Current Practical Applications of Diagnostic Immunohistochemistry in Breast Pathology

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Abstract: In recent years, immunohistochemistry has assumed an increasingly prominent role in diagnostic breast pathology. Immunohistochemistry is now frequently used in the evaluation of many epithelial proliferations of the breast. Common applications include the use of myoepithelial markers to evaluate for stromal invasion, E-cadherin to distinguish between ductal and lobular neoplasia, high molecular weight cytokeratins to differentiate usual ductal hyperplasia from ductal carcinoma in situ, immunohistochemical profiles to characterize site of origin of metastatic carcinomas, and cytokeratin stains to detect metastases in sentinel lymph nodes. Recent advances, practical considerations, and potential pitfalls in the use of immunohistochemistry in these five diagnostic categories are discussed herein.

(*Am J Surg Pathol* 2004;28:1076–1091)

The majority of diagnoses in breast pathology are rendered successfully based on the evaluation of hematoxylin and eosin-stained slides alone. However, the histologic complexity, varied morphology, and overlapping features of many benign and neoplastic lesions often lead to problems in interpretation. Epithelial proliferations are the most common source of diagnostic difficulty, and they have provided fertile ground for exploration of the potential benefits of immunohistochemistry. In this review, recent advances in the use of immunohistochemistry in diagnostic breast pathology are presented in the context of five major topics: 1) determination of stromal invasion, 2) distinction between ductal and lobular neoplasia, 3) differentiation of usual ductal hyperplasia from ductal carcinoma in situ, 4) characterization of metastatic adenocarcinomas, and 5) evaluation of sentinel lymph nodes. The evaluation of estrogen receptor, progesterone receptor, Her-2/neu, and other prognostic and therapeutic markers in breast carcinoma is not discussed.

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ASSESSMENT OF STROMAL INVASION

The surgical pathologist not infrequently faces situations in which the unequivocal diagnosis of invasion, or absence thereof, is difficult on routine histologic sections. For example, the distorted glands of benign radial scar may be mistaken for invasive tubular carcinoma, and vice versa. Carcinoma in situ involving lobules or sclerosing adenosis can closely mimic the growth pattern of invasive carcinoma. High-grade ductal carcinoma in situ can be distorted by periductal sclerosis and inflammation such that it mimics the irregular nests of invasive carcinoma. Conversely, certain invasive carcinomas, such as solid papillary and cribriform carcinomas, typically invade as rounded nests that resemble carcinoma in situ.

Immunohistochemical markers are now commonly used to distinguish benign and in situ proliferations from invasive carcinoma. The approach takes advantage of the fact that like normal ducts, almost all benign breast lesions and in situ carcinomas have a peripheral layer of myoepithelial cells and basement membrane (Fig. 1). Stromal invasion occurs when malignant epithelial cells extend beyond the myoepithelial cell layer and break through the basement membrane. Earlier investigators used antibodies to basement membrane components such as collagen IV and laminin to differentiate between in situ and invasive carcinomas.[1,2] This approach met with only limited success, however, since invasive tumor cells are also capable of synthesizing basement membrane.

Myoepithelial cells, on the other hand, are almost invariably absent from invasive tumor cell nests and present around benign and in situ lesions. Because myoepithelial cells can be difficult to detect on routine sections, especially when they are attenuated in the setting of in situ carcinoma, immunohistochemical stains for myoepithelial markers can be helpful. Commonly used myoepithelial markers include smooth muscle actin, calponin, smooth muscle myosin heavy chain, and p63. These four markers are all highly sensitive for myoepithelial cells but have varying specificities (Table 1).

Smooth Muscle Actin

Smooth muscle actin (SMA) is strongly positive in breast myoepithelial cells (Fig. 2A) and is widely used for their detection. The major drawback to SMA is that it also strongly

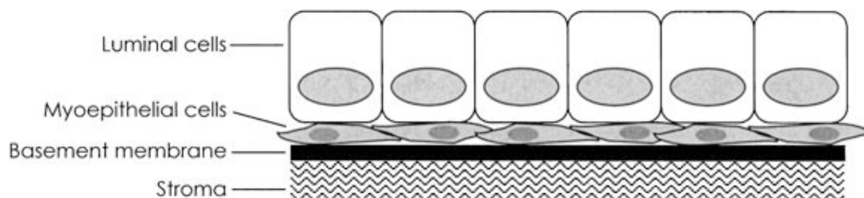

FIGURE 1. In the normal ducts and acini of the breast, central luminal epithelial cells are surrounded by a peripheral layer of myoepithelial cells and basement membrane. This arrangement is also seen in nearly all benign breast lesions and carcinomas in situ. Microglandular adenosis, a benign proliferation lacking myoepithelial cells, constitutes the only known exception.

labels myofibroblasts present in the reactive stroma of invasive carcinoma, ductal carcinoma in situ, and sclerosing lesions (Fig. 3A). When juxtaposed to tumor cell nests, flattened SMA-positive myofibroblasts mimic the staining pattern of myoepithelial cells. This may lead to the false conclusion that myoepithelial cells are present and consequent underrecognition of stromal invasion. SMA also labels blood vessels, and small vessels abutting tumor cells can lead to a similar diagnostic problem. Bona fide myoepithelial cells demonstrate a slight bulging of their cell bodies toward the luminal epithelial cells, unlike myofibroblasts or blood vessels. SMA is easiest to interpret when used on lesions with minimal reactive stroma. Uncommonly, SMA may stain scattered epithelial cells in usual ductal hyperplasia or invasive carcinoma.[3–5]

Calponin

Calponin is a smooth muscle-restricted contraction regulatory protein that is expressed in more fully differentiated smooth muscle cells. Like SMA, it is a highly sensitive marker for myoepithelial cells (Fig. 2B). Unlike SMA, it demonstrates only moderate cross-reactivity to myofibroblasts.[6] Although calponin stains myofibroblasts in 76% to 90% of cases, the actual number of labeled myofibroblasts is typically less than 25% of those labeled by SMA (Fig. 3B).[6,7] Calponin also stains blood vessels, and in rare cases invasive tumor cells may show focal calponin positivity.[4]

Smooth Muscle Myosin Heavy Chain

Smooth muscle myosin heavy chain (SM-MHC) is a structural component of myosin. Like calponin, it is considered a marker of more terminally differentiated smooth muscle cells. The sensitivity of SM-MHC is reported to be equal to or slightly less than that of SMA and calponin (Fig. 2C).[6,7] SM-MHC demonstrates much less cross-reactivity to myofibroblasts than either SMA or calponin, with only 7% to 8% of cases showing staining of rare myofibroblasts (Fig. 3C).[6–8] Although SM-MHC also labels blood vessels, the relative lack of myofibroblast staining eliminates many of the pitfalls associated with the interpretation of myoepithelial markers. Therefore, SM-MHC is a very useful marker for detecting breast myoepithelial cells, with an excellent balance of sensitivity and specificity.

p63

p63, a homologue of p53, is involved in many key developmental events and is expressed in the basal epithelia of multiple organs. In the breast, it is a sensitive and relatively specific marker for myoepithelial cells. Because p63 is localized to the nucleus, positive staining of myoepithelial cells results in a discontinuous "dotted line" pattern around benign glands and in situ carcinomas (Fig. 4A). The gaps between positive nuclei are augmented when the myoepithelial layer is attenuated, as is seen in some in situ carcinomas (Fig. 4B). The main advantage of p63 is its specificity. It is not expressed in myofibroblasts or blood vessels, therefore circumventing the diagnostic pitfalls associated with smooth muscle-related myoepithelial markers. Although p63 may label scattered cells in usual ductal hyperplasia and tumor cells in 5% to 12% of invasive carcinomas, the staining of epithelial cells is usually focal and weaker than the staining of myoepithelial cells.[7,9–11] Tumor cell reactivity is seen more often in poorly differentiated carcinomas or those showing evidence of squamous differentiation.[9,11] Because labeled tumor cells are usually

TABLE 1. Commonly Used Myoepithelial Markers

Marker	Location	Myoepithelial Cells	Myofibroblasts	Vessels	Epithelial Cells
SMA	Cytoplasmic	+++++	+++	+++	Rare +
Calponin	Cytoplasmic	+++++	++	+++	Rare +
SM-MHC	Cytoplasmic	++++	+	+++	
p63	Nuclear	++++	−	−	Occasional +

SMA, smooth muscle actin; SM-MHC, smooth muscle myosin heavy chain.

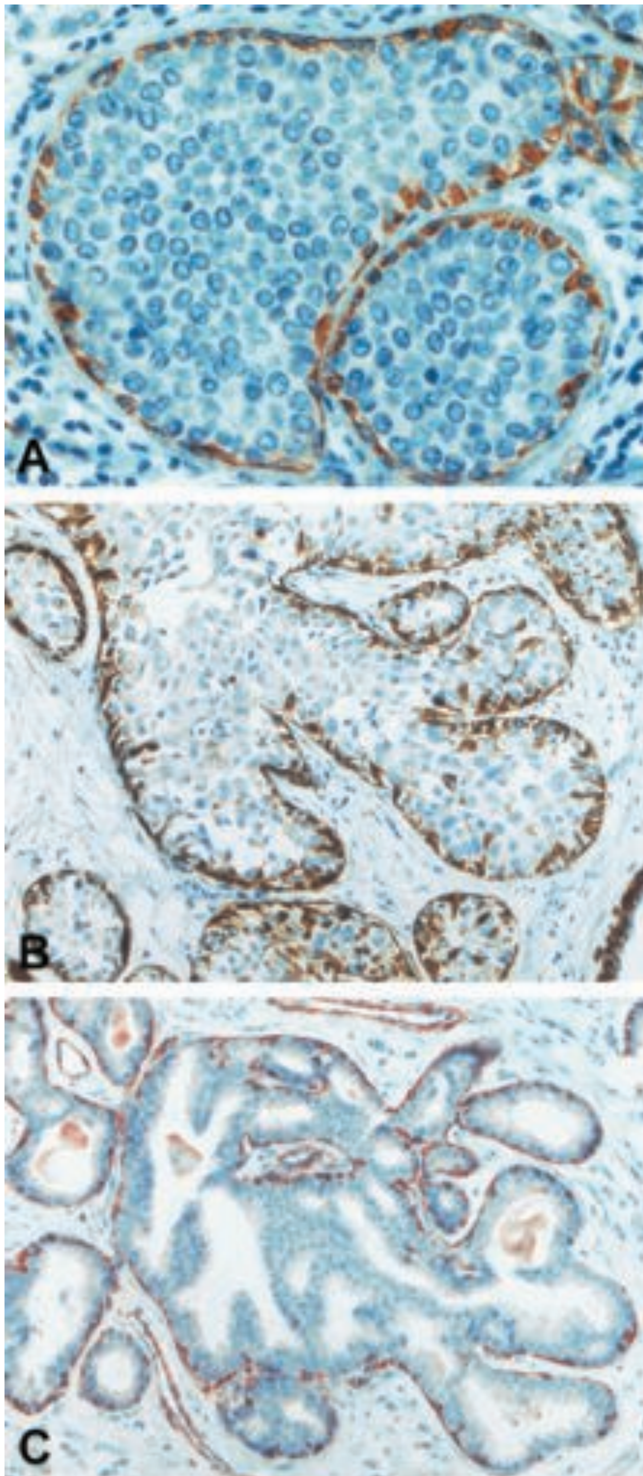

FIGURE 2. Smooth muscle-related myoepithelial markers as an aid in confirming a noninvasive process. Myoepithelial cells surrounding carcinoma in situ are clearly highlighted with smooth muscle actin (**A**), calponin (**B**), and smooth muscle myosin heavy chain (**C**). Note staining of blood vessels in C.

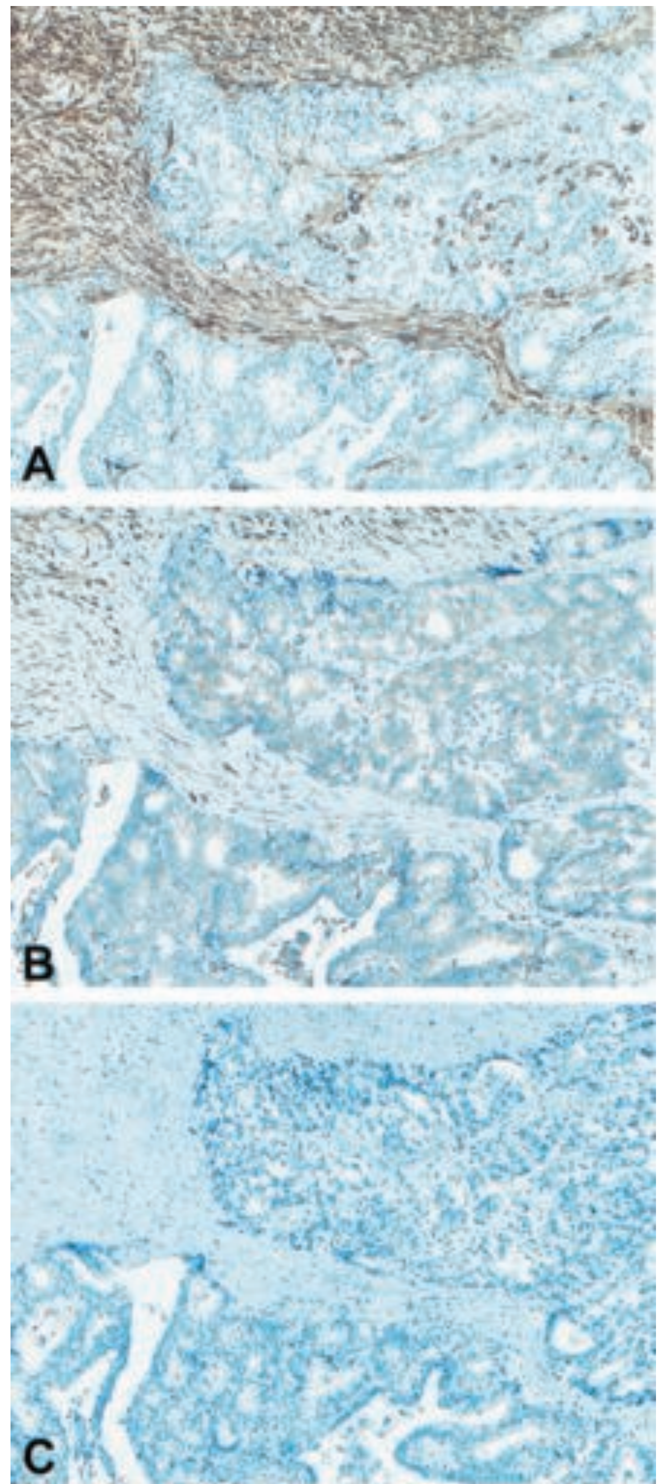

FIGURE 3. Staining of myofibroblasts by smooth muscle-related myoepithelial markers in an example of invasive carcinoma. **A**, Extensive myofibroblast staining in the stroma precludes reliable interpretation of the smooth muscle actin stain. **B**, Calponin labels fewer myofibroblasts than smooth muscle actin. There is also some nonspecific background staining in this example. **C**, Smooth muscle myosin heavy chain shows no reactivity with myofibroblasts, allowing one to appreciate the lack of myoepithelial cells around the invasive tumor. In a small percentage of cases, smooth muscle myosin heavy chain will stain rare myofibroblasts.

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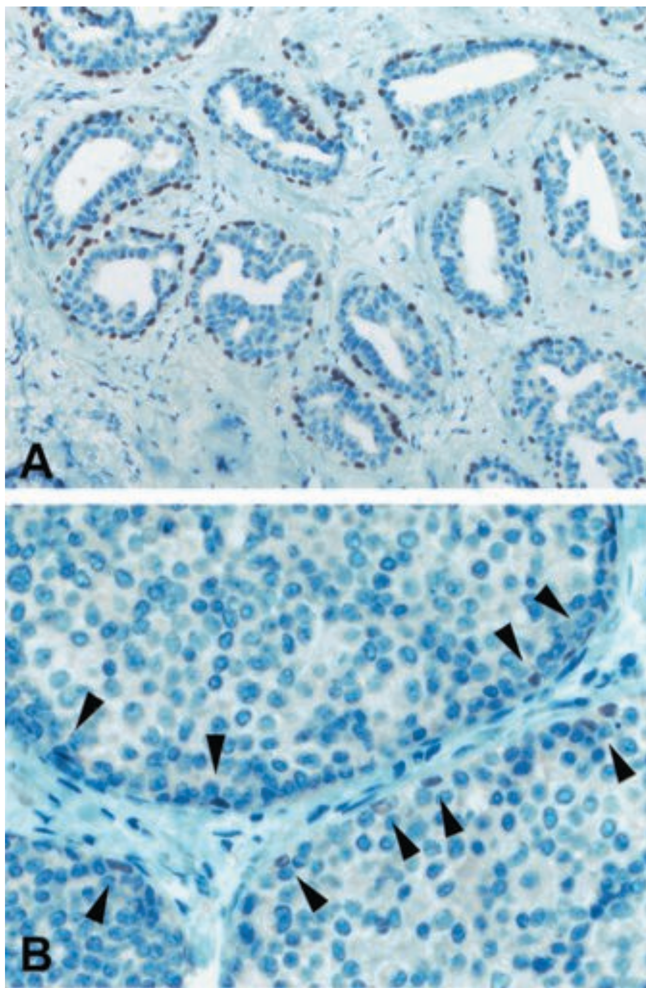

FIGURE 4. p63 staining of myoepithelial cells. **A**, Because p63 is a nuclear stain, positive myoepithelial cells appear as a "dotted line." **B**, In some examples of carcinoma in situ, the myoepithelial cells (arrowheads) are markedly attenuated.

readily identifiable as such, they are rarely confused with myoepithelial cells. Overall, p63 has high sensitivity and specificity for myoepithelial cells and is a very useful marker.

OTHER MYOEPITHELIAL MARKERS

S-100

S-100 was one of the earliest markers used to detect breast myoepithelial cells. Because of only moderate sensitivity and frequent reactivity in both normal and neoplastic luminal epithelial cells,[12] S-100 is no longer recommended for this purpose.

CD10

CD10, or CALLA, is expressed in breast myoepithelial cells.[13] CD10 is also positive in myofibroblasts, but the degree of cross-reactivity is less than that seen with SMA. CD10 does not stain blood vessels. In our experience, CD10 is somewhat less sensitive than the other commonly used myoepithelial markers.

High Molecular Weight Cytokeratins

High molecular weight cytokeratins have been investigated as potential myoepithelial markers. In particular, cytokeratin 5 has been reported to be a highly specific myoepithelial marker in the differentiation of in situ from invasive carcinomas.[8,14] Its specificity in other contexts, though, is limited by variable staining of luminal epithelial cells and strong positivity in usual ductal hyperplasia.[15,16] High molecular weight cytokeratins also have a low sensitivity for myoepithelial cells, which hampers their diagnostic utility.

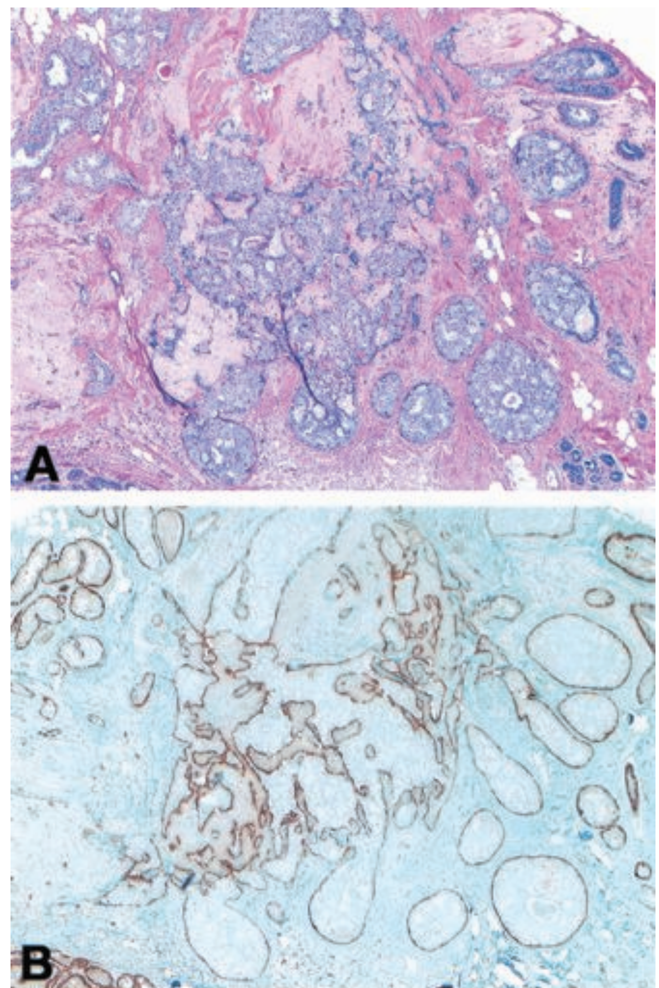

FIGURE 5. Ductal carcinoma in situ involving a radial scar. **A**, The glandular distortion present in the central nidus of the radial scar raises concern for stromal invasion. **B**, A calponin immunostain demonstrates that the tumor is entirely surrounded by myoepithelial cells, supporting a diagnosis of carcinoma in situ.

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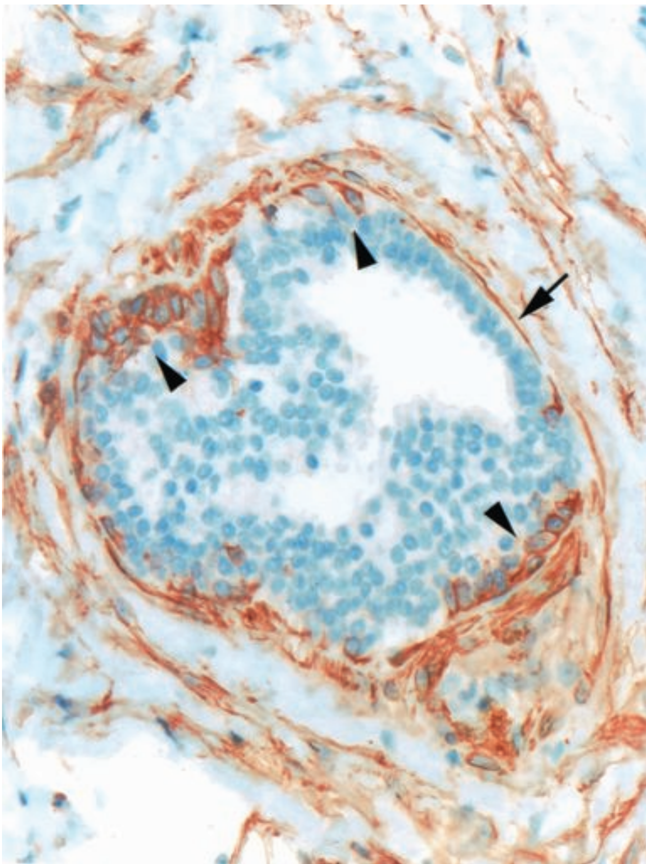

FIGURE 6. Contrast of smooth muscle actin staining of myoepithelial cells and myofibroblasts. Myoepithelial cells show strong cytoplasmic staining. Their cell bodies bulge toward and interdigitate between the luminal cells (arrowheads). In contrast, myofibroblasts are stretched along the outside of the duct (arrow), without evidence of interdigitation between the luminal cells, and are also present in the peripheral stroma.

Novel Markers

Maspin, Wilms' tumor-1, and P-cadherin have all recently been reported to label breast myoepithelial cells.[17,18] All of these markers can label epithelial cells as well, complicating their interpretation.[18–20] These markers are not currently in widespread use, and their diagnostic utility in this context remains to be defined.

COMMENTS ON THE INTERPRETATION OF MYOEPITHELIAL MARKERS

■ *"The absent are never without fault, nor the present without excuse." — Benjamin Franklin*

Detecting Absence

Detecting the absence of something is more problematic than detecting its presence. When immunohistochemical stains fail to reveal myoepithelial cells around tumor, the diagnosis of stromal invasion is supported. However, this interpretation is complicated by a small degree of uncertainty as to whether the myoepithelial cells are truly absent or whether they are merely markedly attenuated and out of the plane of section. The latter is a decidedly uncommon, but theoretically possible, scenario. Reassuring features supporting a genuine lack of myoepithelial cells include medium to large tumor nests without detectable myoepithelial cells, multiple tumor nests without detectable myoepithelial cells, and lack of reactivity with two different myoepithelial markers.

Detecting Presence

Myoepithelial markers suffer from less ambiguity when used to confirm a benign or in situ interpretation (Fig. 5). The positively-staining myoepithelial cells are generally easy to

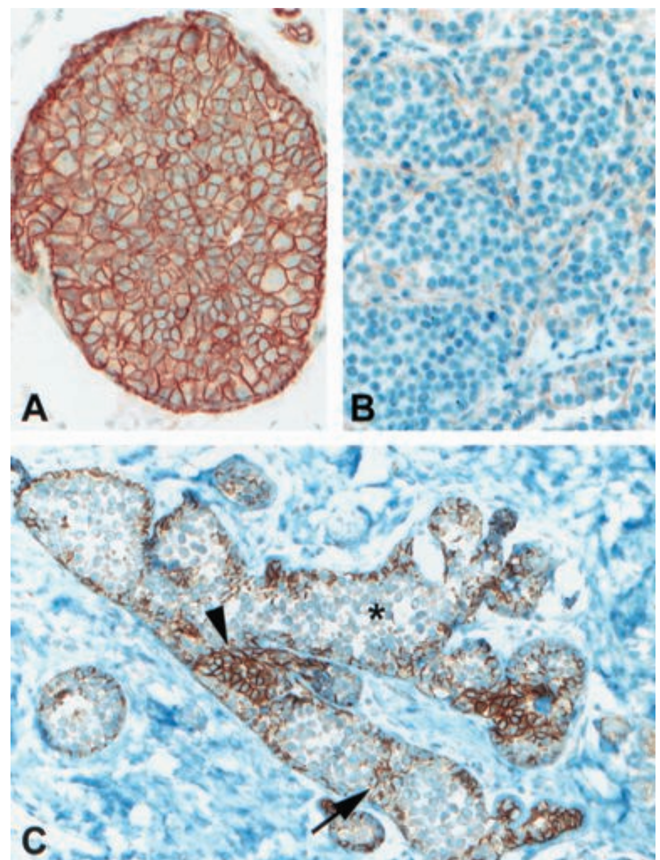

FIGURE 7. E-cadherin in the evaluation of solid carcinoma in situ. **A**, Ductal carcinoma in situ shows strong membranous staining for E-cadherin. **B**, Lobular carcinoma in situ, in contrast, is negative. Surrounding myoepithelial cells show faint, granular staining. **C**, E-cadherin-positive entrapped luminal cells (arrowhead) and myoepithelial cells (arrow) may give the initial impression of tumor cell reactivity, but careful high-power evaluation will disclose that the positive cells are morphologically distinct from the negative lobular neoplasia cells (*).

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detect. As discussed above, however, staining of myofibroblasts and blood vessels with smooth muscle-related markers may mimic the staining pattern of myoepithelial cells. The use of antibodies with less cross-reactivity, such as p63 and SM-MHC, is helpful in avoiding "false positive" interpretations. Features supporting genuine myoepithelial cell staining include a slight bulging of the positive cells toward the luminal epithelial cells (Fig. 6) and a lack of myofibroblasts in the surrounding stroma ("clean background").

Special Subtypes of Invasive Carcinoma

A few subtypes of invasive carcinoma demonstrate myoepithelial differentiation and will therefore stain for myoepithelial markers. These include adenoid cystic carcinoma, low-grade adenosquamous carcinoma, malignant adenomyoepithelioma, and malignant myoepithelioma.[9,21,22] Metaplastic carcinomas, including spindle cell carcinomas, may also stain for myoepithelial markers.[22] Of all these tumors, low-grade adenosquamous carcinoma is the one most likely to cause interpretative difficulty when myoepithelial markers are used to evaluate stromal invasion. Myoepithelial markers can stain the periphery of the invasive adenosquamous tumor nests, simulating an intact myoepithelial cell layer. Awareness of myoepithelial differentiation in these tumors helps to avoid misinterpretation of these foci as benign or carcinoma in situ.

Avoidance of Pitfalls

To circumvent some of the pitfalls in the interpretation of myoepithelial markers, it is helpful to use two different antibodies. p63 and SMM-HC complement each other well. If these two stains yield unclear results, the slightly more sensitive but less specific markers calponin and SMA can be used. The optimal antibody also depends upon the type of lesion being evaluated. If reactive stroma is present, p63 is an excellent choice because it does not stain myofibroblasts or blood vessels. However, p63 is less adroit at highlighting architecture in small glandular proliferations such as sclerosing adenosis, and in these cases a cytoplasmic marker such as SMA may be easier to interpret.

DUCTAL VERSUS LOBULAR

Most cases of carcinoma in situ are readily classified as either ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS) on the basis of cytologic and architectural features. However, some carcinomas in situ, particularly those with a solid growth pattern, have ambiguous features that are not definitively ductal or lobular. Such cases are problematic not only from the standpoint of pathologic classification but also from a therapeutic standpoint, as DCIS and LCIS are managed quite differently.

In recent years, the use of antibodies to detect expression of the cell adhesion molecule E-cadherin has proved to be a valuable tool for distinguishing DCIS from LCIS.[23–26] In almost all cases of DCIS, E-cadherin demonstrates linear, membranous staining of the neoplastic cells (Fig. 7A) . In contrast, LCIS is nearly always negative for membranous E-cadherin (Fig. 7B). The loss of E-cadherin expression in lobular carcinomas appears to be due to somatic mutation of the E-cadherin gene in some cases.[27–31] In the normal breast, E-cadherin demonstrates strong membrane staining of luminal cells and more granular membrane staining of myoepithelial cells. One pitfall in the interpretation of E-cadherin stains occurs when residual luminal cells and/or myoepithelial cells are intermixed with LCIS. E-cadherin positivity in these benign cells may give the false impression of membrane staining of the neoplastic cells (Fig. 7C). Generally, the intermixed benign cells are focal in distribution and have a different morphology than the LCIS cells, and careful study will reveal that the positive staining does not completely encircle the neoplastic cells. Correlation with the corresponding hematoxylin and eosin-stained slides is helpful in such instances.

Some authors have suggested the use of the high molecular weight cytokeratin 34βE12 (K903) in conjunction with E-cadherin to differentiate between DCIS and LCIS.[32] The use of cytokeratin 34βE12 requires strict adherence to protocol to avoid false-negative results.[32] The expected staining profiles for DCIS and LCIS are opposite: DCIS is positive for E-cadherin and shows negative or reduced staining for cytokeratin 34βE12, while LCIS is negative for E-cadherin and positive for cytokeratin 34βE12 (Table 2). Using these two antibodies, Bratthauer et al were able to classify 23 of 50 ambiguous cases as either DCIS or LCIS.[32] The remaining cases were either positive for both markers or negative for both markers. Because the clinical behavior of such double-positive or double-negative carcinomas has not been studied, it is unclear whether they represent entities distinct from DCIS and LCIS. Although Bratthauer et al have detected strong cytokeratin 34βE12 expression in all their examined cases of classic LCIS,[32,33] other authors have seen occasional examples with reduced staining.[16] Cytokeratin 34βE12 may be useful in cases where E-cadherin stains are not definitive, but E-cadherin currently remains the stain of choice and closest to a "gold standard" in the evaluation of ambiguous carcinomas in situ.

Although the different staining patterns of E-cadherin in DCIS and LCIS have been striking and consistent in many studies,[23–25] there are reported rare exceptions. Gupta et al described 5 cases of E-cadherin-negative DCIS,[34] although subsequent studies have found all DCIS cases to be E-cadherin-

TABLE 2. E-cadherin and High Molecular Weight Cytokeratin Expression in DCIS and LCIS

Antibody	DCIS	LCIS
E-cadherin	+	−
Cytokeratin 34βE12	− or reduced (~90%)[41]	+

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positive.[23–25] Some authors have also noted reduced staining in some examples of DCIS,[23,24,34–37] whereas others have not.[25] It is possible that these varying results are due to methodological differences.[25] Additionally, 4% to 14% of LCIS is reported to express focal membranous E-cadherin,[23,24,31,38,39] although this has not been a uniform finding.[25] E-cadherin staining in these cases is typically weaker than that seen in normal epithelium or DCIS, and it is found only focally within a background of E-cadherin-negative carcinoma in situ that appears morphologically consistent with LCIS. The biologic significance of this patchy reactivity is unclear, although it is possible it represents evidence of focal ductal differentiation.[39] One study found that patients with E-cadherin-positive LCIS had an increased incidence of subsequent invasive carcinoma and a shorter time period to development of invasive carcinoma, when compared with patients with E-cadherin-negative LCIS.[39] The risk for subsequent carcinoma associated with E-cadherin-positive LCIS was comparable to that for low-grade DCIS. Therefore, E-cadherin-positive LCIS may be the exception that proves the rule, but further studies are needed to substantiate these provocative findings.

Despite these reported exceptions and the relative lack of clinical correlation studies, the sensitivity and specificity of E-cadherin appear high enough that it is reasonable to recommend its use in the delineation of DCIS from LCIS. The majority of ambiguous carcinomas in situ will be able to be classified based on the presence or absence of membranous E-cadherin. A small subset of cases with equivocal morphologic features, however, will demonstrate both E-cadherin-positive and -negative cells.[24–26] In some instances, these cases represent collision tumors between DCIS and LCIS. In other instances, the positive and negative cells are not morphologically distinct, and such lesions may be classified as "carcinoma in situ with combined ductal and lobular features." Although the biologic behavior of these combined carcinomas is unknown, they have generally been managed as DCIS.

USUAL DUCTAL HYPERPLASIA VERSUS DUCTAL CARCINOMA IN SITU

High molecular weight cytokeratins can be helpful in distinguishing usual ductal hyperplasia from ductal carcinoma in situ (Table 3). Ninety to 100% of usual ductal hyperplasias are strongly positive for cytokeratin 34βE12, which detects a common epitope on cytokeratins 1, 5, 10, and 14. In contrast, cytokeratin 34βE12 expression is lost or markedly reduced in 81% to 100% of ductal carcinomas in situ and 80% to 100% of atypical ductal hyperplasias.[16,40,41] It is likely that in this context cytokeratin 34βE12 is in large part reacting with cytokeratin 5, as antibodies to cytokeratin 5/6 show a similar expression pattern. Eighty-eight to 100% of usual ductal hyperplasias are strongly positive for cytokeratin 5/6 (Fig. 8A), in contrast to loss of expression in 96 to 100% of ductal carcinomas in situ (Fig. 8B) and 80 to 92% of atypical ductal hyperplasias.[16,42] Cytokeratin 5/6 shows less reactivity than cytokeratin 34βE12 in ductal carcinoma in situ[16] and, therefore, may be easier to interpret in this differential diagnosis.

Lobular carcinoma in situ is strongly positive for cytokeratin 34βE12 in 80% to 100% of cases, often with a perinuclear staining pattern.[16,32] However, 83% to100% of lobular carcinomas in situ and 74% of atypical lobular hyperplasias are negative for cytokeratin 5/6.[16,42] Therefore, cytokeratins 34βE12 and 5/6 do not yield parallel findings in lobular neoplasia, and it appears that cytokeratin 34βE12 detects a cytokeratin other than 5 in this context.[33]

In the normal breast, cytokeratins 34βE12 and 5/6 demonstrate variable positivity in luminal epithelial cells and myoepithelial cells.[16,41,42] These benign cells may be a source of positivity in a background of carcinoma in situ, and care should be taken not to interpret these as positive tumor cells. Because many normal epithelial cells and a small percentage of usual ductal hyperplasias are negative for these antigens, the absence of high molecular weight cytokeratin expression alone is not diagnostic of atypia or malignancy. Conversely, a positive immunoreaction does not necessarily indicate a benign process, as a small percentage of ductal carcinomas in situ are positive for these markers and lobular carcinoma in situ is typically positive for cytokeratin 34βE12. Therefore, although high molecular weight cytokeratins may be useful in the evaluation of difficult intraductal proliferations, these antibodies do not represent a "gold standard" and must be interpreted in conjunction with the morphology on hematoxylin and eosin-stained sections.

High molecular weight cytokeratins are unlikely to be helpful in the differential diagnosis of columnar cell prolifera-

TABLE 3. High Molecular Weight Cytokeratin Expression in Benign, Atypical, and Malignant Proliferations[16,32,40–42]

Cytokeratin	UDH (%)	ADH (%)	DCIS (%)	LCIS (%)
34βE12	+++ (90–100)	−/+ (80–100)	−/+ (81–100)	+++ (80–100)
5/6	+++ (88–100)	− (80–92)	− (96–100)	− (83–100)

UDH, usual ductal hyperplasia; ADH, atypical ductal hyperplasia; −/+, absent or reduced staining.

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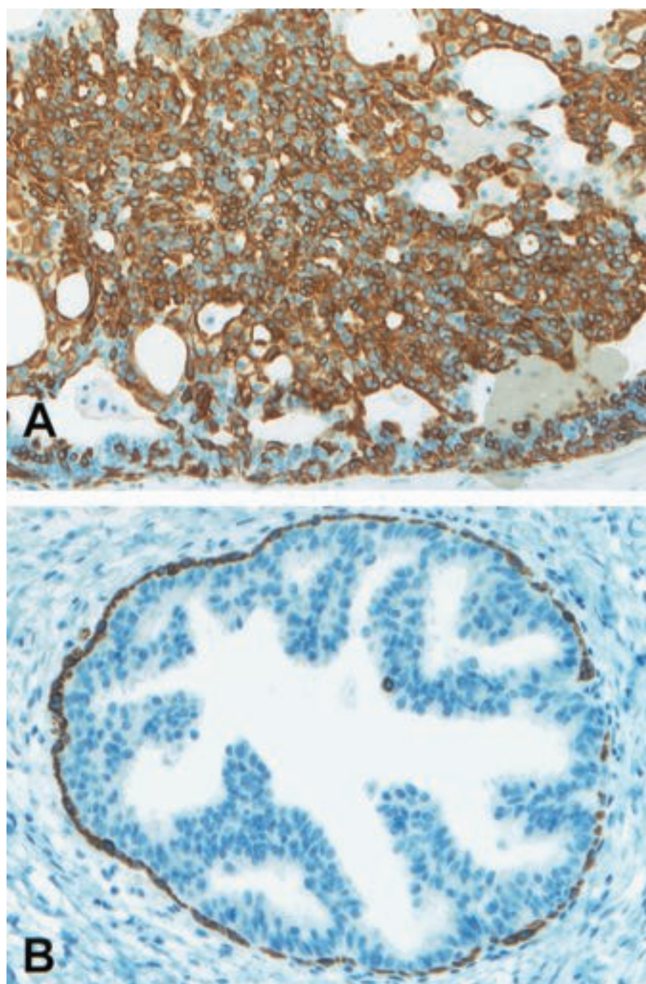

FIGURE 8. High molecular weight cytokeratins in intraductal proliferations. Cytokeratin 5/6 is strongly positive in usual ductal hyperplasia (**A**) but is negative in the neoplastic cells of ductal carcinoma in situ (**B**). Note the positive myoepithelial cells and single residual benign luminal cell in B.

tions. Raju et al noted that cytokeratin 34βE12 is often negative in nonatypical columnar cells adjacent to usual ductal hyperplasia,[40] and Otterbach et al mention but do not elaborate on their observation that columnar cells, regardless of atypia, are negative for cytokeratin 5/6.[42] Carlo et al also found that both nonatypical and atypical columnar cell proliferations demonstrate loss or markedly reduced expression of high molecular weight cytokeratins.[43] Although useful in the evaluation of noncolumnar intraductal proliferations, high molecular weight cytokeratins do not appear to distinguish between nonatypical and atypical columnar cells.

CHARACTERIZATON OF METASTATIC ADENOCARCINOMAS

Breast cancer commonly metastasizes, and distinguishing metastatic breast carcinoma from a primary tumor at another site can be difficult. This diagnostic dilemma is most often encountered in the lung and ovaries. Additionally, breast carcinoma is frequently a consideration in the workup of metastases of unknown primary, both in the aforementioned organs as well as other diverse sites.

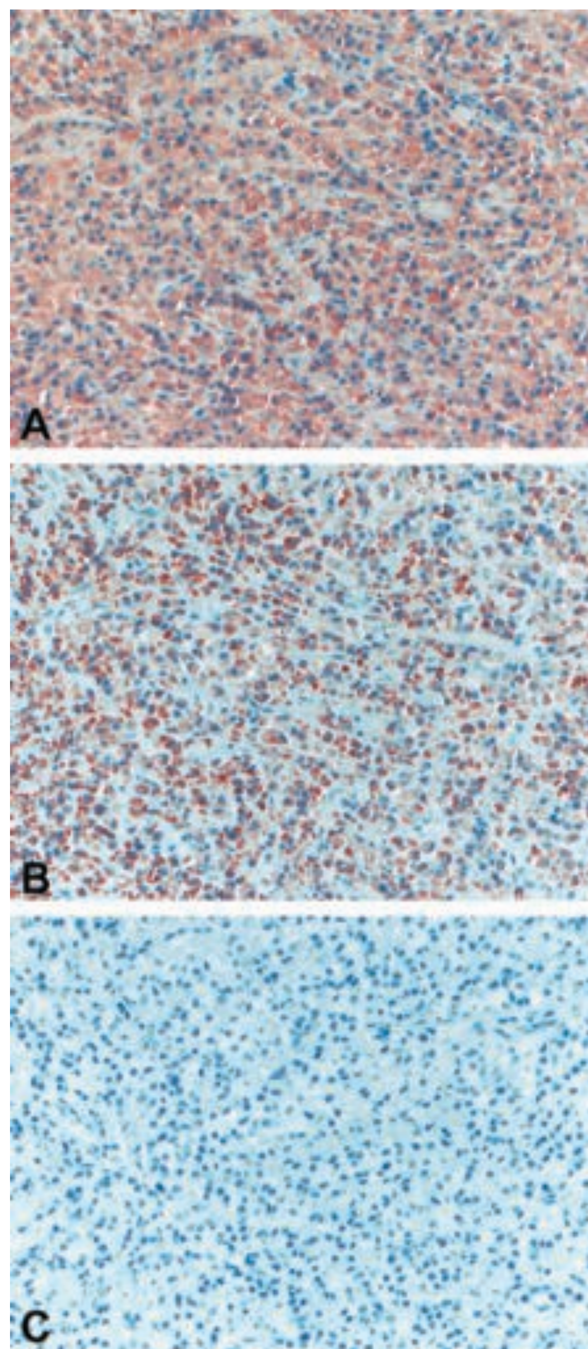

FIGURE 9. Immunohistochemistry in confirming diagnosis of metastatic breast carcinoma. The majority of breast carcinomas are positive for gross cystic disease fluid protein-15 (**A**), and almost all are cytokeratin 7-positive (**B**) and cytokeratin 20-negative (**C**).

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The breast itself is an uncommon site of metastatic disease. Cutaneous melanoma is the most common extramammary solid malignancy to metastasize to the breast. Pulmonary, ovarian, gastric, and renal carcinomas are also common sources of metastases to the breast, as is prostatic carcinoma in males.[44–53] Most patients with metastasis to the breast have known and widely disseminated disease, but in 24% to 40% of cases the breast lesion is the first presentation of an occult malignancy.[44,45,48,51]

Clinical history and comparison with prior tumor slides are more helpful than any special study in discriminating between carcinomas of breast and non-breast origin. Not infrequently, though, the clinical history is not revealing or the prior slides are not available, and in these cases selected immunohistochemical stains can be of benefit.

Gross Cystic Disease Fluid Protein-15

Gross Cystic Disease Fluid Protein-15 (GCDFP-15) is a marker of apocrine differentiation that is expressed in 62% to 77% of breast carcinomas (Fig. 9A), as well as in salivary gland and skin adnexal tumors.[54–56] It is only rarely positive in other malignancies, which include those of the prostate (10%), ovary (4%), stomach (5%), lung (6%), kidney (3%), and bladder (2%).[54,55] When salivary gland, skin adnexal, and prostatic adenocarcinomas are excluded from analysis, a positive immunoreaction with GCDFP-15 is 98% to 99% specific for breast origin.[54,55] A negative result, however, does not exclude a breast origin since, as noted above, a significant proportion of mammary adenocarcinomas do not express GCDFP-15.

Cytokeratins 7 and 20

The Cytokeratin (CK) 7 and 20 profile is not useful for distinguishing among breast, nonmucinous pulmonary, and nonmucinous ovarian adenocarcinomas, as these are all typically CK7+/CK20− (Fig. 9B, C; Table 4).[57,58] Unlike breast carcinomas, though, the majority of gastrointestinal, pancreatobiliary, and mucinous ovarian adenocarcinomas are CK20+. A CK7–/CK20+ profile is highly suggestive of colorectal origin;[57,58] only isolated breast carcinomas have a similar staining pattern.[57,59] The majority of gastric carcinomas also express CK20, with a CK7−/CK20+ pattern in 33% to 37% and a CK7+/CK20+ pattern 13% to 38% of tumors.[57,58,60] Approximately two thirds of pancreatobiliary carcinomas are CK7+/CK20+[57,58] as are up to 93% of mucinous ovarian carcinomas,[57,60,61] but only a small percentage (up to 11%) of breast carcinomas show this double-positive immunoprofile.[60]

Although many mucinous carcinomas from different sites overlap in their immunohistochemical profiles, often being CK7+/CK20+,[60–63] the majority of mucinous breast carcinomas appear to follow the CK7+/CK20− expression pattern of their nonmucinous counterparts.[57,59,64] The rare mucinous cystadenocarcinoma of the breast is also CK7+/CK20−.[65] Signet-ring cell carcinomas, in particular, raise the possibility of metastatic disease, and cytokeratin immunostains may shed some light on their site of origin. In one study, all 22 gastrointestinal signet-ring cell carcinomas were CK20+, compared with only 2 of 79 breast lobular carcinomas.[66] However, CK20 expression is not a uniform feature of gastrointestinal signet ring cell carcinomas, as another study found that 44% of gastric signet ring cell carcinomas were CK7+/CK20−, the same profile seen in the majority of breast carcinomas.[67] Overall, the presence of CK20 positivity in an adenocarcinoma is highly suggestive of non-breast origin but must be considered in conjunction with the clinical history, morphology, and other immunohistochemical stains.

TABLE 4. Predominant CK7/CK20 Profiles of Various Adenocarcinomas[57,58,60,61]

Immunoprofile	Tumor Type	% With Profile
CK7+/CD20−	Breast adenocarcinoma, ductal and lobular	82–96
	Pulmonary adenocarcinoma	74–90
	Nonmucinous ovarian adenocarcinoma	93–100
	Endometrial adenocarcinoma	80–100
CK7−/CK20+	Colorectal adenocarcinoma	75–95
CK7+/CK20+	Pancreatic adenocarcinoma	48–65
	Mucinous ovarian adenocarcinoma	44–93
CK7−/CK20−	Prostatic adenocarcinoma	62–100

Estrogen Receptor

Estrogen receptor (ER) is commonly expressed in breast and gynecologic malignancies. In a study of metastatic adenocarcinoma in body fluids, the sensitivity and specificity for ER in discriminating breast adenocarcinoma from other adenocarcinomas was 52% and 72%, respectively.[68] Kaufmann et al reported a sensitivity and specificity of 63% and 95%, but in their study only 34% of ovarian tumors were positive for ER.[10] Most studies have found only rare to no expression of ER in lung carcinoma,[55,68–71] although a single study reported ER expression in up to 80% of primary lung carcinomas using the 6F11 clone.[72] The latter results have not been replicated by others.[55,68,71] Some gastric carcinomas are also reported to express ER by standard immunohistochemical evaluation.[71,73,74] Overall, an ER-positive tumor is most likely to be of breast or gynecologic origin. Distinction between these two sites then relies upon clinical history, morphology, and selected use of other immunohistochemical stains, such as GCDFP-15 and Wilms' tumor-1.

Wilms' Tumor-1

Wilms' tumor-1 (WT-1) is a transcription factor that is strongly expressed in the nuclei of 88% to 100% of extrauter-

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ine serous carcinomas and 82% of ovarian transitional cell carcinomas.[68,75–80] In contrast, 93% to 100% of breast carcinomas are negative for nuclear WT-1.[68,81,82] Although a single study found WT-1 positivity in 57% of breast carcinomas evaluated by immunohistochemistry,[20] this result has not been replicated.[68,81,82] WT-1 therefore appears to be a promising marker for distinguishing breast from ovarian serous or transitional cell carcinoma.[68] WT-1 is not a general marker for ovarian surface epithelial-stromal tumors, however, as it is only weakly positive or completely negative in mucinous, clear cell, and endometrioid subtypes of ovarian adenocarcinoma.[68,75,76,78,80] Nearly all other carcinomas examined to date have been negative for WT-1.[68,76,77,81,83,84] Although one report demonstrated positivity in 15% of lung adenocarcinomas,[85] the majority of studies have shown uniform WT-1 negativity in these tumors.[68,81,84,86] This contrasts with 72% to 95% positivity in mesotheliomas; thus, WT-1 also appears to be a useful marker for discriminating between lung adenocarcinoma and mesothelioma.[81,83,84,86–88] Only one case each of melanoma and renal cell carcinoma has been reported to express nuclear WT-1.[83] Poor tissue fixation may result in false-negative results.[85]

Thyroid Transcription Factor-1

TTF-1 is a useful marker for pulmonary adenocarcinoma and thyroid neoplasms.[89] Nuclear staining is considered positive, and cytoplasmic staining is disregarded for diagnostic purposes.[90] The majority of studies report that 57% to 76% of pulmonary adenocarcinomas are positive for TTF-1,[90–95] although in one study only 27% were positive.[96] The experience in the cytology literature has been variable, with 19% to 79% of pulmonary adenocarcinomas demonstrating TTF-1 expression.[97–101] It appears that pulmonary mucinous adenocarcinomas, particularly mucinous bronchioloalveolar carcinomas, are largely negative for TTF-1.[92,93,95] Pulmonary signet-ring cell carcinomas are often positive, although only a handful of cases have been tested as these are rare tumors.[102] Expression of TTF-1 ranges from 0% to 38% in pulmonary squamous cell carcinomas and 0% to 26% in pulmonary large cell carcinomas.[89] It is positive in the majority of pulmonary small cell carcinomas, but it is also positive in up to 80% of extrapulmonary small cell carcinomas.[89,103,104] The specificity for lung origin is nearly 100% when small cell carcinomas and thyroid neoplasms are excluded, as nearly all other carcinomas are negative for TTF-1.[90] No breast carcinomas have been positive to date,[91,92,93,98,99,100,101,105] and only very rare gastric, colonic, and endometrial adenocarcinomas have been reported to stain for nuclear TTF-1.[91,94] As long as thyroid neoplasms and small cell carcinoma are morphologically excluded, a positive reaction with TTF-1 strongly supports a lung origin. TTF-1 is of limited diagnostic utility in the evaluation of mucinous adenocarcinomas, however, since most pulmonary and extrapulmonary tumors of this type are negative.

CEA and CA-125

Although CEA is often considered a marker of colonic and pulmonary adenocarcinomas and CA-125 a marker of ovarian adenocarcinoma, both of these can be positive in a significant proportion of breast adenocarcinomas. Up to 40% of breast adenocarcinomas express CEA and up to 23% express CA-125,[56] therefore limiting the specificity of these two antigens in determining site of origin. However, a negative result with CEA or CA-125 generally favors a noncolonic or nonovarian origin, respectively.[56,106]

COMMON PROBLEMS IN DIFFERENTIAL DIAGNOSIS

None of these markers is entirely site-specific, and a panel of antibodies is recommended when trying to determine the origin of a carcinoma. The choice of antibodies should be guided by the specific differential diagnosis raised by evaluation of hematoxylin and eosin-stained slides. The following comments address immunohistochemical stains that are most informative in common diagnostic situations. These studies are only suggestive or supportive of certain sites of origin and must be considered in the context of the clinical presentation, history, and morphology.

Breast Versus Lung

Discriminating between breast and pulmonary adenocarcinoma is a common problem in the evaluation of solitary lung lesions in patients with a history of breast cancer and in the workup of metastases of unknown primary. The most useful markers are GCDFP-15 and TTF-1 (Fig. 10). A positive reaction for GCDFP-15 is strongly suggestive of a breast primary, but a negative reaction is noninformative. TTF-1 reactivity is strongly suggestive of a lung primary, but a negative reaction does not exclude lung origin.

Breast Versus Ovary

Breast and ovarian malignancies are common in the same patient population, particularly in those women who harbor BRCA mutations. The most useful markers to distinguish between the two malignancies are GCDFP-15 and sometimes WT-1 (Fig. 11). A positive reaction for GCDFP-15 is consistent with a breast primary, but a negative reaction is noninformative. WT-1 is useful for distinguishing breast from ovarian serous or transitional cell carcinoma, with a positive reaction supporting an ovarian primary and a negative reaction favoring a breast primary. WT-1 is of limited utility in differentiating breast carcinoma from ovarian mucinous, clear cell, or endometrioid carcinoma, since all are largely negative for WT-1. In these cases, clinical information and morphology must be relied upon. Ovarian mucinous cystadenocarcinoma is not commonly in the differential diagnosis of breast carcinoma, but CK20 reactivity in this particular setting supports an ovarian

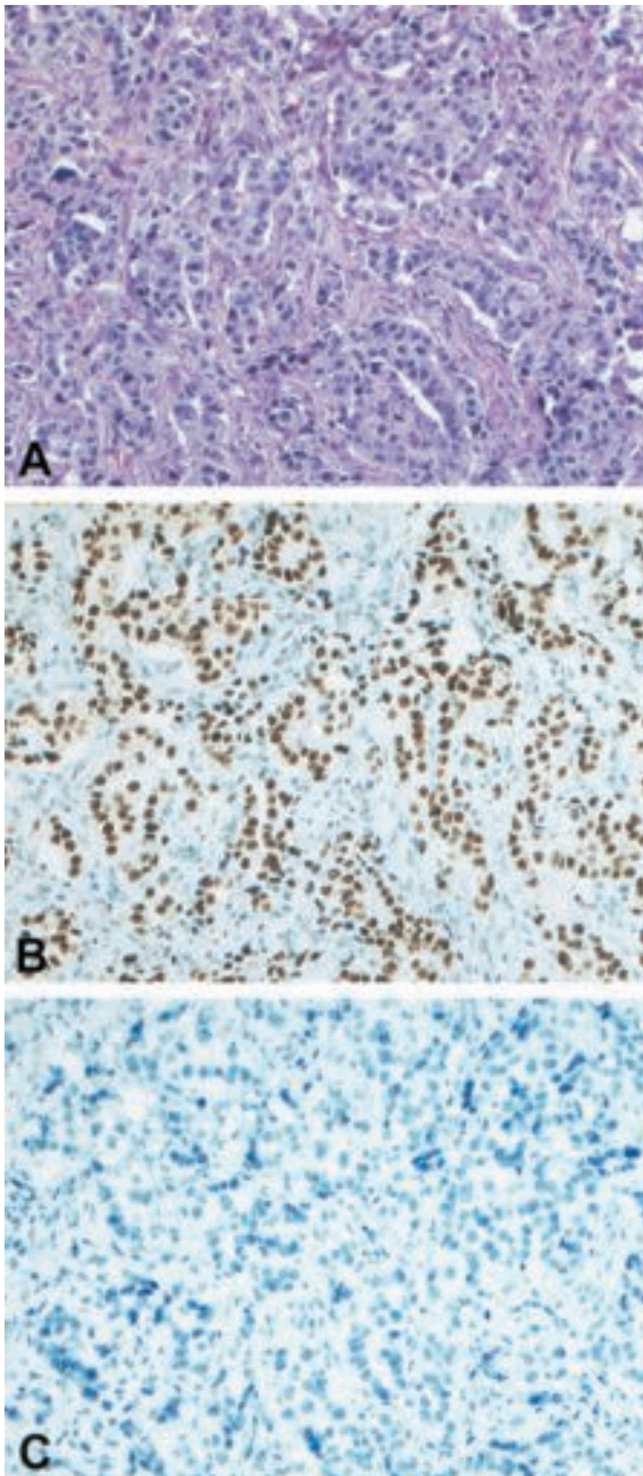

FIGURE 10. Immunohistochemistry in distinction of nonmammary versus mammary carcinoma in patients with history of both. **A**, Metastatic adenocarcinoma in the humerus of a patient with a lung mass and a history of invasive ductal carcinoma. Nuclear reactivity for thyroid transcription factor-1 (**B**) and a negative reaction for gross cystic disease fluid protein-15 (**C**) strongly support a lung origin.

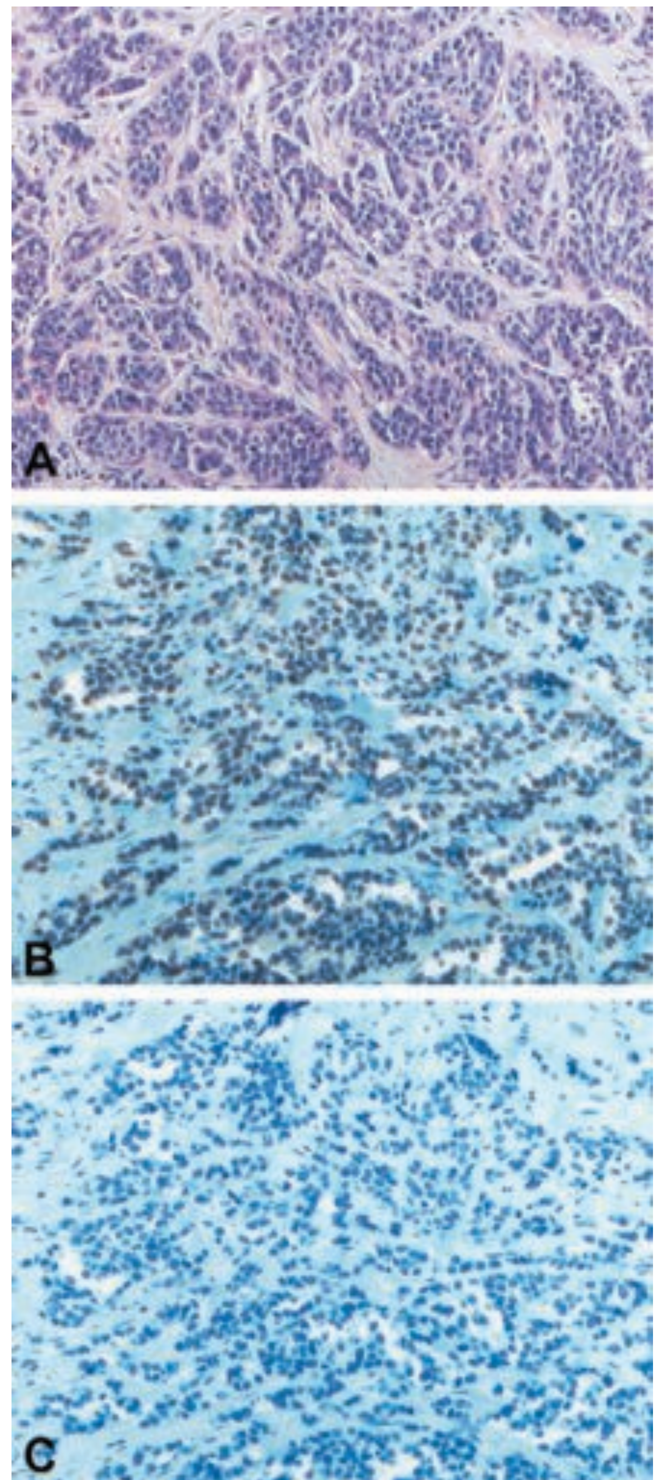

FIGURE 11. Immunohistochemistry in distinction of primary versus metastatic carcinoma in the breast. **A**, Breast mass in a patient with a history of ovarian serous carcinoma. The morphologic features are compatible with poorly differentiated breast carcinoma as well as high-grade serous carcinoma. Nuclear reactivity for Wilms' tumor-1 (**B**) and a negative reaction for gross cystic disease fluid protein-15 (**C**) support an ovarian origin.

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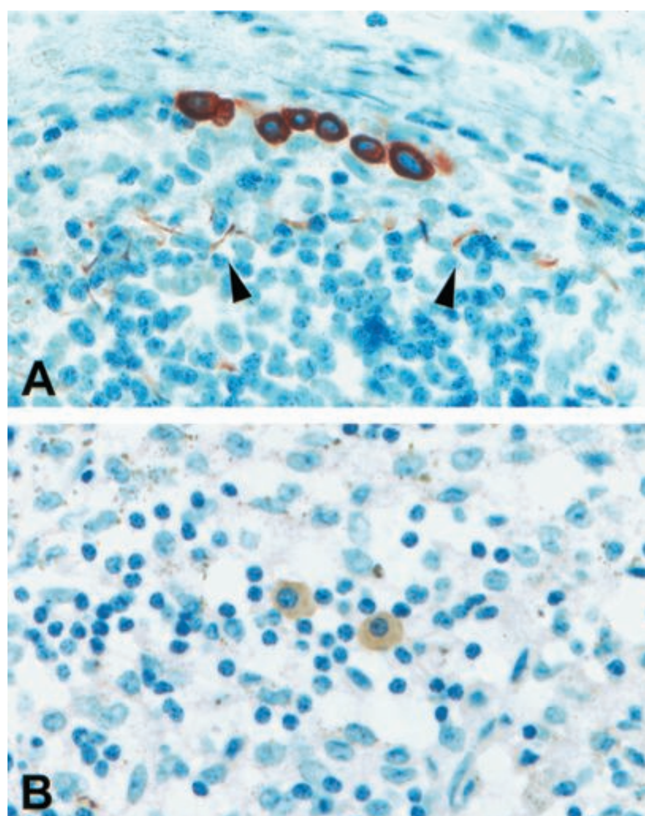

FIGURE 12. Cytokeratin cross-reactivity in lymph nodes. **A**, The reticulum cells show a wispy linear pattern of staining (arrowheads), in contrast to the strong cytoplasmic staining of the metastatic lobular carcinoma cells above. **B**, Plasma cells may stain weakly for cytokeratin. Their faint reactivity and nuclear morphology allow one to distinguish them from tumor cells.

origin. A positive reaction for CA-125 is not helpful in distinguishing breast from ovarian carcinoma, but a negative reaction tends to favor breast origin.

Breast Versus Stomach

Signet-ring cell carcinomas often raise the differential diagnosis of a breast versus a gastric primary. The most useful markers are GCDFP-15, ER, and CK20. A positive reaction for GCDFP-15 is consistent with a breast primary, but a negative reaction is noninformative. An ER+ signet-ring cell carcinoma is more likely to be of breast origin, and a CK20+ tumor is more likely to be of gastric origin. A CK20+/ ER+ signet-ring cell carcinoma is more likely to be of breast origin.[66] A negative reaction for all three of these antibodies is noninformative.

Breast Versus Melanoma

Metastatic melanoma to the breast can be particularly deceptive, mimicking a high-grade ductal carcinoma with a diffuse growth pattern. The presence of melanin pigment is diagnostically helpful, but some lesions are amelanotic. Positive reactions for HMB-45 and MART-1, and a negative reaction for cytokeratin, are diagnostic of melanoma in this setting. S-100 is of limited value, as both melanoma and breast carcinoma can be positive.

Caveat Concerning Immunoprofiles of Primary and Metastatic Tumors

Most metastatic tumors retain the same immunoprofile as their primary tumors, but in unusual instances expression of an antigen may be lost or gained. In these cases, comparison with the morphology of the original tumor is crucial.

SENTINEL LYMPH NODE EVALUATION

Sentinel lymph node biopsy is an accurate predictor of regional lymph node status[107] and is rapidly replacing axillary lymph node dissection in the management of early stage breast cancer. Because sentinel nodes are more likely to contain metastatic disease than non-sentinel nodes, and because their

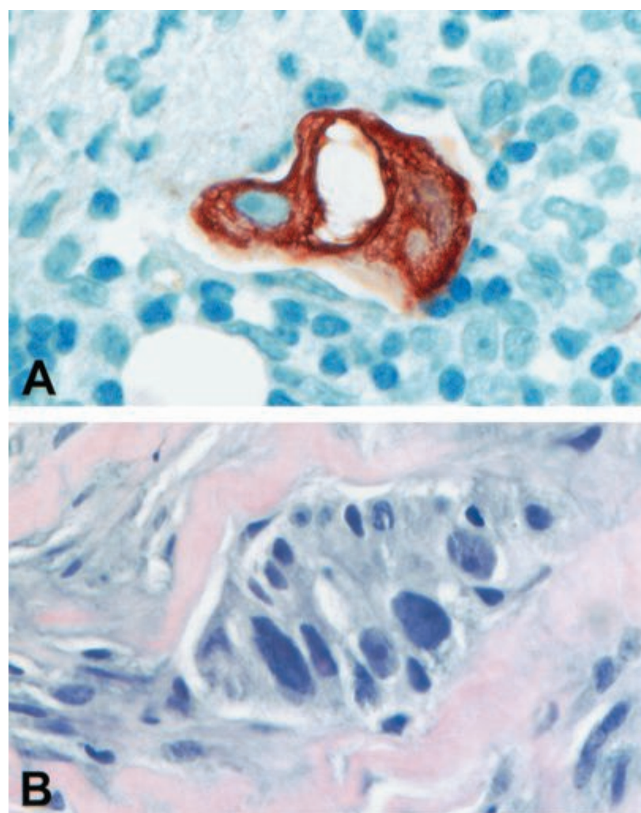

FIGURE 13. Isolated tumor cells detected by cytokeratin immunostains. **A**, Tumor cells in a lymph node demonstrate strong, fibrillar cytokeratin staining of their cytoplasm with clearly demarcated, nonreactive nuclei. **B**, The nuclear features are similar to those of the primary tumor.

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status determines whether or not completion axillary dissection is performed, many pathologists go beyond the traditional single hematoxylin and eosin-stained section when evaluating sentinel nodes. Multiple step levels, cytokeratin immunostains, and/or molecular diagnostics are all variously used. This has led to an increased detection of micrometastases and isolated tumor cells.[107,108] Because the prognostic significance of metastases ≤0.2 cm detected only by immunohistochemical or molecular methods is not established, the College of American Pathologists currently recommends classification of sentinel nodes by hematoxylin and eosin-stained slides, and a single microscopic section is considered sufficient for evaluation.[109] Nonetheless, at some institutions, including our own, immunohistochemistry is routinely used in the evaluation of sentinel lymph nodes. Long-term outcome studies will determine whether or not this type of evaluation should become part of the standard of care.

The minimal objective in the analysis of sentinel nodes is the detection of metastases larger than 0.2 cm (macrometastases). All sentinel nodes should be serially sectioned as close to 0.2 cm in thickness as possible and entirely submitted for histologic evaluation. It is debatable whether sectioning parallel or perpendicular to the long axis is more likely to detect metastases.[108,110,111] In our practice, we initially evaluate all sentinel nodes with one hematoxylin and eosin-stained section per block. If this is negative for metastatic disease, we then evaluate three cytokeratin-immunostained levels per block. Cytokeratin immunostains are more sensitive for the detection of small volume disease, serve as a quality assurance mechanism against missed metastases, and are ultimately more time-efficient to evaluate than hematoxylin and eosin-stained levels. Current American Joint Committee on Cancer staging criteria include a special identifier "i+" to indicate when metastatic deposits ≤0.2 cm are detected only on immunohistochemical stains.[112,113] Metastases larger than 0.2 cm are considered N1 regardless of the method of detection.

If one chooses to use cytokeratin immunostains in the evaluation of sentinel nodes, it is important to be aware of several potential pitfalls in interpretation. Reticulum cells are frequently positive for cytokeratin, particularly when Cam5.2 or pan-cytokeratin is used,[114,115] but much less so when AE1/AE3 or AE1 alone is used.[114–116] The reticulum cells show a fine, linear pattern of staining and are interspersed among the lymphocytes (Fig. 12A). They lack the more abundant cytoplasm, atypical nuclei, and tendency to cluster characteristic of tumor cells. Plasma cells may also weakly stain for cytokeratin in up to 10% of cases (Fig. 12B),[114,115] and in very rare cases histiocytes may show a faint blush when stained for cytokeratin. This cross-reactivity emphasizes the need to evaluate the morphology of any cytokeratin-positive cells. True tumor cells are typically located in the subcapsular or interfollicular sinuses, are round or polygonal in contour, have strong fibrillar cytokeratin staining of their cytoplasm, and have clearly negative nuclei that are morphologically similar to the nuclei of the primary tumor (Fig. 13).

False-negative results using cytokeratin immunostains have also been reported. In a study by Weaver et al, missed metastases ranged from 0.001 to 0.003 cm in size, were overlooked on light microscopy, and were detected by an automated image analysis system.[117] Factors such as human fatigue, incomplete section screening, and variable staining were thought to contribute to missed metastases on light microscopy.

In summary, immunohistochemistry can be a powerful tool for resolving many common diagnostic problems in breast pathology. Its successful use depends upon an understanding of the appropriate situations in which to use certain antibodies, as well as an understanding of the limitations of those antibodies. Because of the complex nature of breast pathology, attention should be paid to the cytologic and architectural features of immunoreactive and nonreactive cells. Careful correlation with the histologic findings will help one avoid many of the pitfalls associated with the interpretation of these stains.

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