The Role of Immunohistochemistry in Breast Pathology

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The current practice of breast pathology is virtually unthinkable without the use of immunohistochemistry (IHC) [1, 2]. However, this indispensable diagnostic technique is burdened by numerous issues. This chapter is intended to outline the main uses of IHC in breast pathology. Furthermore, the problems and pitfalls inherent in the use of this technique are briefly discussed. Some common histopathological dilemmas—including usual *versus* atypical hyperplasia, benign *versus* malignant papillary lesions, and pseudoinvasive *versus* microinvasive carcinoma, etc. are considered. Also, issues relating to sentinel lymph node assessment, "surrogate" molecular classification, and workup of metastatic carcinomas in breast are briefly discussed. The role of immunostaining in assessing prognostic and predictive markers (including ER and HER2) of breast carcinoma are also concisely reviewed.

In general, the main advantages of IHC are as follows: (a) ready applicability to routinely processed, formalin-fixed and paraffin-embedded tissues; (b) ability to correlate with histological sections; and (c) increasing sensitivity and specificity of antibodies.

IHC technique is complex, and the problems inherent in its practice are complicated—and beyond the scope of this chapter [3]. However, in sum, meticulous attention to standardized procedures, ubiquitous use of controls, and regular verification of reactivity patterns of various antibodies ensure successful technical application of IHC to breast pathology.

There are several causes of diagnostic misinterpretation in IHC preparations. The most important of these are (a) cross-reactivity of antibodies (e.g., smooth muscle actin reacts not only with myoepithelial cells but also with myofibroblasts); (b) entrapment of normal tissues amid the lesion (e.g., presence of residual E-cadherin-positive ductal cells amid lobular carcinoma in situ); (c) release of protein from normal cells after invasion by carcinoma (e.g., diffusion of myoglobin released from injured skeletal muscle fibers after invasion by breast carcinoma cells, resulting in myoglobin-positivity of the latter); and (d) unfamiliarity with specific patterns of immunoreactivity (e.g., "aberrant" E-cadherin staining in pleomorphic lobular carcinoma in situ).

Despite the near-ubiquitous use of immunohistochemistry and increasing sophistication of the technique, the significance of routine histopathological evaluation of H&E-stained sections cannot be underestimated. Ideally, the examination of a contemporaneous H&E section should be standard practice whenever immunostains are requested for diagnostic purposes [4].

All benign glands in the breast (from acini to lactiferous ducts) have three layers. The luminal layer is comprised of a single layer of epithelial cells. The next layer is the myoepithelial layer. Below the myoepithelial cell layer is the basement membrane (Fig. 17.1).

The luminal cells are immunoreactive for the so-called "luminal" keratins (CK7, CK8, CK18), E-cadherin, ER, and PR. Myoepithelial cells are positive for so-called "basal" keratins (CK5, CK5/6, CK14, CK17) and are also positive for p63, p40, smooth muscle actin (SMA), calponin, desmin, and smooth muscle myosin-heavy chain (SMM-HC). Immunostains for basement membrane, collagen IV and laminin can be difficult to assess since both of these immunostains can cross-react with other stromal elements. As an alternative, reticulin histochemical stain can be used to highlight the basement membrane.

The three aforementioned layers are almost always present in the normal breast and in nearly all benign conditions. The myoepithelial cell layer is inexplicably absent in some benign apocrine glands of the breast [5, 6]. The myoepithelial layer is also absent in microglandular adenosis (MGA)—a peculiar "triple-negative" lesion of the breast. There is emerging evidence that MGA is a low-grade invasive carcinoma [7].

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Hormone receptors, i.e., estrogen receptor (ER) and progesterone receptors (PR), are variably positive in the epithelial cells (approximately 5–25%, moderate-to-strong) of benign breast glands. Myoepithelial cells are invariably ER (−) and PR (−), whereas myofibroblasts are typically ER (+) and PR (+). The proportion and intensity of hormone receptor staining in "normal" breast epithelial cells depend mainly upon the patient's age and menstrual phase. Certain types of breast epithelial cells are relatively more predictable in this regard: columnar cells are almost always strongly and diffusely ER (+) and PR (+), and apocrine metaplastic cells are almost always ER (−) and PR (−) [8]. Benign and malignant apocrine cells are typically also positive for androgen receptors (AR) [9].

The mammary stroma comprises mainly adipose tissue, myofibroblasts, fibroblasts, and blood vessels. Smooth muscle bundles are present around lactiferous ducts of the nipple and hair follicles of overlying skin. The individual components of the stroma show immunohistochemical reactivities characteristic of each structure (e.g., myofibroblasts are positive for CD34, etc.).

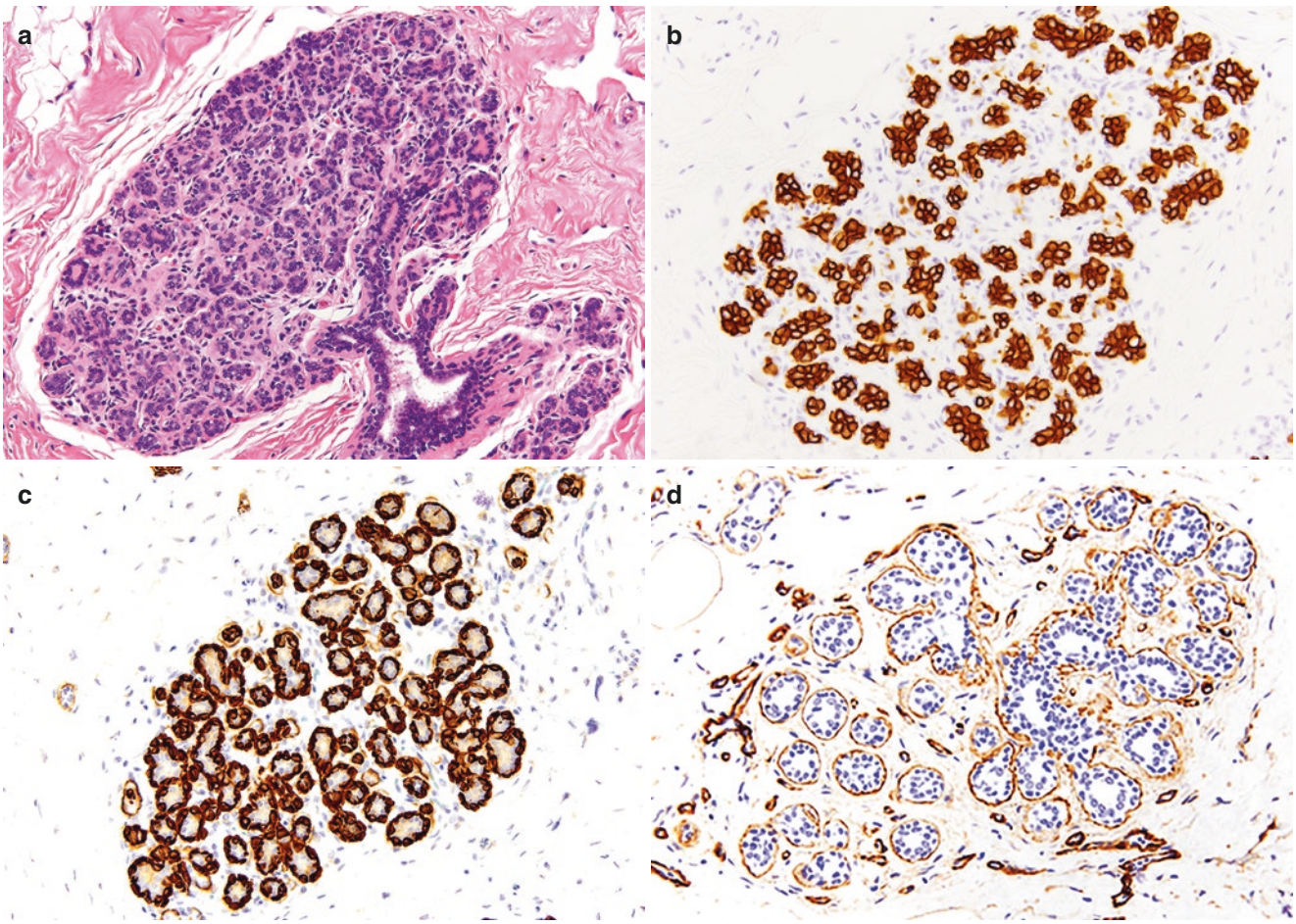

Fig. 17.1 Structure of inactive terminal duct lobular unit (TDLU) of breast. (**a**) This TDLU is from an adult female. Almost all benign glands in the breast have three layers, which may not be evident on routinely stained sections (H&E). (**b**), The luminal layer comprises of a single layer of cytokeratin-positive epithelial cells (CK AE1/AE3). (**c**) The abluminal layer comprises of smooth muscle myosin-positive myoepithelial layer (SMM). (**d**) Below the abluminal myoepithelial layer is the linear basement membrane, which stains for reticulin, collagen IV, and laminin (laminin)

17.1 Usual Ductal Hyperplasia, Atypical Ductal Hyperplasia and Ductal Carcinoma In Situ

The correct interpretation of proliferative epithelial lesions is possibly the most common diagnostic dilemma in everyday pathology practice. Although there are well-established criteria for various degrees of epithelial proliferation (including for usual, florid, and atypical hyperplasia, as well as for low-grade intraductal carcinoma), these criteria can be rather difficult to apply in practice. Immunostains—especially for high molecular weight-cytokeratins (HMW-CK) and estrogen receptors (ER)—can be helpful in this regard. Proliferation marker (i.e., Ki-67) is unhelpful in the differential diagnosis of proliferative epithelial lesions.

HMW-CK (i.e., CK5, CK5/6 and 34BE12/K903), can be regarded as a marker of epithelial "differentiation," and is positive in benign breast epithelium and in UDH. Conversely, atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS) show "loss of differentiation" and consequently lose reactivity with HMW-CK. HMW-CK positivity is observed in most epithelial cells of florid ductal hyperplasia in a heterogeneous pattern that is described as "mosaic-like" (Fig. 17.2). Furthermore, florid ductal hyperplasia is usually focally and weakly ER (+). In more than 90% of cases, ADH and low-grade DCIS are strongly and diffusely ER (+) and HMW-CK (−) (Fig. 17.3). ADH and low-grade DCIS cannot be distinguished based on immunoreactivity patterns with HMW-CK and ER—therefore, established histopathological criteria must be used to render the diagnosis [10–12]. High-grade DCIS (including the so-called "basal-like" DCIS) does not usually present a diagnostic problem. HER2 is positive (3+, on a scale of 0–3+) and ER is negative in a large proportion of high-grade DCIS.

HMW-CK immunostaining is *not* helpful in the differential diagnosis of usual ductal hyperplasia (UDH) versus atypical ductal hyperplasia (ADH) in three specific settings: (a) proliferative columnar cell lesions; (b) proliferative apocrine lesions; and (c) some papillary lesions. Furthermore, columnar cell change and columnar cell hyperplasia are almost always strongly ER (+), and nearly all apocrine lesions (including atypical apocrine hyperplasia and apocrine DCIS) are usually ER (−). In these situations, diagnostic evaluation of the lesions on H&E-stained sections must be relied upon.

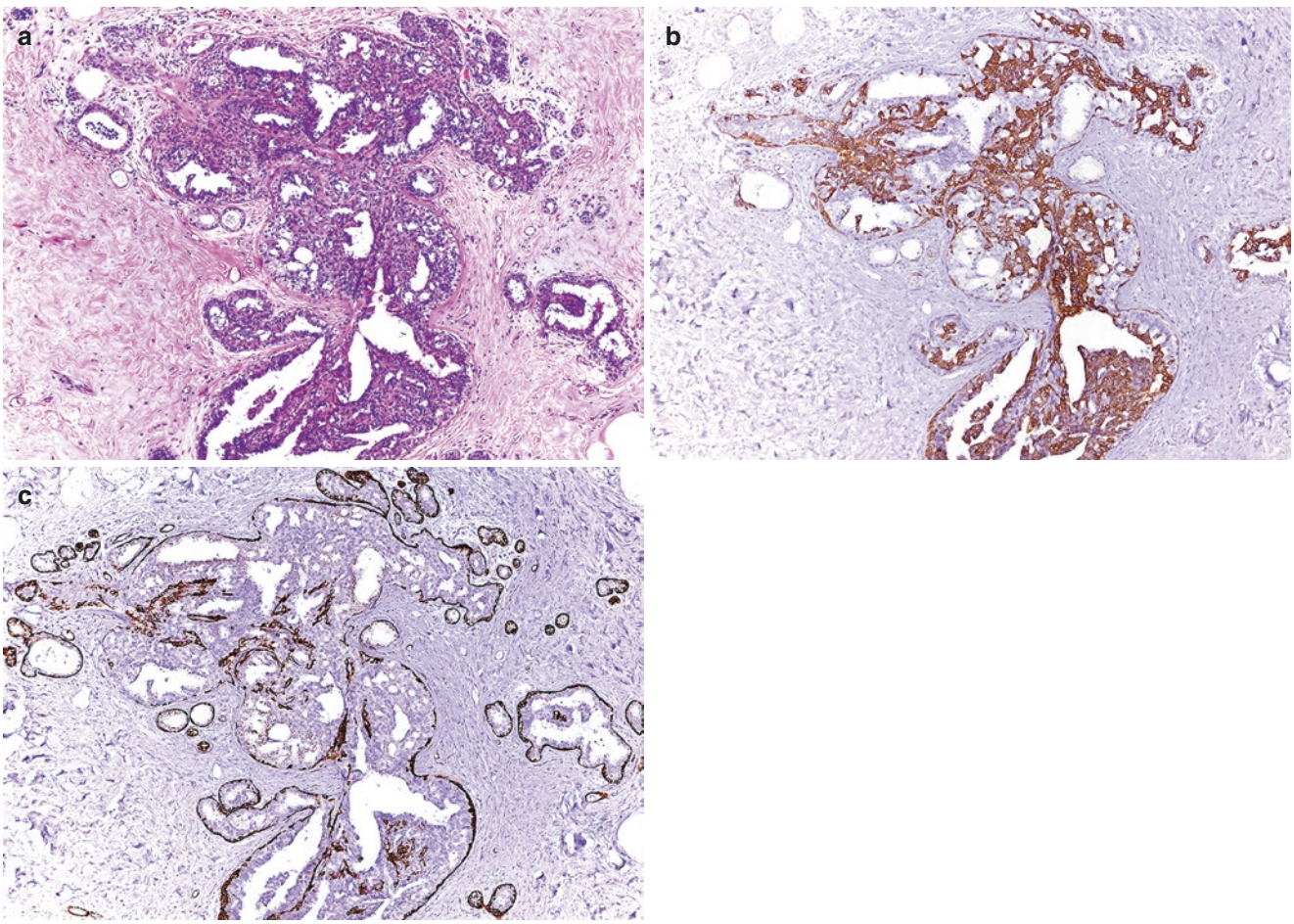

Fig. 17.2 Florid ductal hyperplasia. (**a**) Florid hyperplasia is characterized by exuberant epithelial proliferation (H&E). (**b**) CK 5/6 positivity is observed in some epithelial cells of florid ductal hyperplasia in a heterogeneous pattern that is described as "mosaic-like" (CK 5/6). (**c**) Smooth muscle myosin highlights the presence of myoepithelial cells within and around florid hyperplasia (SMM)

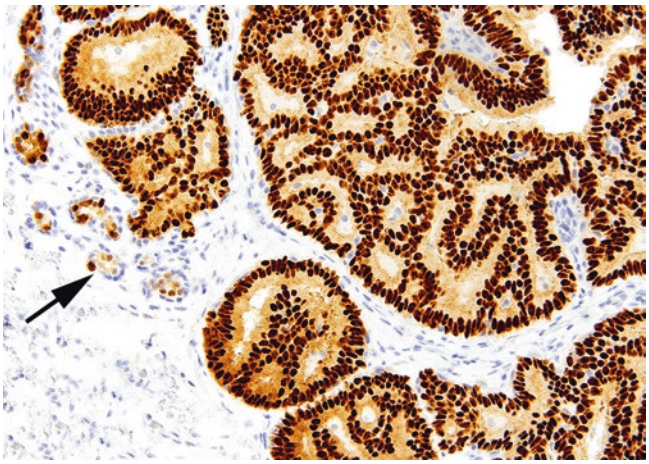

Fig. 17.3 Estrogen receptor (ER) in ductal carcinoma in situ (DCIS). A low-grade DCIS is characterized by strong and diffuse ER-positivity. Note patchy and weaker ER-staining (arrow) in benign glands (ER)

17.2 Lobular Versus Ductal Differentiation of Epithelial Lesions

The distinction between "lobular" and "ductal" carcinomas has clinical implications—especially with regard to differences in anatomical distribution, disease management, risk-stratification, and metastatic pattern. IHC, specifically E-cadherin, can play a vital role in this differential diagnosis. Cadherins (of which E-cadherin is among the best-studied) are a group of transmembrane glycoproteins located in desmosomes, and form complexes with catenins. The latter control several processes including cell migration, differentiation, and proliferation.

E-cadherin is present in benign ductal structures and ductal lesions, and is absent in lobular lesions (Fig. 17.4). Notably, E-cadherin positivity in ductal lesions is present only along the cytoplasmic membrane of the lesional cells. All ductal lesions (including usual ductal hyperplasia, ADH, DCIS, and invasive ductal carcinoma, the latter being called now as invasive carcinoma of NST type) show immunoreactivity with E-cadherin. Lobular lesions (including atypical lobular hyperplasia [ALH], lobular carcinoma in situ [LCIS] and invasive lobular carcinoma) are negative for E-cadherin.

p120 catenin (generally referred to as p120) binds with E-cadherin to form a stable cadherin-catenin complex. This complex is essential for formation of intercellular junctions. Absence of E-cadherin explains the "loss of cohesiveness" in lobular lesions. When E-cadherin is absent, the cytoplasmic pool of p120 increases. It follows that in normal ducts and in ductal lesions, p120 shows cytoplasmic membrane staining, and in lobular lesions with absent or non-functional E-cadherin, p120 localizes within the cytoplasm (Fig. 17.5). Notably, p120 reactivity in ADH and DCIS is similar to that seen with E-cadherin, i.e., positivity is present along the cytoplasmic membrane; and, in lobular lesions, p120 localizes within the cytoplasm—and not along the cytoplasmic membrane. p120 enhances diagnostic accuracy by virtue of being a "positive" stain for lobular carcinoma. Use of E-cadherin and p120 together reduces the rate of equivocal E-cadherin staining [13].

Invasive lobular carcinoma of the classic and pleomorphic types, as well as LCIS of the classic, florid, and pleomorphic types, are all negative for E-cadherin. Rarely, "aberrant" E-cadherin immunoreactivity (i.e., granular "dot-like" cytoplasmic staining) can be encountered in some lobular lesions—particularly in pleomorphic variants of LCIS and invasive lobular carcinoma (Fig. 17.6) [14]. Other immunostains that have been used to distinguish lobular and ductal lesions, with questionable reliability, include beta-catenin (usually negative in lobular lesions) and HMW-CK (usually positive in lobular lesions in a distinctive "perinuclear" pattern). In practice, E-cadherin suffices to establish the diagnosis. p120 immunostain can be additionally evaluated in cases considered equivocal on E-cadherin. Occasionally, invasive

ductal carcinoma can display a lobular-like ("single-file") architectural pattern, at least focally. In most such cases, E-cadherin immunostain can unequivocally establish ductal differentiation (Fig. 17.7).

Rarely, LCIS can coexist with collagenous spherulosis; the resultant lesion can simulate cribriform type of intraductal carcinoma. E-cadherin immunostain can be particularly helpful in this regard [15].

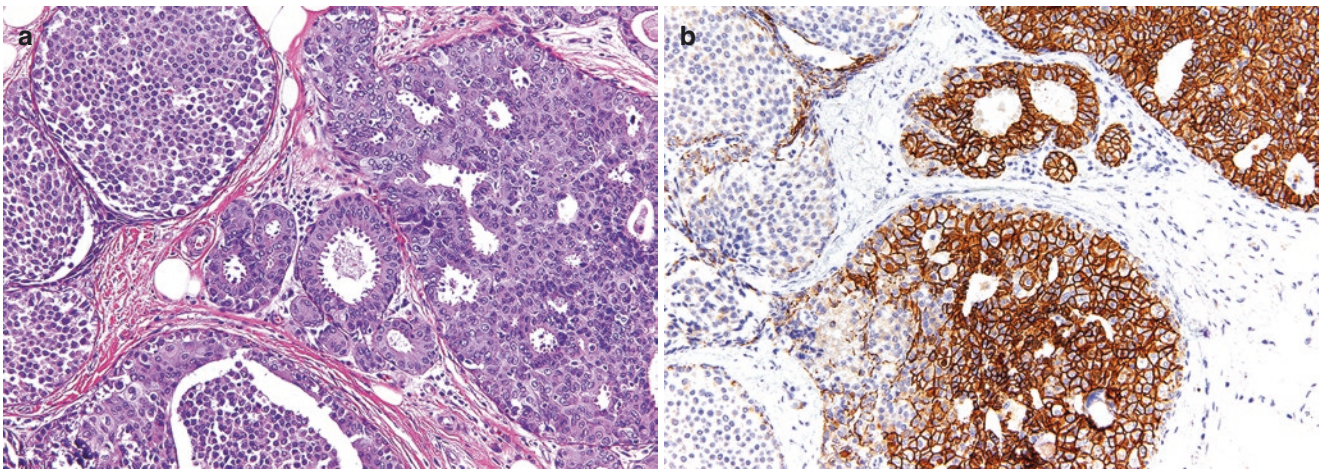

Fig. 17.4 Use of E-cadherin in diagnosing lobular carcinoma in situ (LCIS) and ductal carcinoma in situ (DCIS). (**a**) LCIS is evident on the left, and DCIS is seen on the right (H&E). (**b**) E-cadherin is negative in LCIS and positive in DCIS (E-cadherin)

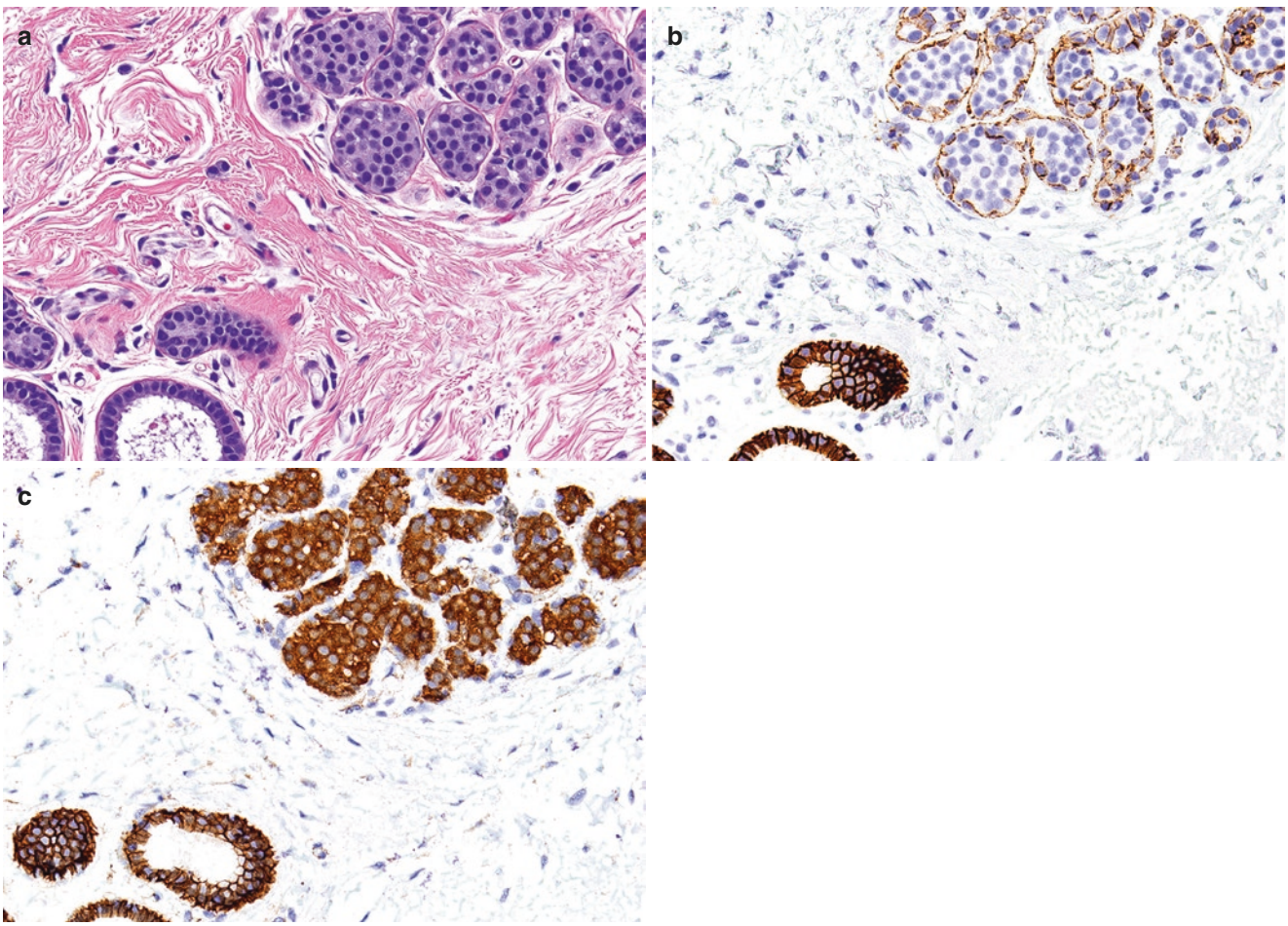

Fig. 17.5 Use of E-cadherin and p120 in diagnosing lobular carcinoma in situ (LCIS). (**a**) LCIS of classic type is seen on upper-right, columnar cell change is present on lower-left (H&E). (**b**) E-cadherin is negative in LCIS, and cystic columnar cell change is positive (E-cadherin). (**c**) p120 is seen to localize within the cytoplasm of LCIS cells, and is positive along the cytoplasmic membrane of columnar cell change (p120)

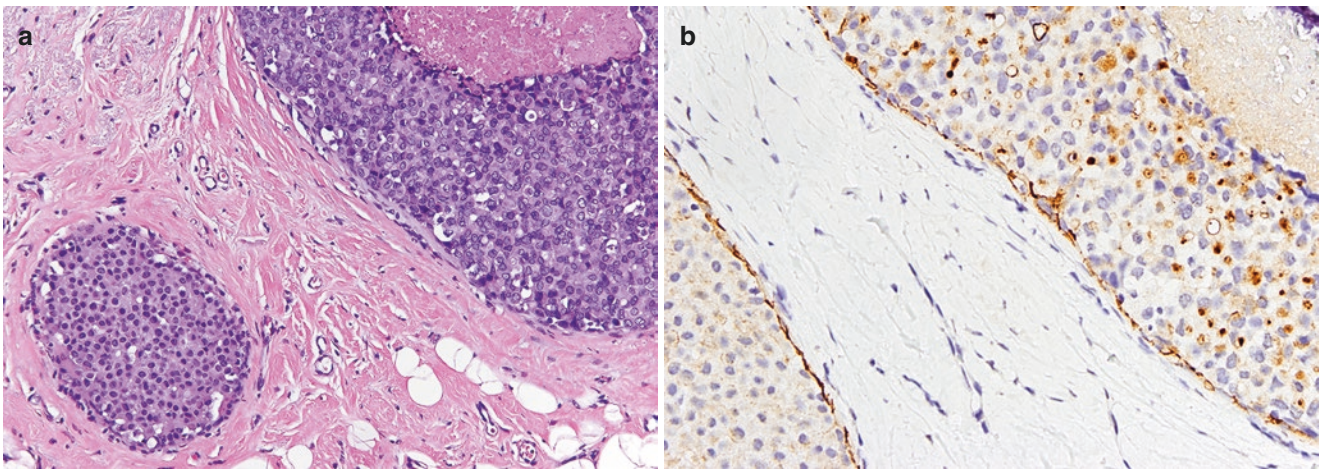

Fig. 17.6 E-cadherin staining in pleomorphic and classic types of lobular carcinoma in situ (LCIS). (**a**) LCIS of pleomorphic type (with "pleomorphic" high-grade nuclei) is seen on upper right, LCIS of classic type is present on lower left (H&E). (**b**) E-cadherin is negative in classic LCIS, and shows "aberrant" E-cadherin immunoreactivity (i.e., granular "dot-like" cytoplasmic staining) in pleomorphic LCIS (E-cadherin)

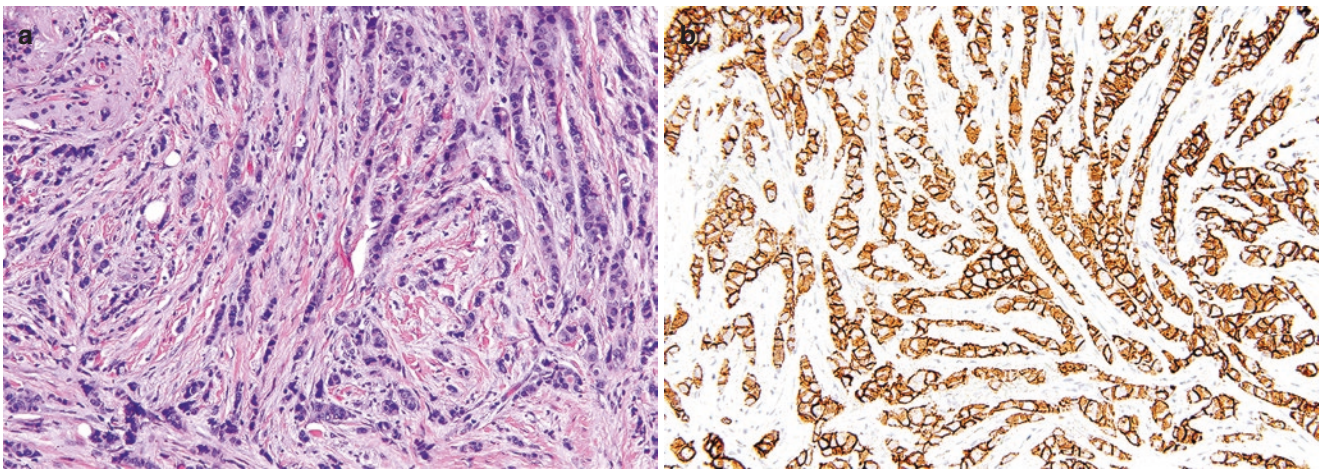

Fig. 17.7 E-cadherin staining in invasive ductal carcinoma. (**a**) Invasive ductal carcinoma with lobular-like ("single-file") architectural pattern (H&E). (**b**) E-cadherin shows positivity in the malignant cells—unequivocally establishing ductal differentiation thereof (E-cadherin)

17.3 Papillary Neoplasms

Immunostains can be helpful in the assessment of some, but not all, papillary lesions of the breast. It must be emphasized that the histological and cytological appearance of papillary lesions on H&E-stained sections is paramount to the diagnosis [16, 17].

Myoepithelial cells typically line fibrovascular stalks (papillae) within benign intraductal papilloma, and also line the perimeter (wall) of an intraductal papilloma. Myoepithelial cells can be diminished or absent in the papillae and/or in the walls of various types of papillary carcinomas. On this premise, IHC for myoepithelial cells (i.e., actin, CD10, calponin, myosin, p40, p63, etc.) are helpful in the evaluation of such lesions. Combination of IHC stains, such as p63 and myosin (in a dual stain, which combines nuclear and cytoplasmic reactivity within the myoepithelial cells) is helpful in demonstrating myoepithelial cells; and the addition of a cytokeratin immunostain to this combination (in a triple stain) can be even more helpful by highlighting the juxtaposition of epithelial and myoepithelial cells within a papillary lesion.

The two fundamental questions to be answered in the assessment of any papillary lesion are (a) Is carcinoma present? and (b) Is invasive carcinoma present?

Myoepithelial cells are uniformly present within the papillae and along the wall of all **benign intraductal papilloma** (Fig. 17.8). In benign papillomas, ER is only sporadically positive. HMW-CK can show a "mosaic-like" staining pattern in the hyperplastic epithelial cells of a benign papilloma.

In an **atypical papilloma**, there is focal ADH within the papilloma. The criteria for diagnosing ADH within a papilloma should be the same within a papilloma as outside it. The myoepithelial cells are usually diminished within the ADH portion of a papilloma, and are present all around the wall of the papilloma.

The criteria for diagnosing **focal DCIS within a papilloma** should be the same within a papilloma as outside of it. The myoepithelial cells are usually absent in the DCIS portion of a papilloma, but are present all around the perimeter (wall) of the papilloma.

Intraductal papillary carcinoma (i.e., papillary DCIS) can be diagnosed when the entire lesion is considered cytologically and histologically malignant, and there is no evidence of invasive carcinoma. The architectural pattern of the DCIS is usually entirely papillary, and sometimes there is a secondary cribriform growth pattern therein. The myoepithelial cells are typically present around the wall of the intraductal papillary carcinoma (Fig. 17.9).

Encysted, encapsulated, intracystic, and solid-papillary carcinomas are all circumscribed papillary carcinomas that usually (but not always) lack myoepithelial cells within the lesion, i.e., in the papillae, and may or may not lack myoepithelial cells in the wall, i.e., at the perimeter. In the absence of frankly invasive carcinoma, these carcinomas behave in an indolent manner. Encysted/encapsulated/intracystic and solid-papillary are terms that essentially imply *non-invasive papillary carcinoma*, and these terms have been used sometimes synonymously and occasionally interchangeably, although some differences have been described between these entities [18, 19]. Cyst formation is prominent in encysted/encapsulated/intracystic papillary lesions, and solid-papillary lesions are characterized by relatively "solid" epithelial proliferation. Often, the only clue to the solid-papillary nature of the lesion is the subtle presence of fibrovascular cores therein. Myoepithelial cells are usually absent within and around the perimeter of these lesions; however, occasionally rare myoepithelial cells can be identified on IHC (in either location). Neuroendocrine differentiation (as evidenced by CD56, chromogranin and synaptophysin immunoreactivity) can be encountered in approximately one-half of solid-papillary carcinomas of the breast.

In sum, myoepithelial cells can be demonstrated to be present around the perimeter of intraductal papilloma, atypical papilloma, papilloma with DCIS, and papillary DCIS. Myoepithelial immunostains show little or no reactivity within and around the perimeter of encysted/encapsulated/intracystic and solid-papillary carcinomas (Fig. 17.10).

The assessment of invasion in papillary carcinomas can be difficult. In general, non-invasive papillary carcinomas have smooth and rounded outer contours, and invasive papillary carcinomas have irregular and jagged outer contours (Fig. 17.11). There may be some degree of stromal reaction around some foci of invasive papillary carcinomas. As outlined above, the absence of myoepithelial cells at the perimeter of papillary carcinomas, as evidenced by IHC, cannot be regarded *per se* as being diagnostic of invasive carcinoma.

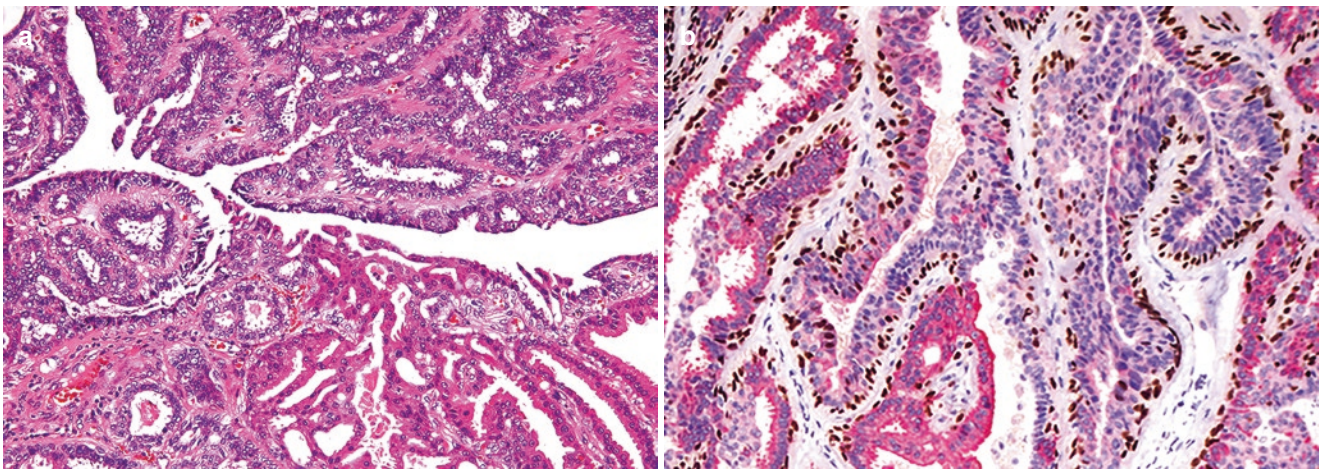

Fig. 17.8 Intraductal papilloma. (**a**) The lesion is characterized by relatively thick fibrovascular cores lined by bland epithelial cells, some of which exhibit apocrine metaplastic cells (H&E). (**b**) Myoepithelial cells are decorated by p63 immunostain (cells with brown staining nuclei) and epithelial cells are stained by cytokeratin immunostain (cells with red-staining cytoplasm); (p63 + CK AE1/AE3 double stain)

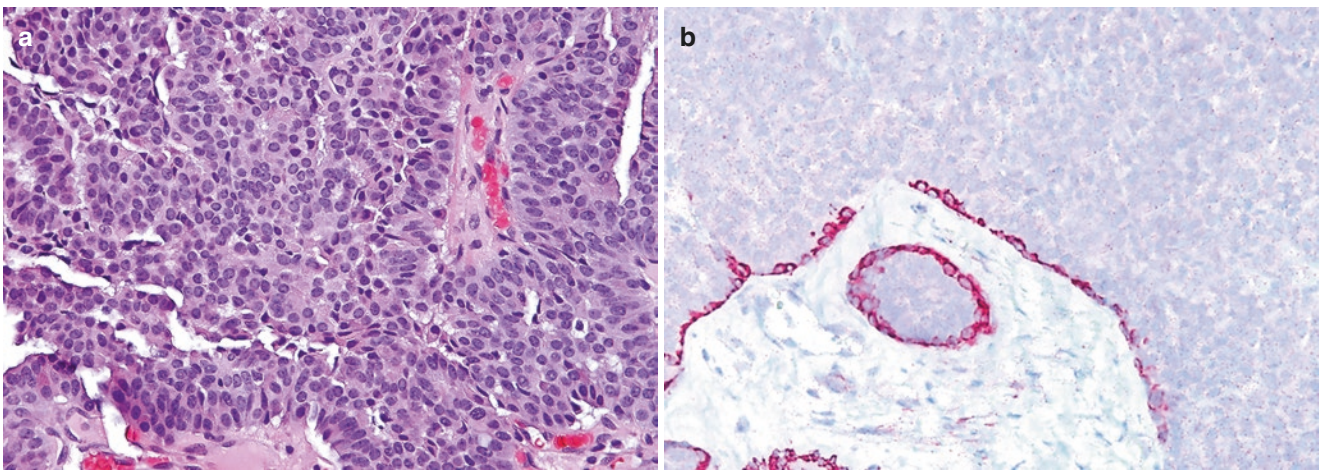

Fig. 17.9 Intraductal papillary carcinoma. (**a**) Intraductal papillary carcinoma (i.e., papillary DCIS) can be diagnosed when the entire lesion is considered cytologically and histologically malignant, and there is no evidence of invasive carcinoma. (**b**) Myoepithelial cells are present around the wall of the intraductal papillary carcinoma (Myosin)

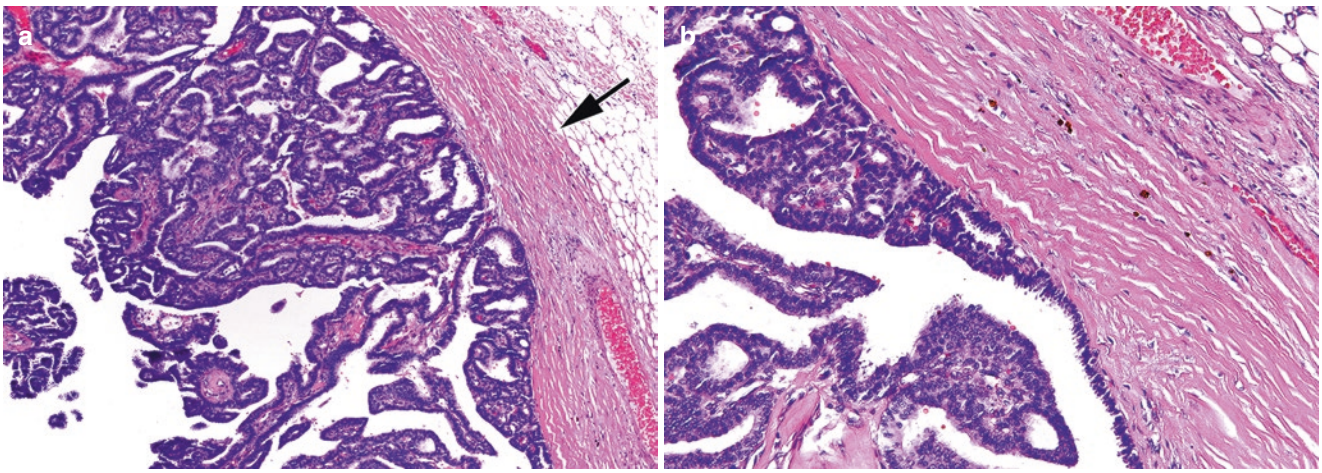

Fig. 17.10 Encapsulated papillary carcinoma. (**a**) The thick "capsule" is present around the papillary carcinoma (arrow). Immunostains for myoepithelial cells were negative (not shown) within and around the perimeter of these lesions. (**b**) Detail of the "capsule"

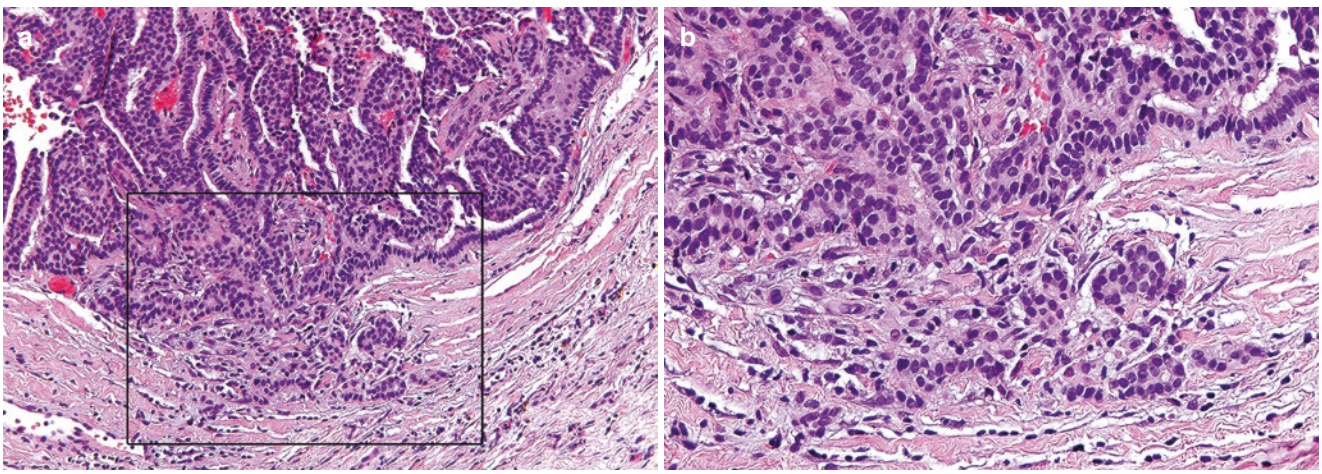

Fig. 17.11 Microinvasive papillary carcinoma. (**a**) The microinvasive papillary carcinoma has irregular and jagged outer contours (box). Noninvasive papillary carcinomas have smooth and rounded outer contours. (**b**) Detail of microinvasive carcinoma

17.4 Sclerosing Lesions

Because of its infiltrative appearance, sclerosing adenosis has a likelihood of being misdiagnosed as invasive carcinoma. The most important diagnostic feature of sclerosing adenosis is that retains a round configuration at low-power magnification. The lesion is more cellular centrally than peripherally, and the glands at the perimeter are dilated relative to those at the center; thus, the lesion has an appearance of a "pinwheel". Histologically, the proliferating glands are lined by two cell types: epithelial and myoepithelial. Occasionally, one component or the other may predominate. Rarely, the myoepithelial cell may show "myoid" or clear change. When the epithelial cells within sclerosing adenosis show apocrine change, the lesion can be referred to as apocrine adenosis. The participation of epithelial cells can be highlighted by cytokeratin immunostains, and that of myoepithelial cells can be demonstrated *via* myoepithelial cells—although the latter may appear to be absent in the center of sclerosing adenosis [20]. Most myoepithelial markers (*except* p63 and p40, both of which are nuclear markers) are immunoreactive in those vascular walls that have smooth muscle and should not be mistaken for myoepithelial cell staining.

Other sclerotic lesions that can display a pseudoinfiltrative appearance include radial scar, sclerosing papilloma, florid papillomatosis of nipple ("nipple adenoma"), and subareolar sclerosing ductal hyperplasia [21]. These lesions can be evaluated by myoepithelial stains if there is any suspicion for invasive carcinoma on H&E-stained slides (Fig. 17.12).

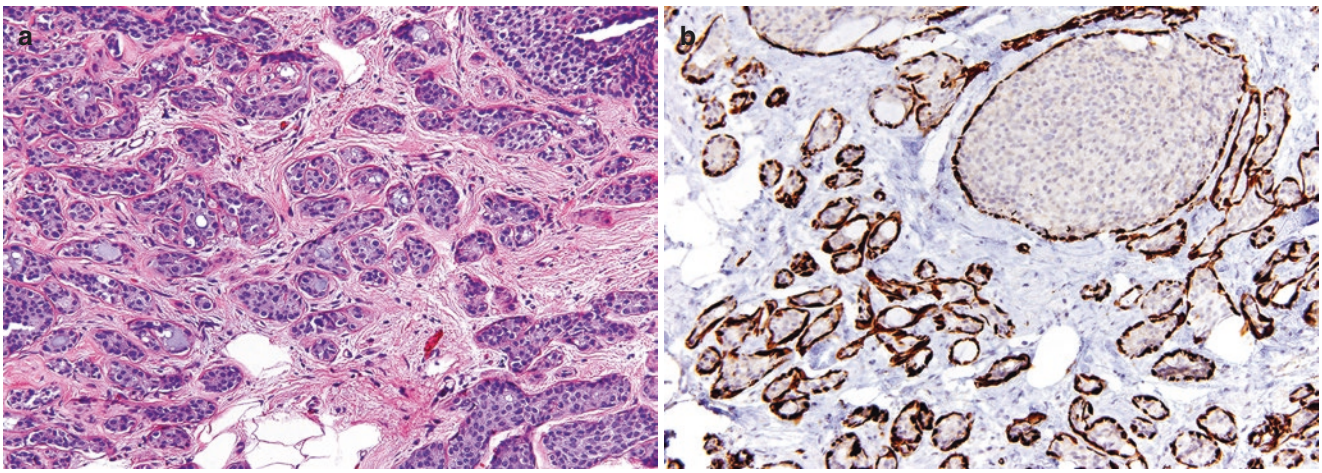

Fig. 17.12 Sclerosing adenosis associated with ductal carcinoma in situ (DCIS). (**a**) Sclerosing adenosis displays a pseudoinfiltrative appearance. Note uniform epithelial cells of DCIS, with intermediate-grade nuclei, inhabiting sclerosing adenosis. (**b**) Smooth muscle myosin (SMM) stain for myoepithelial stains shows complete investment of DCIS cells by myoepithelial cells (SMM). DCIS cells were positive for E-cadherin (not shown)

17.5 Paget Disease of Nipple

The diagnosis of Paget disease of nipple (PDN) may not need immunohistochemical confirmation in cases in which the histopathological features thereof are overt and clinical features (i.e., eczema-like appearance with discolored, oozing, or encrusted nipple or areola) are supportive. However, immunohistochemical confirmation is desirable whenever the diagnosis of PDN is equivocal on routine H&E examination. The majority of PDN cases show the following profile: the neoplastic Paget cells are CK7 (+) and HER2 (+) (Fig. 17.13). Immunoreactivity for ER, PR, GATA3, CEA, and GCDFP15 cannot be relied upon to establish the diagnosis of PDN [22, 23].

Two extremely rare malignancies that involve the nipple may be considered in the differential diagnosis of PDN: Bowen disease (squamous cell carcinoma in situ of skin) and melanoma. Bowen disease is CK7 (−) and p63 (+), and melanomas are CK (−), HMB45 (+), MelanA (+), and MITF1 (+). Toker cells are often included in the differential diagnosis of PDN; however, these cells are cytologically bland, with clear cytoplasm and inconspicuous nucleoli. Toker cells, present in 10% of normal nipples, are typically CK7 (+) and HER2 (−). Rarely, these cells can be relatively large and bear atypical-appearing nuclei [24].

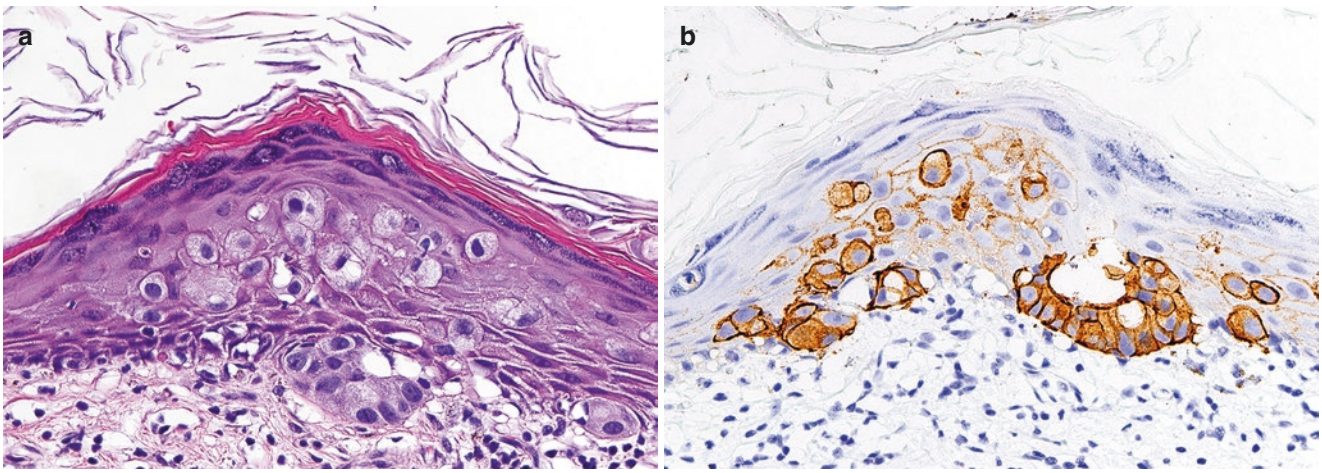

Fig. 17.13 Paget disease of nipple (PDN). (**a**) The intraepidermal carcinoma cells of PDN show marked cytological atypia. (**b**) PDN cells show strong immunoreactivity with HER2 (Hercept)

17.6 Assessment of Invasion

Myoepithelial cells and basement membrane components (i.e., laminin and collagen IV) show a continuously linear pattern of immunoreactivity around benign sclerosing lesions and in non-invasive carcinomas, and are absent around invasive carcinomas (Figs. 17.14, 17.15, and 17.16). Myoepithelial cells can be absent around microglandular adenosis (MGA), and also around some rare apocrine glands. Notably, approximately 5% of DCIS, particularly those of the papillary type, lack myoepithelial cells—at least as these can be demonstrated by immunostains.

Cross-reactivity of SMA and some other myoepithelial markers with myofibroblasts makes identification of myoepithelial cells difficult in some cases of DCIS—especially in cases with marked periductal stromal desmoplasia. Table 17.1 shows the presence and degree of immunohistochemical cross-reactivity of various myoepithelial markers with myofibroblasts and blood vessels.

p63 is a nuclear stain, and it results in apparent "gaps" in myoepithelial cell immunoreactivity. Thus, in the context of DCIS, any nuclear staining around nests of carcinoma should be interpreted as evidence of myoepithelial cell presence. p63 can also be immunoreactive in poorly-differentiated carcinoma.

Microinvasive lobular carcinoma can be particularly difficult to diagnose because often there is minimal stromal reaction (Fig. 17.17). Furthermore, the finding of a few bland microinvasive lobular carcinoma cells amid stromal fibrosis can be particularly subtle, and these cells can be highlighted by cytokeratin immunostain [25].

CK AE1/AE3 immunostain is useful in assessing the *extent* of invasion—particularly in invasive lobular carcinoma (Fig. 17.18). The malignant cells of the latter bear low-grade nuclei that blend imperceptibly amid stromal cells and lymphocytes, and can be difficult to ascertain on H&E-stained histological sections. CK can be especially helpful in detecting invasive carcinoma status-post chemotherapy, and in unequivocal assessment of margins.

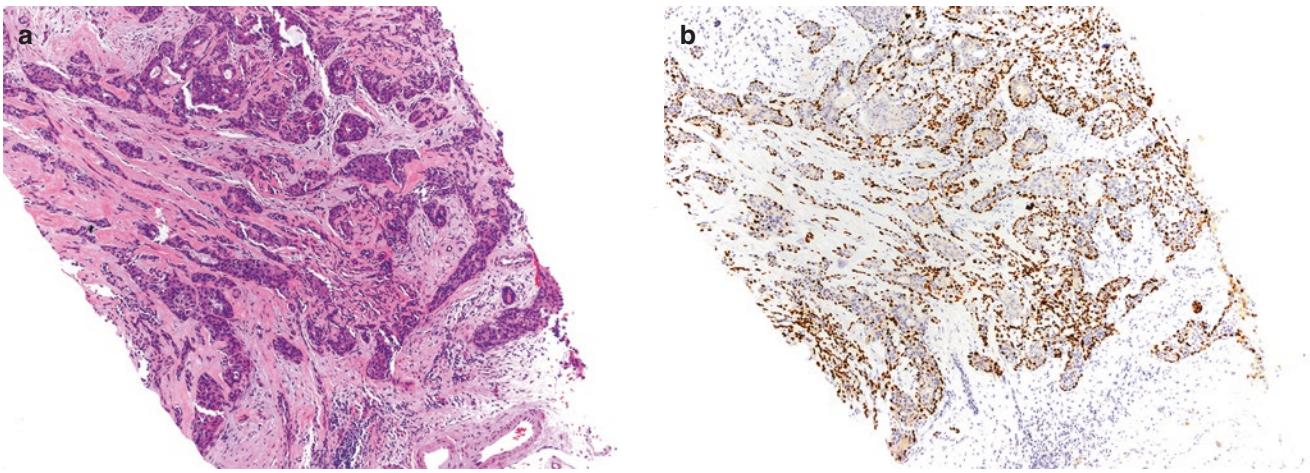

Fig. 17.14 Complex sclerosing papillary lesion. (**a**) Complex sclerosing lesion displays a pseudoinfiltrative appearance. (**b**) Myoepithelial cells show a continuously linear pattern of immunoreactivity with smooth muscle myosin around the sclerosing lesions (SMM)

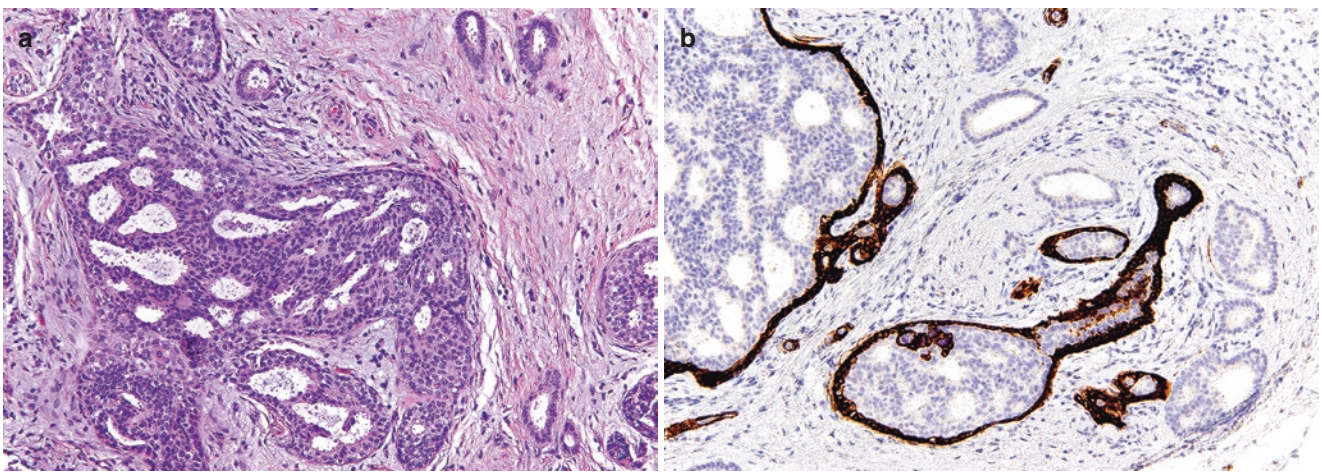

Fig. 17.15 Ductal carcinoma in situ (DCIS) and invasive ductal carcinoma. (**a**) DCIS of cribriform type is associated with invasive ductal carcinoma. (**b**) DCIS shows smooth muscle myosin-positive myoepithelial cells, and absence thereof around invasive carcinoma (SMM)

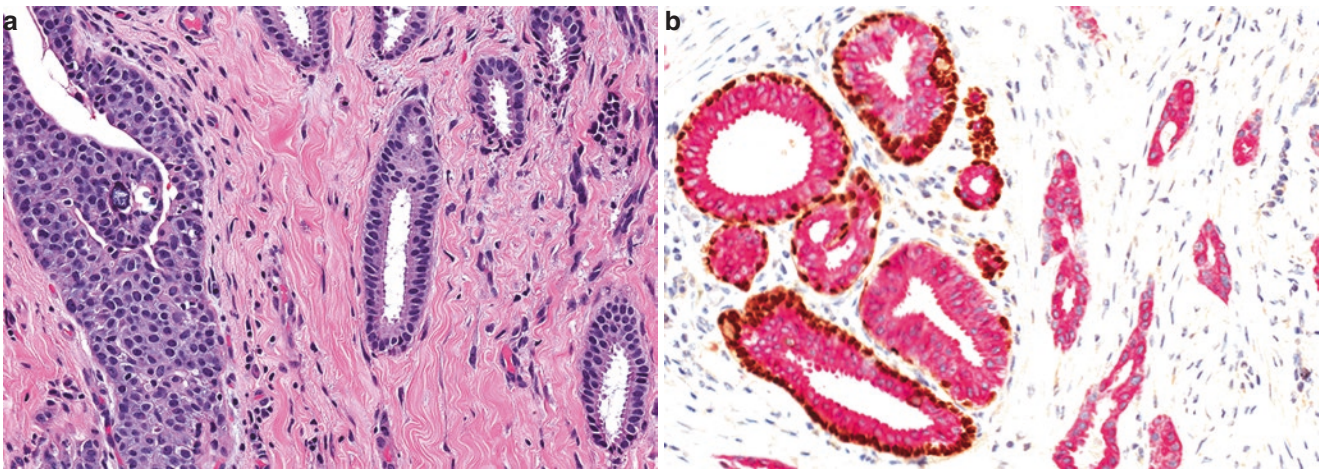

Fig. 17.16 Ductal carcinoma in situ and invasive ductal carcinoma. (**a**) DCIS of cribriform type is associated with invasive ductal carcinoma. (**b**) DCIS shows positivity of myoepithelial cells on combined smooth muscle myosin (SMM) and p63 stain. The malignant epithelial cells show red cytokeratin-positivity, SMM stain shows brown cytoplasmic staining, and p63 shows brown nuclear staining, of myoepithelial cells. No staining of myoepithelial cells is observed around invasive carcinoma (combined cytokeratin+SMM + p63 stain)

Table 17.1 Myoepithelial immunohistochemical stains and cross-reactivity thereof with various other types of cells

	Reactivity in myoepithelial cells	Localization in myoepithelial cells	Reactivity in myofibroblasts	Reactivity in blood vessel walls	Reactivity in epithelial cells
Calponin	++	Cytoplasmic	Uncommon	+	–
CD10	++	Cytoplasmic	Uncommon	+	–
S100p	+	Cytoplasmic	Variable	–	+/–
SMA	++	Cytoplasmic	++	++	–
SMM-HC	++	Cytoplasmic	Uncommon	+	–
p40	++	Nuclear	–	–	–[a]
p63	++	Nuclear	–	–	–[a]

SMA smooth muscle actin, *SMM-HC* smooth muscle myosin-heavy chain
[a]May be positive in rare high-grade carcinoma cells

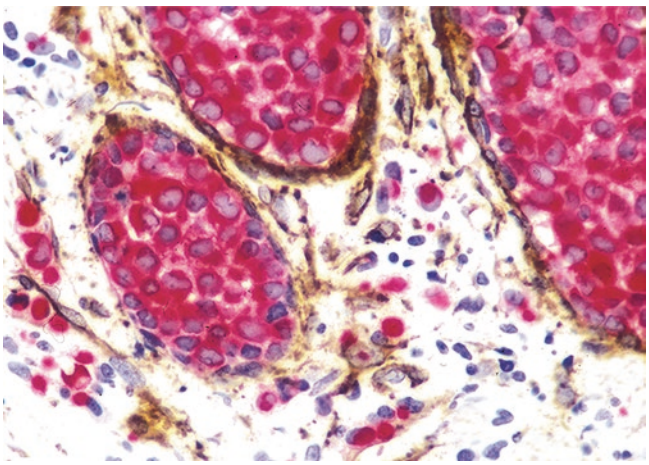

Fig. 17.17 Microinvasive lobular carcinoma. Myoepithelial cells around the in situ carcinoma are decorated by smooth muscle actin (SMA, brown) immunostain, and the malignant epithelial cells are stained by cytokeratin (CK AE1/AE3, red). (SMA + AE1/AE3 double stain). The microinvasive and in situ carcinoma cells were not negative for E-cadherin (not shown)

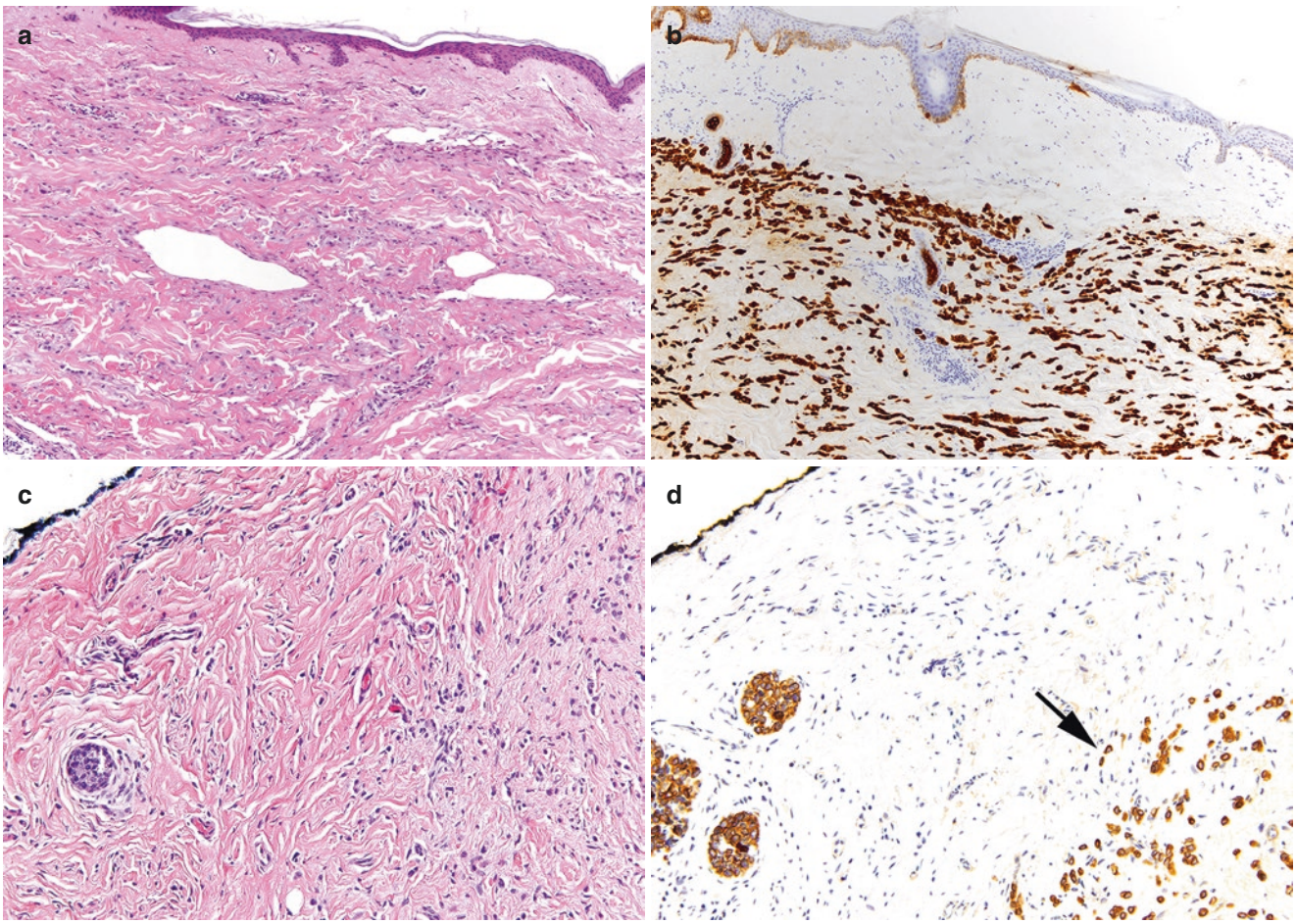

Fig. 17.18 Cytokeratin highlights invasive lobular carcinoma. (**a**, **b**) Dermal invasion by invasive lobular carcinoma (**a**) is highlighted by CK AE1/AE3 immunostain (**b**, CK AE1/AE3). (**c**, **d**) Subtle invasive lobular carcinoma in breast tissue (**c**) is highlighted by CK AE1/AE3 immunostain (arrow). Margin-negativity is confirmed by CK AE1/AE3 stain in this case (**d**, CK AE1/AE3)

17.7 Spindle Cell Lesions

Spindle cell lesions of the breast include a wide variety of benign, borderline, and malignant lesions. The benign lesions include scars, pseudoangiomatous stromal hyperplasia (PASH), myofibroblastoma, and benign phyllodes tumor. The malignant lesions include metaplastic spindle cell sarcoma and malignant phyllodes tumor. Borderline malignant spindle cell lesions include borderline phyllodes tumors and fibromatosis. Immunostains can be helpful in the differential diagnosis; however, the key to diagnosis is the correct interpretation of H&E-stained sections [26].

Spindle cell metaplastic carcinoma is almost always immunoreactive (at least focally) with p63, p40, and one of various cytokeratins—especially HMW-CK (Fig. 17.19). A panel of cytokeratins (e.g., CK-K903, Cam 5.2, MNF116, AE1/AE3) should be used, as the tumor can be focally reactive with all or any one of these. Rarely, p63 and cytokeratin can be immunoreactive in some phyllodes tumors and mammary sarcomas. Spindle cell metaplastic carcinoma can also be immunoreactive for SMA, and are negative for CD34, BCL-2, ER, PR, and HER2. It should be remembered that approximately 30% of metaplastic spindle cell carcinomas are positive for beta-catenin; and that spindle cell metaplastic carcinoma can appear to be cytologically as well as architecturally bland, and appear to be "fibromatosis-like."

The stromal cells of phyllodes tumors are variably positive for CD34, BCL-2, actin, and desmin. Immunostain for proliferation marker Ki-67 is unhelpful in grading of fibroepithelial tumors—owing to tumoral heterogeneity. BCL-2 reactivity is typical of low-grade phyllodes tumor. Malignant phyllodes tumors can be negative for CD34, and can be rarely positive for p40, p63 and HMW-CK [27, 28]. Rarely malignant phyllodes tumors can show focal nuclear staining with beta-catenin—a point worth remembering in the differential diagnosis of fibromatosis. Most cases of mammary fibromatosis are immunoreactive for *nuclear* localization of beta-catenin and for *cytoplasmic* localization of SMA; and are non-reactive for cytokeratins, p63, S100p, CD31, CD34 and ER (Fig. 17.20). Myofibroblastomas and PASH (both being myofibroblastic) are reactive for CD34, desmin, actin, BCL-2, CD99, ER, and PR (Fig. 17.21). The H&E appearance and ER-immunoreactivity of myofibroblastoma can lead to the mistaken diagnosis of invasive lobular carcinoma [29].

p63, a homologue of the tumor suppressor protein p53, is a popular immunostain used in the breast (and in other organs) for the detection of myoepithelial cells. p63 is also useful to detect "myoepithelial" differentiation in metaplastic spindle carcinoma and as a marker of squamous differentiation in low-grade adenosquamous carcinoma. p63 shows nuclear reactivity in the constituent peripheral squamous epithelial cells of cell clusters in low-grade adenosquamous carcinoma (LGASC)—and can possibly lead to its erroneous interpretation as a benign sclerotic lesion with squamous metaplasia (Fig. 17.22). A p40 antibody directed against an N-terminal truncated form of the p63 protein is essentially similar to p63 in its immunoreactivity pattern. Rarely, p63 and p40 may focally stain rare high-grade carcinoma cells.

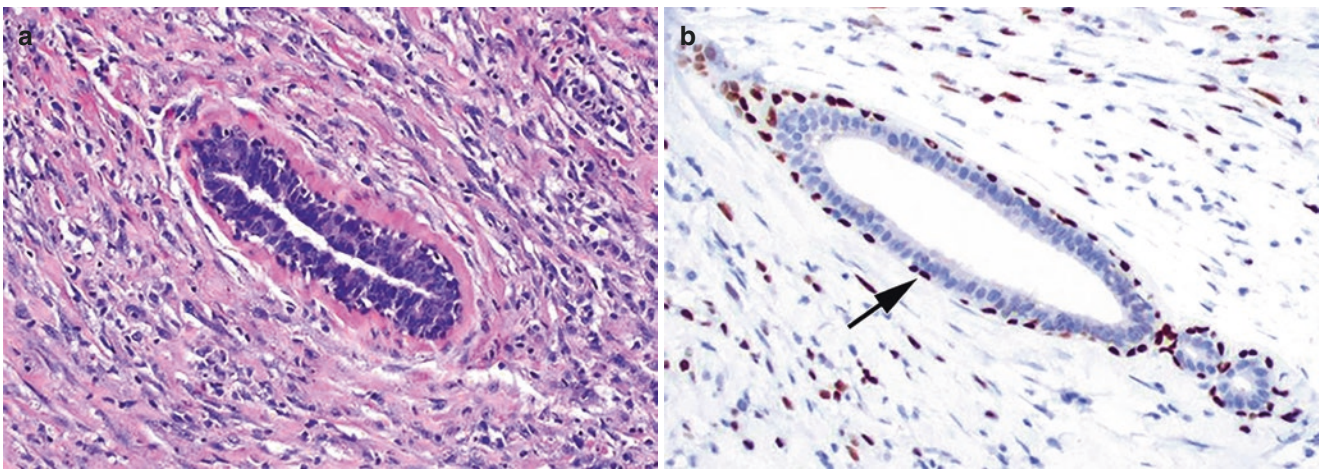

Fig. 17.19 Spindle cell metaplastic carcinoma. (**a**) Spindle cell metaplastic carcinoma infiltrating around a benign duct. (**b**) The metaplastic carcinoma cells are immunoreactive with p63. Note staining of normal myoepithelial cell nuclei of the normal duct by p63 (arrow) (p63)

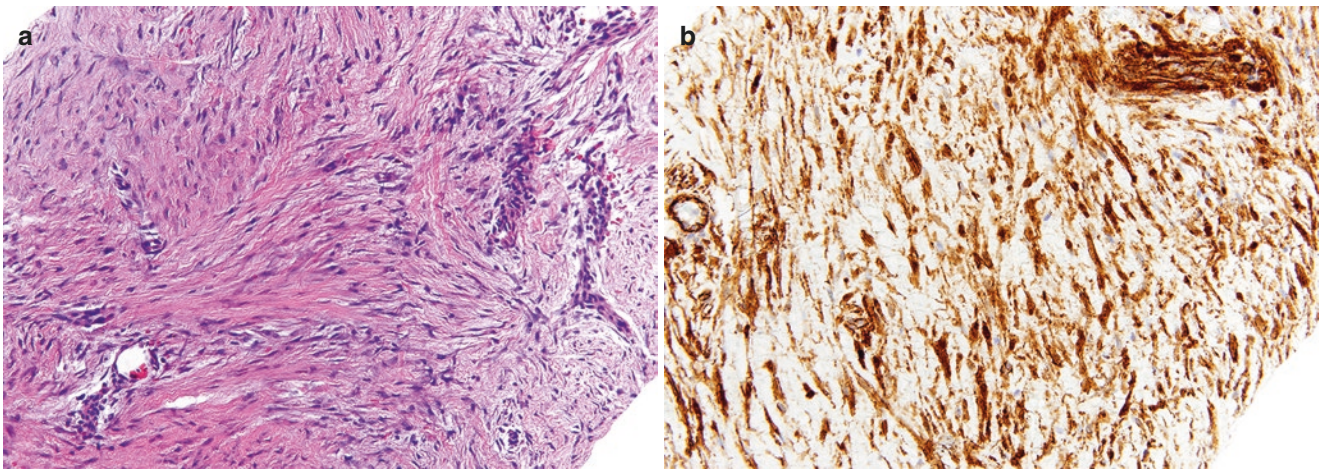

Fig. 17.20 Mammary fibromatosis. (**a**) The typical broad fascicles of "wavy" spindle cells characterize mammary fibromatosis. (**b**) The lesional cells of fibromatosis show nuclear and cytoplasmic staining with beta-catenin (Beta-catenin)

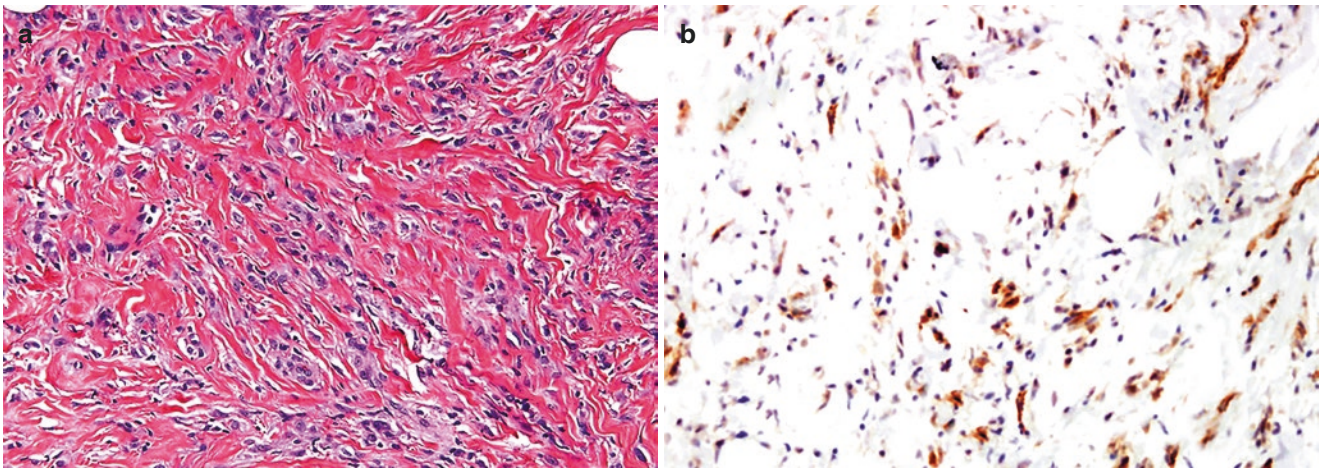

Fig. 17.21 Myofibroblastoma. (**a**) Dense bundles of bland spindle cells lie amid dense collagenous stroma. (**b**) The lesional cells of myofibroblastoma show cytoplasmic staining with CD34 (CD34)

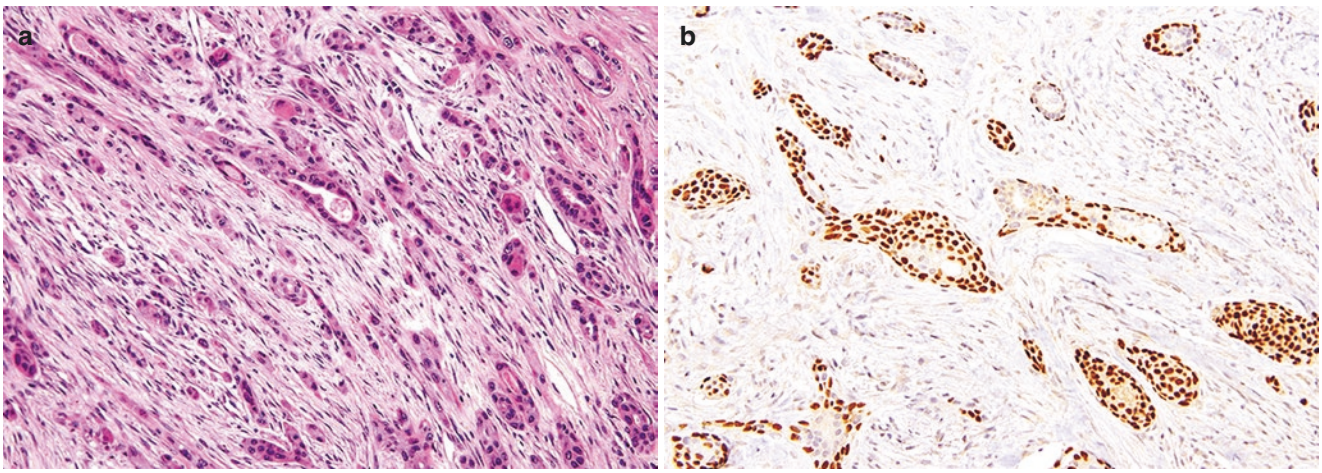

Fig. 17.22 Low-grade adenosquamous carcinoma (LGASC). (**a**) The typical combined "adeno" and "squamous" components of LGASC are evident. (**b**) The neoplastic cells of LGASC show p63-reactivity in the neoplastic squamous cells as well as in some of neoplastic stromal cells. This pattern of p63 staining can lead to erroneous interpretation of LGASC as a benign sclerotic lesion with squamous metaplasia (p63)

17.8 Lymphovascular Channel Involvement

The finding of lymphovascular invasion (LVI) by tumor cells has prognostic significance; however, this important finding can be simulated by tissue retraction. The latter can occur around clusters of in situ or invasive carcinoma in formalin-fixed paraffin-embedded sections [30].

The finding of LVI can be confirmed by adhering to the following criteria: (a) the focus of LVI should be outside the edge of the carcinoma; (b) the tumor emboli should not exactly conform to the space in which they lie; (c) the space should be lined by endothelial cells; and (d) the space is usually accompanied by an artery and vein in its immediate vicinity [31]. The presence of endothelial cells can be confirmed by the use a panel of endothelial markers (e.g., CD31 and D2–40). Lymphovascular endothelia are immunoreactive for CD31, D2–40, ERG, FL1, WT1, and Factor VIII (Fig. 17.23). D2–40 is thought to be specific and sensitive for lymphatic endothelia, and CD31 for vascular endothelia [32]. Notably, D2–40 can be faintly immunoreactive (in a "smudged" pattern) in myoepithelial cells [33].

LVI by tumor cells can also be simulated by artefactual displacement of cells following a needling procedure. In these cases, the artefactual displacement of tumor cell clusters usually appears to be displaced in a linear manner amid granulation tissue along the healing biopsy tract—often accompanied by myoepithelial cells.

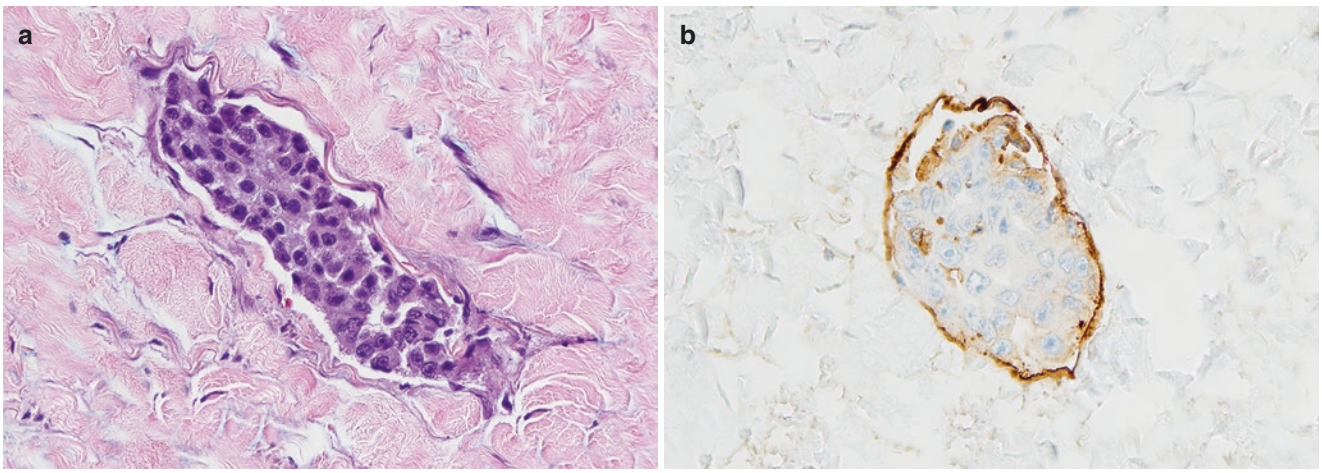

Fig. 17.23 Lymphovascular involvement (LVI) by tumor cells. (**a**) This focus is suspicious for LVI. (**b**) The presence of endothelial cells around the carcinoma cells can be confirmed by the use of CD31 (shown here), D2–40, ERG, FL1, WT1 and Factor VIII (CD31)

17.9 Sentinel Lymph Node

The pathological evaluation of sentinel lymph nodes (SLN) in almost all cases of invasive carcinomas is the standard of care [34, 35]. The SLN should be serially sectioned at 2 mm intervals, and entirely submitted for histopathologial evaluation. H&E-stained sections should be carefully evaluated, and any "suspicious" finding should be further assessed *via* a cytokeratin AE1/3 immunostain (Fig. 17.24). Low molecular weight-cytokeratin (i.e., Cam 5.2) can stain dendritic reticular cells within the lymph node.

Although immunohistochemical staining by cytokeratin is not recommended in the routine processing of SLN examination, a cytokeratin immunostain can be employed in cases of invasive *lobular* carcinoma as even relatively large metastatic tumor aggregates may be missed on H&E examination alone. Furthermore, certain histological findings (e.g., histiocytes, endosalpingiosis, megakaryocytes, etc.) can simulate metastatic carcinoma in SLNs [36–39]. In particular, nevus cell aggregates (positive for S-100 protein and A103/MART1) can be mistaken for micrometastatic carcinoma (Fig. 17.25). In such cases, immunohistochemical confirmation with an *appropriate* immunostain (e.g., CD68 for histocytes) is desirable [40, 41].

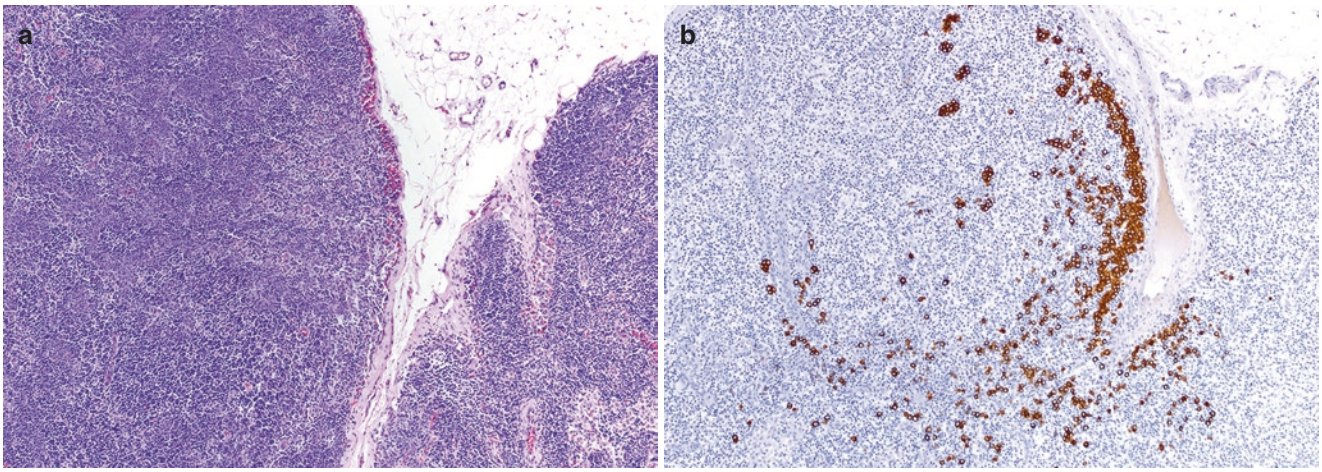

Fig. 17.24 Sentinel lymph node (SLN) with metastatic lobular carcinoma. (**a**) This SLN appears "negative" on H&E. (**b**) CK AE1/AE3 staining of SLN shows more than 200 immunoreactive cells—confirmatory of micrometastasis (CK AE1/AE3)

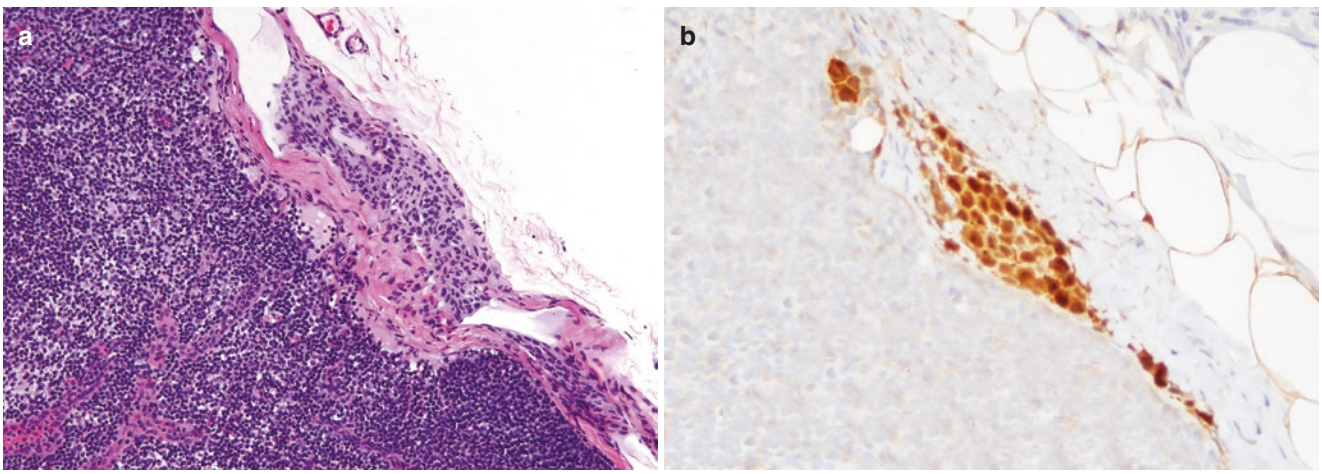

Fig. 17.25 Nevus cell aggregate (NCA) in sentinel lymph node. (**a**) This example of NCA simulates metastatic carcinoma cells. (**b**) S100 protein immunostain can be diagnostic in this regard (S100)

17.10 Metastatic Malignancies

Metastatic neoplasms to the breast can be mistaken, clinically and pathologically, for primary neoplasms [42, 43]. Unless there is a clinical history of another primary neoplasm, the pathologist may not even consider metastatic malignancy in the differential diagnosis.

Most primary breast carcinomas display the following immunoprofile: CK7 (+), GATA3 (+), GCDFP15 (+), mammoglabin (+), ER (+), CK20 (−), TTF1 (−). PAX8 (−), Wilms' tumor protein 1, and WT1 (−). GCDFP-15 is the most specific marker of breast carcinoma, and mammoglobin is a more sensitive marker of breast carcinoma than GCDFP-15; however, GCDFP15 and mammoglobin can be non-reactive in approximately one-quarter of breast carcinomas. Hormone receptors can be present in endometrial, ovarian, and lung primaries. Mammoglobin can also be positive in endometrial carcinomas. GATA3 is reactive in most breast carcinomas (except in about one-third of "triple-negative" carcinomas) [44].

The most common metastatic neoplasms to the breast include lung and melanoma. In most cases, TTF-1 can confirm a primary lung carcinoma. Melanoma markers are negative in breast carcinoma; however, S100 protein can be positive.

Occasionally breast carcinomas need to be distinguished from Mullerian carcinomas (Fig. 17.26). Both groups of carcinomas are CK7 (+) and CK20 (−). However, breast carcinomas are characteristically GATA3 (+), PAX8 (−), and WT-1 (−), and most Mullerian carcinomas are GATA3 (−), PAX8 (+), and WT-1 (+). WT1 can be immunoreactive in some forms of invasive mucinous carcinoma of the breast.

In a metastatic setting, it may be difficult to differentiate between breast, skin, and salivary gland primaries. It is noteworthy that these tumors can show rather similar immunohistochemical results. GCDFP-15 is generally negative in sweat gland carcinomas, CEA is negative in breast carcinomas, and ER is usually negative in salivary gland carcinoma.

In general, a panel of antibodies ought to be used in the workup of metastatic tumors to the breast. Immunohistochemistry can play a role in establishing a non-mammary primary. Reliance on a single antibody to establish any diagnoses (e.g., ER to establish a breast primary) can be misleading.

Malignant lymphoma can involve the breast, usually as part of systemic involvement, and rarely primary—either *de novo* or in association with an implant [45].

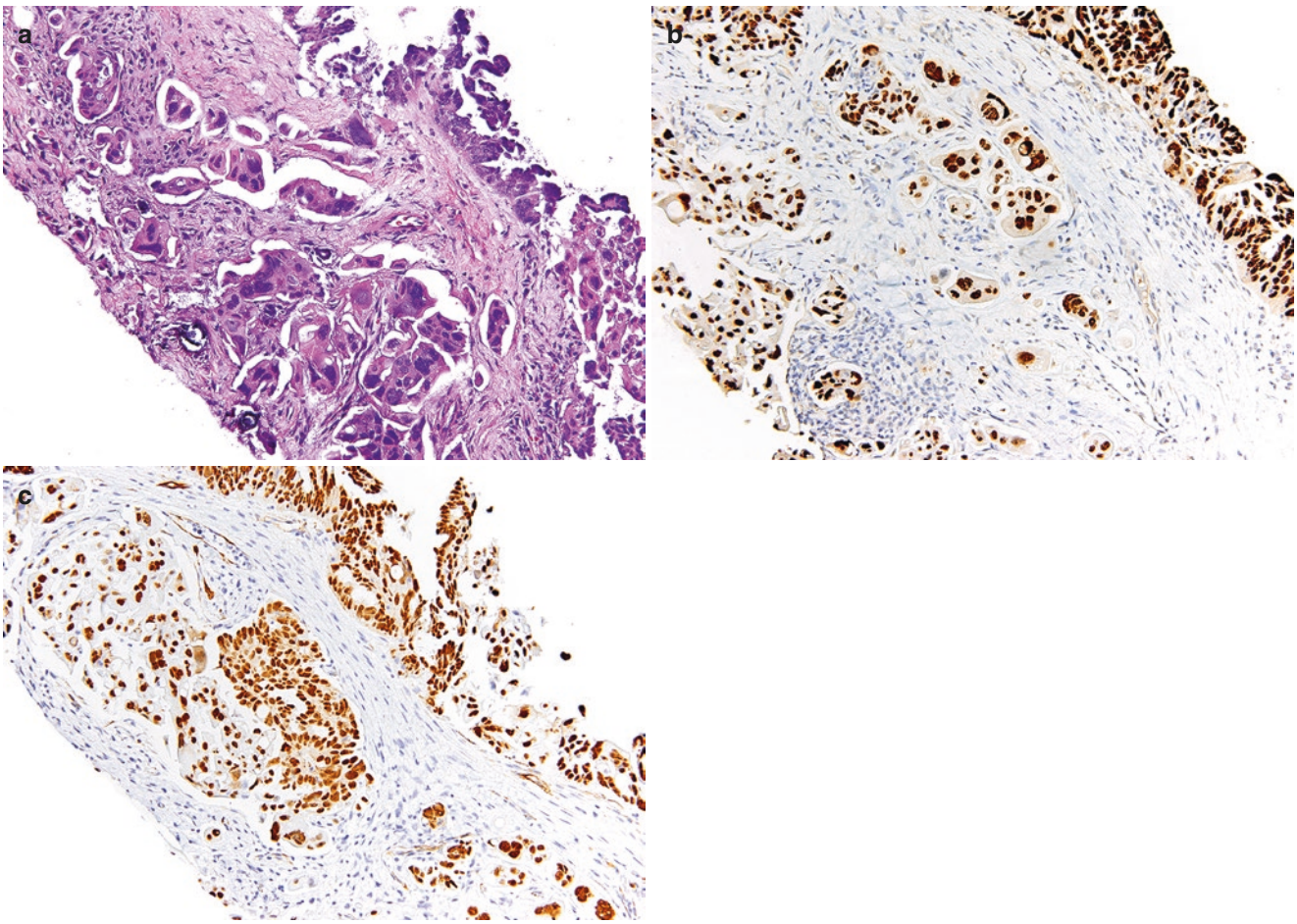

Fig. 17.26 Metastatic Mullerian carcinoma to the breast. (**a**) Note micropapillary architecture of the tumor, "hobnail" appearance of the individual tumor cells, and presence of psammomatous-type calcification. (**b**) This Mullerian carcinoma was positive for both WT1 and PAX8, and negative for GATA3 (PAX8)

17.11 Hormone Receptors and HER2

In the context of oncological pathology, prognostic factors must be differentiated from predictive factors. A prognostic factor is a measure that correlates with disease-free or overall survival in the absence of systemic therapy and is, thus, able to correlate with the natural history of a particular malignancy (e.g., size of invasive carcinoma). A predictive factor is a measure that is associated with response to a given therapy (e.g., HER2). Some factors, such as ER and HER2, should be regarded as both prognostic and predictive (Fig. 17.27). Only the most significant aspects of hormone receptor and HER2 testing via immunostaining are discussed in this section.

Approximately 80% of invasive breast carcinomas are ER (+), and an ER (−) rate of more than 30% in a particular laboratory may be indicative of a technical problem with the assay. In general, PR-positivity parallels ER-positivity. Adequate tissue fixation, appropriate selection of tissue block, and optimal use of control are necessary to obtain excellent results of these tests.

The two parameters that should be evaluated in IHC preparations of ER and PR are the proportion of the tumor cell nuclei stained and the intensity of the staining [46]. These parameters (proportion and intensity) should be reported separately, or the two can be combined using the composite Allred, Quick-score, or H-score systems. Image analysis can be used to assess the results of staining; however, most reporting is being done visually (by "eyeballing"). Although a threshold of >1% immunoreactivity in a carcinoma is considered positive for both ER and PR, there is emerging evidence that <9% immunoreactivity should be regarded as weakly positive (since this result indicates suboptimal response to therapy) [47].

HER2 overexpression (3+, on a scale of 0 to 3+) is observed in approximately 25% of invasive breast carcinomas. Most high-grade DCIS (with "comedo" necrosis) show 3+ reactivity for HER2. On the other hand, it is extremely uncommon for low-grade invasive tumors (including tubular and classic lobular carcinomas) to be HER2 (+). 2+ reactivity in a case is regarded as equivocal, and should be confirmed through FISH testing. Cases negative for HER2 stain either 0 or 1+. 3+ HER2 staining is regarded as positive, and this result need not be confirmed by FISH testing [48].

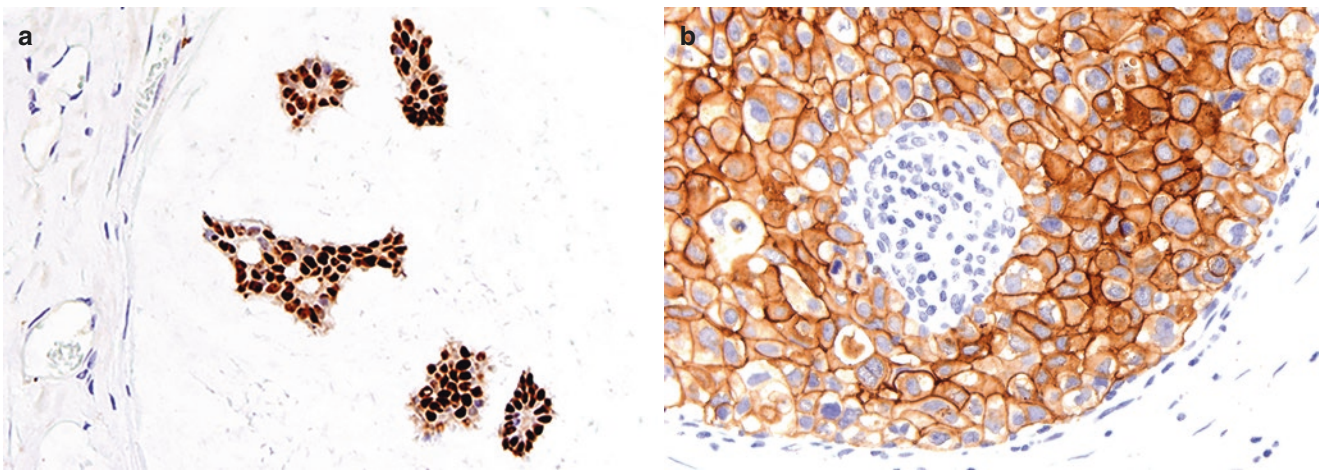

Fig. 17.27 "Biomarkers" in breast carcinoma. (**a**) Estrogen receptor (ER) is strongly and diffusely positive in this invasive mucinous carcinoma (ER). (**b**) HER2 shows 3+ reactivity (on a scale of 0 to 3+) in high-grade ductal carcinoma in situ (Hercept)

17.12 Surrogate Markers for Molecular Classification

Efforts to divide breast carcinomas into distinct groups based on similarities in gene expression profiles using microarray platforms has rapidly evolved into molecular classification thereof. This classification basically divides breast carcinoma into four groups:

- **Luminal A** (with high expression of hormone receptors as well as associated genes, and with the best prognosis)
- **Luminal B** (moderate expression of hormone receptors as well as associated genes, and relatively higher expression of proliferation genes)
- **HER2** (high expression of *HER2* and other genes in amplicon on 17q12)
- **Basal-like** (with low expression of hormone receptors and *HER2* genes, and with the worst prognosis)

Since resources limitations preclude the application of molecular classification of breast carcinomas in each case, efforts have been made to devise a surrogate classification utilizing IHC markers [49, 50].

Although there are limitations to applying IHC markers to molecular classification (e.g., non-standard approach to the assessment of proliferation rate), the following immunoprofiles correspond best to the four groups:

- Luminal A tumors are ER (+), PR (+), and HER2 (−), with a low proliferation rate (<15%).
- Luminal B tumors are ER (+), PR (+), and HER2 (−/+), and are of higher nuclear grade with high proliferation rate (>15%) than luminal A tumors. "Triple-positive" tumors, i.e., ER (+), PR (+) and HER2 (+), belong to luminal B group.
- HER2 group are, as the name implies, HER2 (+), and are ER (−) and PR (−).
- "Triple-negative" tumors are, as the name implies, ER (−), PR (−), and HER2 (−). Most "basal" carcinomas are triple-negative; and in addition, this group of cases are CK5 (+), EGFR (+), and p63 (+). CK5 (a marker for the basal, i.e., myoepithelial layer of the breast glands) is considered the most sensitive immunostain for the identification of "basal" breast carcinomas.

At the present time, the aforementioned surrogate IHC-based molecular classification of breast carcinoma is being used clinically for management purposes in *selected* cases [51]; however, evolutionary refinement in surrogate molecular classification is surely to be expected.

Conclusions

Immunohistochemistry has become an integral part of breast pathology. This *science* can be used to effectively confirm, refine, or refute various pathological diagnoses; however, the appropriate and cost-effective use of immunostains is an *art* that can be mastered—by way of awareness of its advantages, disadvantages, and pitfalls in interpretation.

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