokeratins [CK] 5 and 6 and 34\(\beta\)E12) in conjunction with estrogen receptor (ER) IHC may be helpful in these borderline cases. Atypical proliferative lesions and in situ carcinoma lack staining with such keratins or they show occasional staining alone. By contrast, a strong and diffuse staining pattern indicates a benign process (Fig 1A).

When using these stains for the diagnosis of atypia in our practice, we have found that a potential pitfall is that cells with apocrine differentiation are generally negative. This finding is important when diagnosing atypia in columnar cell changes, which characteristically have apocrine features (Fig 1B).

In this setting, the pathologist should rely on cytologic and architectural features on H & E examination alone. ER is sometimes used because, in the setting of benign breast tissue, ER shows scattered positivity alone in lobules and ducts. Conversely, in atypical and low-grade neoplastic processes such as ADH or low-grade ductal carcinoma in situ (DCIS), this stain tends to be diffusely and strongly positive (Fig 1C and 1D).

It is important to highlight that these IHC stains help classify a proliferative lesion into benign or atypical, and routine histological criteria must be followed to distinguish between ADH and DCIS.

**In Situ and Invasive Carcinoma**

The presence or absence of a myoepithelial cell layer around carcinoma cells is the basis for dividing tumors into in situ and invasive types. In most cases, distinguishing in situ from invasive carcinoma is straightforward. However, myoepithelial cell markers can be useful in diagnosing microinvasion, particularly in the setting of extensive high-grade DCIS or prominent inflammatory infiltrate associated with DCIS. In addition, certain entities are known to cause diagnostic challenges due to their complex architectural features. Invasive carcinoma or DCIS involving adenosis, invasive cribriform carcinoma or cribriform DCIS, and invasive carcinoma or solid papillary carcinoma are select examples (Fig 1E and 1F).

Any single myoepithelial cell marker is imperfect due to cross-reactivity with stromal myofibroblasts, vascular smooth muscle cells, or luminal epithelial cells. Therefore, at least 2 markers should be used in combination. The most appropriate combinations...
recommended in the literature have been a nuclear stain (p63) together with a cytoplasmic stain, such as smooth muscle myosin heavy chain, smooth muscle actin, or calponin. Among the cytoplasmic markers, smooth muscle myosin heavy chain appears to be more specific but is less sensitive than calponin. P63 has the advantage of being a clean and easy-to-read stain and it has good specificity rates in the setting of breast cancer. However, its staining pattern can be discontinuous, particularly around distended ducts with carcinoma in situ. In addition, most metaplastic carcinomas and, rarely, ductal carcinomas stain positively for p63. Despite this cross-reactivity, even when breast carcinomas stain positive for p63, staining tends to be patchy and of less intensity than adjacent myoepithelial cells.

Recent studies have also shown that qualitative differences may exist with regard to the myoepithelial cell markers expressed in different lesions; specifically, myoepithelial cells surrounding DCIS as well as benign sclerosing lesions have phenotypic alterations compared with myoepithelial cells surrounding normal ducts and lobules. P63, smooth muscle myosin heavy chain, and calponin all show reduced expression in these cases, potentially leading to the erroneous diagnosis of invasive carcinoma. In particular, the myoepithelial cells layer in small-sized biopsy specimens may not be present in the plane of the section. In addition, expansion of the ducts associated with DCIS could result in “attenuation” of the myoepithelial cell layer, and, in such a scenario, reactivity with myoepithelial markers may be absent. Paying careful attention to the type of lesion in question and using a panel that includes several myoepithelial cell markers could help avoid such potential diagnostic errors.

Myoepithelial cell markers have also been used to classify papillary lesions. Benign papillary lesions are characterized by a continuous layer of myoepithelial cells in the fibrovascular cores and at the periphery of the lesion. Although papillary carcinomas lack myoepithelial cells inside the lesion, the hallmark of in situ carcinomas is their presence at the periphery. Unusual entities, such as solid papillary carcinoma and encapsulated papillary carcinoma, can be devoid of “demonstrable” myoepithelial cells at the periphery. Therefore, discussion regarding the nature of these tumors has been taking place for some time, with some researchers noting that solid papillary carcinoma and encapsulated papillary carcinoma tend to behave as in situ carcinomas and, thus, are classified as such. Paying attention to morphological features on H & E and interpreting IHC stains in the correct context are key to making the correct diagnosis in such cases.

Microglandular adenosis is a benign lesion that defies the general rule of benign glands being surrounded by a layer of myoepithelial cells. Absence of a myoepithelial cell layer surrounding the glands, together with their tendency for haphazard growth into adipose tissue, makes this entity prone to being misdiagnosed as invasive carcinoma, specifically the tubular type.

Paying careful attention to subtle morphological features such as the commonly present, colloid-like secretions within the glandular lumina, as well as positivity of the epithelial cells for S100 can help in making the correct diagnosis. Of note, these lesions are negative for ER and progesterone receptor (PR), which would be unexpected in low-grade tubular carcinoma. This “unexpected” immunoprofile should raise questions in cases misdiagnosed as invasive carcinoma.

**Classification of Tumor Type**

**Ductal and Lobular Carcinoma**

Invasive and in situ carcinomas in the breast are divided into 2 main groups: ductal and lobular carcinomas. These 2 carcinoma types have major differences in anatomical distributions, long-term risk stratifications, and metastatic patterns. Consequently, to perform an appropriate preoperative workup, patient counseling, and treatment planning, an accurate diagnosis on CNB specimens is necessary.

IHC staining for E-cadherin, which is an adhesion molecule expressed in carcinomas of the ductal type, has long been used by pathologists. Ductal carcinomas show a strong membranous staining pattern, whereas lobular carcinomas are either negative or rarely display an aberrant staining pattern (Fig 1G and 1H). A small subset of lobular carcinomas (in particular, the pleomorphic type) can be positive for E-cadherin, and E-cadherin can show granular cytoplasmic staining in some lobular carcinomas. This is a potential pitfall in tumor classification because the finding is sometimes misinterpreted as positive staining. Careful attention to H & E findings as well as the location and the relative intensity of staining between tumor cells and benign ducts are all helpful in making the correct diagnosis.

The combination of E-cadherin and p120 (a catenin that binds to E-cadherin on the cell membrane and is essential for the formation of tight junctions) is superior to using E-cadherin alone when facing ambiguous cases. Ductal carcinomas will have a membranous pattern of staining, whereas lobular carcinomas show cytoplasmic staining. Because ductal and lobular carcinomas can be distinguished on H & E alone in the majority of cases or with the use of E-cadherin alone, we recommend using p120 for difficult-to-diagnose cases alone.
**Special-Type Carcinomas and Spindle Cell Tumors**

Typically, special-type carcinomas are diagnosed based on their morphological features on H & E examination. However, occasionally, IHC stains can be helpful in this setting, particularly for spindle cell tumors (Fig 2A–C). Recognizing metaplastic carcinoma without an obvious epithelial or heterologous element is difficult — and sometimes impossible — on H & E alone, and this is especially true for limited samples such as CNB. The differential diagnosis of spindle cell metaplastic carcinoma includes other spindle cell lesions, such as phyllodes tumor, fibromatosis, angiosarcoma, and spindle cell melanoma, among others. Keratin expression in metaplastic tumors can be heterogeneous and focal; therefore, a battery of CK stains (both pan-keratins and high-molecular-weight keratins), epithelial membrane antigen, p63, melanoma markers (eg, S100, melan A, HMB45), and additional markers to rule out other sarcomas (as needed) should be used in difficult cases.18-21

The identification of basal-like carcinomas is important in premenopausal young women because this type of tumor may be associated with hereditary breast and ovarian cancers and carries a poor prognosis.4 Most of basal-like carcinomas are triple negative (negative for ER, PR, and HER2), and variably express basal-type keratins (CK5/6, CK14, CK17), luminal-type keratin CK8/18, epidermal growth factor receptor, vimentin, and p53.18,19,22 This panel of immunostains can be used in clinical practice and represents a less costly, more readily available methodology in which to classify these tumors when compared with other commercially available assays.

**Paget Disease of the Nipple**

Most cases of Paget disease of the nipple are associated with underlying breast carcinoma (mainly DCIS); therefore, the diagnosis can be made on H & E. However, biopsy specimens in patients without a detectable underlying mass can be diagnostically challenging. The main differential diagnosis in such a rare setting includes melanoma and squamous cell carcinoma (Bowen disease).

CK7 immunostain is positive in almost all cases of Paget disease and is negative in squamous cell carcinoma. Typically, Paget cells express the same IHC characteristics of the underlying carcinoma.3 Because the underlying malignancy is frequently of a high-grade type, ER, PR negative, HER2-positive DCIS, HER2 immunostain is useful and positive in up to 90% of Paget cases. Therefore, the use of CK7 and HER2 is considered the most appropriate combination for confirming the diagnosis.23,24 Conversely, ER and PR immunostains are of no value in the differentiation of this entity from nonmammary lesions.

Select pitfalls must be kept in mind when evaluating CK7 immunostain. Both Toker and Merkel cells may be positive for CK7.25,26 Moreover, intraepidermal CK7-positive cells may represent an extension of benign lactiferous ducts into the nipple.3 Careful attention to the distribution and volume of positive cells may help distinguishing these benign entities from Paget disease. Melanoma can be ruled out by a panel of at least 2 immunostains, with the understanding that S100 is sometimes positive in Paget disease.27 Squamous cell carcinomas are negative for CK7 and positive for pan-keratin, high-molecular-weight keratins, and p63.
Metastatic Setting
Although breast cancer commonly metastasizes to other organs, metastases to the breast are rare and account for 0.5% to 2.0% of all breast malignancies.28 In most cases, a history of malignancy is known. Melanoma is one of the most common tumors metastasizing to the breast, followed by carcinomas of lung and those of gynecological origin.29 Absence of an in situ component as well as clinical history of another malignancy should raise suspicion for metastasis rather than primary breast carcinoma. The distinction is crucial because therapeutic management differs in these cases.

A commonly encountered situation is metastatic carcinoma of unknown origin to a nonbreast site. In these cases, metastatic breast carcinoma is usually in the differential diagnosis (Fig 2D). Breast carcinomas are likely to be CK7 positive and CK20 negative. However, a similar profile is seen in lung and gynecological tract carcinomas. Positivity for ER and PR can be helpful; however, these stains can also be positive in other tumors — in particular, carcinomas of a gynecological origin are positive for ER and PR (in which case, Wilms tumor 1 and paired box 8 can be helpful). When the differential diagnosis includes lung adenocarcinoma, thyroid transcription factor 1 and napsin-A, which are both positive in lung adenocarcinomas, can be used.4

No single stain is unique to the breast. Gross cystic disease fluid protein 15 (GCDFP15) is relatively specific for the breast (also positive in salivary gland tumors and adnexal tumors of the skin), but it lacks sensitivity and shows a patchy staining, which becomes problematic in small-sized biopsy specimens. Mammaglobin is more sensitive than GCDFP15 but it lacks specificity. A combination of the 2 stains may be superior to either one alone; however, even when used in combination, up to 30% of tumors can be negative for both.30 Of note, neither stain can be used to differentiate breast carcinoma from adnexal tumors of the skin (sweat gland carcinomas) or those of salivary gland duct origin. To distinguish between primary breast carcinoma and sweat gland carcinoma, a panel of IHC that includes mammaglobin, GCDFP15, p63 (strong expression in the latter group), and basal cytokeratins (CK5, CK14, and CK17) has been suggested as having highly sensitivity and specificity rates.31

GATA-binding protein 3 (GATA3) IHC is highly sensitive and fairly specific for breast carcinoma if urothelial carcinoma is not in the differential diagnosis. Rarely, staining for GATA3 has been reported in endometrioid adenocarcinoma.32,33 Of note, the rate of positive staining in breast carcinomas is highest in well-differentiated, ER-positive tumors, including lobular carcinomas (Fig 2E and 2F).

Prognostic/Predictive Immunohistochemical Stains
Hormone Receptors and HER2 Status
Hormone receptor and HER2 testing should be performed on all primary invasive breast carcinomas, as well as in recurrent or metastatic tumors. Multiple preanalytic and analytic factors can affect test results; however, this review only briefly summarizes the analytic component of these tests based on guidelines from the American Society of Clinical Oncology and the College of American Pathologists.34,35

All cases with at least 1% positive tumor cells for ER and PR have been associated with clinical response; therefore, they are classified as positive (Fig 2G).31 The College of American Pathologists requires providing quantitative information regarding the extent of positivity in all pathology reports.34 Quantification can be performed by simply providing the proportion of positive cells or using the Allred score or H score systems, both of which use the intensity and percentage of positive cells.35

Staining patterns/intensities of HER2 immunostain have been categorized into 3 groups. Positive (3+) staining is defined by complete, intense, circumferential membrane staining (Fig 2H). Negative results include cases categorized as 1+ characterized as incomplete membrane staining that is faint/barely visible and in more than 10% of the invasive tumor cells, and 0 characterized by no staining or membranous staining that is incomplete and is faint/barely perceptible and within 10% or less of the invasive tumor cells.35 Equivocal cases (2+) are those that display circumferential membrane staining that is incomplete, weak/moderate, or both and within less than 10% of the invasive tumor cells or complete and circumferential membrane staining that is intense and within 10% or less of invasive tumor cells.35 If results are equivocal, then reflex testing should be performed using an alternate assay (ie, in situ hybridization if IHC was used as the initial test).35

Conclusions
Immunohistochemical (IHC) stains provide information and aid in the differential diagnosis of challenging epithelial lesions of the breast. In addition, IHC can be used to obtain information relating to prognostic/predictive markers crucial for the treatment and prognostic assessment of patients with breast carcinoma.

IHC stains should be used selectively and judiciously and interpreted with the differential diagnoses and pitfalls in mind. Hematoxylin and eosin continues to be the most appropriate tool for diagnosing breast epithelial lesions.
References