Practical Anatomic Pathology
Series Editors: Fan Lin · Ximing J. Yang

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Practical Breast Pathology

Frequently Asked Questions

Springer

Practical Anatomic Pathology

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The proposed Book Series will be designed to provide a comprehensive, practical and state-of-the art review and update of the major issues and challenges specific to each subspecialty field of surgical pathology in a question and answer (Q&A) format. Making an accurate diagnosis especially from a limited sample can be quite challenging, yet crucial to patient care. The proposed Book Series, using the most current and evidence-based resources, will 1) focus on frequently asked questions in surgical pathology in day-to-day practice; 2) provide quick, accurate, terse, and useful answers to many practical questions encountered in daily practice; 3) emphasize the importance of a triple test (clinical, radiologic, and histologic correlation); 4) delineate how to appropriately utilize immunohistochemistry, in situ hybridization and molecular tests; and 5) minimize any potential diagnostic pitfalls in surgical pathology. These books will also include highly practical presentations of typical case scenarios seen in an anatomic pathology laboratory. These will be in the form of case presentations with step-by-step expert analysis. Sample cases would include common but challenging situations, such as evaluation of well-differentiated malignant tumors vs. benign/reactive lesions; distinction of two benign entities; sub-classification of a malignant tumor; identification of newly described tumor and non-tumor entities; workup of a tumor of unknown origin; and implementation of best practice in immunohistochemistry and molecular testing in a difficult case. The Q&A format will be well accepted, especially by junior pathologists, for several reasons: 1) this is the most practical and effective way to deliver information to a new generation of pathologists accustomed to using the Internet as a resource and, therefore, comfortable and familiar with a Q&A learning environment; 2) it's impossible to memorialize and digest massive amounts of new information about new entities, new and revised classifications, molecular pathology, diagnostic IHC, and the therapeutic implications of each entity by reading large textbooks; 3) sub-specialization is a very popular practice model highly demanded by many clinicians; and 4) time is very precious for a practicing pathologist because of increasing workloads in recent years following U.S. health care reforms. This Book Series will meet all of the above expectations. These books will be written by established and recognized experts in their specialty fields and will provide a unique and valuable resource in the field of surgical pathology, both for those currently in training and for those already in clinical practice at various skill levels. It does not seek to duplicate or completely replace other large standard textbooks; rather, it will be a new, comprehensive yet concise and practical resource on these timely and critical topics.

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Practical Breast Pathology

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Practical Anatomic Pathology
ISBN 978-3-030-16517-8 ISBN 978-3-030-16518-5 (eBook)
https://doi.org/10.1007/978-3-030-16518-5

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

To my husband, Zhongyue Jiang, PhD, and to our beloved daughter, Sharon, for their love, inspiration and continuous support"

Yan Peng

To my husband, Bruce Xu, and two daughters, Sara and Lora, for their unconditional love and support

Ping Tang

Preface

With recent understanding of breast tumor biology and rapid advances in breast cancer treatment, timely updates on diagnostic surgical pathology of breast disease become keenly necessary and important. The purpose of this book is to provide a practical, evidence-based, up-to-date, problem-solving guide to frequently encountered diagnostic problems, challenges, and controversies in breast pathology and associated molecular pathology practice, with emphasis on addressing diagnostic issues that have significant impact on clinical management.

This textbook is based on a PubMed (US National Library of Medicine, Bethesda, Maryland) literature review and the editors' and chapter authors' personal experiences. It consists of ten chapters with an abundance of color photomicrographs and images. The book is organized in a question-and-answer format accompanied by case presentations. We hope this format will facilitate finding answers to frequently asked questions in breast pathology. The book also discusses genetic alterations and molecular abnormalities in breast cancer and commonly encountered interpretation dilemmas regarding immunohistochemistry in breast cancer and metastatic cancer to the breast, with a focus on prognostic and predictive tumor biomarkers. In addition, the book covers some uncommon, diagnostically challenging breast lesions.

We hope that practicing pathologists and pathologists-in-training will find this book helpful for efficiently solving diagnostic problems in their daily practice in breast pathology.

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Acknowledgments

We are grateful to all the contributing chapter authors for working with us on this book and for making this collaboration a memorable and rewarding experience.

We are honored to have been invited to contribute to this volume in the Practical Anatomic Pathology series, which is edited by Dr. Fan Lin and Dr. Ximing J. Yang and published by Springer.

We greatly appreciate our outstanding mentors—Dr. David Dabbs, Dr. Steven Hajdu, and Dr. Daryl Carter—for their teaching and guidance during our breast pathology training.

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Intraductal Proliferative Disease of the Breast

1

Xiuzhen Duan, Yihong Wang, Hua Guo, and Ping Tang

List of Frequently Asked Questions

1. **What is intraductal proliferative disease?**

Intraductal proliferative diseases are diverse groups of proliferations typically originating in and confined to the terminal ductal–lobular unit (TDLU). They are associated with an increased risk of subsequent development of breast cancer of different magnitudes [1]. They often include usual ductal hyperplasia (UDH); columnar cell lesions (CCLs): columnar cell change (CCC), columnar cell hyperplasia (CCH), and flat epithelial atypia (FEA); atypical ductal hyperplasia (ADH); and ductal carcinoma in situ (DCIS). Atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS) are also part of this group, and they will be discussed separately.

2. **What is the clinical presentation of intraductal proliferative disease?**

No clinical features are specifically correlated with this group of diseases, because most of these lesions are microscopic in size and not palpable, with the exception of some DCIS that can present as a mass lesion.

3. **What is the imaging finding of intraductal proliferative disease?**

Imaging findings are not specific. The most frequent imaging findings are microcalcification, architecture distortion, or mass/nodule. Those lesions are often assessed as a Breast Imaging Reporting and Data System (BIRADS) category 4, which is an indication for biopsy [2, 3].

4. **What are the clinical implications of intraductal proliferative diseases? Why is it important to differentiate them from one another?**

Lesions of low-grade breast neoplastic pathways are a group of high-risk lesions including columnar cell lesions, ALH/LCIS, ADH, and low-grade DCIS. They often coexist and share morphologic, immunophenotypic, and genetic characteristics. Patients with ADH and low-grade DCIS were found to have a four- to ten-fold increased risk of breast cancer over the general population. Notably, these high-risk lesions are not only markers of future carcinoma but also indicators of concurrent carcinoma missed due to biopsy sampling. The practical implication for pathologists is to correctly identify these lesions and promptly search for other coexisting lesions. Although the management of lesions such as FEA is currently debatable, core needle biopsy (CNB) diagnosis of ADH (management by surgical excision) is done in most institutions. A multidisciplinary management with radiology–pathology correlation is advocated [4, 5].

5. **What are the typical gross characteristics of intraductal proliferative disease?**

There are no specific gross tissue findings associated with UDH, CCC, FEA, or ADH except for rare cases involving

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Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5_1

mass lesions such as radial scar, sclerosing adenosis, or papilloma.

6. **What is the most effective way to sample the surgical specimen?**

These lesions are often nonpalpable and excised by guided wire localization. It is recommended to submit the entire specimen for microscopic evaluation if the specimen can fit into 20 cassettes. If the specimen is larger, the most effective way to sample the surgical specimen is to sample the area/lesion of interest (a clip or biopsy site). Serially slice the specimen and submit the entire area of lesion with focus on the relationship with surrounding breast tissue and surgical margins. If the patient has a prior biopsy of ADH or DCIS and there is no grossly visible lesion, the entire specimen should be mapped and examined in order to estimate the extent of DCIS if present [6].

7. **What is the clinical–pathologic correlation? What is considered concordance?**

Core needle biopsy (CNB) is the preferred, initial, minimally invasive diagnostic procedure for nonpalpable breast lesions or palpable breast masses. Concordance assessment of the histologic, imaging, and clinical findings determines further management. For this reason, CNB findings require correlation with imaging and clinical findings to determine concordance, and to either exclude the diagnosis of a malignancy by further histological evaluation or to establish a formal plan of follow-up through risk-based, shared decision-making with the patient.

If CNB was performed for mammographic calcifications, then radiographic and microscopic confirmation of calcifications in the specimen should be documented; otherwise, further efforts to identify and excise them are indicated. If imaging reveals features suspicious for malignancy – such as a spiculated or irregular mass or architectural distortion – and histology reveals a lesion – such as invasive carcinoma, fat necrosis, or radial sclerosing lesion – it is considered concordant after radiology and clinical correlation [7–9].

8. **What is considered discordance? What is the next step for a non-concordant case?**

Discordance refers to the situation in which a breast CNB demonstrates benign histology, while the clinical or imaging findings are suspicious for malignancy. If there is discordance between imaging and pathology, histological evaluation is still needed. This can be accomplished either by repeat CNB or surgical excision [9].

9. **What is UDH and what are its key histologic features?** (Fig. 1.1a–f)

UDH is characterized by a solid or fenestrated proliferation of epithelial cells that often show streaming growth, particularly in the center of involved spaces [1, 10]. The cytologic features are heterogeneous epithelial cell proliferation; variability in size, shape, and orientation; poorly defined cell borders; and variation in size, shape, and placement of nuclei. The architectural features are fenestrated, micropapillary (gynecomastoid type), and solid growth patterns; formation of irregularly shaped oval, angulated, or slit-like spaces; and streaming or swirling appearance.

10. **What IHC markers can be helpful for the diagnosis for UDH?** (Fig. 1.2a–f)

Immunohistochemistry mosaic patterns of expression of high-molecular-weight cytokeratins such as CK5/6 and variable expression of estrogen receptor (ER) are the most helpful markers for differential diagnosis of UDH from ADH/LG DCIS and columnar cell lesions [11].

11. **What are the underlying molecular changes associated with UDH?**

Molecular genetic studies have identified loss of heterozygosity (LOH) at several loci in UDH; however, there are no consistent genetic alterations for UDH. Epidemiologic studies have shown that UDH is associated with a slightly increased general breast cancer risk. Currently, UDH is not viewed as a precursor lesion of DCIS [12, 13].

12. **How does one differentiate UDH from grade 2 DCIS?**

Grade 2 DCIS or intermediate-grade DCIS refers to a group of DCIS that cannot be assigned readily to the high– or low–nuclear grade categories. The nuclei are moderate pleomorphic, less than in high-grade type, but lack the uniformity and typically are larger than those seen in the low-grade type.

Histologically, the micropapillary and solid pattern of DCIS can be difficult to distinguish from the gynecomastoid and solid pattern of UDH. Oftentimes, the cytologic and architectural features are of little help because variation in cell size and shape and even secondary irregular lumina may be found in both lesions. Micropapillary DCIS is characterized by bulbous tips in which the nuclei are enlarged throughout the micropapillations. In contrast, the micropapilla of gynecomastoid UDH have a broad base with a narrower, pinched tip and the nuclei are larger at the base and smaller at the tip. In those cases, careful morphologic observation is required, and, when needed, demonstration of CK5/6 mosaic pattern of immuno-

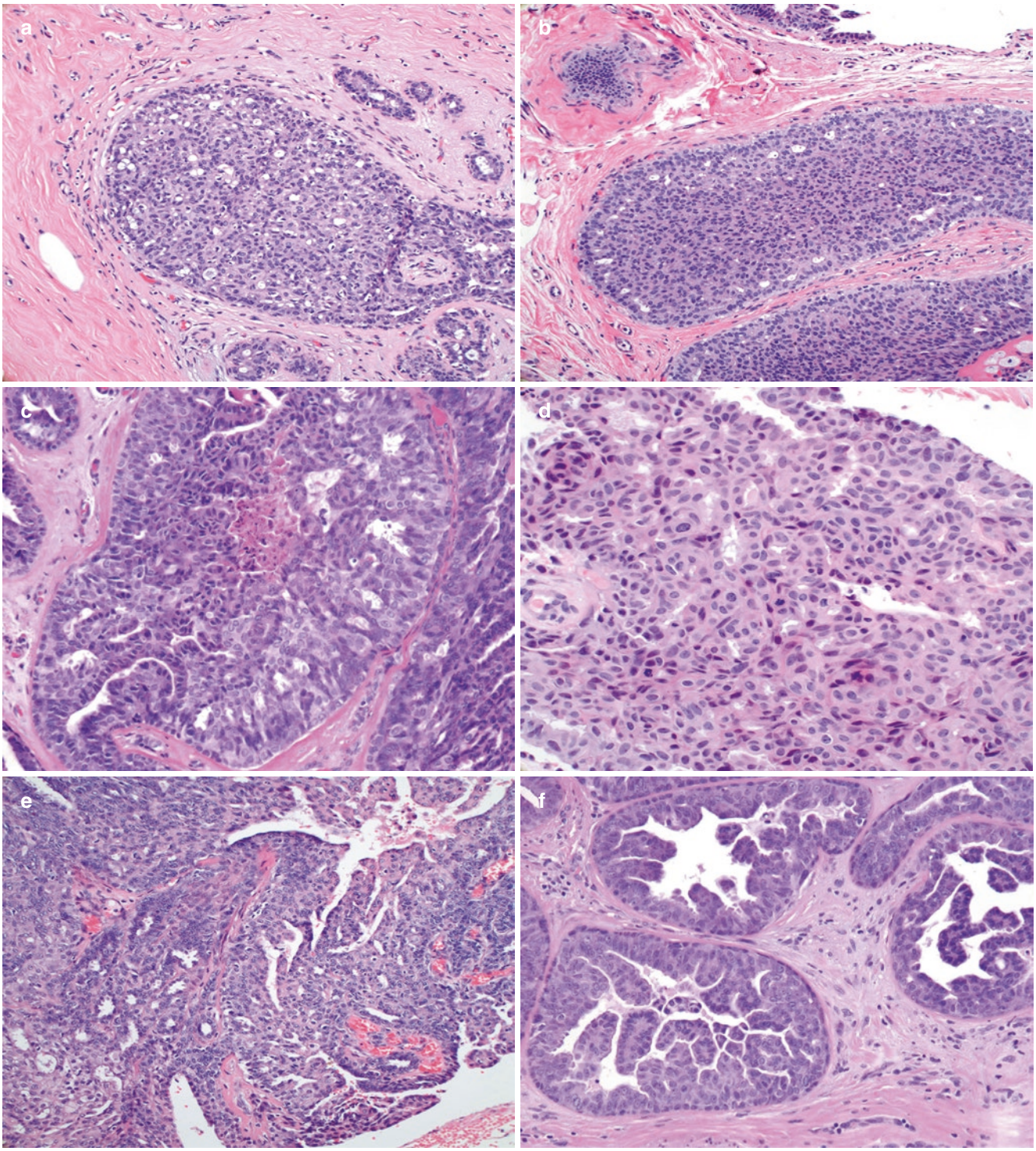

Fig. 1.1 UDH H&E stains. Intraductal proliferation of a mixed population of cells (**a**); streaming pattern of proliferation (**b**); with focal central necrosis (**c**); with mitosis (**d**); involved in papilloma (**e**); and involved in gynecomastia (**f**)

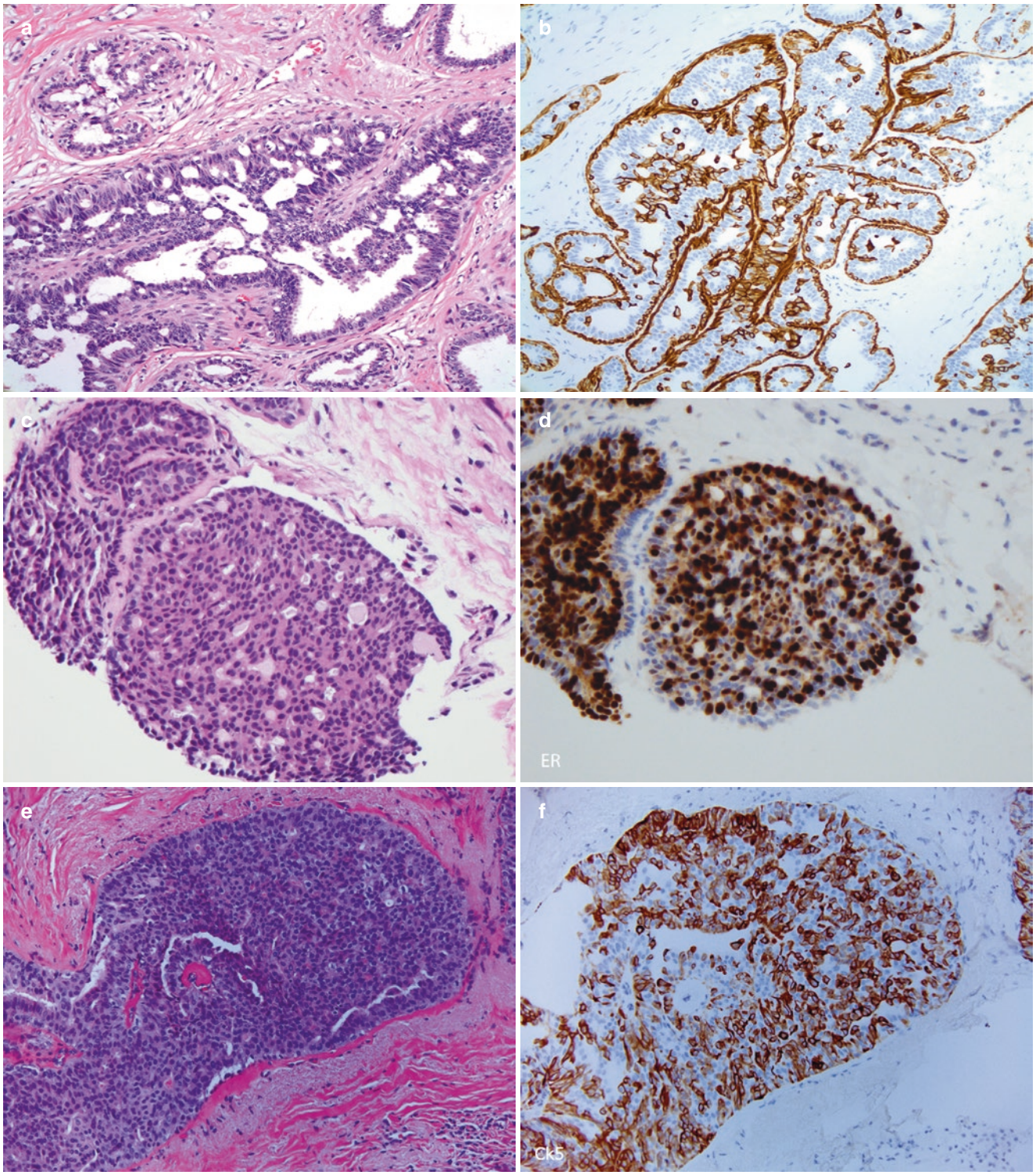

Fig. 1.2 UDH and IHC for CK5/6. Proliferation into the lumen (**a**), with cribriform-like pattern (**c**) and with solid proliferation (**e**); and their corresponding mosaic pattern of ER staining (**b**, **d**, **f**)

histochemistry is indicative of UDH whereas grade 2 DCIS shows a distinct CK5/6 negative expression [11].

LCIS variants such as florid lobular carcinoma in situ (FLCIS) refers to a proliferative lesion that exhibits cell dyshesion, low to intermediate nuclear grade, and massive expansion of the acini [14]. The differential diagnosis between solid pattern of UDH and FLCIS can sometimes be difficult. Histologically, the solid pattern of UDH usually

contains some fenestration at the periphery that may be the clue to the nature of the epithelial proliferation, whereas loss of polarity, loss of cell–cell cohesion, intracytoplasmic vacuoles, and the absence of microacini should raise concern for lobular neoplasia. Immunohistochemistry loss of E-cadherin can be used to confirm the presence of lobular neoplasia.

13. How does one differentiate UDH from basal-like DCIS?

A small proportion of high-grade DCIS exhibits basal-like characteristics with a triple negative phenotype, expression of basal cytokeratins (such as CK5/6, CK14, CK17, etc.), and/or EGFR compared to non-triple-negative high-grade DCIS. Both UDH and basal-like DCIS can express high-molecular cytokeratins; however, UDH has a unique mosaic pattern of expression with variable ER, while basal-like DCIS is negative for ER [15, 16].

14. Can UDH have necrosis? Punctate and central?

UDH can have focal necrosis, but rarely has central necrosis [13].

15. Can UDH have mitosis?

Yes, but this is not common.

16. What types of breast lesions can UDH be associated with?

UDH can associate with any types of breast lesions. It is often found against a background of normal tissue, adenosis, or in association with a group of benign intraductal proliferative breast diseases such as columnar cell lesions, ADH, and DCIS. It may be observed in the context of benign tumor–forming lesions such as radial scar/radial sclerosing lesion, or an intraductal papilloma. Florid UDH in an intraductal papilloma often shows a syncytial or streaming pattern with secondary, slit-like lumina or fenestrations. The proliferating epithelial cells are typically heterogeneous and overlapping or irregularly spaced, and have indistinct cell borders and variably sized nuclei with frequent nuclear grooves and pseudonuclear inclusions.

17. What is CCC and what are its key histologic features?

Columnar cell lesions (CCLs) of the breast are a spectrum of benign to atypical entities, which are characterized by enlarged terminal duct lobular units (TDLUs) with variably dilated acini lined by single or multilayered columnar-shaped epithelial cells, which may have apical snouts. There are usually secretions and/or microcalcifications inside the lumen. CCLs are increasingly being encountered in breast biopsies with association of microcalcifications which are detected on mammographic screening [17]. CCLs have been described and classified under a variety of names by different authors. The World Health Organization (WHO) Working Group on the Pathology and Genetics of Tumors of the Breast currently categorizes them as columnar cell change (CCC), columnar cell hyperplasia (CCH), and flat epithelial atypia (FEA) referring to previously called CCC or CCH with atypia [1].

The involved acini of CCC usually have irregular contours and are lined by one or two layers of columnar epithelial cells with uniform, ovoid to elongated nuclei regularly oriented perpendicular to the basement membrane, with evenly dispersed fine chromatin and inconspicuous nucleoli. CCH also has variably dilated acini with epithelium lining showing similar cytologic features to CCC but is composed of cellular stratification or tufting more than two cell layers thick. Crowding or overlapping of the nuclei in the proliferative foci may give the impression of nuclear hyperchromasia [18]. Therefore, low-power view often exhibits a distinct eye-catching blue color of the columnar arrangement. However, there should be no true atypical micropapillary structure for CCH [19]. Sometimes, exaggerated apical cytoplasmic snouts may give a hobnailed appearance.

18. What IHC markers can be helpful for the diagnosis of CCC/CCH/FEA?

The full spectrum of columnar cell lesions – including CCC, CCH, and FEA – shares similar immunophenotypic features with low-grade DCIS. The lining epithelium expresses low-molecular-weight cytokeratins (LMW-CKs) such as CK7, CK8, CK18, and CK19, and broad-spectrum cytokeratin AE1/AE3, but is negative for high-molecular-weight cytokeratins (HMW-CKs) such as 34βE12 (CK903), CK5 or CK5/6, and CK14 [20]. CCLs are typically strongly positive for E-cadherin. A majority of cells exhibit intense and diffuse immunoreactivity to estrogen receptor (ER) and progesterone receptor (PR), but negative for HER2 [18, 21]. They also often express gross cystic disease fluid protein-15 (GCDFP-15) and Bcl-2. Proliferation rate as indicated by Ki-67 staining is low.

19. What are the underlying molecular changes associated with CCC?

By microdissection approach, low level of allelic imbalance and recurrent 16q loss have been demonstrated in CCLs [22]. No mutational changes are found in simple CCLs without atypia [7, 8]. Some examples of CCH have shown to exhibit loss of heterozygosity (LOH) at chromosome 9q and 10q. Studies showed progressive accumulation of allelic damage

in CCLs with atypia, DCIS, and invasive carcinoma, with a fractional mutation percentage increasing progressively from CCH through invasive carcinoma. Allelic loss damage appeared to preferentially target loci at 9q, 10q, 19p, 16q, 17p, and 17q [20, 22]. Recurrent changes were identified as loss on 16q, 17p, and X and gains on 15q, 16p, and 19 [5]. These findings raise the possibility that CCL may be the precursor lesion for low grade DCIS and low grade IDC. Additional investigations are needed to further characterize the features of CCLs.

20. **How does one differentiate CCC from UDH?** (Fig. 1.3a–c)

CCC and CCH are both featured by variably dilated TDLU acini containing secretory material. From low-power view, it may be mistaken for microcysts from fibrocystic changes, which are typically lined by attenuated, cuboidal, or apocrine epithelium [18]. Occasionally, CCLs may show more proliferative changes: especially when areas of cellular stratification or tufting from CCH become broad and multiple layered, then the lesion may mimic UDH. The gynecomastoid pattern of micropapillations in UDH may have similar features to the tufts seen in CCH, including broad-based papillae and narrow-pinched tip. The lining cells of CCH always have pronounced ovoid to elongated nuclei regularly oriented perpendicular to the basement membrane, with apical snouts. CK5/6 is strongly positive in mixed population of UDH and negative in CCH. ER shows heterogeneously low positive in UDH, while showing intense and diffuse positivity in CCC. However, spending effort to make a distinction between CCH and UDH is "of no diagnostic importance" according to some authors [18].

21. **Can CCC have necrosis? Punctate and central?**

Apical snouts and luminal secretions may be present, but usually not prominent or exaggerated in CCC. In CCH, apical snouts or luminal secretions can become exaggerated and prominent. True intraluminal necrosis is very rare in CCC. When present, high-power view to exclude possible atypia is necessary.

22. **Can CCC have mitosis?**

CCC has fine nuclear chromatin. Nucleoli and mitotic figures are exceedingly rare or absent. The Ki67 index was significantly lower in CCC (mean, 0.1%) than in normal TDLUs (mean, 2.4%) [21].

23. **What types of breast lesions can CCC be associated with?**

Atypical lobular hyperplasia (ALH) or LCIS frequently accompany columnar cell abnormalities, and tubular carcinoma may also be present, which composes a triad termed "Rosen Triad." The observation is that patients with tubular carcinoma of the breast often had foci of CCC distributed in surrounding tissue or sometimes even merging with the carcinomatous lesions [21]. LCIS may sometimes be present also. CCLs are increasing being encountered in breast biopsies with association of microcalcifications detected on

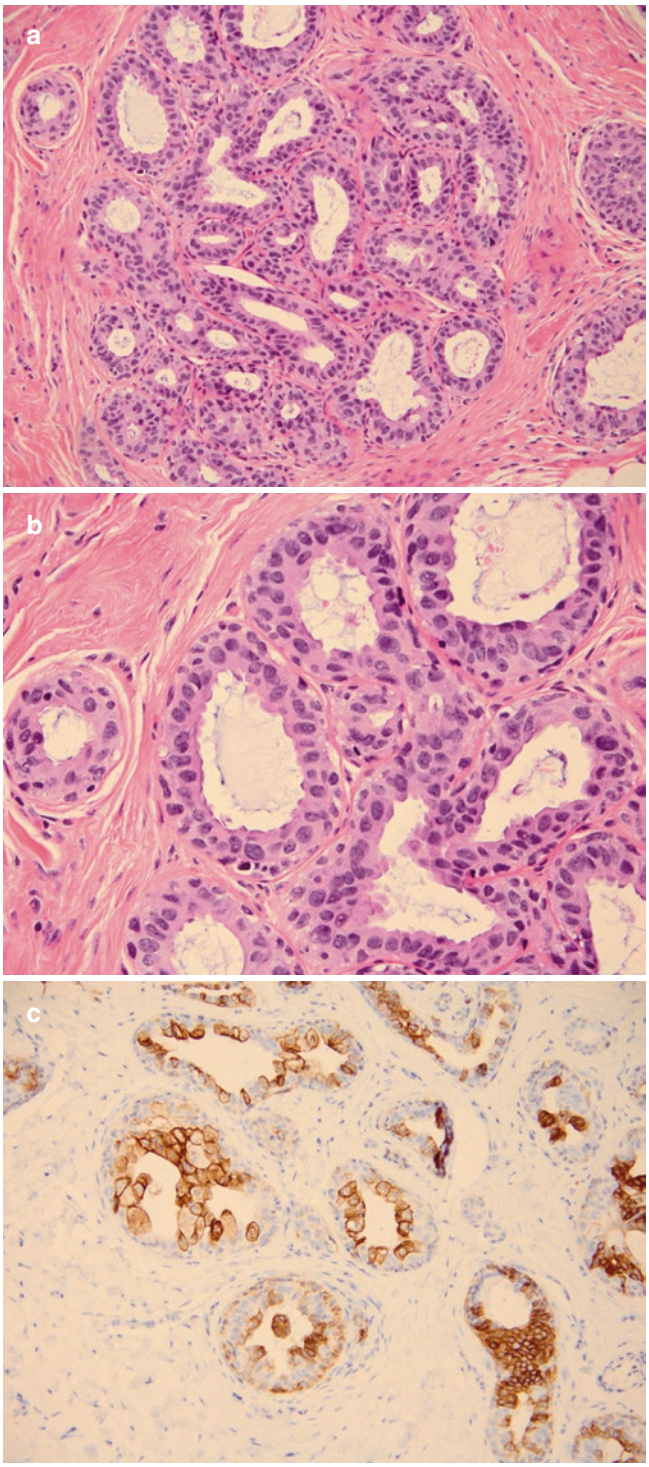

Fig. 1.3 UDH – blunt duct adenosis. Dilated acinar structures in TDUL (**a**) with larger plump cells without polarity (**b**) can be overlapping and retain CK5/6 stain (**c**)

mammographic screening. Although we should be aware of the possibility of coincidental tubular carcinoma, it is not demonstrated in most women, and the risk of subsequent tubular carcinoma is poorly documented [21]. Follow-up studies suggested that CCC is associated with a mild (~ 1.5 fold) increase in breast cancer risk. However, this increased risk is not clearly independent of the risk associated with the concurrent proliferative disease such as UDH [18].

24. **What is FEA and what are its key histologic features?** (Fig. 1.4a–f)

Flat epithelial atypia (FEA) is a neoplastic alteration of the enlarged dilated TDLUs, characterized by replacement of the native epithelial cells by one to several layers of relatively round or oval monotonous cells which have loss of polarity and increased nuclear/cytoplasmic ratio [23]. The nuclei are evenly distributed and align the basement membrane, and the cytological atypia resembles the cells diagnostic for G1-DCIS. The nuclear chromatin may be slightly clumping, irregular, and vacuolated, and margination may be present with visible prominent nucleoli. Mitotic figures may be seen [23].

Since the interobserver and intraobserver agreement is poor for the diagnosis of FEA, careful investigation of all CCLs at medium to high magnification is necessary to detect any cytological atypia [17]. More importantly, recognition of true ADH or even low-grade DCIS is crucial.

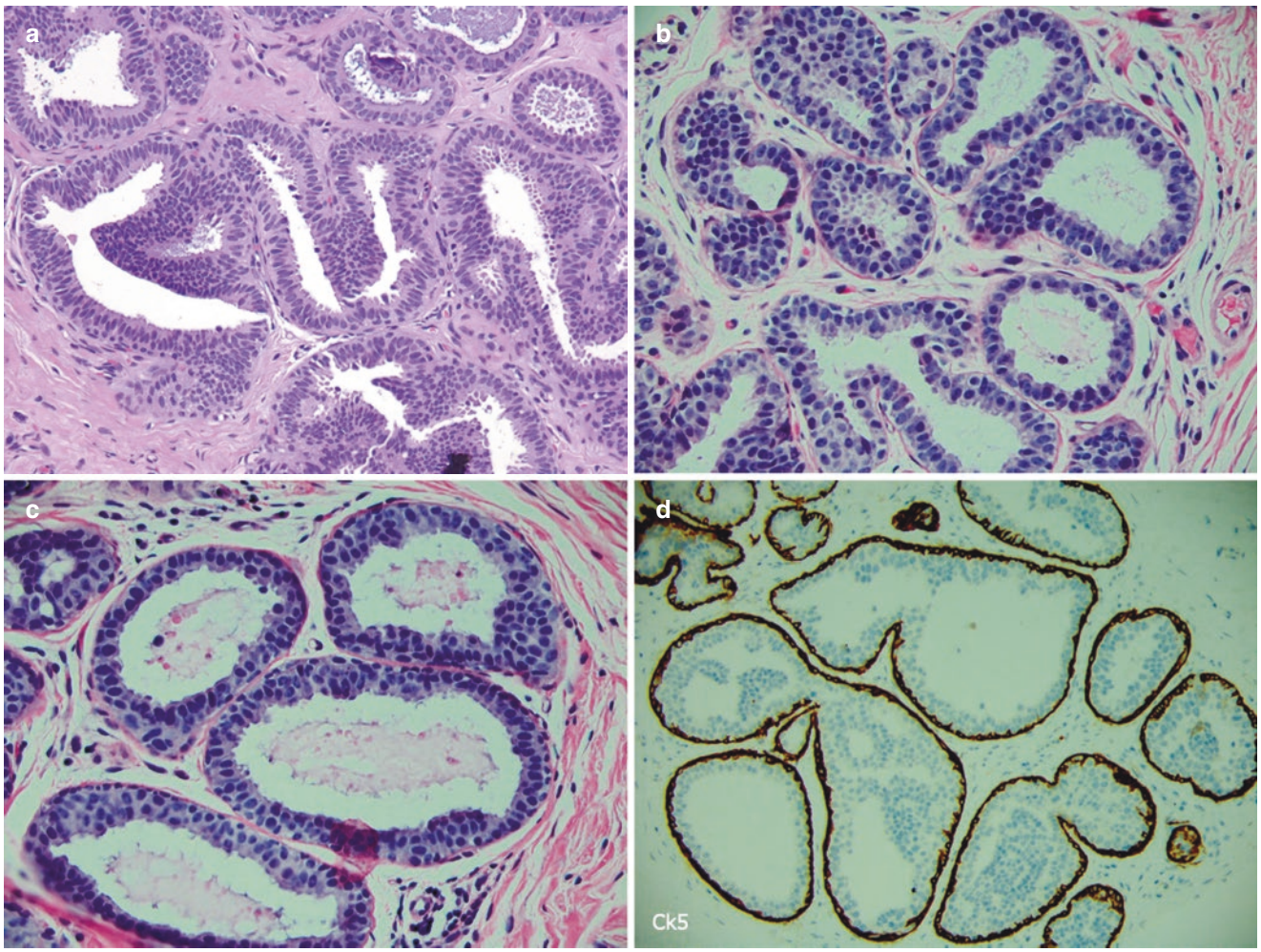

Fig. 1.4 CCC and FEA. CCC with TDLU with dilated acinar spaces and columnar cells perpendicular to BM (**a**). EFA with round cells lost polarity and lack prominent secondary structures (**b**, **c**). Negative for CK5/6 (**d**), negative for p63 (**e**), and uniformly positive for ER (**f**)

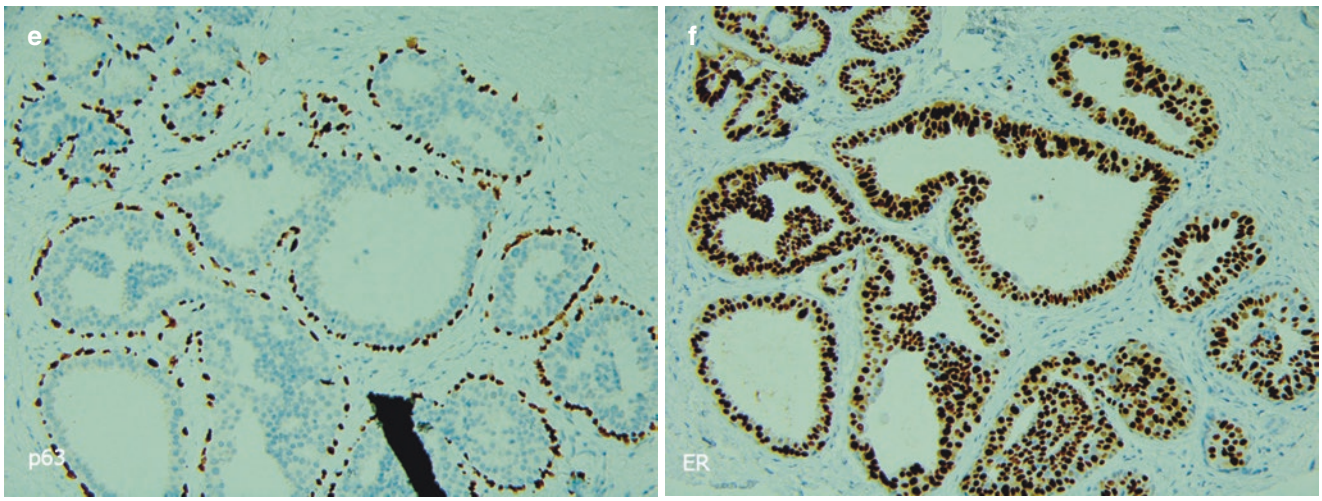

Fig. 1.4 (continued)

25. **How does one differentiate UDH from FEA?**

In contrast to mixed population and overlapping nuclei features of UDH, FEA is characterized by epithelial cells that are more cuboidal and rounded monomorphic nuclei with loss of polarization, which resemble those seen in low-grade DCIS. The gynecomastoid pattern of micropapillations in UDH may have similar features to the tufts seen in CCH, including broad-based papillae and narrow-pinched tip. However, in the majority of times with multiple epithelial cell layers, the lining epithelium in FEA remains flat with no architectural atypia [19].

Immunohistochemical stain for CK5/6 and ER shows distinct difference between UDH and FEA. CK5/6 is strongly positive in mixed population of UDH and negative in FEA. ER shows heterogeneously low positive in UDH, while appearing intense and showing diffuse positivity in FEA.

26. **What are the underlying molecular changes associated with FEA?**

Some genetic studies have indicated that FEA is a clonal lesion and shares genetic alterations with low-grade DCIS and tubular carcinoma, such as LOH at loci chromosome 16q, allelic loss, or damage to 9q, 10q, 17p, and 17q [22, 24]. However, the available follow-up studies of patients with FEA demonstrated an extremely low risk of subsequent progression to invasive breast cancer when present as an isolated lesion [18, 25]. Thus, World Health Organization (WHO) Working Group on the Pathology and Genetics of Tumors of Breast recommended that FEA should not be treated as equivalent to ADH or ALH [1].

27. **How does one differentiate FEA from ADH?**

Although the low-grade atypical lining epithelial cells in FEA may "form mounds, tufts, or short, abortive micropapillations," complex architectural patterns should not be seen [3]. The complex architectural patterns which indicate ADH include secondary architecture such as rigid Roman bridges, bars and arcades, cellular tufts with well-developed, club-shaped micropapillations, or cribriforming growth pattern with cell polarization around the lumens [18]. Whether to diagnose ADH or low-grade DCIS will depend on the details (quality and quantity) of the architectural and cytological atypia.

When a flat lesion shows high-grade lining epithelium with pleomorphic and marked nuclear atypia, even with a single cell layer, a clinging-type high-grade DCIS diagnosis should be rendered. For such a case, other histological features of high-grade DCIS are usually easy to find. Rarely, columnar cell lesion with intermediate-grade cytological atypia without complex architectural patterns may be encountered in breast specimens. Currently, there is no consensus on how to best classify the lesion. A diagnosis of FEA accompanied by a comment indicating that the degree of nuclear atypia is greater than that typically seen in FEA is recommended [18].

28. **Can FEA have necrosis? Punctate and central?**

Apical snouts and luminal secretions may be present in FEA, occasionally becoming exaggerated and prominent with a hobnailed appearance. Intraluminal punctate necrosis or apoptosis can be present. However, central comedonecrosis is extremely rare. When central necrosis is identified, the degree of epithelial atypia needs to be carefully investigated to rule out clinging growth pattern of high-grade DCIS or undersampled DCIS.

29. **Can FEA have mitosis?**

Mitotic figures may be seen but are uncommon [18]. Studies have shown that Ki-67 proliferative index was significantly higher in FEA (8.2%) than in CCLs without atypia and nor-

mal TDLUs, similar to that of low-grade DCIS (8.9%), but was significantly lower than the proliferation rate in intermediate- to high-grade DCIS (25.4%) [26].

30. **What types of breast lesions can be associated with FEA?**

FEA is reported to occur in 0.7–12.2% of percutaneous breast biopsies obtained for mammographic calcifications [27]. FEA significantly has been often detected in conjunction with a lesion of higher concern, including ALH, LCIS, ADH, and low-grade DCIS, and has been associated with tubular carcinomas [21]. The presence of FEA should trigger a careful search for areas with diagnostic features of these lesions, especially invasive tubular carcinomas. However, the upgrade rates to carcinoma among the current follow-up studies varied from 0 to 42%. FEA identified at the surgical resection margins is not an indication for additional surgery [25, 27–29]. The approach to the diagnosis of FEA on a core biopsy specimen has been controversial; such a situation should be subject to radiologic–pathologic correlation to determine the need for surgical excision [18, 30, 31].

31. **What is ADH and what are its key histologic features?** (Fig. 1.5a–i)

Atypical ductal hyperplasia (ADH), by definition, is a proliferation of uniform and monomorphic epithelial cells, evenly distributed as solid nests or well-formed architectures within terminal-duct lobular units. In another words, ADH is a proliferative epithelial lesion, with some but not all architectural and cytologic features of low-grade ductal carcinoma in situ. The key histologic features of ADH include both cytologic and architectural alterations. These lesions are usually small and may be multicentric like low-grade DCIS. The cytologic features of ADH at least partially resemble those of low-grade DCIS. Cells in ADH are relatively small and uniform in shape and size. They appear monomorphic and clonal with nearly normal chromatin pattern and may be mixed with cells of usual-type ductal epithelial hyperplasia. Nuclear

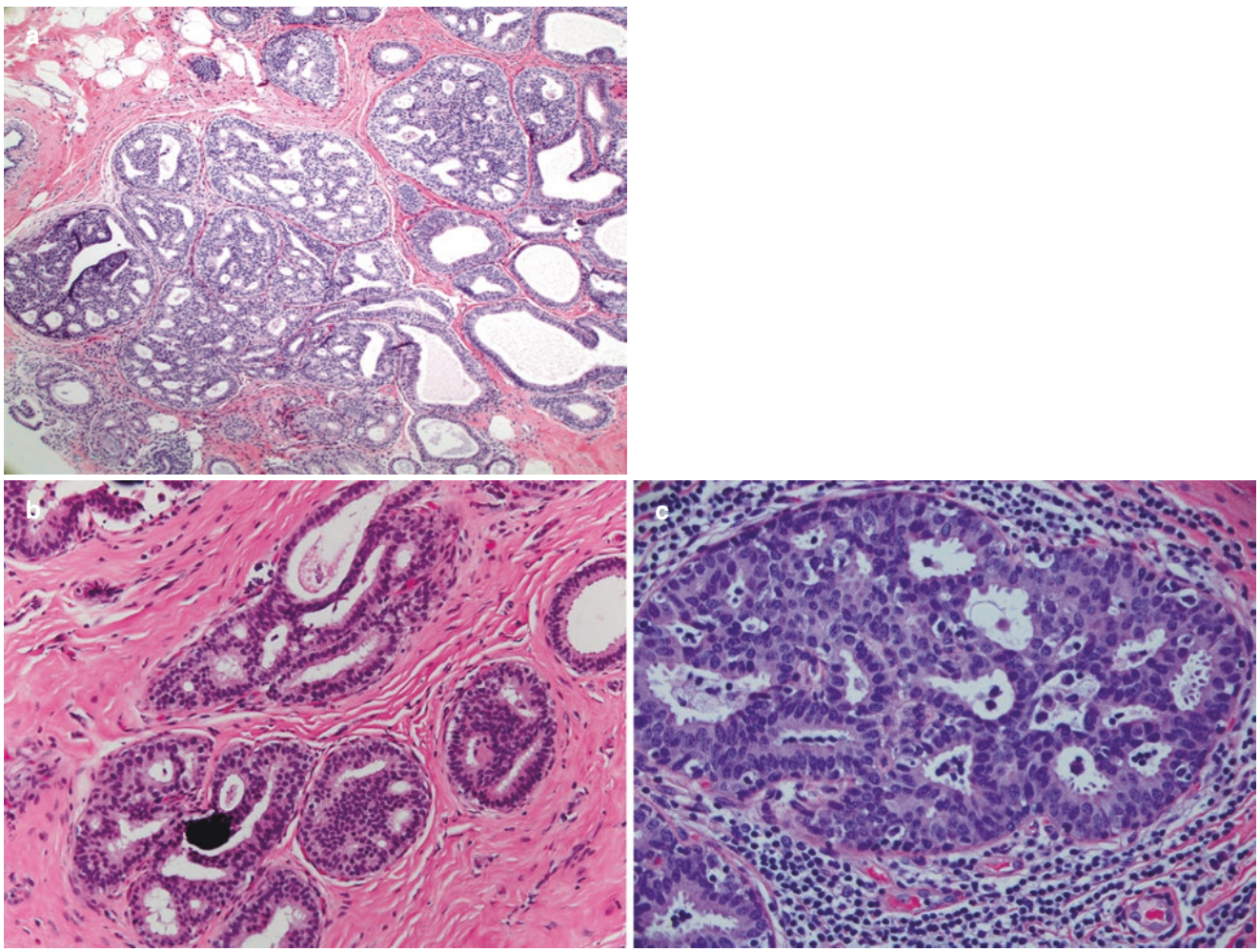

Fig. 1.5 ADH. Partially involved glands (**a**), associated with calcium (**b**), and irregular spaces (**c**). The ADH cells can be overlapping (**d**), look like a mixed population (**f**), and streaming (**h**), but they are uniformly lacking of stains for CK5 (**e**, **g**, **i**)

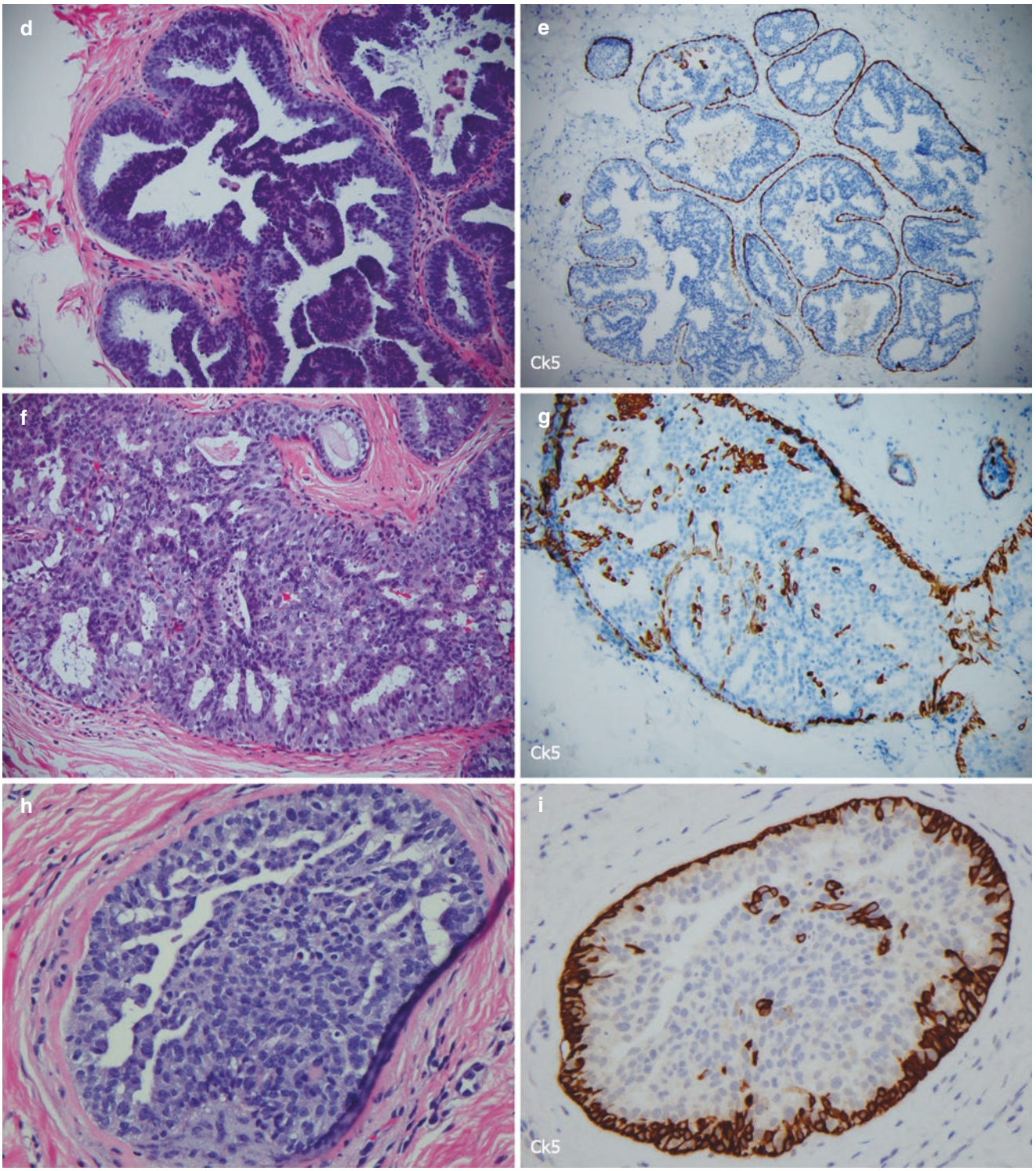

Fig. 1.5 (continued)

atypia is mild and mitosis is uncommon. Necrosis – especially central necrosis – is usually not associated with ADH. Architecturally, ADH can be solid: evenly distributed uniform cells fill the lumen of terminal ducts and lobules without overlapping or streaming. They may also form clear-cut round or oval shaped spaces, such as cribriform pattern with polarized lumen, micropapillae, small tufts, or ridged Roman bridging, such as in DCIS [32–35].

32. What IHC markers can be helpful for the diagnosis for ADH?

The diagnosis of ADH is mainly based on morphology of cytologic and architectural patterns. There are no immunologic markers for the diagnosis of ADH. That being said, some IHC markers can be helpful if the differential diagnoses are benign ductal hyperplasia and ADH. Estrogen receptor (ER) is usually diffusely and strongly positive, and CK5/6 is negative or scattered cells positive in ADH. On the other hand, hyperplastic cells of UDH are also positive for ER but the staining pattern is not diffuse and the staining intensity is variable. Cells in UDH usually show patchy or mosaic staining pattern for CK5/6 [11, 36, 37].

33. What are the underlying molecular changes associated with ADH?

There are very few studies on the molecular alteration of pure ADH. The diagnosis of ADH is often associated with low-grade DCIS and/or invasive ductal carcinoma, and the morphology of these lesions is similar in the same specimen. Most studies on molecular alterations of ADH are based on tissue with combine ADH and DCIS or invasive carcinoma. These studies found genomic similarity and nearly identical molecular alteration and chromosome imbalances in ADH, and associated DCIS or invasive carcinoma in the same specimens. Reported molecular changes include aneuploidy and LOH in at least one focus with loss of chromosome 16 being the most common. Other molecular changes include loss at 17p and 11q13, and gains at 1q [38–40].

34. How does one differentiate ADH from UDH?

The differences of ADH and UDH are mainly in their morphology, and immunostains can be of some help in difficult cases. The morphologic differences between ADH and UDH include different cytologic and architectural features. The cytologic features of ADH at least partially resemble those of low-grade DCIS. Cells in ADH are small, uniform, and monomorphic with nearly normal chromatin pattern and may be mixed with cells of usual-type ductal epithelial hyperplasia. Mitoses and necrosis are rare. Architecturally, cells in ADH are evenly distributed without overlapping or streaming. They also form solid nests or well-formed round- or oval-shaped spaces including cribriform, micropapillae, small tufts, or rigid Roman bridging, similar to that in DCIS. Cytologic features of UDH are different from those in ADH. UDH consists of heterologous cell populations, and the nuclei of these cells are small and variable in size and shape. They may have inconspicuous nucleoli and have rare mitoses. Cells in UDH tend to be crowded and haphazardly placed, with streaming and swirling. The growth pattern of UDH can be solid, micropapillary, or fenestrated. Fenestrated structures in UDH are ill formed, irregular shaped, or slit like.

With regard to immunohistochemistry, estrogen receptor (ER) is usually diffusely and strongly positive and CK5/6 is negative or scattered cells positive in ADH. On the other hand, hyperplastic cells of UDH are also positive for ER, but the staining pattern is not diffuse and the staining intensity is variable. Cells in UDH usually show patchy or mosaic staining pattern for CK5/6 [32–35, 41, 42].

35. How does one differentiate ADH from FEA?

These two lesions are different in both architecture and cytology but do share some similarities. Architecturally, the terminal ductal lobular units (TDLU) are usually dilated, without formation of cribriform, micropapillary or other types of structure in FEA, while ADH can form different architectures including solid growth pattern, Roman bridging, blunt micropapillae, or cribriform formation. Cytologically, these two lesions share some cytologic similarity in that cells in both lesions are low grade with mild nuclear atypia. However, cells lining the dilated ducts in FEA are usually single layered and may be crowded; the morphology of these cells is enlarged cuboidal shaped or rounded with columnar configuration while cells in ADH are more uniform and can form a solid nest. There are no IHC markers to differentiate ADH from FEA. Cells in both lesions are positive for ER, usually uniform and strong. They are negative for CK5/6 [32–35, 43–45].

36. How does one differentiate ADH from grade 1 DCIS?

ADH resembles grade 1 DCIS both in cytology and architecture; the differences of these two lesions are both qualitative and quantitative, both in cytology and in architectures. Qualitatively, to make a diagnosis of low-grade DCIS, the lesion needs to fulfill all features of ductal carcinoma in situ, that is, cytologically, atypical tumor cells are uniform, and, monomorphic, no streaming, or overlapping. Architecturally, these cells may form a solid pattern or sharply defined cribriform, micropapillae, or rigid Roman bridging. These atypical cells and well-formed architectures need to fill the entire duct. The other criteria are quantitative, which requires the size of lesional tissue fulfilling

Table 1.1 Differences of UDH, FEA, ADH, and grade 1 DCIS

	UDH	FEA	ADH	Grade 1 DCIS
Cytology	Multiple types of mixed cell populations; no nuclear atypia, normal pattern chromatin, mitosis can be seen	Enlarged cuboidal shaped or rounded cells with columnar configuration, fine chromatin pattern and rare mitosis, maybe crowded	Uniform and monomorphic cells; fine chromatin, inconspicuous nucleoli, rare mitosis, may be mixed with normal cells	Uniform and monomorphic cells, fine chromatin, inconspicuous nucleoli, rare mitosis
Architecture	Slit-like, streaming, and overlapping arrangement	Dilated acinar structure in terminal ductal lobular unit with single layer lining cells that have loss of polarity	Complex secondary structures such as rigid bars, bulbous micropapillae, round punctate space, and rigid Roman bridging	Complex secondary structures such as rigid bars, bulbous micropapillae, round punctate space, and rigid Roman bridging
Size	No size limit, can be rare or extensive	No size limit	<2 ducts or <2 mm	>2 ducts or >2 mm
Immunostains	Variable positive for ER, mosaic pattern CK5/6	Diffuse and strong positive for ER and negative for CK5/6	Diffuse and strong positive for ER and negative for CK5/6	Diffuse and strong positive for ER and negative for CK5/6
Risk of cancer	Slight	Mild increase	Moderate	High risk

the above criteria either involving two ducts or measuring at least 2 mm in length. The diagnosis of ADH is made when ducts are only partially involved by carcinoma cells, only one duct is fully involved, or the involved area is <2 mm [21, 32–35, 46]. See Table 1.1.

37. **Can ADH have necrosis? Punctate and central?**

ADH is usually associated with low-grade DCIS. Central necrosis and punctate necrosis are usually associated with high-grade DCIS, but they can be rarely seen in ADH. The presence of necrosis or punctate necrosis does not change the diagnosis of ADH [32–35].

38. **Can ADH have mitosis?**

Mitotic figures can be identified in ADH, but they are uncommon. There may be more mitoses in UDH compared to ADH [32–35].

39. **What types of breast lesions can ADH be associated with?**

Atypical ductal hyperplasia (ADH) is not a type of breast cancer; instead, it is a marker indicating an increased risk for developing breast cancer in the future. The risk of developing cancer includes both breasts but the risk is higher on the ipsilateral breast. ADH is often associated with breast cancer, most likely ductal carcinoma, either in situ or invasive. The diagnosis of ADH is most often treated by surgical excision or lumpectomy, and the diagnosis of ADH can be upgraded to DCIS or invasive carcinoma. The possibility of finding carcinoma on surgery depends on the amount of ADH identified on core biopsy and on associated radiologic findings. The more ADH identified on core needle biopsy, the higher chance of cancer identified on the surgical specimen. The rate of cancer identified on surgical specimen varied by a large range; however, estimated upgrading of carcinoma on surgical specimen is 30% based on two large studies [47–51].

40. **What is grade 1 DCIS and what are its key histologic features?** (Fig. 1.6a–c)

Ductal carcinoma in situ (DCIS) is a type of in situ breast carcinoma in which tumor cells originate from ductal epithelium and are confined within ductal–lobular units without stromal invasion. The morphology of these tumor cells ranges from monotonous with mild cytologic atypia to cells with marked nuclear pleomorphism. Tumor cells in grade 1 DCIS are monomorphic with only mild cytologic atypia. Nuclei are uniform size with inconspicuous nucleoli. Mitoses and necrosis are rare. It may be associated with microcalcifications. To make a diagnosis of grade 1 DCIS, we need to see that 1. qualitatively, tumor cells fill the entire ducts, without overlapping or streaming, or they form punch-out spaces, cribriform, or micropapillary patterns; and 2. these changes need to involve at least two ducts or involve 2 mm or above of the area. Architectural patterns of DCIS grades 1–3 can be solid, cribriform, micropapillary, or comedo types [45, 46].

41. **What IHC markers can be helpful for the diagnosis for grade 1 DCIS?**

There are no IHC markers for making a diagnosis of grade 1 DCIS. However, if the differential diagnosis is usual ductal hyperplasia, IHC of CK5/6 and ER can be of some help. Grade 1 DCIS like ADH is usually negative for CK5/6 and strong and diffusely positive for ER [11, 52].

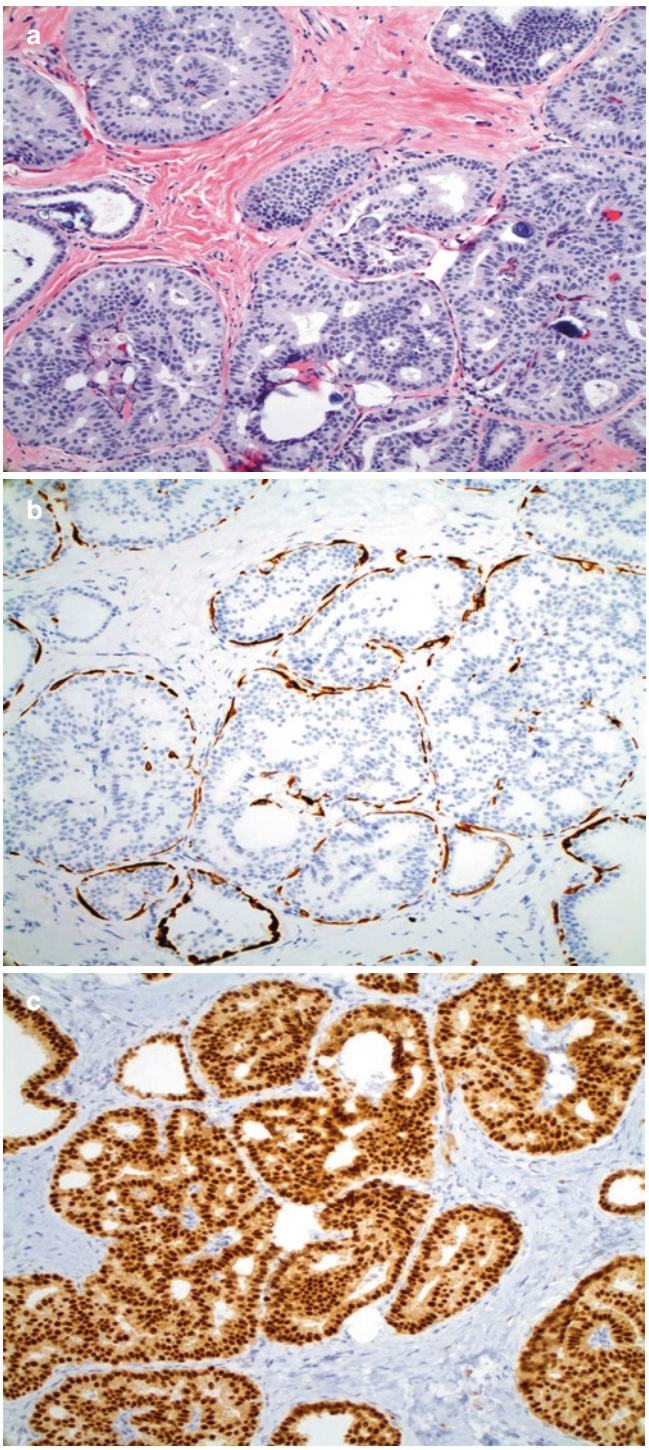

Fig. 1.6 G1 DCIS. Low-grade and monotonous neoplastic cells >2 mm or 2 spaces (**a**), negative for CK5/6 (**b**), and uniformly positive for ER (**c**)

42. What are the underlying molecular changes associated with grade 1 DCIS?

Molecular changes associated with grade 1 DCIS are similar to those of ADH, including aneuploidy, LOH, chromosomal losses at 16q, 17p, and 11q13, and gains at 1q. Loss of chromosome 16 is the most common alteration [38–42].

43. How does one differentiate grade 1 DCIS from UDH?

The differences between DCIS and UDH are also mainly in their morphology. Immunostains can be of some help in difficult cases. The morphologic differences between grade 1 DCIS and UDH include different cytologic and architectural features. Cytologically, tumor cells appear uniform, monotonous, and clonal with nearly fine chromatin pattern and inconspicuous nucleoli. Mitoses are not common and necrosis, especially central necrosis, is rare. Architecturally, cells in DCIS form solid nests or well-formed round or oval-shaped spaces including cribriform, micropapillae, small tufts, or rigid Roman bridging. Cytologic features of UDH are different from those of DCIS. UDH consists of heterologous cell populations, and the nuclei of these cells are small and variable in size and shape. These cells may have inconspicuous nucleoli and have rare mitoses. They tend to be crowded and haphazardly placed, with streaming and swirling. The growth pattern of UDH can be solid, micropapillary, or fenestrated. Fenestrated structures in UDH are ill formed, irregular shaped, or slit like. The difference of IHC in grade 1 DCIS vs UDH is the same as that of ADH vs UDH [11, 41, 42, 51].

44. How does one differentiate grade 1 DCIS from grade 2 DCIS?

Nuclear size and morphology: Tumor cells in grade 1 DCIS are small and uniform (1–2x of normal breast epithelial cells). They have small nuclei, normal chromatin pattern and inconspicuous nucleoli. Mitoses and necrosis are very rare. The features of tumor cells in grade 2 DCIS are in-between grade 1 and grade 3 DCIS (>2.5x of normal breast epithelial cells). They are not as uniform as that of grade 1 DCIS. Tumor cells in grade 2 DCIS show mild to moderate variation in cell shape and size. Chromatin is variably coarse and may have prominent nucleoli.

45. How does one differentiate grade 1 DCIS from florid LCIS? (Figs. 1.7a–e, 1.8a–c, 1.9a–d)

Grade 1 DCIS cells are small, uniform, and cohesive, and form solid nests, cribriform, micropapillary, and other architectures. Florid LCIS consists of classic lobular cells that are small, uniform, and noncohesive. These cells present in marked distended TDLU and occasionally are associated with microcalcifications and central necrosis. Frequently, they are associated with invasive lobular carcinoma. When suspicious, a confirmation IHC for lack of E-cadherin staining is recommended for diagnosis. Occasionally, low-grade DCIS can mimic LCIS (involves TDLU, termed cancerization

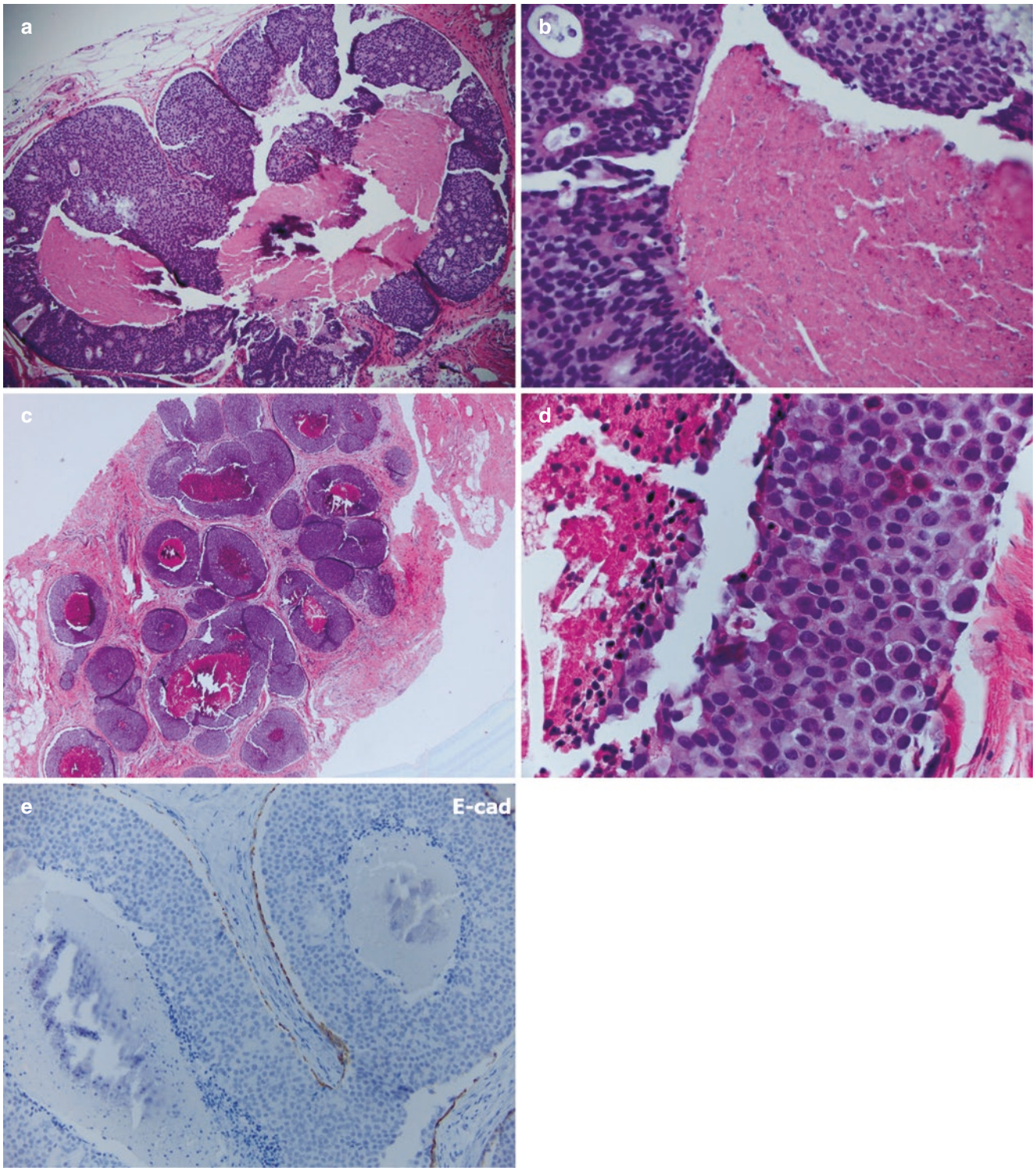

Fig. 1.7 G1 DCIS and florid LCIS. GI DCIS, solid and cribriform patterns and with central necrosis (**a**, **b**). FLCIS with classic lobular cells and central necrosis (**c**, **d**) and negative for E-cadherin (**e**)

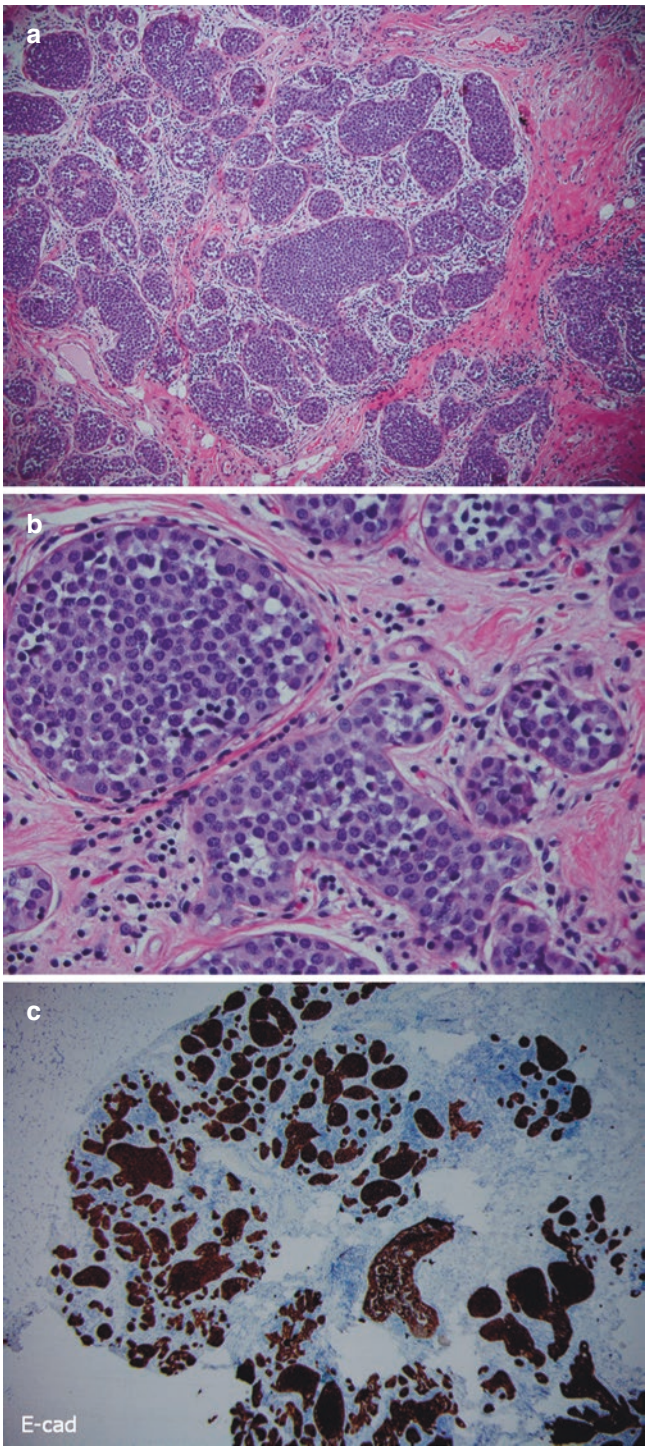

Fig. 1.8 G1 DCIS mimics LCIS in TDLU. G1 DCIS involving TDLU (**a**, **b**) and showing strong positive membrane staining for E-cadherin (**c**)

of the lobules), and DCIS and LCIS could coexist in the same ductal space [21, 46].

46. **Can grade 1 DCIS have necrosis?**

Grade 1 DCIS usually does not have necrosis, especially central necrosis; however, it occasionally can have necrosis, especially punctate necrosis. The presence of punctate necrosis does not change the diagnosis of grade 1 DCIS. Mitoses are rare in grade 1 DCIS; however, it does present. Grade 1 DCIS can associate with invasive ductal breast cancer, low grade [21, 46].

47. **What is grade 3 DCIS and what are its key histologic features?** (Fig. 1.10a, b)?

Grade 3 DCIS is a high-grade in situ ductal carcinoma. Tumor cells are large with pleomorphic nuclei, coarse clumped chromatin, and prominent nucleoli. Mitoses are common and often atypical. Grade 3 DCIS may present as solid pattern, cribriform, or micropapillary types with necrosis and calcifications; it is often associated with marked periductal desmoplastic changes, which is not a feature of lower-grade DCIS. Comedo-type necrosis associated with necrotic debris and calcifications are common. Key features of grade 3 DCIS are high-grade pleomorphic nuclei (>2.5× of normal breast epithelial cells). The size of grade 3 DCIS is usually large, more than 5 mm; however, the diagnosis does not have a size limit [21, 46].

48. **What IHC markers can be helpful for the diagnosis of grade 3 DCIS?**

The diagnosis of grade 3 DCIS is made by morphology: high-grade pleomorphic nuclear features associated with central necrosis and calcifications. These tumors are often negative for ER and PR and positive for Her-2. Sometimes, the tumor cells are positive for CK5/6 and EGFR, defining the basal-like subtype of DCIS. No definitive IHC markers are helpful in the diagnosis.

49. **What are the underlying molecular changes associated with grade 3 DCIS?**

The molecular profile of grade 3 DCIS is different from grade 1 DCIS both quantitatively and qualitatively and is similar to that of high-grade invasive ductal carcinoma. In general, 24.5% of patients with grade 3 DCIS carry germline mutation of BRCA1 and BRCA2. They tend to be ER and PR negative and Her-2 positive, and have a p53 mutation. Grade 3 DCIS is usually aneuploid with complex genetic patterns. It frequently has a loss of 11q, 14q, 8q, and 13q and gains of 17q, 5p, and 8q. It also has amplifications of 17q12, 17q22–24, 6q22, 8q22, 11q13, and 20q13 [53–56].

50. **How does one differentiate grade 3 DCIS from ADH?**

Cytologic and nuclear features: Cells in ADH are uniform without variation in size and shape. Nuclei are small with nearly normal pattern chromatin, inconspicuous nucleoli, and very infrequent mitoses. On the other hand, tumor cells in grade 3 are large, pleomorphic, and hyperchromatic, with

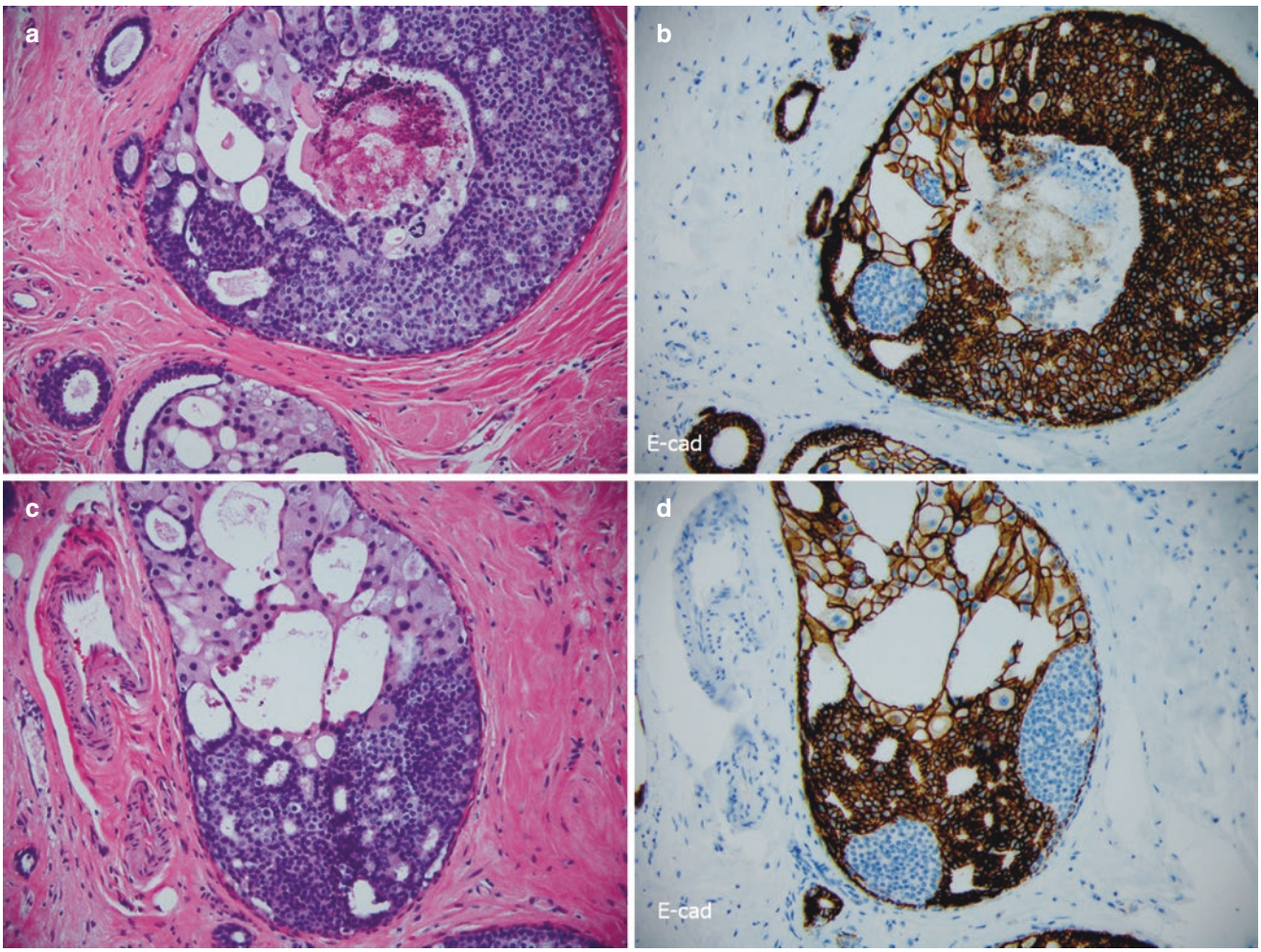

Fig. 1.9 (**a**–**d**) G1 DCIS admix with LCIS. DCIS and LCIS involving single glandular spaces (HE and E-cadherin)

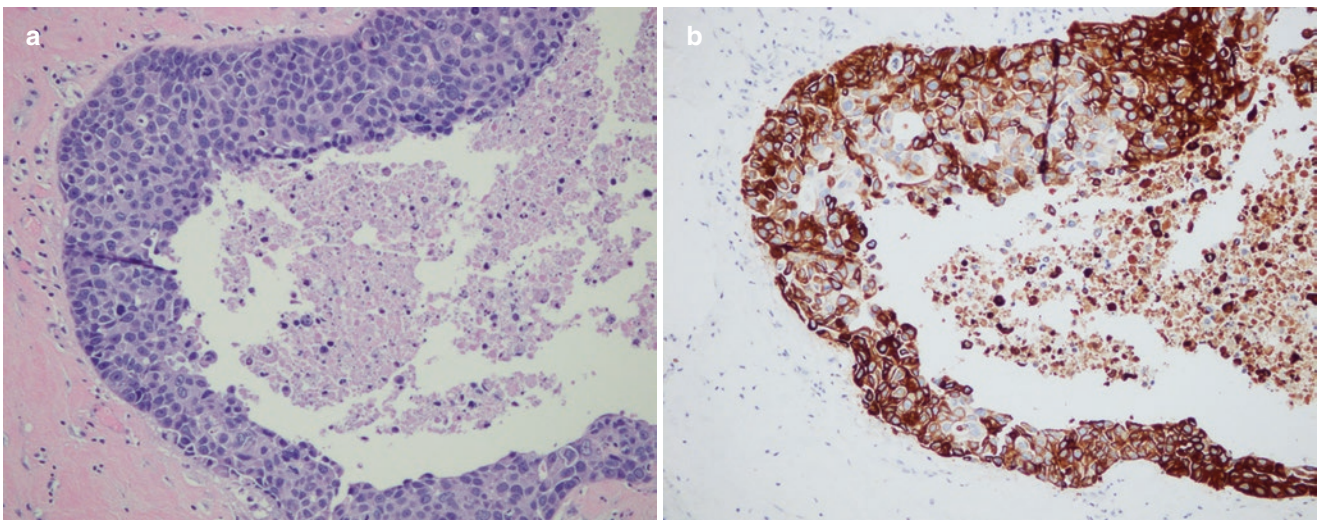

Fig. 1.10 (**a**, **b**) G3 DCIS with central necrosis and positive CK5/6 staining

prominent nucleoli and brisk mitoses. Necrosis, especially central necrosis associated with microcalcifications, is common.

Tumor markers: ADH is usually diffusely and strongly positive for ER and PR while grade 3 DCIS is usually negative for ER and PR and positive for Her-2 [21, 32–35, 46].

51. How does one differentiate grade 3 DCIS from UDH?

Cytologic features: In UDH, lesion cells are heterologous mixed cell populations; nuclei are small and variable in size and shape. They may have inconspicuous nucleoli and rare mitoses. Cells tend to be haphazardly placed and crowded with streaming and swirling. The growth pattern can be solid, micropapillary, or fenestrated. Fenestrated structures in UDH are ill formed, irregular shaped, or slit like. Tumor cells in grade 3 are large, pleomorphic, and hyperchromatic, with prominent nucleoli and brisk mitoses. Necrosis, especially central necrosis associated with microcalcifications, is common. The growth pattern of grade 3 DCIS can be solid, irregular shaped, cribriform, or micropapillary, often associated with central punctate necrosis and calcification.

IHC: ER staining is of variable intensity and of mosaic pattern staining for CK5/6 in UDH, while most grade 3 DCIS is negative for ER and negative or positive for CK5/6 [21, 41, 42, 46].

52. How does one differentiate grade 3 DCIS from grade 2 DCIS?

Grade 3 DCIS and grade 2 DCIS are separated by their nuclear and cytoplasmic features and architectures. Tumor cells in grade 3 are large, often more than three times that of RBC; nuclei are pleomorphic and hyperchromatic, with course clumped chromatin, prominent nucleoli, and brisk mitoses. On the other hand, tumor cells in grade 2 DCIS show mild to moderate variation in size and shape. They have variable course chromatin, inconspicuous nucleoli, and rare mitoses. Central punctate necrosis with necrotic debris and calcifications are common in grade 3 DCIS, while these changes are not as common in grade 2 DCIS. Additionally, the diagnosis of grade 3 DCIS does not have a size requirement. Simply put, features of tumor cells in grade 2 DCIS are in-between grade 1 DCIS and grade 3 DCIS but closer to grade 1 DCIS [21, 46].

53. What is mammary Paget's disease and what are its key histologic features? (Fig. 1.11a–d)

Mammary Paget's disease (MPD) is a rare type of breast cancer involving the nipple and surrounding areola, in which glandular tumor cells are located within squamous epithelium. It accounts for 1–3% of all primary breast tumors. It was first described by British doctor Sir James Paget in 1874, who noted a relationship between a chronic eczematous disease of the nipple and areola and intraductal and invasive breast cancer.

Paget's disease of the breast most often occurs in postmenopausal women. A large majority of patients (>95%) with MPD have underlying breast cancers, either DCIS or invasive ductal carcinoma. Symptoms of Paget's disease include eczematous rash, flaky or scaly skin lesion, and nipple discharge with straw colored or bloody fluid. These symptoms usually affect the nipple and areola and later spread to the remaining breast. Diagnosis is established by punch biopsy.

Histology of MPD: Glandular tumor cells present within the epidermis of the nipple and areola and may extend to adjacent skin. These tumor cells (Paget cells) are usually large and round, with abundant pale cytoplasm. They have large nuclei with large and prominent nucleoli. Paget cells can present as isolated single cells, small clusters, or packed clusters. Involved epidermis is thickened with papillomatosis and enlargement of the interpapillary ridges. Hyperkeratosis, parakeratosis, and marked inflammation may also occur and may be associated with surface ulcer [57–60].

54. What IHC markers can be helpful for the diagnosis of MPD?

The immunohistological pattern of Paget cells is the same as the underlying breast cancer. Paget cells are usually positive for low-molecular-weight cytokeratin, such as cytokeratin 7 and CAM 5.2. They are also positive for immunostaining of CEA and for special stain of PAS and diastase resistance. MPD is most often associated with high-grade carcinoma, and most MPDs are also positive for Her-2 and less than half of them express ER and PR. By contrast, surrounding squamous cells are positive for CK5/6 and p63 and negative for low-molecular-weight keratin, CEA and PASD [58, 61, 62].

55. How does one differentiate MPD from nipple melanoma?

To differentiate MPD from melanoma can be difficult clinically and might have to be done through histopathology. The morphology of MPD and nipple melanoma is similar in that tumor cells can present within the epidermis, and melanin can be present in both tumors; however, the pattern of distribution of tumor cells within the epidermis is different. Tumor cells of melanoma tend to form nests along the dermo-epidermal junction with extension into papillary dermis whereas tumor cells of MPD are usually distributed more diffusely. Tumor cells in MPD may form acini which is not seen in melanoma. Immunohistochemical stains can be of help to differentiate these entities in difficult cases. Cells in melanoma are positive for melanocytic markers and negative for markers of cytokeratin, PASD and CEA, while Paget cells are negative for melanocytic markers but positive for markers of cytokeratin, PASD and CEA (Table 1.2) [63–65].

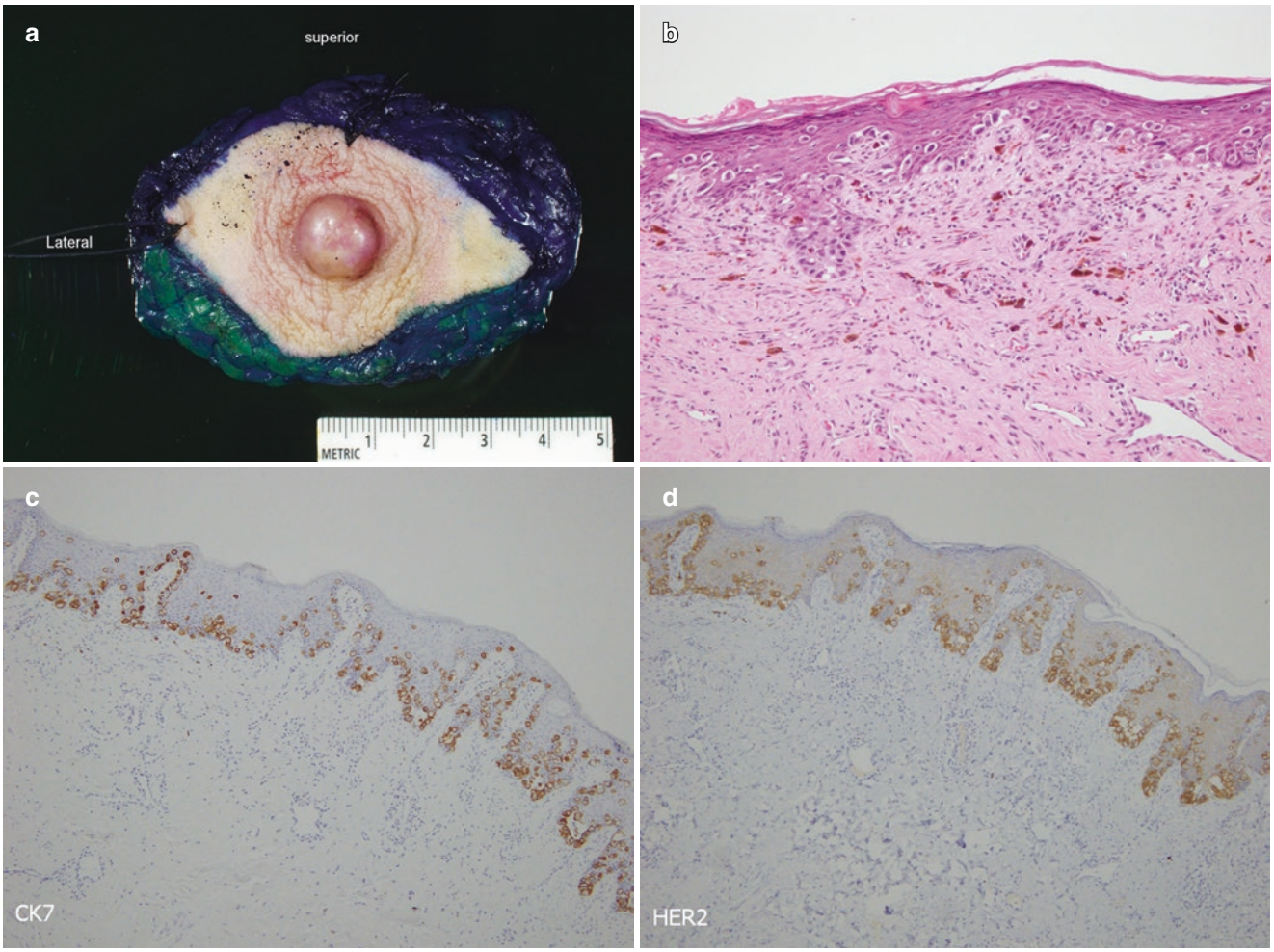

Fig. 1.11 Mammary Paget disease (MPD). MPD grossly involved in nipple (**a**). The large tumor cells scattered in epidermis (**b**) are positive for CK7 (**c**) and HER2 (**d**)

Table 1.2 Immunohistochemical stains differentiate cells in melanoma, Paget cells, and squamous cells

	Melanocytic markers (HMB45, S100, Mela A, and Sox10	Low-molecular-weight CK (CK7 and Cam 5.2)	CK5/6 and p63	PASD and CEA
Paget's disease	N	P	N	P
Melanoma	P	N	N	N
Squamous cells	N	N	P	N

56. What types of breast lesions can be associated with MPD?

MPD is associated with either DCIS or IDC in the great majority of cases. More than 95% of MPD is associated with underlying breast cancer, invasive or in situ carcinoma. Immunostaining pattern and molecular changes of MPD are the same as the underlying breast cancer.

57. How does one stage MPD?

MPD itself is an intraepithelial carcinoma in the great majority of cases. The staging of MPD depends on its underlying carcinoma since >95% of MPD have underlying carcinoma.

Case Presentations

Case 1: FDH with Necrosis (Fig. 1.12a–d)

History: A 40-year-old female with calcification on screening mammogram underwent core needle biopsy
Histology: Intraductal proliferation with central necrosis; cells are relatively uniform and not overlapping
IHC: ER: positive 70%, PR: positive 60%, P63: peripheral stains
DDx: Consider DCIS, G2

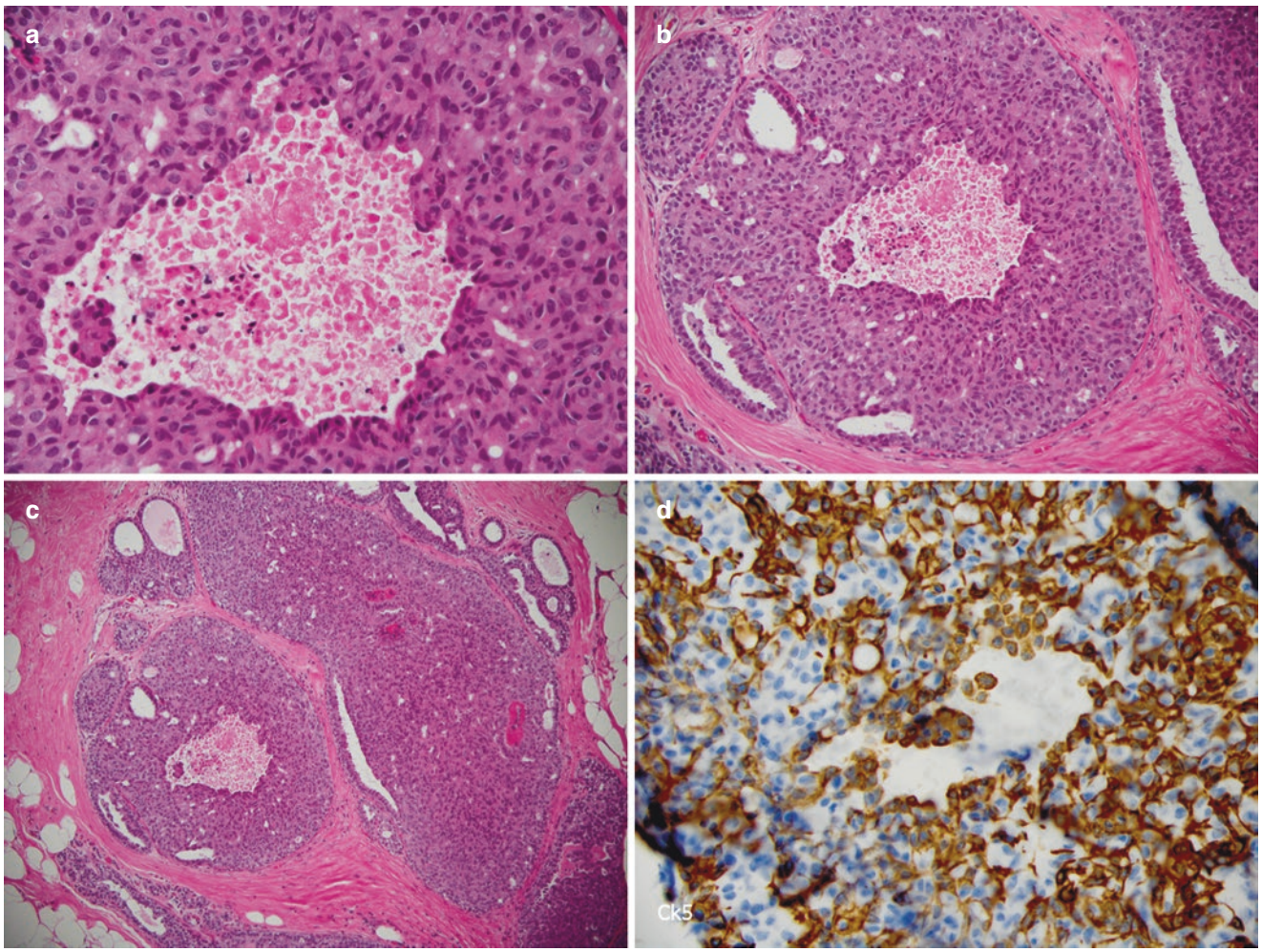

Fig. 1.12 Case 1: FUDH with necrosis. High-power view (40×) shows central necrosis with enlarged epithelial cells, mimic grade 2 DCIS (**a**); lower-power view (20×) shows the cells with features of florid ductal hyperplasia (disorganizing and overlapping with irregular spaces) (**b**), and a 10× view shows that the FUDH is part of intraductal papillary lesion (**c**). The mosaic staining pattern for CK5/6 supports the diagnosis of FUDH (**d**)

Next Steps:
- CK5/6: positive with mosaic pattern
- ER/PR staining pattern: positive with variable intensity

Final Diagnosis:
- FDH with necrosis

Take-Home Messages:
- Central necrosis can occur in UDH
- CK5, not p63 is a best marker for UDH

Case 2: Blunt Duct Adenosis mimic FEA (Fig. 1.13a–d)

History: A 40-year-old female with calcification on screening mammogram underwent core needle biopsy
Histology: Lobular centric proliferation with enlarged acinar structures; cells are enlarged and without polarity; apocrine snouts can be seen; no secondary structures

DDx: CCC vs G2 DCIS vs others
Next Steps:
- CK5

Final Diagnosis:
- Blunt duct adenosis

Take-Home Messages:
- Overlapping with no clear cytoplasmic membrane
- Myoepithelial layer usually prominent
- Mixed population identified by CK5

Case 3: G1-DCIS with Attenuated Myoepithelial Cells (Fig. 1.14a–f)

History: A 40-year-old female with mass on screening mammogram underwent core needle biopsy
Histology: DCIS, low-grade and cribriform pattern

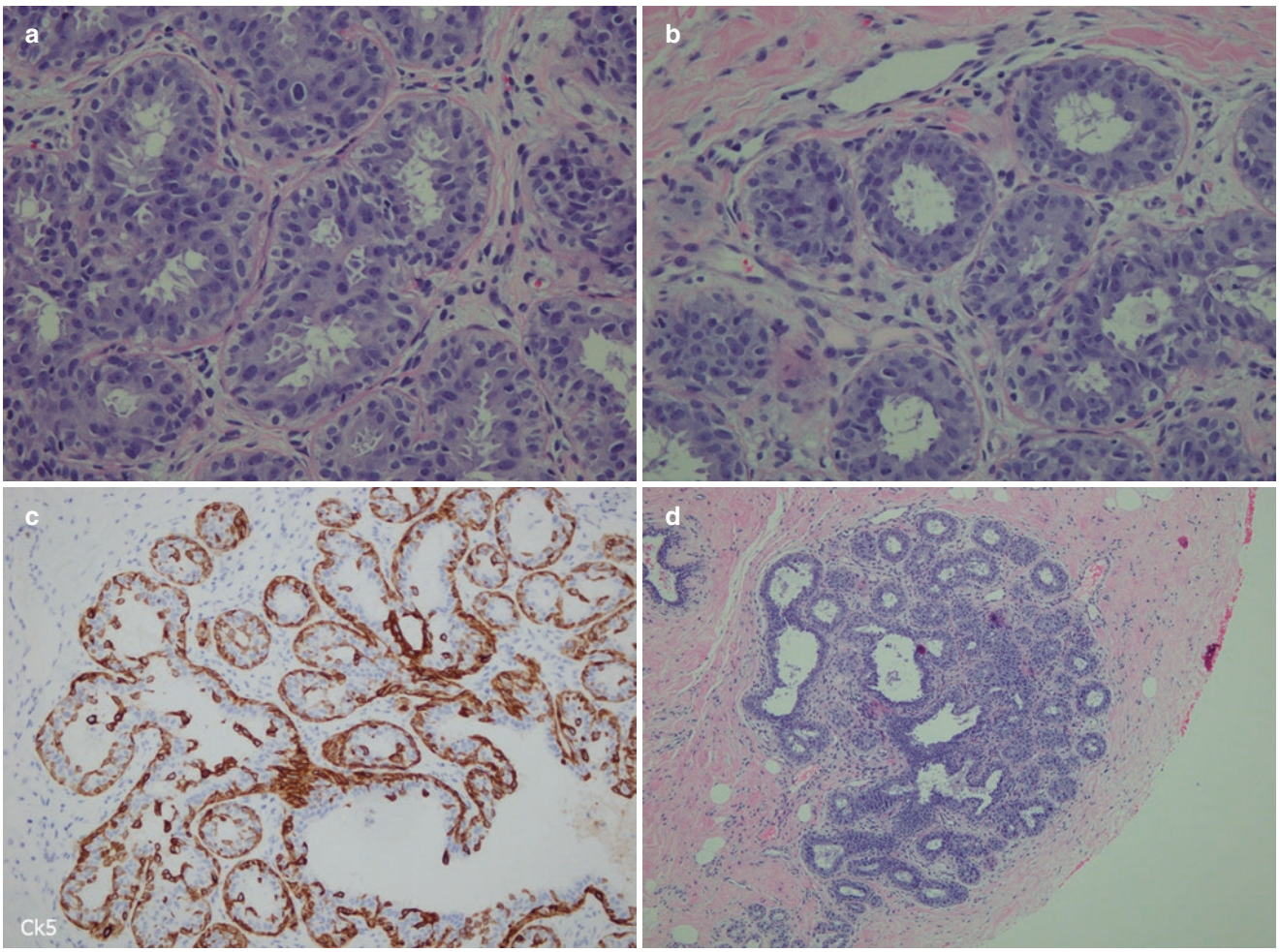

Fig. 1.13 Case 2: Blunt duct adenosis vs FEA. This is a lobular centric epithelial proliferation with all features of UDH, such as enlarged epithelial cells that are overlapping, with different orientations and without prominent cell borders (**a**, **b**); the mixed population of epithelium is highlighted by IHC analysis for CK5/6, which is different from CCC that is lacking CK5/6 positive luminal cells (**c**). A low-power view demonstrates the lobular centric architecture (**d**)

IHC: ER and PR: strongly positive
Next Steps:

- Surgical resection, IHC for myoepithelial cells

Final Diagnosis:

- DCIS

Take-Home Messages:

- Although attenuated myoepithelial cells most often occur in high-grade DCIS, they can occur in low-grade DCIS as well.
- Some myoepithelial marker is better than others; thus, use a panel of markers instend of single marker.

Case 4: DCIS Versus IDC (Fig. 1.15a–e)

History: A 40-year-old female with calcification on screening mammogram underwent core needle biopsy
Histology: Extensive solid high-grade DCIS with central necrosis; no prominent cancerization of the lobule present; focal areas (>1 mm) with small angulated glands in a desmoplastic background, with necrosis, and suspicious of invasion; areas of microinvasion (<1 mm) also present and one positive LN
DDx:

- IDC, multifocal, <1 mm to 5 mm
- Then:
- send to HER2 FISH, no invasive cancer

Next Steps:

- MEP present in the 5 mm focus; thus it is not IDC, but DCIS with sclerotic changes
- Tumor markers repeated on the mets

Final Diagnosis:

- IDC, microinvasive (m), with positive node

Take-Home Messages:

- Cancerization of lobule with sclerotic changes can mimic IDC
- Do not assume that IDC and DCIS in the same patient are always morphologically different

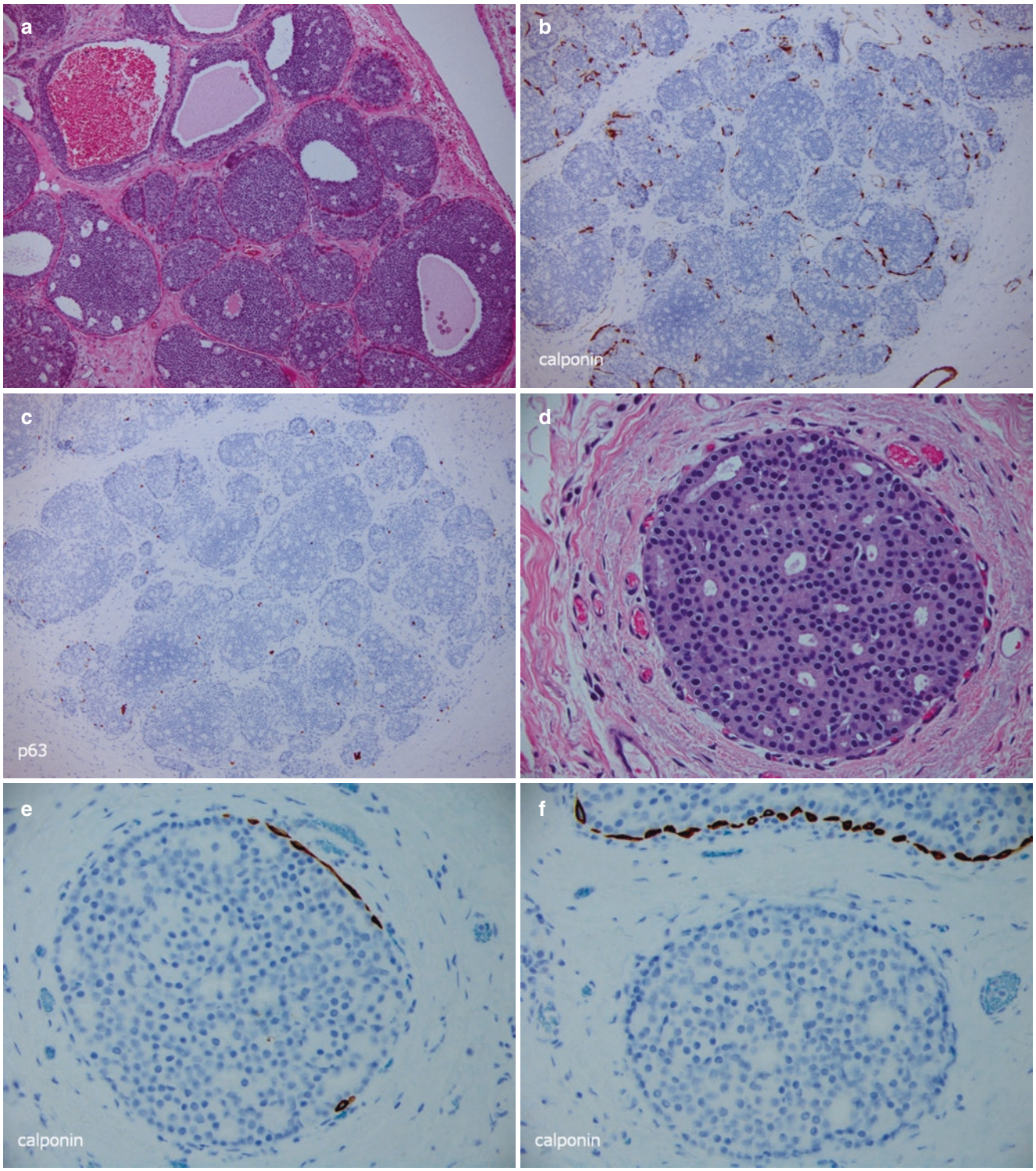

Fig. 1.14 Case 3: LG-DCIS with attenuated myoepithelial cells. A low-grade DCIS with cribriform and solid patterns (**a**), with low-power views of IHC analyses for myoepithelial markers calponin (**b**) and p63 (**c**), which demonstrates the marked attenuated staining patterns, especially for p63. A high-power view (40X) confirms the low-grade nuclei for this DCIS (**d**), while the calponin stains show complete presence (E-upper) and absence (E-lower) and partial presence (**f**) of staining patterns

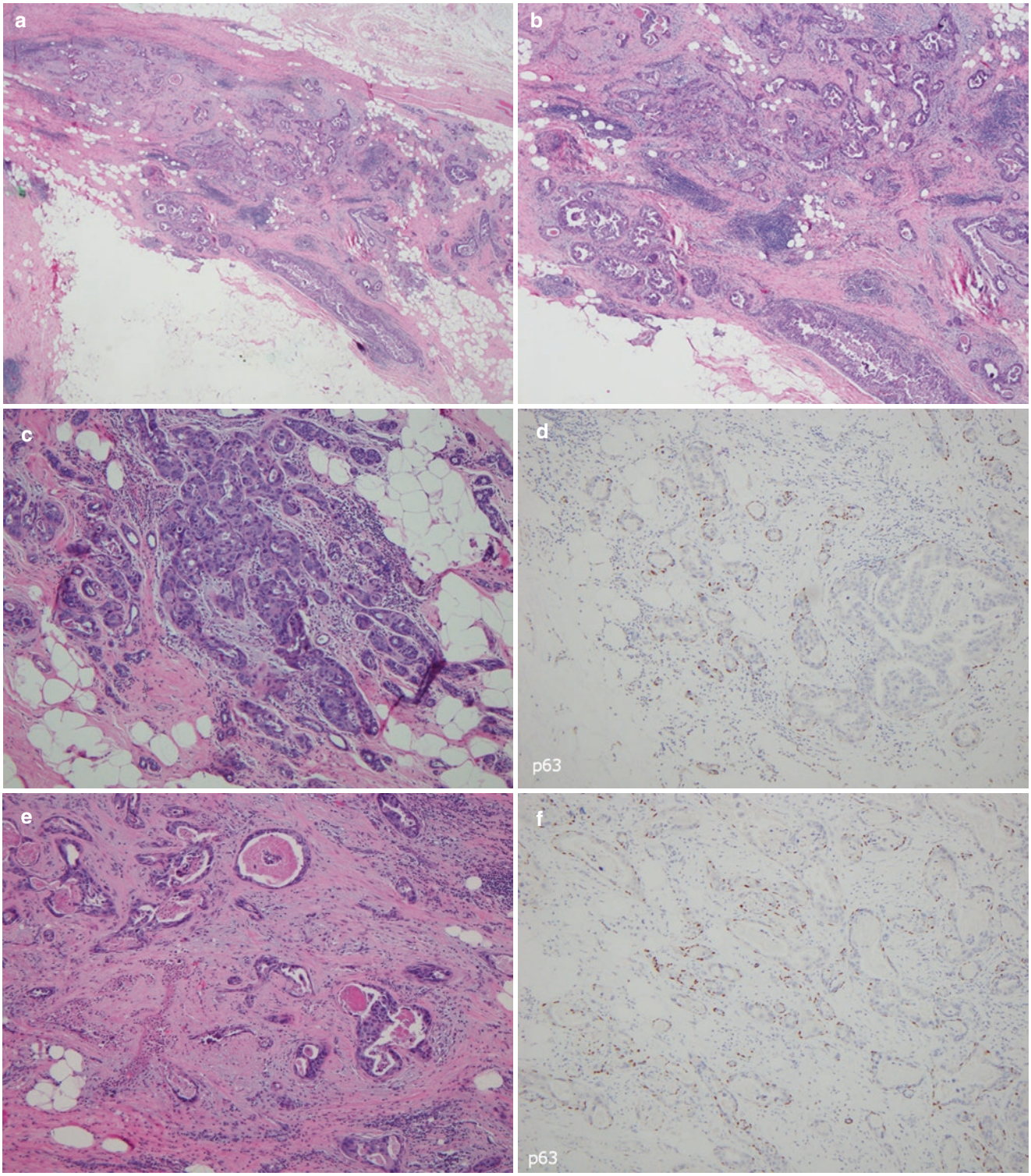

Fig. 1.15 Case 4: DCIS vs IDC. Low-power view shows an area of small infiltrative glands with some larger glands with central necrosis at the lower edge of the lesion, likely representing a mixed lesion with both IDC and DCIS components (**a**). Higher power shows that these small glands are angulated, with necrosis and associated with desmoplastic stroma, all features suggesting an invasive process (**b**, **c**); however, p63 staining clearly demonstrates the presence of myoepithelial cells around these glands, proving that this is an in situ lesion, i.e., a sclerotic appearance of DCIS with cancerization of a lobule; a second look at (**a**) proved the lobular central appearance of this area (**d**). A separate area of classic cancerization of the lobule by DCIS is shown for comparison (**e**, **f**)

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Invasive Ductal Carcinoma (NOS) of the Breast

2

Xiaoxian Li, Zaibo Li, Xiaoyan Cui, and Yan Peng

List of Frequently Asked Questions

1. **Should I worry about metastatic carcinoma to the breast without in situ component?**

Metastatic carcinoma to the breast is always a concern when there is no ductal carcinoma in situ (DCIS) component identified in the breast. Carcinoma from other origins metastasizing to the breast is not common, and it happens in less than 2% of cases [1, 2]. The most common metastatic tumors to the breast are malignant melanoma and cancers from lung, ovary, stomach, and other gastrointestinal organs such as kidney, thyroid, and cervix. Prostate carcinoma metastasis to the male breast was also reported [1, 2]. Metastatic tumor to the breast commonly forms a single mass lesion [2]. Occasionally, metastatic tumor to the breast can be the initial clinical presentation [3]. Some morphologic features may provide diagnostic clues: for example, clear cell morphology in metastatic renal cell carcinoma, papillary structure and psammoma bodies in metastatic ovarian serous carcinoma, and pigmentation in metastatic melanoma. However, these morphological features may not be present or overlap with those of primary breast carcinomas. Clinical information is critical in these cases.

Immunohistochemistry (IHC) studies can be helpful. GATA3, mammaglobin, GCDFP-15, and estrogen receptor (ER) are useful markers to identify breast carcinoma. It should be noted that expression of all these markers is reduced partially or completely in ER-negative breast carcinomas. Triple-negative breast carcinoma (TNBC) could lose all of these markers [4, 5]. On the other hand, a majority of these markers are not specific for primary breast carcinoma. GATA3 can express in urothelial carcinoma and many others [4, 6, 7]. GCDFP-15 expression can be found in a subset of lung carcinomas, although it is considered as a relatively specific marker for a breast origin [8]. ER expression is commonly seen in gynecological tumors [9]. Mammaglobin is a more sensitive marker than GCDFP-15 for detecting breast carcinoma but has lower specificity than GCDFP-15 [10–12]. IHC markers commonly used to identify non-breast primary tumors can be expressed in breast carcinoma. Breast carcinoma can be occasionally positive for TTF-1, S-100, and WT-1 [10, 13, 14]. Breast carcinoma is generally negative for PAX-8 expression [15, 16], which is helpful to differentiate breast carcinoma from thyroid, kidney, and Müllerian carcinomas. Although a majority of the breast carcinomas are cytokeratin (CK)7 positive, up to 10% are negative for CK7 [17].

In summary, metastatic tumors from other origins to the breast are rare. Clinical information is always a key component to offer accurate diagnosis. When in doubt, an initial IHC panel including GATA-3, ER, mammaglobin, TTF-1, and PAX-8 would be helpful. In an appropriate clinical setting, if an unknown primary tumor is positive for GATA3 and ER and negative for TTF-1 and PAX-8, it is almost certain that it is a primary breast carcinoma. Sometimes, distinguishing TNBC from poorly differentiated metastatic carcinoma to the breast can be challenging since TNBC can lose the expression of all the breast carcinoma markers; clinical and radiographic correlation and a broader IHC panel are essential to make a correct diagnosis. IHC studies may have very limited value to differentiate breast carcinoma from skin adnexal tumors; clinical

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Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5_2

presentation may be helpful in such situations. Rarely, high-grade lymphoma and epithelioid sarcoma such as angiosarcoma can be seen in the breast.

2. Are all triple-negative breast cancers (TNBCs) basal-like subtype in the molecular classification?

Triple-negative breast cancer (TNBC) is a clinical classification characterized by negative estrogen receptor (ER) and progesterone receptor (PR) expression, and the absence of HER2 protein overexpression and HER2 gene amplification. Basal-like breast cancer is an intrinsic subtype based on gene expression profile [18]. By gene expression profile studies, breast cancers have at least four intrinsic subtypes including Luminal A, Luminal B, HER2 enriched, and basal like [18]. Basal-like breast cancers have high expression of basal-type cytokeratins (CK5, 6, and 17) and are associated with a high frequency of p53 mutation. The majority of *BRCA* gene mutation–related breast cancers are basal-like subtype [19].

Recent studies identified another subtype by gene profile study: claudin-low subtype [20]. Claudin-low breast cancers are characterized by downregulation of tight junction proteins and high expression of genes associated with epithelial–mesenchymal transition (EMT) and breast cancer stem cells [19, 20]. The claudin-low subtype includes the majority of the spindle sarcomatoid metaplastic carcinoma and is resistant to conventional chemotherapy. The majority of basal-like and claudin-low subtypes are clinically TNBC but the overlap is not 100%. Approximately 50–80% of TNBCs are basal like, 10–30% are HER2 enriched, and a small number of TNBCs are even luminal subtypes [19, 21]. Most TNBCs with CK5/6 and epidermal growth factor receptor expression are basal-like subtype [22]. Therefore, there is a significant overlap between basal-like and TNBC subtypes, but they are not completely inclusive of each other. So far, treatments are based on the status of ER, PR, and HER2 expression determined by pathologists using immunohistochemistry or fluorescence in situ hybridization (FISH) analysis. Clinical trials are ongoing to tailor the best therapies for different subtypes of breast cancer including TNBC.

3. What is the significance of neuroendocrine differentiation in invasive carcinoma?

Primary mammary carcinoma with neuroendocrine features is not uncommon. The 2003 World Health Organization (WHO) Pathology and Genetics of Tumours of the Breast and Female Genital Organs defines primary neuroendocrine carcinoma as 50% of the tumor cells showing neuroendocrine differentiation with positive expression of either synaptophysin or chromogranin [23]. By this criterion, primary mammary neuroendocrine carcinoma is rare and represents <1% of breast carcinoma. However, this criterion was removed from the 2012 WHO Classification of Tumors of the Breast, which simply defines invasive carcinoma with neuroendocrine differentiation as "all tumors express neuroendocrine markers to a greater or a lesser degree" [24]. The 2012 WHO tumor classification indicates that up to 30% of invasive carcinoma of no special type (NST) shows neuroendocrine differentiation (with any percentage of tumor cells). However, the exact proportion of breast carcinomas with neuroendocrine differentiation is not clear due to lack of studies. The neuroendocrine differentiation is more common in solid papillary carcinoma and invasive mucinous carcinoma. Wei and coauthors studied 74 patients with primary mammary carcinoma with neuroendocrine differentiation using the criterion by 2003 WHO tumor classification [25]. They found that most of the mammary carcinomas with neuroendocrine features were ER positive (91.9%) or PR positive (68.9%). Only 2.7% were HER2 positive. They showed that mammary carcinoma with neuroendocrine differentiation had worse recurrence-free survival and overall survival than invasive carcinoma of NST even when controlled for age, ethnicity, tumor stage, surgical procedure, and HER2 status. However, such observation was not seen in other studies [26]. Until more solid evidence from prospective studies are available, clinicians currently treat breast carcinoma with neuroendocrine features in the same fashion as they treat breast carcinoma of NST. However, breast carcinoma with neuroendocrine differentiation should be differentiated from metastatic neuroendocrine carcinoma of other origins.

4. What is microinvasive carcinoma?

Microinvasive carcinoma is defined as invasive carcinoma that measures no more than 1 mm in the greatest dimension. Tumor with multiple foci of microinvasive carcinoma is still staged as microinvasive carcinoma (pT1mi). The size of each focus should not be added up. However, when two or more foci are close, deeper H&E levels should be obtained to rule out the possibility of a larger invasive carcinoma, which may change tumor pathology stage and patient managements. Microinvasion is characterized by stromal infiltration of irregular tumor clusters or single tumor cells. Although the presence of myoepithelial cells precludes the diagnosis of microinvasion, the absence of myoepithelial cells does not warrant a diagnosis of microinvasion. High-grade ductal carcinoma in situ (DCIS) with distorted architecture or tangential cut may result in small tumor cell clusters without myoepithelial cells. These tumor clusters generally have a regular and rounded contour. A diagnosis of suspicious for microinvasive carcinoma could be rendered when it is difficult to give definite diagnosis, and surgeons will generally treat the patient as positive for microinvasion. See Fig. 2.1a–c showing microinvasive carcinoma.

5. What is the prognosis of microinvasive carcinoma?

Microinvasive carcinoma has excellent prognosis. Patients with DCIS and associated microinvasion have similar recurrence and overall survival as patients with DCIS only [27–30].

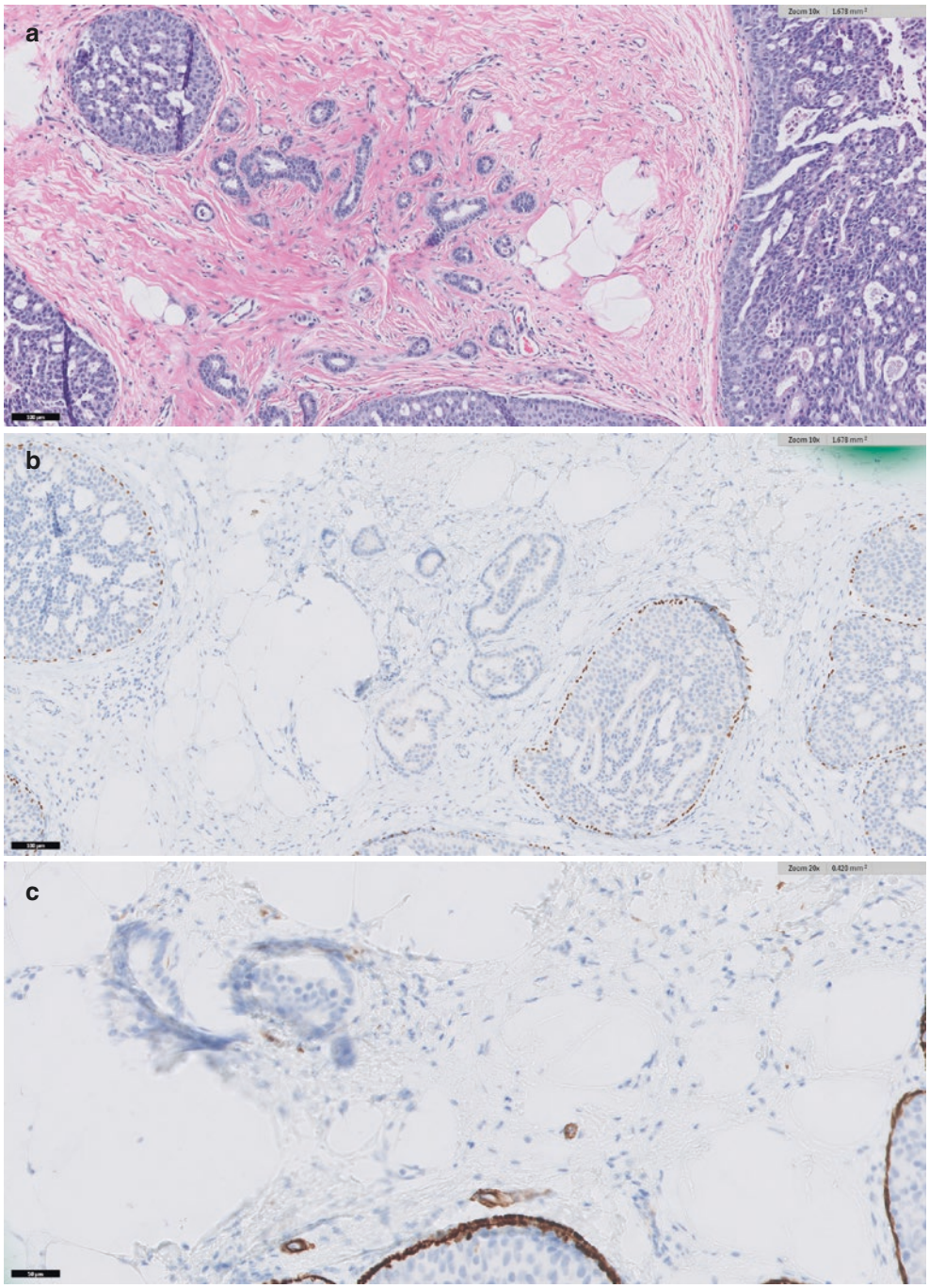

Fig. 2.1 Microinvasive carcinoma microinvasion is present at the periphery of DCIS and the size is less than 1.0 mm (**a**); immunostains for p40 (**b**) and SMMS (**c**) show the presence of myoepithelial cells around DCIS and the absence of myoepithelial cells around microinvasion

Compared to DCIS with single focus of microinvasion, DCIS with multifocal microinvasive carcinoma does not have an increased risk of axillary lymph node metastasis or worse patient outcomes [30, 31].

6. **Should a sentinel lymph node biopsy be performed in patients with microinvasive carcinoma?**

Sentinel lymph node biopsy in patients with microinvasive carcinoma is controversial. Approximately 3–15% of microinvasive carcinomas have a lymph node metastasis [30–34]. The majority of the lymph node metastases are either isolated tumor cells (ITCs) or micrometastasis. The benefit of sentinel lymph node biopsy in patients with microinvasive carcinoma is unclear. There is no prospective clinical trial comparing the prognosis in patients with or without sentinel lymph node biopsy. The rate of lymph node macrometastasis is low in DCIS cases with only microinvasion. Pathologists should extensively sample the specimen to avoid missing a large invasive carcinoma in such situations.

7. **Should I order ER, PR, and HER2 on microinvasive carcinoma?**

ER and PR should be ordered on ductal carcinoma in situ (DCIS) component in cases with DCIS and associated microinvasion. However, HER2 testing is generally not ordered on microinvasive carcinoma unless in clinical trials. DCIS with microinvasion has similar rates of recurrence and/or distant metastasis as DCIS without microinvasion [27–30]. HER2 expression in the microinvasive carcinoma is not associated with recurrence [35]. There is no evidence to treat microinvasive carcinoma with anti-HER2 therapies. Therefore, HER2 test is not recommended on microinvasive carcinoma without lymph node macrometastasis to avoid overtreatment. In cases of microinvasive carcinoma and lymph node macrometastasis, HER2 test could be ordered on the nodal metastatic carcinoma.

8. **Should microinvasive carcinoma be treated with chemotherapy?**

DCIS with microinvasion has a similar prognosis as DCIS without microinvasion [27–30]. Chemotherapy is generally not recommended in patients with microinvasive carcinoma. However, physicians may treat patients with chemotherapy when microinvasive carcinoma is associated with lymph node macrometastasis.

9. **What is the prognosis of ductal carcinoma in situ (DCIS) with multifocal microinvasive carcinoma?**

The number of focus of microinvasive carcinoma does not adversely impact prognosis compared to single focus of microinvasive carcinoma. Matsen and colleagues reviewed 414 cases with ductal carcinoma in situ (DCIS) with microinvasion [31]. Of the 414 cases, 235 (57%) had one focus of microinvasion and 179 (43%) had 2 or more foci of microinvasion. Lymph node macrometastasis was found in 1.4% of the cases and micrometastasis in 6.3%. The frequency of lymph node metastasis (micro or macro) was not different between cases with 1 focus and 2 or more foci of microinvasion [31]. Shatat and colleagues examined 40 cases with DCIS with microinvasive carcinoma. Twenty-eight of the 40 cases had 3 or less foci of microinvasive carcinoma, 5 had 4–9 foci, and 3 had 10 or more foci of microinvasive carcinoma. A majority of the cases had high-grade DCIS. Lymph node status was available in 35 cases: three had isolated tumor cells (ITCs) in the lymph node (the patients had 3, 3, and 10 foci of microinvasion in the breast, respectively); 1 had micrometastasis (the patient had 4 foci of microinvasion in the breast), and 1 had macrometastasis (the patient had 1 focus of microinvasion in the breast). These studies indicate that the status of nodal metastasis is not associated with the number of foci of microinvasion in the breast. Compared to patients with a single focus of microinvasive carcinoma, patients with multiple foci of microinvasive carcinoma have similar nodal metastasis rate and prognosis [30, 31].

10. **Can estrogen receptor (ER)-positive breast cancer be a high Nottingham histologic grade?**

ER-positive breast cancers are generally low or intermediate grade (Nottingham histologic grade 1 or 2). However, Nottingham grade 3 ER-positive breast cancer can be seen. By molecular classification, ER-positive breast cancer includes Luminal A and Luminal B subtypes. The Luminal A is generally ER+/HER2- with low proliferation, and the Luminal B is ER+/HER2+ or ER+/HER2- with high proliferation index. Compared with the Luminal A breast cancer, the Luminal B subtype is more aggressive and may benefit from chemotherapy.

11. **When should Oncotype DX® (Genomic Health, Redwood City, CA, USA) test be ordered?**

Oncotype DX recurrence score (RS) test measures mRNA expression levels of 21 genes including 16 cancer related genes and 5 housekeeping genes from formalin-fixed paraffin-embedded (FFPE) tissues containing invasive breast cancer [36]. The RS ranges from 0 to 100. The scores are categorized into low (score < 18), intermediate (18–30), and high (>30). The American Society of Clinical Oncology (ASCO) recommended that the RS may be used in ER/PR-positive, HER2-negative, and lymph node–negative breast cancer [37]. The recommendation is based on several large prospective clinical trials [38–40]. ASCO does not recommend using the RS in patients with ER-positive, HER2-negative, and lymph node–positive breast cancer [37]. The RxPONDER trial (Rx for Positive Node, Endocrine Responsive Breast Cancer) is accruing patients in an attempt to set up a cutoff of the RS for potential benefits of chemotherapy in those patients with one to three positive lymph nodes. The ASCO also recommended not using the RS to guide treatments in patients with HER2-positive breast cancer. [37]

12. **What are the other tests in estrogen receptor-positive breast cancers?**

Although Oncotype DX recurrence score (RS) test is the most widely used molecular test for ER-positive breast cancers, there are other tests available for these tumors, including MammaPrint (Agendia, Amsterdam, The Netherlands), PAM50, Breast Cancer Index® (Biotheranostics, San Diego, CA, USA), Genomic grade index, EndoPredict® (Myriad, Salt Lake City, UT, USA), IHC4 assay, and Magee Equations™ [36, 37, 41]. ASCO recommends using some of these tests in ER-positive, HER2-negative, and lymph node–negative breast cancers. [37] It should be noted that the Magee equation has a very high concordance rate with the RS [41] and it is available online: http://path.upmc.edu/onlineTools/MageeEquations.html.

13. **Should Ki-67 be ordered in ER-positive breast cancer?**

ER-positive breast cancers are classified as Luminal A and Luminal B subtypes by gene expression profiling. The Luminal B breast cancer is characterized by high proliferative index. Patients with Luminal B type of ER-positive breast cancer may benefit from chemotherapy. The 2011 St. Gallen Breast Cancer Consensus Conference suggested using 14% as a cutoff to separate Luminal A from Luminal B subtype [42]. However, there is no universally accepted Ki-67 cutoff because of the low reproducibility [43, 44]. Although Ki-67 is not recommended to be used as the sole parameter to guide treatment for ER-positive, HER2-negative breast cancer patients [37], as part of tumor profile, it may provide additional information about tumor biology and tumor aggressiveness.

14. **Where should we look for lymphovascular invasion in invasive breast cancer?**

Lymphovascular invasion in breast carcinoma is associated with distant metastasis and survival independent of lymph node metastasis [45–47]. It is not necessary to separate tumor emboli in blood vessels or lymphatic channels. The lymphovascular invasion should be evaluated outside the invasive carcinoma border and is usually found within 1–2 mm distance from the invasive tumor front. The tumor cells in the vessels generally do not conform to the exact contour of the vessels, and endothelial cells should be seen lining the lymphovascular spaces. The lymphovascular invasion should not be evaluated within the invasive carcinoma; the significance of lymphovascular invasion within the carcinoma is not clear.

15. **How do we measure the size of invasive carcinoma arising from solid papillary carcinoma of the breast?**

Solid papillary carcinoma is a well circumscribed tumor with compact tumor cells. Delicate fibrovascular networks are seen within the tumor. The tumor cells can have neuroendocrine differentiation and mucin production. The majority of the solid papillary carcinomas are ER positive, PR positive, and HER2 negative [48, 49]. Solid papillary carcinoma can have myoepithelial cells at the periphery. However, some very expanded tumors may lack myoepithelial cells at the periphery [50]. It is thought that the myoepithelial cells in a very expanded solid papillary carcinoma are too attenuated to be detected. Solid papillary carcinoma is generally considered an in situ carcinoma although rare cases can have lymph node metastasis [50]. The invasive carcinoma arising from solid papillary carcinoma generally has frank infiltrating growth pattern and stromal invasion. For pathologic staging purpose, the size of invasive carcinoma should be measured on the invasive component only and should not include the well-circumscribed portion of solid papillary carcinoma even when the myoepithelial cells are absent. Overestimating the invasive carcinoma size may result in false upstage of the tumor. See Fig. 2.2a–c showing invasive carcinoma arising from solid papillary carcinoma.

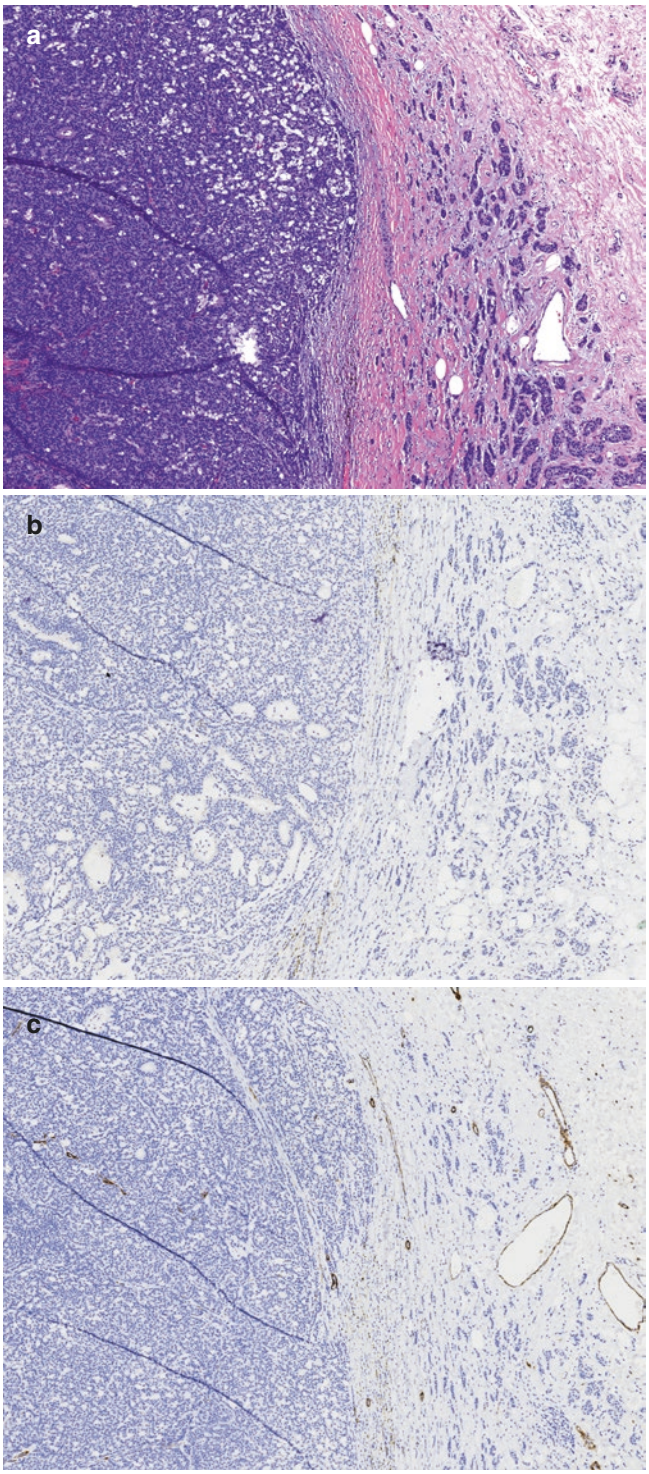

Fig. 2.2 Invasive carcinoma arising from solid papillary carcinoma. Invasion is present at the periphery of solid papillary carcinoma (**a**); immunostains for p40 (**b**) and SMMS (**c**) show the absence of myoepithelial cells around both solid papillary carcinoma and invasive carcinoma components

16. **How do we evaluate tumor response to neoadjuvant therapy in invasive breast carcinoma?**

Triple-negative and HER2-positive breast cancers are more likely to be treated with neoadjuvant (pre-surgery) chemotherapy (NACT). The purposes of the treatment are to downstage the tumor, pursue better cosmetic outcome, and, importantly, evaluate tumor response to targeted therapy or chemotherapy in breast resection specimens. Three parameters are included to evaluate breast cancer response to the NACT: tumor bed size in two dimensions, residual invasive carcinoma cellularity, and lymph node status. Of the three parameters, lymph node status has the highest weight in predicting cancer prognosis. There are four categories of residual cancer burden (RCB) indexed from 0 to III. RCB-0 is also referred to as pathologic complete response (pCR), which is defined as no residual invasive carcinoma in the breast and lymph node at the time of surgery. Residual carcinoma in situ in the breast is allowed in the pCR category. Cancers in the RCB-I class have minimal amount of residual cancer burden in the breast and lymph nodes. Breast cancer patients in both the pCR or the RCB-I category generally have good prognosis [51, 52]. More information about residual cancer burden can be found at: http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3

17. **How do we measure residual cancer tumor bed in a surgical breast specimen post-NACT?**

Because the evaluation of breast cancer to NACT has been widely used to predict patient prognosis and by the U.S. Food and Drug Administration (FDA) to approve new drugs, it is imperative to standardize the protocol for evaluation of breast specimens post-NACT. Recommendations were given by an international multidisciplinary working group organized by the Breast Cancer International Group and the North American Breast Cancer Group (BIG-NABCG) [53]. Recommendations are summarized as follows:

- Post-neoadjuvant systemic therapy specimen should be correlated with pretreatment clinical and imaging findings for pretreatment tumor size and location. A biopsy clip placement is strongly recommended at the time of diagnostic biopsy.
- Systematic sampling of areas to include the largest cross section of the tumor bed is preferable to exhaustive sampling. Five blocks of the largest cross section of the tumor bed, with a maximum of ~25 blocks, are sufficient. Mapping of sections taken is strongly recommended.
- If multiple lesions are seen grossly, sections should be taken from each lesion and additional sections should be taken in between these lesions to determine if they are truly multiple lesions.
- Residual disease may have scattered multiple tumor foci in a large tumor bed. It is recommended to document the two dimensions of the largest cross section of the area involved by residual invasive and average residual tumor cellularity.
- All surgically removed lymph nodes must be entirely submitted.
- The number of positive lymph nodes, micro- and macrometastases, size of largest metastasis, and isolated tumor cells should be documented.
- Lymph nodes with treatment effect only without residual carcinoma should be regarded as negative lymph nodes.
- Residual lymphovascular invasion should be documented and is not classified as pCR.

Repeat testing of ER, PR, and HER2 is not required but may be helpful.

18. **How should breast carcinoma be staged after neoadjuvant therapy?**

The eighth edition of the American Joint Cancer Committee (AJCC) cancer staging manual recommends staging residual breast cancer after NACT based on the largest focus when multi-foci are seen [54]. The residual cancer burden (RCB) method measures the largest dimension of the area involved by viable cancer cells [53]. The prognostic value of the RCB tumor bed size has been validated in several studies [51, 52]. The RCB tumor bed evaluation is likely a better method to assess degree of tumor response to NACT, compared to the AJCC staging system. To avoid confusion stemming from the two different methodologies, an explanation note in the pathology report would be helpful for the patients and clinicians.

19. **If there is no residual carcinoma identified in breast resection specimens post neoadjuvant therapy, should the entire specimen be submitted?**

The international multidisciplinary working group organized by the Breast Cancer International Group-North American Breast Cancer Group (BIG-NABCG) recommends taking five sections of the largest tumor bed in a slice. Sampling of adjacent fibrotic areas with a total of 25 sections should suffice [53]. However, it is critical to correlate with breast imaging studies to identify the location of the pretreatment cancer and biopsy clips. When a tumor has a good response to NACT, it may be hard to grossly identify the tumor area, and the biopsy clip can migrate from where it is initially placed. Therefore, it is also important to recognize the histologic findings and features of tumor bed on microscopic evaluation to ensure that the correct tumor bed area is sampled. The tumor bed usually shows treatment effects including myxoid changes, chronic inflammation, abundant foamy macrophages, and hemosiderin deposition in the stroma. If these

tumor bed changes cannot be found, completely submitting the entire possible tumor bed area for further microscopic evaluation merits consideration.

20. How do we define pathologic complete response (pCR)?

Currently in the United States, pathologic complete response (pCR) is defined as no invasive carcinoma in the breast and no lymph node metastasis at the time of surgery after neoadjuvant therapy [19, 51, 52]. Patients with pCR have good prognosis. If only residual carcinoma in situ is seen in the breast without any invasive component and the lymph nodes are negative, the tumor is still classified as pCR. Occasionally, tumor emboli in lymphovascular spaces are the only finding in breast resection specimens after neoadjuvant therapy. Cheng and colleagues identified 6 patients with only lymphovascular invasion after neoadjuvant therapy. Five of the 6 patients developed distant metastasis [55]. Rabban and colleagues identified six patients with pure lymphovascular invasion in the breast without any residual invasive carcinoma. Five of the 6 patients also had lymph node metastasis at the time of surgery, and four developed distant metastasis [56]. Although the patient samples were small, these studies suggested that patients with residual pure lymphovascular invasion post-NACT may have a worse prognosis than those without any residual carcinoma in the breast or lymphovascular space. Therefore, breast cancers with residual pure lymphovascular invasion after NACT are currently not classified as pCR [53].

21. What is claudin-low breast cancer?

Breast cancer is no longer regarded as a single disease. Gene expression studies have classified breast carcinoma into intrinsic subtypes including Luminal A, Luminal B, HER2-enriched, and basal-like subtypes [18]. The basal-like subtype has a high expression of cytokeratins 5, 6, and 17, which are usually expressed in the basal epithelial cells of normal tissues. The majority of the basal-like subtype tumors are triple-negative breast cancer. Recent studies identified a new subtype of breast cancer by gene expression profiling, the claudin-low subtype [20, 57]. The claudin-low breast cancer is characterized by downregulation of tight junction proteins including E-cadherin and some claudins, and is reportedly associated with poor survival. The claudin-low subtype has a high expression of epithelial–mesenchymal transition (EMT), immune response, and breast cancer stem cell–related genes [20, 57]. Claudin-low tumors generally display a triple-negative phenotype; however, only a small number of triple-negative breast cancers are claudin-low tumors. This subtype of breast cancer includes a majority of the spindle cell sarcomatoid metaplastic carcinoma [57, 58]. Similar to the breast cancer stem cells, which are resistant to conventional chemotherapy [59], the spindle cell metaplastic carcinoma generally does not respond to chemotherapy and has a worse prognosis when compared to other subtypes of triple-negative breast cancer [60, 61].

22. Is triple-negative breast cancer a homogenous disease group?

The gene expression profiling studies classified breast cancer into Luminal A, Luminal B, HER2-enriched, and basal-like subtypes. The majority of the basal-like tumors have triple-negative immunophenotype. Further studies discovered that triple-negative breast cancer (TNBC is not a homogenous disease group. Initial studies identified 6 molecular subtypes of TNBC by gene profiling [62]. The six defined subtypes were basal-like 1 and 2 (BL1 and BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem like (MSL), and luminal androgen receptor (LAR). By laser capture microdissection studies, the same group later found that the transcripts in the IM and MSL subtypes were contaminations from infiltrating lymphocytes and peritumoral stromal cells [63]. Therefore, the subtypes of TNBC were refined from six to four subtypes, referred to as TNBCtype-4 [63]. The LAR subtype has the intrinsic luminal gene signature and low proliferative rate with higher AR expression by immunohistochemistry (IHC) studies. The majority of BL1 and some BL2 breast cancers share similar gene signature with the intrinsic basal-like subtype and are associated with *BRCA* mutations. The different TNBC subtypes had different clinical outcomes [62, 64]. Masuda and coauthors showed that the BL1 subtype had the highest rate of pathological complete response (pCR) (52%) when treated with neoadjuvant chemotherapy. The LAR had a low pCR rate (10%) but the best overall survival. The M subtype had the worst survival [64]. TNBC is a heterogeneous disease and has a complex cancer biology. This concept helps understand that TNBC is difficult to treat and has the worst outcome compared to other subtypes of breast cancer. Developing effective targeted therapy for patients with TNBC is critical to improve their survival.

23. What is the significance of tumor infiltrating lymphocytes (TILs)?

The immune system constantly scrutinizes abnormal cells and helps to control cancer initiation and development [65]. There is mounting evidence showing the important role of tumor infiltrating lymphocytes (TILs) in breast cancer prognosis and response to therapies [66–69]. In addition to the innate surveillance role of the immune system, immunological response to control residual disease may be triggered by radiotherapy or chemotherapy [70, 71]. It seems that TILs are associated with better therapy response, disease-free survival (DFS), and overall survival in triple-negative and HER2-

positive breast cancers but not in ER-positive cancer [66–69, 72–74]. High levels of TILs are significantly associated with high pathologic complete response (pCR) rate in both triple-negative and HER2-positive breast cancers in neoadjuvant therapy settings [69, 73, 74]. High TIL levels are also associated with better disease-free survival and overall survival in triple-negative and HER2-positive breast cancers in adjuvant therapy settings [67, 68, 72]. Although currently TILs are not recommended as a marker to prescribe or withhold chemotherapy in breast cancer treatment [37], it is conceivable that in the near future, evaluation of TILs may become an integral part of the pathologic breast cancer protocol checklist.

24. How do we score tumor infiltrating lymphocytes (TILs)?

With the emerging role of tumor infiltrating lymphocytes (TILs) in breast cancer response to therapy and prognosis, an international TILs working group was convened and proposed recommendations to standardize evaluation of TILs in breast cancer in clinical practice, translational research, and clinical trials [72]. Recommendations are summarized as follows:

- Stromal TIL evaluation is easier and more reproducible than intratumoral TIL assessment and therefore should be used.
- TIL evaluation is reported as % stromal TILs, which is the area of stromal tissue occupied by mononuclear inflammatory cells over the total stromal area.
- TILs should be evaluated within the invasive carcinoma border but not outside the invasive carcinoma nor around ductal carcinoma in situ and normal breast tissue components.
- Exclude areas with crush artifacts, necrosis, or biopsy site changes.
- All mononuclear cells including lymphocytes and plasma cells should be scored, but polymorphonuclear leukocytes should be excluded.
- Average TILs in the tumor area but not hot spots should be used in the TIL evaluation.
- One formalin fixed paraffin-embedded (FFPE) section (4–5 μm thick, magnification x 200–400) per case is sufficient and full sections are preferred over biopsies.
- TIL evaluation should be assessed as a continuous parameter.

25. What is the clinical significance of reporting extranodal extension?

Extranodal extension is defined as tumor cells invading through the lymph node capsule into the surrounding soft tissue. Extranodal extension is measured by the largest dimension in the tissue outside the nodal capsule. Extranodal extension is associated with increased tumor burden in the axillary lymph nodes [75, 76]. Extranodal extension larger than 2 mm is especially associated with increased axillary lymph node metastasis. Therefore, the presence and size of extranodal extension should be reported. Reporting the presence and size of extranodal extension is especially important for stage T1 and T2 tumors with <3 positive lymph nodes. These tumors meet the criteria of the American College of Surgeons Oncology Group (ACOSOG) Z0011 trial [75–77]. Patients who meet the Z0011 trial criteria may undergo lumpectomy and whole breast radiation therapy without axillary dissection even with one or two positive axillary lymph nodes. However, if these patients have an extranodal extension (especially larger than 2 mm in size), axillary lymph node dissection may be needed to improve outcomes. [75, 76]

26. What is HER2 intratumoral heterogeneity (ITH) and what is the associated clinical significance?

HER2 intratumoral heterogeneity (ITH) describes the coexistence of multiple tumor cell subpopulations with varying HER2 status within the same tumor, and has been found in up to 40% of breast cancers [78–81]. Previously, HER2 genetic heterogeneity was categorized into either of two types based on heterogeneity distribution: clustered (regional) heterogeneity, which is defined as segregated HER2 amplified tumor cells and non-amplified HER2 tumor cell populations; mosaic (intermixed) heterogeneity, which is defined as comingled HER2 amplified and non-amplified tumor cells [82]. Studies have demonstrated a wide range of prevalence of HER2 ITH in breast cancer, ranging from 5% to 40% [83–87]. However, all these studies investigated HER2 ITH on the basis of only one method, either HER2 immunohistochemistry (IHC) or in situ hybridization (ISH) assay. Our recent studies have demonstrated that it is both easier and more accurate to examine HER2 ITH by a novel gene protein assay (GPA), which combines HER2 IHC and ISH on a single slide to allow identification of discordance between HER2 gene amplification and protein overexpression [88, 89]. A recent study by Kurozumi and coauthors demonstrated that 17.2% (34/198) of HER2 IHC– and HER2 ISH–negative cases showed ITH by GPA [90]. They categorized the patterns of the HER2 protein and HER2 gene status analyzed by GPA into six types: (A) IHC & ISH positive, (B) IHC positive & ISH negative, (C) IHC equivocal & ISH positive, (D) IHC equivocal & ISH negative, (E) IHC negative & ISH positive, and (F) IHC & ISH negative. Cases with at least two of the six types of HER2 gene and protein status combinations were categorized as HER2 heterogeneous [90].

HER2 ITH may contribute to inaccurate assessment of HER2 status, affecting treatment decisions, and thereby patients' clinical outcome. Reported findings in regard to this issue remain sparse. In one study, there was no significant difference in disease-free survival (DFS) in patients with ITH in up to 30% of HER2 positive tumor cells as compared to those without ITH [91]. Another study demonstrated that HER2 ITH was independently associated with inferior DFS as compared with tumors with homogeneous HER2 amplification. It has been reported that HER2 ITH might be associated with reduced DFS in primary and metastatic HER2-positive breast cancers treated with adjuvant trastuzumab [78, 92, 93]. Our recent data have demonstrated that HER2 ITH is associated with incomplete response to anti-HER2 neoadjuvant chemotherapy and this association is independent of other factors [94].

27. What is Magee equation recurrence score (RS)?

Magee equations are derived by linear regression analysis using several pathologic variables and semiquantitative immunohistochemical results of ER, PR, HER-2, and Ki-67 to calculate an RS that highly correlates with the Oncotype DX RS and provides similar information as Oncotype DX [41, 95]. Three Magee equations (#1, #2, and #3) using different combinations of standard histopathologic variables (Nottingham score (NS), ER, PR, HER-2, Ki-67, and tumor size) have been previously reported to predict the Oncotype DX RS, available at the website of University Pittsburgh Medical Center Magee Women's Hospital pathology department: http://path.upmc.edu/onlineTools/mageeequations.html. Many studies have demonstrated excellent concordance between the Magee RS and Oncotype DX RS [96–98].

28. Is it necessary to order Oncotype Dx on all ER-positive early stage invasive carcinoma of the breast?

The Magee equation uses standard histopathologic and immunohistochemical variables and could identify a portion of ER-positive breast cancer patients who may not need Oncotype DX for RS. The data from our recent study demonstrated cases with a Magee equation RS >30 or ≤11 can be accurately classified in the same categories by the Oncotype DX test [96]. Therefore, these patients with either modified Magee equation RS < 11 or >30 are unlikely to benefit from Oncotype DX test. Similar analysis has also been proposed by a previous study using a screening algorithm with the combination of Magee equations, histologic criteria and biomarker results to identify potential cases unlikely to benefit from the Oncotype DX [98]. Our data show up to 12% of Oncotype DX eligible cases which may not need Oncotype DX.

Additionally, several specific histologic types of breast carcinoma including invasive tubular carcinoma (ITC), invasive mucinous carcinoma (IMC), and classical invasive lobular carcinoma (CILC) are low-grade tumors and are associated with a good prognosis. Almost all low-grade invasive breast carcinomas are ER-positive, PR-positive, and HER2-negative and therefore may undergo Oncotype DX testing according to the National Comprehensive Cancer Network (NCCN) guidelines. Studies including ours have indicated that these special types of tumors are unlikely to have a high RS score [97]. However, if clinicians do not feel comfortable making a management decision without an RS, the Magee equation may be an alternative method to stratify these low-grade invasive breast carcinomas. If the Magee equation RS is less than 18, Oncotype DX testing is unlikely to add any more information. It should be noted that a tumor with prominent inflammation may have a false high Oncotype DX score.

29. Should the tumor profile (ER, PR, and HER2) be repeated in breast specimens post-neoadjuvant therapy?

Currently, there are no guidelines regarding whether residual tumor after neoadjuvant therapy should be retested for breast cancer biomarkers. Studies have demonstrated wide ranges of discrepancy of biomarker status [99–105]. The median frequencies of change reported in literatures are 13% for ER, 21% for PR, and 12% for HER2 [99–105]. There are much higher rates of changes from positive to negative in all three biomarkers than that from negative to positive. The average rates of changes from negative to positive are 7% for ER, 7% for PR, and 3% for HER2 [99–105]. Some of the changes in biomarker status are likely due to the receipt of neoadjuvant therapy (loss of ER after hormone therapy or loss of HER2 after trastuzumab); others may be caused by intratumoral heterogeneity, different antibody clones, variability in pathologist's interpretation, and/or specimen handling and processing.

A few studies have assessed clinical outcomes and management changes in patients with biomarker status change after neoadjuvant therapy, but the results are controversial [101, 104]. Some studies have suggested that repeat testing may yield prognostic information [101, 104]; however, others have observed no survival difference in patients with the biomarker changes versus those without changes [104]. Although the change of biomarkers from negative to positive may potentially impact clinical management, the observed difference is usually small (e.g., ER/PR negative-to-positive changes usually show low percentage of weak staining).

In summary, there are no guidelines and recommendations for retesting breast biomarkers in residual tumors after neoadjuvant therapy. Currently, whether to retest the breast cancer biomarker is institution dependent.

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Invasive Carcinoma of the Breast: Special Types

3

Zaibo Li, Xiaoyan Cui, Xiaoxian Li, and Yan Peng

List of Frequently Asked Questions

1. **What is invasive cribriform carcinoma and what are its key diagnostic features?**

Invasive cribriform carcinoma is characterized histologically by the cribriform growth pattern of the invasive carcinoma [1, 2]. The glands are morphologically similar to those of cribriform-type ductal carcinoma in situ (DCIS), manifested as fenestrated, rounded, or angulate infiltrating glands. The tumor cells are usually low to intermediate nuclear grade. Mucinous secretion is sometimes present within the lumens, as well as microcalcifications. The surrounding stroma is often fibroblastic, sometimes associated with osteoclast-like giant cells [3]. Pure invasive cribriform carcinoma has >90% of the tumor with this cribriform morphology. Areas of tubular growth pattern are commonly seen, and those with minor tubular component (<50%) are also included in this category. If the minor component is of another morphological type other than tubular pattern, they are regarded as being "mixed type" [1, 2].

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2. **What is the most common tumor profile status of invasive cribriform carcinoma?**

As a well-differentiated carcinoma, invasive cribriform carcinoma is usually estrogen receptor (ER) positive, progesterone receptor (PR) positive, and human epidermal growth factor receptor 2 (HER2) negative. See Fig. 3.1a–g.

3. **Does invasive cribriform carcinoma have a better prognosis compared to other types of breast cancer?**

Compared to invasive ductal carcinoma of no special type, invasive cribriform carcinoma has a better and favorable prognosis [1, 2].

4. **What is tubular carcinoma and what are its key diagnostic features?**

Tubular carcinoma is characterized by haphazardly arranged small tubules that closely resemble normal ductules. The tubules are angulated, oval or round in shape, lined by a single layer of epithelial cells with low-grade nuclear atypia and enclosed in an open lumen. There is no consensus for required proportion (75–100%) of tubule formation for the diagnosis of tubular carcinoma. But practically, ≥90% is needed to render a diagnosis of pure tubular carcinoma. When the tubular component involves <90% of the tumor, it is referred to as a "mixed" tubular carcinoma or invasive ductal carcinoma of no special type with tubular features. Complex architecture, marked nuclear pleomorphism, and high mitotic activity are contradictions for the diagnosis of tubular carcinoma [4].

Most tubular carcinomas are 2 cm or less in size. Under low-power examination, the stroma admixed with tubular carcinoma is usually desmoplastic or fibroelastotic, different

Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5_3

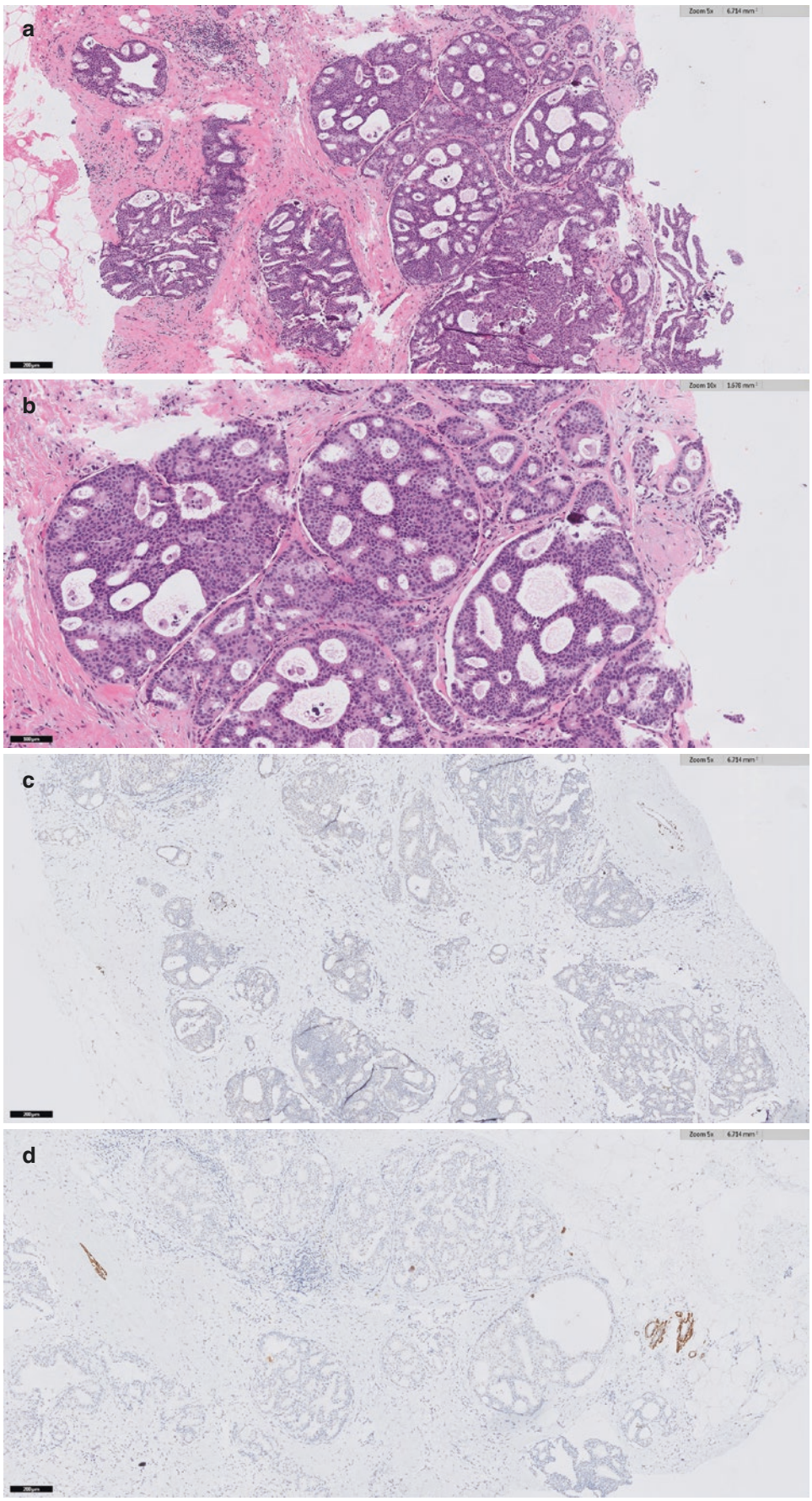


Fig. 3.1 Invasive cribriform carcinoma. Carcinoma cells grow in cribriform pattern with microcalcification (**a**). Carcinoma cells show low-grade nuclei at high magnification (**b**). p40 (**c**), SMMS (**d**), and CK5 (**e**) immunostains show the absence of myoepithelial cell layers around carcinoma cells. Carcinoma cells are strongly and diffusely positive for ER (**f**) and PR (**g**)

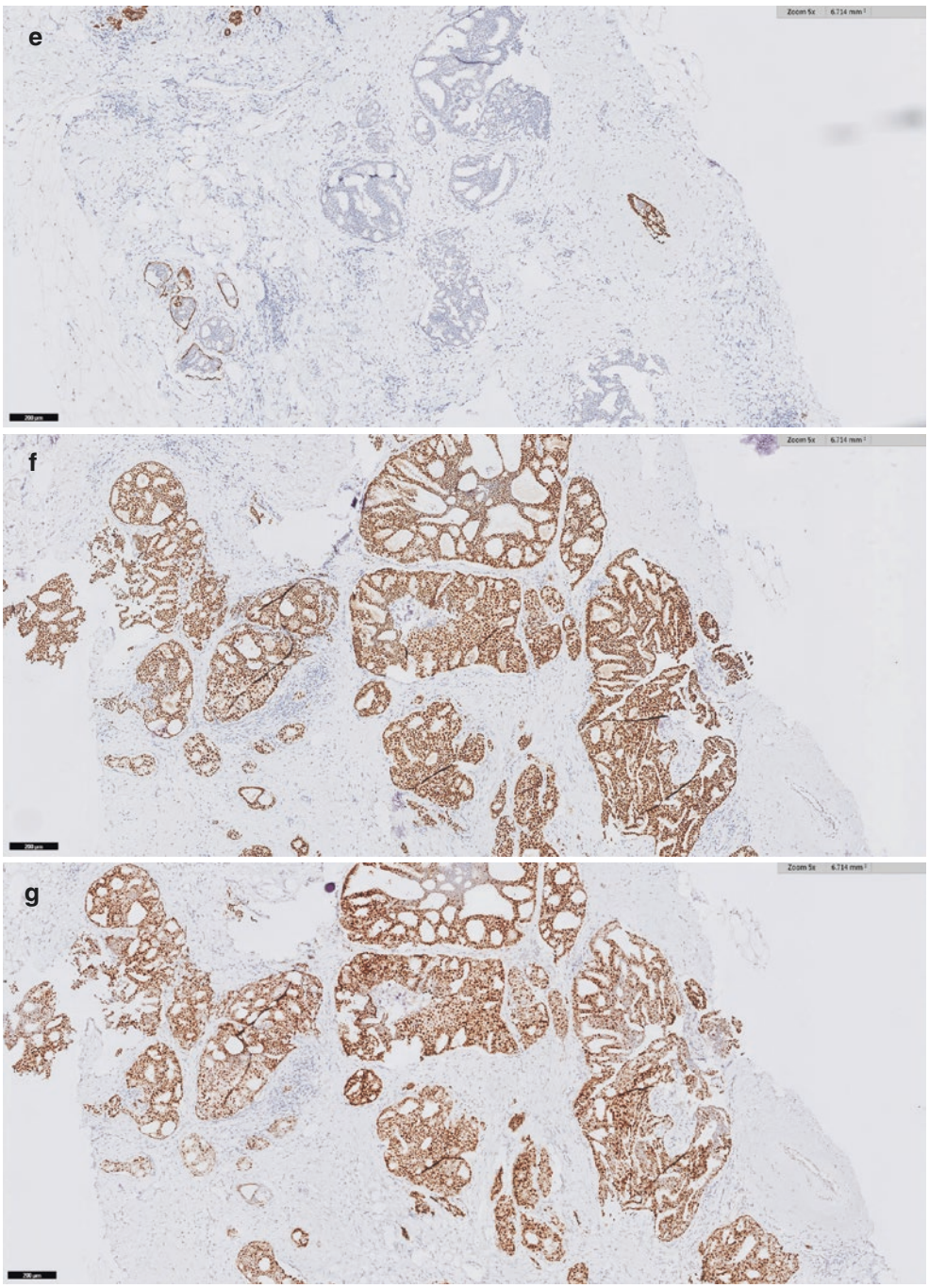


Fig. 3.1 (continued)

from the surrounding benign breast stroma, providing a useful clue for the diagnosis.

Tubular carcinoma is frequently associated with columnar cell lesions, ranging from columnar cell change (CCC), columnar cell hyperplasia (CCH), to CCC or CCH with atypia. DCIS and LCIS are also seen. DCIS arising in the background of CCC often has a low nuclear grade and cribriform or micropapillary architecture. The commonly associated CCC, LCIS (classic type), and invasive tubular carcinoma have been referred to as the "Rosen triad" [5].

5. **What is the most common tumor profile status of tubular carcinoma?**

Tubular carcinoma is usually ER positive, PR positive, and HER2 negative. See Fig. 3.2a–d.

6. **Does tubular carcinoma have the best prognosis among all types of breast cancer?**

Tubular carcinoma has an excellent prognosis. Most studies suggest that patients with tubular carcinoma have a longer disease-free survival than patients with invasive ductal carcinoma of no specific type. In some studies, it is comparable to that of age-matched set of women without breast cancer or the general population [4, 6].

7. **What is mucinous carcinoma and what are its key diagnostic features?**

Mucinous carcinoma is characterized by clusters of tumor cells floating in a pool of extracellular mucin. The relative proportion of mucin and tumor cells is variable. The diagnosis of pure mucinous carcinoma is reserved for at least 90% of the tumor showing mucinous component. Those in which the mucinous component comprising 50–90% of the lesions are regarded as "mixed" mucinous carcinoma. Invasive ductal carcinomas with less than 50% of the mucinous component are best referred to as having focal mucinous differentiation.

Pure mucinous carcinoma is uncommon and accounts for about 2% of invasive breast carcinomas [4]. The mean age of patients with invasive mucinous carcinoma is in general older (mean age is 71 years) than those with breast cancer of no special type [7].

Pure mucinous carcinoma grossly has a characteristic gelatinous and glistening appearance on the cut surface due to the presence of abundant extracellular mucin. Microscopically, the tumor cells form small clusters, large sheets, or with papillary or cribriform configurations floating in the pool of mucin. The tumor cells are usually low to intermediate nuclear grade. High nuclear grade is rare and should be emphasized in the diagnostic pathology report because the clinical course may be worse than usual pure mucinous carcinoma. The periphery of most tumors is characterized by a pushing border due to their slow growth. When assessing the margin status, the presence of extracellular mucin without tumor cells at the margin is considered positive.

Based on the morphology, mucinous carcinoma has been subclassified as type A and type B [8]. Overall, mucin is more abundant in type A than in type B tumors, which show hypercellularity. Type B tumors also show frequent neuroendocrine differentiation. Currently, no clinical implications have been noted in separating these subtypes, and the subtyping is barely mentioned in routine diagnosis.

A micropapillary variant of pure mucinous carcinoma has been reported [9, 10]. The tumor cells form micropapillae. Epithelial membrane antigen (EMA) immunostain is positive in the outer surface of the micropapillae, indicating the reversed polarity of the epithelium, similar to that in invasive micropapillary carcinoma of the breast. The variant seems to have a more aggressive behavior than conventional pure mucinous carcinoma and has a higher frequency of lymph node metastasis.

Radiographically, mucinous carcinoma usually mimics fibroadenoma, a common benign breast tumor.

8. **What is the most common tumor profile status of mucinous carcinoma?**

The majority of pure mucinous carcinomas are ER positive, PR positive, and HER2 negative. Mucinous carcinomas predominantly express MUC2 and MUC6 [11]. See Fig. 3.3a, b.

9. **Does mucinous carcinoma have a better prognosis compared to other types of breast cancer?**

Invasive mucinous carcinoma has a favorable prognosis compared to breast cancer of no special type. The accumulation of extracellular mucin serves as a barrier to the spread of tumor cells. Major prognostic factors are similar to most types of breast carcinoma. Nodal status is the most significant prognostic factor; others include age at diagnosis, tumor size, status of PR expression, and nuclear grade.

10. **What is invasive micropapillary carcinoma and what are its key diagnostic features?**

Invasive micropapillary carcinoma is characterized by tumor cells forming micropapillae and tubuloalveolar or morule-like clusters without fibrovascular cores, surrounded by clear stromal space. There is no universal criterion to distinguish mixed and pure invasive micropapillary carcinoma. In practice, pure invasive micropapillary carcinoma refers to those with at least 75% of the tumor showing micropapillary configuration. The cell clusters display reversed polarity with the luminal aspect of the cells present on the outer surface of the clusters close to the stroma. This can be well demonstrated by epithelial membrane antigen (EMA) immunostaining the cell membrane facing toward the stroma. MUC1, like EMA, also stains the similar pattern. The nuclear grade of invasive micropapillary carcinoma is usually intermediate to high. The clear spaces around the tumor cells mimic lymphovascular invasion, but they are not lined by endothelial cells. They are usually attributed to artifacts during tissue processing. However, invasive micropapillary carcinomas do have a higher frequency of lymphovascular invasion [12], and the tumor emboli in the lymphovascular spaces show similar micropapillary morphology.

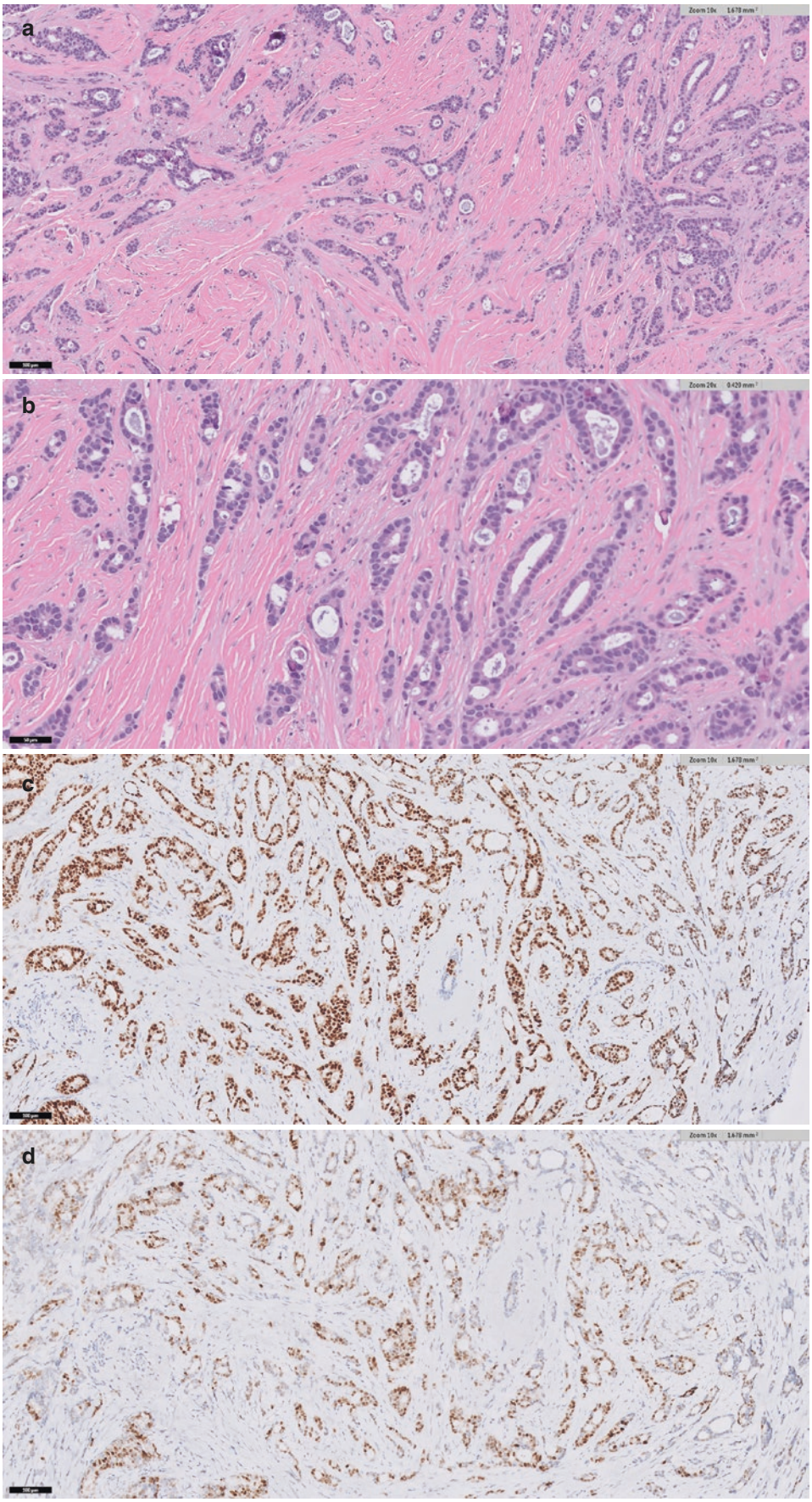


Fig. 3.2 Invasive tubular carcinoma. (**a**) The majority of carcinoma cells grow in tubules, which are angulated, irregular, and infiltrating into the surrounding stroma. (**b**) Carcinoma cells show low-grade nuclei at high magnification. (**c**) Positive for ER (strong and diffuse). (**d**) Positive for PR (variable)

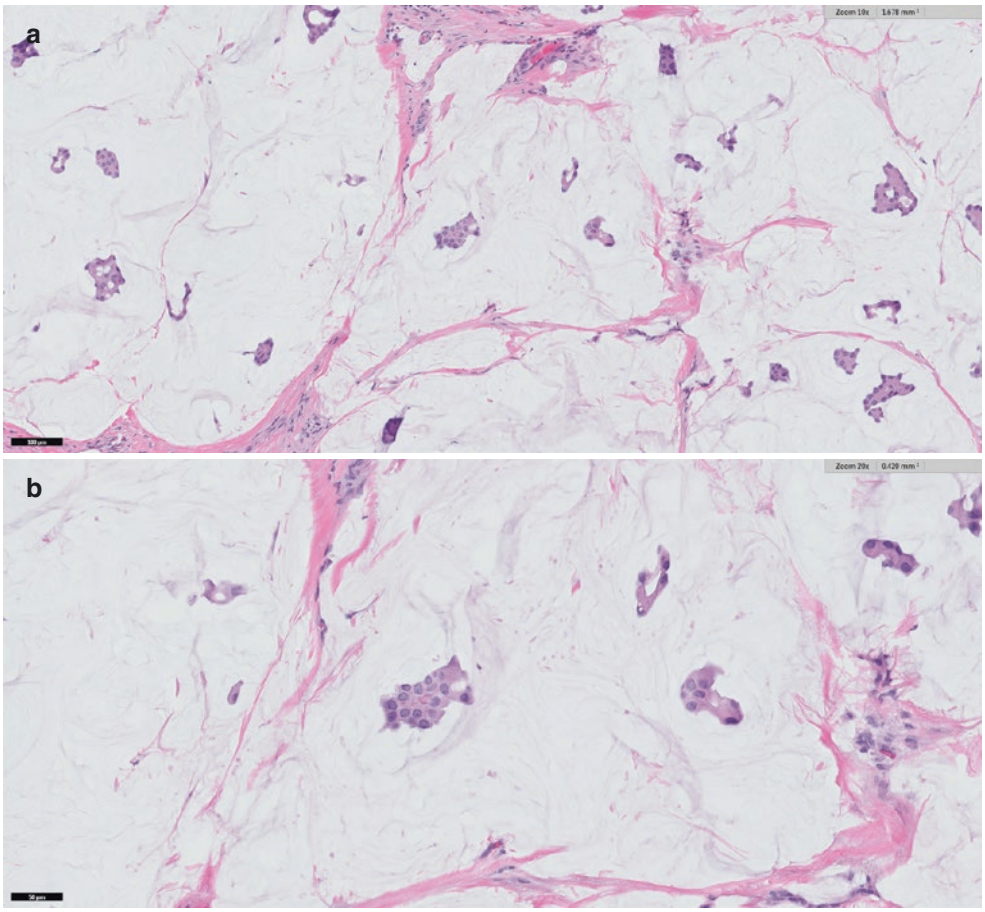


Fig. 3.3 Invasive mucinous carcinoma. (**a**) Small clusters of carcinoma cells are floating in a mucinous pool. (**b**) Carcinoma cells show low-grade nuclei at high magnification

11. **What is the most common tumor profile status of invasive micropapillary carcinoma?**

Most invasive micropapillary carcinomas are positive for ER and PR. HER2 protein is variably overexpressed in a fraction of tumors. See Fig. 3.4a, b.

12. **Does invasive micropapillary carcinoma have an increased risk for nodal metastasis and a worse prognosis compared to other types of breast cancer?**

When compared with invasive ductal carcinoma of no special type, invasive micropapillary carcinoma has a higher frequency of lymphovascular invasion and lymph node metastasis, and more lymph nodes are involved [13]. Patients usually have a significantly shorter disease-free survival (DFS) and overall survival (OS) [14]. But when stratified for the number of involved lymph nodes and other prognostic factors, patients seem to have similar survival rates to those with non-micropapillary invasive ductal carcinoma [15]. Unlike other special-type breast carcinomas, the poor prognosis associated with this entity appears to be the same whether the micropapillary component is present focally or diffusely within a tumor [15].

13. **What is invasive apocrine carcinoma and what are its key diagnostic features?**

Invasive apocrine carcinoma is composed of tumor cells with apocrine differentiation of tumor cells, characterized with abundant densely eosinophilic, granular, or vacuolated cytoplasm, large nuclei, and often prominent nucleoli. Compared to benign apocrine cells, apocrine tumor cells demonstrate an increase in nuclear size, significant nuclear pleomorphism, irregular nuclear membrane, hyperchromatic nuclei, and one or more macronucleoli. Features of cytoplasm are similar to those in the benign apocrine cells. Pure apocrine carcinoma is reserved for a tumor consisting of almost all malignant apocrine cells. If only a portion (>10%) of the tumor consists of malignant apocrine cells, then it can be considered as invasive carcinoma with apocrine differentiation. Apocrine differentiation can be seen in up to 30% of all breast cancers [16].

It has been reported that some benign cystic and papillary apocrine lesions show little or no detectable surrounding myoepithelial cells [17]. Without cytological atypia, the absence of immunoreactive myoepithelial layer is not an absolute criterion for the diagnosis of invasive apocrine carcinoma.

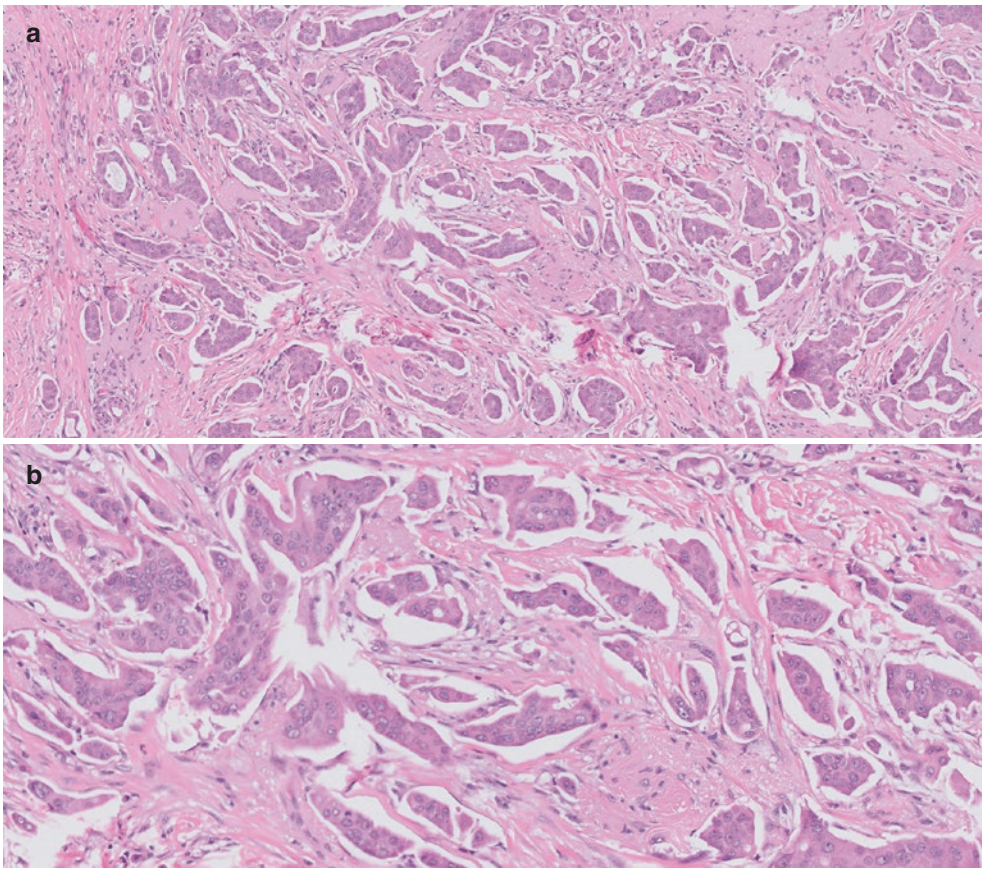


Fig. 3.4 Invasive micropapillary carcinoma. (**a**) Carcinoma cells grow in a micropapillary pattern without fibrovascular cores. There are empty spaces around the clusters of carcinoma cells. (**b**) Carcinoma cells show variable grade nuclei at high magnification

14. **Is there any difference on the tumor profile in apocrine carcinoma compared to other types of breast cancer?**

Most invasive apocrine carcinomas are negative for ER and PR, but positive for androgen receptor (AR). Some studies have regarded only tumors with apocrine morphology and ER-negative, PR-negative, and AR-positive immunoprofile as pure apocrine carcinoma. About half of pure apocrine carcinomas are HER2 negative and the remaining HER2 positive [18]. Immunohistochemical studies may be used to confirm the diagnostic impression of apocrine differentiation but are not essential to establish the morphological diagnosis of apocrine carcinoma. See Fig. 3.5a, b.

GCDFP-15 (BRST-2) immunostain is positive in a high percentage of invasive apocrine carcinoma [16].

15. **Does invasive apocrine carcinoma carry a worse prognosis than other types of breast cancer?**

The prognosis of invasive apocrine carcinoma is related to tumor grade, size, lymph node status, and tumor stage, similar pathologic parameters as those of non-apocrine breast carcinomas.

16. **What is mammary carcinoma with osteoclast-like giant cells and what are its key diagnostic features?**

Carcinomas with osteoclast-like giant cells are characterized by the presence of multinucleated osteoclast-like giant cells in the stroma. These cells are non-neoplastic, while the carcinomatous components can be a variety of histological types. Frequently, the carcinomatous components are invasive ductal carcinoma of no special type with a cribriform growth pattern, but other histological types such as lobular, squamous, papillary, mucinous, and metaplastic carcinoma have also been reported [19–22]. Grossly, the tumors display red-brown to dark-brown color, which is due to the presence of hemorrhage and hemosiderin-laden macrophages in the tumors. Microscopically, the giant cells are variable in size as well as the number of nuclei. They are cytologically bland with no mitotic activity. Hemorrhage in the stroma is commonly seen in the tumor, which may be a clue for the presence of osteoclast-giant cells under low-power examination. These giant cells may also be present in metastatic and recurrent tumors.

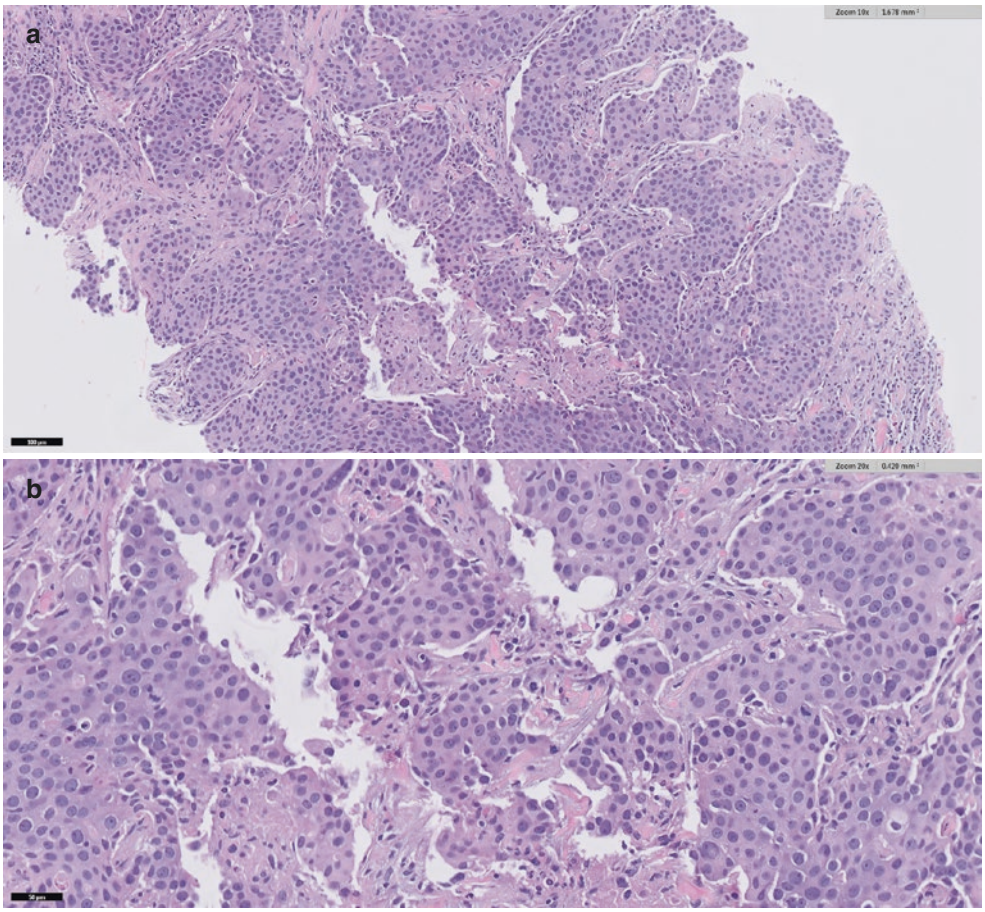


Fig. 3.5 Invasive apocrine carcinoma. (**a**) Carcinoma cells grow in solid nests with minimal intervening stroma. (**b**) Carcinoma cells show intermediate to high nuclear pleomorphism with abundant eosinophilic cytoplasm at high magnification

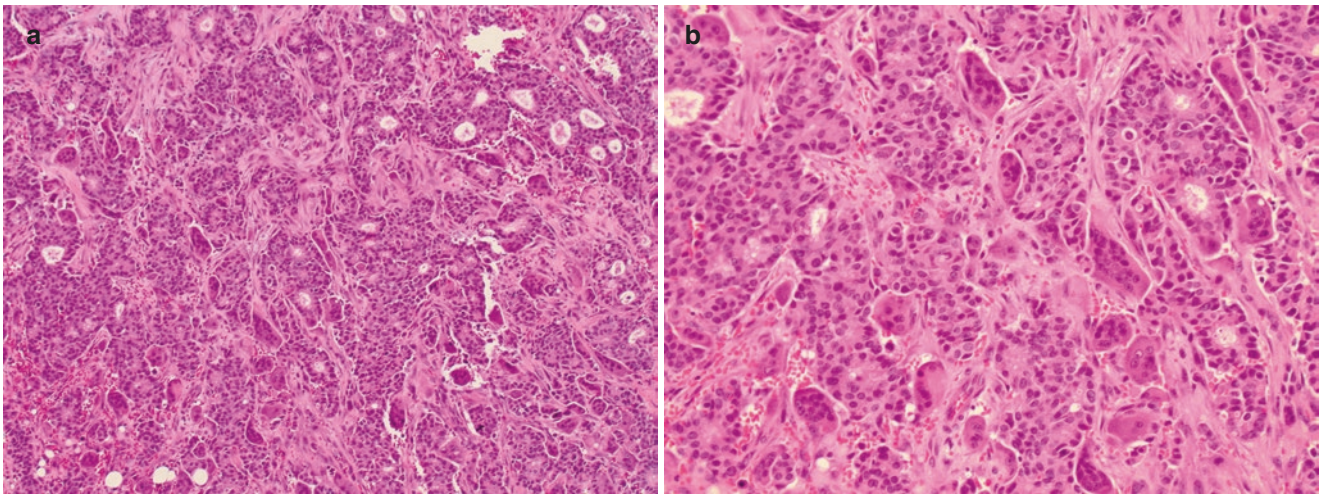


Fig. 3.6 Invasive mammary carcinoma with osteoclast-like giant cells. (**a**) Carcinoma cells grow in solid nests intermixed with osteoclast-like giant cells. (**b**) Osteoclast-like giant cells are large with abundant cytoplasm, multiple nuclei, and prominent nucleoli

17. **What is the tumor profile status of mammary carcinoma with osteoclast-like giant cells?**

The osteoclast-like giant cells in the carcinoma are of histiocytic lineage, which express CD68, acid phosphatase, nonspecific esterase, and lysozyme, and are negative for S100, actin, and keratin [23, 24]. See Fig. 3.6a, b. However, the mechanism by which they are formed is still unknown.

The tumor profile status of the carcinoma depends on the histological type of its carcinomatous component.

18. **What is the prognosis of mammary carcinoma with osteoclast-like giant cells?**

Lymph node metastases are seen in about one third of the cases, and the 5-year survival rate is around 70% [24]. The presence of osteoclast-like giant cells does not carry any specific prognostic implications. Prognosis is related to the histologic and immunophenotypic features of the associated carcinoma.

19. **What is invasive ductal carcinoma with medullary features and what are its key diagnostic features?**

In the 2003 World Health Organization (WHO) Classification of Tumors of the Breast, medullary carcinoma was defined as a "well circumscribed carcinoma composed of poorly differentiated cells with scant stroma and prominent lymphoid infiltration" [25]. The classical morphologic features include a well-circumscribed smooth rounded pushing border, a syncytial growth pattern greater than 75% of the tumor (broad anastomosing sheets of tumor cells with indistinct cell borders), diffuse lymphoplasmacytic infiltrates within the tumor, and, at greater than 75% of the tumor periphery, a high degree of nuclear pleomorphism, prominent nucleoli, and a brisk mitotic activity. Breast fibroglandular tissue should not be present within the invasive carcinoma [26]. In the most recent 2012 edition of the WHO classification, the term of this entity was revised to invasive ductal carcinoma with medullary features (see Fig. 3.7a, b), which also includes "atypical medullary carcinoma" referring to tumors that do not fulfill all the diagnostic criteria [27].

20. **What is the most common tumor profile and genomic abnormality of invasive ductal carcinoma with medullary features?**

Invasive ductal carcinomas with medullary features are often triple-negative breast cancers with a basal-like phenotype expressing CK5/6, CK14, and EGFR [28–31]. These tumors are often associated with *BRCA1* mutations (in up to 60% of tumors), whereas less frequently associated with *BRCA2* mutations [27]. About 11% of patients showed *BRCA1*germline mutations. In addition, invasive ductal carcinoma with medullary features shows more frequent genomic instabilities, aneuploid or polyploid, and *p53* mutations than invasive ductal carcinoma NOS [27].

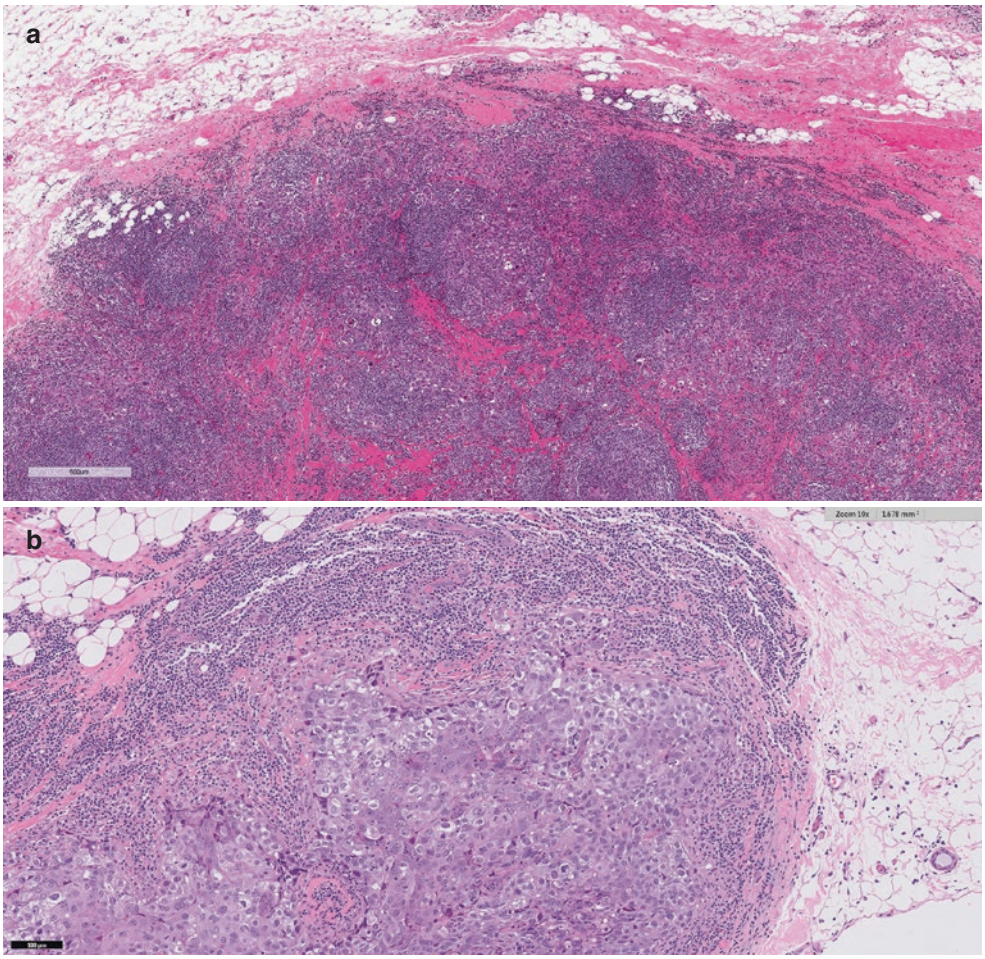


Fig. 3.7 Invasive ductal carcinoma with medullary features. (**a**) Carcinoma with well-circumscribed border and prominent lymphocytic infiltrates at the periphery. (**b**) Syncytial growth pattern of anaplastic tumor cells admixed with lymphoplasmacytic cells

21. Does invasive ductal carcinoma with medullary features carry a better prognosis?

Invasive ductal carcinoma with medullary features has been considered a distinctive subgroup of triple-negative carcinomas with a favorable prognosis despite its high-grade morphology. However, it is necessary to adhere to strict morphologic criteria for the diagnosis of this tumor in order to predict its better prognosis [31–36].

Recently, it has been reported that breast invasive carcinomas with prominent lymphocytic infiltrates (also called tumor infiltrating lymphocytes, or TILs) have better prognosis and response to neoadjuvant chemotherapy, especially in high-grade HER2-positive and triple-negative breast carcinoma [37–39]. The relatively good outcome seen in patients with this tumor may result from prominent lymphocytic infiltrates rather than an inherently better prognosis. Therefore, most breast pathologists prefer to diagnose invasive ductal carcinoma with medullary features as a basal-like triple-negative carcinoma with prominent lymphocytic infiltrates.

22. What is invasive carcinoma with neuroendocrine features and what are its key diagnostic features?

In the 2003 WHO classification, neuroendocrine carcinomas of the breast were divided into solid neuroendocrine carcinoma, small cell/oat cell carcinoma, and large cell neuroendocrine carcinoma [25]. In the 2012 WHO classification, the term of the tumor was revised to carcinomas with neuroendocrine features, which was defined as carcinomas with neuroendocrine differentiation exhibiting morphology similar to that of neuroendocrine tumors of the lung and gastrointestinal tract. No definitive threshold for neuroendocrine marker positivity was required [27].

Histologically, these tumors can be classified into three categories: well-differentiated neuroendocrine tumor (WD-NET), poorly differentiated neuroendocrine carcinoma/small cell carcinoma (PD-NEC/SCC), and invasive breast carcinoma with neuroendocrine differentiation (IBC-NED). Morphologically, WD-NET consists of cellular solid expansile nests and trabeculae separated by delicate fibrovascular stroma, similar to NET from other sites. The tumor cells are usually spindled, plasmacytoid, or polygonal with abundant granular or clear vacuolated cytoplasm [40–42]. The nuclear features include classic smooth nuclear borders and salt-and-pepper chromatin seen in carcinoids of other sites. PD-NEC/SCC is morphologically identical to its counterpart in other sites, consisting of densely packed hyperchromatic cells with scant cytoplasm and crushing artifact. Mitotic activity and necrosis are common [43–47]. IBC-NED can show variable morphology with only subtle cytologic/nuclear features of neuroendocrine differentiation. Neuroendocrine differentiation has been demonstrated in up to 30% of invasive ductal carcinomas, most commonly in mucinous carcinoma or solid papillary carcinoma [46]. Neuroendocrine differentiation can also be seen in invasive lobular carcinoma, especially alveolar variant [43]. See Figs. 3.8a–h and 3.9a–f.

23. What is the most common immunoprofile of invasive carcinoma with neuroendocrine features?

The diagnosis of neuroendocrine tumor usually requires demonstrating the expression of neuroendocrine markers. Synaptophysin and chromogranin A are the most commonly used neuroendocrine markers, with synaptophysin as the most sensitive and chromogranin A as the most specific immunohistochemical marker. Other neuroendocrine markers such as neuron-specific enolase (NSE) and CD56 may also be used, with less sensitivity and specificity. Neuroendocrine markers are usually diffusely positive in WD-NET and PD-NEC/SCC, while patchy and focal in IBC-NED. There is only limited information available regarding the expression of biomarkers (tumor profile) in invasive carcinomas with neuroendocrine features. Available data suggest that these tumors are most commonly ER positive, PR positive, and HER2 negative [27]. ER and PR are positive in the majority of WD-NETs and in greater than 50% of PD-NECs, but variable in IBC-NEDs [43–47]. Similar to SCCs of other sites, primary SCCs of the breast can show expression of thyroid transcription factor −1 (TTF-1) [47].

24. Do the neuroendocrine features of invasive carcinoma play a role in prognosis and treatment decision?

No specific guidelines exist for grading breast carcinomas with neuroendocrine features, and the 2012 WHO classification states that grading is unlikely to be clinically significant [27]. Currently carcinomas with neuroendocrine features of the breast are staged, histologically graded, and treated similarly to invasive carcinomas of no special type. The use of endocrine therapy or HER2 targeted therapy depends on the status of the tumor's ER, PR, and HER2 expressions [27]. No consensus has been reached on the prognosis for this group of tumors. Although many studies demonstrate a poor prognosis for breast carcinomas with neuroendocrine features, the results are conflicting, likely due to varying inclusion criteria [44, 47–50].

25. What is secretory carcinoma of the breast and what are its key diagnostic features?

Secretory carcinoma is a rare, special type of invasive carcinoma with a solid, microcystic, and tubular architecture and large amounts of extracellular and intracellular secretions. Historically, secretory carcinoma was known as "juvenile breast carcinoma" as it was originally identified

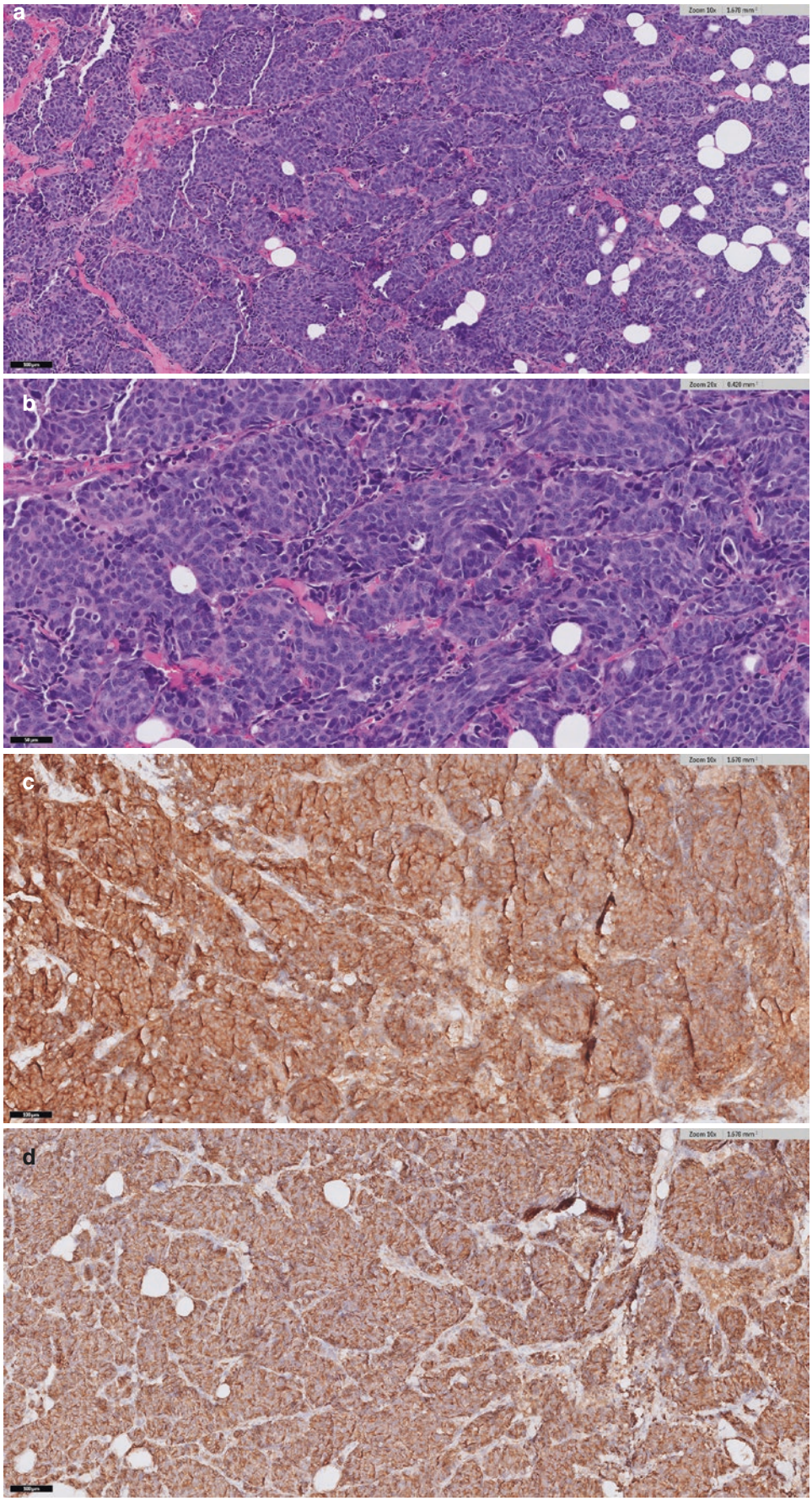
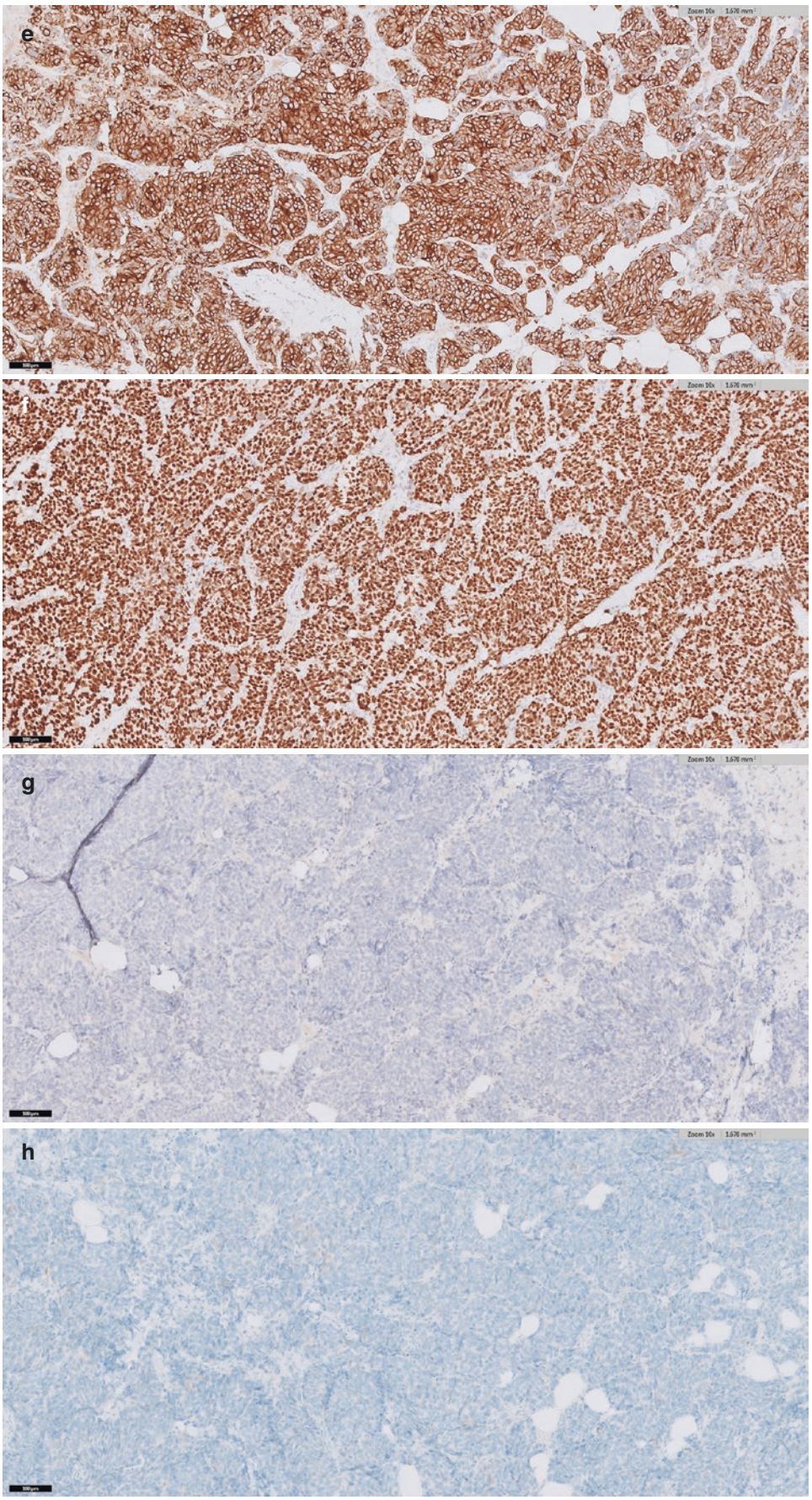


Fig. 3.8 Invasive ductal carcinoma with neuroendocrine features. The tumor is composed of epithelial cells in trabecular growth pattern (**a**). Neuroendocrine nuclear features are appreciated at high magnification (**b**). The tumor cells are positive for synaptophysin (**c**), chromogranin A (**d**), CK7 (**e**), and ER (**f**). The tumor cells are negative for PR (**g**) and HER2 (0-1+) (**h**)



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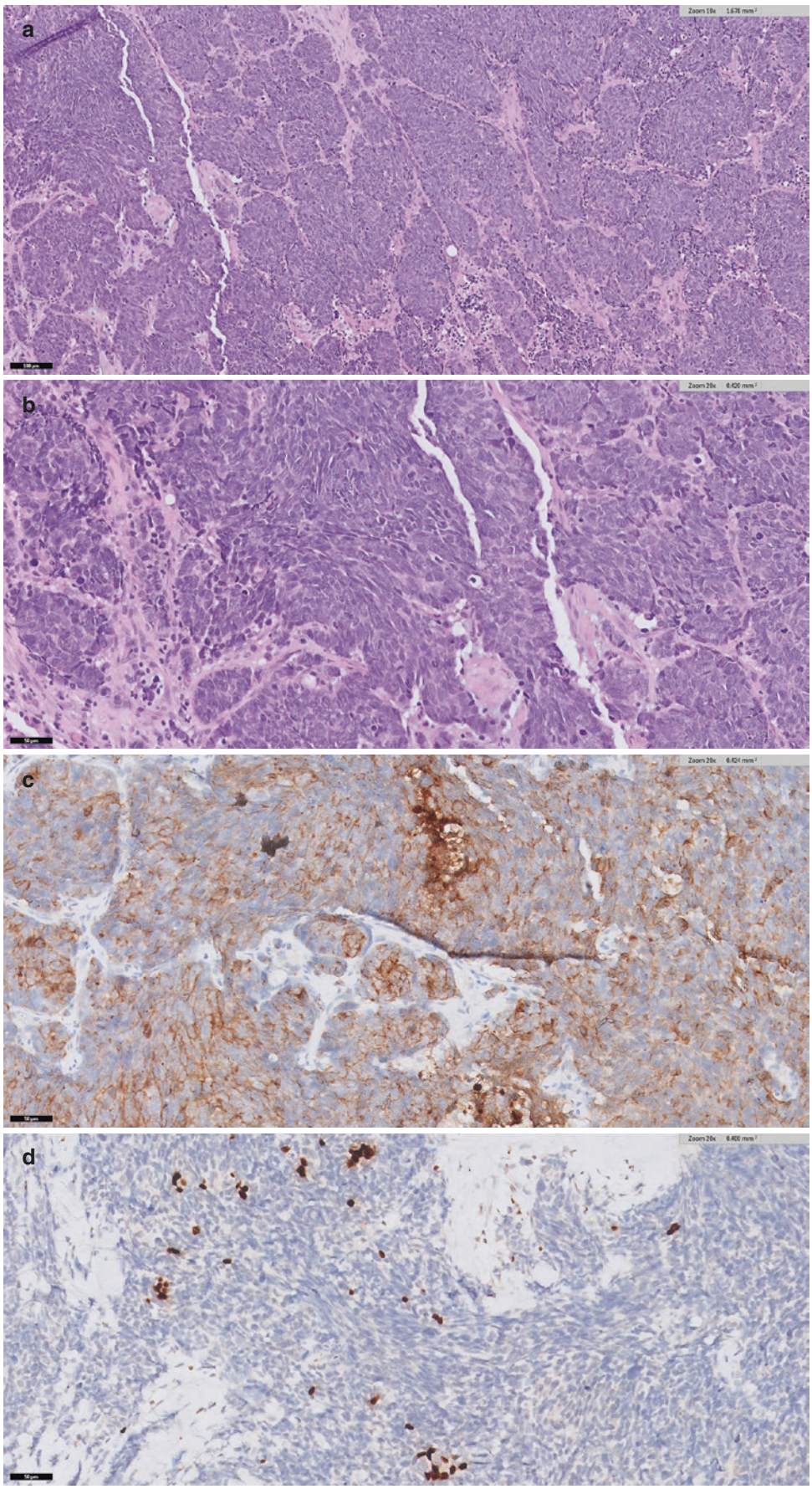
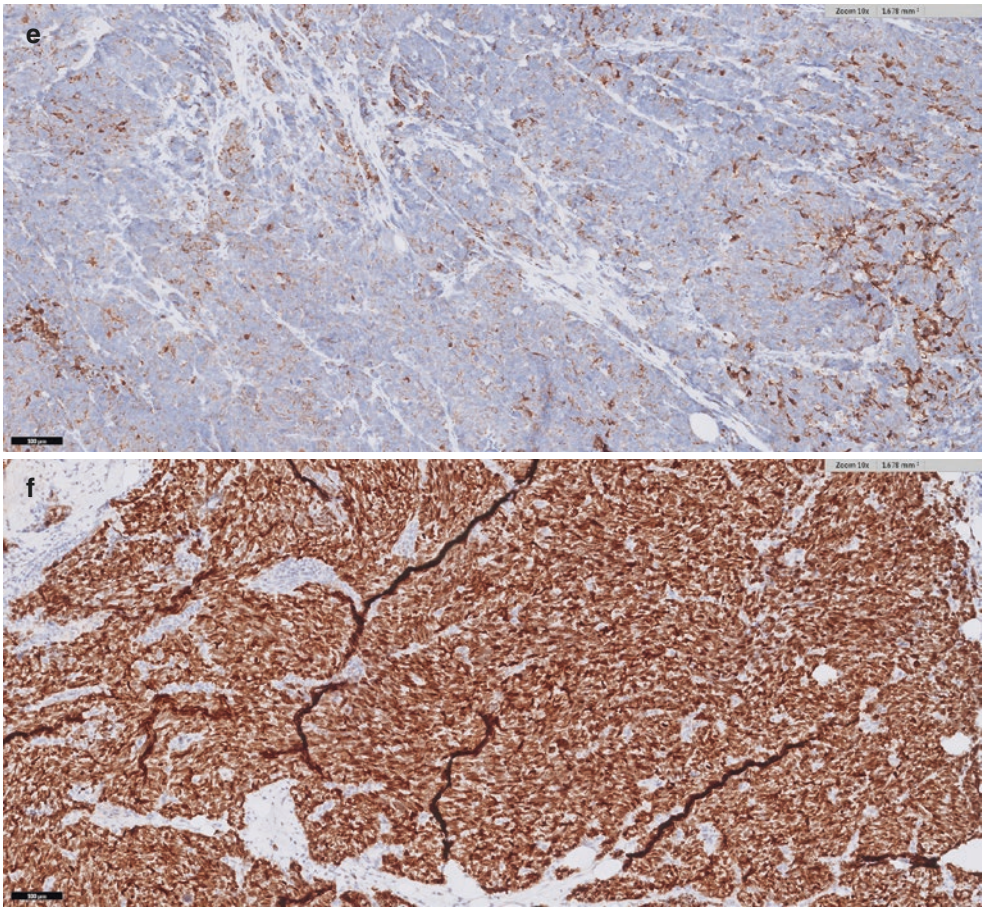


Fig. 3.9 Small cell carcinoma of the breast. The tumor is composed of nests of malignant epithelial cells with high nuclear to cytoplasmic ratio and hyperchromatic nuclei (**a**). Neuroendocrine nuclear features and nuclear molding (**b**). The tumor cells are positive for chromogranin A (**c**), GATA3 (very focal) (**d**), CK7 (patchy) (**e**), and TTF-1 (diffuse) (**f**)

Fig. 3.9 (continued)



in young patients. However, it has been reported in patients in a wide range of age (3–87 years) and a median age of 25 [27, 51].

Secretory carcinomas are composed of well-circumscribed nodules with tumor cells growing in three patterns: solid, microcystic, and tubular patterns. The microcystic pattern shows multiple small cysts resembling thyroid follicles. The tubular pattern shows tubules with lumen containing secretions. Most tumors contain all three patterns with various combinations. Tumor cells are usually uniform with round or angulated contour, mild nuclear atypia, and finely granular or vacuolated cytoplasm containing dense eosinophilic secretions. Signet ring cells can be present. Extracellular eosinophilic secretions are present within the lumens of tubules or microcysts. The eosinophilic secretions are positive for Periodic acid–Schiff (PAS), PAS diastase, and Alcian blue. Ductal carcinoma in situ with similar secretory features can be seen together with invasive secretory carcinoma [51–53]. See Fig. 3.10a, b.

26. What is the most common tumor profile status of secretory carcinoma?

Secretory carcinoma is significantly more common in females and usually presents as a mobile, palpable lesion in the subareolar region. Radiological breast imaging shows a well-circumscribed mass with regular margins, which can be easily mistaken as a fibroadenoma in young patients.

Like adenoid cystic carcinoma, secretory carcinoma is typically a low-grade triple-negative carcinoma with a basallike phenotype with expression of high-molecular-weight cytokeratins (CK5/6, 34E12, CK14, CK17), EGFR, and c-kit. Ki67 proliferative index is low (<15%). The carcinoma cells are also positive for S100 (strong and

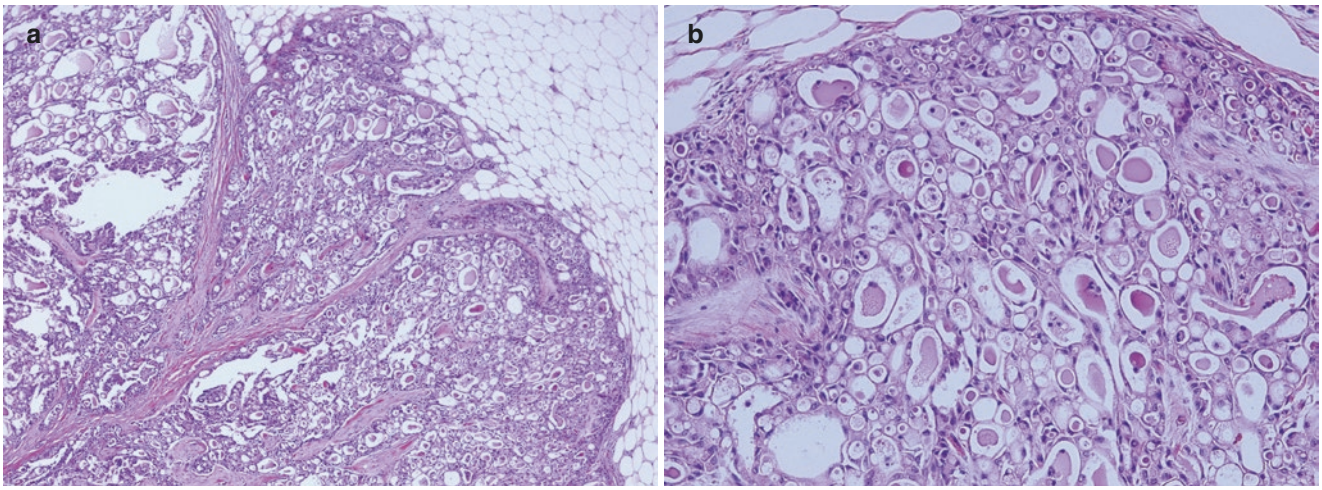


Fig. 3.10 Secretory carcinoma of the breast. (**a**) The tumor is composed of irregular lobules of eosinophilic cells separated by band-like fibroconnective tissue. (**b**) Dense eosinophilic secretion is intermixed with cytologically bland tumor cells. (Courtesy of Dr. Shi Wei, University of Alabama at Birmingham)

diffuse) and mammaglobin but negative for GCDFP-15 [54, 55].

27. **Is secretory carcinoma associated with a better prognosis?**

Secretory carcinoma usually manifests as an indolent, well-circumscribed mobile lump with excellent prognosis. Axillary lymph node metastases may occur but rarely involve more than three lymph nodes. Secretory carcinoma should not be confused with invasive ductal carcinoma with apocrine features, which is more common and has a more aggressive behavior [54].

28. **What is genetic abnormality in secretory carcinoma?**

Secretory carcinoma is characterized with chromosomal translocation t(12:15), resulting in the *ETV6NTRK3* fusion gene. The *ETV6* (*TEL) oncogene* encodes a transcription factor involved in development. The same translocation t(12:15) leading to *ETV6NTRK3* fusion gene also occurs in congenital fibrosarcoma and mesoblastic nephroma. FISH for the *ETV6* break apart probe or RT-PCR for the *ETV6NTRK6* fusion gene is a diagnostic tool for these tumors [56].

29. **What is adenoid cystic carcinoma of the breast and what are its key diagnostic features? How does one differentiate this entity from its counterpart in the head and neck?**

Adenoid cystic carcinoma (ACC) of the breast, an analogue to its counterpart in the salivary gland, accounts for only about 0.1% of all breast carcinomas. ACC predominantly affects postmenopausal women with a median age of 60 years in contrast to triple-negative invasive ductal carcinoma of no special type, which usually affects younger patients (<50 years) [27, 57, 58].

Similar to ACC of the salivary gland, mammary ACC is also composed of two populations of cells: glandular luminal cells and basaloid cells, with three growth patterns: tubular, cribriform, and solid. The basaloid cells have myoepithelial features. Eosinophilic hyaline or mucoid material may be seen in the lumen of cribriform structures and tubules. Carcinoma cells are usually small with scant cytoplasm and vesicular nuclei without prominent nucleoli. The mitotic activity is low [59]. Nottingham histologic grading system is also used for ACC of the breast. The solid variant of ACC is a high-grade variant with a more aggressive behavior. Tumor cells in this variant are larger with moderate to marked nuclear pleomorphism and increased mitotic activity [60]. Mammary ACC is a triple-negative breast cancer with a basallike phenotype. However, unlike most basal-like breast cancers that are high grade with an aggressive clinical course, mammary ACC except solid variant is usually low grade with an indolent clinical course.

ACC of the breast is morphologically similar to ACC of the salivary gland. Recent studies reveal that both mammary and salivary gland ACCs share a recurrent translocation t(6:9) which leads to the chimeric fusion gene *MYBNFIB* and may explain the phenotypic similarity [61, 62]. Clinical history is important to make a diagnosis of ACC of the breast instead of a metastasis from head/neck ACC.

ACCs should be graded using the standard Nottingham grading system with most exhibiting mild to moderate nuclear pleomorphism and low to moderate mitotic activity. As a result, most are classified as histologic grade one or two depending on the proportion of solid areas. See Fig. 3.11a–h.

30. **What is the immunohistochemical profile of adenoid cystic carcinoma of the breast?**

Mammary ACC cells are typically negative for ER, PR, and HER2 expressions; however, rarely they may exhibit weak ER or PR staining. Similar to ACC of the salivary gland, the basaloid cells of ACC of the breast are typically positive for myoepithelial markers (p40, p63, smooth muscle myosin, calponin, and S-100), basal cytokeratins (CK5 or CK5/6, CK14, and CK17), and epidermal growth factor receptor

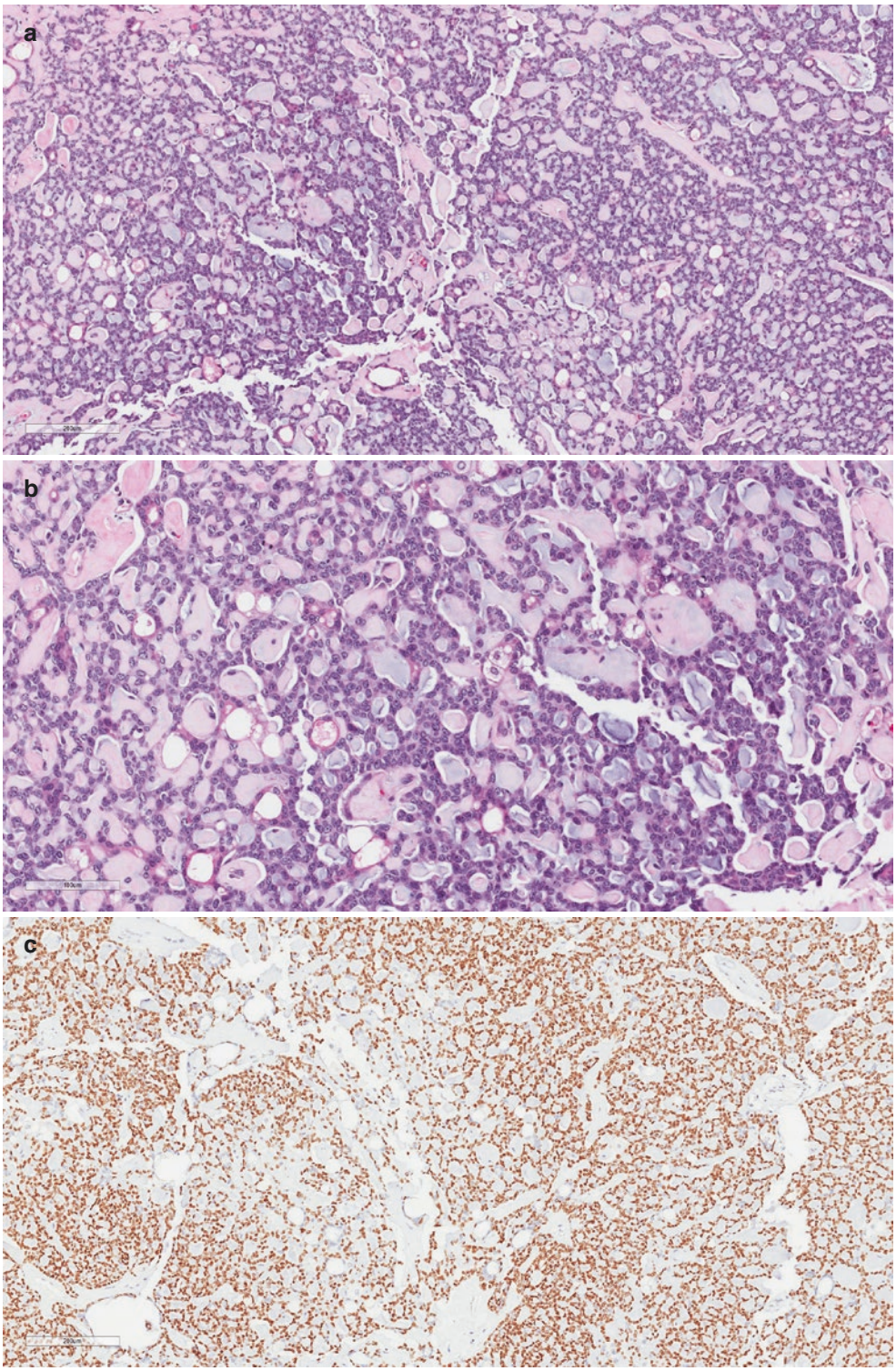
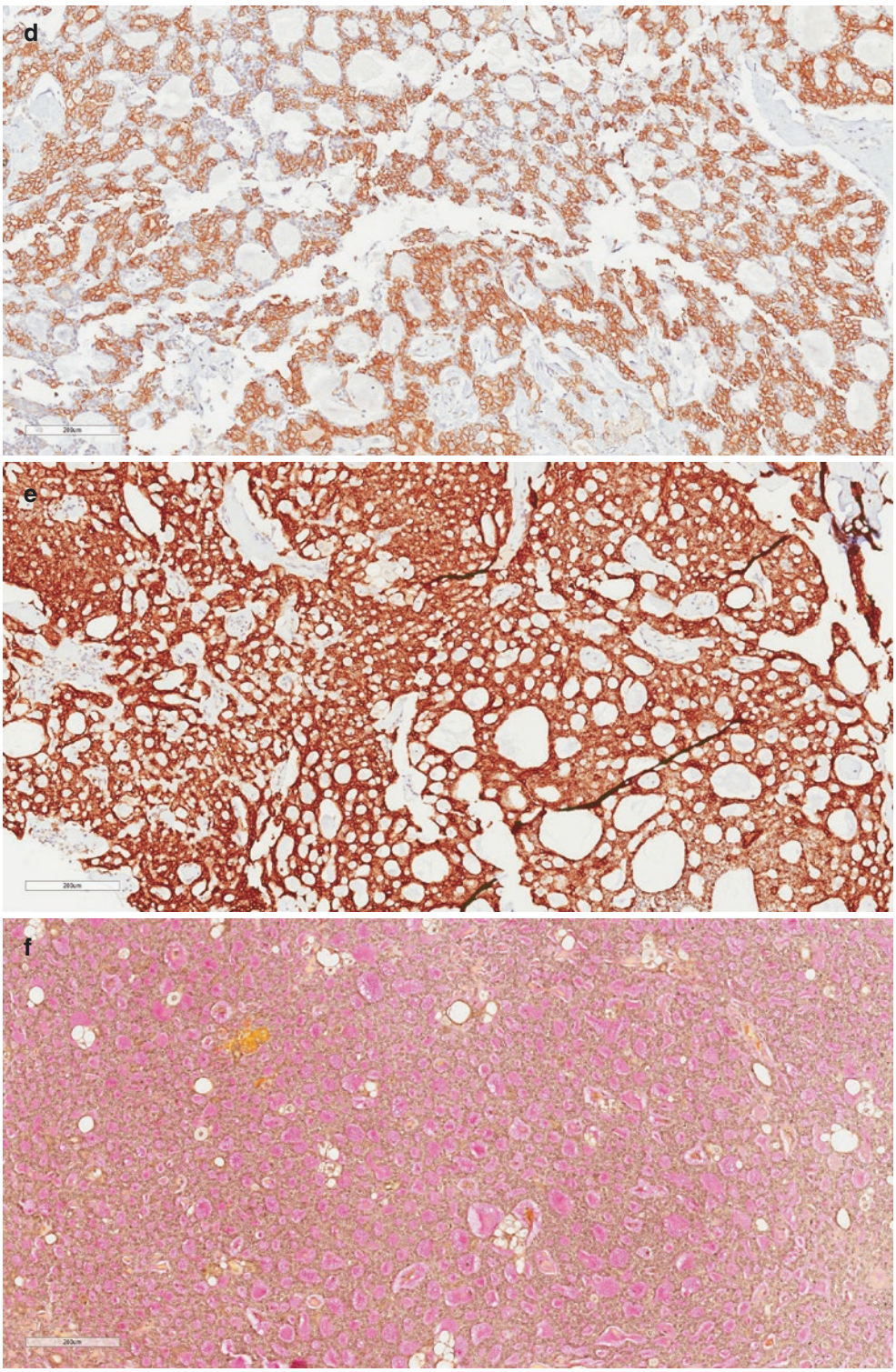


Fig. 3.11 Adenoid cystic carcinoma of the breast. Invasive cribriform nests of carcinoma cells surrounded by desmoplastic stroma (**a**). Cribriform nests with eosinophilic globular material (**b**). P63 stains basaloid cells of the tumor (**c**). Glandular luminal cells of the tumor are diffusely positive for c-Kit (CD117) protein (**d**) and CK5 (**e**). Mucicarmine stains the eosinophilic globular material (**f**). The tumor cells are negative for ER (**g**) and PR (**h**)



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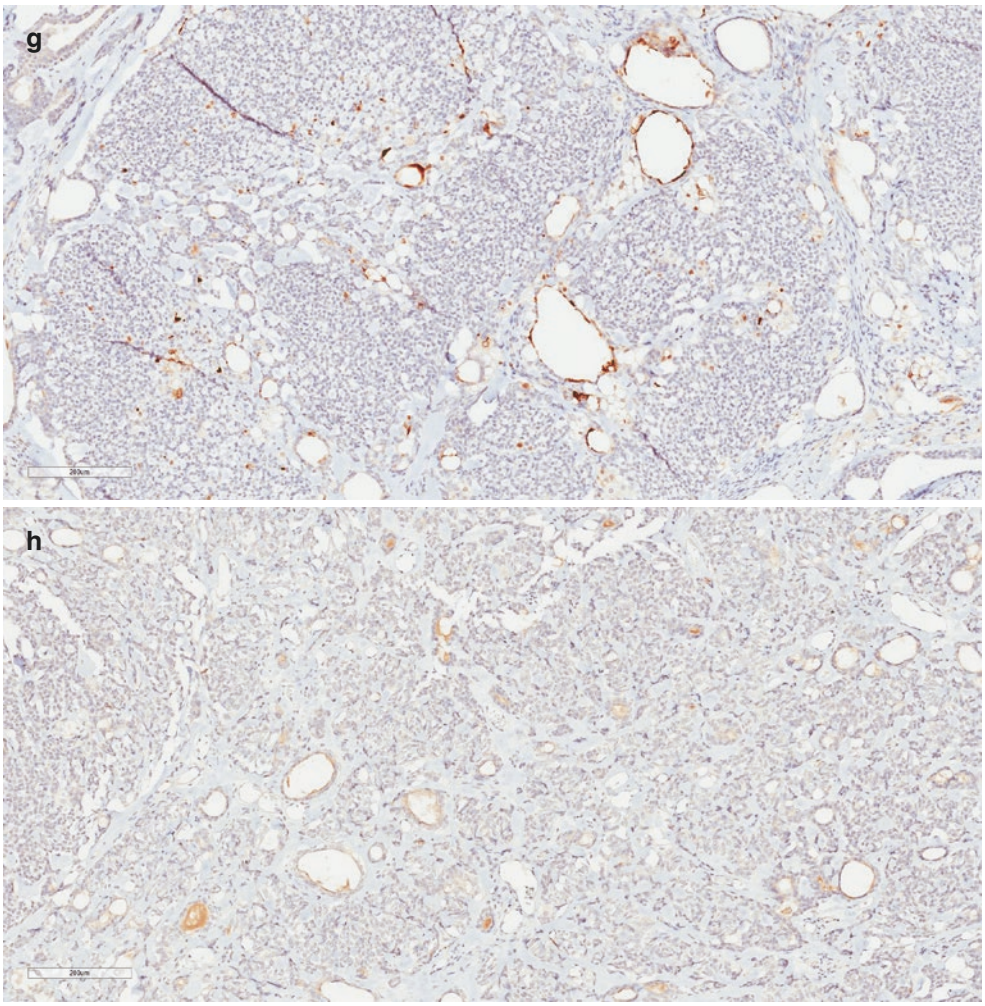


(EGFR) [63]. The glandular luminal cells are usually positive for CK7, CK8/18, epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), and c-Kit (CD117) [64]. Interestingly, the CK5 or CK5/6 can be diffusely positive in the glandular luminal cells as well [27]. The proliferative index labeled with Ki-67 is usually low but can be variable depending on the variants or grading of the tumors. The immunohistochemical profile of the mammary ACC is very similar to that of the basal-like triple-negative breast carcinoma (TNBC); however, prognosis of mammary ACC is better than that of basal-like TNBC [65–67]. Androgen receptor (AR) is negative in ACCs, but positive in around 30% of basal-like TNBCs [68].

31. What are the molecular features of adenoid cystic carcinoma of the breast?

Similar to ACCs of the salivary gland, ACCs of the breast also demonstrate recurrent t(6;9)(q22-23;p23-24) translocation with a *MYB-NFIB* gene fusion, resulting in an oncogenic fusion protein with transcription factor function [61, 69, 70]. This finding is confirmed with MYB RNA overexpression, which can be demonstrated by in situ hybridization. Besides MYB translocation, other genomic alterations in ACC of the breast include gains of 1p36.12-p35.3, 11p15.5, 12p13.31, 16p13.3, and 19p13 and losses of 6p25.3-q26 and 9p11.1-q21.11 [69].

32. Is adenoid cystic carcinoma associated with a better prognosis?

ACC of the breast is usually indolent as a localized disease with a low frequency of axillary lymph node metastasis (<8%) [71]. However, the solid variant of mammary ACCs has relatively higher incidence of the nodal metastases than classical ACCs, which may indicate a more aggressive behavior [72]. Distant metastases may occasionally occur in patients with ACC of the breast (<20%), most commonly to the lung or the bone [71, 73].

A breast-conserving surgical approach with or without radiotherapy is usually recommended for the treatment of ACC. Most studies have demonstrated an excellent clinical outcome with 10-year survival exceeding 90% after the treatment. Patients with mammary ACC have a prolonged and

indolent clinical course even when they present with local recurrence or distant metastasis [74, 75].

In ACCs of the salivary glands, MYB expression has been associated with a better survival compared with MYB-negative ACCs [76]. However, the association of MYB expression with survival remains unknown in patients with ACC of the breast.

33. What are the differential diagnoses for adenoid cystic carcinoma of the breast?

The differential diagnosis of ACC includes other types of invasive breast carcinomas and intraductal lesions that have a cribriform growth pattern and collagenous spherulosis. Invasive cribriform carcinoma can be confused with ACC, but the cribriform carcinoma has only one cell type and has glandular lumina without the mucinous or basement membrane material. In addition, most other types of breast carcinomas with a cribriform growth pattern are ER and PR positive and do not express p63 or c-kit. Collagenous spherulosis, a benign breast lesion, can also be confused with ACC, especially as the p63 is expressed in both lesions. However, ckit should not be expressed in collagenous spherulosis and can be helpful in the differential diagnosis. Another potential pitfall is with ACCs that have a predominantly nonclassical growth pattern, such as the solid variant, which can be confused with a higher-grade breast carcinoma. p63, EGFR, and c-kit may not be useful as these markers can be positive in high-grade invasive ductal carcinoma. In such cases, the FISH split-apart or fusion probes to detect the t(6;9) rearrangement and/or RTPCR for the *MYBNFIB* fusion gene may be needed to establish the diagnosis of ACC.

34. What are the current classification and subtypes of metaplastic breast carcinoma?

Metaplastic carcinoma (MC) of the breast represents 0.25–1% of all breast cancers diagnosed annually [27]. Based on the 2012 World Health Organization classification of Tumors of the Breast, MC is classified based on the histological features of tumor cells: (1) purely epithelial components (low-grade adenosquamous carcinoma, fibromatosis-like metaplastic carcinoma, squamous cell carcinoma, and spindle cell carcinoma) and (2) mixed epithelial and mesenchymal components (metaplastic carcinoma with mesenchymal differentiation and mixed metaplastic carcinoma) [27].

35. What is low-grade adenosquamous carcinoma of the breast and what are its key diagnostic features?

Low-grade adenosquamous carcinoma is an uncommon variant of metaplastic carcinoma with a good prognosis [77, 78]. Morphologically, round or comma-shaped infiltrating ducts are admixed with foci of squamous differentiation. The lumens of the ducts are usually compressed. Eosinophilic material or keratin may be present in the lumens. The tumor cells show low-grade nuclear features. The tumor stroma can be edematous or sclerotic and have variable spindle cells, but the cellularity of the stroma around the epithelial nests is often increased [79, 80]. See Fig. 3.12a–g.

36. What are the differential diagnoses for low-grade adenosquamous carcinoma of the breast?

The differential diagnosis of low-grade adenosquamous carcinoma includes benign breast lesions, such as sclerosing adenosis, squamous metaplasia or syringomatous adenoma of the nipple, and malignant lesions such as invasive tubular carcinoma. The absence of myoepithelial cells demonstrated by immunostains (SMMS, p40 or p63) will help to exclude benign lesions [78]. The intramammary parenchymal location of low-grade adenosquamous carcinoma is important to differentiate it from syringomatous adenoma of the nipple. Demonstrating squamous differentiation in low-grade adenosquamous carcinoma by careful sampling and histologic examination is important to differentiate it from the invasive tubular carcinoma [80].

37. What is fibromatosis-like metaplastic carcinoma and what are its key diagnostic features?

Fibromatosis-like spindle cell carcinoma (FLSCC) is a recently described low-grade variant of metaplastic carcinoma with a favorable prognosis [81, 82]. FLSCC grossly presents as a firm and white mass, and the cut surface shows a fibrous, gray-white nodular parenchyma. Microscopically, FLSCC shows the proliferation of cytologically bland, low-grade, spindled, fibroblast-like cells and stellate myofibroblast-like cells, resembling fibromatosis. The cellularity of proliferation of neoplastic cells is variable among FLSCCs. The neoplastic spindle cells show minimal nuclear atypia and pale eosinophilic cytoplasm; the nuclei vary from thin, slender, spindled nuclei with tapered ends to more plump, round to oval nuclei with discrete nucleoli. The tumor border is usually infiltrative with broad, finger-like projections into the surrounding tissue. Neoplastic squamous or glandular epithelial elements may be present but should be less than 5% of the total tumor volume. FLSCC may also show collagenous stroma, similar to fibromatosis. The presence of small, cohesive clusters of fusiform to polygonal epithelioid cells scattered among the spindle cells is a defining and characteristic histologic feature for FLSCC [83, 84]. See Fig. 3.13a–i.

A panel of immunohistochemical stains is generally needed to demonstrate the epithelial origin of the spindle cells in FLSCCs in order to differentiate it from other spin-

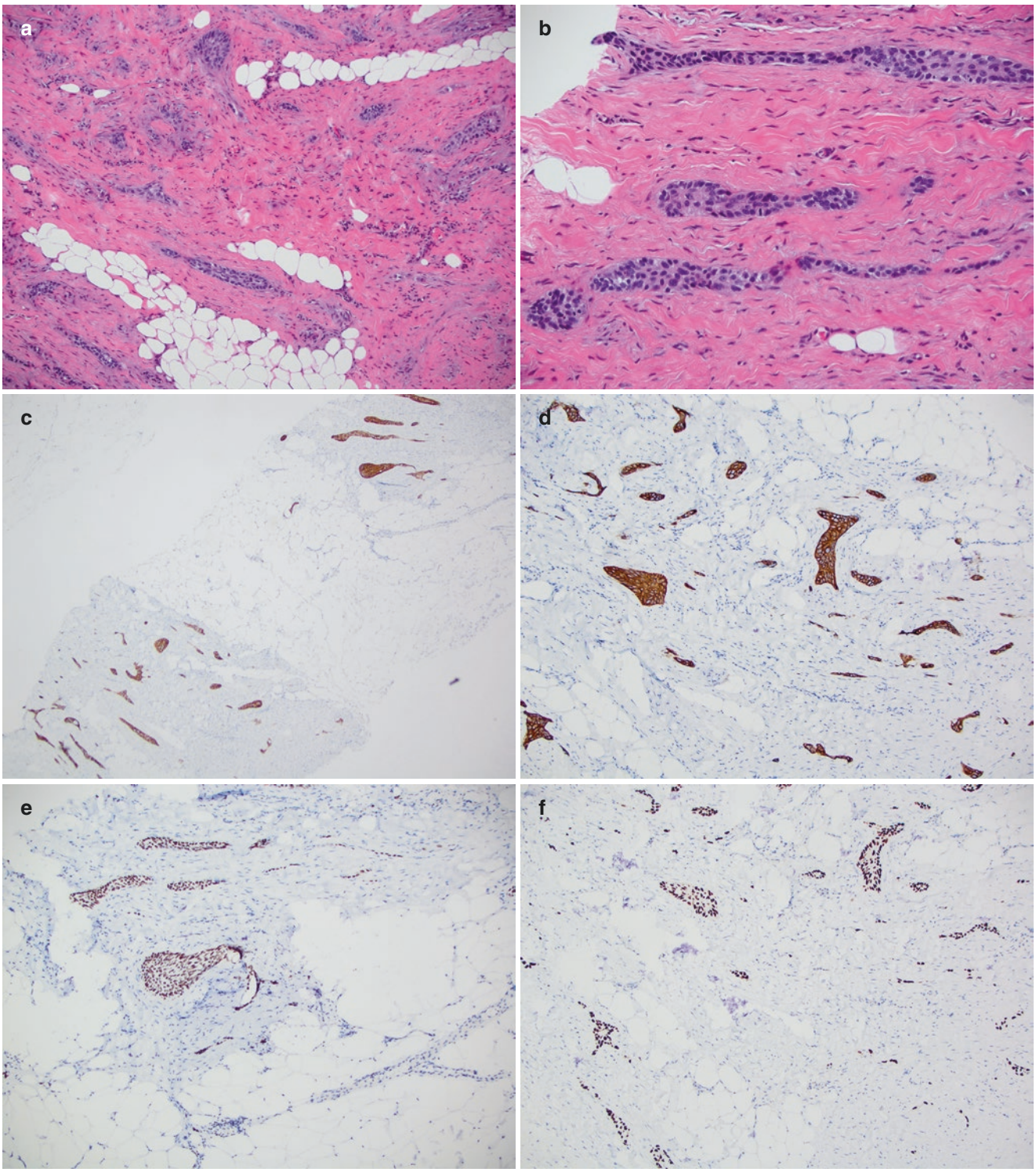


Fig. 3.12 Low-grade adenosquamous carcinoma of the breast. Infiltrating solid glandular structures of carcinoma cells into surrounding stroma (**a**). The tumor cells are low grade, bland looking with both squamous and glandular differentiation (**b**). The tumor cells are diffusely positive for BerEP4 (**c**), CK5 (**d**), GATA3 (**e**), and p63 (**f**). SMMS stain shows loss of myoepithelial cells around the tumor (**g**)

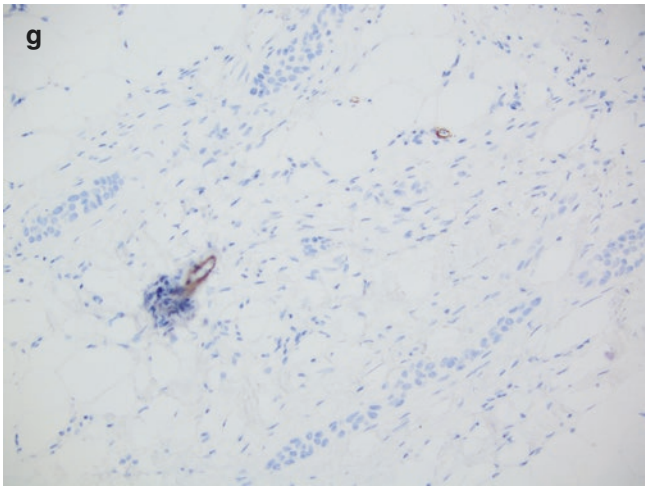


Fig. 3.12 (continued)

dle cell lesions of the breast. The cytokeratin immunohistochemical stains can include antibodies against broad-spectrum cytokeratins (AE1/AE3 and pankeratin), basal cytokeratins (CK5, 34βE12, and CK14), and luminal cytokeratins (CK7, CK19, and CAM 5.2). The spindle cell component and small clusters of epithelioid cells usually exhibit immunoreactivity for basal cytokeratins, but no to focal immunoreactivity for luminal cytokeratins. It has been suggested that the neoplastic spindle cells actually demonstrate an immunoprofile more compatible with myoepithelial differentiation with immunoreactivity of basal cytokeratins (34βE12, CK14, and CK5) and myoepithelial markers (smooth muscle actin, S100, p63 and p40). A study has demonstrated that p63 was strongly positive in 87% of metaplastic carcinomas and was positive in all metaplastic carcinomas with spindle cell and/or squamous differentiation [85]. The neoplastic spindle cells of FLSCCs are negative for smooth muscle myosin heavy chain (SMMHC) and epithelial membrane antigen (EMA); the proliferation index of FLSCC is typically low with less than 5% of Ki-67 staining [84, 86].

38. What are the differential diagnoses for fibromatosis-like metaplastic carcinoma?

The main differential diagnoses for FLSCC include nodular fasciitis and fibromatosis. Nodular fasciitis is a benign proliferative lesion containing fibroblasts and myofibroblasts in myxoid stroma with prominent vasculature. The lesion is very rarely seen in the breast and should be diagnosed only after extensive sectioning and with negative cytokeratin staining. Fibromatosis is a clonal proliferation of benign-appearing fibroblasts and myofibroblasts with an infiltrative growth pattern. The spindle cells in fibromatosis are negative for cytokeratins but show positive staining of beta-catenin in the nuclei. Other differential diagnoses include myofibroblastoma and pseudoangiomatous stromal hyperplasia (PASH). Myofibroblastoma is a rare benign proliferation of myofibroblasts. Myofibroblastomas were originally reported to occur more frequently in males, but recent data suggest they are equally frequent between males and females [87]. Histologically, myofibroblastomas are composed of bland-appearing spindle cells in short haphazard fascicles separated by collagen bands. Patchy perivascular chronic inflammatory infiltrates are characteristic findings. The myofibroblast cells are positive for vimentin and variably positive for desmin, CD34, smooth muscle actin, estrogen receptor, progesterone receptor, and Bcl-2, but negative for cytokeratins [88, 89]. PASH is a benign lesion with anastomosing empty, slit-like pseudovascular spaces lined by myofibroblasts (not endothelial cells) in a dense collagenous stroma. Similar to those in the myofibroblastoma, the spindle cells in the PASH are positive for vimentin and variably positive for desmin, CD34, and smooth muscle actin, but negative for cytokeratins [90].

39. What is squamous cell carcinoma of the breast and what are its key diagnostic features?

Metaplastic squamous cell carcinomas can be pure or mixed with other forms of invasive carcinoma. Pure squamous cell carcinomas in the breast are rare. More commonly, squamous differentiation is identified coexisting with invasive ductal carcinomas and carcinomas with medullary features. Squamous cell carcinomas usually present as cystic lesions with squamous lining cells showing variable atypia and nuclear pleomorphism. The tumor cells can show sheets, cords, or nests of proliferation infiltrating into the stroma with a prominent stromal reaction and lymphocytic response [27]. See Fig. 3.14a–f.

40. What are the differential diagnoses for squamous cell carcinoma of the breast?

If the tumor is composed entirely of malignant squamous cells, a metastasis from another site, especially skin, lung, or head/neck region, must be ruled out before making the diagnosis of mammary squamous cell carcinoma. The other differential diagnosis is mucoepidermoid car-

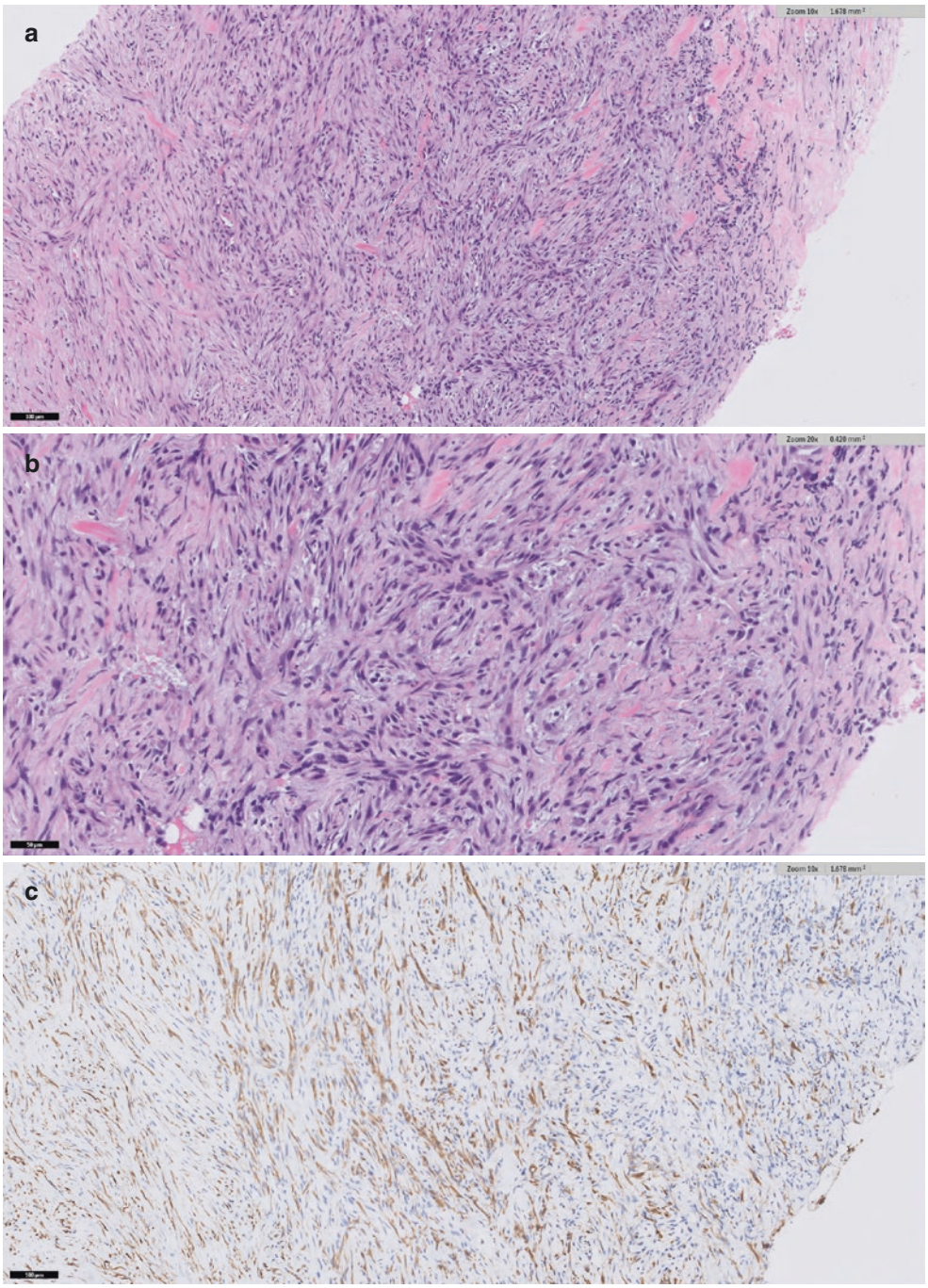
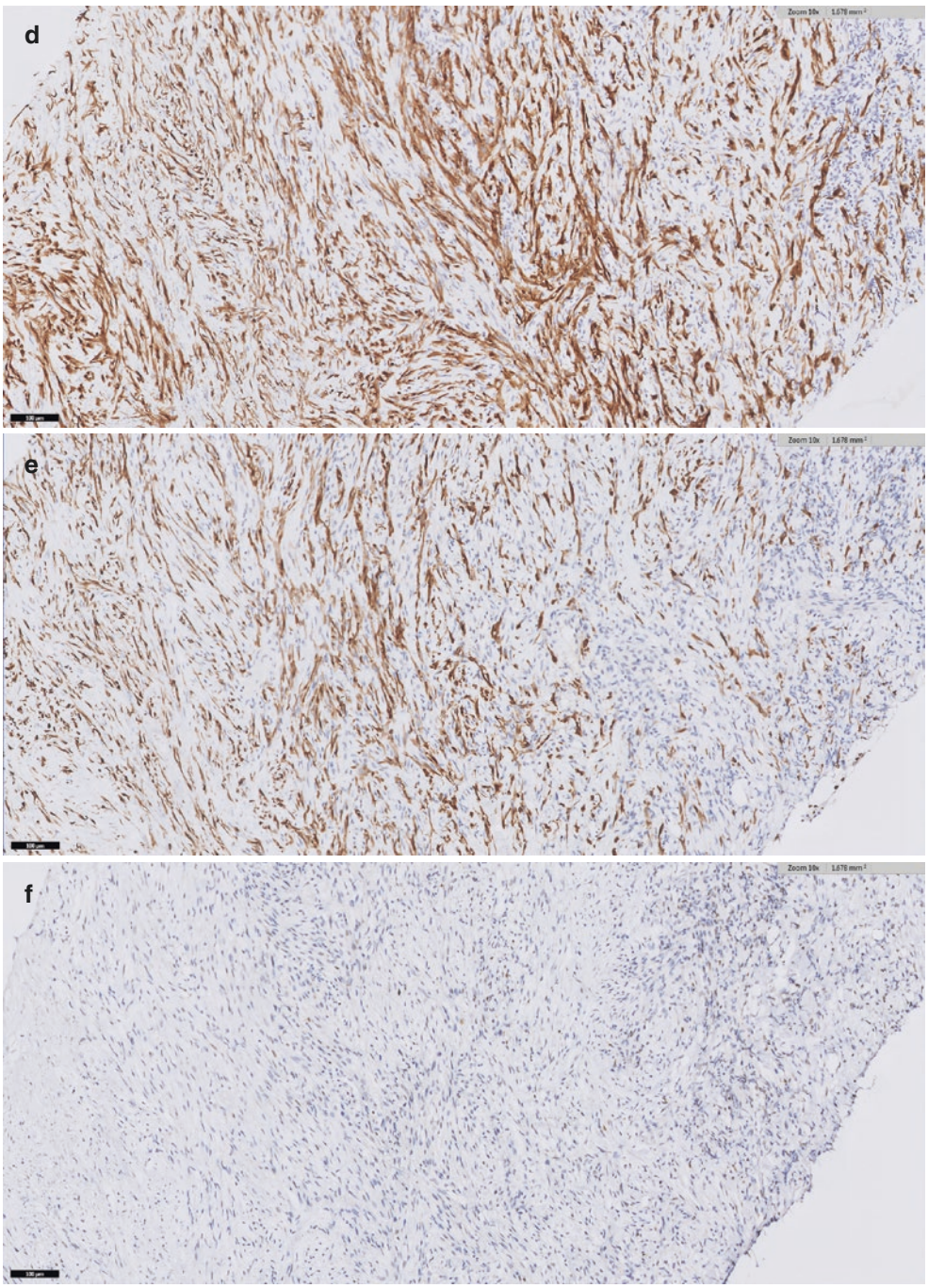


Fig. 3.13 Low-grade fibromatosis-like spindle cell carcinoma (FLSCC). Broad infiltrative projections of the tumor extending into the surrounding soft tissue (**a**). The tumor is composed of cytologically bland cells with thin and spindled to round or oval nuclei (**b**). The tumor cells are positive for cytokeratin AE1/AE3 (**c**), MNF116 (**d**), and CK5 (**e**) and positive for CK7 (**f**). The tumor cells are focally positive for GATA3 (**g**) and negative for desmin (**h**) and ER (**i**)



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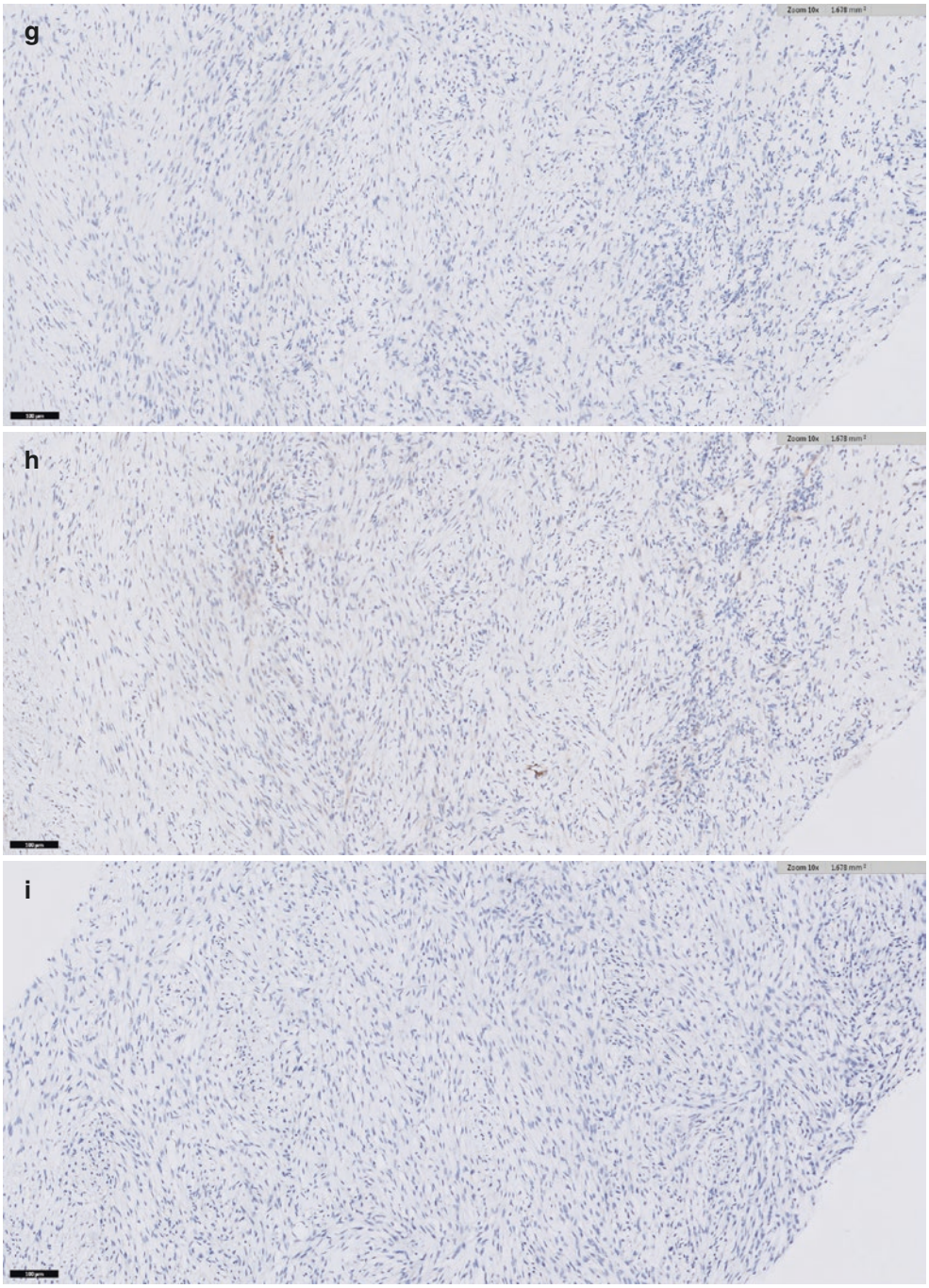


cinoma (both low- and high-grade types), which usually shows extracellular or intracellular mucin [91, 92]. Squamous metaplasia in the breast varies from syringoma-like differentiation to inconspicuous foci in largely glandular lesions. Keratinizing cysts are uncommon, but small osteocartilaginous foci can be seen [77]. Some benign squamous lesions in the breast may also get into the differential diagnosis of squamous cell carcinoma of the breast, including posttraumatic lobular squamous metaplasia [93], mixed squamous–mucous cysts [94], squamous metaplasia in gynecomastia [95], Zuska's disease (squamous metaplasia of lactiferous ducts), and infarction with squamous metaplasia of intraductal papilloma [96, 97].

41. **What is spindle cell carcinoma (sarcomatoid carcinoma) of the breast and what are its key diagnostic features?**

Spindle cell carcinomas of the breast can be pure or mixed with other components, such as glandular, heterologous, or squamous elements [98, 99]. These tumors are composed of atypical spindle cells in a growth pattern of long fascicles (herringbone or interwoven pattern) or short fascicles (storiform). The atypical spindle cells can range from bland appearing to highly pleomorphic. The cytoplasm can range from elongated to plump spindle and the nuclei can range from bland-looking to apparently pleomorphic. Mitotic rate can be variable among spindle cell carcinomas of the breast. The spindle cells infiltrate into the surrounding stroma with entrapped benign ducts and lobules [100]. Nottingham grading is not applicable to metaplastic spindle cell carcinomas [27].

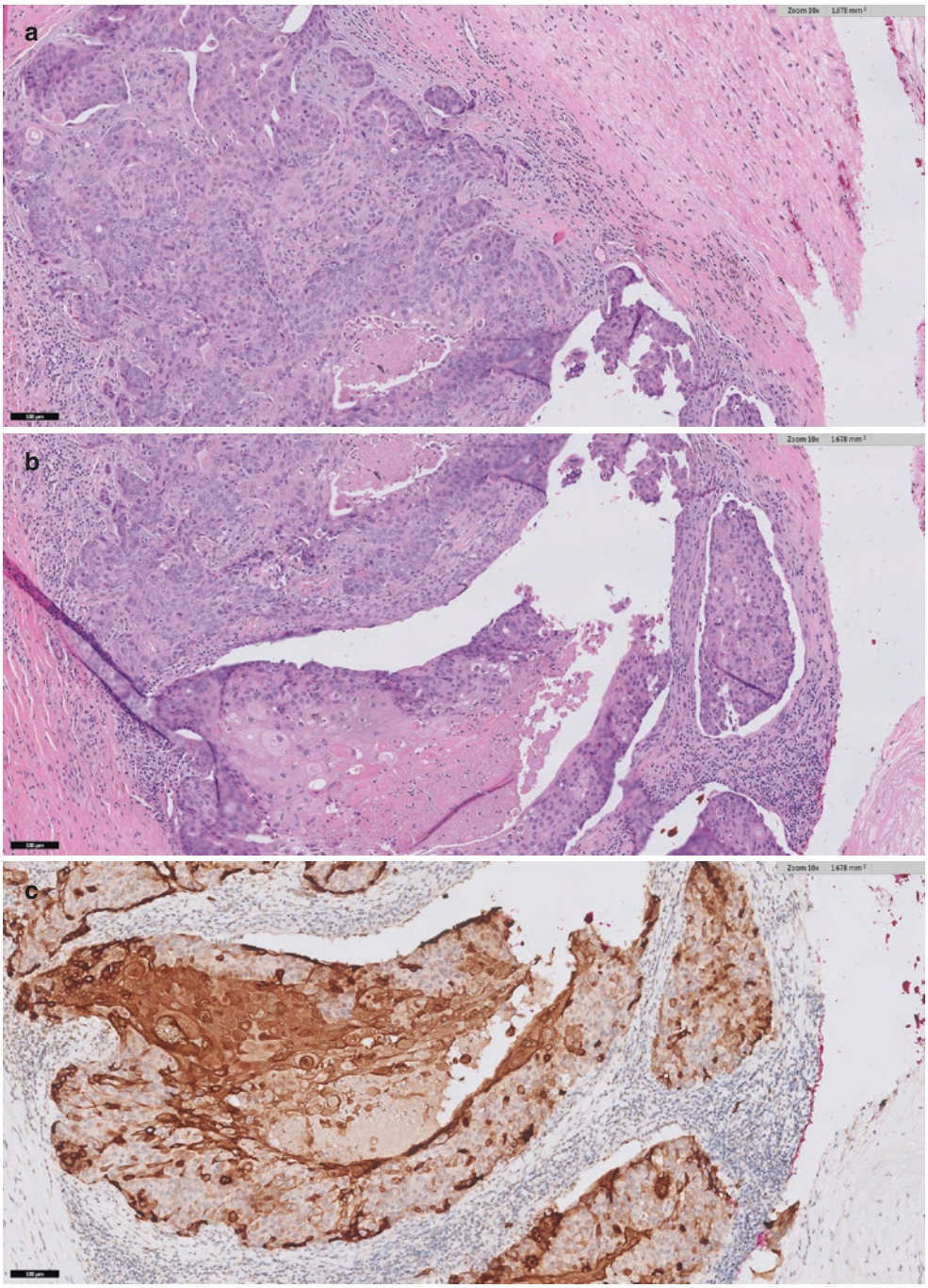
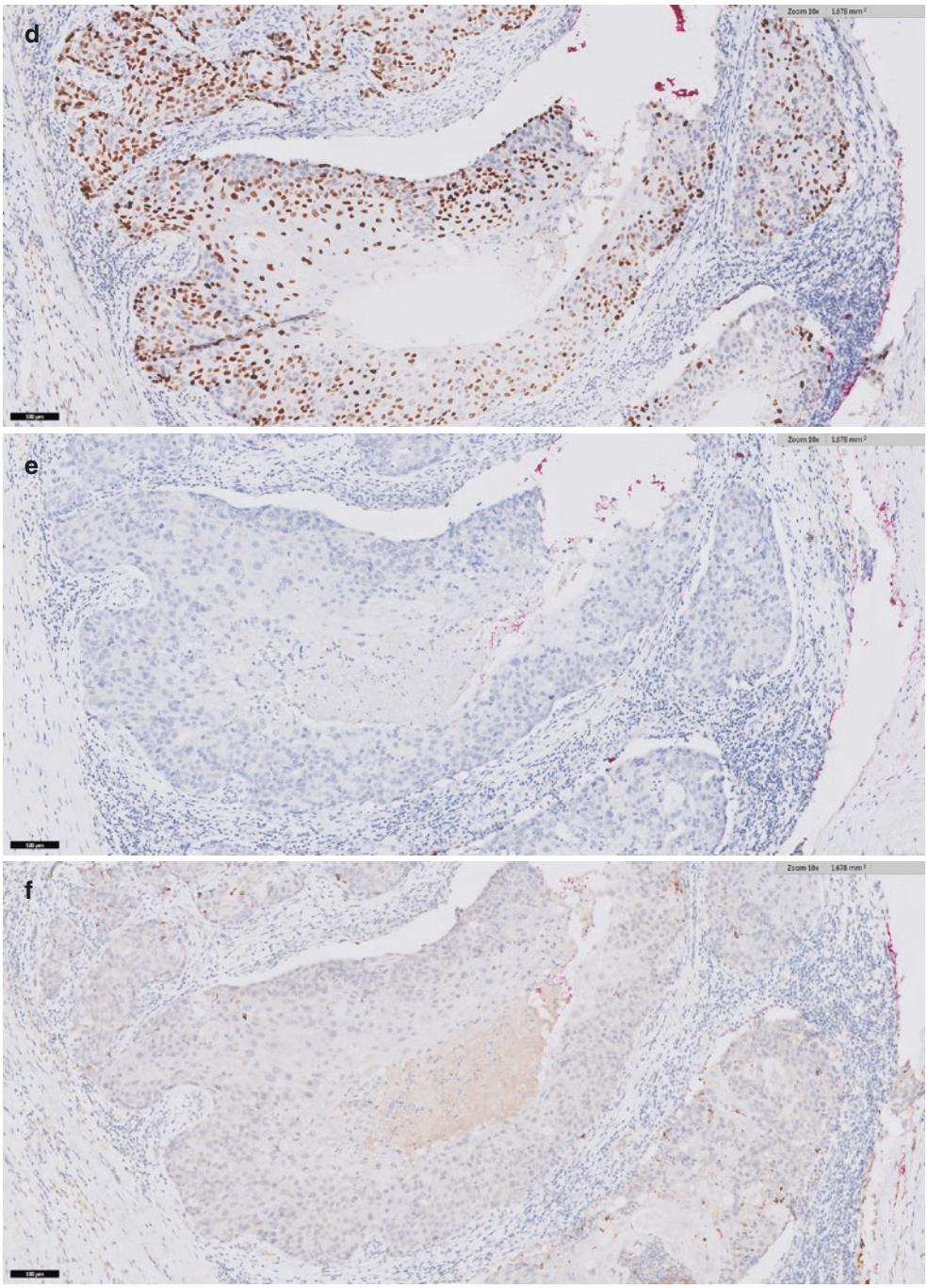


Fig. 3.14 Metaplastic squamous cell carcinoma of the breast. Invasive carcinoma with both squamous differentiation and ductal differentiation with focal necrosis and lymphocytic response (**a**). Squamous carcinoma cells show nuclear pleomorphism and keratinization (**b**). The tumor cells are diffusely positive for CK5 (**c**) and p40 (**d**) and negative for ER (**e**) and PR (**f**)

Fig. 3.14 (continued)



Spindle cell carcinomas of the breast can coexist with an epithelial component of invasive ductal carcinoma or ductal carcinoma in situ. For any lesion with pure spindle cells, a suspicion for metaplastic spindle cell carcinoma must be high so that immunostains for epithelial differentiation should be performed. A panel of cytokeratins is often necessary with a broad spectrum of cytokeratins, including high-molecular-weight cytokeratins (CK5/6 and 34βE12) and low-molecular-weight cytokeratins. The neoplastic spindle cells usually express myoepithelial markers such as p63, p40, smooth muscle actin, and muscle specific actin. Similar to other subtypes of metaplastic carcinoma, spindle cell carcinomas are generally negative for ER, PR, and HER2 [27, 100]. See Fig. 3.15a–i.

42. What are the differential diagnoses for spindle cell carcinomas of the breast?

For spindle cell carcinomas of the breast, the main differential diagnoses include malignant phyllodes tumor with prominent spindle cell overgrowth, sarcomas (angiosarcoma, fibrosar-

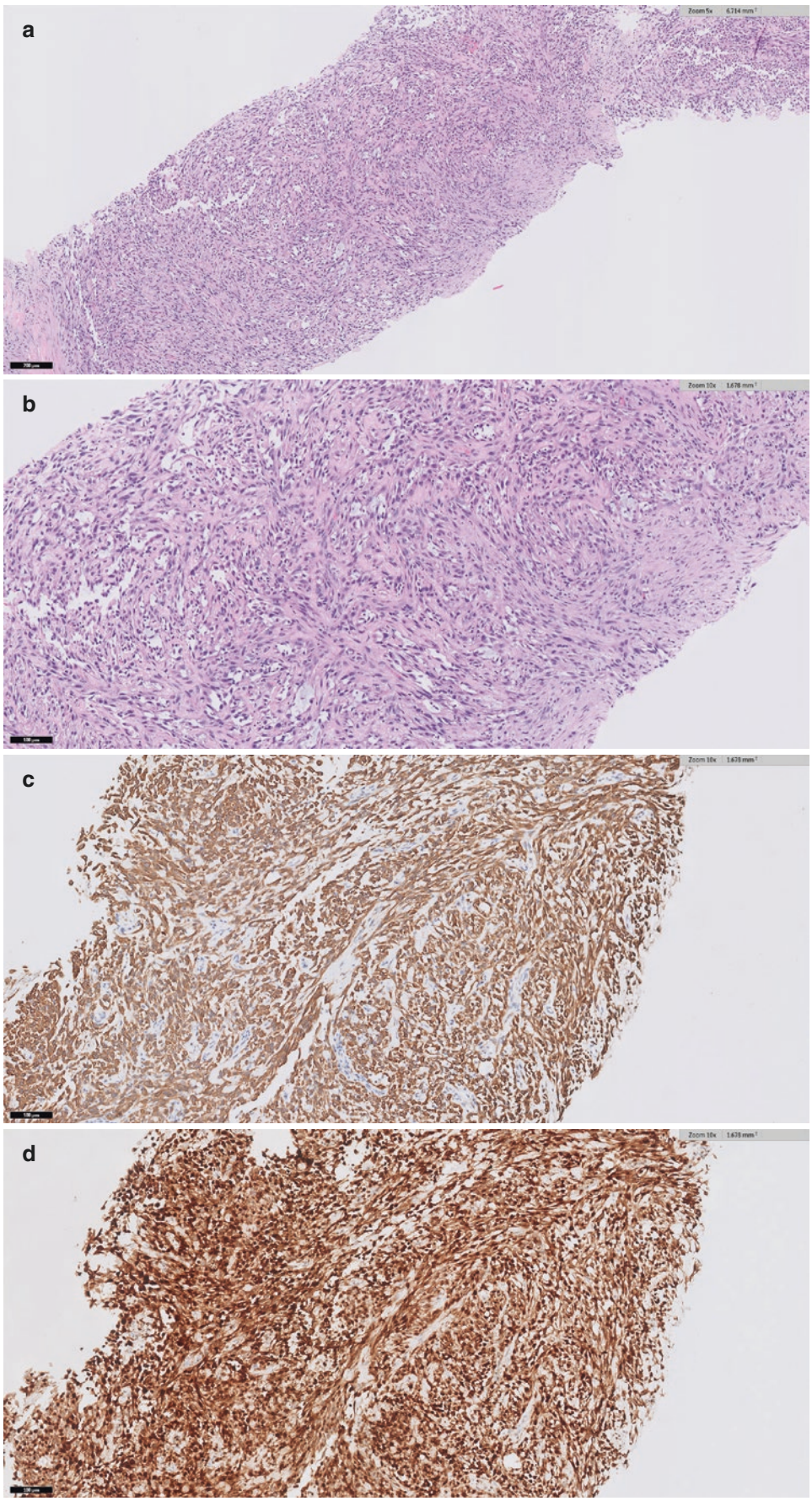
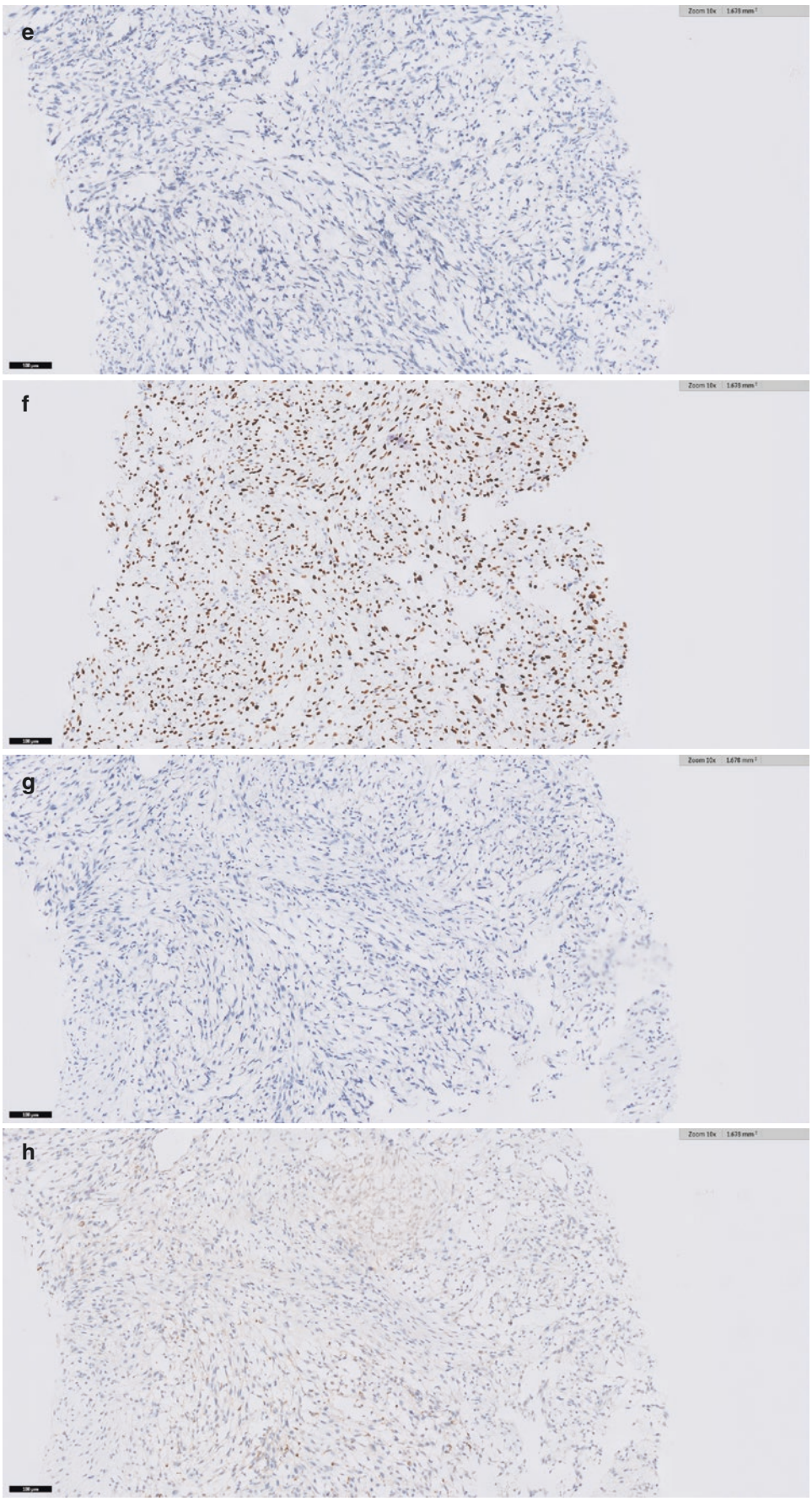


Fig. 3.15 Spindle cell carcinoma of the breast. The tumor is composed of atypical spindle cells in a growth pattern of long fascicles with desmoplastic stromal reaction (**a**). The atypical spindle cells are mildly to moderately pleomorphic and the cytoplasm is mostly elongated to plump spindly (**b**). The tumor cells are diffusely positive for cytokeratin AE1/AE3 (**c**) and CAM5.2 (**d**) and negative for CK7 (**e**). The tumor cells are also diffusely positive for p40 (**f**) and negative for ER (**g**), PR (**h**), and HER2 (**i**)



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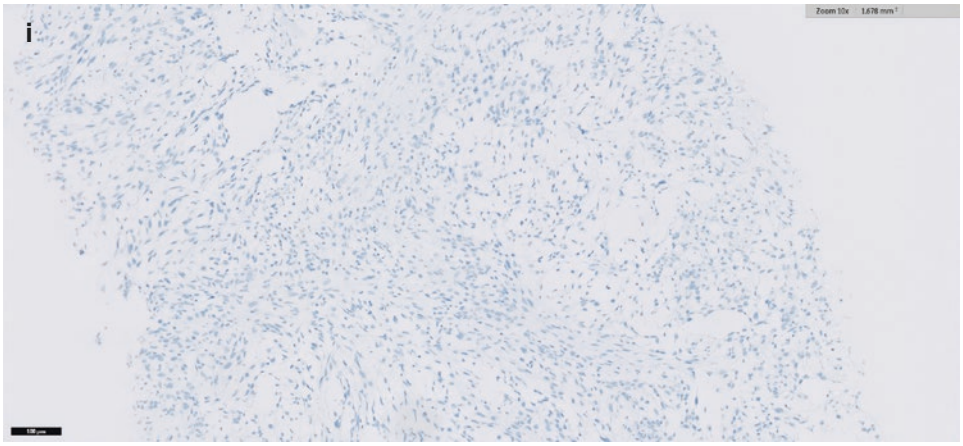

coma, etc.), and benign spindle cell lesions, such as fibromatosis and PASH. Extensive sampling to identify malignant epithelial component is important, and epithelial immunohistochemical markers such as cytokeratins and p63/p40 are almost always necessary to make the diagnosis. The leaf-like architecture is characteristic of phyllodes tumor. The stromal spindle cell proliferation in the phyllodes tumor is generally negative for cytokeratins and positive for CD34. The spindle cells of fibromatosis usually show nuclear staining for beta-catenin but negative staining for cytokeratins [27, 100].

43. What is metaplastic carcinomas with mesenchymal differentiation and what are its key diagnostic features?

Metaplastic carcinomas with mesenchymal differentiation contain mesenchymal elements (cartilage, bone, rhabdoid, or a chondromyxoid matrix) admixed with carcinomatous components [101]. The osseous and chondroid elements can appear histologically benign or frankly malignant with an appearance of chondrosarcoma or osteosarcoma [101]. Extensive sampling may be necessary to identify epithelial components. At the same time, a broad panel of cytokeratins may also be necessary to reveal the epithelial component when no apparent glandular component is present. Metaplastic breast carcinomas with mesenchymal differentiation originate from carcinomas that undergo sarcomatous transitions as a result of further genetic instability or mutations, and the identical clonality of the carcinomatous and mesenchymal components has been confirmed. The term "matrix-producing carcinoma" was historically used for a subtype of metaplastic carcinomas with mesenchymal differentiation, which usually contains chondroid differentiation or chondromyxoid matrix [102]. See Fig. 3.16a–c. Similar to other subtypes of metaplastic carcinomas, metaplastic carcinomas with mesenchymal differentiation are also negative for ER, PR, and HER2 [27].

44. What are the differential diagnoses for metaplastic carcinomas with mesenchymal differentiation?

The main differential diagnoses of metaplastic carcinoma with mesenchymal differentiation are sarcomas. Primary breast sarcomas are exceedingly rare and most frequently arise in association with a phyllodes tumor. To make a distinction between these two entities, extensive sampling is usually necessary to identify either malignant epithelial component for diagnosis of metaplastic carcinoma with mesenchymal differentiation or benign-appearing epithelial component and/or leaf-like architecture for phyllodes tumor in cases with predominantly sarcomatous proliferation [27].

45. What is the prognosis of most metaplastic carcinomas? Do all metaplastic carcinomas carry a bad prognosis?

Due to the heterogeneity of metaplastic carcinoma, the prognosis largely depends upon the histologic features. Some low-grade subtypes of metaplastic carcinomas such as low-grade adenosquamous carcinoma or fibromatosis-like metaplastic carcinoma usually have a favorable prognosis with only local recurrence and rare distant metastases, while others (high-grade spindle cell carcinoma, metaplastic carcinoma with mesenchymal differentiation, or squamous cell carcinoma) have an aggressive clinical course with poor outcomes.

In general, patients with metaplastic carcinoma have larger tumors with negative hormone receptor status and less involvement of the regional lymph nodes [103]. However, even in the absence of lymph node metastasis, distant metastasis to the brain and lungs can occur [104, 105]. The prognosis of fibromatosis-like metaplastic carcinoma parallels that of fibromatosis, suggesting that wide excision with clear margins or simple mastectomy without axillary lymph node dissections should be sufficient for

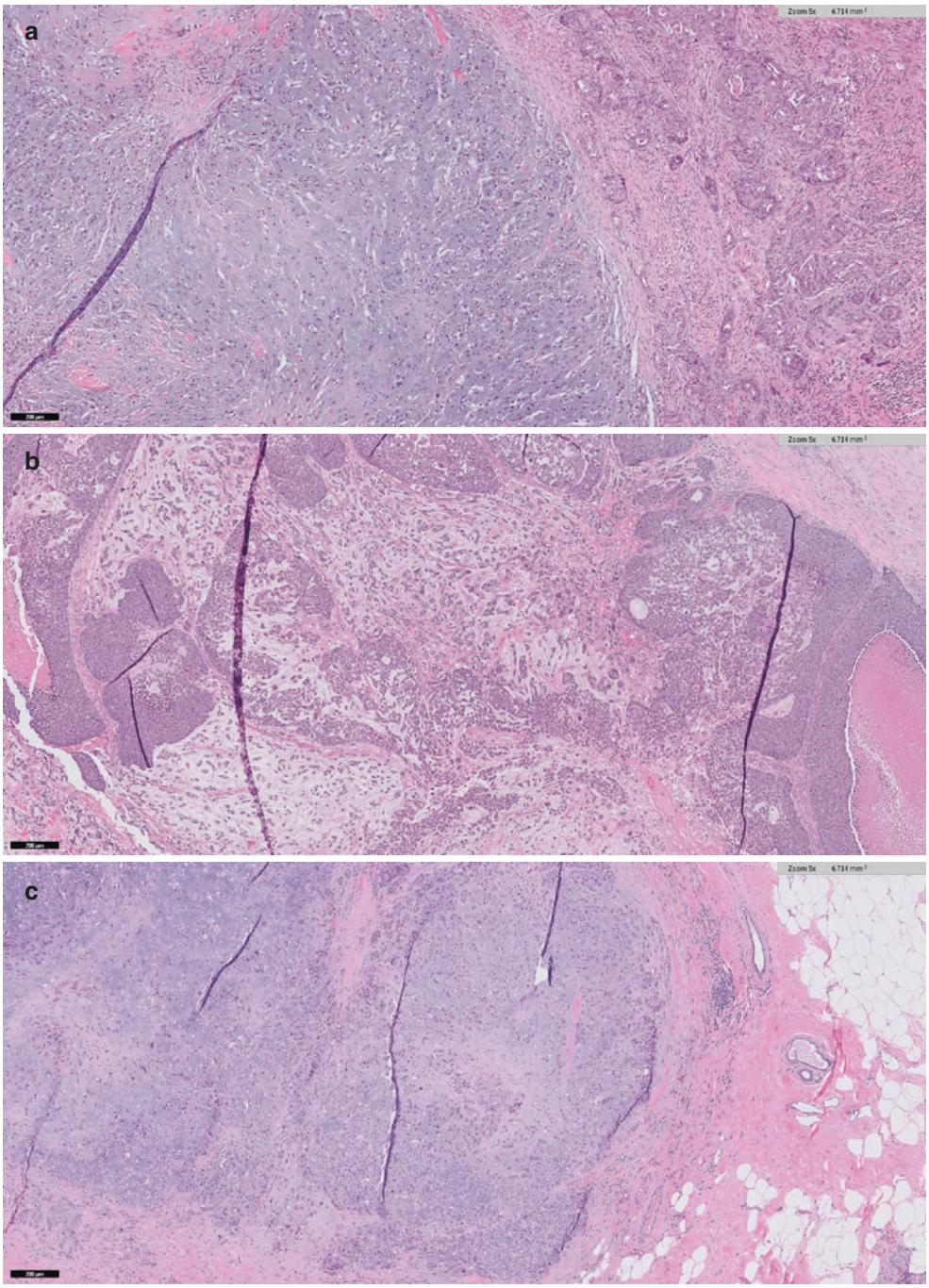

Fig. 3.16 Metaplastic carcinoma with mesenchymal differentiation. Metaplastic carcinoma with chondromyxoid matrix (**a**). Metaplastic carcinoma with chondromyxoid matrix intermixed with malignant epithelial cells (**b**). Metaplastic carcinoma with chondromyxoid matrix intermixed with malignant mesenchymal cells (**c**)

initial treatment of FLSCC; chemotherapy and radiation therapy may not be needed. However, the data are limited and more studies are warranted. On the other hand, patients with high-grade metaplastic carcinomas usually have a relatively poor prognosis and should be treated like Nottingham grade 3 invasive ductal carcinoma of the breast [106–108].

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Lobular Breast Lesions

4

Megan L. Troxell, Yun An Chen, Jing Yu, Debra M. Ikeda, and Kimberly H. Allison

List of Frequently Asked Questions

Invasive Lobular Carcinoma

1. **What are the differences in clinical, radiological, and pathologic gross presentations, clinical management, and prognosis in invasive lobular carcinoma as compared to invasive ductal carcinoma?**

See Table 4.1. Also see questions 3 through 6 and question 44 for further details.

2. **What is the incidence of invasive lobular carcinoma?**

- Invasive lobular is the second most common subtype of female breast cancer, after ductal or so-called "no special type" breast carcinoma.
- Invasive lobular carcinoma accounts for 5–15% of invasive breast carcinomas based on current World Health Organization (WHO) estimates [1]; other sources cite closer to 5% incidence [2].

Table 4.1 Clinical features of invasive lobular as compared to invasive ductal carcinoma

Clinical feature	Lobular	Ductal
Focality	Often multifocal	Usually unifocal
Radiological imaging	Mass, architectural distortion, non-mass enhancement (MRI), or occult	Mass, architectural distortion, or calcifications. Non-mass enhancement (MRI)
Gross pathology	Actual size of tumor often larger than what is estimated on gross and radiological examination	Typically forms a dominant grossly evident mass
Management after biopsy	MRI may be performed to evaluate extent of disease; resect to negative margins	Neoadjuvant chemotherapy may be given for tumor of high grade, Her2+, or triple-negative receptor status; resect to negative margins
Distant metastasis	Bone, GI, GYN	ER+: Bone (and other organs) ER-: Brain, lung, liver

- The incidence of invasive lobular carcinoma has been estimated at 10.6/100,000 US women (based on registry data from 1999 to 2004) [3]. The incidence of lobular carcinoma was noted to decline over this interval, possibly due to declining use of hormone replacement therapy [3, 4].

3. **How does invasive lobular carcinoma typically present clinically?**

Invasive lobular cancer may present in several ways:

- It is most commonly detected on screening imaging studies, although it can be imaging occult.
- It may come to attention as a clinically palpable breast mass or masses. (It is commonly multifocal.)

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Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5_4

- It may present as nipple retraction or, if advanced, with breast retraction. However, Paget's disease of the nipple is usually caused by a ductal rather than lobular process.
- If it has spread to lymph nodes, it may present with palpable, hard axillary lymph nodes.
- Occult lobular carcinoma can present with metastatic disease to other organ sites, such as bone, abdominal organs, and serosal surfaces of the gynecologic and gastrointestinal tract. It is the most common type of breast cancer to present with distant metastases from an occult primary.

4. **What are the typical features of invasive lobular carcinoma on breast imaging (mammography, ultrasound, MRI)?**

Based on its growth pattern, interaction with surrounding tissue, paucity of microcalcifications, and often slow rate of growth, invasive lobular carcinoma may be relatively more difficult to detect as compared to other subtypes of breast cancer [1, 5–7]. It may even be imaging occult on some studies [5–7].

- By mammography, invasive lobular carcinoma tends to present as some form of architectural finding, such as mass, spiculated mass, asymmetric density, or architectural distortion (Figs. 4.1a–g and 4.2a–g) [1, 5, 7]. Calcifications were noted in about 1/3 in one study [5].
- The sensitivity of mammography for lobular carcinoma detection has been quoted as widely variable (34–92%) [6].
- Digital breast tomosynthesis (DBT) is a newer variation of X-ray mammography in which a series of angled digital mammographic views are reconstructed to produce a three-dimensional view of the breast. It is said to enhance

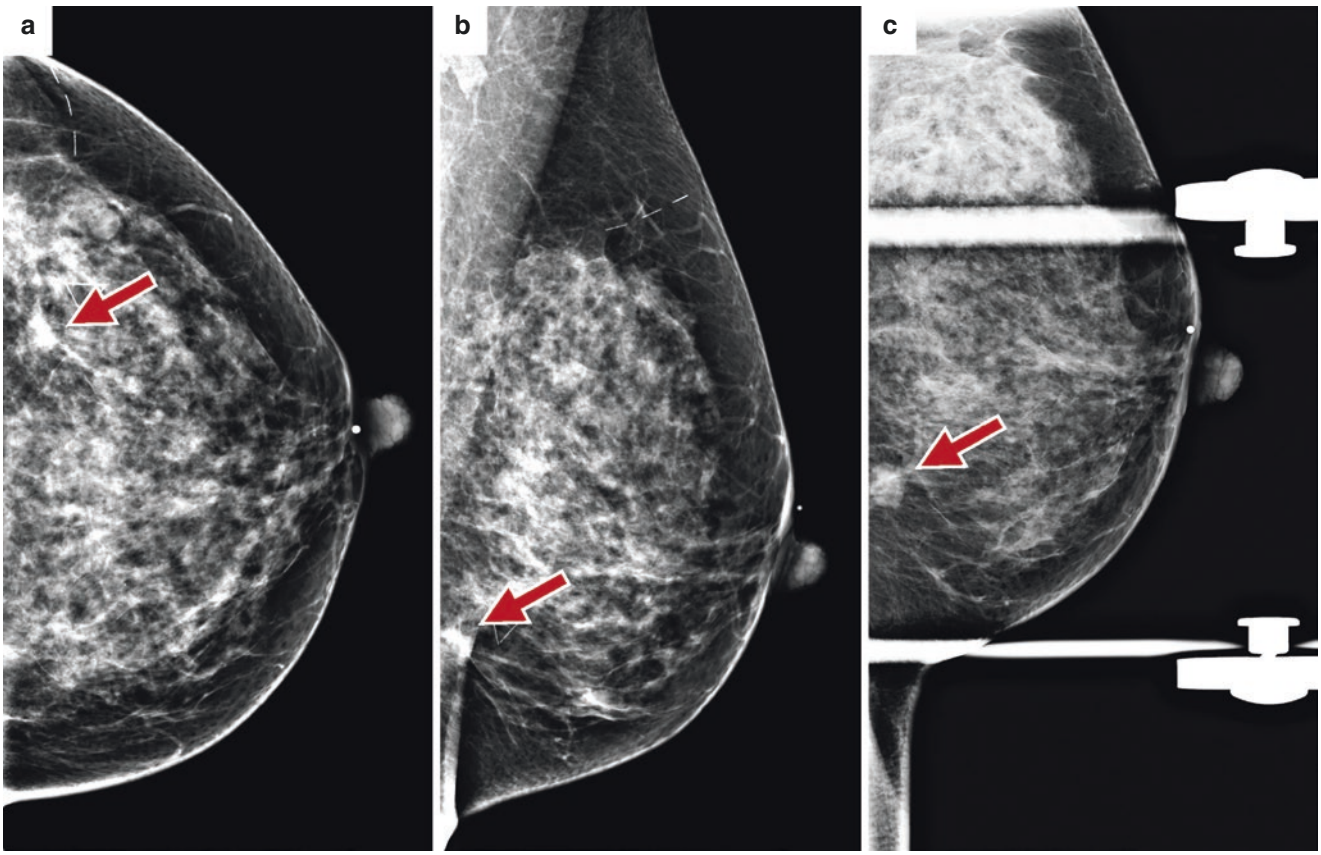


Fig. 4.1 Invasive lobular carcinoma, breast imaging. A 47-year-old woman who noticed a lump in her lower left breast at the 5:30 position with associated skin dimpling. Craniocaudal (**a**) and mediolateral oblique (**b**) full-field digital mammograms (FFDM) of a left breast palpable mass. There is a two-view 8 mm focal asymmetry (arrow) with associated skin tethering. Note the linear scar marker in the upper outer left breast from a remote benign excisional biopsy. A skin marker above the nipple shows the superior extent of the areola. (**c**) FFDM spot compression lateral medial mammogram clearly shows a round, irregular mass against the chest wall. (**d**) Real-time breast ultrasound shows an irregular, spiculated hypoechoic mass with acoustic shadowing and nonparallel orientation, with probable skin invasion, suspicious for cancer. (**e**) Axial contrast-enhanced breast MRI shows a 1.7 cm irregular spiculated mass at the 5:00 position with skin tethering and skin invasion. The lesion is larger on MRI as compared to mammography. Kinetic analysis showed fast initial and washout delayed enhancement, suspicious for cancer. US-guided core biopsy with marker placement was performed, followed by lumpectomy. (**f**) Histologic sections of the lumpectomy specimen demonstrate an infiltrative breast cancer with direct dermal invasion at low power; skin is at left (10×). (**g**) Higher power demonstrates dyscohesive architecture and cytologic features of invasive lobular carcinoma (200×). E-cadherin negative (not shown)

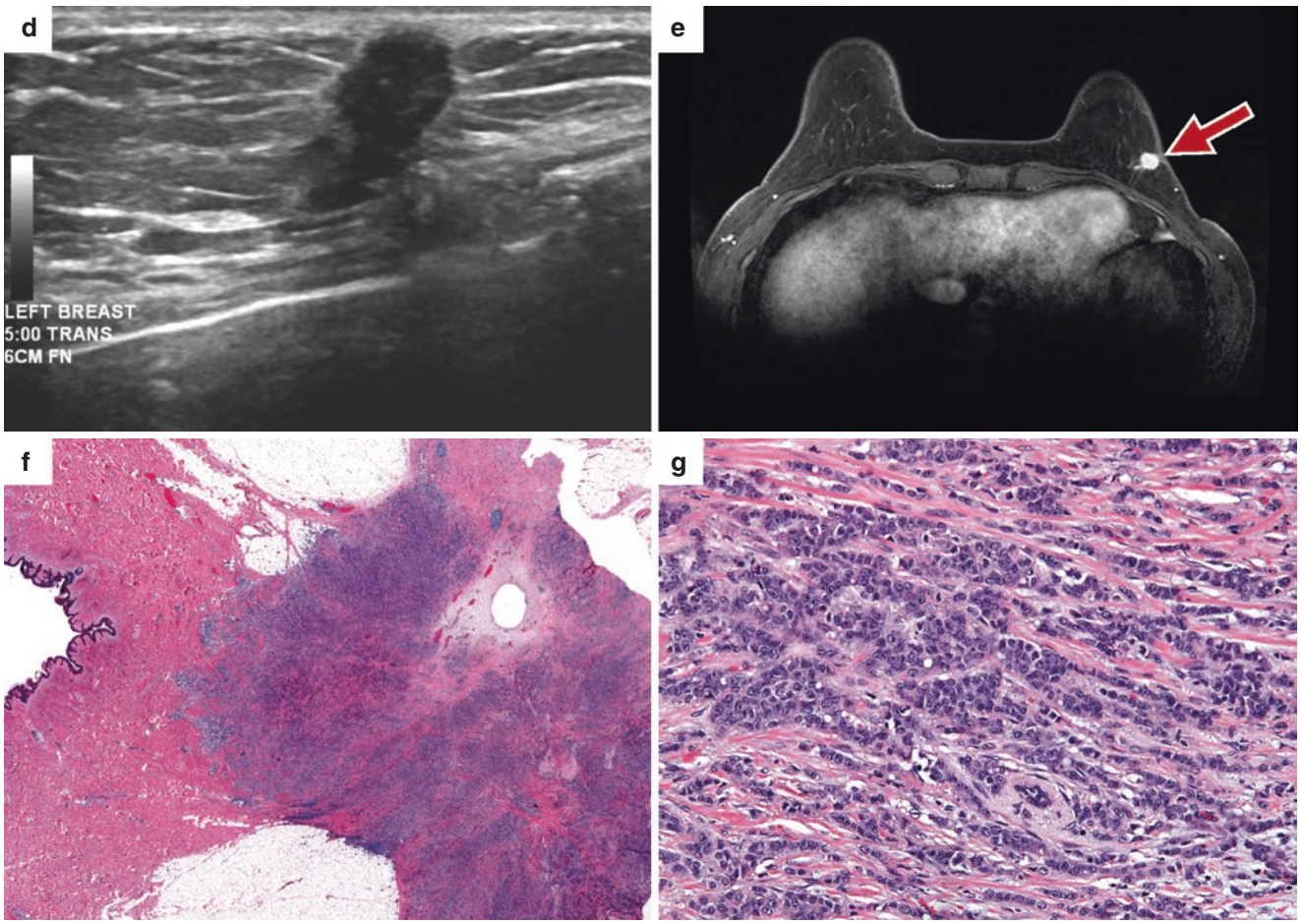


Fig. 4.1 (continued)

detection of architectural distortions and increase conspicuity of invasive lobular carcinoma [8].

- By ultrasound, invasive lobular carcinoma is most often seen as a hypoechoic irregular area, or mass, with poorly defined borders and posterior acoustic shadowing [1, 5, 6]. One study reported mostly isoechoic lesions [6]. However, small lesions may be relatively occult (Figs. 4.1a–g and 4.2a–g).
- The sensitivity of ultrasound for lobular carcinoma detection is about 68–98% [6].
- Magnetic resonance imaging (MRI, used with intravenous gadolinium contrast) has the highest sensitivity for lobular carcinoma detection. Invasive lobular carcinomas may appear on MRI as mass-like lesions, often with spiculated margins, frequently with multiple lesions. They also can present as enhancing areas (termed "non-mass enhancement") and with the enhancement further described as focal, regional, ductal, clumped, segmental, or heterogeneous (Figs. 4.1a–g and 4.2a–g) [5, 6].
- The sensitivity of MRI for lobular carcinoma detection has been cited as 93–95% [6].
- Breast-specific gamma imaging (BSGI) utilizes a radiotracer, often ^{99m}Tc sestamibi, to highlight metabolically active sites and has shown high sensitivity in small studies [5], yet it is not universally accepted.
- Invasive lobular carcinoma is frequently undersized by each of these imaging modalities, as compared to final surgical pathology extent [8]. MRI tends to demonstrate the largest tumor extent yet may still underestimate in some cases.
- Breast imagers and pathologists should be wary of multiple lesions in the setting of lobular differentiation. In some institutions, patients diagnosed with lobular carcinoma undergo additional imaging, especially MRI, before surgical planning. Gross tissue sampling in pathology may also need to be more extensive to determine the extent of a lobular carcinoma.
- Additional breast imaging of lobular carcinoma is illustrated with Cases 1, 4, and 5 at the end of this chapter.

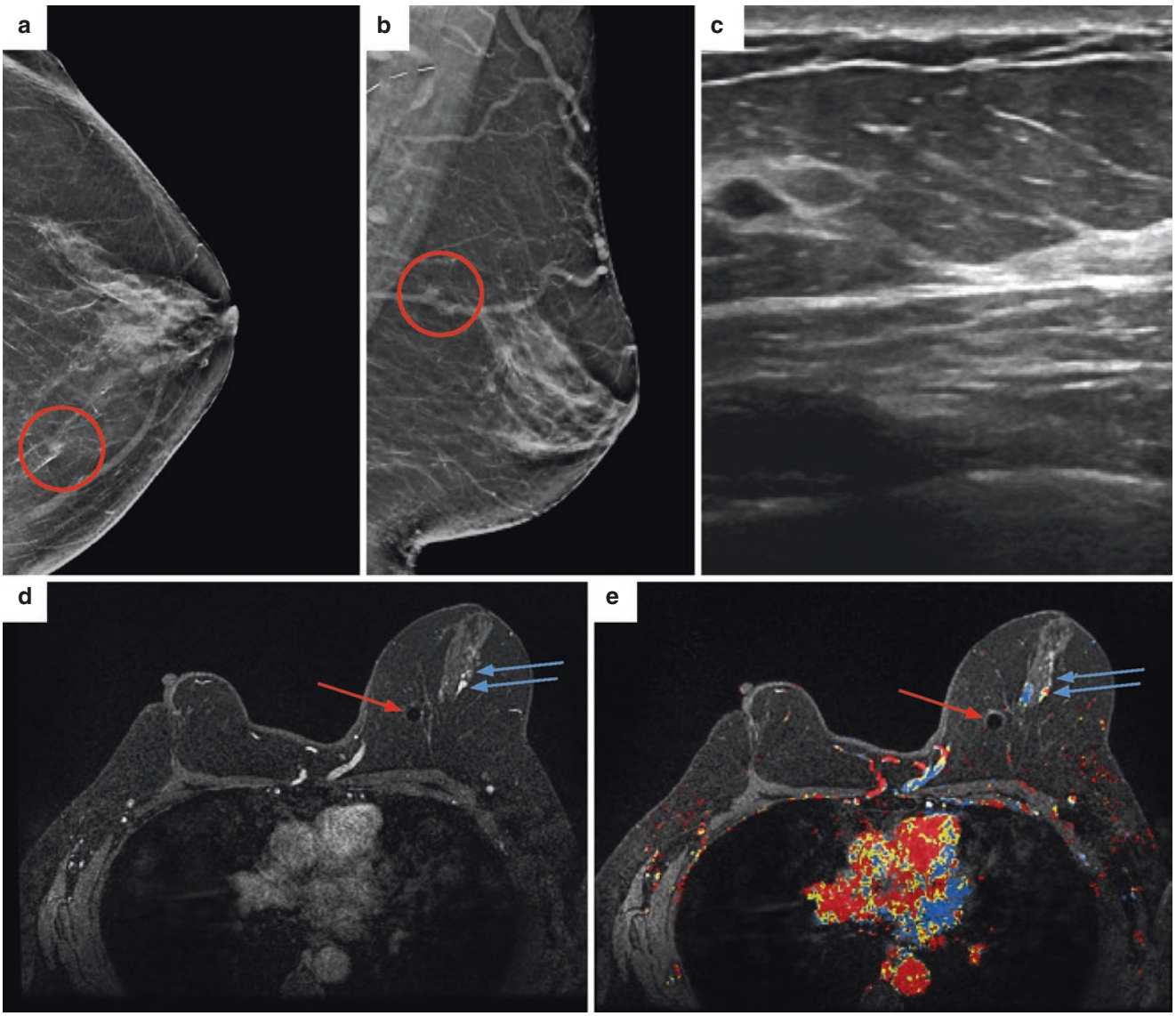


Fig. 4.2 Multifocal invasive lobular carcinoma, breast imaging. Woman with history of contralateral right breast invasive lobular carcinoma (ILC), stage IIB (T2, N1) diagnosed 13 years earlier, treated with lumpectomy and radiation therapy, now with new imaging finding. Craniocaudal (**a**) and mediolateral oblique (**b**) digital synthetic mammograms show a 5 mm ill-defined mass in the upper inner left breast, posterior depth (circles). In (B) the mass appears just superior to a prominent vessel. (**c**) Real-time breast ultrasound shows an irregular hypoechoic mass with an echogenic halo corresponding to the mammographic mass. US-guided core biopsy with marker placement was performed, followed by breast MRI. (**d**) Axial contrast-enhanced breast MRI shows postlumpectomy change in the right breast; in the medial left breast there is a signal void (single red arrow) from the metallic marker showing the location of the biopsied ILC. There also is linear non-mass enhancement (NME) in the retroareolar region (double blue arrows), occult on mammography. (**e**) Computer-aided detection (CAD) MRI image shows fast initial and late washout kinetics (red color), in the NME (double blue arrow), suspicious for cancer. (**f**) Core biopsy of the mammographically detected mass demonstrates a densely cellular tumor composed of sheets of dyscohesive cells (40× magnification). (**g**) Core biopsy of the MRI-detected NME shows a somewhat less cellular infiltrative carcinoma (40× magnification). Both tumors were E-cadherin negative (not shown) with abundant pink-pale cytoplasm and rather pleomorphic nuclei. Predictive markers from this case can be seen in Fig. 4.19

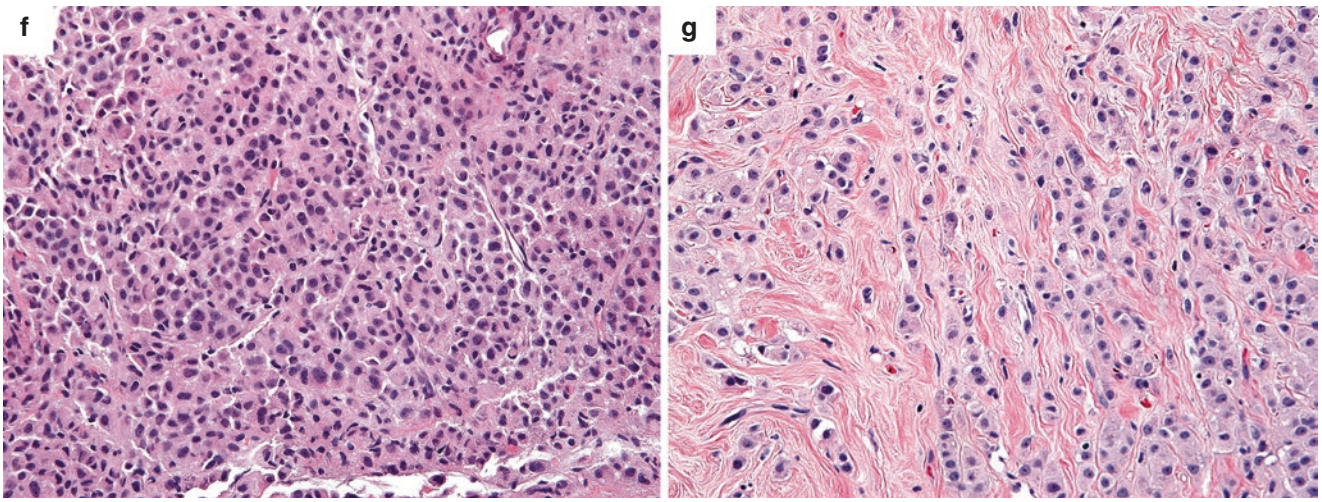


Fig. 4.2 (continued)

5. **What are the typical management steps after a diagnosis of invasive lobular carcinoma on core biopsy?**

The initial treatment of invasive lobular carcinoma shares many similarities with that of invasive breast carcinoma of other types.

- Based on clinical and radiologic features, imaging evaluation of axillary lymph nodes may be performed. If suspicious nodes are identified, they are usually sampled by fine needle aspiration (FNA) or core biopsy.
- Patients diagnosed with lobular carcinoma may undergo additional imaging, especially MRI, before surgical planning.
- Surgical resection is often the first step in definitive treatment (lumpectomy or mastectomy with axillary node sampling).
- A subset of patients may receive adjuvant therapy before surgery (neoadjuvant) in an effort to shrink the cancer and facilitate less aggressive surgery. For lobular carcinoma, this may entail endocrine therapy (neoendocrine) for a prolonged period, or a standard course of cytotoxic chemotherapy. Estrogen receptor-positive invasive carcinomas with low proliferation fraction, including most lobular carcinomas, generally do not show robust response to cytotoxic chemotherapy [7, 9, 10]. Rates of pathologic complete response (pCR) are quite low for classic invasive lobular carcinoma (6%) [7, 10].

6. **What are the gross pathologic features of invasive lobular carcinoma?**

- Invasive lobular carcinoma may form a hard, gritty spiculated mass on cut section.
- Many lobular carcinomas are difficult to identify grossly. Synthesis of visual inspection, palpation of the incised or serially sectioned specimen, specimen X-ray, and clinical imaging findings may be needed to identify the carcinoma, especially in a mastectomy specimen.
- Identification of the prior biopsy site or deployed tissue marker ("clip") can be very helpful in locating the region of the tumor. Prior biopsy site may appear as a small (or rarely large) area of hemorrhage or fat necrosis.

7. **How should a resected invasive lobular carcinoma be sampled for histologic review?**

Invasive cancers need to be sampled such that all data pertinent to diagnosis, treatment, and staging are apparent on resulting histologic slides. As lobular cancers may often be larger than grossly or mammographically apparent, generous but judicious sampling is recommended (Figs. 4.3 and 4.4).

- Correlate with imaging findings before grossing the specimen. Check tumor location, presumed size, focality, and lymph node status. If a specimen radiograph of a lumpectomy was obtained, there may be comment on areas of margin concern.

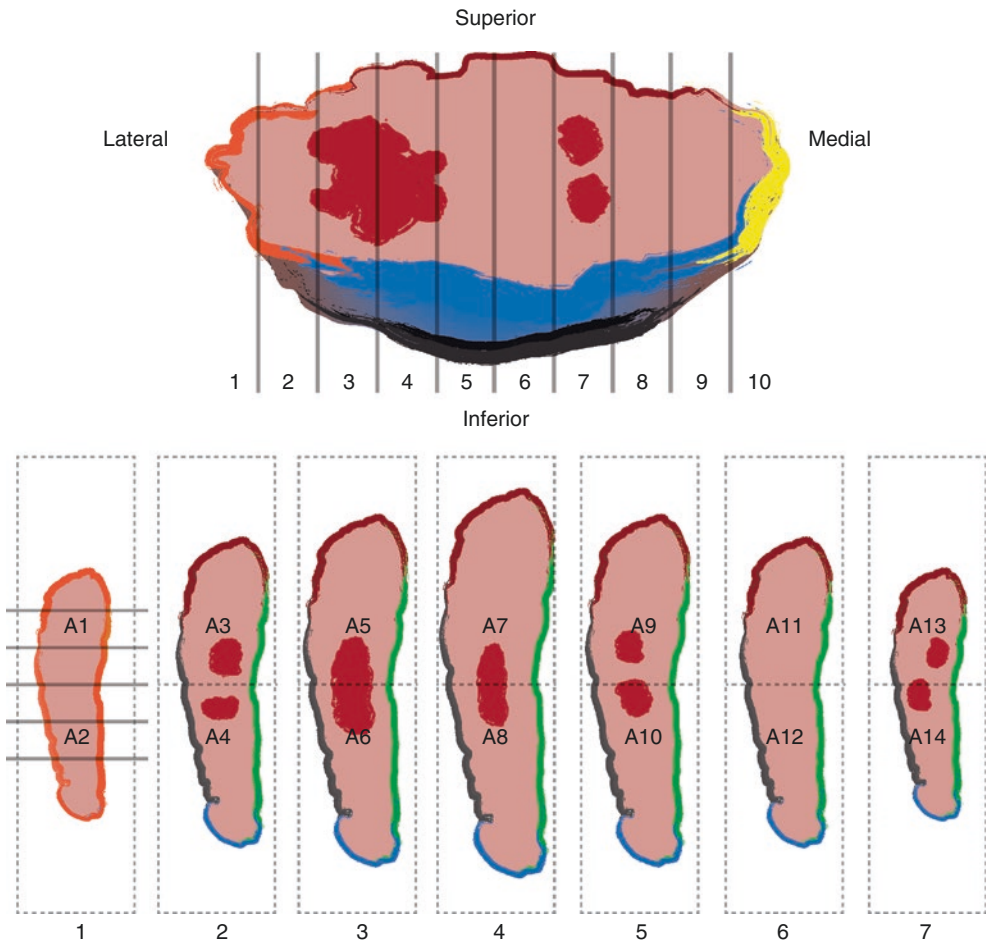


Fig. 4.3 Grossing with cassette mapping. Top diagrams slicing of a breast specimen, after inking, perpendicular to the longest axis. Bottom: the slices are laid out in order, with orientation preserved, and the specimen is X-rayed or photographed. This is used to create a map of tissue sampling, with cassette numbers superimposed. The first and last sections should be sectioned perpendicularly (macroslices 8–10 not shown). The tumor size for staging would be derived from composite of A5 + A6, or the span across macroslices 2–5 (4 slices multiplied by average slice thickness), and should be correlated with size by radiology and gross assessment. Multifocal carcinomas are sampled in macrosection7, slides A13 + A14

- Mapping location of tissue cassettes directly onto an X-ray or photograph of the sliced specimen is most helpful in documenting the tumor sizes of complex specimens (Figs. 4.3 and 4.4) [13].
- The largest contiguous histologic span of tumor will be reported and used for staging purposes. An annotated map should ensure that size can be accurately calculated based on the histologic findings in relation to the gross sampling.
- It is recommended to sample from sections beyond the grossly obvious extent of tumor to ensure adequate size estimation.
- If the cancer only involves a single slide, the size can be measured on the single slide. However, most often it involves multiple sections, and either a composite section or span across multiple sections will need to be used in size calculations.
- For a composite section of a single slice, the tumor can be outlined with a dotting pen on each glass slide, and the adjacent slides juxtaposed to measure extent across the composite.
- Most often, the tumor dimension across multiple tissue slices (e.g., medial-lateral) is the largest dimension.
- The size of a cancer involving multiple slices can then be calculated by multiplying the number of slices involved by the slice thickness (0.4–0.5 cm as recommended above) [13].
- The average thickness of each slice can also be estimated by dividing the overall specimen dimension by the total number of slices [13].
- However, small errors in slice thickness estimates can result in significant over- or underestimations if many slices are involved, so calculated size should be reconciled with imaging findings and gross exam.
- If there is more than one tumor mass, sample in-between them to determine whether they are connected by occult invasive tumor.
- Sample other areas of gross abnormality and/or imaging density.
- Carefully dissect axillary tissue (separate specimens or attached to mastectomy) for lymph nodes; submit all candidate nodes.

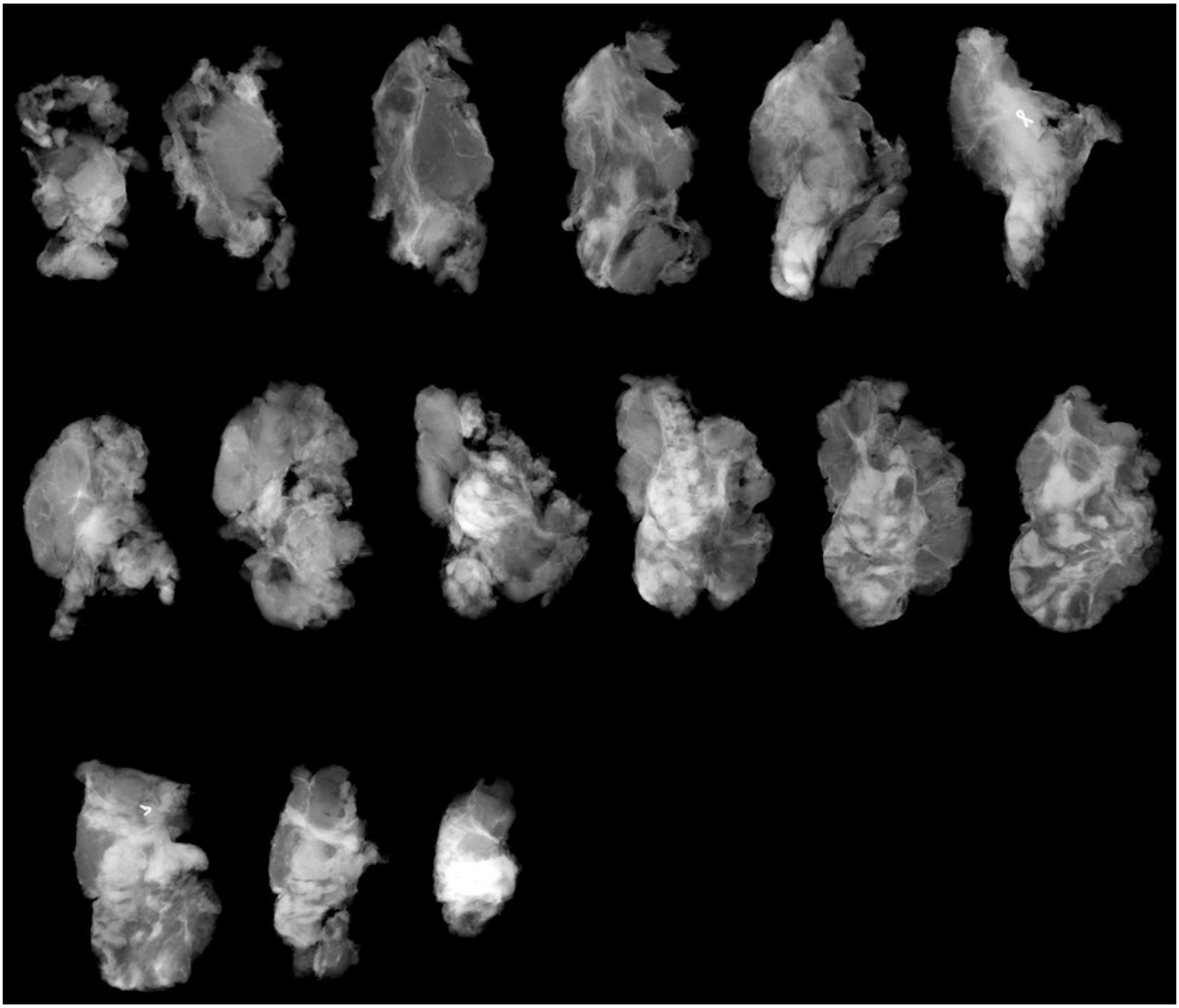


Fig. 4.4 Radiograph of sectioned surgical specimen. Breast imaging studies demonstrated two masses, both biopsy-proven invasive lobular carcinoma: 1.3 × 1.1 cm at the ribbon-shaped marker (upper right) and 1.1 × 0.8 cm at the wing-shaped marker (bottom left). In the surgical specimen. Masses were ill-defined by gross examination and specimen radiography. Histologic sections demonstrated lobular carcinoma continuously involving the entire span between markers (4 cm), along with a microinvasive satellite lesion in a separately submitted margin specimen (not shown). The cassette map can be superimposed on a specimen radiograph or photograph, as diagramed in Fig. 4.3

8. **Does lobular carcinoma originate from breast lobules?**

- A putative precursor lesion, LCIS, was first identified in and named by its involvement of lobules. However, this is a misnomer, and the assumption that lobular carcinoma arises in lobules is thought to be incorrect. (See question 55.)
- Lobular and low-grade ductal carcinomas are both thought to originate from the terminal duct lobular unit [14].
- Invasive lobular carcinoma shares molecular similarities with the ductal carcinomas in the low-grade "luminal" (or ER-positive) neoplasia pathway [15]. (See question 35.)

9. **What are the characteristic cytologic features of classic invasive lobular carcinoma** [1, 2, 9, 16, 17]**?**

- Bland dyscohesive, small cells (Figs. 4.5a–f and 4.6a–i)
- Central or eccentric nuclei, often round, sometimes compressed or squared off by adjacent cells
- Inconspicuous nucleoli
- Pale cytoplasm, usually scant (even plasmacytoid)

- Occasionally intracytoplasmic mucin vacuoles
- Must be distinguished from plasma cells and/or lymphocytes

10. **What are the characteristic architectural features of classic invasive lobular carcinoma** [1, 2, 9, 16, 17]**?**

- "Single file" cells infiltrating tissue (Figs. 4.5a–f and 4.6a–i).
- Linearly arranged cords of cells, 1–2 cells thick.
- Concentric cords around normal ducts, or lobules, imparting a so-called "targetoid" architecture.
- Near absence of duct, gland, or tubule formation (although these can rarely form).
- Relative lack of stromal desmoplasia.
- Density of lobular carcinoma cells varies greatly from case to case.
- Associated ALH/LCIS, which often demonstrates a "pagetoid" growth pattern.

11. **What are type A, type B, and pleomorphic lobular cells?**

- Type A: classic lobular cells with small nuclei as described above (grade 1, Fig. 4.7a–c) [15–19].
- Type B: slightly larger cells with more variation in nuclear and cell size (larger) and shape, often with paler chromatin and/or small nucleoli, more like grade 2 nuclei (twice the size of lymphocyte nuclei, Fig. 4.7a–c) [15–19].
- Pleomorphic: lobular cells with grade 3 nuclei or grade 2 nuclei with increased mitotic rate. Cells and nuclei are markedly larger than those of classic lobular carcinoma, with nuclei that are more hyperchromatic, with prominent nucleoli (Fig. 4.7a–c) [1, 2, 9, 15, 20]. (See question 13.)

12. **What are the morphologic variants of invasive lobular carcinoma?**

The World Health Organization (WHO) classification describes solid, alveolar, pleomorphic, tubulolobular, and mixed variants of lobular carcinoma (Figs. 4.8a–f and 4.9a–d) [1]. Most are architectural variants, whereas some are based on cytologic characteristics. The recognition of these variants can be helpful because they can mimic ductal (or no special type) carcinoma and may benefit from confirmatory studies. (See questions 14, 23.) In addition, the pleomorphic, solid, and alveolar subtypes have been associated with more aggressive features (such as HER2 positivity or higher proliferative rates) and worse clinical outcomes [1, 2, 21]. (See question 13 for pleomorphic subtype.)

Architectural variants (Fig. 4.8a–f) (usually have typical cytologic features as described in question 10):

- Solid: confluent sheetlike growth of invasive lobular cells, or large nests. May have higher mitotic rate or greater nuclear pleomorphism (grade 2 nuclei) [1, 2, 15, 21]
- Alveolar: globular arrangements of at least 20 cells [1, 2, 15, 21]
- Trabecular: cords (trabeculae) 2–3 cells thick [2, 21]
- Tubulolobular: admixed tubular growth pattern (see question 23 for discussion of immunostaining) [1, 2, 15]
- Mixed: admixture of classic and variant pattern

Cytologic variants (Fig. 4.9a–d) (usually have single file architecture or can have any of the above patterns):

- Pleomorphic: Greater degree of cellular atypia and pleomorphism and higher mitotic rate than classic lobular carcinoma [1, 2, 9, 15, 20] (See question 13.):
  - Generally grade 3 nuclei or grade 2 nuclei with abundant mitoses.
  - Cells and nuclei are markedly larger than those classic lobular carcinoma, nuclei more hyperchromatic, with prominent nucleoli.
  - Recognizable lobular features include dyscohesion and central to eccentric nuclei that are somewhat round.
  - Despite the terminology, nuclear pleomorphism (cell to cell variation) is less than that of a high-grade ductal (or no special type) breast carcinoma, and nuclei remain somewhat round in our experience [20]. (See question 11.)
  - May have apocrine features (abundant eosinophilic cytoplasm, GCDFP-15 and androgen receptor positive, molecular apocrine) [2, 16, 22].
- Signet ring: Dyscohesive lobular cells with prominent intracytoplasmic mucin vacuoles [1, 16]
- Histiocytoid: Dyscohesive lobular cells with abundant pale finely granular cytoplasm [2, 9, 23]:
  - Histiocytoid lobular may mimic reactive histiocytes, histiocytic neoplasm, or granular cell tumor and be difficult to recognize as carcinoma on H&E sections [23].
  - Many histiocytoid examples are androgen receptor (AR) positive, strongly GCDFP-15 positive, and thought to have apocrine features [16, 22, 23].
- Signet ring (or histiocytoid) variants more likely to have pleomorphic cytologic features [15, 23]

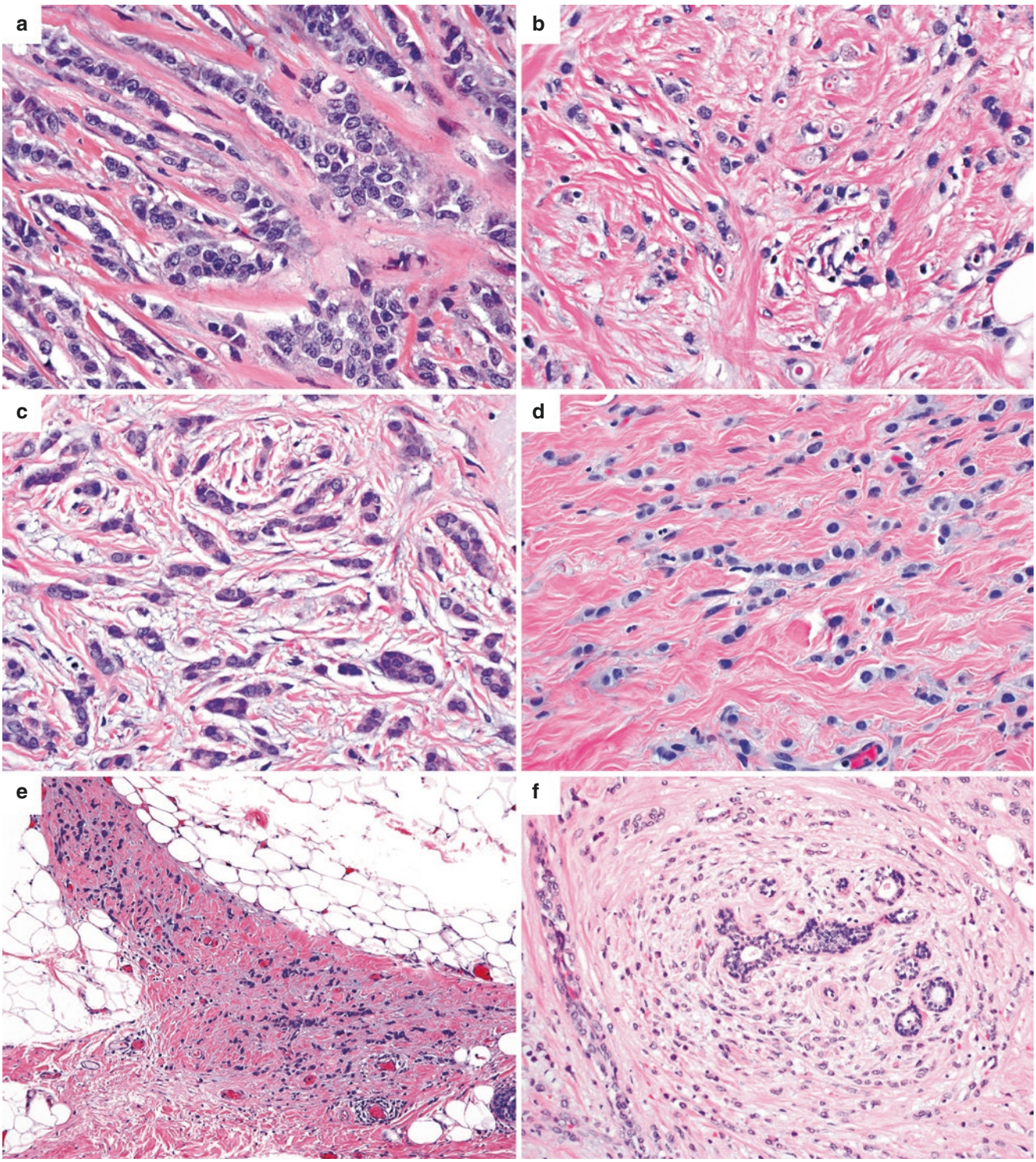


Fig. 4.5 Classic histology of invasive lobular carcinoma. (**a**) Densely cellular example of invasive lobular carcinoma with scant cytoplasm, slightly irregular nuclei and cord-trabecular architecture. (**b**) Dyscohesive invasive lobular carcinoma with cytoplasmic mucin vacuoles. Mucin droplets appears as a pink dot in intracytoplasmic lumens. (**c**) Invasive lobular carcinoma with single file cells and rudimentary tubules in edematous stroma. (**d**) Less cellular example with pale cytoplasm and dyscohesive single file cells in sclerotic stroma (**a**–**d** 400×). (**e**) Lobular carcinoma infiltrates as scattered cells within a fibrous septal area. Such foci are relatively occult radiologically (100× magnification). (**f**) Lobular carcinoma infiltrates around normal structures in a "targetoid" pattern (200× magnification)

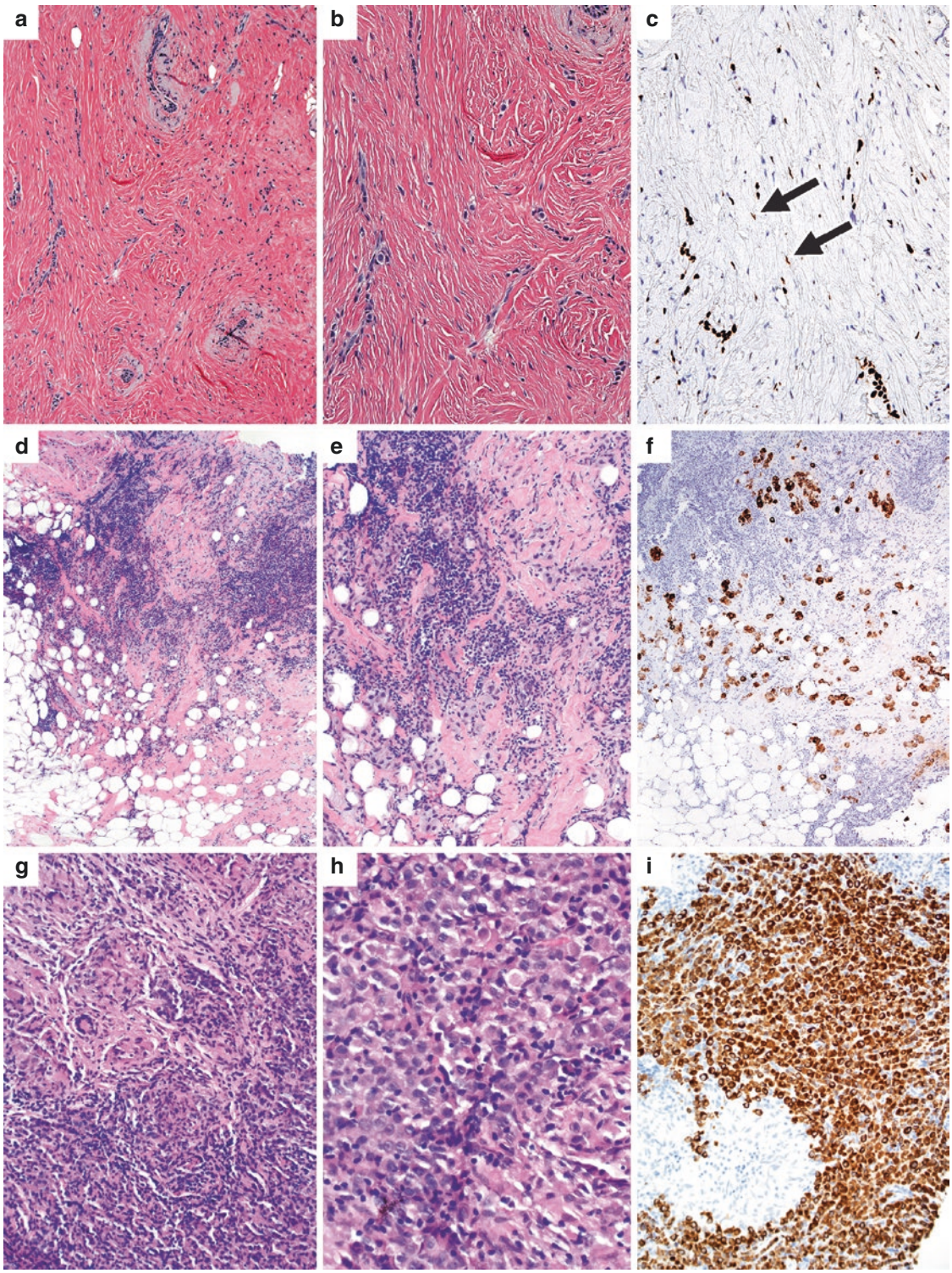


Fig. 4.6 Lobular carcinoma can be difficult to identify when paucicellular, admixed with inflammation or granulomas. (**a**) Densely hyalinized breast stroma with atrophic duct (top), prominent vessels and a few linear collections of cells (100×). (**b**) Higher power demonstrates chains of dyscohesive epithelioid cells suspicious for lobular carcinoma (left, 200×). (**c**) Estrogen receptor (ER) staining is positive in epithelioid cell clusters in a nearby but unmatched field (200×). While this result is compatible with lobular carcinoma, stromal cells may also be ER positive (arrows); keratin staining is more specific and definitive. (**d**) Breast core biopsy with inflammation (100×). (**e**) Higher-power view reveals population of pale epithelioid cells in upper right and lower center (200×). (**f**) Keratin stain confirms invasive carcinoma with dispersed cells (100×); E-cadherin was negative (not shown). (**g**) This core biopsy demonstrated granulomatous inflammation (top), apparently with associated epithelioid histiocytes (bottom, 100×). (**h**) High-power view shows that the pink mononuclear cells have uniform round nuclei, suspicious for carcinoma rather than epithelioid histiocytes. A vague granuloma is at lower right (400×). (**i**) Keratin staining reveals nearly confluent dyscohesive invasive lobular carcinoma cells between granulomas. E-cadherin was negative (not shown) (100×)

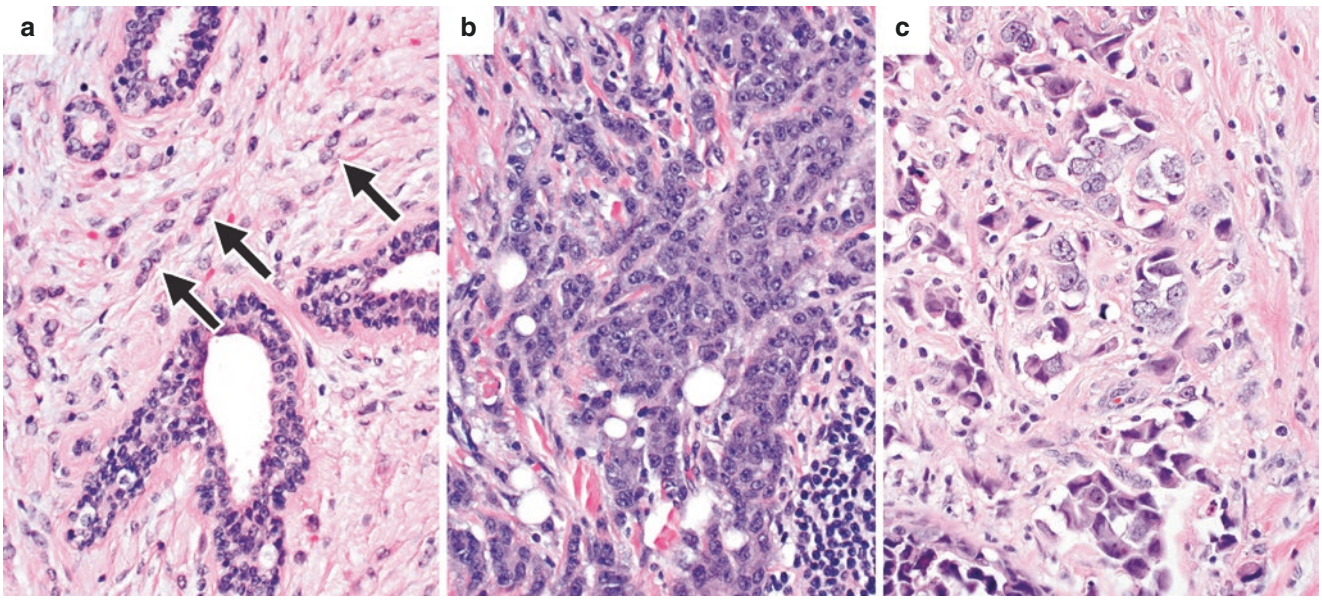


Fig. 4.7 Cytologic heterogeneity of invasive lobular carcinoma. (**a**) Infiltrating cells with small nuclei, no nuclei and scant cytoplasm (arrows, examples). Compare tumor cell size to normal breast glands ("Type A," 400×). (**b**) Invasive lobular carcinoma with larger slightly vesicular nuclei, and nuclei still with scant cytoplasm. Compare nuclear size to lymphocytes at bottom right ("Type B," 400×). (**c**) Pleomorphic lobular carcinoma with grade 3 vesicular nuclei. Nucleoli are apparent but not especially large in this example. A few lymphocytes and stromal cells are at far left (400×)

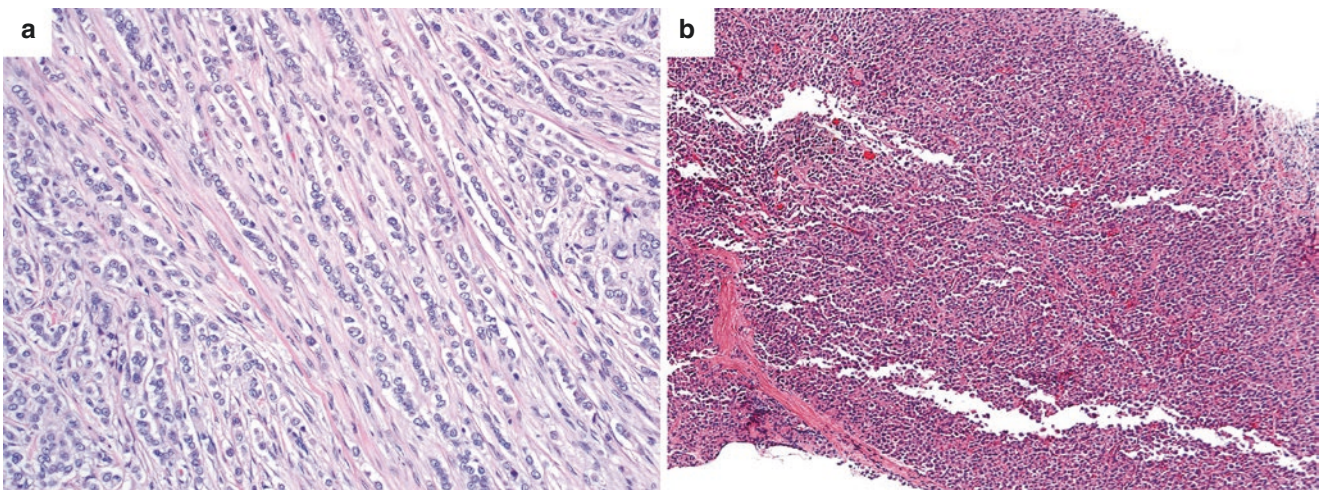


Fig. 4.8 Architectural variants of invasive lobular carcinoma. (**a**) Densely cellular carcinoma demonstrating the classic single file architecture (200×). (**b**) Solid variant characterized by sheets of tumor cells (40×). (**c**) Higher power of the solid invasive lobular carcinoma shown in b; these tumor cells are also rather pleomorphic (200×). (**d**) Invasive lobular carcinoma with alveolar architecture upper left and trabecular architecture lower right (200×). (**e**) Another example with alveolar architecture (200×). (**f**) Rudimentary tubule formation in a lobular carcinoma (200×)

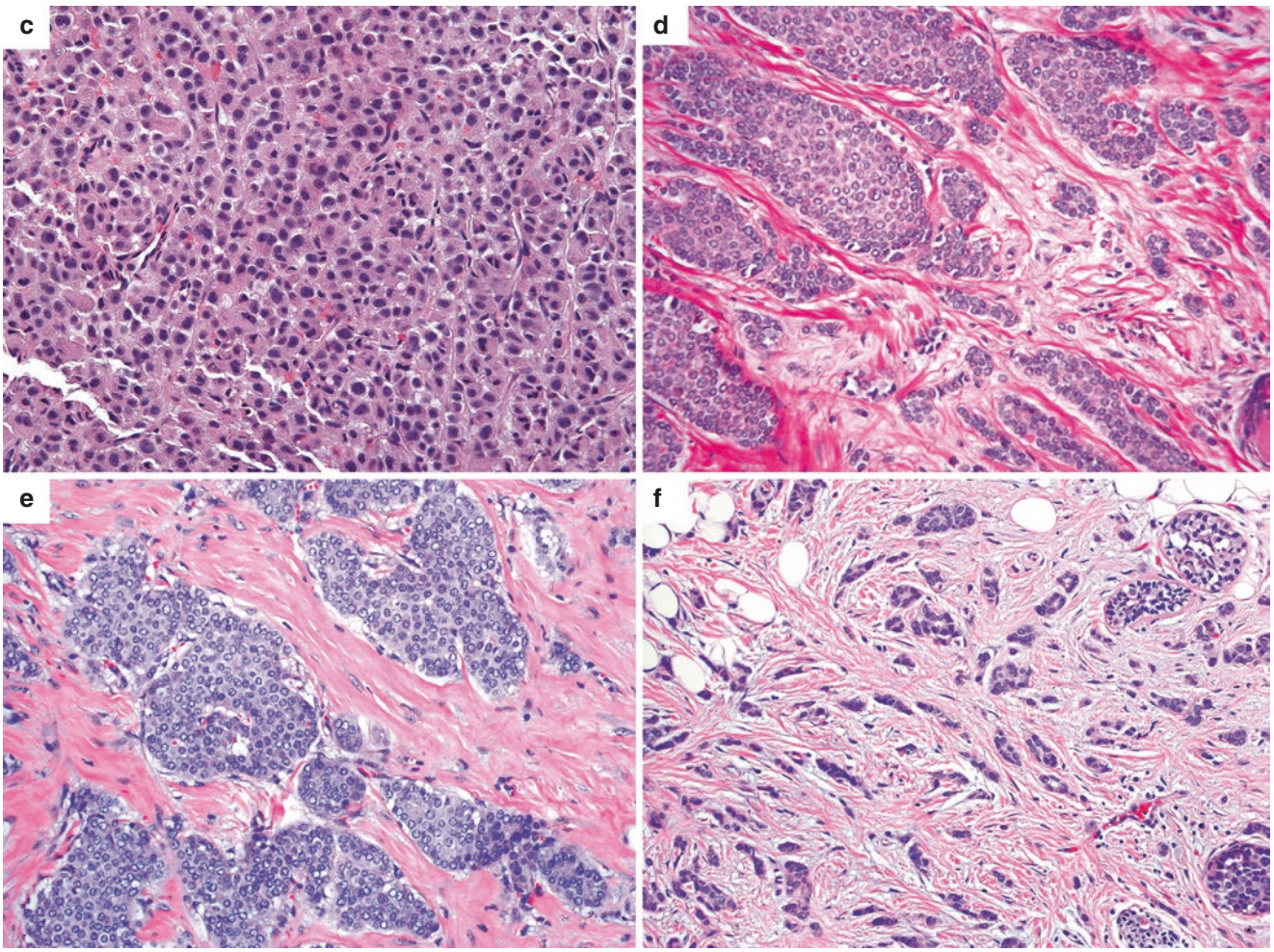


Fig. 4.8 (continued)

13. **Why is it important to recognize pleomorphic invasive lobular carcinoma?**

- Pleomorphic invasive lobular carcinoma has higher nuclear grade, higher mitotic rate, and thus higher overall grade than classic lobular carcinoma. Because of its high grade, it can mimic a ductal carcinoma [1, 2, 9, 16].
- Higher-grade, more proliferative cancers may receive benefit from chemotherapy.
- Pleomorphic lobular carcinomas can have lower levels of hormone receptor expression than classic invasive lobular carcinoma (and sometimes are ER and PR negative) [9, 22, 24–26].
- Pleomorphic lobular carcinomas have a HER2 positivity rate more similar to ductal carcinomas (between 10% and 20%). When HER2 positive, patients should be offered HER2-targeted therapies (in addition to chemotherapy) [15, 22, 24–26]. *HER2* mutations have also been found in pleomorphic lobular carcinoma [9, 24].
- Pleomorphic lobular carcinoma shares more aggressive molecular features with high-grade ductal (or no special type) breast carcinoma such as gains of chromosome 8q24, 17q12, and 20q13, among others [2, 9, 15, 26–28].
- Pleomorphic lobular carcinoma behaves more aggressively with higher recurrence rate and lower 10-year survival [1, 2, 7, 15, 22].

14. **How is invasive lobular carcinoma distinguished from invasive ductal (or "no special type") carcinoma?**

Invasive lobular carcinoma has characteristic architectural and cytologic features, described in questions 9 and 10. Together, these are often quite distinctive from other types of breast cancer, as listed in Table 4.2 and illustrated in Fig. 4.10a–f. Variants of lobular carcinoma are listed in question 12.

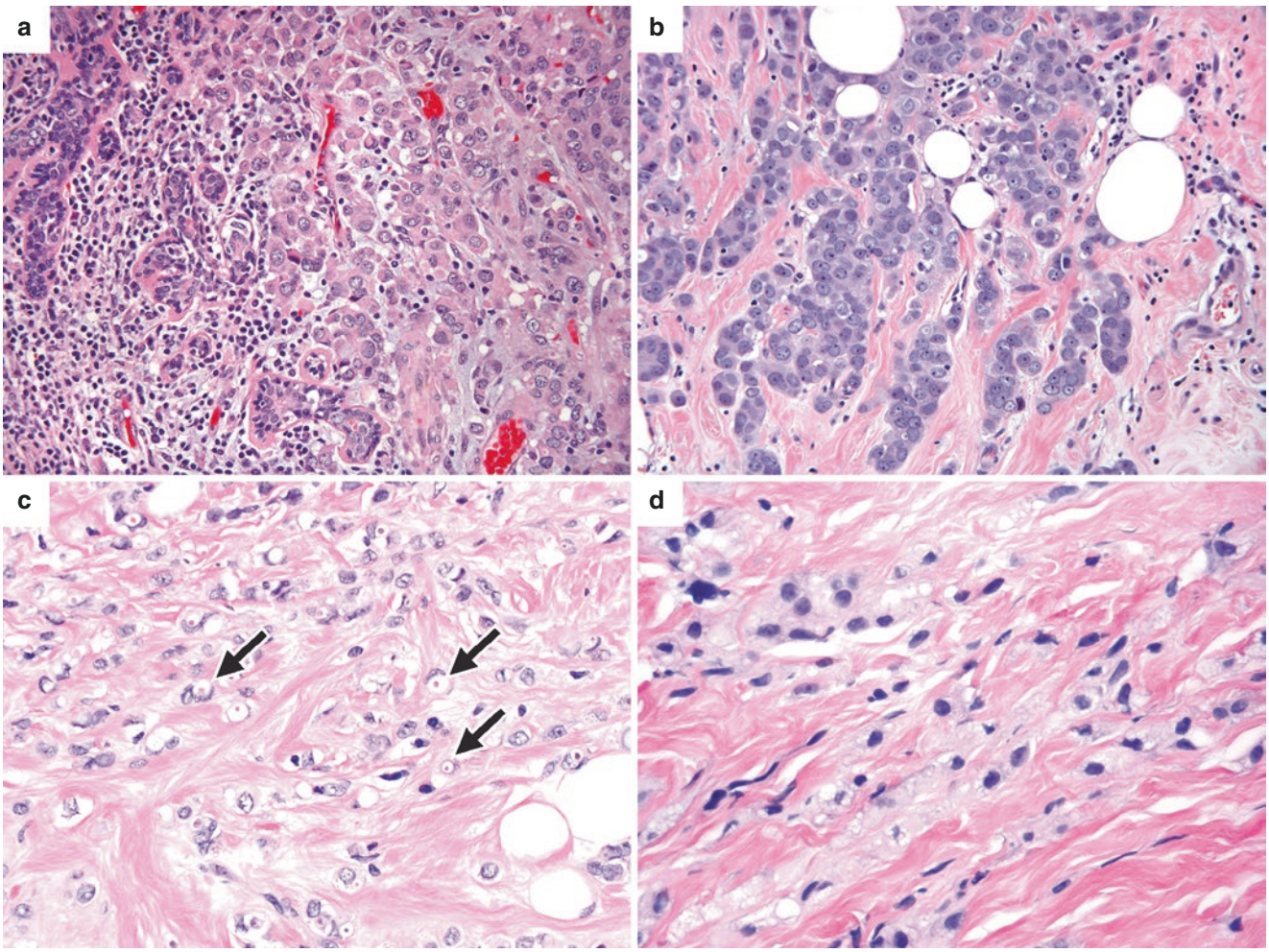


Fig. 4.9 Cytologic variants of invasive lobular carcinoma. (**a**) Pleomorphic lobular carcinoma. Nuclei are very large as compared to normal breast epithelium and lymphocytes at left. Dyscohesive pleomorphic lobular cells have abundant eosinophilic cytoplasm, suggestive of apocrine features (400×). (**b**) Pleomorphic lobular carcinoma with large nuclei and nucleoli (compare to lymphocytes) and scant cytoplasm (400×). (**c**) Lobular carcinoma with signet ring cells; enlarged nuclei are distorted by mucin vacuoles (arrows, 400×). (**d**) Lobular carcinoma with abundant pale foamy "histiocytoid" cytoplasm and at least grade 2 nuclei

Table 4.2 Histopathologic features of invasive lobular as compared to invasive ductal carcinomas

Feature	Classic lobular	Low-grade ductal/NST	High-grade ductal/NST
Architecture	Single file cells, no glands	Well-formed glands (tubule score 1–2)	Usually sheets, large nests
Cell cohesion	Dyscohesive	Cohesive	Usually cohesive
Cell polarization	None, central or eccentric nuclei	Columnar with polarized nuclei	Variable
Cell size	Small cells, scant to moderate cytoplasm	Small to moderate size, often more cytoplasm	Large, variable cytoplasm
Nuclei (see question 18)	Grade 1–2, round regular	Grade 1–2, regular, less round	Grade 2–3, large, hyperchromatic, irregular shape
Nucleoli	Inconspicuous	Inconspicuous	Variable (often large)
E-cadherin (Table 4.3, question 23)	Negative	Positive along basolateral cell-cell membranes	Positive along basolateral cell-cell membranes, lost in small subset
p120 (Table 4.3, question 23)	Cytoplasmic	Positive along basolateral cell-cell membranes	Positive along basolateral cell-cell membranes

NST no special type

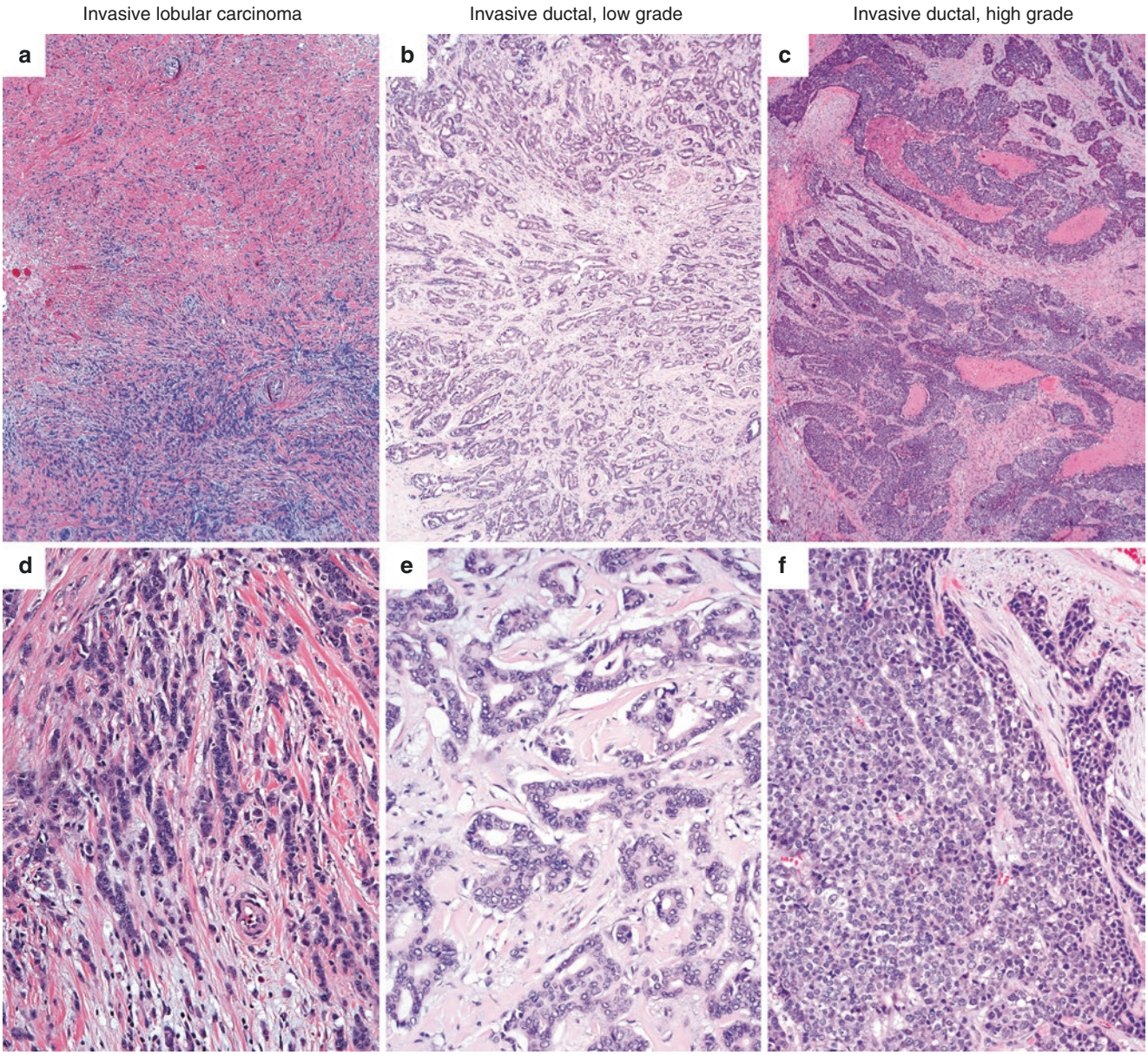


Fig. 4.10 Comparison of classic invasive lobular carcinoma, low-grade invasive ductal (no special type), and high-grade invasive ductal (no special type) carcinoma. (**a**) Low-power view of invasive lobular carcinoma with single and single file cells and areas of variable density (40×). (**b**) Low power of low-grade invasive ductal carcinoma; note prominent tubule formation (40×). (**c**) Low-power view of high-grade ductal carcinoma with large sheets of tumor, some with necrosis; these can have highly variable morphology (40×). (**d**) Invasive lobular carcinoma at higher power. Note single file cells, low-grade cytology, and stromal desmoplasia in this case (200×). (**e**) Low-grade ductal carcinoma at higher power has low nuclear grade and well-formed tubules. (**f**) High-grade ductal carcinoma with high nuclear/cytoplasmic ratio and abundant mitotic figures; high-grade ductal carcinoma can have highly variable morphology

15. Are intracytoplasmic mucin droplets specific for lobular carcinoma?

No, ductal (or no special type) breast carcinomas may also have intracytoplasmic mucin, although this feature is more common in lobular carcinoma (Figs. 4.5a–f and 4.9a–d).

16. Does invasive lobular carcinoma always have "single file" architecture?

No, variant invasive lobular carcinoma includes solid, trabecular, and alveolar architecture. (See question 12, Fig. 4.8a–f.)

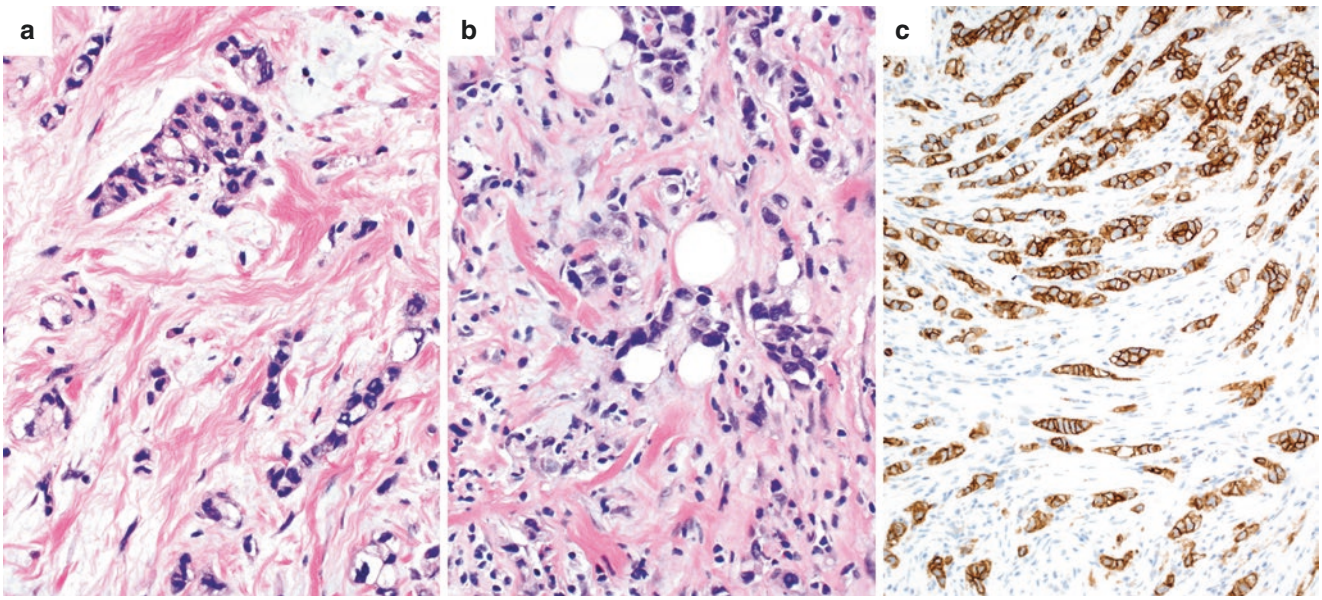


Fig. 4.11 Grade 3 invasive ductal carcinoma with focal single cell architecture. (**a**) Ductal architecture is seen in upper portion, with single cell and cord-like infiltration at bottom (400×). (**b**) Nuclear contour and hyperchromasia is typical of ductal carcinoma, without cytology of lobular or pleomorphic lobular differentiation. (**c**) E-cadherin is strongly positive (membranous) throughout the tumor, consistent with morphologic impression of invasive ductal carcinoma

17. **Can invasive ductal (or "no special type") carcinoma ever have "single file" architecture?**

Yes, invasive ductal carcinoma may also invade as "single file" cells. Cytologic features with the addition of immunohistochemistry when needed can help make this distinction (Fig. 4.11a–c). (For immunohistochemistry, see question 23.)

18. **How is invasive lobular carcinoma graded?**

Every invasive lobular carcinoma should be graded according to the current WHO criteria, also termed Elston & Ellis, Nottingham, or modified Scarff-Bloom-Richardson grade [29]. Points are assigned for each of the following three features:

- Tubule and gland formation.
  - 1: majority of carcinoma (>75%)
  - 2: moderate (10–75%)
  - 3: little or no (<10%)
- Nuclear pleomorphism.
  - 1: small regular uniform cells
  - 2: moderate increase in size and variability
  - 3: marked variation
- Mitotic rate: see microscope field diameter calibration in WHO or CAP checklists.
  - 1: low
  - 2: moderate
  - 3: high
- Assign overall grade based on sum of points.
  - Grade 1: 3–5 points
  - Grade 2: 6–7 points
  - Grade 3: 8–9 points

Most classic lobular carcinomas receive scores of 5 or 6 in the above schema, on the cusp of grade 1–grade 2 [9, 15]. Dyscohesion is a hallmark of lobular carcinoma, so most are assigned 3 points for tubules; classic lobular carcinomas have a low mitotic rate (1 point). Thus, the nuclear score largely influences the final grading. Pleomorphic lobular carcinomas have higher nuclear grade and higher mitotic rate and should calculate to grade 3.

19. **What are the diagnostic criteria for microinvasive lobular carcinoma?**

Microinvasive carcinoma is defined as invasive carcinoma measuring less than or equal to 1 mm (0.1 cm), regardless of subtype [30–32]. Small invasive lobular carcinomas can be very difficult to recognize histologically. An area appearing to have increased stromal cellularity should be examined at high power to rule out an invasive lobular carcinoma or other subtle findings. Small or low cellularity lobular carcinomas may mimic plasma cells or other inflammatory cells. Keratin stains can be used to highlight infiltrating lobular cells and confirm the diagnosis [9].

20. **What molecular mechanisms are responsible for the characteristic architectural features of lobular carcinoma?**

Loss or dysfunction of the cell-cell adhesion molecule E-cadherin is characteristic of lobular carcinoma [1, 2, 9, 14–16, 27, 32–40].

- E-cadherin (Epithelial-cadherin) is a transmembrane protein that forms calcium-dependent homodimers between cadherin molecules on adjacent cells [14, 32–40].
- E-cadherin is an integral part of adherens junctions and links to the cytoskeleton through catenins [14, 32–40].
  - alpha-catenin
  - beta-catenin
  - gamma-catenin (also called plakoglobin)
  - p120 catenin
- E-cadherin is encoded by the *CDH1* gene.
- E-cadherin and catenins also influence cell polarization and motility [14, 40].

21. **Are there heritable mutations associated with risk of lobular carcinoma?**

Germline mutation in *CDH1* (16q22.1) encoding E-cadherin is associated with hereditary diffuse gastric cancer and lobular carcinoma of the breast [35, 41, 42]. In these families, lobular breast cancer has lesser incidence and mortality than gastric cancer [41]. Recently, hereditary lobular breast cancer without gastric cancer has been described [42].

22. **What are the mechanisms of E-cadherin loss or downregulation in lobular carcinoma?**

There are several mechanisms of E-cadherin downregulation [1, 9, 14, 15, 27, 35, 43–45].

- A frameshift mutation in the *CDH1* gene (50–65% of cases) resulting in E-cadherin protein truncation, often in combination with changes listed below.
- Loss of heterozygosity of the other allele (>90% of cases); 16q is frequently lost in low-grade invasive breast cancers.
- Epigenetic silencing (promoter methylation), transcriptional repression (less common).

23. **How are E-cadherin and catenin immunohistochemistry utilized to discriminate lobular from ductal differentiation?**

Lobular carcinoma is characterized by loss of functional E-cadherin, and E-cadherin immunohistochemistry is a helpful ancillary tool in differentiating invasive ductal and invasive lobular carcinoma (Figs. 4.12 and 4.13a–h, Table 4.3) [1, 2, 14–16, 27, 36, 39, 40].

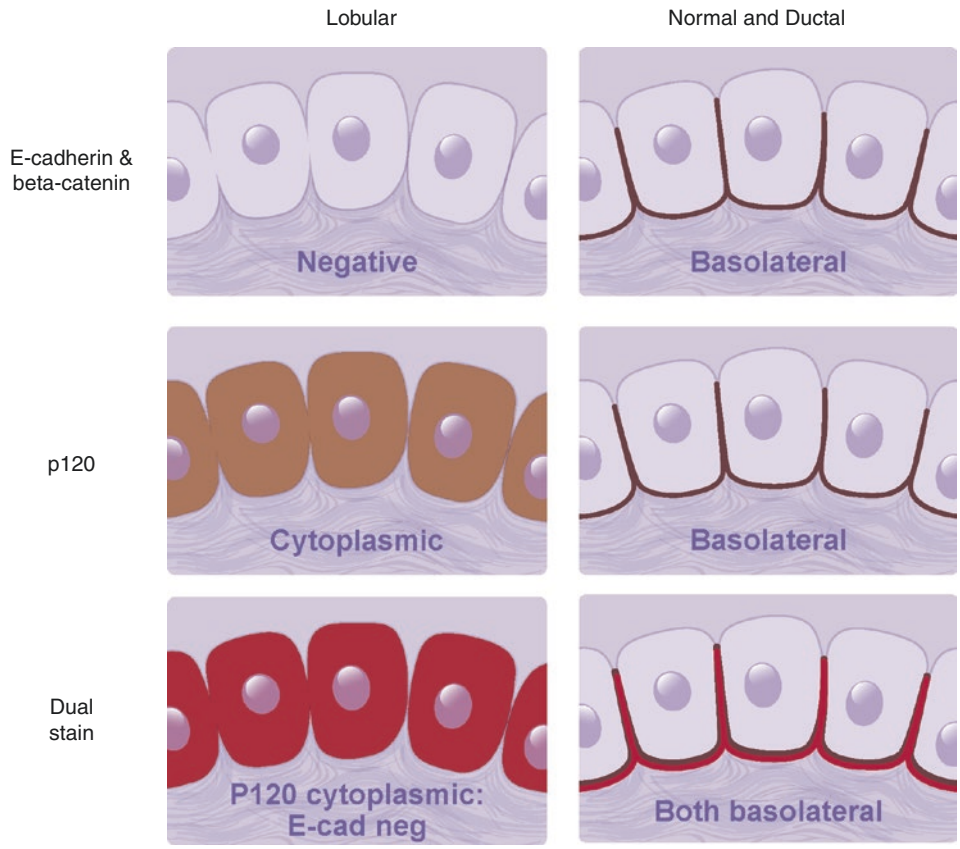


Fig. 4.12 Immunohistochemical staining patterns in ductal and lobular differentiation. Top row: E-cadherin and beta-catenin are localized to basolateral cell membranes in normal and ductal cells, but are negative or weak in invasive lobular carcinoma and ALH/LCIS. Middle row: p120 catenin localizes to basolateral cell membranes in normal breast and ductal cells. In lobular lesions, p120 catenin is relocalized to the cytoplasm (cytoplasmic granular), without membrane staining. Bottom row: expected staining with E-cadherin (brown), p120 (red) dual stain

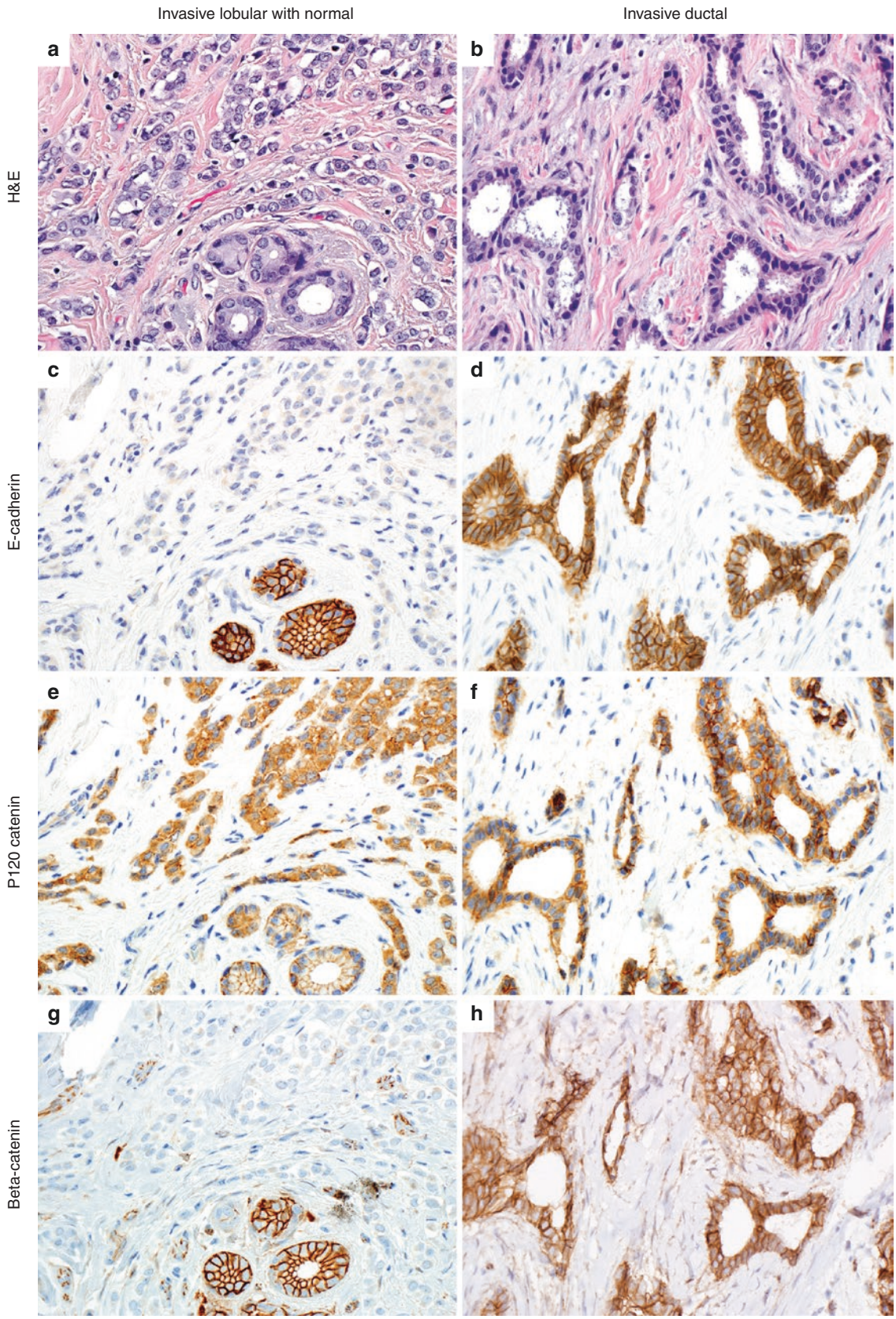


Fig. 4.13 Immunophenotype of classic invasive lobular and invasive ductal carcinoma. Left column: invasive lobular carcinoma with focal normal acini (bottom center of each panel). Serial sections stained with (**a**) H&E, (**c**) E-cadherin, (**e**) p120 catenin, (**g**) and beta-catenin (all 400×). In typical lobular carcinomas, E-cadherin and beta-catenin are negative, while p120 catenin demonstrates granular cytoplasmic staining without membranous accentuation. All three proteins have membranous expression in normal acini. Right column: invasive ductal carcinoma in serial sections including (**b**) H&E, (**d**) and cell membrane localization of E-cadherin, (**f**) p120 catenin, (**h**) and beta-catenin, all membranous (all 400×)

- E-cadherin is normally localized to basolateral cell membranes. Normal ducts or acini serve as a good comparative control.
- Negative, fragmented, or very weak E-cadherin staining is generally seen in lobular carcinoma, with caveats below [1, 2, 14–16, 27, 36, 39, 40].

Table 4.3 E-cadherin and catenin staining of lobular as compared to ductal proliferations (also see Figs. 4.12, 4.13, 4.14, 4.15, 4.16, 4.17, 4.18, 4.34, and 4.36)

Immunostain	Lobular	Ductal
E-cadherin	Absent or weak	Membrane
p120	Cytoplasmic, granular, no membrane staining	Membrane
Beta-catenin	Absent or weak	Membrane

Data from Refs. [1, 2, 14–16, 27, 36, 39, 40]

- Loss or dysfunction of E-cadherin results in loss or redistribution of cytoplasmic catenins (beta-catenin, p120 catenin, respectively) (Fig. 4.12), also amenable to immunohistochemistry, with p120 more widely published than beta-catenin [1, 2, 14–16, 27, 37, 39, 40, 46–52].
- Caveats include:
  - The interpretation E-cadherin staining is not always straightforward. Complete absence is not required for a lobular diagnosis. These patterns are also compatible with lobular differentiation [14, 16, 40, 51–53].
    - Membranous E-cadherin staining that is markedly weaker than normal internal control is aberrant, favoring lobular.
    - Partial or fragmented E-cadherin staining.
    - Cytoplasmic or Golgi pattern E-cadherin staining (Fig. 4.14a–d) [14, 40, 51–53].

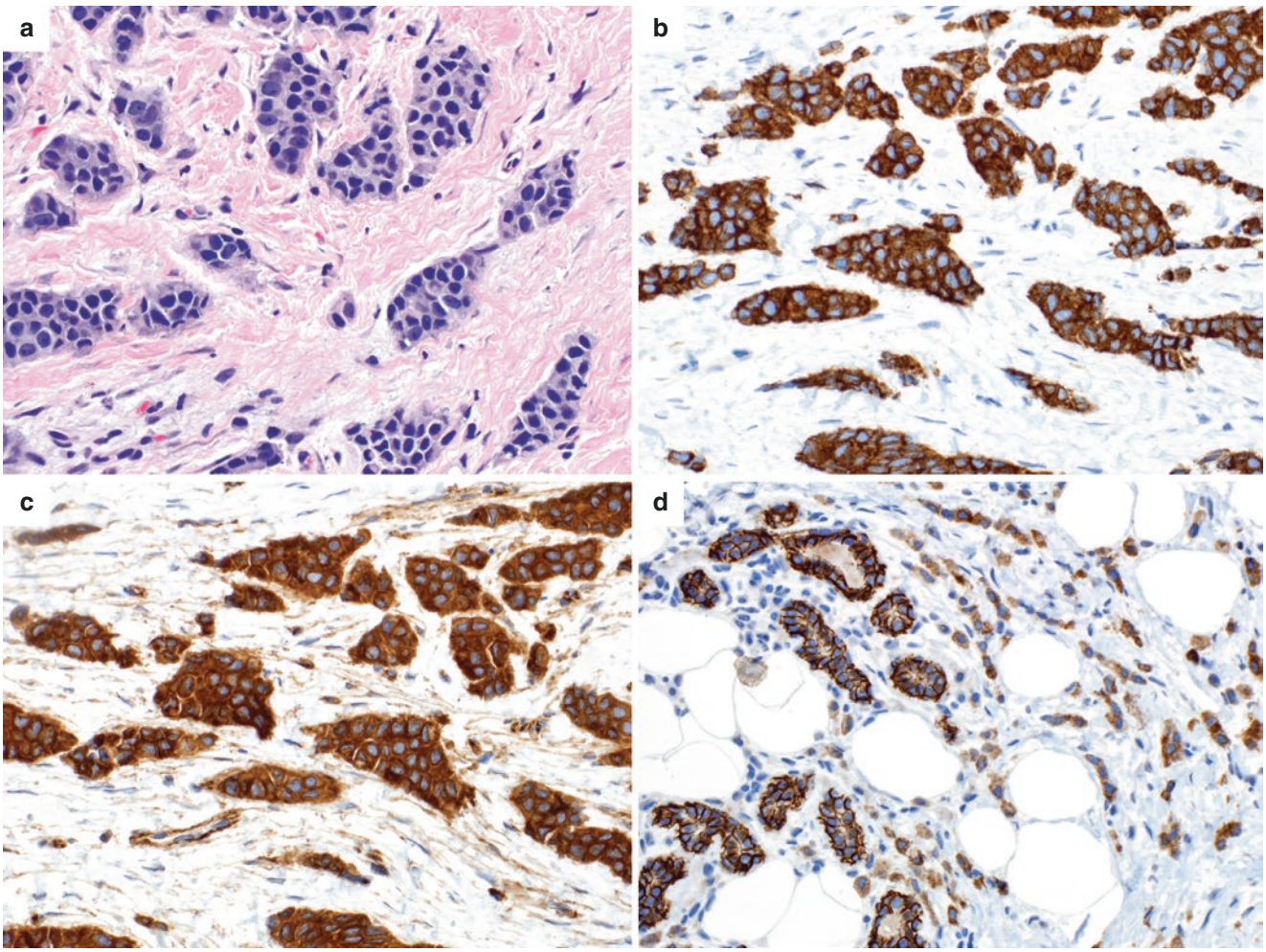


Fig. 4.14 Aberrant E-cadherin with cytoplasmic localization. (**a**) H&E sections demonstrate invasive carcinoma with cords and alveolar architecture. (**b**) E-cadherin is strongly positive in the carcinoma, but is even throughout the cytoplasm, without membrane localization. This can be difficult to recognize in cases with scant cytoplasm. (**c**) Cytoplasmic p120 localization confirms E-cadherin dysfunction and lobular differentiation. (**d**) In another focus of invasive lobular carcinoma from the same case, cytoplasmic E-cadherin staining is weaker in the dyscohesive single file cells (upper right), and the pattern of staining contrasts with that of normal alveoli in the left portion of the field

- A small fraction of lobular carcinomas may show positive E-cadherin membrane expression (Fig. 4.15a–d) [14, 16, 40, 51, 52]. (See question 25.)
  - We generally perform E-cadherin staining first and if results are unexpected add catenin (p120) staining to resolve unusual cases.
  - Aberrant catenin staining (non-membranous) is consistent with lobular carcinoma.
- Rarely, nonfunctional E-cadherin may be cytoplasmic or perinuclear (Golgi). This pattern supports lobular carcinoma, and catenins will be redistributed. This emphasizes the importance of the *pattern* of staining (Fig. 4.14a–d).
- Several studies affirmed that many tubulolobular carcinomas are E-cadherin positive (membranous) and are thus best classified as invasive ductal carcinoma [54–56]. However, we have seen E-cadherin-negative examples of tubulolobular carcinoma and prefer to classify these as lobular (Fig. 4.16a–d).
- The interpretation of cytoplasmic and membranous p120 staining is uncertain and should be considered equivocal.
- Some commercial stain cocktails include E-cadherin and p120 catenin (Fig. 4.12).
  - Staining conditions for multi-stains often represent a compromise and are not ideal for either antibody.
  - Care must be taken with internal controls and interpretation. An overly strong red stain may mask weak brown or vice versa.

24. **Must E-cadherin immunostaining be performed in order to render a diagnosis of invasive lobular carcinoma?**

No, it is not essential to demonstrate loss of E-cadherin in diagnosing lobular carcinoma [57]. The diagnosis can be made based on typical architectural and cytologic features. Immunostaining may be helpful if there is morphologic

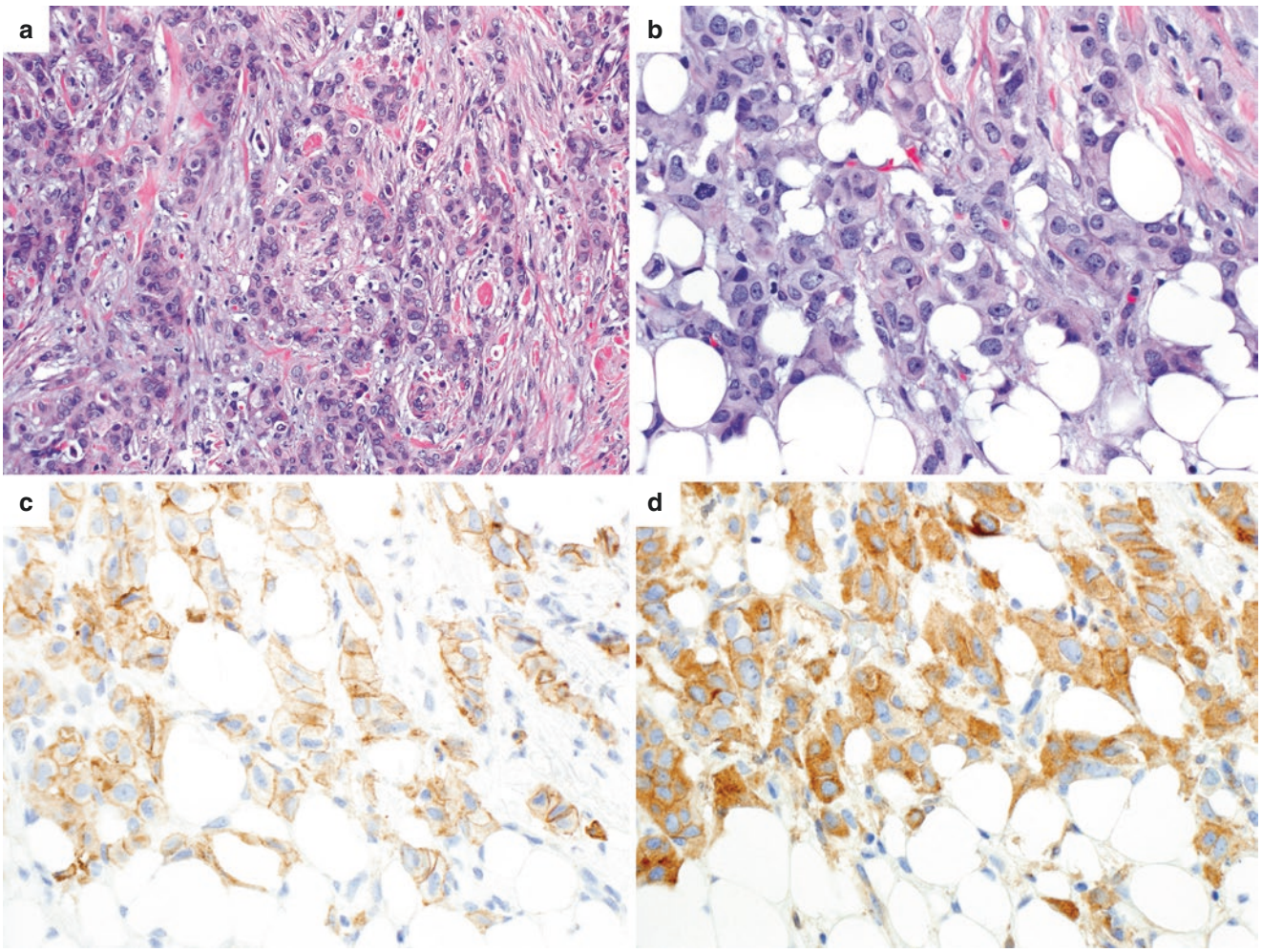


Fig. 4.15 Invasive lobular carcinoma with membranous E-cadherin. (**a**) This carcinoma has architectural and cytologic features of invasive lobular carcinoma (200×). (**b**) Higher power demonstrates at least grade 2 nuclei and the leading edge infiltrating adipose tissue (400×). (**c**) E-cadherin is localized to cell membranes, although weak-moderate in intensity (400×). (**d**) p120 has cytoplasmic localization, indicating dysfunction of E-cadherin complex, supporting lobular differentiation in a serial section (400×). Beta-catenin was also negative (not shown)

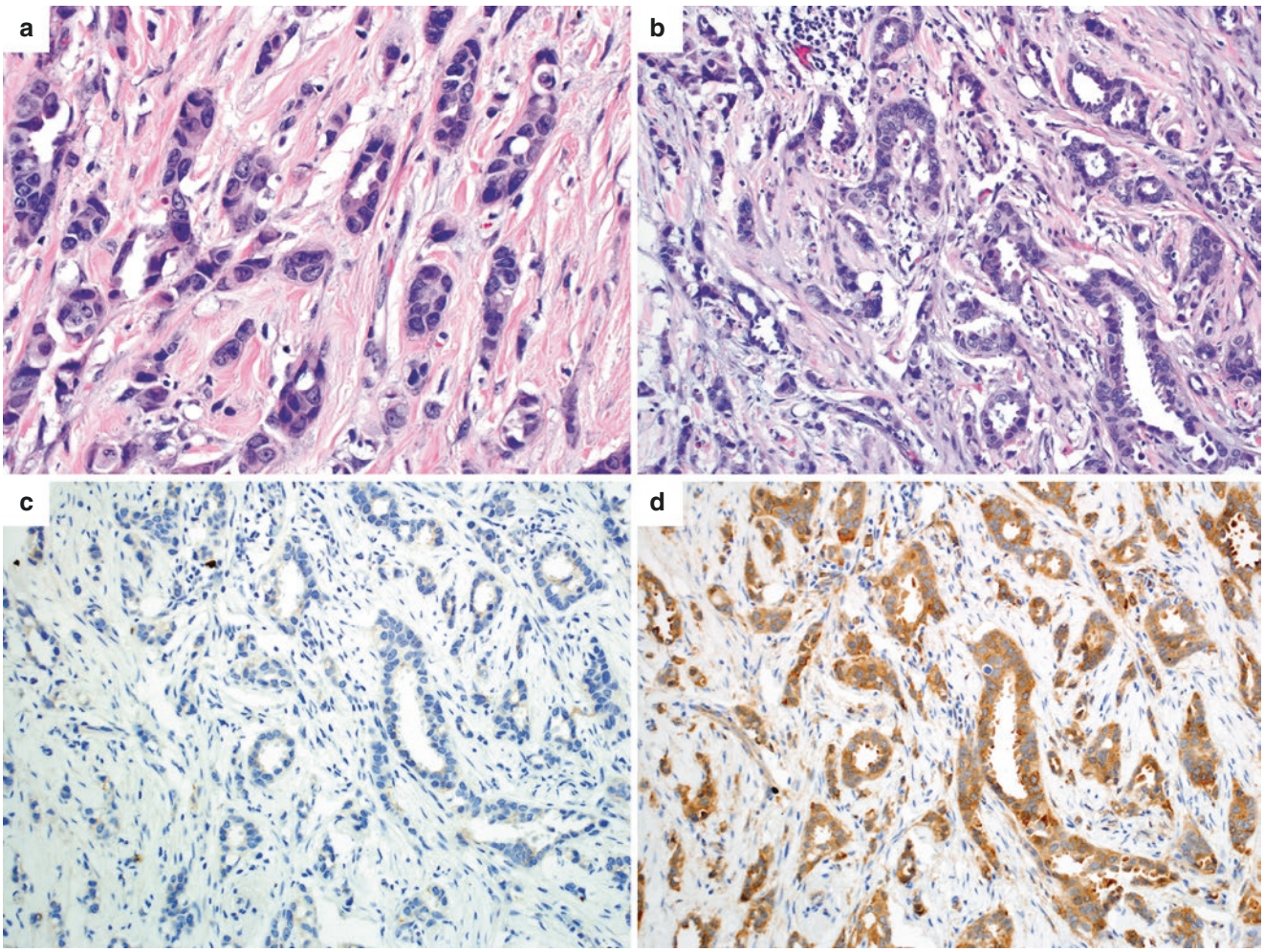


Fig. 4.16 Tubulolobular carcinoma. (**a**) High power demonstrates rudimentary tubules with lumens (400×). (**b**) Another area with more well-formed tubules (200×); serial sections for immunostaining are negative for E-cadherin (**c**) and have cytoplasmic p120 catenin (**d**), a lobular pattern. Classic lobular architecture was seen in some areas of this carcinoma (not shown)

doubt or mixed features (sheet or trabecular architecture, nuclear irregularities, etc.) [14, 40].

25. Can a diagnosis of lobular carcinoma ever be made if E-cadherin is positive?

A small fraction of lobular carcinomas may maintain membranous E-cadherin expression, without proper function (15% in one study) [14, 16, 40, 51, 52]. Catenin staining (especially p120, also beta-catenin) should be helpful in resolving these cases (Fig. 4.15a–d). In our experience, the majority of carcinomas with lobular-like morphology and positive E-cadherin (membrane) also have membranous catenin staining (ductal pattern).

26. Can a diagnosis of ductal carcinoma ever be made if E-cadherin is negative?

A small fraction of high-grade ductal carcinomas may also lose E-cadherin (5–10%) [9, 32, 33, 58]. While a useful immunostain, loss of E-cadherin is neither necessary nor sufficient for diagnosis of lobular carcinoma.

27. How is mixed ductal lobular carcinoma defined?

The designation mixed ductal lobular carcinoma has been used differently in different centers in different eras [1, 9, 16, 57, 59].

- The WHO monograph does not explicitly define this terminology, but discusses that the mixed terminology may be used if the special type (lobular) makes up 10–90% of the cancer recognized special type (such as lobular) in 10–49% of the tumor [1]. The WHO definition differs for the in situ situation. (See question 75.)
- We agree with Naidoo's definition of mixed ductal and lobular carcinoma as "A tumour is regarded as being of mixed type, for example, ILC and ductal/no special type (IDC/NST), if unequivocal separate areas of both

morphological types are present and not if the lesion shows indeterminate features" (Fig. 4.17a–d) [57].
  - In one study using this definition, mixed tumors showed metastatic patterns similar to invasive lobular carcinoma [59].
  - In another study using different definitions, invasive ductal carcinoma with lobular features had clinical and biologic characteristics more similar to invasive lobular carcinoma (ductal, E-cadherin positive, with areas of small cells dispersed, in linear cords, or loose aggregates) [60].
- If the carcinoma shows intermediate or indeterminate features, we use diagnostic terminology and an explanatory comment, such as:
  - Invasive mammary carcinoma
  - Invasive carcinoma with ductal and lobular *features*
  - Invasive ductal carcinoma with lobular *features*
  - Invasive ductal carcinoma with lobular *growth pattern*
- Intermediate or indeterminate features may include [16, 60]:
  - Ductal cytology with single file/cord architecture
  - Lobular cytology and single file/cord architecture with occasional tubule formation
  - Lobular cytology and/or single file/cord architecture, E-cadherin positive (Fig. 4.18a–d)
  - Focal presence or absence of E-cadherin staining, weak or equivocal E-cadherin/catenin staining
  - Uncertain morphology without available E-cadherin stain (especially at core biopsy)
  - For E-cadherin and other ancillary immunostaining (see question 23)
- A recent molecular study categorized "mixed" histology tumors as predominantly lobular or ductal based on mRNA expression profile [43].

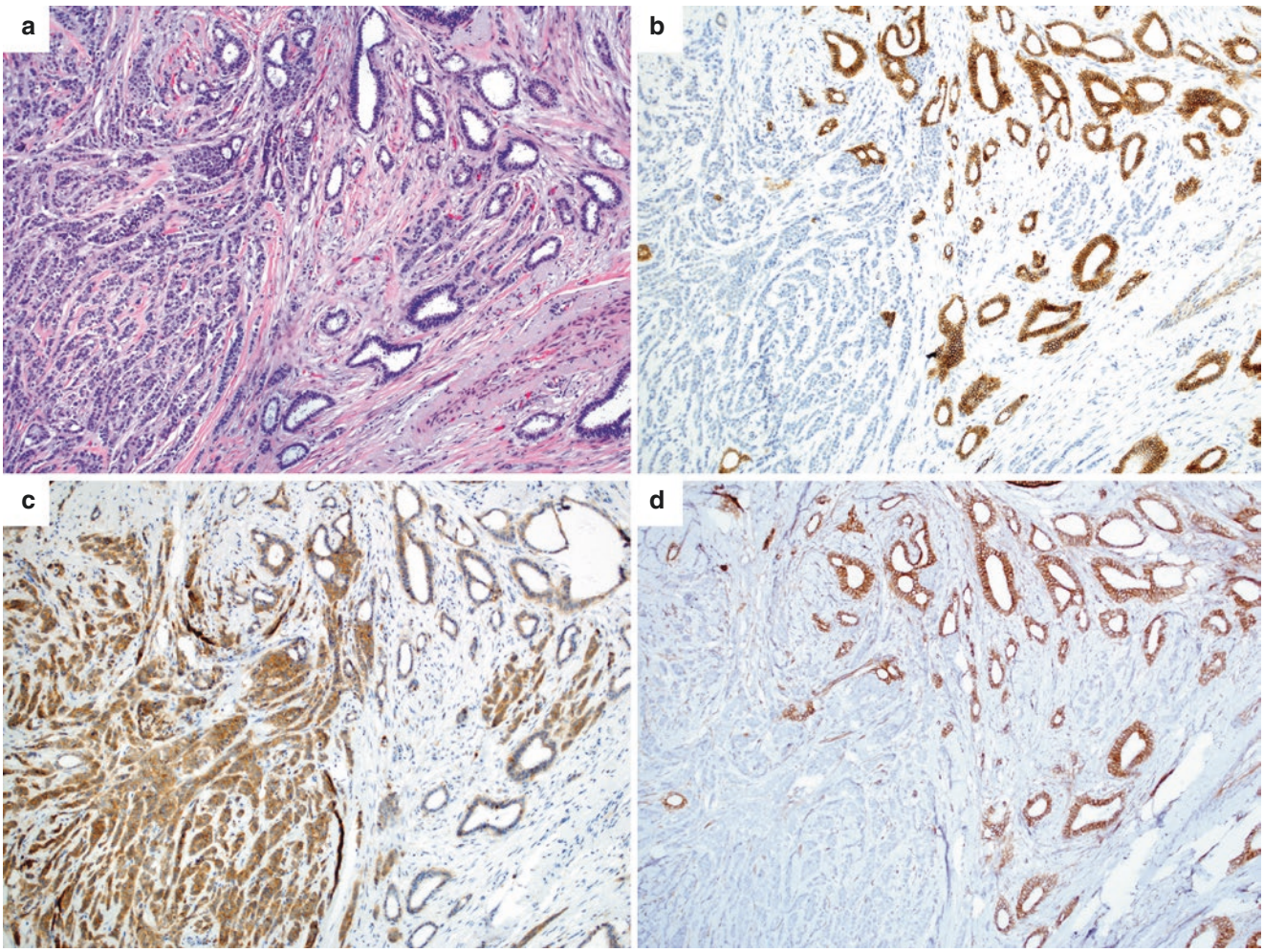


Fig. 4.17 Mixed ductal and lobular carcinoma (WHO definition). (**a**) H&E demonstrating a single tumor with areas of single file and cord-like architecture (lobular, left) and glandular architecture (ductal, right), with concordant immunohistochemistry. (**b**) E-cadherin demonstrates membrane staining in ductal area and is negative in lobular (left). (**c**) p120 catenin is cytoplasmic in lobular carcinoma (left), with weak membrane staining in the ductal component of this microscopic field; membrane staining was stronger in other ductal areas of the tumor. (**d**) Beta-catenin shows a pattern similar to E-cadherin. (all 100×)

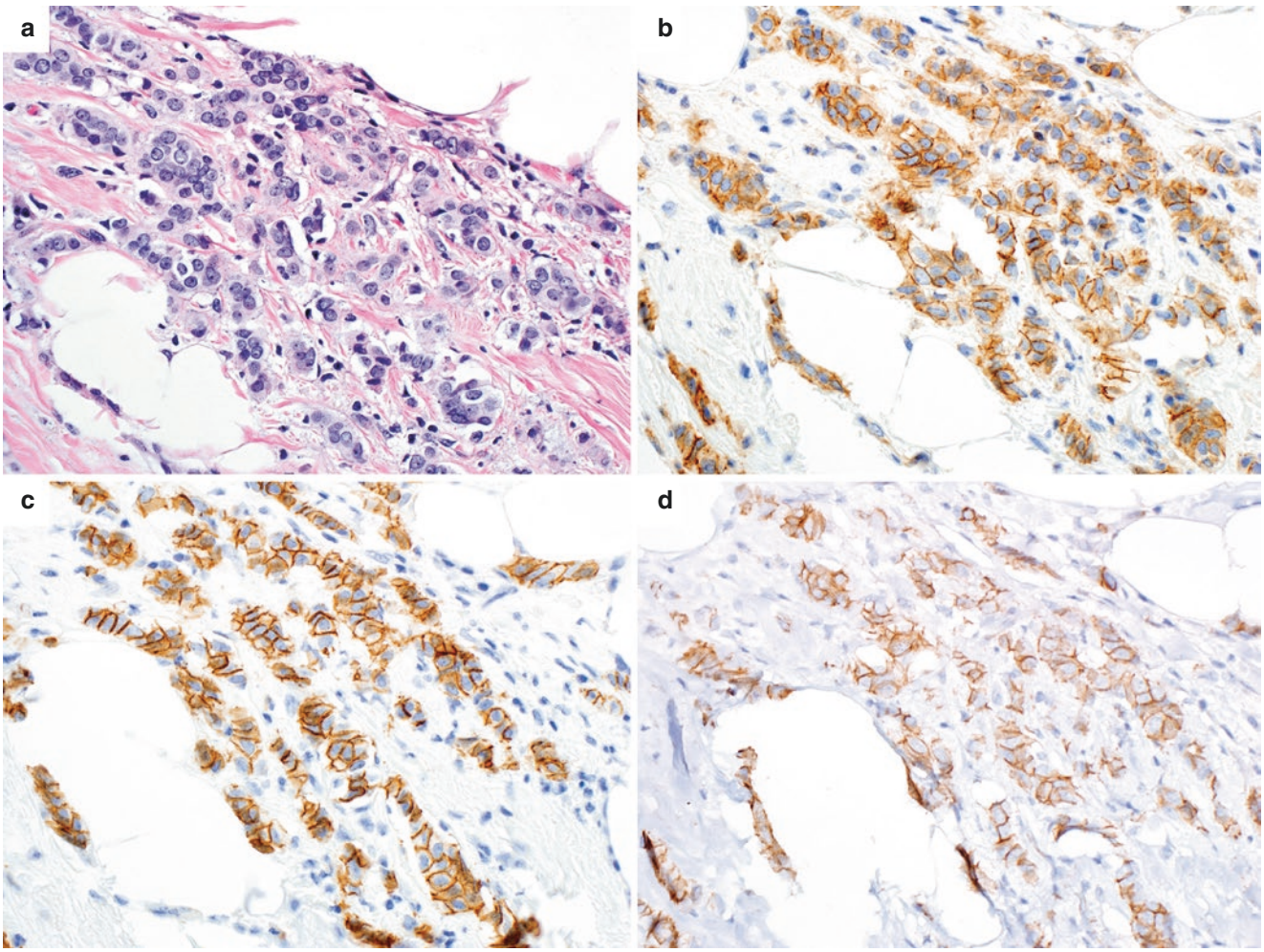


Fig. 4.18 Invasive ductal carcinoma with lobular growth pattern. (**a**) High-power H&E-stained section demonstrating lobular cytology and single file or small clusters of cells without tubule formation. (**b**) E-cadherin demonstrates membrane staining. (**c**) p120 also demonstrates membrane localization, unlike the case shown in Fig. 4.15. (**d**) Beta-catenin is positive (membranous). The immunohistochemistry supports ductal differentiation. This could be described as invasive ductal carcinoma or invasive ductal carcinoma with lobular growth pattern or with lobular features (all 400×)

28. How is invasive lobular carcinoma staged?

Invasive lobular carcinoma is staged similarly to other breast cancers, using the AJCC TNM system [1, 30, 31]. A simplified version of pathologic TNM categories is listed below; please refer to the AJCC manual or CAP staging checklists for full detail [30, 31]. The 8th edition was updated to incorporate data on hormone receptor, Her2, proliferation rate, and Oncotype DX recurrence scores into an overall prognostic stage in extensive tables which can be found in the AJCC manual [30, 31]. However, the anatomic TNM staging also still applies.

- pT – Tumor (primary)
  - TX: Cannot be assessed.
  - T0: No evidence of primary tumor.
  - Tis: Carcinoma in situ.
  - T1: Tumor 2 cm or less in greatest dimension.
    - T1mi: 0.1 cm or less (microinvasive)
    - T1a: more than 0.1 up to 0.5 cm
    - T1b: more than 0.5 cm up to 1 cm
    - T1c: more than 1 cm up to 2 cm
  - T2: Tumor more than 2 cm up to 5 cm in greatest dimension.
  - T3: Tumor more than 5 cm in greatest dimension.
  - T4: Tumor of any size with direct extension to chest wall and/or skin (ulceration or skin nodules). See AJCC manual for further details and substaging [30, 31].
- pN – Nodes (regional) (see question 43)
  - NX: Cannot be assessed.
  - N0: No regional lymph node metastasis or only isolated tumor cells (clusters of tumor cells not more than 0.02 cm = 0.2 mm and less than 200 cells; see question 40).
    - pN0(i+): ITCs only

  - N1: Micrometastasis or metastasis in 1–3 axillary ipsilateral; see AJCC for internal mammary nodes [30, 31].
    - N1mi: larger than 0.02 cm (0.2 mm) and/or more than 200 cells, but none larger than 0.2 cm (2 mm), micrometastasis.
    - N1a: metastasis in 1–3 axillary lymph nodes, including at least one larger than 0.2 cm (2 mm).
    - N1b,c: internal mammary nodes involved; see AJCC manual [30, 31].
  - N2: Metastasis in 4–9 axillary lymph nodes, including at least one larger than 0.2 cm (2 mm); see AJCC for internal mammary nodes [30, 31].
  - N3: Metastasis in ten or more axillary lymph nodes, including at least one larger than 0.2 cm (2 mm); see AJCC for internal mammary nodes [30, 31].
- pM – distant Metastasis
  - M0: No distant metastasis
  - M1: Distant metastasis

29. How should surgical margins be reported for invasive lobular carcinoma?

- Tumor resection specimens (lumpectomy, partial mastectomy, mastectomy) should be oriented and inked to preserve margin status as described in question 7.
- Ideally, the closest approach of invasive tumor to each surgical margin should be reported (distance in cm or mm).
  - If tumor is well removed from margin (greater than 0.5 cm, greater than 1 cm), exact distance may not need to be stated.
  - If carcinoma is within 0.5 cm of margin, stating distance of tumor to margin in millimeters is recommended, without further qualification or interpretation (without positive/negative).
  - Designation of a margin as positive or involved by carcinoma is reserved for cancer present at surgical margin (ink on carcinoma, carcinoma at cauterized specimen margin, distance to margin =0) [61].
  - If a margin is positive, the extent of margin involvement should be described, best with a distance measurement.
- In cases with a main lumpectomy specimen and separately submitted final margins, practices may differ.
  - Some groups provide status of specimen margins of the main specimen and along with diagnosis and margins for each separately submitted specimens.
  - Other groups prefer to synthesize results of all separate specimens and report "final" margin status.

30. What typically defines acceptable surgical margins for invasive lobular carcinoma?

Recent consensus criteria have proposed that adequate excision for invasive breast carcinoma of any type is "no tumor on ink." It specifically addressed lobular carcinoma in summary point 6: "Wider negative margins than no ink on tumor are not indicated for invasive lobular carcinoma (ILC)" [61].

However, in practice these guidelines are still considered controversial for cases with <1–2 mm margins that may not be receiving additional therapies. Multiple clinical, imaging, and pathologic factors should be considered in the setting of multiple close margins in an invasive lobular carcinoma.

31. What is the typical hormone receptor/Her2 profile and intrinsic type of classic invasive lobular carcinoma?

Most classic invasive lobular carcinomas are estrogen receptor (ER) positive and Her2 negative, consistent with the so-called "luminal" intrinsic type, as also demonstrated by gene expression analysis [1, 2, 7, 15, 27, 45, 62–65]. Those with low proliferation (often also strongly progesterone receptor (PR) positive) are luminal A (see case 2), whereas those lobular cancers with higher proliferation are luminal B (and may be low or negative PR) [1, 2, 7, 15, 27, 45, 62–65]. Reviews cite 95% ER positivity and 60–70% PR positivity [1, 2, 7, 15, 21, 27, 45].

32. What is the typical hormone receptor/Her2 profile and intrinsic type of pleomorphic invasive lobular carcinoma?

As compared to classic lobular carcinoma, pleomorphic lobular is less likely to be ER positive [1] and more likely to be Her2 positive (Fig. 4.19a–d) [15, 22, 24, 25]. (See question 13 and case 6.) Thus, few pleomorphic lobular cancers would be of luminal A type, while some would be luminal B or Her2.

33. Can the Oncotype DX assay be applied to invasive lobular carcinoma?

Yes, the Oncotype DX assay is being applied to invasive lobular carcinomas.

- The Oncotype assay is a commercial RT-PCR assay offered by Genomic Health (Redwood City, CA, USA) for ER-positive, Her2-negative invasive carcinomas limited to the breast or with low lymph node disease burden.
- This assay evaluates mRNA expression levels of 16 genes along with 5 housekeeping genes; based on those results, a "recurrence score" is mathematically calculated which has been shown to have utility in predicting outcome with tamoxifen-alone treatment, in predicting benefit of chemotherapy, and has been incorporated into NCCN treatment guidelines and the eighth edition AJCC staging [45, 66, 67].

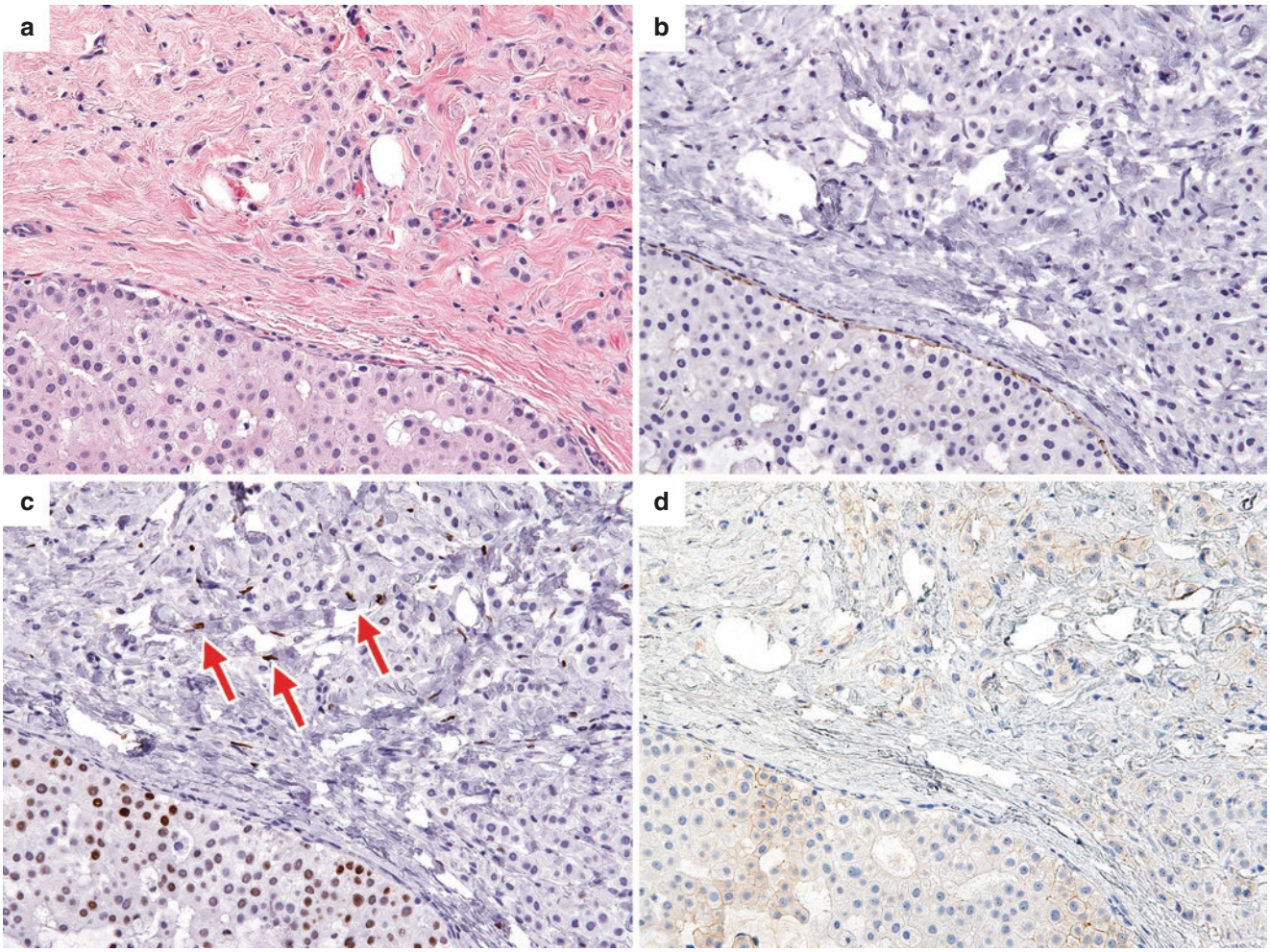


Fig. 4.19 Hormone receptor studies in pleomorphic lobular carcinoma. (**a**) H&E stain shows invasive pleomorphic lobular carcinoma (top) and carcinoma in situ (bottom, 200×). (**b**) E-cadherin is negative in both components, supporting lobular differentiation, despite the residual intraductal spaces. The staining at the edge of the involved duct represents compressed myoepithelial cells. (**c**) Estrogen receptor shows variable positive staining in LCIS (bottom) but is negative in the invasive carcinoma. The positive nuclei are spindle stromal cell nuclei, which should not be overinterpreted as positivity in carcinoma (red arrows, examples). (**d**) Her2/neu immunohistochemistry is negative (1+ barely perceptible membrane staining, all 200×)

- Most initial studies were done on invasive ductal or no special type breast carcinoma. There are no outcome studies of lobular carcinoma, yet lobular carcinomas frequently fall into this ER+ Her2- group.
- Recent small single-center studies demonstrate the vast majority of classic invasive carcinomas fall into the low- and intermediate-risk group or have recurrence scores below 25, different in distribution than cohorts of ductal carcinomas [45, 68–70].

34. How is the mRNA expression profile of classic lobular carcinoma different from low-grade ductal carcinoma?

Classic invasive lobular carcinomas generally belong to the "luminal" intrinsic type of breast cancers, along with low-grade invasive ductal carcinoma. Differences include expression of genes related to cell adhesion (E-cadherin program), actin cytoskeleton remodeling, and cell migration, with other pathways differing by study [27, 43–45, 62–65]. Expression profiling has also suggested subgroupings of lobular carcinomas (immune related, and/or hormone related, reactive, proliferative) [15, 27, 43, 44, 71].

35. Are there recurrent genomic changes in invasive lobular carcinoma (mutations, chromosomal gains/losses)?

Classic invasive lobular carcinoma has genomic features of the "low-grade" breast carcinoma molecular pathway, with the addition of E-cadherin loss. This often includes [1, 9, 15, 27, 43–45, 71]:

- Gain of 1q, 16p
- Loss of 16q (site of *CDH1*, E-cadherin gene)

Enriched in lobular
- PI3Kinase pathway dysregulation:
  - Activating mutations in *PIK3CA* (45%)
  - Activating mutations in *AKT1* (4%)
  - Inactivation of *PTEN* (4%)
- Truncating mutations of *CDH1*
- Frequent mutation of *FOXA1*, *TBX3* (inactivating)
- Increased copy number of *ESR1* (estrogen receptor)
- Paucity of *TP53*, *GATA3* mutations, *ERBB2* amplification (ILC may be slightly enriched in *ERBB2* mutation.)

36. What adjuvant therapy is typically given after resection of invasive lobular carcinoma?

After resection of invasive lobular carcinoma to appropriate negative margins, most patients are recommended to receive:

- Adjuvant hormonal therapy with tamoxifen or especially an aromatase inhibitor for 5 years or longer [7, 9, 15].
- Adjuvant radiation therapy for patients who had breast-conserving surgery (lumpectomy or partial mastectomy).
- Radiation is considered in a subset of patients treated with mastectomy, such as those with large tumors, margin issues, many positive lymph nodes, or other adverse features [7].
- Adjuvant cytotoxic chemotherapy: see question 37.

37. What is the role of cytotoxic chemotherapy in treatment of invasive lobular carcinoma (neoadjuvant, adjuvant)?

- Adjuvant cytotoxic chemotherapy may be considered, but classic lobular carcinoma that is ER positive with low proliferation rate does not respond as well as higher-grade breast carcinomas [2, 7].
- In the neoadjuvant setting (chemotherapy before surgery), the rate of pathologic complete response (pCR) for classic invasive lobular carcinoma is low (6%) and lower than that of ductal carcinoma overall [7].
- Patients with multiple tumors have different responses to neoadjuvant therapy based on tumor biology, for example:
  - Excellent or even complete pathologic response of high-grade "triple-negative" ductal carcinoma
  - Minimal response in concurrent invasive lobular carcinoma (see Case 3)
- Cytotoxic chemotherapy should be considered for lobular carcinomas with higher proliferation and more aggressive biologic profile (for instance, pleomorphic lobular carcinoma).

38. What are the features of lobular carcinoma after neoadjuvant or neoendocrine therapy?

Neoadjuvant therapy is cytotoxic chemotherapy before surgery. Neoendocrine therapy is anti-hormonal therapy such as tamoxifen or aromatase inhibitor prior to surgery (Fig. 4.20a–d).

- Proliferation rate generally decreases.
- Some tumors have minimal response and demonstrate the same tumor cell density and tumor cell cytology after chemotherapy.
- Tumors with good response have decreased cell density.
- Lobular carcinoma cells may become small and pyknotic and closely resemble lymphocytes. Keratin stains may be needed to demonstrate residual tumor cells (Fig. 4.20a–d) [9].
- Carcinomas with complete response have no residual invasive carcinoma in the breast, no intralymphatic tumor, and no carcinoma in lymph nodes. Areas of tumor may be replaced by fibrous tissue in the breast and/or lymph nodes.
- It is essential to know pre-treatment status and correctly identify and thoroughly sample the tumor bed in breast cancer resection specimens.

39. Does invasive lobular carcinoma occur in men?

Yes, invasive lobular carcinoma occurs rarely in men, estimated at about 1% of male breast cancer, comprising a lower proportion than in female breast cancer. A recent SEER study catalogued 155 cases of male lobular breast cancer, with poorer 5- and 10-year unadjusted cancer-specific survival (both 82.9%) as compared to women with lobular breast cancer (91.9% and 84.5%, respectively) [72]. This study documented some treatment differences, as well as more higher-grade lobular cancers, raising the possibility that registry data could have included some misclassified high-grade ductal cancers [72].

Metastatic Lobular Carcinoma

40. How is lobular carcinoma recognized in lymph nodes (Figs. 4.21a–f, and 4.24a–d)?

Metastatic lobular carcinoma shares cytologic features with primary lobular carcinoma and can be problematic to recognize in lymph nodes. Look for the following patterns [2]:

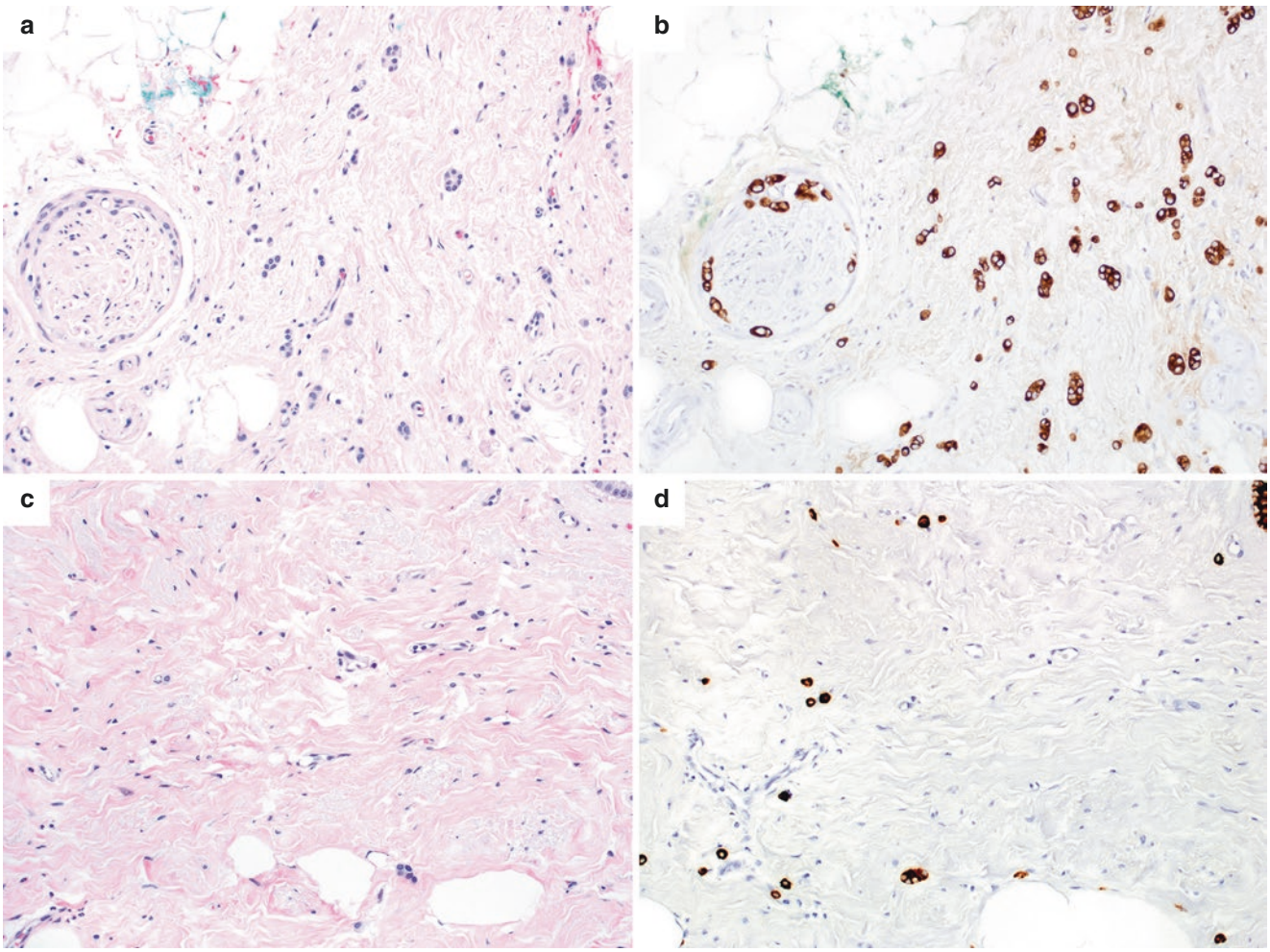


Fig. 4.20 Invasive lobular carcinoma resected after endocrine therapy (neoendocrine therapy). (**a**) Obvious residual tumor cell clusters with perineural invasion (left, 200×). (**b**) Keratin stain of the same area (200×). (**c**) Another area with dense collagenized stroma and minimal increased cellularity; epithelioid tumor cells with minimal cytoplasm are scattered among vascular or stromal cells (200×). (**d**) Keratin stain can help identify the few small residual tumor cells (200×)

- "Pinker" areas of lymph node may represent nodular or diffuse involvement by metastatic lobular carcinoma. Metastatic cells are slightly larger than the background lymphocytes with slightly more pale or pink cytoplasm (Figs. 4.21a–f and 4.24a–d). Eosinophilia may also be due to accompanying collagen fibrosis.
- Small collections of lobular carcinoma in or near sinusoids. These may closely mimic sinusoidal histiocytes. Histiocytes have more filmy, foamy, less eosinophilic cytoplasm as compared to lobular carcinoma and may have more variable nuclear contours (bean-shaped, non-round). Lobular carcinomas have more uniform cytoplasmic density and monotonous nuclei (Figs. 4.22a–f and 4.24a–d). Immunohistochemical staining may be essential to diagnosis.
- Dispersed single cells. These may blend almost imperceptibly with the surrounding nodal tissue and can be confirmed by keratin staining (Figs. 4.23a–d and 4.24a–d).

Immunostaining for keratin is helpful and sometimes essential in confirming or refuting the presence of lobular carcinoma in lymph nodes (Figs. 4.22a–f and 4.24a–d). While keratin staining of sentinel lymph nodes is no longer routinely performed in most centers, keratin staining of nodes in the setting of lobular carcinoma should be considered, especially when [73–76]:

- There is histologic difficulty in discerning histiocytes from potential lobular carcinoma.
- There is histologic concern for small clusters or dispersed lobular carcinoma cells.
- It is important to note that isolated tumor cells are the most common additional finding when using immunohistochemistry to detect metastatic lobular carcinoma; isolated tumor cells have minimal clinically significance currently [73, 74, 77–79]. (See question 43.) Therefore, the routine use of immunohistochemistry to detect isolated tumor cells is not recommended.

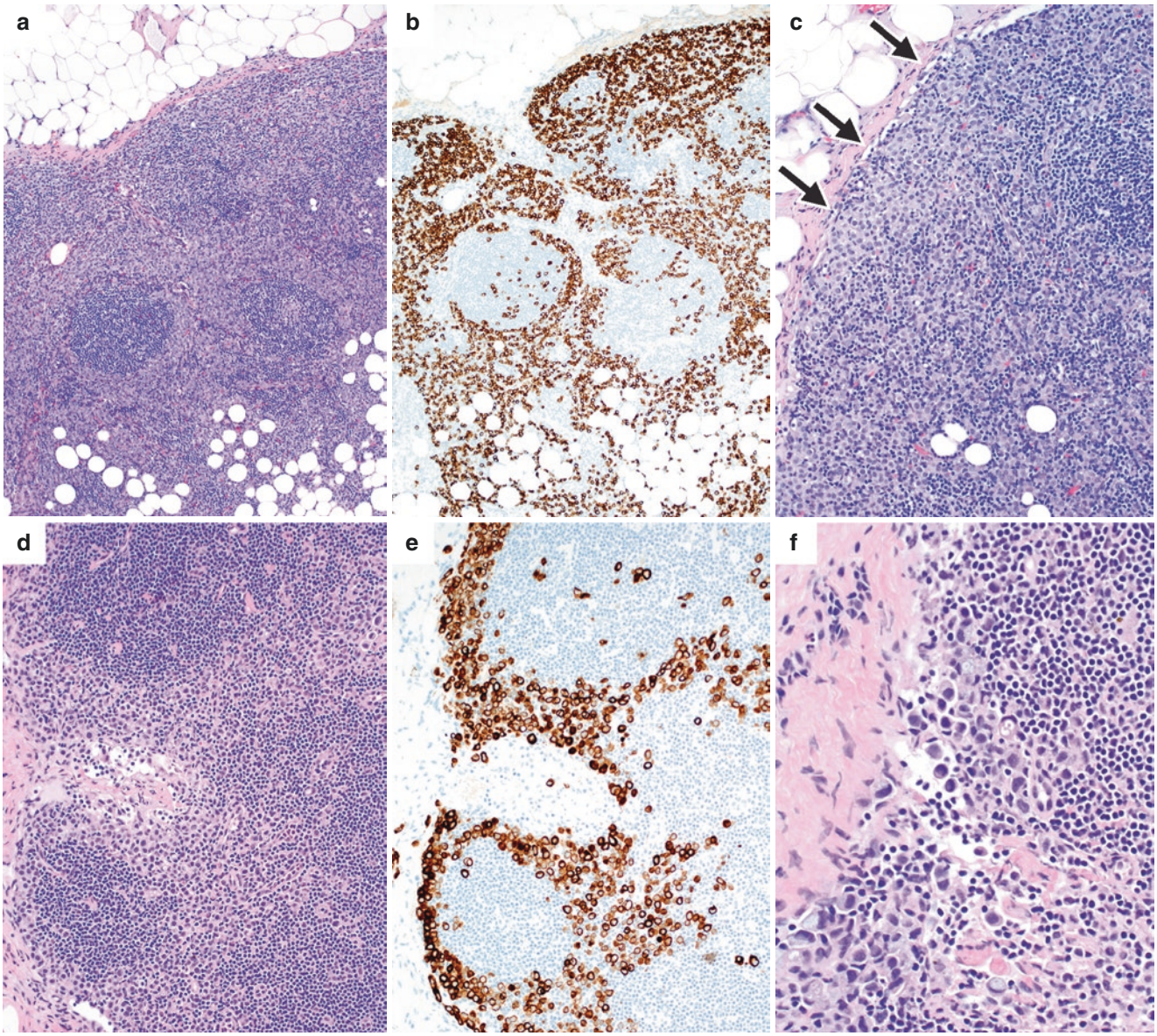


Fig. 4.21 Lymph node involvement by lobular breast carcinoma. (**a**) At low power, this area of the node is unusually pink; there is an obvious interfollicular infiltrate of metastatic lobular carcinoma (100×). (**b**) Keratin staining of a serial section (100×). There is more than 2 mm of confluent involvement by tumor in this node, which is classified as a (macro)metastasis. (**c**) This subcapsular metastasis is also pink but blends in somewhat with the background lymph node (arrows, 200×); higher-power examination reveals lobular carcinoma (not shown). (**d**) Subcapsular and septal infiltrate of dyscohesive atypical cells (200×). (**e**) Keratin stain highlights extent of tumor in a serial section (200×). (**f**) Higher power demonstrates pleomorphic lobular cells, some with intracytoplasmic mucin (400×). This focus is >200 cells and > 0.2 mm, but less than 2 mm, and is categorized as micrometastasis (mi)

- Keratin immunostains are recommended over other breast differentiation or histiocytic histochemical or immunohistochemical stains in this scenario [74]:
  - While lobular carcinomas are ER positive, a subset of nodal lymphocytes, especially germinal center cells, are also ER positive, such that ER is not a specific stain in the context of nodal metastasis [80].
- In interpreting keratin stains, a positive stain will have strong dark staining in cells with size, nuclear morphology, and location compatible with lobular carcinoma [74]. Re-review the H&E sections looking for the cells highlighted by immunohistochemistry.
- Keratin immunostaining pitfalls in axillary lymph nodes include (Fig. 4.23a–d):
  - Dendritic-like cells with fine elongated processes commonly stain for keratin (CAM5.2 more so than AE1/3). These cells may be light brown, but they do not have the correct morphology of lobular carcinoma [74].

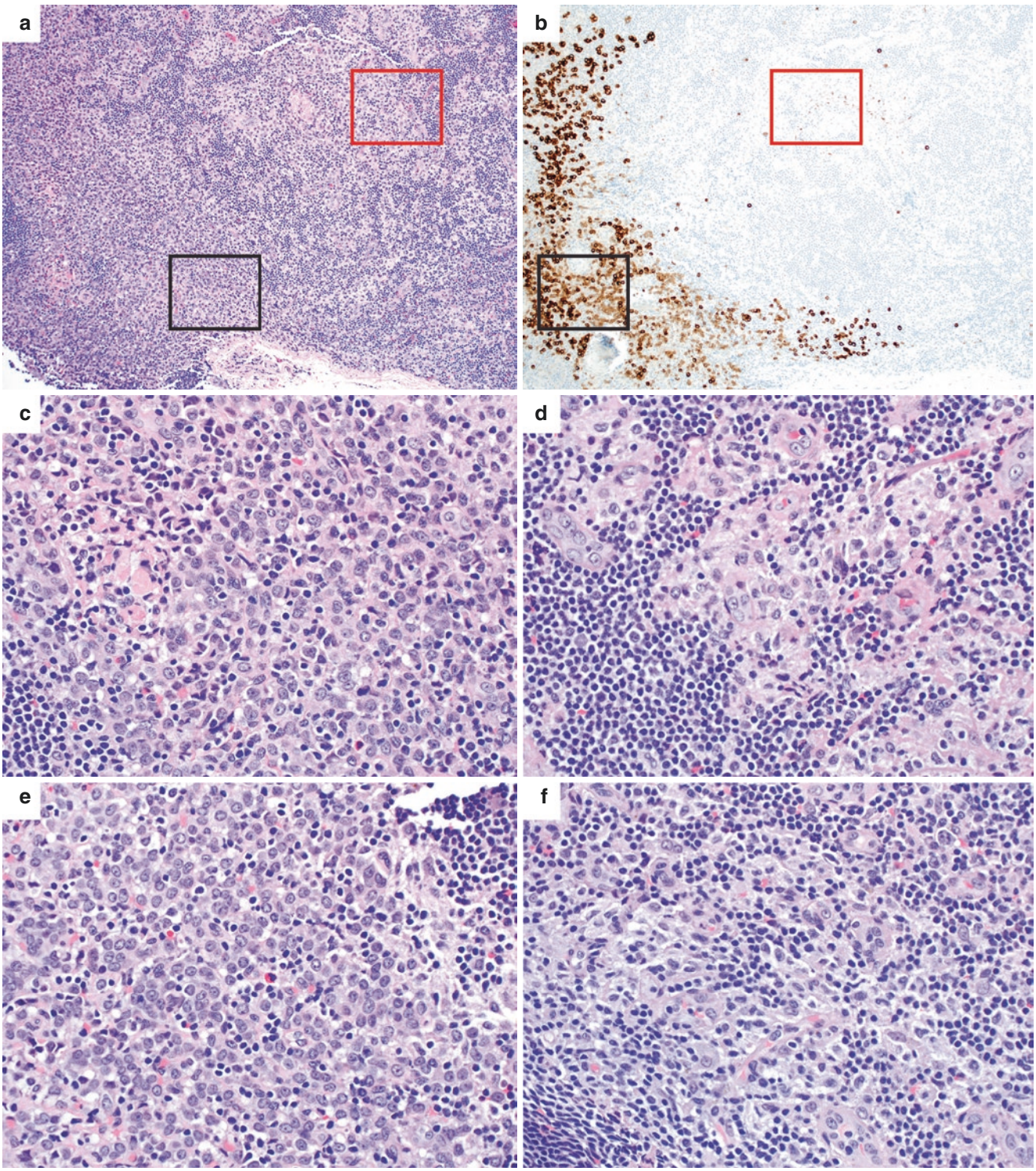


Fig. 4.22 Lymph node involvement by lobular breast carcinoma. (**a**) In this "pink" node, metastatic carcinoma involved one portion (lower left), with abundant histiocytes at right (100×). The extent of tumor was very difficult to discern morphologically and is nicely illustrated in (**b**) with keratin stain (100×). Higher power from the boxed regions is shown below. (**c**) Black box shows carcinoma with monotonous mostly round nuclei and scant cytoplasm. (**d**) Red area shows histiocytes and lymphocytes without carcinoma. Histiocytes have more abundant filmy or foamy cytoplasm as compared to carcinoma, somewhat exaggerated in this field. Additional images of metastatic lobular carcinoma (**e**) and histiocytes (**f**) from other areas of the same case

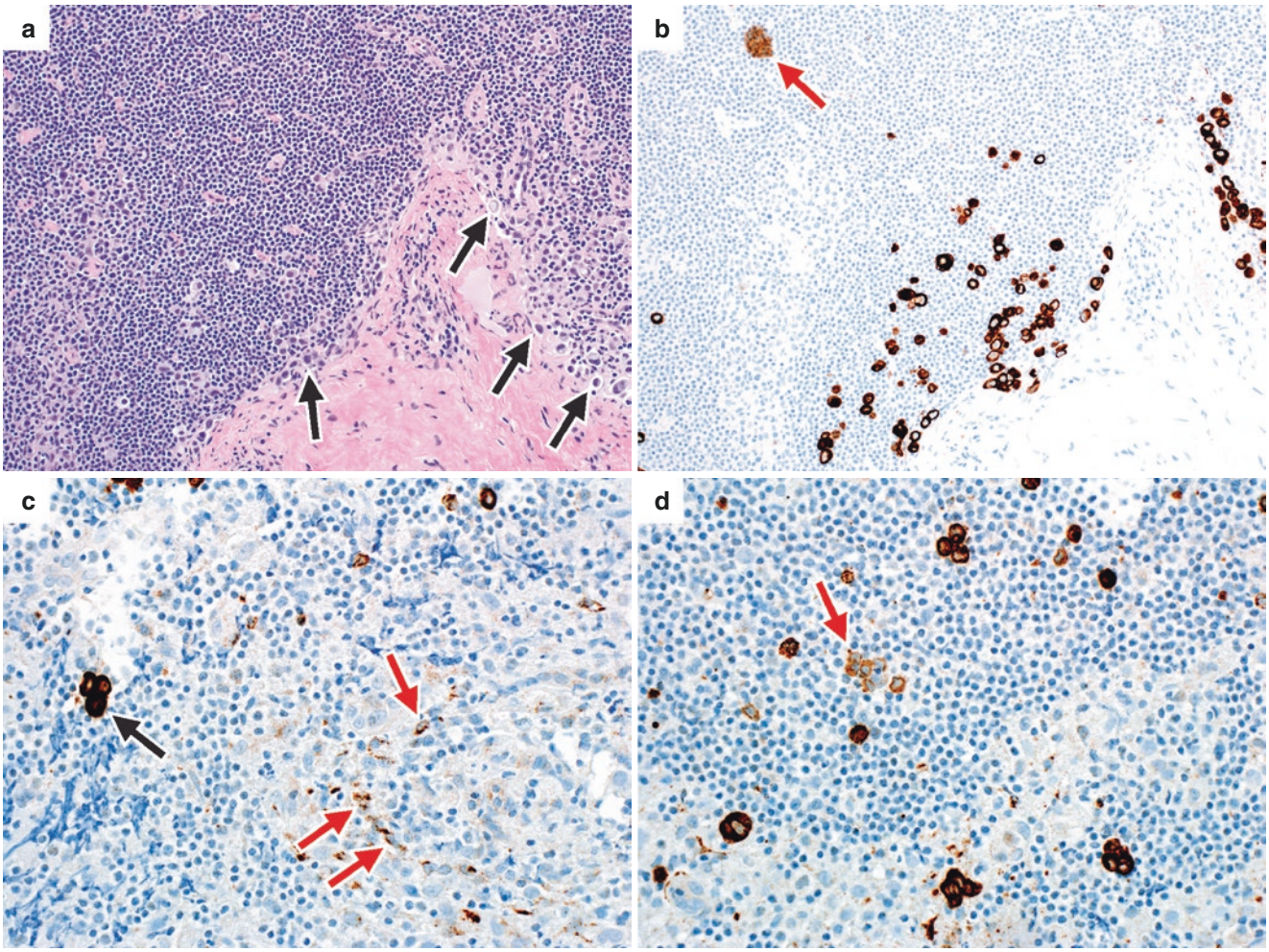


Fig. 4.23 Pitfalls of keratin immunostaining in axillary lymph nodes, with isolated tumor cells. (**a**) Subcapsular tumor cells can be identified by their pleomorphic nuclei, signet ring, and epithelioid features (black arrows, 200×). (**b**) They are highlighted in keratin stain of serial section. The red arrow highlights an extraneous keratin-positive squamous epithelial cell. Note the difference in morphology (larger, no nucleus, out of plane) and staining (pale) as compared to tumor cells in the same field (200×). This node has <200 tumor cells and is classified as isolated tumor cells (itc). The measurement should not include the span of individual cells since they are not confluent (cells not touching one another). This node is not included in the count of positive nodes. (**c**) A cluster of three keratin-positive metastatic lobular carcinoma cells is marked by the black arrow. The keratin stain also weakly labels dendritic cells that have different morphology (red arrows, examples, 400×). (**d**) Numerous clusters and single cells of strongly keratin staining metastatic lobular carcinoma are seen; the red arrow highlights a small cluster of weakly staining plasma cells. This weak staining does not indicate metastatic carcinoma (400×)

- Plasma cells may weakly cross-react with keratin antibodies. These may have morphology close to lobular carcinoma, but should not stain strongly [74].
- Large filmy polygonal usually anucleate cells out of the plane represent squamous cells from handling of the slides prior to staining, coverslipping. These do not have the morphology of lobular carcinoma.

41. Is sentinel lymph node mapping reliable for lobular carcinoma?

Yes, sentinel lymph node mapping is commonly used in invasive lobular carcinoma [74, 81, 82]. In brief, (lymphazurin) blue dye and/or radiocolloid (usually Tc-99m sulfur colloid) is injected at the tumor site or dermis around the areola [74, 81, 82]. After massage and time, the "hot" and/or "blue" lymph nodes are collected as the "first" nodes draining the breast tumor area and thus the most likely to harbor metastatic tumor, if any [74, 81, 82].

42. Are sentinel lymph node frozen sections or touch preps reliable for lobular carcinoma?

Given the generally small nuclei, small cells, and often dyscohesive pattern in metastatic nodes as described in question 40, intraoperative identification of metastatic lobular carcinoma by frozen section or touch preparation can be difficult.

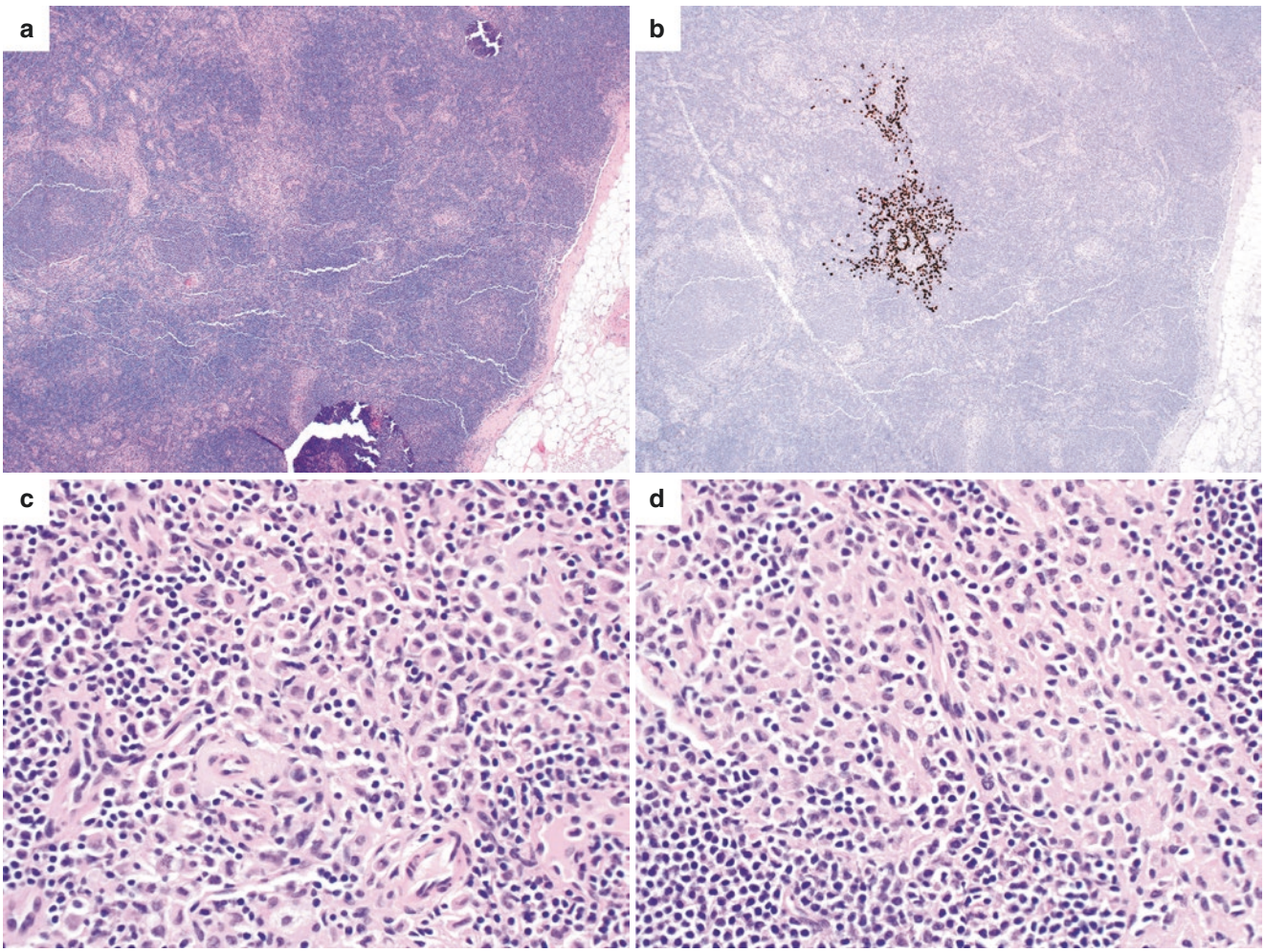


Fig. 4.24 Metastatic lobular carcinoma, scattered cells. (**a**) Dispersed tumor cells are occult at low power, but (**b**) are highlighted by keratin stain of a matched field. (**c**) Carcinoma cells can be discerned at high power in the area identified on keratin stain; they are smaller with more discrete cell boundaries and darker nuclei as compared to histiocytes (**d**) in this section. This lymph node was previously frozen, introducing artifact

- Several groups have reported lower sensitivity of frozen sections for lobular carcinoma [83], while others have reported comparable sensitivity [84].
- Frozen sections should be evaluated with the same criteria as permanent sections. (See question 40.) Intraoperative keratin staining is not available in most centers.
- We and others have found invasive lobular carcinoma particularly difficult to identify in nodal "touch preps" or "imprint" cytology. Some groups disfavor cytologic analysis of lobular carcinoma and preferentially or additionally perform frozen sections [84]. Sensitivity of intraoperative cytology has been published as 41–71% as compared to permanent sections with immunohistochemistry [85, 86]. Granted, some specialized centers have reported equivalent sensitivity for ductal and lobular carcinoma [85, 86].
- Although lobular carcinoma can be easily missed intraoperatively, it is more important not to "overcall" small collections of histiocytes or other constituents as lobular carcinoma.
- In the current era, axillary lymph node dissections are not performed for a major subset of patients even with low axillary metastatic burden, especially if patients will be receiving adjuvant endocrine or radiation therapy [73, 77–79]. Therefore, it is important to let the operating surgeon know when only rare metastatic cells are identified on intraoperative touch preparations or frozen sections.

43. How is metastatic lobular carcinoma measured and staged in lymph nodes?

The lymph nodes in breast cancer are categorized based on size/quantity of nodal tumor deposit regardless of histologic subtype of the primary. Tumor deposits are classified as macrometastasis, micrometastasis, and isolated tumor cells [30, 31].

- It is important to section lymph nodes at 0.2 cm (2 mm) intervals along either the long or short axis to facilitate detection of all macrometastasis [30, 31, 73].
- Each lymph node is classified based on its largest contiguous focus of carcinoma (cells touching one another). Discontinuous foci are not added together, and areas of fibrosis in between tumor cells do not count.
  - Macrometastasis: Tumor deposit larger than 0.2 cm (2 mm, Fig. 4.21b).
  - Micrometastasis (mi): Tumor deposit larger than 0.02 cm (0.2 mm) but not larger than 0.2 cm (2 mm, Fig. 4.21e).
  - Isolated tumor cells (ITC): Small clusters of tumor cells not larger than 0.02 cm (0.2 mm, Fig. 4.23a–d).
  - If only dispersed cells are present, as commonly occurs with lobular carcinoma, more than 200 dispersed cells in lymph node cross section qualify as micrometastasis and less than 200 cells as isolated tumor cells. Pathologist judgment is needed.
- The size of the largest nodal metastasis should be reported, along with any extranodal extension.
- The numbers of nodes with macro- and micrometastasis are added to determine pN stage. Nodes with isolated tumor cells are not added to the total for stage determination [30, 31].
- A simplified version of pathologic N categories is listed in question 28; please refer to the AJCC manual or CAP staging checklists for full detail [30, 31].

44. What are the most common metastatic sites of invasive lobular carcinoma?

Lobular carcinoma has a propensity for metastatic involvement of:

- Abdominal cavity, including gastrointestinal tract (especially gastric), peritoneum, ovaries, and uterus (endometrium) [1, 2, 7, 9, 87] (Fig. 4.25a–f).
- Meninges [1, 2, 87].
- Skin [1, 7], periorbital area [9].
- Bone, liver (equal to or more so than ductal) [1, 2, 9].
  - Widely dispersed single cells can be difficult to identify in bone marrow biopsies and may require keratin stains, especially in the background of cellular marrow (Fig. 4.26a–d) [2].
- Involves lung less frequently than ductal, no special type [1, 2, 7].
- Metastatic disease may rarely be the first presentation of lobular breast carcinoma.

45. Is there a difference in late recurrence between ductal "no special type" and lobular carcinoma?

For stage-matched invasive lobular vs. ductal or no special type invasive breast cancers, classic lobular tends to have:

- Better 5-year disease free and overall survival [2, 7]
- Higher rates of later recurrence (10 years) and worse longer-term survival [2, 7, 9]

46. What immunostains are helpful to diagnose metastatic lobular carcinoma (Figs. 4.25a–f, and 4.27a–f)?

Metastatic lobular carcinoma is usually positive for the following immunomarkers:

- GATA3 (positive in 96% of primary lobular carcinomas) [88–90].
- Mammaglobin (positive in 70% of primary lobular carcinomas) [89].
- GCDFP-15, gross cystic disease fluid protein-15 (positive in 50% of primary lobular carcinomas) [89, 91, 92].
- Estrogen receptor (ER).
- Progesterone receptor (PR).
- Cytokeratin 7 (CK7).
- FOXA1 (forkhead box protein A1) and PELP1 (proline-, glutamate and leucine-rich protein 1) are transcription factors with emerging data in identification of breast cancers [93–95].

None of these are entirely specific for breast cancer including lobular carcinoma, and the immunostain panel should be tailored to the differential diagnosis and location, for example:

- Lung:
  - TTF-1, napsin generally negative in breast carcinoma, with 2.4% of breast carcinomas TTF-1 positive [95, 96]
  - GATA3 positive in about 10% of lung adenocarcinomas [88, 95]

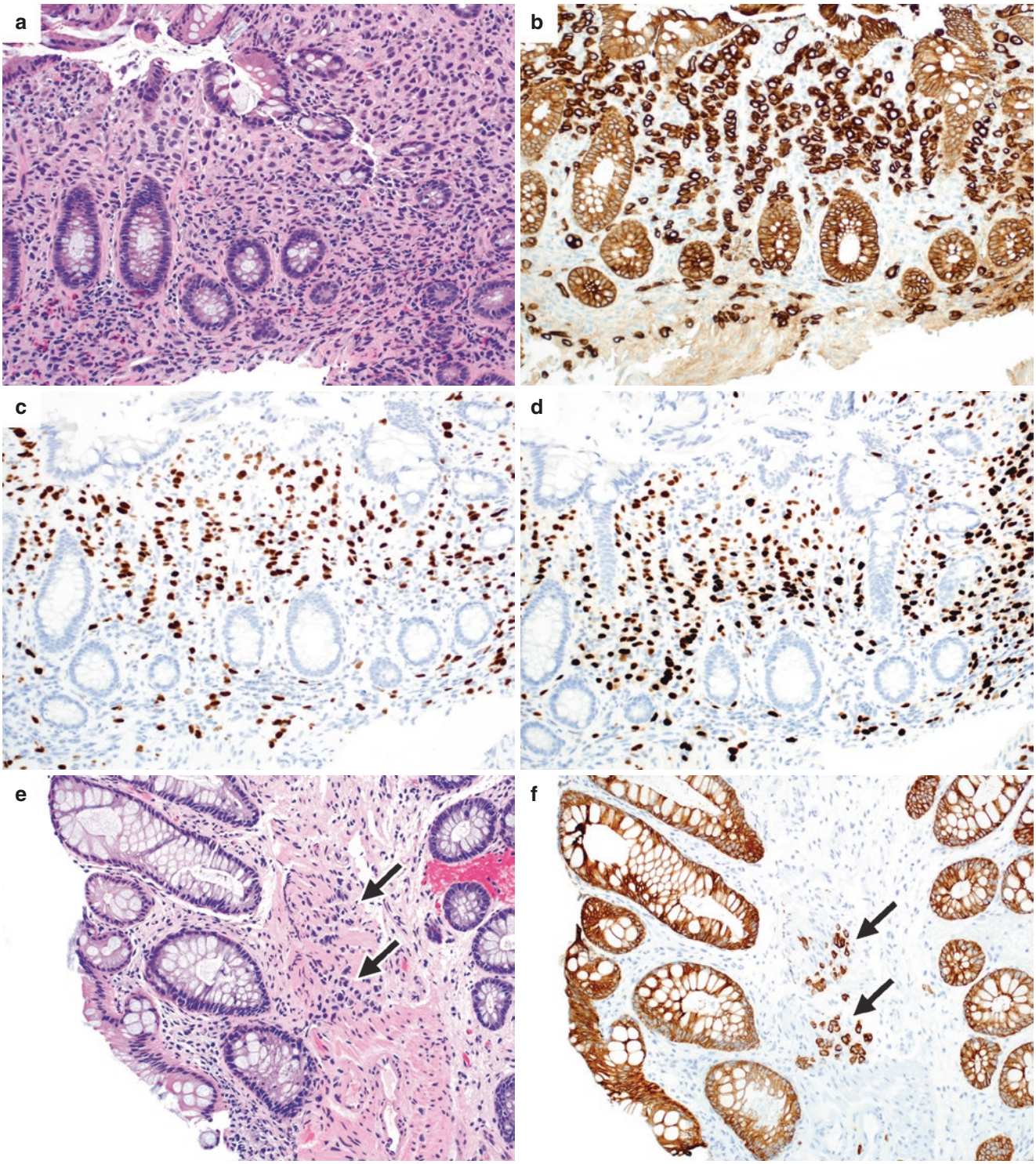


Fig. 4.25 Metastatic lobular breast cancer to colon. The patient was diagnosed with and treated for a 6 cm invasive lobular carcinoma with five positive axillary lymph nodes, 8 years prior. (**a**) Lamina propria is filled with cells with intermediate nuclear grade and scant cytoplasm arranged in files. Immunostains positive for keratin (**b**), GATA3 (**c**), and estrogen receptor (**d**), indicating metastatic breast cancer (all 200×). Lobular carcinoma metastasis in a colon biopsy from another patient is much more subtle. (**e**) The only focus shown on H&E (arrows), keratin positivity (**f**) confirms carcinoma (both 200×)

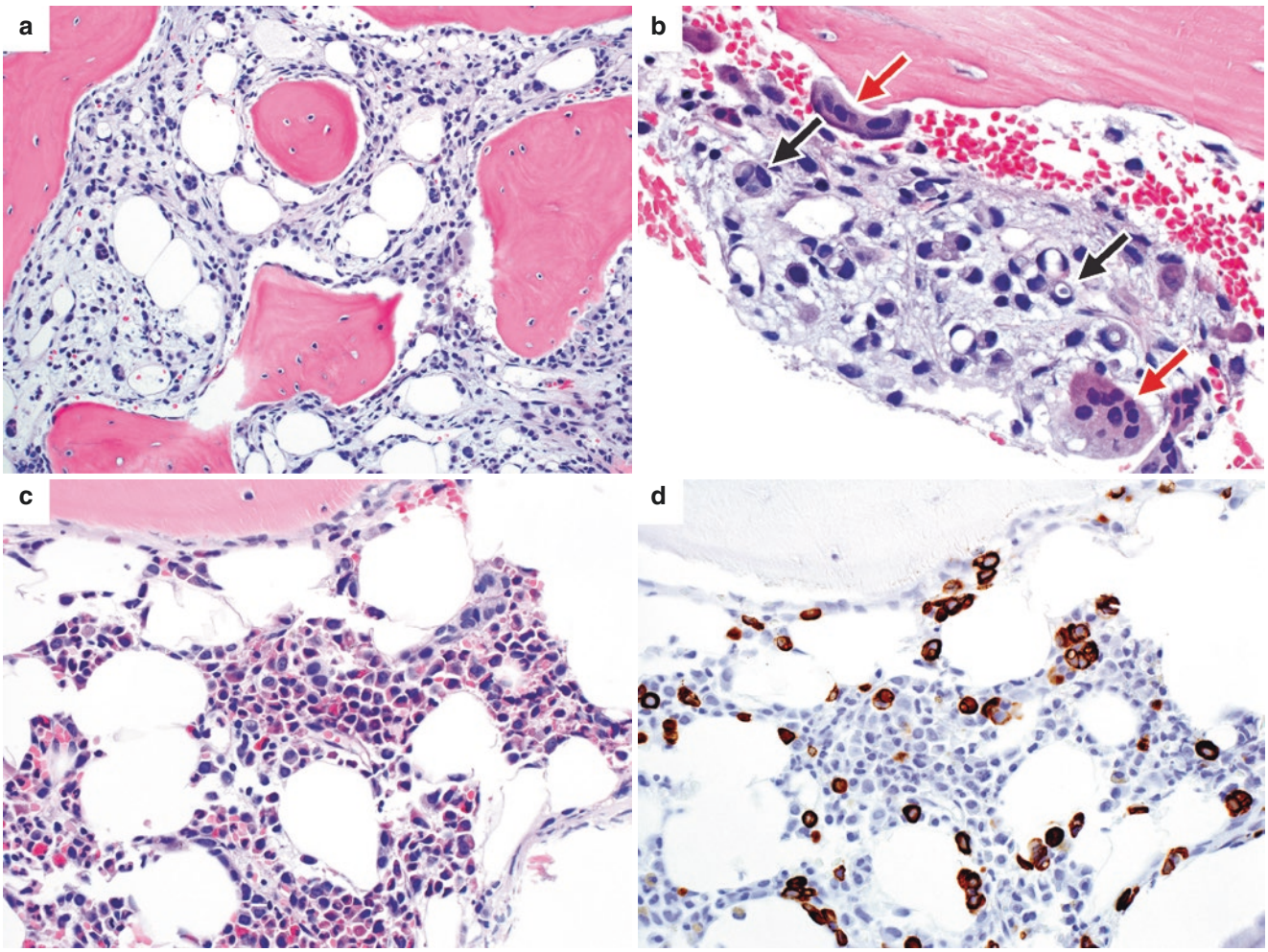


Fig. 4.26 Lobular carcinoma metastatic to bone marrow. (**a**) Obvious tumor infiltrate replacing marrow elements. (**b**) Lobular carcinoma with mucin vacuoles (black arrows) with nearby osteoclasts (red arrows). (**c**) In this cellular marrow, metastatic tumor cells are very subtle. (**d**) Keratin staining highlights metastatic carcinoma in a matched field. Sometimes the carcinoma can only be appreciated with keratin stains

  - ER positive in 10–20% of lung carcinomas, usually weak, focal [92, 97]
- Gastric: distinguish from primary diffuse gastric cancer (many cases E-cadherin negative; see question 47 [98]):
  - ER strongly positive in lobular carcinoma, rarely weakly positive in gastric [92, 99].
  - GATA3 positive in 5% of gastric cancers (unspecified type) [88] and 0/32 signet ring gastric carcinomas in a tissue microarray study [100].
  - Mammaglobin and GCDFP-15 negative in signet ring gastric cancer in a tissue microarray study [101].
  - CDH17 and CDX2 may be positive in gastric [98, 102], no specific gastric carcinoma markers.
  - Gastric carcinoma commonly CK7+ (non-discriminator).
- Gynecologic: metastatic lobular breast cancer can insinuate in cellular ovarian or endometrial stroma and be very hard to detect.
  - Keratin staining may be necessary to demonstrate the population of diffuse single cells within stroma and correctly score other immunostains against the ER+ cellular background.
  - ER is often positive in both and is not useful.
  - PAX8 useful gynecologic marker [103].
  - WT-1 stains ovarian carcinomas, but also 1–8% of breast cancers [97, 104, 105].
  - Mammaglobin positive in up to 40% of endometrial and ovarian carcinomas, mandating caution [106–108]; smaller proportions stain with GCDFP-15 (5–10%) [92, 108].
  - Gynecologic carcinoma commonly CK7+ (non-discriminator).
- Urothelial: metastatic lobular carcinoma must be distinguished from plasmacytoid or signet ring urothelial carcinomas (Fig. 4.27a–f).
  - GATA3 is frequently positive in urothelial carcinomas and is not useful in this differential, including signet ring and plasmacytoid variants [100, 101].
    - GATA3 also stains paraganglia [109].

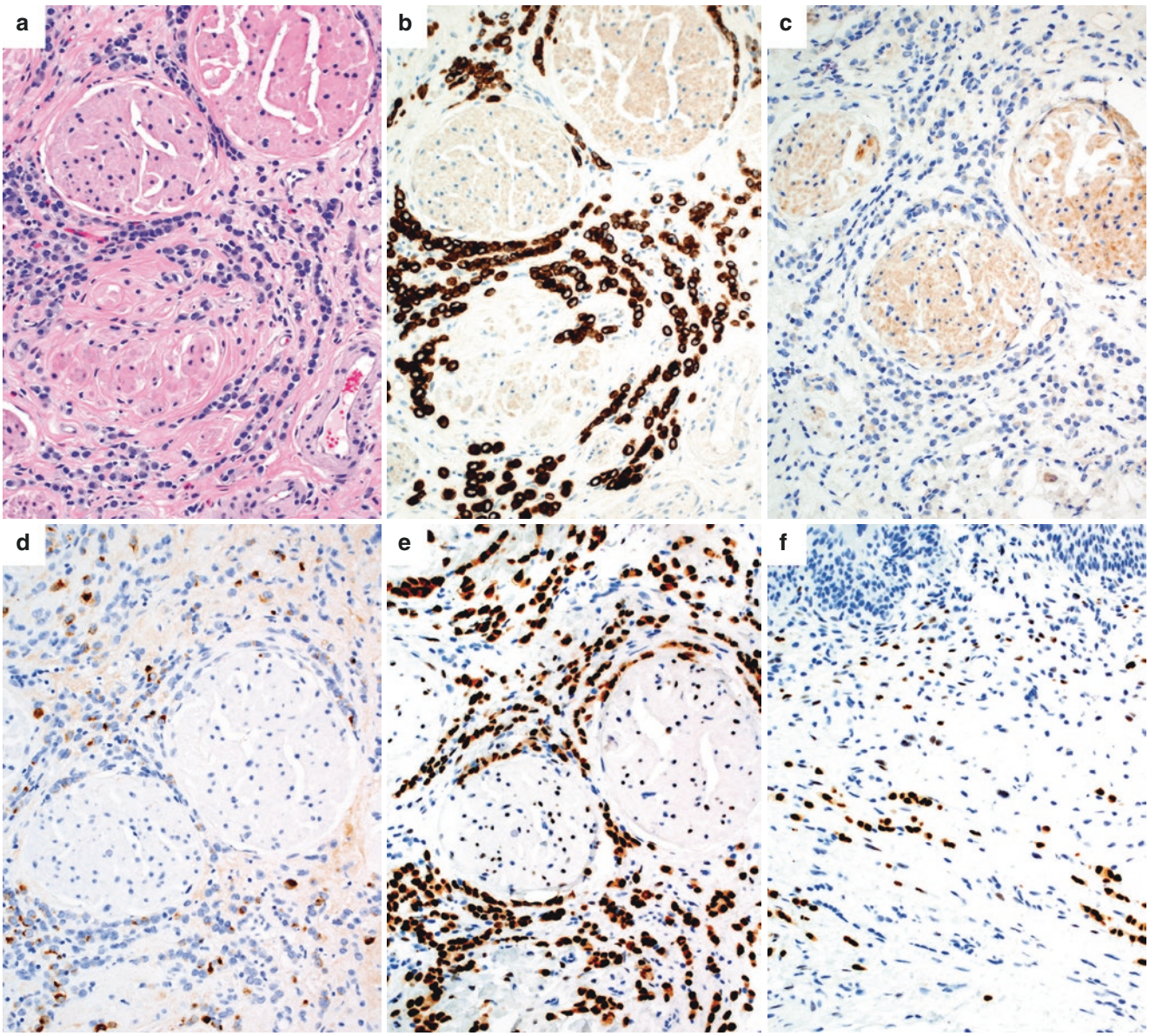


Fig. 4.27 Metastatic lobular carcinoma to the bladder. (**a**) Dyscohesive cells infiltrating around bundles of muscularis propria. The differential diagnosis includes plasmacytoid urothelial carcinoma, metastatic diffuse gastric cancer, as well as lobular carcinoma. The overlying urothelium was normal (not shown). (**b**) Infiltrating cells are keratin positive and E-cadherin negative. (**c**) These stains and GATA3 do not discriminate bladder and metastatic breast carcinoma. (**d**) GCDFP-15 is positive, supporting breast carcinoma. GCDFP-15 often stains breast cancer only focally, as seen here. (**e**) Likewise, estrogen receptor is strongly diffusely positive in the carcinoma cells. (**f**) In this field from the same case, estrogen receptor staining is positive in chains of carcinoma cells at the bottom of the field, yet it also moderately labels smaller stromal nuclei in the superficial lamina propria at top. Other immunostains include CD45 negative in tumor cells and negative progesterone receptor and Her2 (immunohistochemistry score 0, not shown)

  - ER is infrequently positive in such urothelial carcinomas and is a discriminator [100, 101].
    - PR stains 0–15% urothelial carcinomas [100, 101].
  - Mammaglobin is negative in urothelial carcinoma and is recommended [101].
  - GCDFP-15 stains 0–25% of these variant urothelial carcinomas suggesting caution [100, 101].
  - Urothelial carcinoma commonly CK7+ (non-discriminator).

- Hematolymphoid:
  - Keratin immunostaining essential in demonstrating an occult epithelial population amidst hematolymphoid cells (Fig. 4.26a–d)
- Some typical lymphoid markers stain epithelial neoplasms and vice versa:
  - CD138 (syndecan, plasma cell marker) also stains many carcinomas, including at least 40–60% of breast carcinomas [110, 111].

  - GATA3 is positive in T-cells and related neoplasms [88].
- Soft tissue tumors may occasionally mimic
  - Rhabdomyosarcoma: the dyscohesive cells with eccentric pink cytoplasm may occasionally mimic lobular carcinoma. Rhabdomyosarcoma may rarely metastasize to the breast; patient age group rarely overlaps.
    - Rhabdomyosarcoma stains with desmin, myogenin, myoD1, and PAX7 [112, 113].
    - Rhabdomyosarcoma should not stain with keratin or typical breast markers described above.
  - The rare epithelioid hemangioendothelioma (EHE) and sclerosing fibrosarcoma may appear as small dyscohesive pale cells in myxohyaline or fibrotic stroma. Few examples of EHE may be keratin positive.

47. **Can E-cadherin be used to diagnose metastatic lobular carcinoma?**

No, E-cadherin loss is not specific for lobular breast carcinoma. Dyscohesive carcinomas originating in other organ systems are also E-cadherin negative, notably:

- Subset of diffuse gastric cancer [98, 99, 114].
- Plasmacytoid urothelial carcinoma [101, 115].
- Subset of various high-grade carcinomas, especially "signet ring" cell carcinomas [98].
- Metastatic melanoma and other non-epithelial malignancies that can mimic carcinoma.
- E-cadherin could be helpful as part of a panel of stains, to help confirm metastatic lobular differentiation, if other stains demonstrate breast origin, as described above.

Lobular Neoplasia In Situ (ALH/LCIS)

48. **What is the incidence of atypical lobular hyperplasia/lobular carcinoma in situ (ALH/LCIS)?**

- According to the World Health Organization, lobular neoplasia is encountered in 0.5–4% of "otherwise benign" breast biopsies. Other sources report core biopsy incidence of 1–1.5% for ALH/LCIS combined [116–118].
- In two large contemporary studies of reduction mammoplasty specimens, ALH was found in 3–5% of specimens, with LCIS in 0.7–1%, including one pleomorphic LCIS [119, 120].
- In a SEER database analysis from 2000 to 2009, the incidence of LCIS was calculated as 2–2.75/100,000 women [121], reflecting an increase over prior SEER intervals [122].

49. **What are the typical features of ALH/LCIS on breast imaging (mammography, ultrasound, MRI)** (Fig. 4.28a–c)**?**

- Most ALH/LCIS is occult on imaging [123], with lobular neoplasia identified incidentally in biopsies performed for separate adjacent lesions (e.g., calcifications on mammography seen in association with columnar cell change, with incidental LCIS without calcification).
- Between 2% and 35% of ALH/LCIS may have an imaging correlate [123, 124].
- LCIS may appear as mammographic calcifications (Fig. 4.28a–c). These may be of variable morphology, including amorphous, fine, and heterogeneous, and are often grouped. Studies differ in the frequency of coarse or pleomorphic calcifications [14, 123–125].
- Ultrasound findings are described as typically irregular, hypoechoic, avascular mass lesions, with posterior acoustic shadowing [123]. However, oval, circumscribed masses without posterior shadowing have also been described [126].
- MRI findings include mass lesions or non-mass enhancement, with variable kinetics [123, 127].

50. **What are the typical features of pleomorphic LCIS on breast imaging (mammography, ultrasound, MRI)?**

As described below, pleomorphic LCIS is of higher grade than ALH or classic LCIS, and thus imaging features may share more similarity to high-grade DCIS [128].

- In one study, over 80% of identified pleomorphic LCIS had calcifications on mammography (Fig. 4.28a–c) [125, 128].
- MRI findings again include mass lesion, non-mass enhancement, or essentially negative MRI [128].

51. **What are the typical management steps after a diagnosis of ALH/LCIS on core biopsy?**

Management of LCIS/ALH discovered on core biopsy is controversial, especially in the current era advocating against "overtreatment."

- Careful imaging correlation is recommended. Upgrade rates to carcinoma are much lower if LCIS/ALH is "incidental" and it was another sampled lesion that explains the imaging finding, as discussed in question 52 [116, 117, 129].
- Conservative excision is often recommended for patients with classic LCIS on biopsy and should still be recommended for those with pleomorphic or florid LCIS on biopsy [116, 128–132].

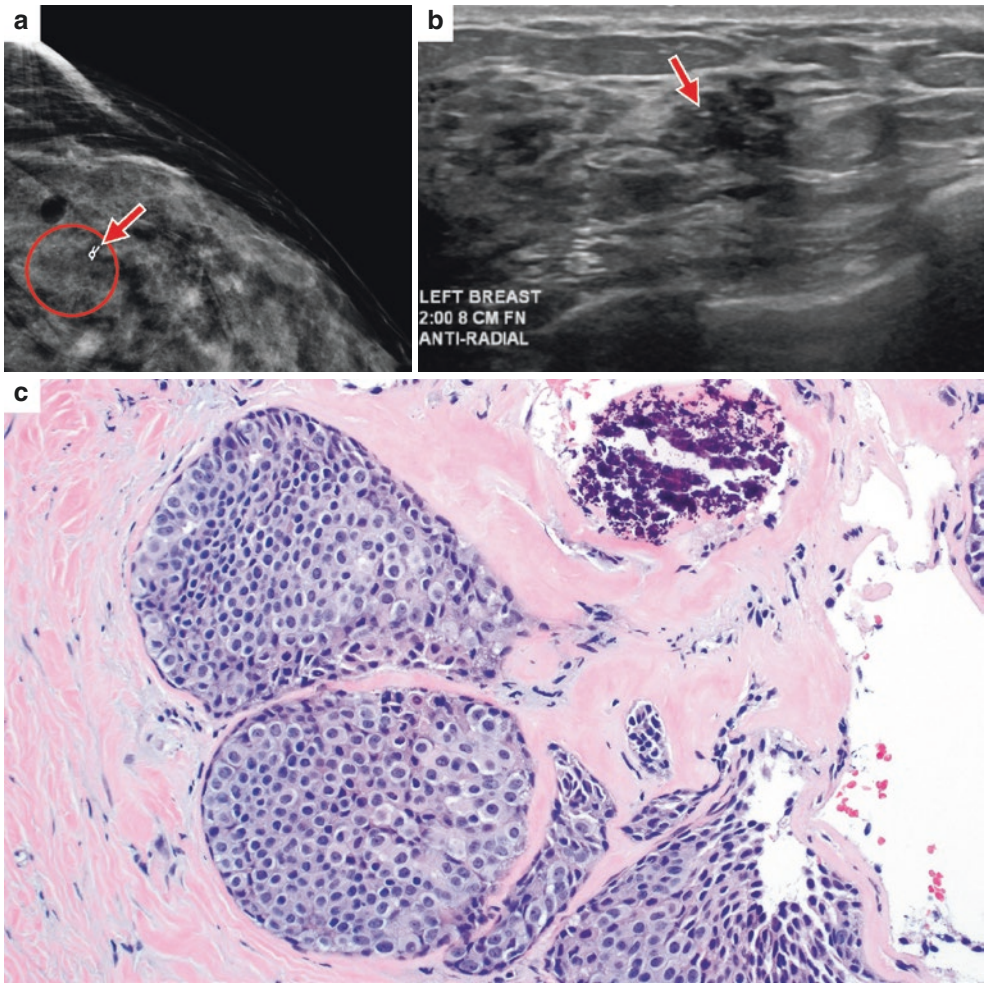


Fig. 4.28 LCIS with calcifications. Calcifications were seen on screening mammography. (**a**) Post-biopsy mammogram shows residual calcifications (circle), air in the biopsy site (above circle), and an omega-shaped post-biopsy marker. (**b**) Real-time breast ultrasound shows an irregular hypoechoic region or ducts with internal calcifications (arrow) in the region of the mammographic mass. (**c**) Histologic sections demonstrate calcification (upper right) immediately adjacent to pleomorphic LCIS (200×), confirmed with negative E-cadherin stain (not shown). Additional radiologic and histologic images from this case can be seen in Case 4, Figs. 4.48 and 4.49

- The management of ALH is even more controversial, given even lower upgrade rates [116, 129, 130].
- A recent consensus guideline statement from the American Society of Breast Surgeons (ASBS), published online, includes:
  - "we no longer advocate *routine* excision of ALH or LCIS when the radiological and pathological diagnoses are concordant, and no other lesions requiring excision are present" [132].
  - Regarding non-classic LCIS variants "these lesions, and pleomorphic LCIS, in particular, should be treated with complete surgical excision, similar to DCIS" [132]. LCIS variants are discussed below in questions 60 and 61.
- The 2017 National Comprehensive Cancer Network (NCCN) recommendations for LCIS are similar, with excision recommended for non-concordant imaging and risk-reducing therapy for LCIS with concordant imaging [133].
- Expected risk of upgrade and potential outcomes should be discussed with the patient, factoring in personal and family history along with imaging and pathologic features. The patient is an important participant in individualized decision-making.
- Close clinical and imaging follow-up is necessary for non-excised lesions [132].

52. How often is invasive carcinoma diagnosed after finding ALH/LCIS on core biopsy?

Rates of upgrade to invasive carcinoma, DCIS, or pleomorphic LCIS vary widely in the literature. In general factors that influence upgrade rates include [117, 121, 134–137]:

- Patient risk factors (personal or family history of breast cancer)
- Extent of imaging findings
- Extent of pathology findings

A recent single-center study with comprehensive review of the literature is summarized in Table 4.4 [117].

53. What is long-term risk of invasive cancer after a diagnosis of ALH? LCIS?

For over a half century, the implications of lobular neoplasia as either a direct precursor of cancer or as a marker of increased cancer risk (so-called risk lesion) have been a matter of considerable debate [116, 129]. Current WHO consensus recognizes lobular neoplasia as both a risk factor and a non-obligate precursor of breast cancer [116].

- Compared to patients without these lesions, the relative risk of subsequent cancer is 3–5-fold for ALH and 6–12-fold for LCIS [116, 129, 136].
- Patients with ALH/LCIS have a 1–2% annual incidence of breast cancer, which is cumulative, even over decades [130, 137–139].
- LCIS shares point mutations as well as genomic gains and losses with a significant fraction of synchronous invasive lobular carcinomas [116, 130, 140–142].

54. Is future risk of invasive carcinoma local (unilateral) or bilateral?

- Early and well-cited studies recognized a bilateral risk of breast cancer, in alignment with the "risk lesion" concept [17, 143].
- Lobular neoplasia itself has a propensity for bilaterality [130].
- More recent larger and longer-term studies have documented a bias toward cancer arising in the same breast as the ALH/LCIS (ipsilateral), with contralateral cancer still prevalent; see Table 4.5 [137, 138, 143–147].

Table 4.4 Upgrade rates of ALH and LCIS on core biopsy

Upgrade rate on excision	ALH %	LCIS %	ALH % incidental only	LCIS % incidental only
Single center	9%	24%	4%	7%
Meta-analysis, mean (range)	9% (0–67%)	18% (0–60%)	Not done	Not done

Data from ref. [117]

55. Does ALH/LCIS originate in lobules?

- ALH/LCIS, as well as many other forms of ductal and columnar in situ proliferation, are believed to originate from the terminal duct lobular unit [14].
- The most recognizable location of ALH/LCIS and its nomenclature may lead to the incorrect assumption that lobular neoplasia arises from breast lobules.

56. What are the characteristic cytologic and architectural features of ALH/classic LCIS?

Cytologic features are as follows (Figs. 4.29a–d and 4.30a–d) [116, 129–131, 134, 139, 148, 149]:

- Small dyscohesive cells.
- Round monotonous nuclei.
- Inconspicuous nucleoli.
- Pale cytoplasm usually scant, round, cuboidal, or polygonal cells.
- Non-polarized cells, though nuclei may be eccentric.
- Occasionally intracytoplasmic mucin vacuoles.
- Similar to invasive lobular carcinoma.
- Variants include clear cell change, apocrine cytoplasm, and (rhabdo)myoid cytology [16].

Architectural features are as follows (Figs. 4.29a–d and 4.30a–d) [116, 129–131, 134, 139, 148, 149]:

- Cells fill and/or expand acini
- May appear dyscohesive or loosely cohesive
- Most often do not form true luminal structures, unless a mixed population
- May extend along ductal structures in a "pagetoid" fashion (see question 65)

Table 4.5 Cancer incidence after ALH/LCIS diagnosis, selected studies

Study	Year published	Number of invasive cancers/number of in situ lobular lesions	Ipsilateral cancer	Contralateral cancer
Rosen [143]	1978	36/99 (LCIS)	53%	44%
Bodian [144]	1996 (updates Haagensen cohort)	55/216	54%	46%
Page [145]	2003	44/237 (ALH)	72%	23%
King [138]	2015	168/1060 (LCIS)	63%	25%
Wong [137]	2017 (SEER) ILC only	1837/ 19,462 368/1837	55% 69%	45% 31%
Coopey [147]	2012	106/784 untreated	61%	39%

Numbers may not total 100% given categorization of bilateral carcinomas
ALH atypical lobular hyperplasia, *ILC* invasive lobular carcinoma, *LCIS* lobular carcinoma in situ

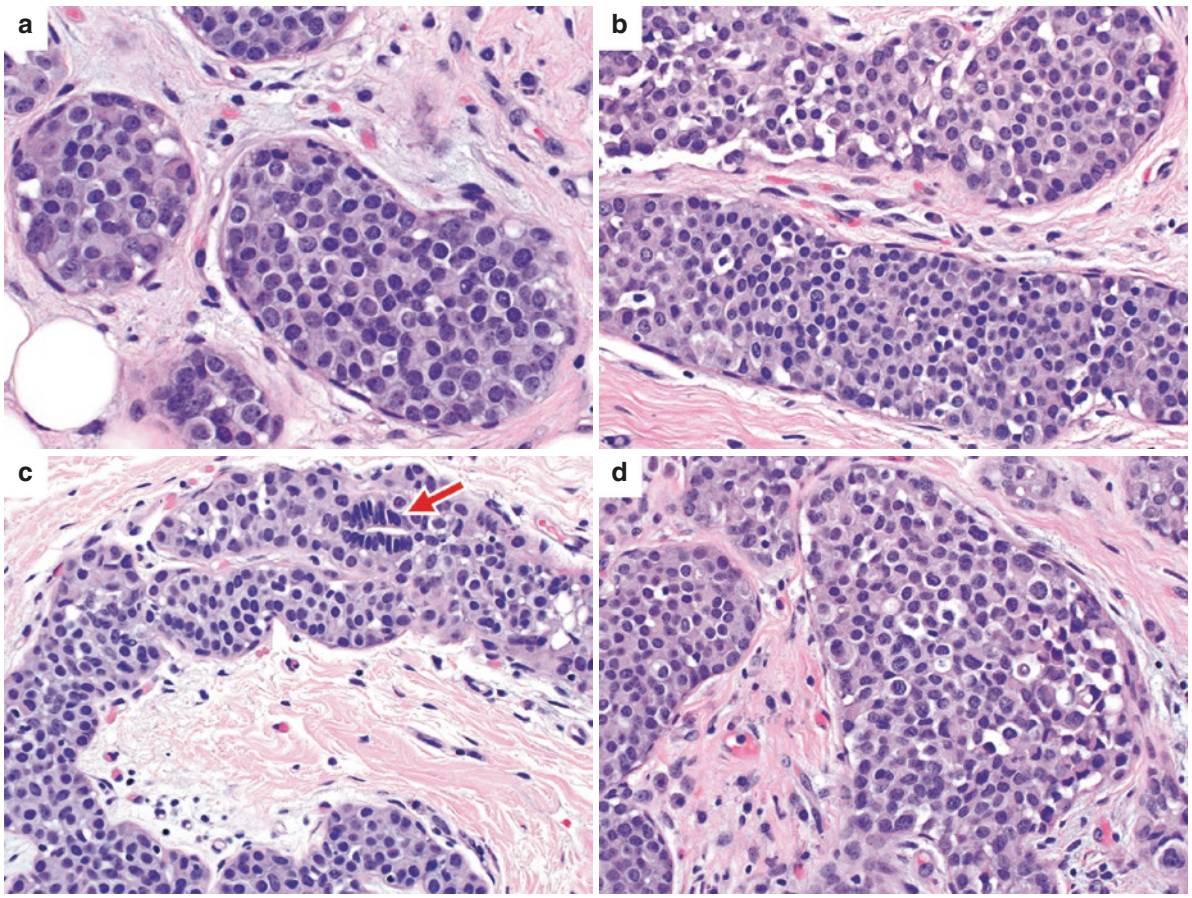


Fig. 4.29 Classic cytologic and architectural features of lobular neoplasia in situ (ALH/LCIS). (**a**) A uniform population of cells with eccentric round monotonous nuclei fills these spaces. Not all of the cells appear dyscohesive; cell-cell membranes are sometimes apparent. (**b**) Two small ductal spaces filled by LCIS. Cells appear dyscohesive in top space (400×). (**c**) A small tortuous ductule involved by ALH. A few remnant luminal (ductal) epithelial cells are marked by the red arrow (400×). (**d**) This focus of LCIS, from the same patient, has somewhat larger more hyperchromatic nuclei

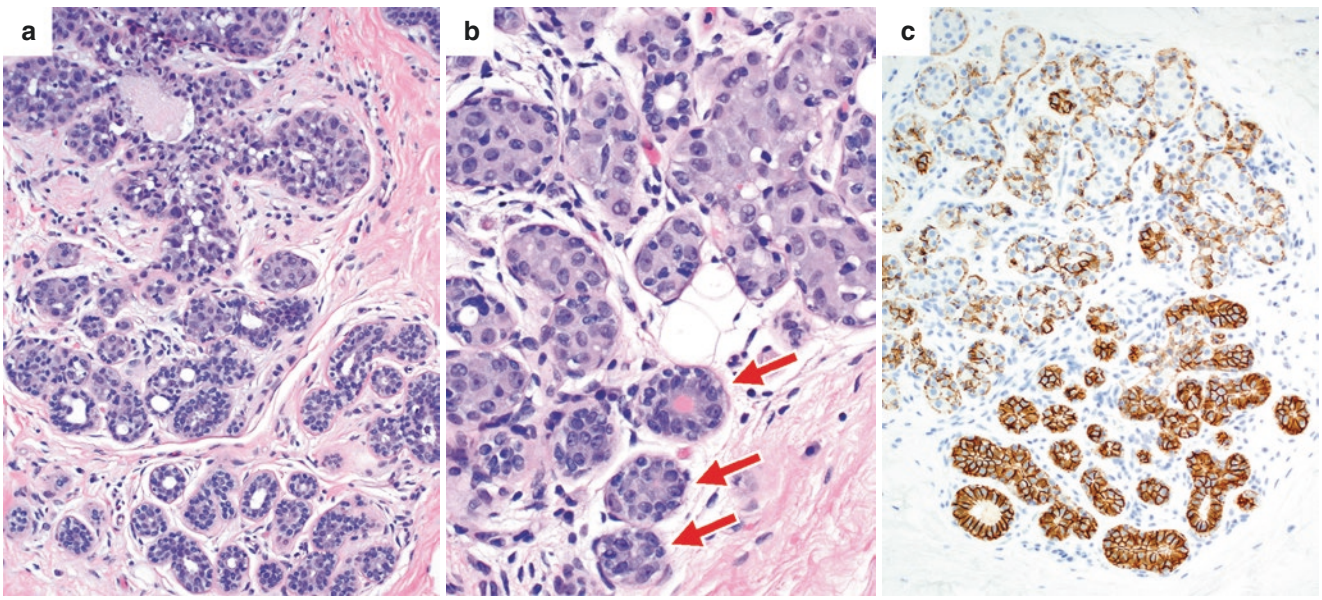


Fig. 4.30 Lobular neoplasia (ALH) partially involving a terminal duct lobular unit. (**a**) The ducts and acini at the top of the field are colonized and mildly expanded by lobular cells. Acini at bottom are uninvolved and appear smaller and less full (200×). (**b**) ALH involves most of the acini in the field; red arrows show uninvolved acini. (**c**) E-cadherin immunostaining is negative in lobular cells within acini at the top of the field, with labeling of a few remnant ductal cells; myoepithelial cells also stain such that E-cadherin outlines many of the acini. E-cadherin shows membranous staining in normal cells at bottom

57. How are ALH and LCIS distinguished from one another?

Both ALH and LCIS are characterized by small uniform lesional cells that show lobular phenotype and loss of cohesion. They are defined and discriminated by extent (Fig. 4.30a–d). The classic studies of Page and colleagues used the following definition:

- LCIS: "At least half of the acini in the lobular unit completely filled, distorted, and distended with characteristic cells….such that no intercellular lumina existed" [134].
  - Various criteria for "distention" include acini with at least eight cells across [14].
  - Other criteria include acinar diameter larger than nearby uninvolved acini [129].
- ALH: less than half of the acini in a lobule are filled and distended. As per Page: "lesions that meet most of the preceding criteria but fail to meet one of the criteria in over 50% of the acini within a lobular unit" [134].
- There are no analogous criteria for lobular neoplasia involving ductal structures. The presence of ductal involvement is not sufficient for a diagnosis of LCIS in our opinion and that of other experts [130, 134].

58. What are the minimal diagnostic criteria for ALH?

- Based on the description of Page and Dupont, minimal criteria for ALH include "clear round cells reminiscent of ALH and LCIS are present within lobular acini" with one of the following [150]:
  - Acinar distention
  - Acinar distortion
  - Increase in number of cells
- There is no entity of "lobular hyperplasia," and this terminology should not be used.

59. What are type A and B cells?

In the 1970s, the Columbia group recognized subtle variations in "classic" lobular neoplasia and categorized them as follows (Fig. 4.31a–c). Although this group soon found the type A/B designation to be overly simplistic, and it remains of no known significance, it has been carried

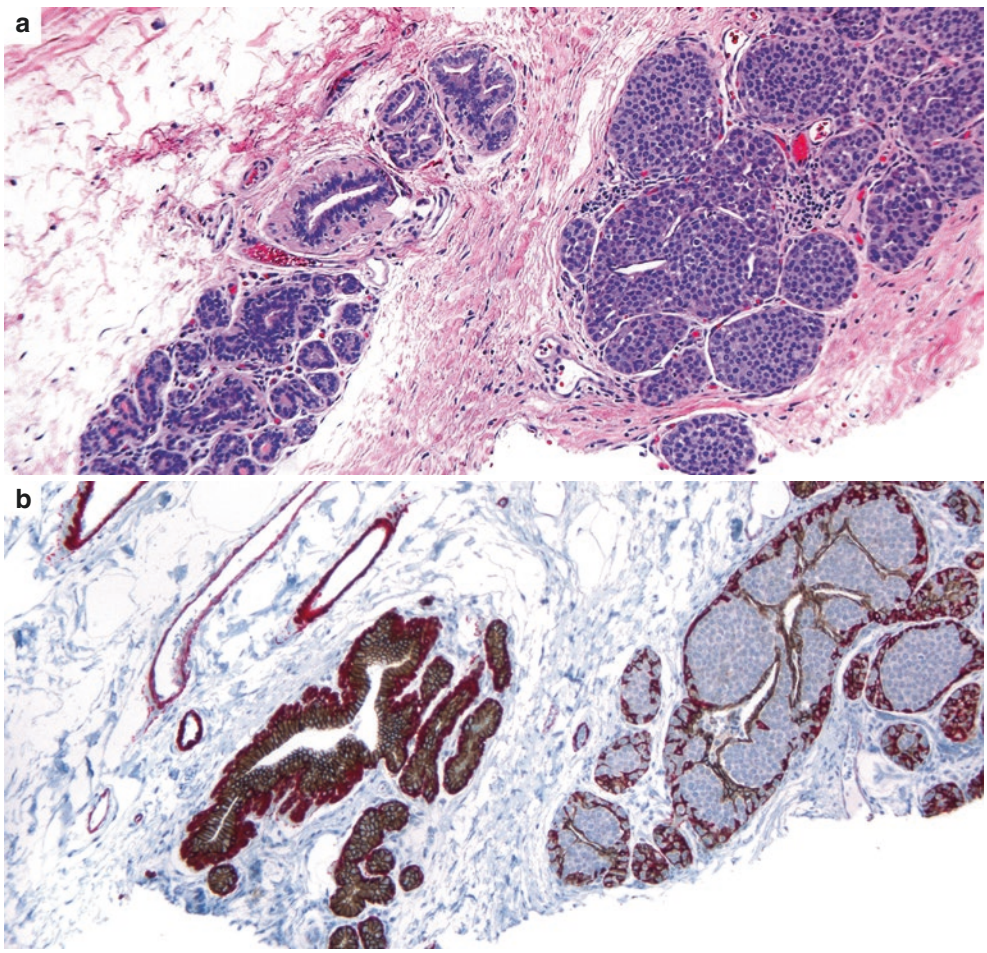


Fig. 4.31 LCIS and normal breast. (**a**) H&E shows LCIS at right and a terminal duct lobular unit at left (100×). (**b**) Dual staining for E-cadherin (brown) and the myoepithelial marker smooth muscle myosin heavy chain (red) of a section deeper in the same block. In the normal duct (left), luminal ductal cells have E-cadherin membrane staining, with underlying myoepithelium in red. In the LCIS (right), attenuated ductal cells are highlighted in brown, while smooth muscle myosin heavy chain (red) outlines the distended acini

through into many textbooks. A parallel nomenclature has been applied to invasive lobular carcinoma [14, 15, 17, 18, 116, 130, 148]:

- Type A: small cells with small uniform nuclei, akin to grade 1 nuclei.
- Type B: slightly larger cells with more variation in nuclear and cell size (larger) and shape, often with paler chromatin and/or small nucleoli, more like grade 2 nuclei (twice the size of a lymphocyte nuclei).
- Both are considered "classic" ALH/LCIS.

60. **What are the morphologic variants of LCIS?**

- Pleomorphic LCIS: Lobular features in larger cells with high-grade nuclei (Figs. 4.31a–c and 4.32a–f) [18, 116, 139, 149, 151, 152]:
  - Large nuclei (>4 lymphocytes), pleomorphic with prominent nucleoli, nuclear membranes may be irregular (grade 3 nuclei).
    - The cell to cell variation and degree of nuclear membrane irregularity is generally less than high-grade DCIS [20].
  - Dyscohesive large cells with eccentric nuclei, often with abundant cytoplasm.
  - Increased mitotic rate [130].
  - Comedonecrosis, central calcification, and massive acinar distention may be seen.
  - No true intercellular lumens.
  - May mimic DCIS (see question 69).
  - Variants include (usually pleomorphic):
    - Apocrine
    - Signet ring
    - Histiocytoid

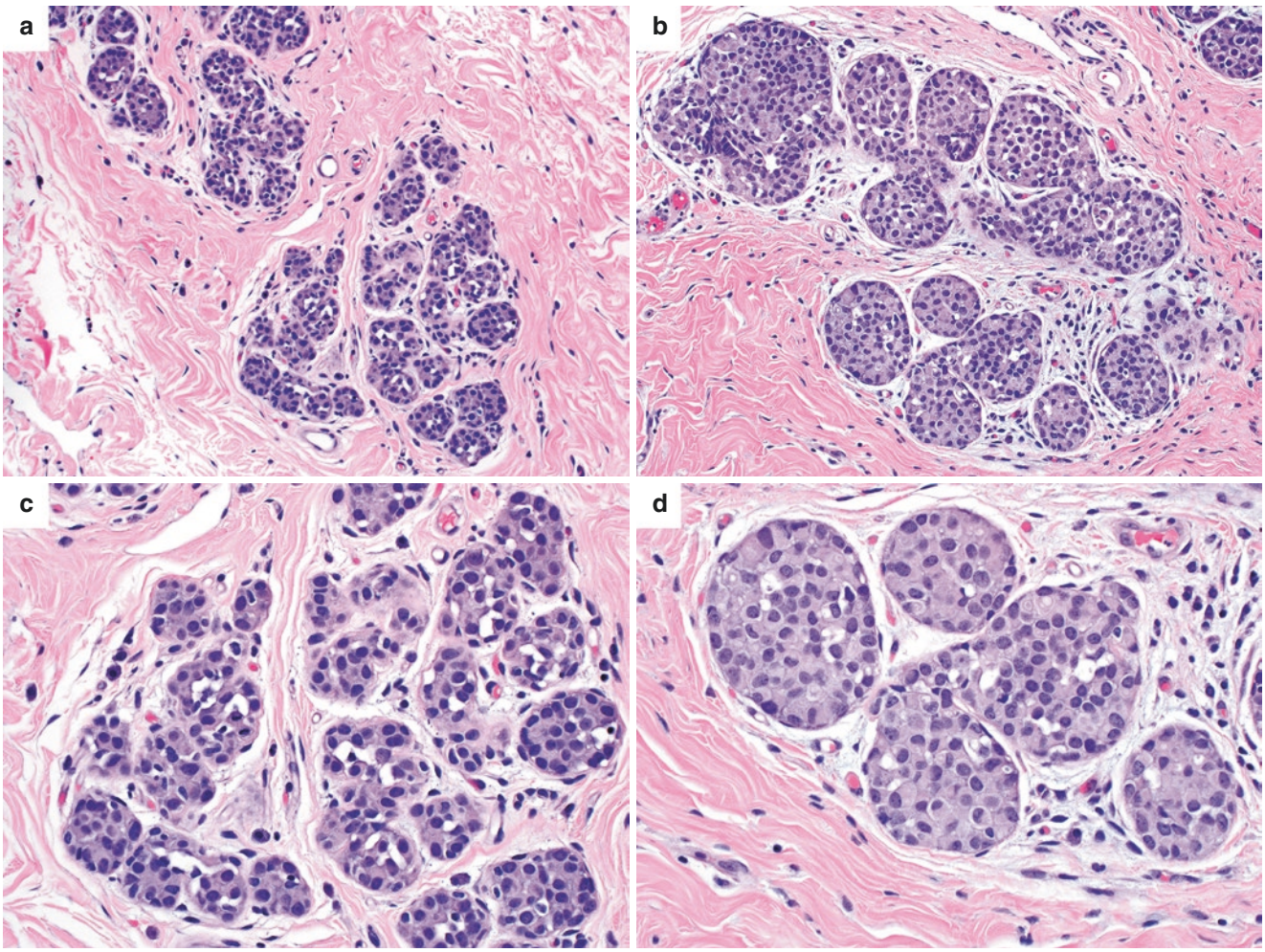


Fig. 4.32 ALH compared to LCIS. (**a**) These acini are colonized by lobular cells, but hardly distended (200×). (**b**) In LCIS, acini are filled and markedly expanded by a similar population (200×). (**c**) ALH at higher power (400×). (**d**) LCIS at high power (400×)

- Florid LCIS: "Classic" LCIS cytology with (Fig. 4.32a–f) [18, 116, 129, 149]:
  - Massive acinar or ductal distention, usually with little or no stroma between markedly distended acini
  - Central necrosis and associated calcifications
- Variants make up about 2–5% of LCIS [152].

61. Why is it important to recognize pleomorphic and florid LCIS?

Although outcome data remain relatively sparse, pleomorphic and florid LCIS have cytologic, architectural, immunophenotypic, molecular, and clinical features of more aggressive in situ neoplasia as compared to classic LCIS [116, 129, 151–156]. Pleomorphic and florid LCIS were likely diagnosed DCIS in past decades and thus treated as such; this management likely remains relevant despite the reclassification.

- Selected data from an excellent study summarizing immunophenotypic and molecular features (comparative genomic hybridization) are listed in Table 4.6 [155].
- Pleomorphic and florid LCIS on core biopsy is frequently associated with invasive carcinoma and/or DCIS at excision [156]. Thus, excision is recommended for pleomorphic, florid, or mass-forming in situ lobular lesions identified on core biopsy [116, 129–131, 139, 149].
  - Recent reviews of the literature calculated a 30% rate for pleomorphic LCIS on core upgrading to invasive carcinoma on excision and 8% upgrade to DCIS on excision [156, 157].
  - Florid LCIS has been associated with concomitant invasive lobular carcinoma in up to 70% of cases, based on small studies [116, 158, 159].

Table 4.6 Immunophenotypic and molecular features of pleomorphic lobular carcinoma

Parameter	LCIS, classic	LCIS, florid	LCIS, pleomorphic apocrine	LCIS, pleomorphic non-apocrine
Estrogen receptor +	100%	92%	23%	100%
Her2+	0%	18%	31%	0%
Ki-67	4.2%	Not done	13.9%	9.9%
Fraction genome altered	0.072	0.109	0.119	0.054
Breakpoints	3.8	11.55	3.15	5.86
Amplifications, #	0.25	2.1	5.00	0.077

Data from Ref. [155]
LCIS lobular carcinoma in situ

- The management of surgical margins in the setting of pleomorphic LCIS is more controversial and is discussed in question 82.
- An explanatory comment in the pathology report is recommended to explain the clinical significance of these variants.

62. Can LCIS have comedonecrosis and calcifications (Figs. 4.28a–c and 4.32a–f)?

Classic LCIS is very rarely associated with comedonecrosis and calcifications. However, florid and pleomorphic LCIS often do have comedonecrosis and/or calcifications and may mimic DCIS on mammography [18, 139, 149].

63. Is LCIS graded?

Grading of LCIS is not required by College of American Pathologists (CAP) or American Joint Commission on Cancer (AJCC). However, it is important to recognize and report LCIS with high-grade nuclei or aggressive features (pleomorphic LCIS, florid LCIS). (See questions 60–62.)

64. How does ALH/LCIS translate to LIN nomenclature?

LIN designates "Lobular Intraepithelial Neoplasia" a nomenclature championed by Tavassoli, Eusebi, and others. The LIN scheme eliminates the name "carcinoma" and acknowledges the qualitative and quantitative spectrum of such lobular proliferations. This nomenclature has not been widely adopted in the USA, but is more broadly applied in Europe. As defined by Tavassoli and colleagues [131, 160]:

- LIN1: "partial or complete replacement…by a proliferation of generally uniform cells with poorly defined margins which may fill, but do not distend, the acinar lumens (in comparison to adjacent uninvolved acini)." Often loosely cohesive proliferation, but with residual acinar lumens.
  - Similar to ALH
- LIN2: "more abundant proliferation of similar cells than in LIN1, which fill and actually distend some or all acini, but the acinar outlines remain distinct….with the persistence of intervening lobular stroma; residual lumens may persist in some acini."
  - Similar to LCIS
- LIN3: proliferation of cells similar to LIN1–2, "but there is a massive degree of acinar distension to the point that the acini appear almost confluent." "When there is necrosis or the proliferating cells are completely of the pleomorphic or signet ring cell type, significant acinar distension is not required."
  - Similar to florid and/or pleomorphic LCIS

65. **How does LCIS/ALH involve ducts** (Fig. 4.33a–e)**?**

Lobular neoplasia usually involves ducts in a so-called "pagetoid" fashion.

- LCIS/ALH undermines the ductal epithelium, colonizing the duct with layer(s) of neoplastic cells between the ductal (luminal) epithelium and myoepithelial cells [14, 129, 130, 139, 149, 150].
- The presence of lobular cells may alter the contour of the duct, resulting in characteristic architecture described as budding, sawtooth, or cloverleaf [14, 129, 130, 139, 149, 150].
  - The basement membrane remains intact.
  - The myoepithelial cell layer is generally continuous, although myoepithelial cells may be seen admixed with lobular cells [129, 130, 161, 162].
- More abundant LCIS/ALH may fill a small duct, resulting in a solid pattern and must be distinguished from low-grade DCIS [130].
- Duct extension of LCIS/ALH may even involve larger or lactiferous ducts.

66. **Does Paget's disease of the nipple arise in association with LCIS?**

LCIS rarely colonizes nipple ducts; in those cases, it could theoretically extend to the squamous epithelium of the epidermal surface [163]. However, this is extremely rare and should prompt further diagnostic confirmation (LCIS vs. DCIS, Toker cells, melanocytes, etc.).

67. **How can pagetoid ductal involvement by ALH/LCIS be distinguished from prominent myoepithelium?**

- Nuclei of myoepithelial cells are generally smaller and darker than ALH/LCIS [129].
- Epithelioid myoepithelial cells are most often present as a single layer, whereas lobular neoplasia may be multilayered [129].
- Immunohistochemistry may be helpful in perplexing foci. (See questions 23 and 71.)
  - Myoepithelial cells:
    - Positive for p63 (nuclear). Usually positive for smooth muscle myosin heavy chain, calponin,

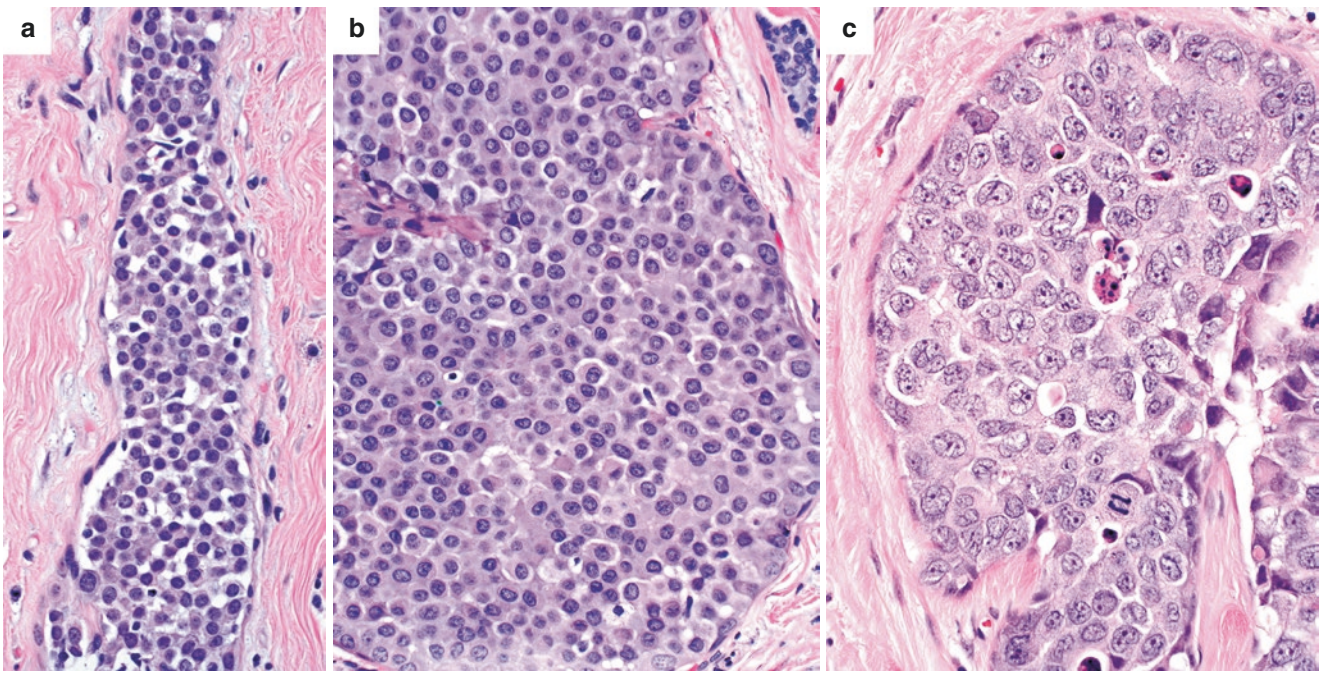


Fig. 4.33 Cytologic heterogeneity of lobular neoplasia in situ. (**a**) Small dyscohesive cells with eccentric small nuclei with fine chromatin ("Type A," 400×). (**b**) Larger dyscohesive cells with moderate amounts of pale cytoplasm, larger vesicular nuclei, and small nucleoli ("Type B," 400×). (**c**) Pleomorphic LCIS. Large dyscohesive cells with eccentric cytoplasm, large vesicular irregular nuclei. Note focal necrosis (central) and a mitotic figure, lower (400×)

although the myoid staining may be diminished if epithelioid [129]. Also positive for CD10, smooth muscle actin (SMA), often D2–40.
    - Positive for E-cadherin, membranous p120
  - ALH/LCIS:
    - Negative for p63 (nuclear), smooth muscle myosin heavy chain, calponin
    - Negative for E-cadherin, cytoplasmic granular p120

68. How is ALH/LCIS distinguished from low-grade DCIS?

Distinguishing features include (Fig. 4.34a–h) [18, 130]:

- ALH/LCIS lacks intraductal architecture such as cribriform or punched out spaces (secondary lumens).
  - Partial involvement or LCIS "colonizing" usual hyperplasia, adenosis, or papillary lesions may leave residual architecture.
- ALH/LCIS is composed of non-polarized, dyscohesive cells, in contrast to low-grade DCIS; DCIS has more distinct cell membranes and cells that polarize around neo-lumens.
- ALH/LCIS more commonly demonstrates intracytoplasmic mucin vacuoles (although DCIS can as well).
- ALH/LCIS may show pagetoid ductal involvement (although DCIS can as well; see question 65).
- Solid pattern low-grade in situ carcinoma can be particularly challenging to determine if ductal or lobular. Subtle microacini and crisp/distinct cell borders favor a ductal process. Immunostains can be helpful in distinguishing lobular from ductal. (See questions 23 and 71 for stain details and pitfalls.)
  - ALH/LCIS is E-cadherin negative (beta-catenin negative, p120 cytoplasmic).
  - DCIS is E-cadherin positive in a membranous pattern (beta-catenin and p120 also membranous).

69. How is pleomorphic LCIS distinguished from high-grade DCIS?

Distinguishing features include (Fig. 4.35a–d) [130]:

- Pleomorphic LCIS lacks intraductal architecture such as cribriform or punched out spaces (secondary lumens).
- Pleomorphic LCIS is composed of non-polarized, high-grade dyscohesive cells.
- Pleomorphic LCIS more commonly demonstrates intracytoplasmic mucin vacuoles.
- Pleomorphic LCIS may be accompanied by classic ALH/LCIS and show pagetoid ductal involvement.
- Pleomorphic LCIS with comedonecrosis and calcification may closely mimic high-grade DCIS, and the possibility of LCIS is important to consider. Immunostains can be especially helpful with this differential. (See Table 4.3 and questions 23 and 71 for stain details and pitfalls.)
  - Pleomorphic LCIS is E-cadherin negative (beta-catenin negative, p120 cytoplasmic).
  - DCIS is E-cadherin positive in a membranous pattern (beta-catenin and p120 also membranous).

70. What molecular mechanisms are responsible for the characteristic features of ALH/LCIS?

Loss or dysfunction of the cell-cell adhesion molecule E-cadherin is apparent in ALH and LCIS, as well as invasive lobular carcinoma. See Fig. 4.36a–e. E-cadherin function is discussed in question 20 [14, 18, 32–40, 116, 129–131, 139, 148, 149, 164, 165].

71. How is E-cadherin and catenin immunohistochemistry utilized to discriminate lobular from ductal differentiation?

ALH/LCIS, like invasive lobular carcinoma, is characterized by loss of functional E-cadherin, and E-cadherin immunohistochemistry is a helpful ancillary tool in differentiating in situ ductal and lobular neoplasia (Table 4.3, Figs. 4.12, 4.34a–h, 4.37a–c, and 4.38a, b) [14, 18, 32–40, 116, 129–131, 139, 148, 149, 164, 165]. Stain interpretation and caveats are thoroughly discussed in question 23. Additional features unique to the in situ situation are below:

- In situ proliferations have surrounding or admixed myoepithelial cells; these have intact membranous E-cadherin and catenin and should not be misconstrued as lesional cell staining [162].
- A small population of ductal cells may be present together with a lobular proliferation; the ductal population would be E-cadherin and catenin positive (membranous) and should be separately scored (Figs. 4.33a–e, 4.37a–c, and 4.38a, b).

72. Must E-cadherin immunostaining be performed in order to render a diagnosis of ALH/LCIS?

No, it is not essential to demonstrate loss of E-cadherin in diagnosing lobular neoplasia [57]. The diagnosis can be made based on typical architectural and cytologic features [14, 40, 57, 116, 129–131, 139, 149].

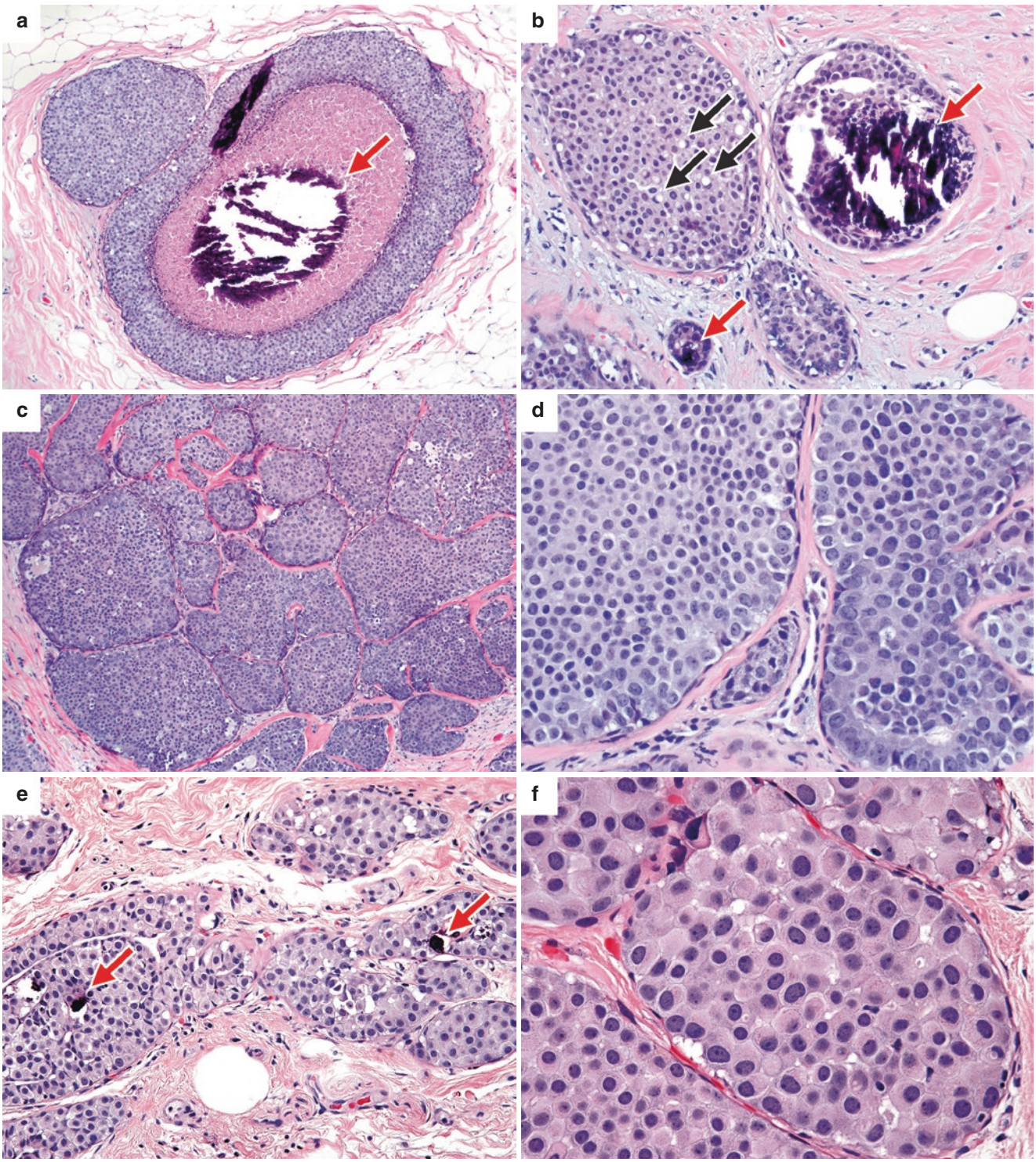


Fig. 4.34 Florid and pleomorphic LCIS. (**a**) Ductal structure floridly distended by pleomorphic LCIS with central comedonecrosis and large calcification (red arrow), E-cadherin negative (not shown, 100×). (**b**) Large and small calcifications (red arrows) associated with pleomorphic LCIS without necrosis. Some cells have intracytoplasmic lumens and signet ring nuclei (examples, black arrows, 200×). (**c**) Florid LCIS with massive acinar distension, so as to be almost confluent (100×). (**d**) Pleomorphic LCIS involves the right space; notice the degree of nuclear enlargement and hyperchromasia compared to the LCIS in the center of the left space (400×). (**e**) This pleomorphic LCIS has associated microcalcifications (arrows, 200×). (**f**) Pleomorphic LCIS with markedly enlarged nuclei, which remain somewhat round and uniform, and abundant eosinophilic cytoplasm, possibly apocrine (400×)

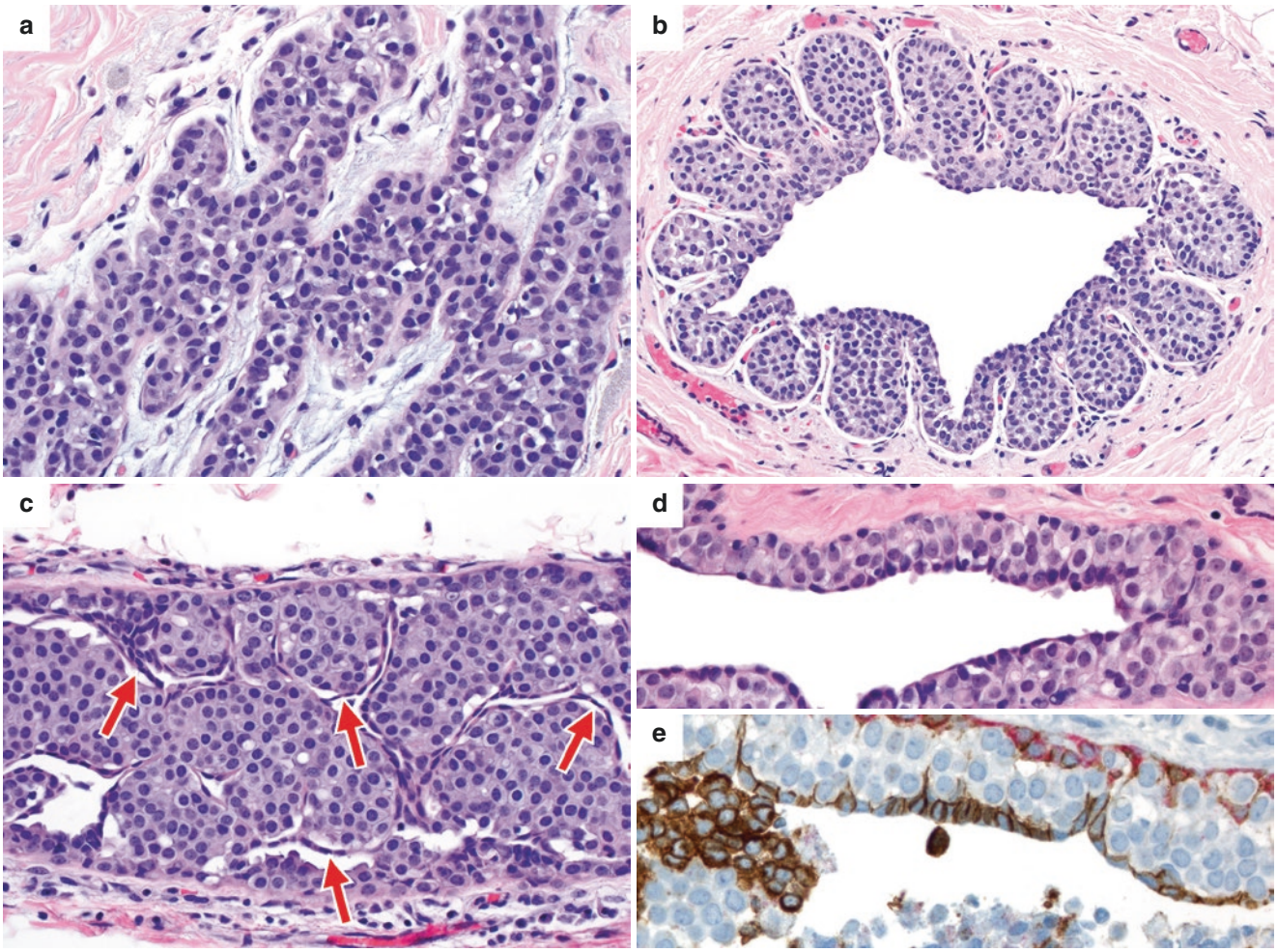


Fig. 4.35 Lobular neoplasia involving ducts. (**a**) Small duct colonized by ALH with "sawtooth" architecture (400×); typical lobular cells are apparent, with some residual lumen (400×). (**b**) Larger duct with blunt buds due to involvement by ALH (200×). Neoplastic lobular cells insinuate between luminal ductal cells and myoepithelium/basement membrane. (**c**) In this small duct, the proliferation of lobular cells protrudes inward, and each nodule is lined by a layer of attenuated ductal cells (red arrows, examples, 400×). (**d**) Small duct colonized by lobular neoplasia (400×). (**e**) Immunostained serial section demonstrating E-cadherin-negative lobular cells between E-cadherin-positive luminal ductal cells (brown stain) and smooth muscle myosin heavy chain-positive myoepithelial cells (red stain; vague brown E-cadherin positivity is also seen in myoepithelial cells, 400×)

73. Are there recurrent genetic changes in ALH/LCIS (mutations, chromosomal gains/losses)?

Like invasive lobular carcinoma, LCIS very frequently contains *CDH1* (E-cadherin), as well as *PIK3CA* mutations [9, 14, 48, 116, 129–131, 139, 149, 166, 167].

- ALH/LCIS are early lesions in the "low-grade" molecular progression pathway, characterized by genomic changes including [9, 48, 140, 166–169]:
  - Loss of 16q (location of *CDH1* gene)
  - Gain of 1q
- LCIS shares point mutations as well as genomic gains and losses with a significant fraction of synchronous invasive lobular carcinoma and DCIS, though less so with invasive ductal carcinoma [116, 140–142, 170].

74. Can ALH/LCIS and DCIS occur together?

Lobular neoplasia and DCIS can be seen within the same specimen and may even coexist within the same acinar or ductal space. This is seen as two discrete populations (Fig. 4.39a–h) [40, 116]:

- One with the cytologic and architectural features of ALH/LCIS.
- Another with different cytologic and/or architectural features of DCIS (of any grade).
- Immunohistochemistry can be helpful to highlight the two distinct populations (Table 4.3).
  - Lobular component: E-cadherin negative (beta-catenin negative, p120 cytoplasmic)

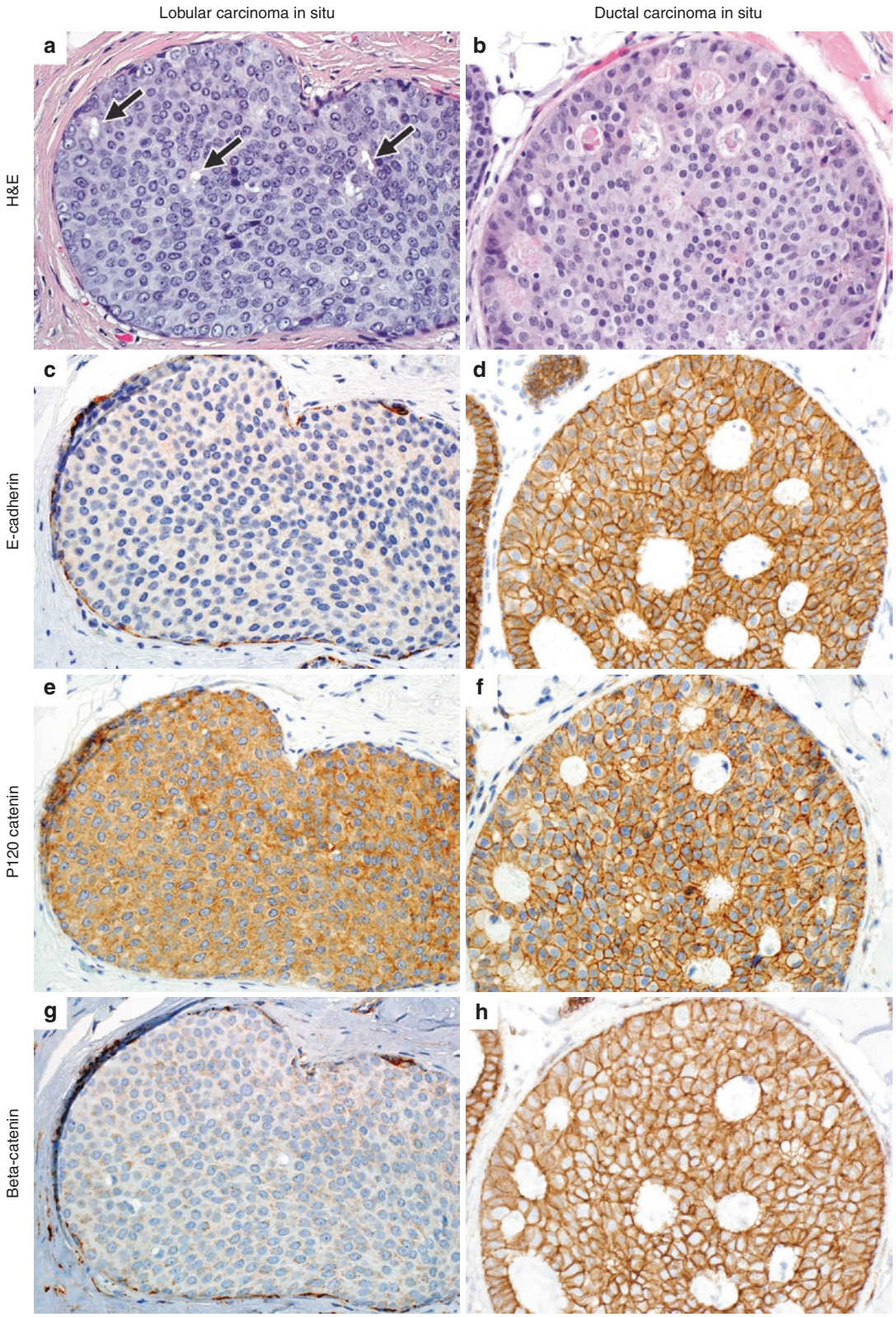


Fig. 4.36 Morphology and immunophenotype of classic LCIS and DCIS. Left column: LCIS with subtle residual spaces (arrows); LCIS is usually solid. Serial sections stained with (**a**) H&E, (**c**) E-cadherin, (**e**) p120 catenin, (**g**) beta-catenin (all 400x). In typical lobular carcinomas, E-cadherin and beta-catenin are negative, while p120 catenin demonstrates cytoplasmic staining, sometimes granular in texture, without membranous accentuation. Right column: DCIS in serial sections. H&E (**b**) shows low-grade cells with some cytologic resemblance, but different architecture than LCIS, including multiple round sharply punched out spaces. There is cell membrane localization of (**d**) E-cadherin, (**f**) p120 catenin, (**h**) beta-catenin. Also see diagrammatic Fig. 4.12

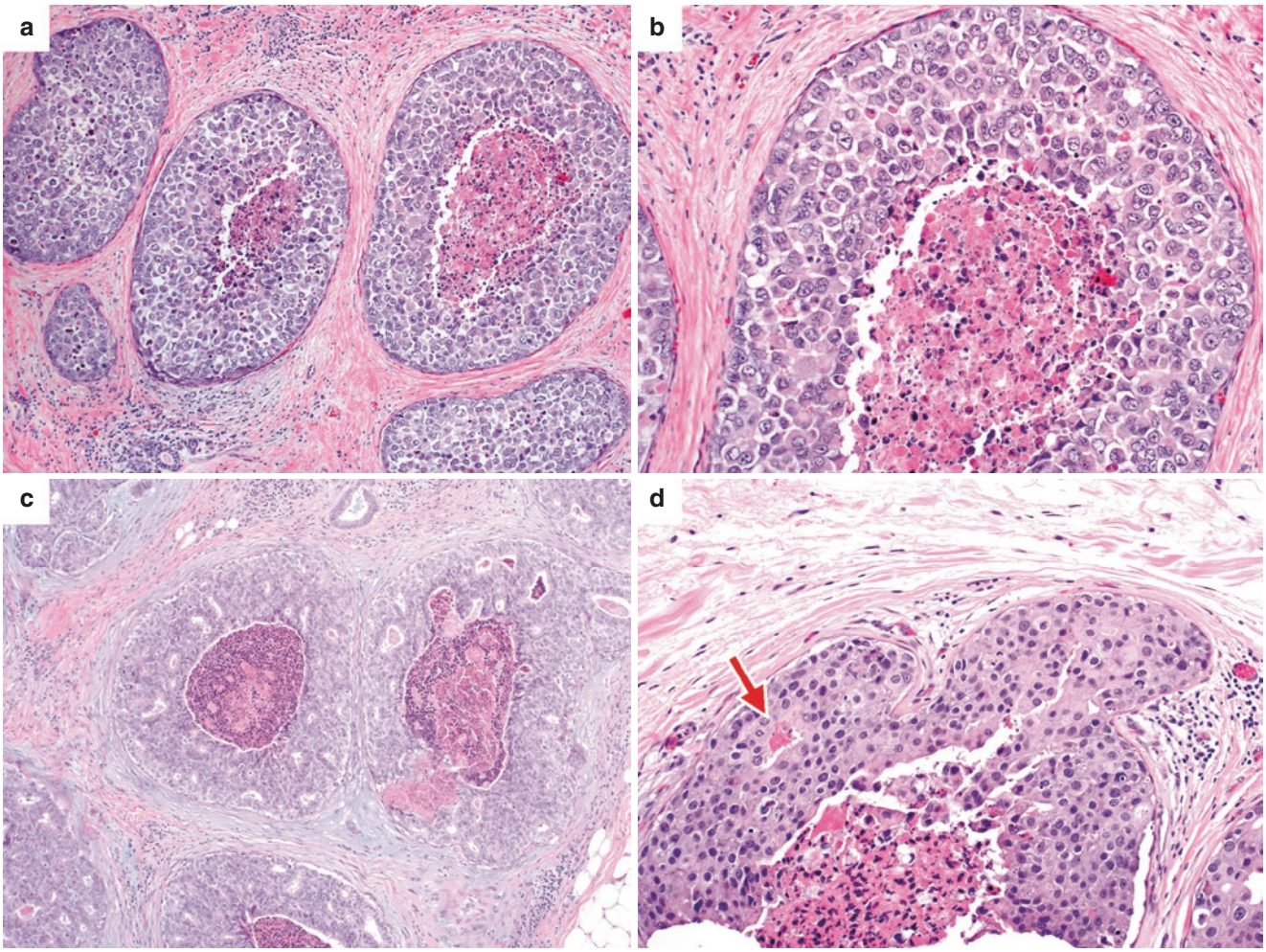


Fig. 4.37 Pleomorphic LCIS compared to DCIS. (**a**) Low-power view of pleomorphic LCIS with ductal spaces markedly distended by a solid proliferation of malignant cells with comedonecrosis (100×). (**b**) Higher power of the left space shows high grade cells with vesicular eccentric nuclei and dyscohesion. The morphology favors pleomorphic LCIS with a differential of high grade DCIS; E-cadherin was negative (not shown), confirming pleomorphic LCIS (200×). (**c**) Cribriform DCIS with comedo necrosis; this architecture would not be seen in LCIS (100×). (**d**) High grade DCIS with comedo necrosis has a rare intraductal lumen (red arrow) and different cytology as compared to pleomorphic LCIS (200×)

  - Ductal component: E-cadherin positive, membranous (beta-catenin and p120 membranous; see questions 23 and 71)
- It is prudent to make separate diagnoses of DCIS and LCIS (or ALH) with an explanatory comment. Alternative nomenclature such as "mixed ductal and lobular lesion" could be considered [116].
- Treatment should follow that of DCIS (unless pleomorphic LCIS is the higher-grade lesion).

75. How is "carcinoma in situ with mixed ductal and lobular features" defined?

The 2012 WHO monograph suggests a diagnosis of "carcinoma in situ with mixed ductal and lobular features" when "after careful assessment of morphologic and immunohistochemical features, a case cannot be definitively classified as DCIS or LCIS" [116].

- Recommendations for management of margins in this scenario are not provided.
- This is a very rare scenario, and additional data are needed. It is unclear whether the above statement is widely accepted, and this concept will likely evolve.
- Bratthauer and colleagues have suggested the nomenclature "Mammary Intraepithelial Neoplasia" (MIN) for this circumstance [160].
- This nomenclature is different than naming of intraductal proliferations that contain discrete areas of E-cadherin-negative cytologically lobular cells and E-cadherin-positive cytologically ductal cells. (See question 74.)

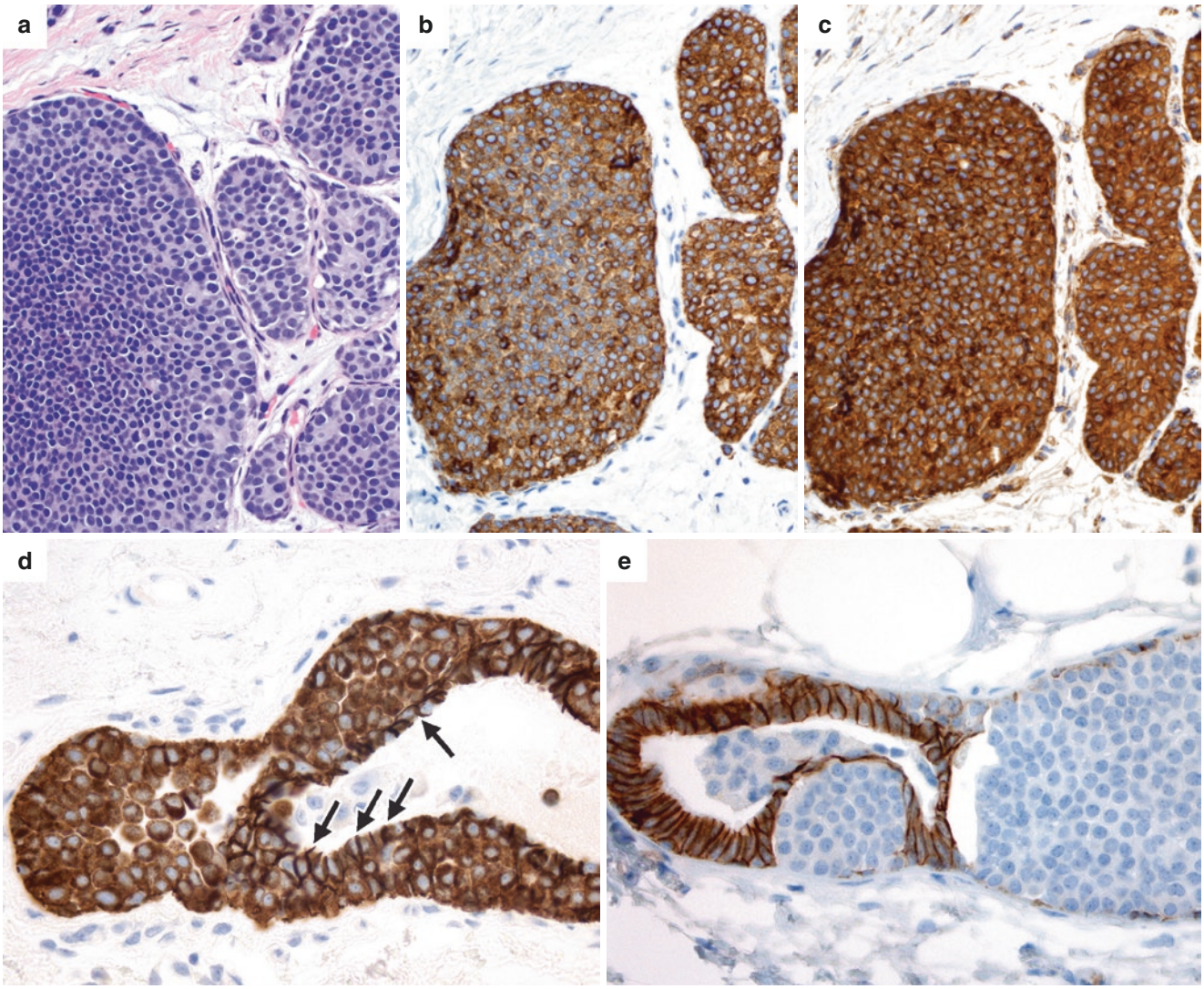
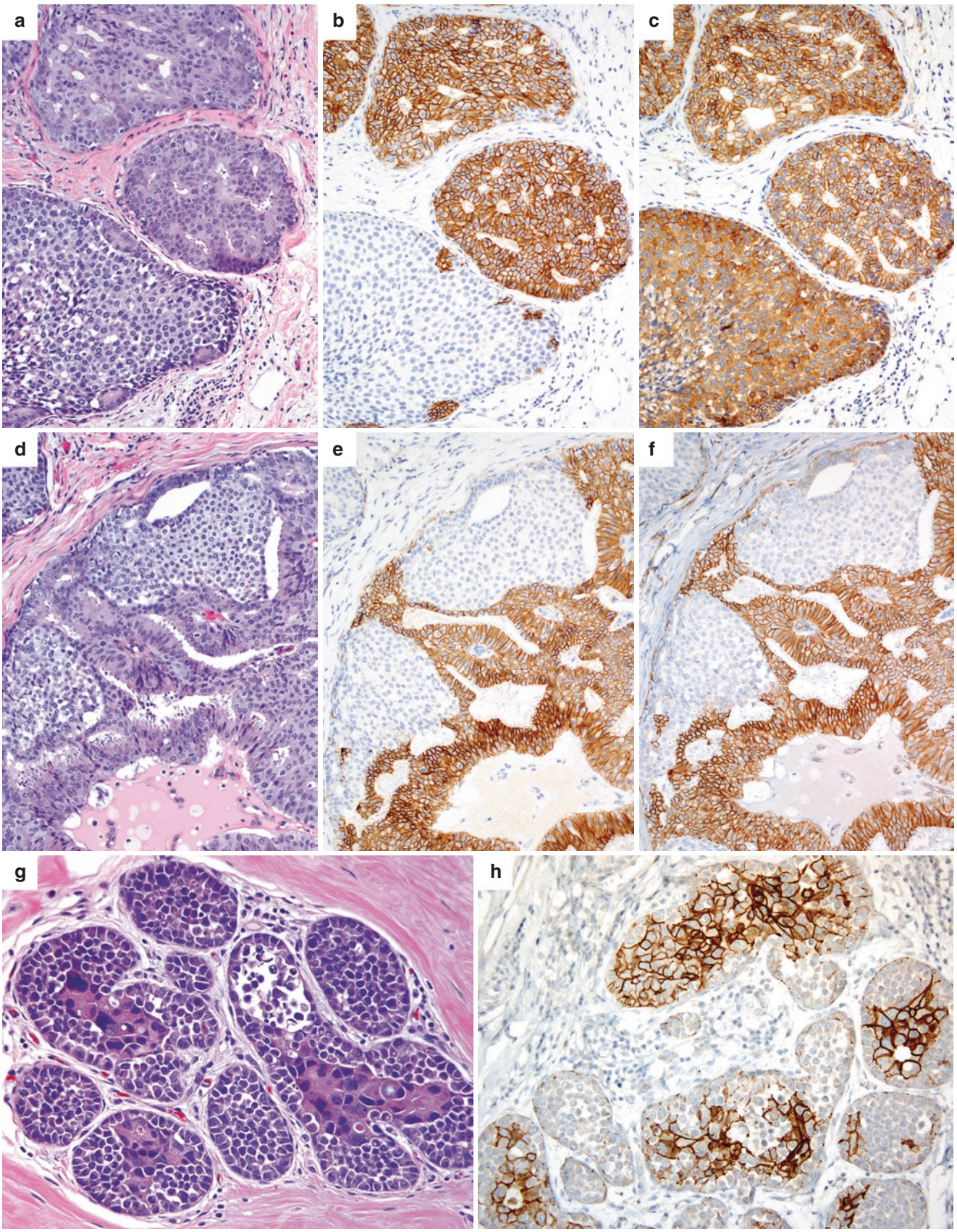


Fig. 4.38 Aberrant E-cadherin with cytoplasmic localization in LCIS. (**a**) H&E sections demonstrate an in situ proliferation. (**b**) E-cadherin is strongly positive in the CIS, but is not localized to the cell membranes. Instead, it is evenly localized through the cytoplasm. (**c**) Cytoplasmic p120 localization confirms E-cadherin dysfunction and lobular differentiation (LCIS). (**d**) Another case of in situ lobular neoplasia with cytoplasmic E-cadherin stain. Remnant ductal epithelium lining the lumen has a subtlety different cell membrane staining (arrow). (**e**) A different E-cadherin antibody is negative in the lobular proliferation of the same case, with the remnant ductal cells showing obvious membrane labeling

Fig. 4.39 LCIS and DCIS together. (**a**) H&E shows intermediate-grade DCIS in the top 2 spaces, with cribriform architecture. The space at bottom left contains a cytologically and architecturally different population of dyscohesive cells with paler cytoplasm; focal DCIS is seen at the periphery (200×). (**b**) E-cadherin staining is positive (membranous) in DCIS and completely negative in LCIS (200×); beta-catenin had the same staining pattern (not shown). (**c**) p120 catenin shows membranous positivity in DCIS, but has cytoplasmic localization (non-membranous) in LCIS (200×). (**d**) Another field from the same case with serpiginous DCIS in a large duct, nodules of pale LCIS are seen at top and left (200×). (**e**) DCIS is E-cadherin positive (membranous), while LCIS is negative (200×). (**f**) Beta-catenin shows the same staining pattern as E-cadherin (200×). p120 was cytoplasmic in the LCIS, though weak (not shown). (**g**) In a different case, highly pleomorphic squamoid DCIS is central and surrounded by pleomorphic LCIS characterized by dyscohesive cells with scant cytoplasm (200×). (**h**) In a different field, E-cadherin stains the central DCIS and is negative in peripheral LCIS (200×)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76. Can LCIS/ALH occur with invasive ductal carcinoma? DCIS with invasive lobular carcinoma?

An estimated 15–25% of DCIS are associated with invasive lobular carcinoma, and likewise, LCIS may be associated with invasive ductal carcinoma (Fig. 4.40a–e) [116, 131, 170, 171]. Low-grade, estrogen receptor-positive DCIS and invasive ductal lesions tend to share genomic changes with synchronous lobular processes [140, 171, 172], whereas high-grade ductal components are less likely to have common genomic alterations.

77. Can LCIS/ALH involve proliferative breast lesions such as sclerosing adenosis, papillary lesions?

Lobular neoplasia can coexist with other breast lesions, seemingly "colonizing" the epithelium (Fig. 4.41a–d). The same diagnostic criteria are applied. Myoepithelial stains may be helpful in ruling out invasive carcinoma if LCIS/ALH involves sclerosing lesions such as sclerosing adenosis or radial scar [14, 40, 116, 129].

78. Can LCIS/ALH involve collagenous spherulosis?

Lobular neoplasia has some propensity for involvement of collagenous spherulosis (Fig. 4.41a–d) [40, 173, 174]. LCIS was identified in 25% of collagenous spherulosis in one series [173]. As such, it can be a difficult mimic of DCIS, given the cribriform-like spaces [116, 129, 173, 174]. Unlike DCIS, collagenous spherulosis with LCIS:

- Contains eosinophilic basement membrane material within the "punched out" spaces.
- Contains a mixed population of myoepithelial cells, ductal epithelial cells, and luminal epithelial cells, with the

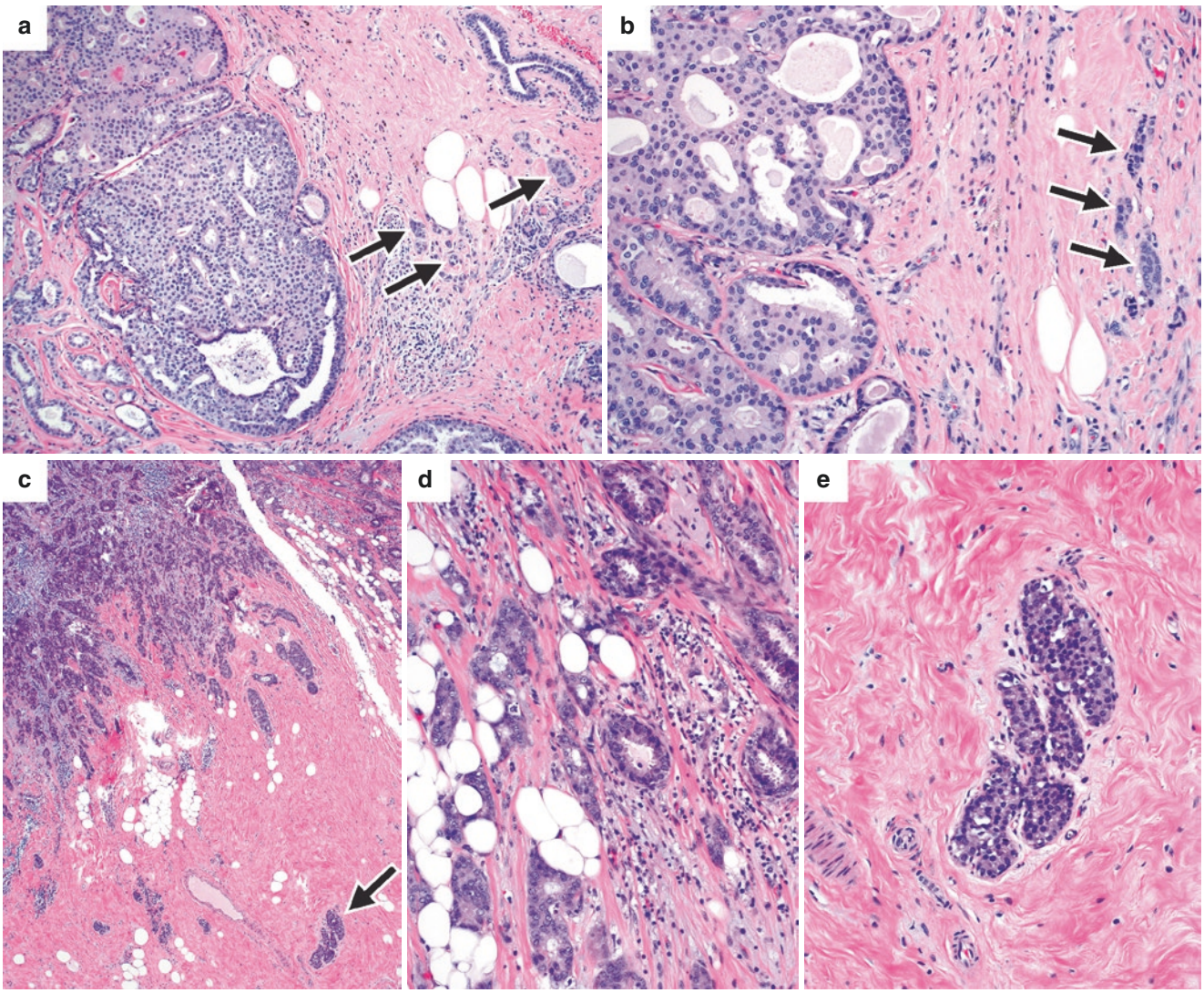

Fig. 4.40 Ductal and lobular lesions in the same specimen. (**a**) Invasive lobular carcinoma (right, black arrows) and low-grade DCIS involving a sclerosing lesion (right, 100×). (**b**) Higher power of another field also shows invasive lobular carcinoma (black arrows) and DCIS (left). (**c**) Invasive ductal carcinoma and ALH (black arrow, 40×). (**d**) Higher power of invasive ductal carcinoma (200×). (**e**) Higher power of ALH (400×)

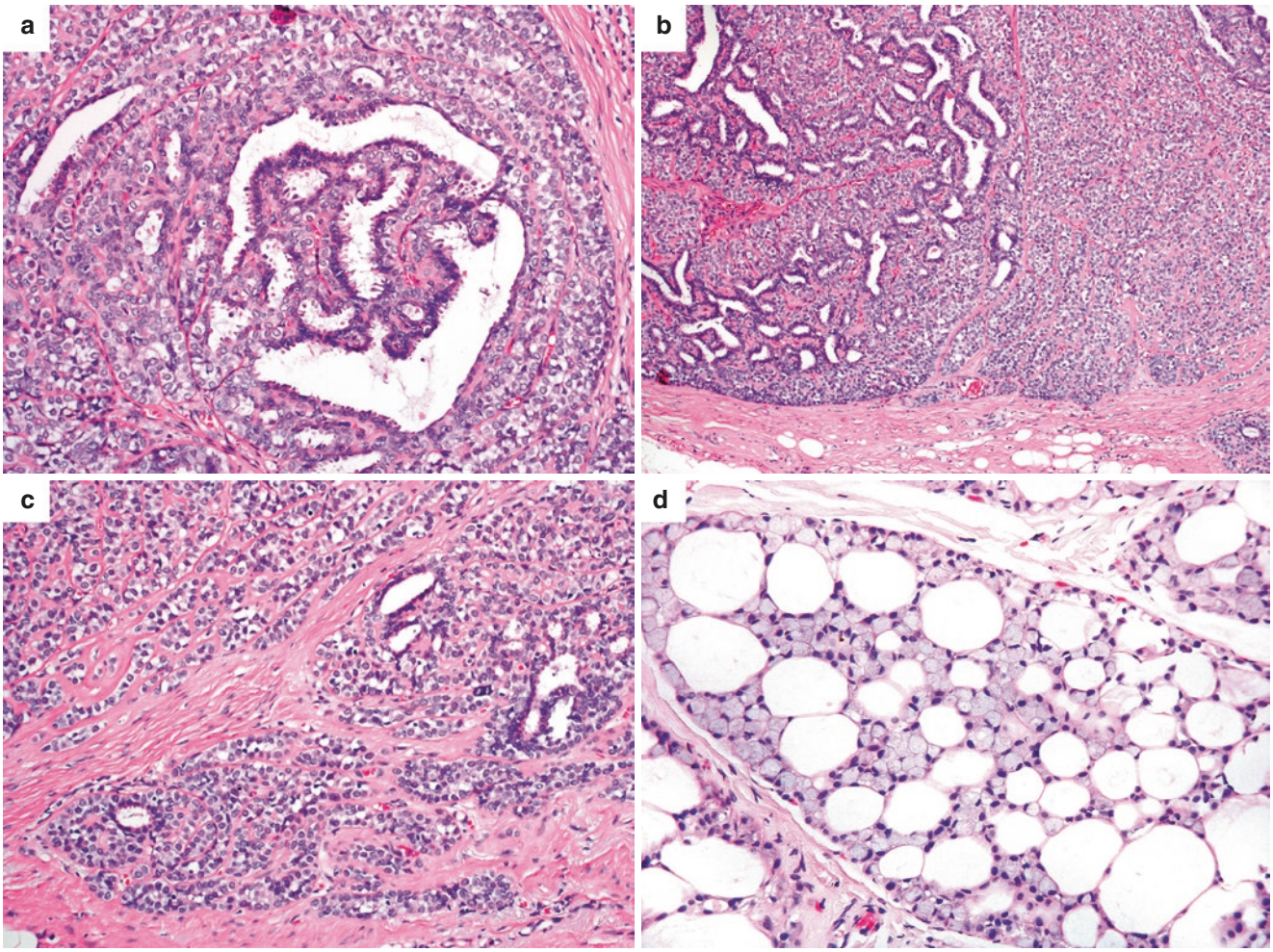

Fig. 4.41 LCIS in involving other proliferative lesions. (**a**) Lobular neoplasia colonizes a papillary lesion. The pale cells underlying the columnar epithelium are lobular (200×). (**b**) LCIS involving adenosis. The border is circumscribed and slit-like architecture with remnant ductal epithelium is seen at left (100×). (**c**) A higher-power view of another area from the same lesion with pale lobular cells throughout. Immunostains would be prudent to rule out an invasive component (not shown, 200×). (**d**) LCIS involving collagenous spherulosis. The punched out spaces are due to the underlying architecture of collagenous spherulosis; the basement membrane component is not apparent in this section. LCIS with cytoplasmic mucin is seen throughout, mixed with the myoepithelial component (400×)

myoepithelial cells surrounding the spaces; these can be highlighted by immunohistochemistry (p63, calponin, smooth muscle myosin heavy chain) [129].
- Contains an E-cadherin-negative (p120 cytoplasmic) population [129]. Care should be taken in stain interpretation, as this will be a mixed population.
- May contain microcalcifications, in up to 40% [174].
- May rarely occur with pleomorphic LCIS [174].
- Collagenous spherulosis is an incidental finding involving one or few ductal spaces [173, 174].

79. Can ALH/LCIS involve fibroepithelial lesions?

Lobular neoplasia may involve the epithelial component of a fibroepithelial lesion [129]. A single-center series documented 18 cases of ALH/LCIS in fibroadenomas (17) or phyllodes tumors (1) [175]. Sin and colleagues reported three examples within phyllodes tumors and along with other cases of concurrent phyllodes and nearby LCIS [176].

80. Is ALH/LCIS typically estrogen receptor positive?

Classic ALH/LCIS is almost invariably estrogen receptor positive [116, 149, 155, 177]. Florid or pleomorphic variants of LCIS have lower rates of estrogen receptor positivity (see question 61, Fig. 4.19a–d, Case 6) [129, 139, 149, 155, 177].

81. How should surgical margins be reported for classic ALH? LCIS? Pleomorphic LCIS?

In general, the relationship of classic ALH and classic LCIS to surgical margin does not need to be reported [116]. Given the higher-grade, more aggressive features of pleomorphic LCIS, and management considerations, documentation of

margin status is recommended for pleomorphic LCIS, florid LCIS, or LCIS with comedonecrosis at margin [116, 139].

82. What typically defines acceptable surgical margins for classic ALH? LCIS? Pleomorphic LCIS?

Traditionally, classic lobular neoplasia at surgical margin did not warrant re-excision. This practice has been reaffirmed by a recent evidence-based expert consensus guideline:

- "Classic lobular carcinoma in situ (LCIS) at the margin is not an indication for re-excision" [61].

However, given the biologic features of pleomorphic LCIS discussed previously (see question 61), there is greater concern for recurrence and subsequent invasive cancer after excision. Published rates vary between 0% and 50%, averaging 10% in a meta-analysis with widely variable length of follow-up, endocrine therapy, and margin status [152, 156, 178]. The management of margins remains uncertain if not controversial.

- The 2014 guideline document refrained from concrete recommendation, awaiting additional data [61].
- Some authors advocate for treatment similar to DCIS, in other words excision to clear or 2 mm margins [157, 179].
- The 2017 version of the NCCN guidelines stated in a footnote "Clinicians may consider complete excision with negative margins for pleomorphic LCIS, but this may lead to high mastectomy rate without proven clinical benefit."
- The WHO monograph and other groups advocate caution in recommending universal excision to negative margins for pleomorphic LCIS [116, 156]. Multifactoral analysis of extent of surgery anticipated, alternate risk management (hormonal therapy if ER positive), pathology review is recommended in such decisions [116].
- WHO 2012 recommends against re-excising margins in cases that contain pleomorphic LCIS, but have only classic LCIS at margin [116].

83. Is adjuvant therapy indicated for classic LCIS/ALH? Pleomorphic LCIS?

Anti-hormonal agents (tamoxifen, raloxifene, and aromatase inhibitors) have been shown to decrease the risk of subsequent breast cancer in patients with LCIS and atypical hyperplasia, lesions which are typically estrogen receptor positive (Table 4.7) [129, 147, 180–182]. Small case series have documented the use of such agents in patients with pleomorphic LCIS, if estrogen receptor positive, but there are no outcome studies [128, 151, 152, 178]. Cytotoxic chemotherapy is not indicated.

Table 4.7 Anti-hormonal therapy decreases risk of subsequent cancer in patients with ALH/LCIS, selected data

Selected ALH/LCIS studies	Subsequent cancer no chemoprevention	Subsequent cancer with chemoprevention
Fisher (NSABP-P1)		56% risk reduction tamoxifen
King (1980–2009 MSKCC)	7% at 5 years 21% at 10 years	3% at 5 years 12% at 10 years
Cuzick (IBIS-I trial)	LCIS: 28% 15-year risk LCIS: 17% 10-year risk	LCIS: 27% tamoxifen 15-year risk LCIS: 5% AI 10-year risk
Cuzick (IBIS-II trial)	LCIS: 17% 10-year risk ALH: 14% 10-year risk	LCIS: 5% AI 10-year risk ALH: 5% AI 10-year risk

Data from Ref. [181]
AI aromatase inhibitor, *ALH* atypical lobular hyperplasia, *LCIS* lobular carcinoma in situ

84. Is radiation therapy indicated for classic LCIS/ALH? Pleomorphic LCIS?

Radiation therapy is not currently recommended for diagnosis of classic LCIS/ALH (Fig. 4.42a, b) [129, 131, 133]. While some patients with pleomorphic LCIS receive radiation therapy [151, 152], there are no data to support this practice [NCCN]. Management considerations for LCIS versus DCIS and invasive carcinomas are summarized in Table 4.8.

85. Does ALH/LCIS occur in men?

Yes, although rare, ALH/LCIS has been reported in men. A recent SEER study documented 16 cases of male LCIS, with 100% 5- and 10-year cancer-specific survival [72].

Case Presentations

Case 1

- *History*: A 76-year-old woman with history of left invasive breast cancer treated with lumpectomy and radiation therapy 17 years previously, left excisional biopsy of encapsulated papillary carcinoma/DCIS 5 years previously. She has a new right breast asymmetry on mammography.
- *Imaging*: See Fig. 4.43a–f. There was a 5 mm asymmetry in the outer right breast on the craniocaudal mammographic view only. On craniocaudal tomosynthesis the mass was more apparent. Breast ultrasound demonstrated 9 mm irregular hypoechoic mass with size and location corresponding to mammographic finding. US-guided core biopsy with marker placement was performed.

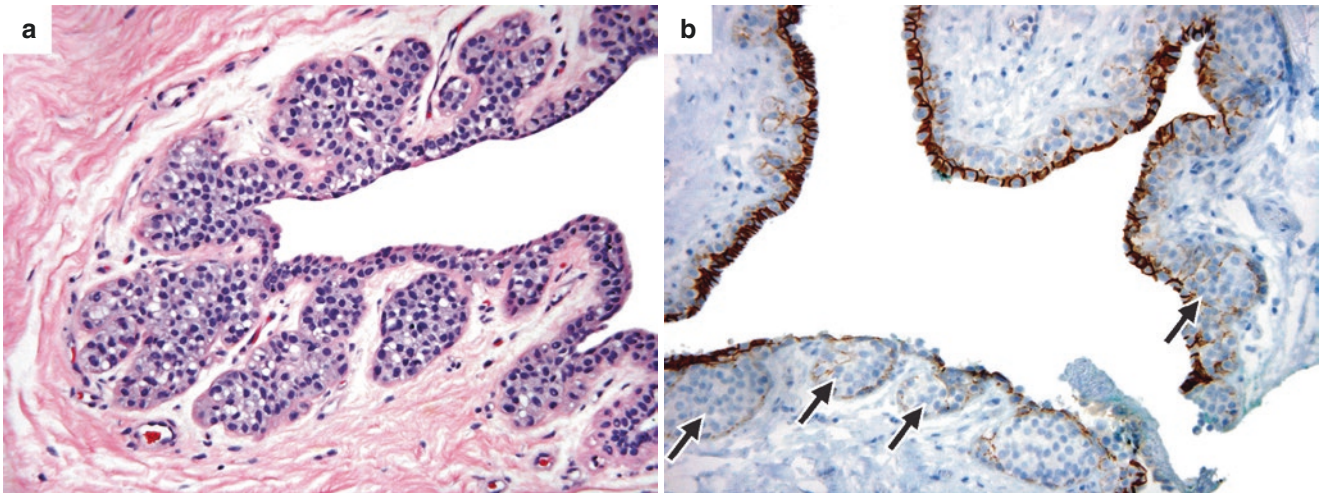

Fig. 4.42 Post-chemotherapy LCIS. (**a**) A ductal structure with pagetoid involvement by LCIS, without discernable treatment effect. (**b**) E-cadherin staining of a similar structure is negative in the buds of lobular cells (black arrows), with membrane staining of the ductal population (200×)

Table 4.8 Management considerations for lobular as compared to ductal in situ and invasive lesions

Lesion	Management after core biopsy diagnosis	Management of positive surgical margin	Radiation therapy in breast conservation	Adjuvant hormonal therapy (if ER+)
Classic LCIS	Often excise (correlate)	LCIS at margin OK	No	Consider
Pleomorphic LCIS	Excise	Consider re-excision	Not standard	Consider
DCIS	Excise	Re-excise (typically to 2 mm margins)	Standard	Standard
Invasive lobular	Excise (consider MRI for extent)	Re-excise (no cancer at ink)	Standard	Standard
Invasive ductal	Excise	Re-excise (no cancer at ink)	Standard	Standard

Clinical, radiologic, and pathologic correlation is recommended in individualizing management

- *Histologic Findings*: See Figs. 4.6a–i and 4.44a–f. Multiple tissue cores each demonstrated a sclerotic area of varying cellularity. High-power examination showed small epithelioid cells with scant cytoplasm.
- *Differential Diagnosis*: Invasive ductal carcinoma, invasive lobular carcinoma, scar with inflammation, scar with plump stromal cells
- *Ancillary Studies*: See Fig. 4.44a–f. The small cells were keratin positive, E-cadherin negative, and estrogen receptor positive (3+ >95%), although not necessary for diagnosis. The remainder of the invasive breast cancer prognostic panel showed PR positive (3+ 80%), Ki-67 5–10%, and Her2/neu negative (0) by immunohistochemistry, non-amplified by FISH.
- *Final Diagnosis*: Invasive lobular carcinoma, grade 1
- *Take-Home Messages*:
  - Invasive lobular carcinoma can be relatively occult on imaging.
  - Paucicellular invasive lobular carcinoma can be subtle in areas of sclerosis.
  - Keratin is the best diagnostic marker if immunostaining is needed for diagnosis.
  - Estrogen receptor is generally positive in classic infiltrating lobular carcinoma, but also stains a subset of stromal cells and lymphocytes and is not specific.

Case 2

- *History*: A 59-year-old woman with family history of breast cancer (3 of 4 sisters in their 50s, both grandmothers), with breast mass and separate area of skin dimpling. Genetic testing was negative on a panel of multiple breast cancer-associated genes.
- *Imaging*: In the left breast at the 6:00 position, 6 cm from the nipple, there is a 1.7 cm irregular mass, overlying the skin dimpling, with prior core biopsy diagnosed as invasive lobular carcinoma. At the area of mass, 3:00 6 cm from the nipple, a prior core biopsy demonstrated LCIS. MRI was not performed. Both areas were excised in separate lumpectomies, with sentinel lymph node biopsy.

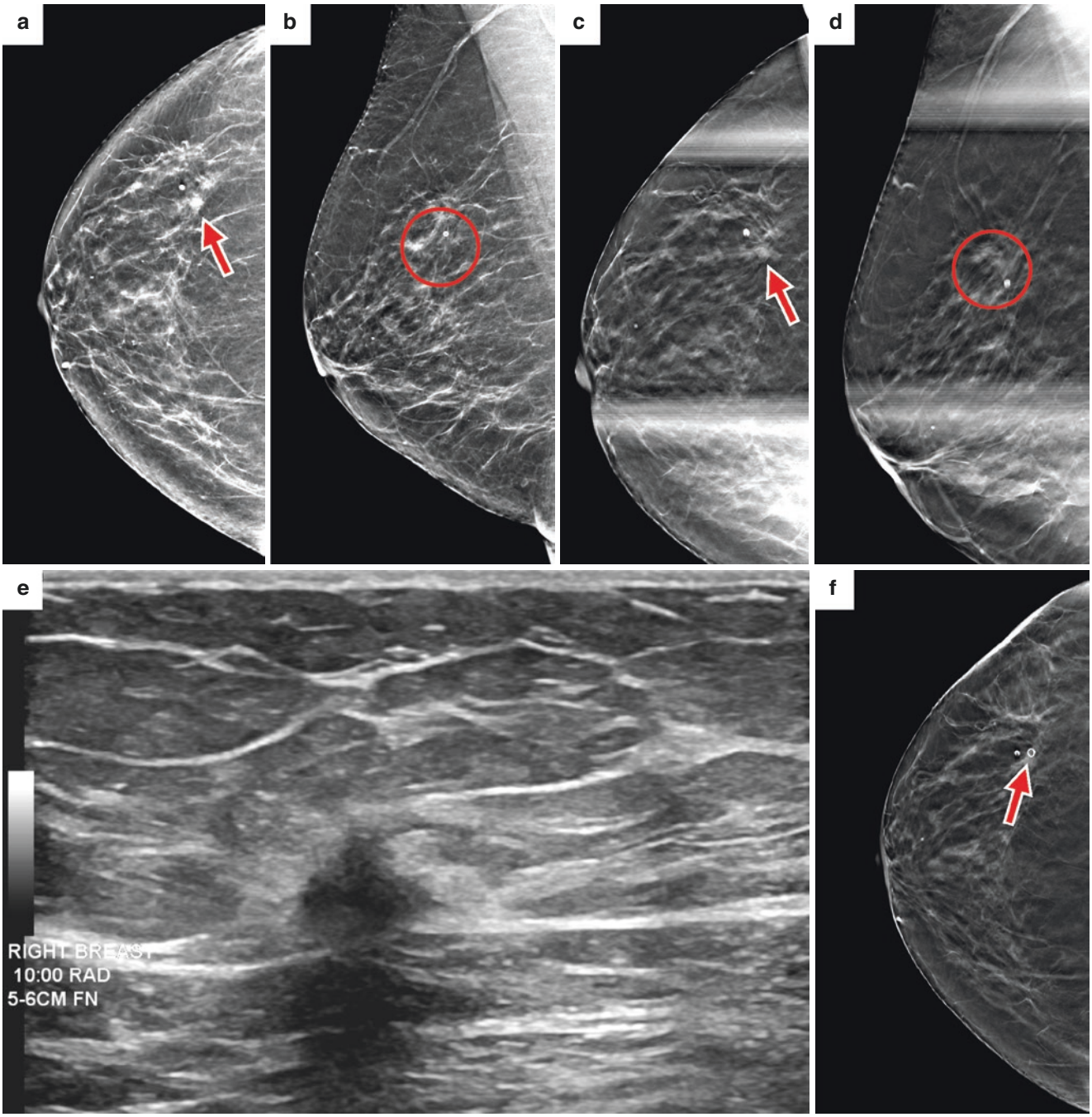

Fig. 4.43 Case 1. Right breast craniocaudal (**a**) and mediolateral oblique (**b**) synthetic digital mammograms show a 5 mm asymmetry in the outer right breast on the craniocaudal view only (arrow), not seen on the MLO view (circle). Craniocaudal tomosynthesis slice (**c**) shows the mass better (arrow), but the spot MLO tomosynthesis slice (**d**) shows only architectural distortion (circle) and no mass in the upper breast. (**e**) Real-time breast ultrasound shows a 9 mm irregular hypoechoic mass with posterior acoustic shadowing corresponding in size and location to the mammographic finding. Ultrasound-guided core biopsy with marker placement. (**f**) After ultrasound-guided core biopsy of the mass, a post-biopsy tomosynthesis craniocaudal mammograms shows the marker (arrow), demonstrating that the mass identified by ultrasound corresponded to the tomosynthesis mass

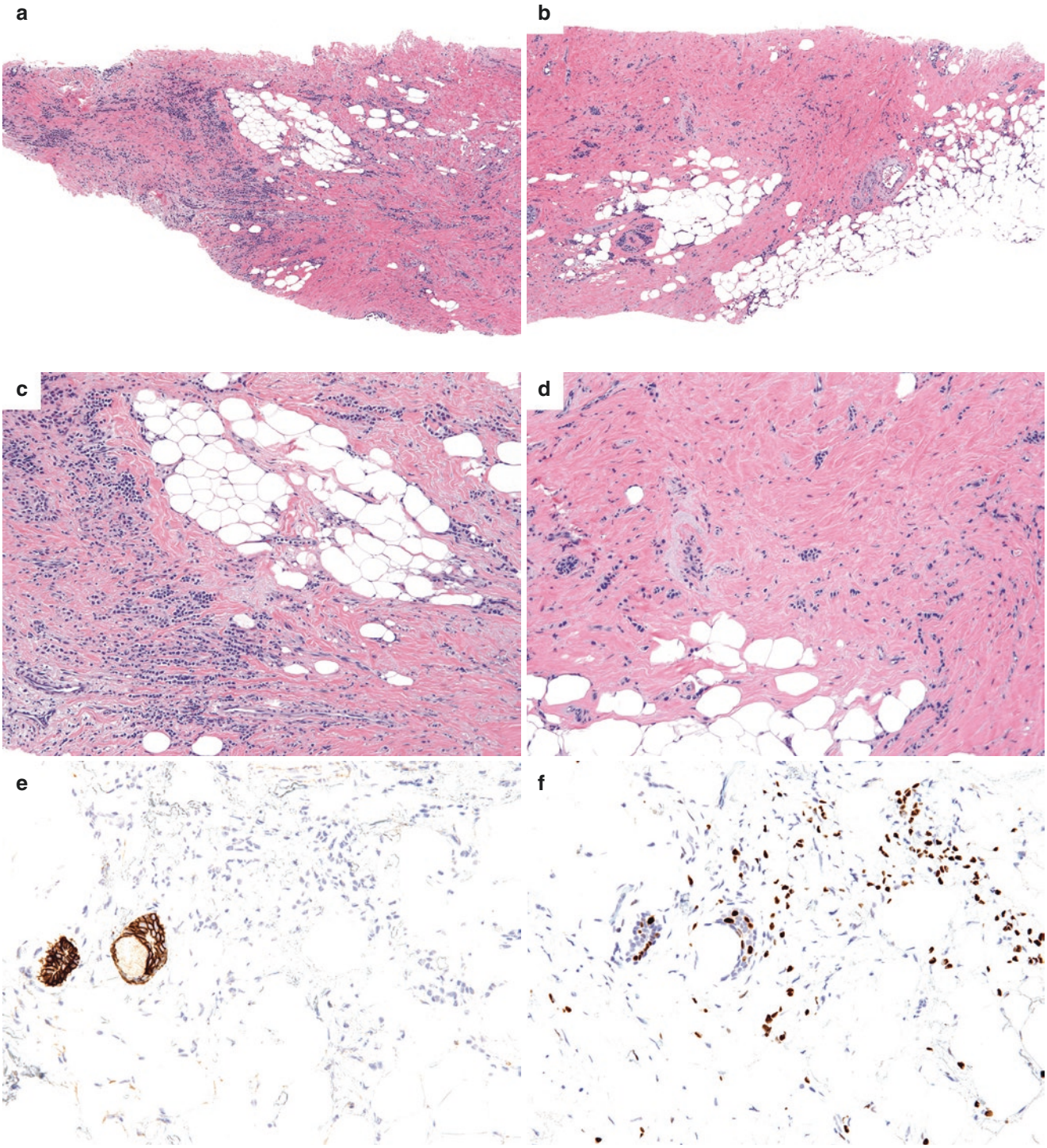

Fig. 4.44 Case 1. Histologic sections of the core biopsy show invasive lobular carcinoma with highly variable tumor cell density in sclerotic stroma. (**a**) Low power of one of the core samples is densely cellular, medium power of the central area (**c**) demonstrates dense areas of small dyscohesive cells, single and in small collections. (**b**) Low power of another core from the same specimen has widely scattered collections of lobular cells in dense stroma against background fatty breast (right), with the central area seen at medium power in (**d**). Also see Fig. 4.6a–c for additional images of the central more sclerotic portion. (**e**) Higher power of a different relatively paucicellular area of the core biopsy shows that tumor cells are E-cadherin negative, with internal control at left (200×), and estrogen receptor (ER) positive (**f**) in a serial section (200×)

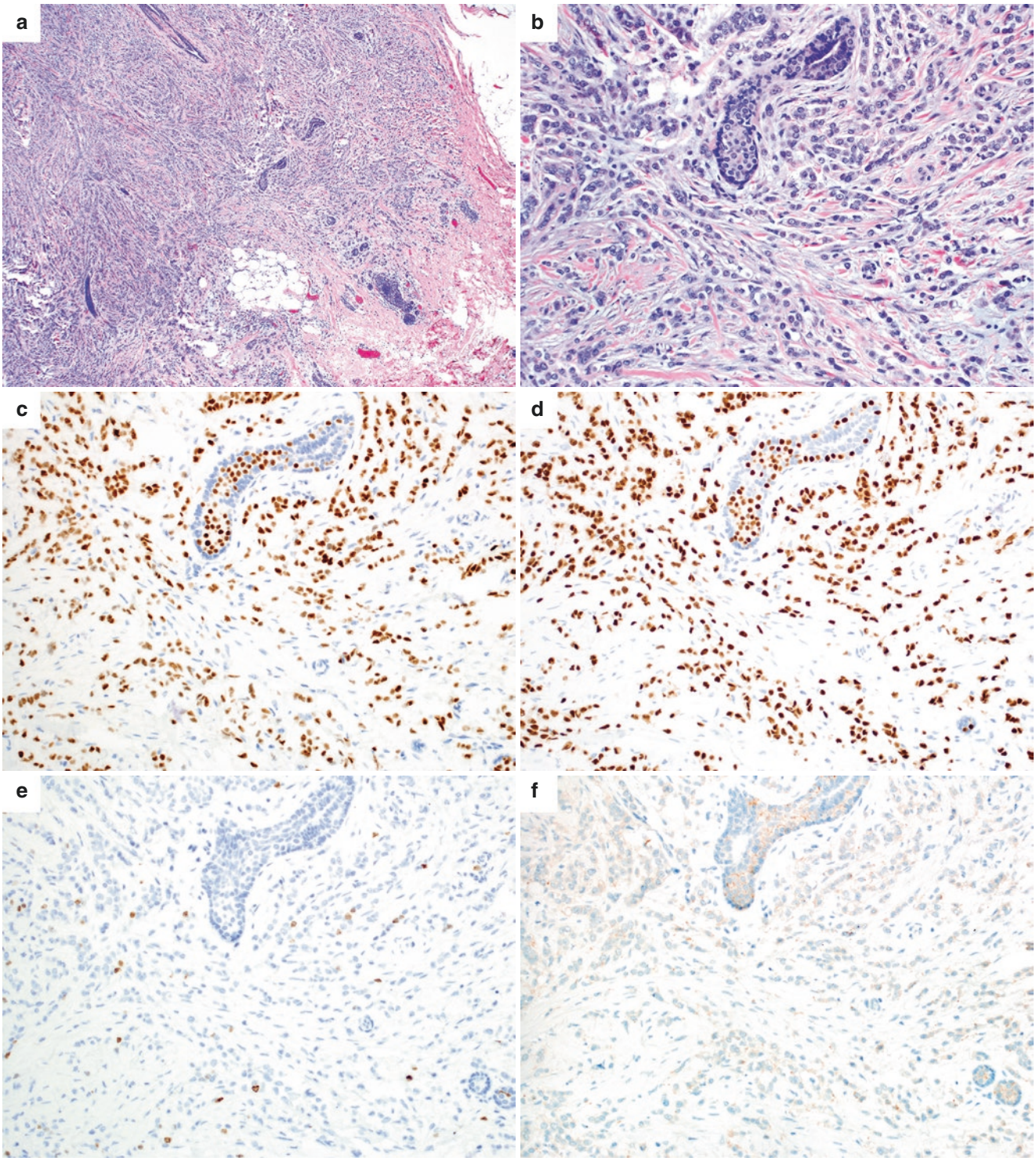

Fig. 4.45 Case 2. Invasive lobular carcinoma. (**a**) One of the lumpectomy specimens contained a 2.4 cm invasive lobular carcinoma (40×). (**b**) Higher power shows single file infiltrating carcinoma and ALH in the small ductule at top center (200×). Serial sections stained for predictive markers are consistent with a "luminal A" phenotype (all 200×). (**c**) Estrogen receptor (ER) positive (3+ > 95%), as is ALH. (**d**) Progesterone receptor (PR) positive (3+ >95%). (**e**) Low Ki-67. (**f**) Negative Her2 (0). E-cadherin staining was not performed

- *Histologic Findings*: See Figs. 4.45a–f and 4.46a–d. The 6:00 lumpectomy contained a 2.4 cm invasive carcinoma, which was densely cellular with grade 2 nuclei, without tubule formation. E-cadherin staining was not performed. ER+, PR+, Her2 negative, and Ki-67 low (5–10%). Multiple sub-2 mm satellite foci of invasive carcinoma were also present. Margins were focally positive. The 3:00 lumpectomy specimen contained multifocal invasive

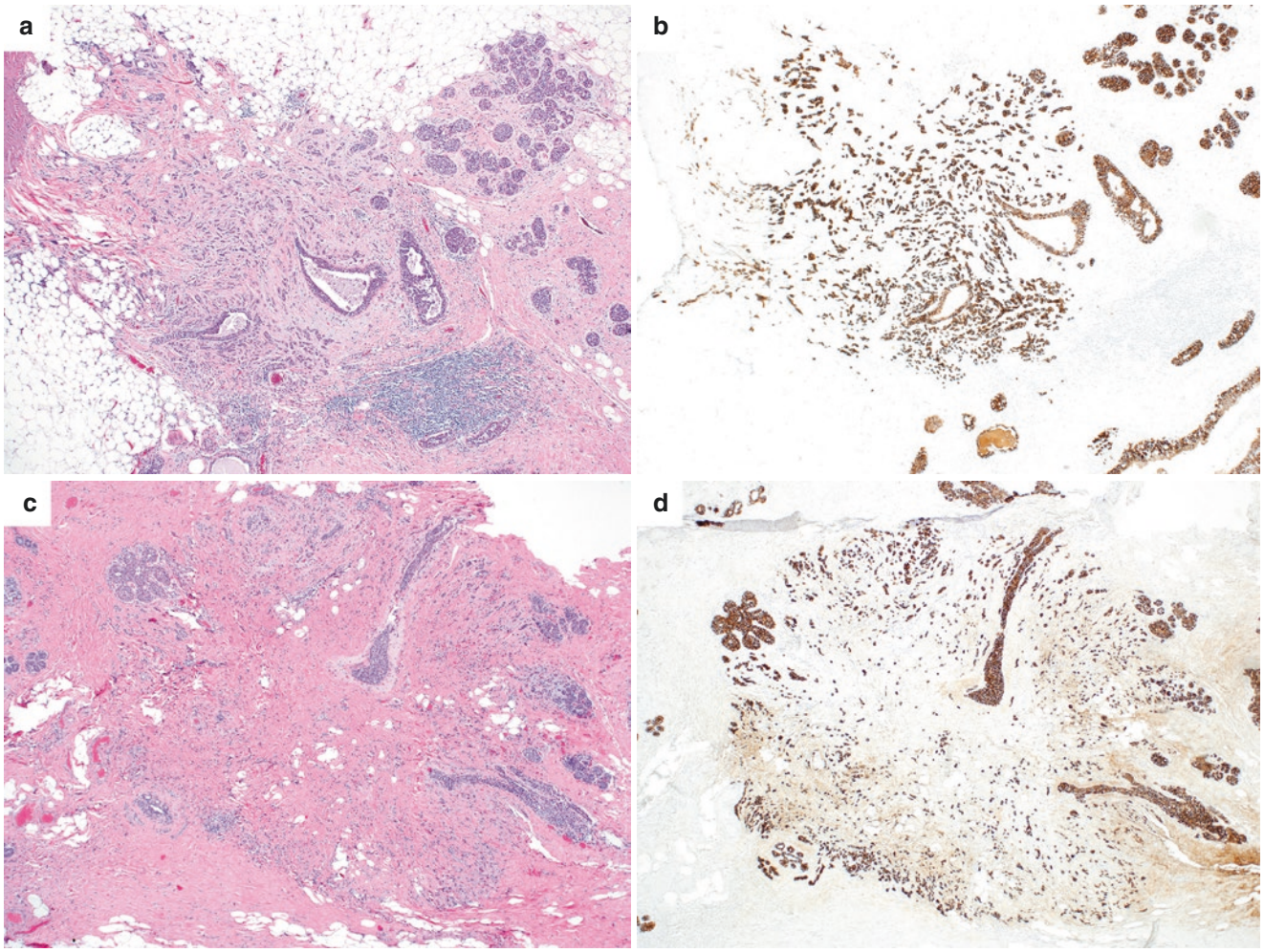

Fig. 4.46 Case 2. Multifocal invasive lobular carcinoma. In addition to the 2.4 cm invasive lobular carcinoma shown in Case 2, Fig. 4.45, there were multiple separate subcentimeter carcinomas, two of which are shown here, similar to the (pink) satellite masses diagramed in Fig. 4.3. (**a**) Low-power view of a discrete infiltrating mass lesion with desmoplastic stroma. The prominent ducts in the center demonstrate mild ductal hyperplasia with a focus of inflammation below. LCIS is seen at upper right (40×). (**b**) Keratin stain highlights the infiltrating lobular carcinoma (40×). (**c**) A second separate focus of invasive tumor in the same histologic slide has more sclerotic stroma and is less cellular. It does not stand out as well on H&E. ALH involves the central duct and the peripheral terminal duct lobular units. (40×). (**d**) Keratin stain again highlights lobular carcinoma

lobular carcinoma, including multiple sub-1 cm tumors, along with LCIS and ALH. Predictive markers were performed on one section containing two discrete tumors, and results were identical to the larger tumor. Margins were focally positive and were later re-excised. Isolated tumor cells were present in one of the three lymph nodes (not shown).

- *Final Diagnosis*: Multifocal invasive lobular carcinoma, grade 2. AJCC stage: pmT2 pN0(i+)
- *Take-Home Messages*:
  - Invasive lobular carcinoma is often multifocal; MRI can be helpful in this setting.
  - E-cadherin immunostaining is not necessary in making a diagnosis of lobular carcinoma.
  - Classic invasive lobular carcinoma is often ER+ PR+ Her2 negative.
  - Lobular carcinoma can rarely be hereditary (*CDH1* or partners), although genetic testing was negative here.

Case 3

- *History*: A 53-year-old woman with multiple palpable breast masses
- *Imaging*: Spiculated right breast mass at 12:00, 2 cm size; adjacent mass at 11:00, 1 cm size; and lobulated right breast mass at 9:00, 1.3 cm size. Right axillary ultrasound identified two suspicious lymph nodes with thickened cortices. After core biopsy, the patient received

chemotherapy: dose dense AC-T (anthracycline/cyclophosphamide followed by taxane) with Her2-targeted agents, namely, trastuzumab and pertuzumab. Further imaging demonstrated near resolution of the 9:00 mass, but minimal change in the 12:00 masses. Right mastectomy was performed and grossly a 2.1 cm mass was identified at 12:00, with a 1.5 cm irregular mass at 9:00.

- *Histologic Findings*: See Fig. 4.47a–h. Note the difference in cytologic, architectural, and immunophenotypes (E-cadherin, ER, PR, Her2) of the two concurrent tumors at the time of core biopsy. After chemotherapy, the grade 3 ductal Her2-positive tumor had completely resolved, leaving only fibrotic tumor bed in the breast and no evidence of metastatic ductal carcinoma in lymph nodes. However, a 3.6 cm invasive lobular carcinoma remained after chemotherapy, with somewhat diminished tumor cellularity, but with lymph node metastases that were not sampled on prior biopsy.
- *Final Diagnosis*: Residual invasive lobular carcinoma, 3.6 cm with metastatic lobular carcinoma in one of three sentinel nodes (ypT2 pN1). Complete pathologic response of invasive ductal carcinoma
- *Take-Home Messages*:

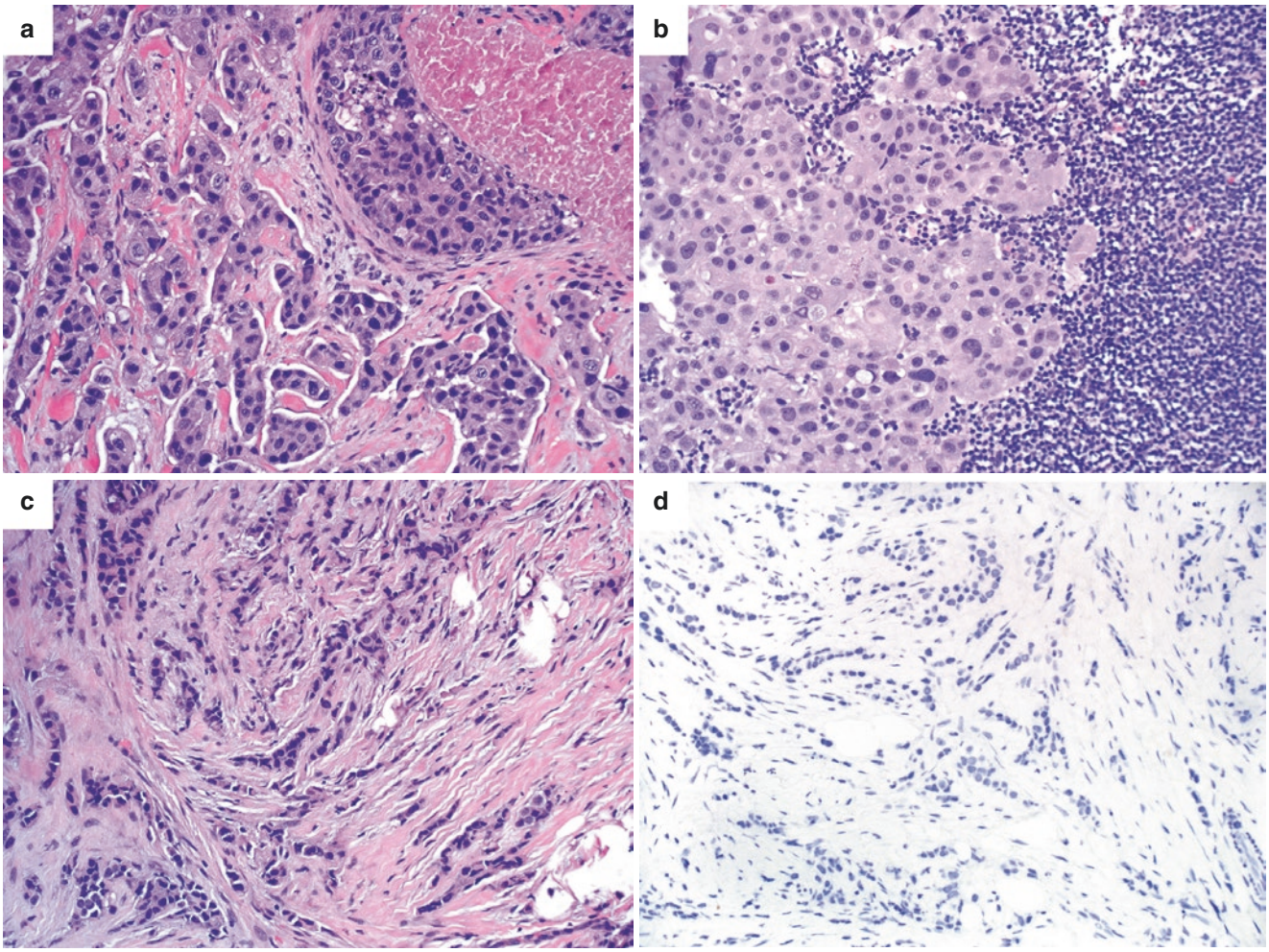

Fig. 4.47 Case 3. Concurrent invasive ductal and lobular carcinoma, treated with chemotherapy. (**a**) Core biopsy of 9:00 mass, demonstrating grade 3 invasive ductal carcinoma, with necrosis (upper right), and (**b**) lymph node metastasis with identical ductal histology. This carcinoma was E-cadherin positive (not shown), weakly positive for ER (1+ 30%), PR negative, and Her2 equivocal by immunohistochemistry and amplified by FISH (Her2/CEP17 = 8, Her2/cell = 22). (**c**) Core biopsy of ipsilateral 12:00 mass with grade 2 invasive lobular carcinoma in sclerotic stroma. (**d**) This carcinoma is E-cadherin negative. Predictive markers: ER positive (2–3+ 80%), PR positive (2–3 + 80%), Her2/neu 1+ IHC, and Her2 FISH negative. (**e**) Tumor bed from site of invasive ductal carcinoma demonstrates complete pathologic response (40×); lymph nodes were negative for ductal carcinoma (not shown). (**f**) At the 12:00 site, there is residual invasive lobular carcinoma, perhaps of lower cellularity than seen on core biopsy (200×). (**g**) Lymph nodes contain residual metastatic lobular carcinoma (100×), seen at higher power (**h**) (200×), but there was no evidence of ductal carcinoma in nodes or breast

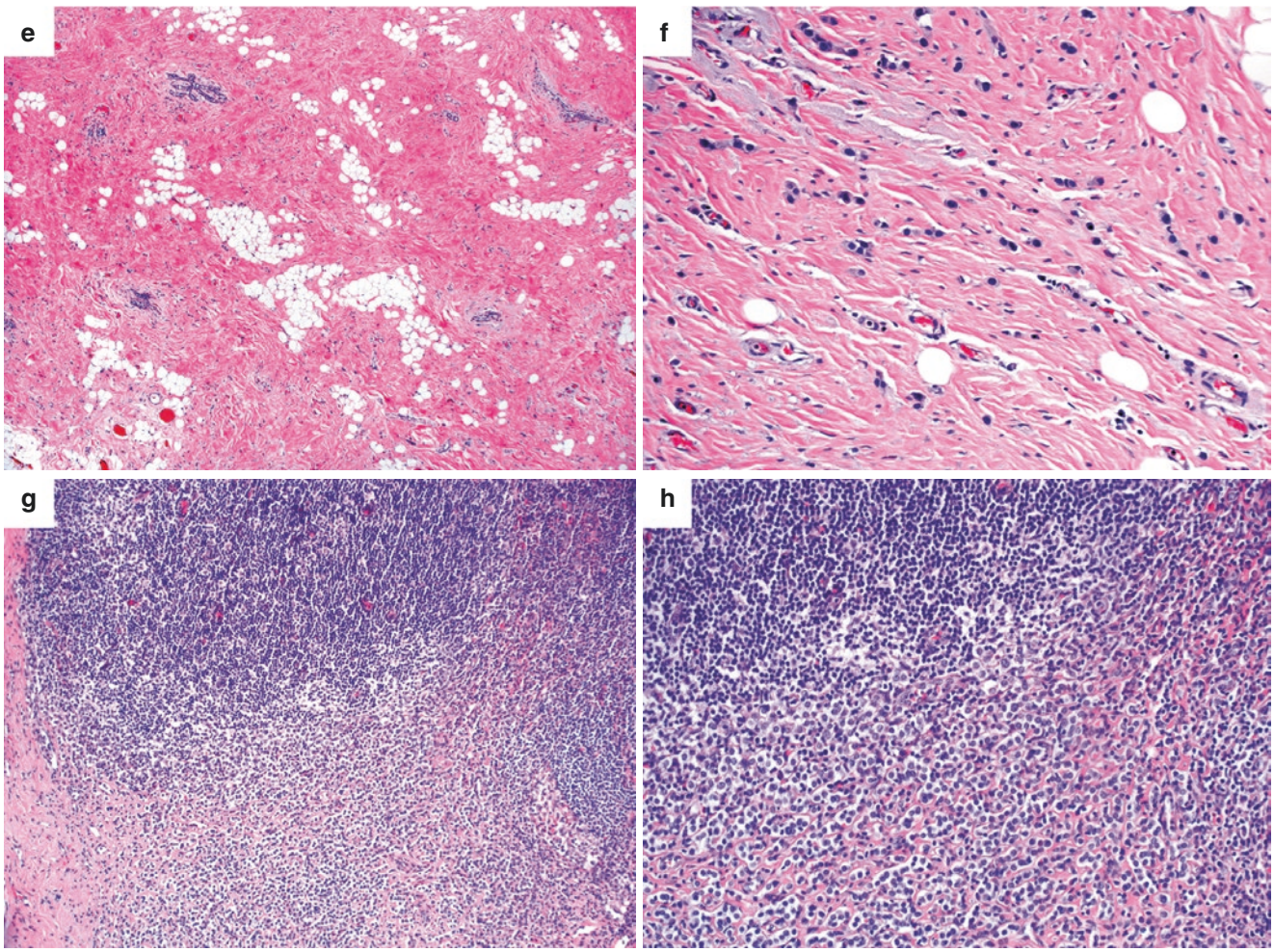

Fig. 4.47 (continued)

- Multiple simultaneous tumors can have disparate biology. Predictive markers may reveal differences.
- Classic lobular carcinoma responds poorly to cytotoxic chemotherapy, whereas high-grade carcinomas often respond better.
- Her2-negative tumors respond poorly to Her2-targeted therapy.

Case 4

- *History*: A 49-year-old premenopausal G1P1 woman with a maternal aunt with breast cancer with suspicious calcifications on mammography
- *Imaging*: See Fig. 4.48a–f. Suspicious 1.2 cm group of punctate and fine pleomorphic calcifications in the upper outer left breast, posterior depth. MRI was performed and showed at least three enhancing masses in the outer left breast in a clumped non-mass enhancement pattern corresponding to, but larger than, the suspicious calcifications on mammography. Stereotactic core biopsy was performed, sampling calcifications, followed by wire localized lumpectomy. The biopsy marker, residual calcifications, and localizing wires can be seen in the specimen radiograph. Grossly, there was a 2 cm mass that was 0.8 cm from the closest margin.
- *Histologic Findings*: See Fig. 4.49a–c. The core biopsy contained classic, florid, and pleomorphic LCIS with calcifications, without invasive carcinoma. The excisional specimen revealed a 0.2 cm focus of invasive lobular carcinoma, grade 2 (poor tubule formation (3), intermediate nuclear grade (2), low mitotic rate (1), total of 6). Predictive marker immunohistochemistry was performed and reported on the invasive component: ER positive (3+ >95%), PR positive (3+ >95%), Ki-67 < 5%, Her2 2+ immunohistochemistry, non-amplified by FISH. There was a background of extensive classic, florid, and pleomorphic LCIS. The pleomorphic LCIS was at one margin and within 0.1 cm of another. Re-excision was performed, containing further classic LCIS. Sentinel lymph node

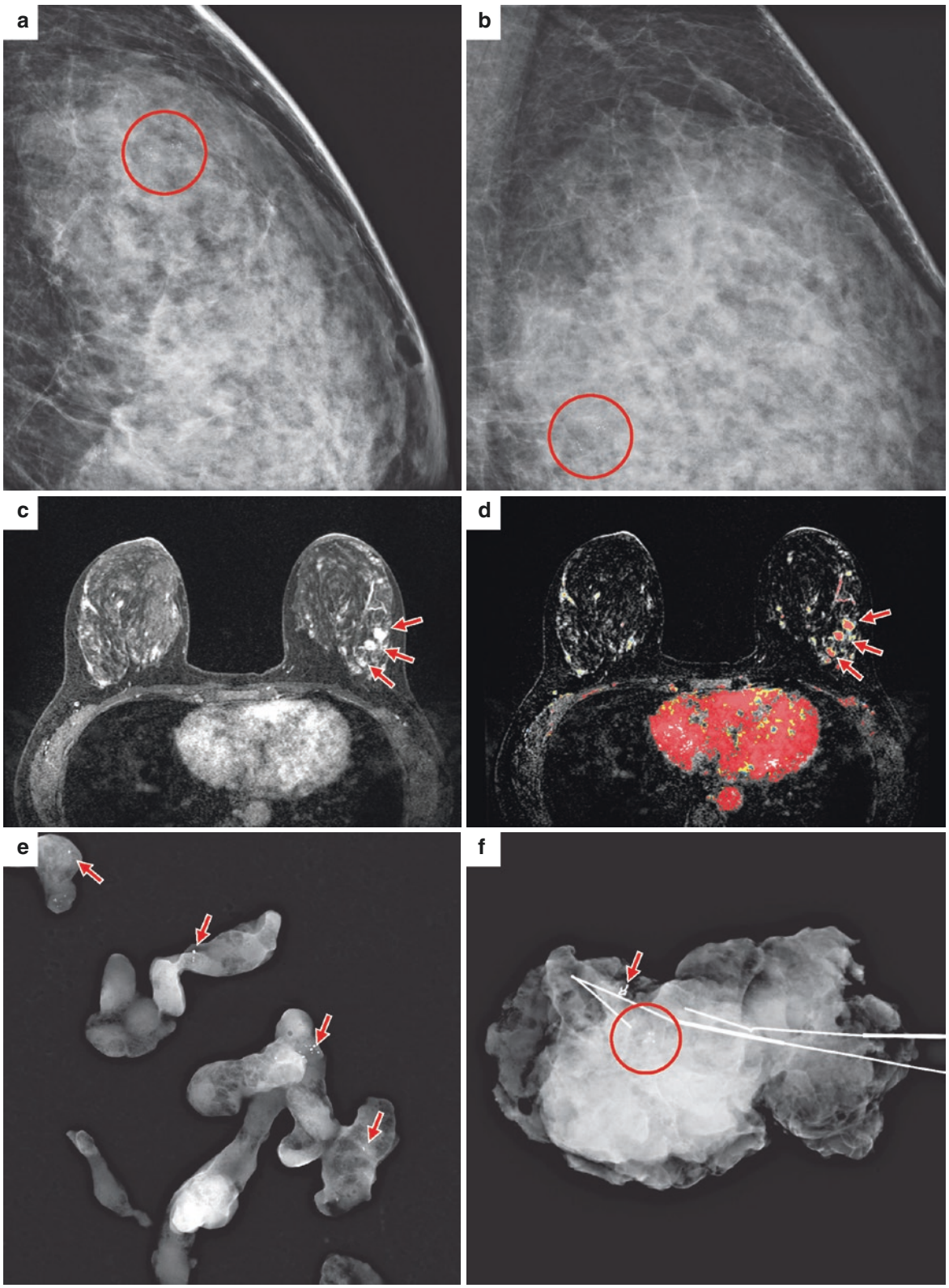

Fig. 4.48 Case 4. Craniocaudal (**a**) and lateral medial (**b**) magnification full-field digital mammograms show a suspicious 1.2 cm group of punctate and fine pleomorphic calcifications in the upper outer left breast, posterior depth (circles). Ultrasound from this can be seen in Fig. 4.28. (**c**) Images from axial contrast-enhanced breast MRI shows marked background parenchymal enhancement and at least 3–4 enhancing masses (arrows) in the outer left breast in a clumped non-mass enhancement pattern corresponding to, but larger than, the suspicious calcifications on mammography. (**d**) Computer-aided detection (CAD) MRI images show fast initial and late washout kinetics (red color), suspicious for cancer. (**e**) Core biopsy specimen radiography shows the targeted calcifications (arrow). Post-biopsy mammogram from this case is shown in Fig. 4.28, including large calcifications. (**f**) Subsequent specimen radiograph from the time of surgery shows the two localization wires, omega marker (arrow) and residual calcifications (circle)

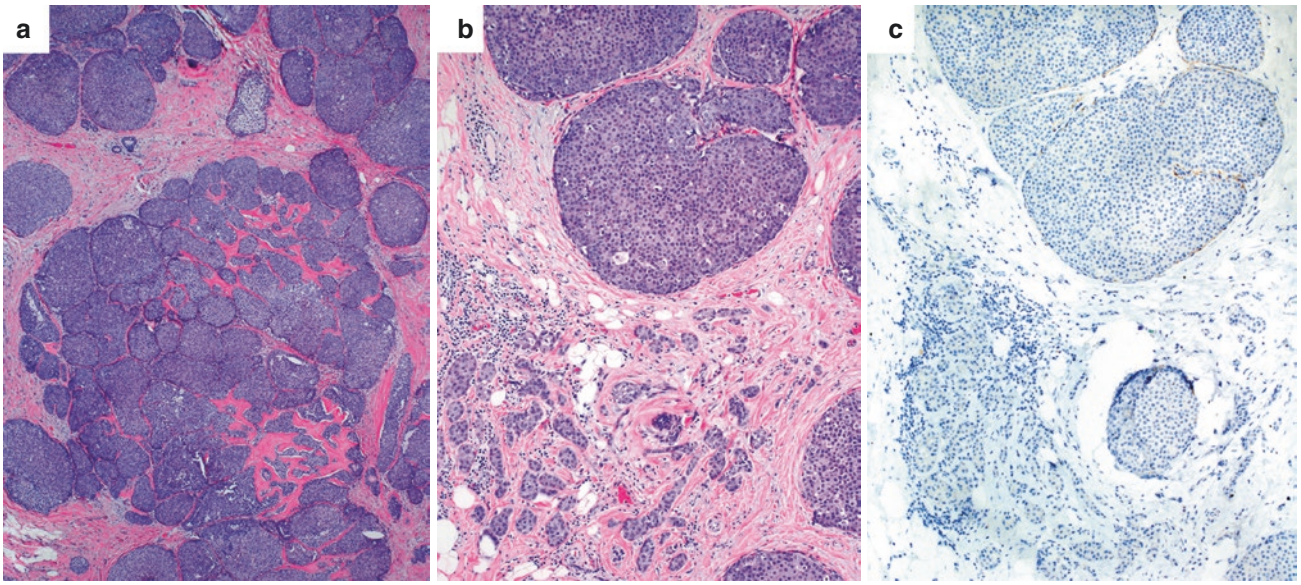

Fig. 4.49 Case 4. Histologic sections from the core biopsy and subsequent excision demonstrated LCIS: classic, florid, and pleomorphic (also see Fig. 4.28), with a small focus of invasive lobular carcinoma apparent only on excision. (**a**) Florid LCIS from the excisional specimen (40×). (**b**) Pleomorphic LCIS at top, and at bottom invasive lobular carcinoma with cords and small nests of infiltrating tumor (100×). (**c**) Serial section with negative E-cadherin immunostain (100×)

biopsy was performed at the time of second surgery and nodes were negative.

- *Final Diagnosis*: Invasive lobular carcinoma, grade 2, 0.2 cm (pT1a pN0), extensive classic, florid, and pleomorphic LCIS
- *Take-Home Messages*:
  - MRI may be helpful in assessing extent of lobular lesions.
  - LCIS on core biopsy may be associated with unsampled invasive carcinoma, especially if abundant or of pleomorphic or florid type.
  - Invasive lobular carcinoma is graded with the WHO criteria.
  - Margins should be reported for pleomorphic LCIS, but need not be reported for ALH or classic LCIS.
  - Management of pleomorphic LCIS margins is controversial, but many clinicians and patients chose to re-excise.

Case 5

- *History*: An 85-year-old female with history of left invasive ductal carcinoma (20 years ago) status post lumpectomy
- *Imaging*: See Fig. 4.50a–h. A focal asymmetry in the upper outer right breast was visualized on the most recent study. In retrospect, there is a slowly developing focal asymmetry up to 2 years before, a pattern commonly seen in ILC on mammography. A core biopsy was performed, followed by surgical excision.
- *Histologic Findings*: See Fig. 4.51a–d. The core biopsy demonstrates collagenous stroma with a haphazard infiltrate of small dyscohesive epithelial cells, characteristic of invasive lobular carcinoma. Predictive marker results: ER positive (3+ >95%), PR positive (2+ 30%), Ki-67 low (<5%), Her2 immunohistochemistry negative (1+), not amplified by FISH. The subsequent resection contained a 2.7 cm focus of similar carcinoma, grade 1 (poor tubule formation (3), low nuclear grade (1), low mitotic rate (1), total of 5 points). Margins were positive, including separately submitted new margin specimens, and were subsequently re-excised.
- *Final Diagnosis*: Invasive lobular carcinoma, grade 1, 2.7 cm (pT2 N0), background of lobular neoplasia in situ
- *Take-Home Messages*:
  - Classic lobular carcinoma grows slowly and may be mammographically occult at first.
  - Invasive lobular carcinoma is graded with the WHO criteria.
  - Invasive lobular carcinoma may be larger than mammographically or grossly recognized.

Case 6

- *History*: A 68-year-old woman with history of left breast DCIS 15 years prior, now with right axillary adenopathy on mammography, but no diagnostic findings in the right breast. A right axillary lymph node FNA was performed and demonstrated metastatic carcinoma, consistent with breast primary.

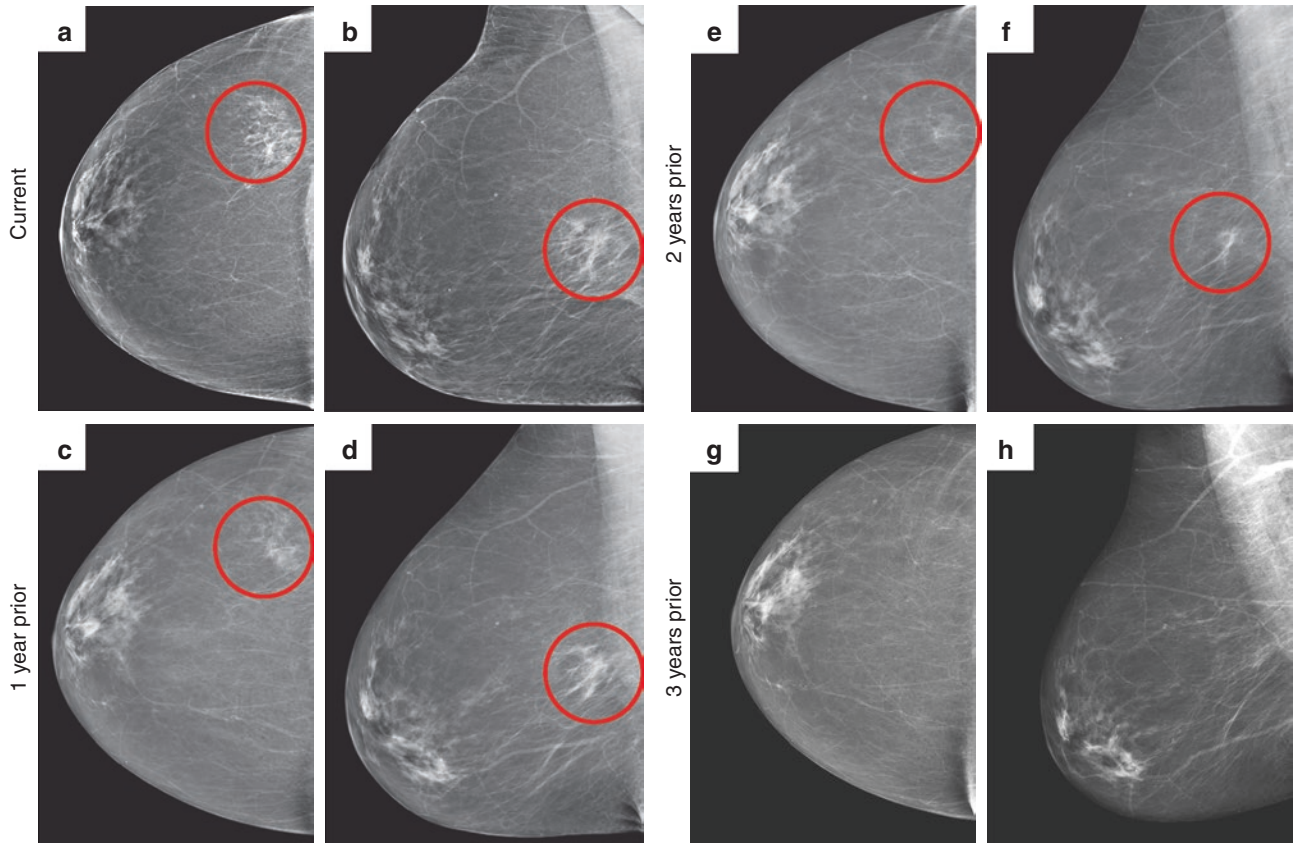

Fig. 4.50 Case 5. There is a focal asymmetry in the upper outer right breast on the most recent study. In retrospect, there is a slowly developing focal asymmetry up to 2 years before, a pattern commonly seen in ILC on mammography. In the most recent study on craniocaudal (**a**) and mediolateral (**b**), there is a focal asymmetry in the right upper outer breast (circle). (**c, d**) One year prior, there was asymmetry in the right upper outer breast (circle) that had grown on the subsequent study. (**e, f**) Two years prior, there was asymmetry in the right upper outer breast (circle) that was new since the year before (**g, h**)

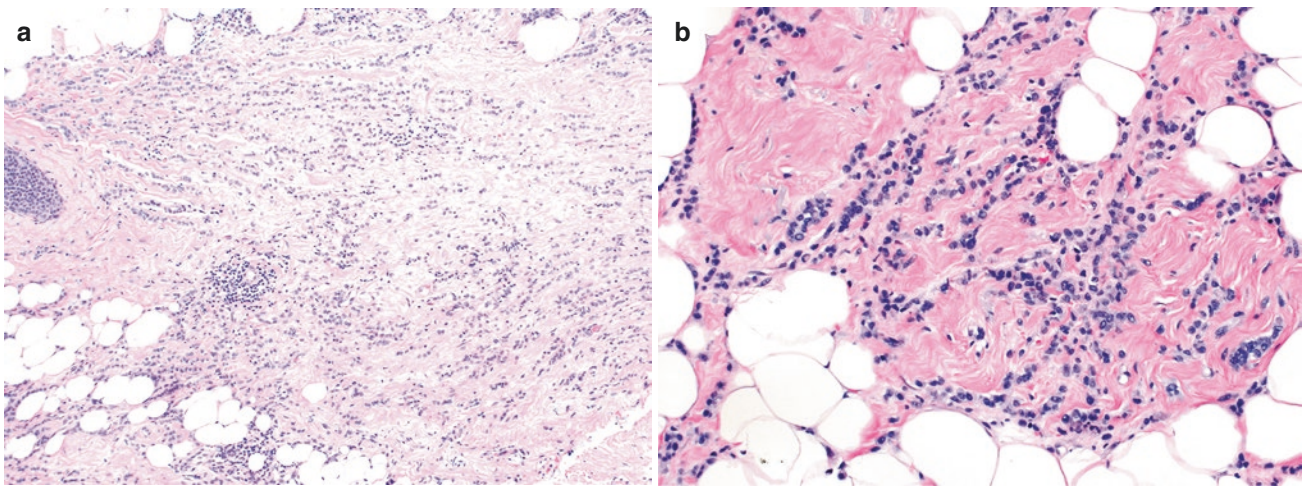

Fig. 4.51 Case 5. Histologic sections from the core biopsy and surgical specimen. (**a**) Core biopsy invasive lobular carcinoma in collagenous stroma, with ALH at left (100×). (**b**) Higher power of single file cells in collagenous and adipocytic stroma (200×). (**c**) The extent of invasive carcinoma was also difficult to determine at the time of surgery; margins were positive for invasive lobular carcinoma (tumor at ink, bottom, 40×). (**d**) Higher power of lobular carcinoma in the surgical specimen (200×)

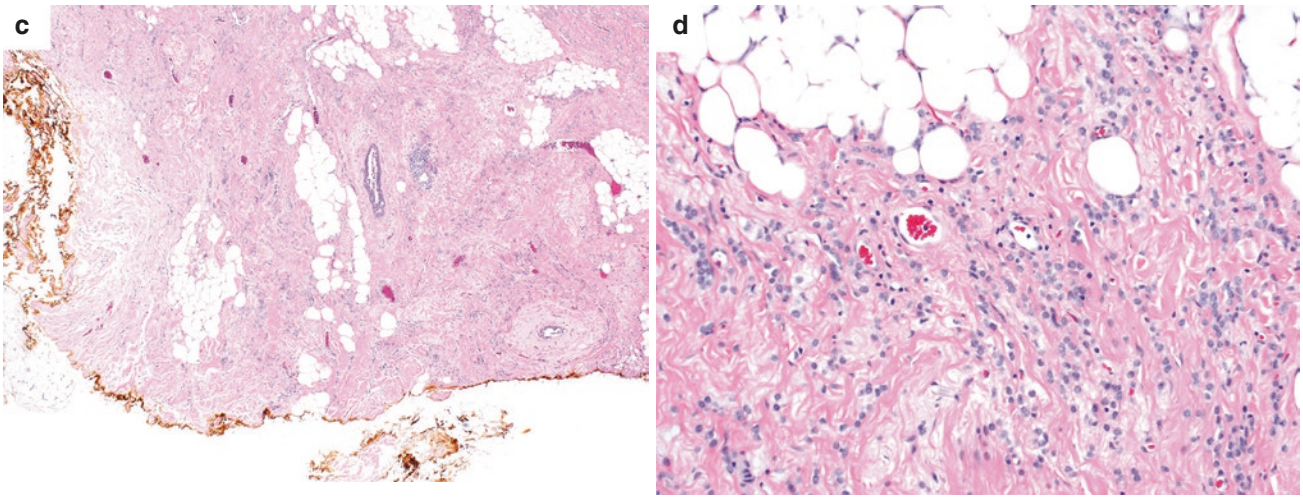

Fig. 4.51 (continued)

- *Imaging*: Right breast MRI demonstrated mass with abnormal enhancement, and core biopsy was performed.
- *Histologic Findings*: See Fig. 4.52a–d. Biopsy showed cords and small sheets of plump invasive carcinoma cells with grade 2 nuclei. E-cadherin was negative with positive internal control; however, given the pleomorphic morphology along with ER negativity (predictive markers: ER negative (0), PR negative (0), Ki-67 20%, Her2 equivocal (2+ immunohistochemistry, Her2/CEP17 ratio = 1.9, Her2/cell = 5)), a diagnosis of invasive mammary carcinoma was made, with classification deferred to the excisional specimen.
- The post-chemotherapy specimen is shown in Fig. 4.53a–f, with only a small nodule of fairly high tumor cellularity remaining in the middle of a 1.8 cm tumor bed containing scattered individual tumor cells. Tumor cells had more abundant pale cytoplasm after chemotherapy and were again E-cadherin negative. One lymph node was largely replaced by fibrosis, consistent with an area of treated tumor, with a few residual isolated tumor cells. According to the AJCC eighth edition, the span of fibrosis is not measured in determination of nodal stage in the post-chemotherapy setting, but rather the size of the largest tumor deposit.
- *Final Diagnosis*:
  - Residual invasive pleomorphic lobular carcinoma, 1.8 cm size (ypT1c pN0(i+)
- *Subsequent History*: Four years later, the patient presented with a palpable right axillary mass; core biopsy is shown in Fig. 4.54a–f. Both the morphology and predictive markers were similar to the primary tumor, with minimal ER staining, and Her2 FISH now scored as low amplified. The morphology, together with the strong diffuse androgen receptor staining, is suggestive of apocrine differentiation in this pleomorphic lobular carcinoma, consistent with recurrence.
- *Take-Home Messages*:
  - Pleomorphic lobular carcinoma may have good response to chemotherapy.
  - Pleomorphic lobular carcinoma is more often hormone receptor negative with higher proliferation than classic lobular carcinoma.
  - Pleomorphic lobular carcinoma may behave more aggressively.
  - A subset of pleomorphic lobular carcinomas, and pleomorphic LCIS, show apocrine features.

Acknowledgments The authors would like to thank Norm Cyr for providing expert assistance with figures.

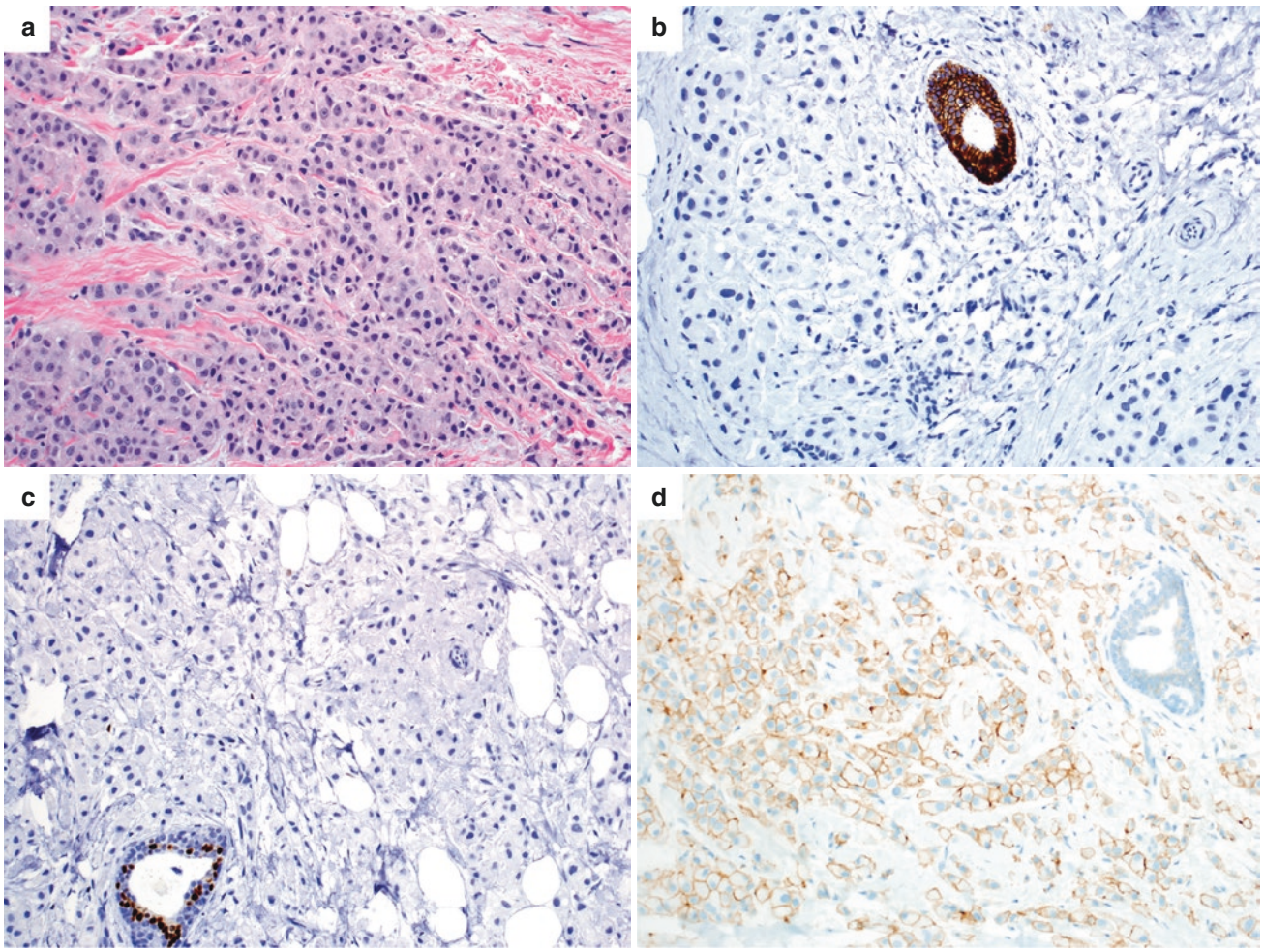

Fig. 4.52 Case 6. Diagnostic core biopsy. (**a**) Cords and small sheets of plump invasive carcinoma cells with grade 2 nuclei (200×). (**b**) E-cadherin negative with positive internal control (normal duct, upper, 200×). This core biopsy was diagnosed as invasive mammary carcinoma with concern for pleomorphic lobular carcinoma and final classification deferred to resection. (**c**) Estrogen receptor negative with positive internal control (bottom left, 200×). (**d**) Her2/neu immunohistochemistry 2+, FISH equivocal (Her2/CEP17 ratio 1.9, Her2/cell = 5)

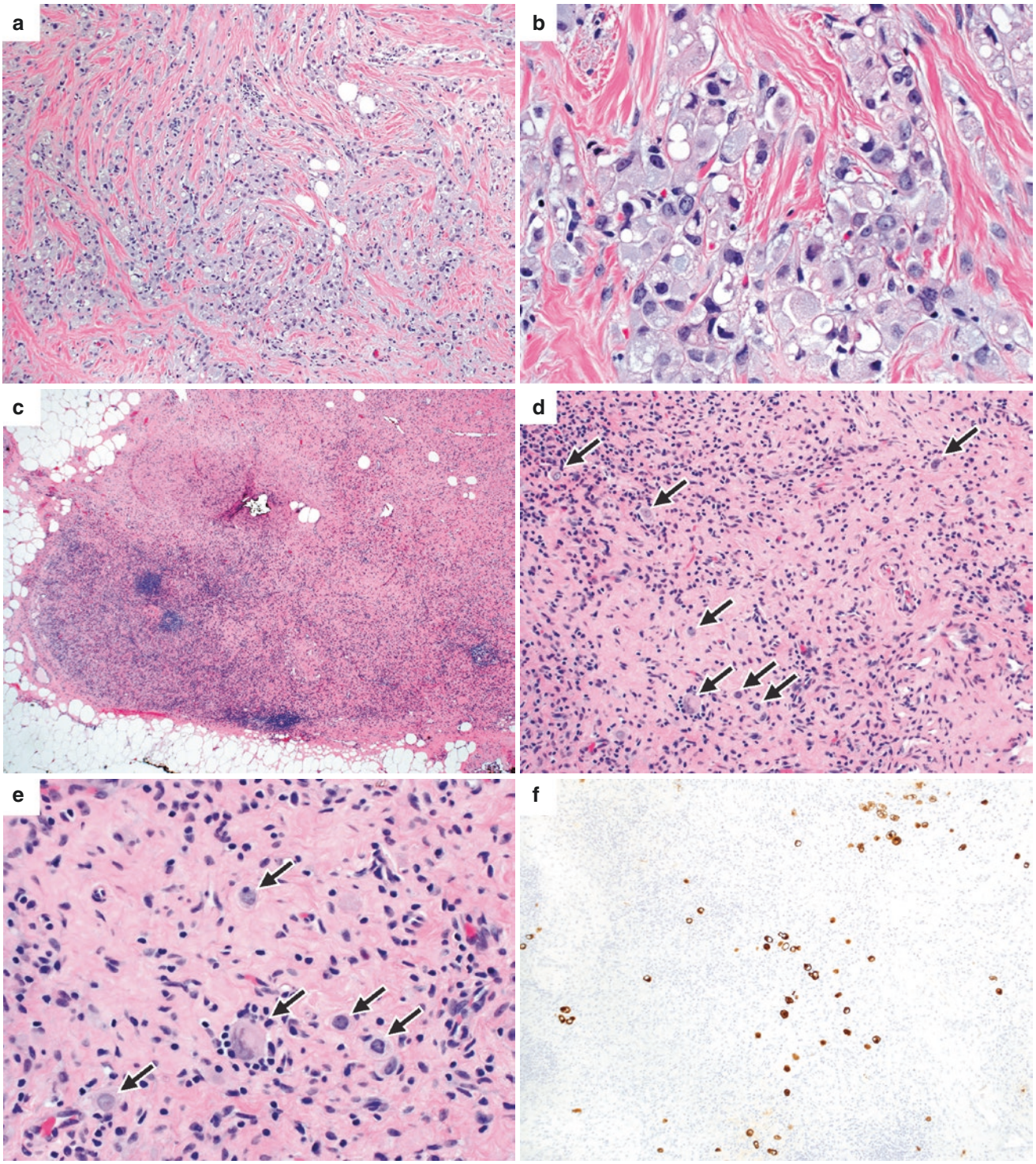

Fig. 4.53 Case 6. Post-chemotherapy pathology. (**a**) There was one small nodule of residual invasive carcinoma 0.4 cm (100×), within a 1.8 cm span of scattered single cells in tumor bed (not shown). (**b**) High power shows eccentric nuclei and more abundant histiocytoid cytoplasm as compared to original biopsy (400×), again E-cadherin negative (not shown). (**c**) Axillary lymph node with treatment effect and residual isolated tumor cells. At low power, the lymph node appears largely fibrotic (40×). Tumor cells are apparent at higher power on H&E 200× (**d**), 400× (**e**) (arrows), and with keratin stain (**f**) (100×)

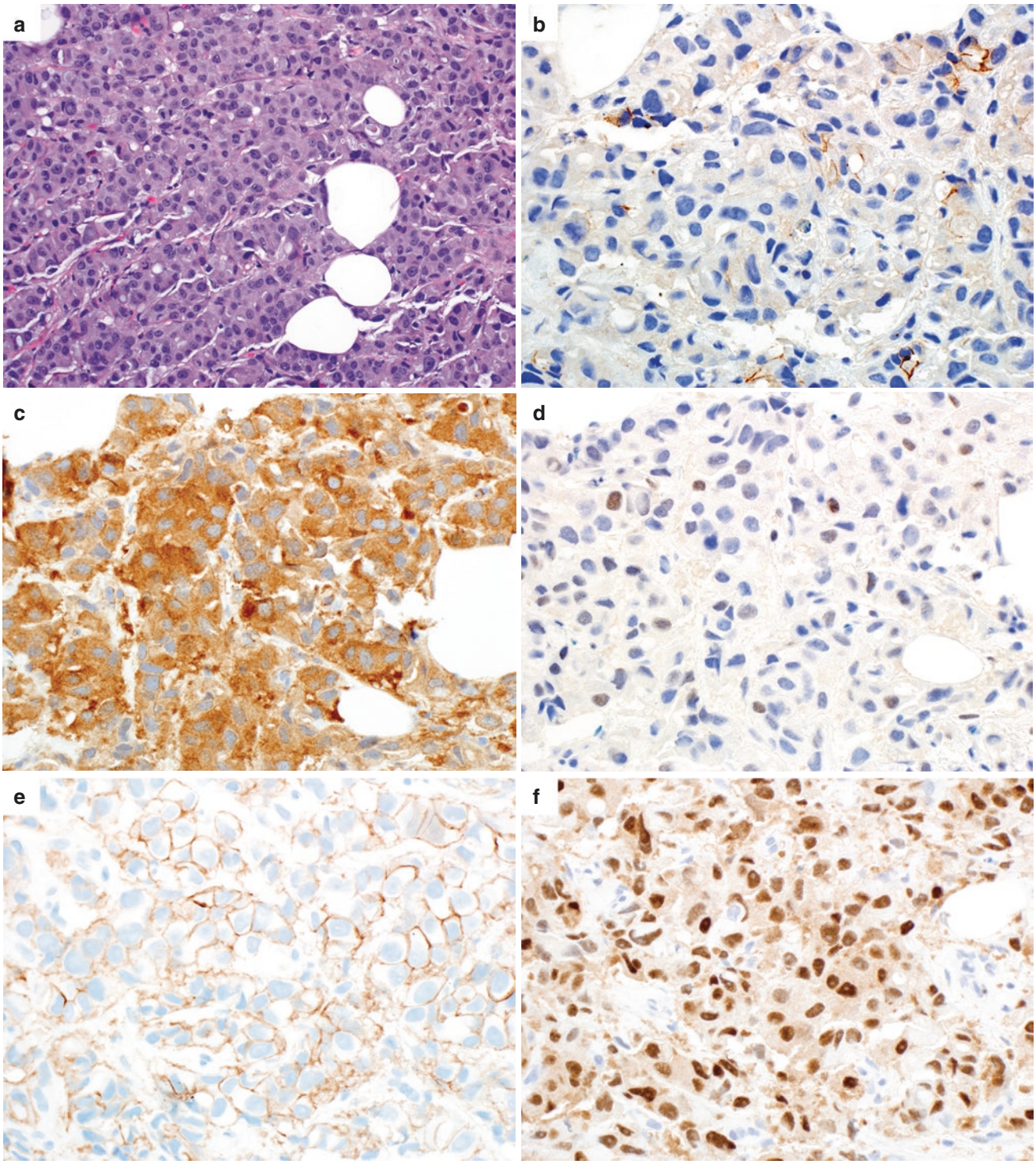

Fig. 4.54 Case 6. Recurrent carcinoma. (**a**) H&E section demonstrates sheets and cords of cells with grade 3 nuclei and moderate amounts of eosinophilic cytoplasm, infiltrating fat (200×). (**b**) E-cadherin is negative (400×) and p120 is cytoplasmic (**c**), supporting lobular differentiation (400×). (**d**) Estrogen receptor shows weak staining in only a subset of nuclei; PR stains similarly (not shown). (**e**) Her2/neu immunohistochemistry was weak to focally moderate circumferential staining (2+, shown here, 400×); HER2 FISH studies were low amplified (Her2/CEP17 = 2.2, Her2/cell = 5). (**f**) Androgen receptor immunohistochemistry was strong and diffuse (400×), consistent with a Her2+ pleomorphic apocrine lobular carcinoma

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Papillary Lesions of the Breast (IDP, IDPC, EPC, SPC)

5

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List of Frequently Asked Questions

1. **What are papillary lesions** [1]**?**

Papillary lesions encompass a broad spectrum of diseases, spanning from benign intraductal papilloma (IDP) to malignant lesions, namely, intraductal papillary carcinoma (IDPC), encapsulated papillary carcinoma (EPC), and solid papillary carcinoma (SPC). The characteristic pathologic features in all papillary lesions are the intraductal epithelial proliferation around fibrovascular cores arising from the ductal wall. As papillary lesions are derived from the ducts, a layer of intervening myoepithelial cells is retained between the epithelial cell layer and the fibrovascular cores, especially in the benign lesions. However, it may be lost to various extents in atypical to malignant papillary lesions. The classification between different papillary lesions is dependent on the presence of cytological/architectural atypia and the presence of myoepithelial layers. The term "micropapillary" should not be confused with "papillary." The former is composed of small, hollow, or morula-like clusters of cancer cells but not encasing true fibrovascular cores.

2. **What is the clinical presentation of papillary lesions?**

Clinically, papillary lesions may present with nipple discharge, either blood stained or serosanguinous, originating from the same lactiferous duct opening. There are few, if any, specific clinical features differentiating between benign and malignant papillary lesions. Solid papilloma may be more common in perimenopausal women and less common with a palpable mass, whereas patients with papillary carcinoma are typically older and palpable masses are more frequent.

3. **What are the imaging findings of papillary lesions** [2–6]**?**

The mammographic appearance of papilloma is usually a rounded or ovoid, well-circumscribed retroareolar mass, sometimes with ductal dilatation. Smaller lesions may be mammographically occult. Calcifications are uncommon. Ultrasonography (US) of papilloma typically shows solid mural nodule within a dilated duct. A cystic component may be seen, particularly in the larger, central lesions. It may be hypervascular on color Doppler. Occasionally, a benign papilloma can possess speculated margins on imaging, mimicking a malignant disease. IDPC when detected in imaging may show pleomorphic calcifications and architectural distortion on mammography and ill-defined hypoechoic mass or calcifications on US. Other malignant papillary lesions appear as oval circumscribed solitary or clustered masses that may be associated with microcalcifications on mammography. The lesions may be single or multiple, circumscribed solid or complex mixed cystic and solid masses on US. They are vascular and tend to bleed centrally resulting in intracystic fluid-debris levels. Magnetic resonance imaging (MRI) for papillary lesions may show variable enhancement patterns. It has a high sensitivity, but low specificity, and is useful in establishing the extent and distribution of multiple lesions. A nonparallel orientation, echogenic halo, posterior acoustic enhancement, and associated calcification are reported to be more frequent in malignant lesions. However, due to overlapping findings, imaging is unreliable in differentiating between benign and malignant papillary lesions.

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Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5_5

4. **What are the typical gross characteristics of papillary lesions** [7]**?**

Papillary lesions have variable sizes, ranging from a few millimeters to several centimeters. Palpable IDPs vary from soft to firm with dense sclerotic foci. Focal areas of hemorrhage and necrosis are common particularly in larger lesions. Careful gross examination may show the lesions lying within dilated ducts. There are no specific macroscopic features for IDPC. For EPC, a friable mass within a cystic cavity may be appreciated. SPC may be observed as a whitish gray or yellowish brown fleshy firm or soft nodular circumscribed mass on gross examination.

5. **What are the histologic features of intraductal papilloma** [1]**?** (Fig. 5.1a, b)

IDPs are characterized by a cohesive but arborescent structure composed of fibrovascular cores covered by myoepithelial cells and epithelial cells. The myoepithelial layer can be attenuated. The epithelial component may consist of one layer of cuboidal/columnar cells or show different degrees of hyperplasia, particularly florid epithelial hyperplasia or metaplasia (apocrine, squamous). As the lesion is intraductal in origin, there is also an intact layer of myoepithelial cells around the surrounding ductal wall. Identification of myoepithelial cells in both compartments helps to confirm the diagnosis. The fibrovascular cores are generally considered to be broad and fibrous (compared to IDPCs, which possess long and slender fibrovascular cores).

6. **What are the benign changes that can be seen in IDP** [8, 9]**?** (Fig. 5.2a, b)

The epithelial layer of IDP may show different degrees of hyperplasia, particularly florid epithelial hyperplasia or metaplasia (apocrine, squamous). Apocrine change and squamous metaplasia are mostly associated with areas of infraction. Very infrequently, mucinous clear cell changes and sebaceous

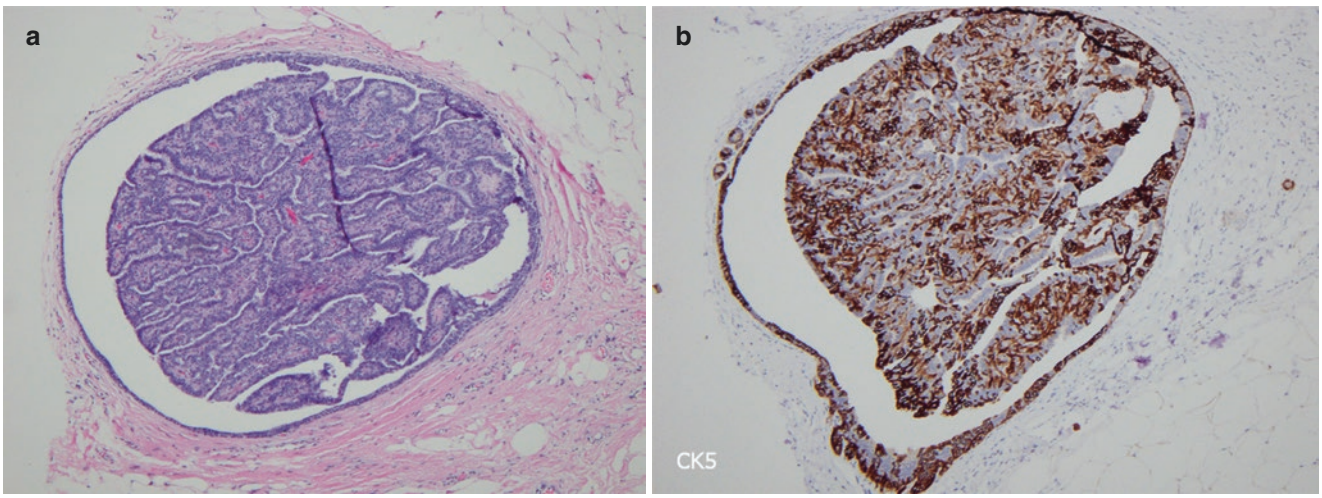


Fig. 5.1 IDP. Intraductal papilloma with HE stain (**a**) and CK5/6 stain (**b**)

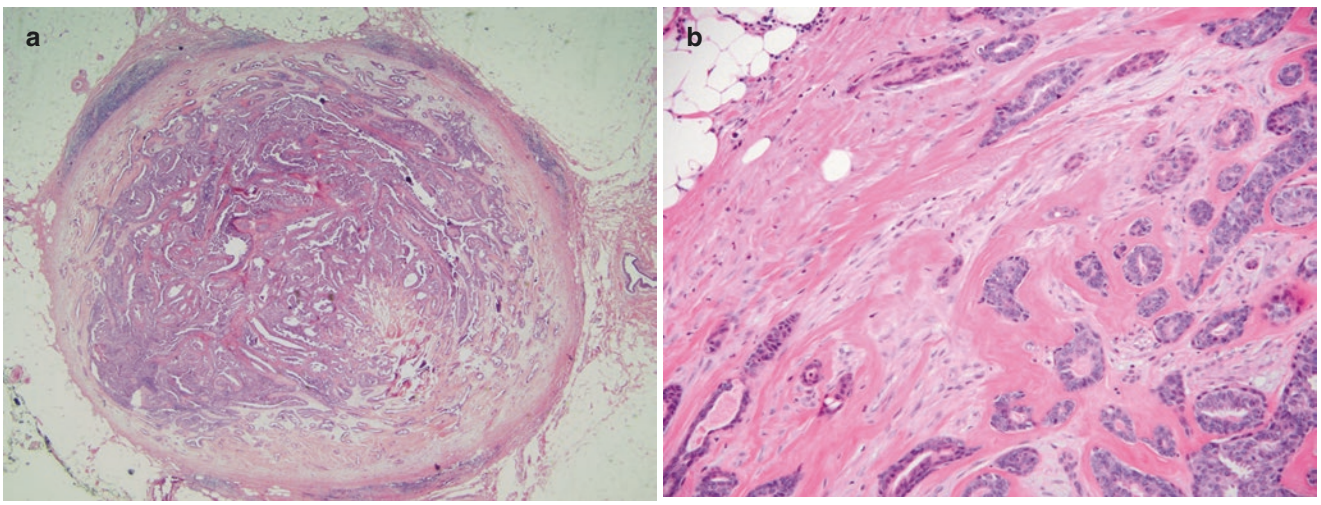


Fig. 5.2 IDP with sclerotic changes. Intraductal papilloma with sclerosis, low-power view (**a**) and high-power view at the periphery with "infiltrative glands" (**b**)

metaplasia may be seen. Other changes that can occur in IDPs include hemorrhage, infarction, and stromal fibrosis. Hemorrhage or infraction may occur secondary to a needling procedure or due to torsion of fibrovascular cores. The changes can be very extensive so that the papillary architecture may be obscured, and the terms "sclerosed papillomas" or "ductal adenomas" are coined in these extreme cases.

7. **What is atypical papilloma** [1, 10, 11]**?** (Fig. 5.3a–d)

IDPs can display focal atypical epithelial proliferation composed of a monotonous cell population. In earlier studies, atypical papilloma has been used to describe papilloma with 10 to <30% low-grade atypical epithelial hyperplasia, but this terminology is not in current use. The current recommended terminology is IDP with atypical duct hyperplasia (ADH) or ductal carcinoma in situ (DCIS). For the diagnosis, the WHO Work Group recommends size-based criteria. A lesion with low-grade atypical hyperplasia <3 mm in the greatest dimension will be classified as papilloma with ADH. A diagnosis of DCIS involving a papilloma will be made if the proliferation is low grade and 3 mm or larger, or is high grade.

8. **When is a diagnosis of IDP with atypia or DCIS made** [1, 11]**?**

The latest recommendation from WHO on the diagnosis for IDP with atypia is based on the extent of atypical epithelial proliferation. IDP with atypia is diagnosed when the atypical epithelial proliferation is <3 mm, while IDP with DCIS will be diagnosed when this atypical population is greater than 3 mm or high grade. (See also question 7.)

9. **How can one differentiate IDP from IDPC** [12–21]**?** (Fig. 5.4a–f)

IDPC shows ducts and/or TDLU filled with slender, branching fibrovascular stalks covered by a monotonous neoplastic epithelial cell population. In contrast, IDP is composed of benign epithelial cells showing more heterogeneous morphology with variable degree of intraductal hyperplasia. The papillae in IDPC are typically thin and delicate with minimal stromal fibrosis when compared with the board blunt fronds of IDP. The neoplastic epithelial cells in IDPC may form micropapillary, cribriform, or solid structures obliterating

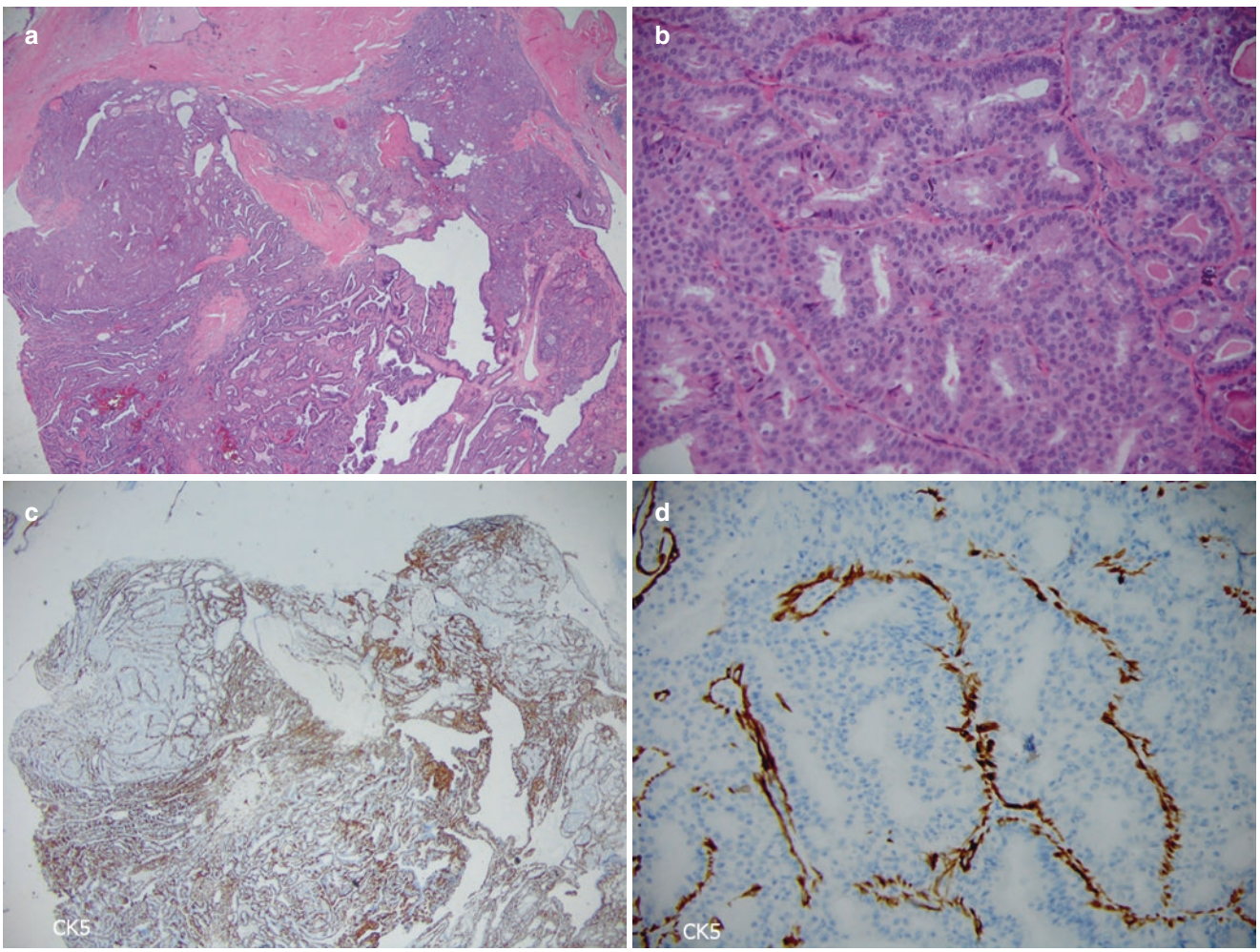


Fig. 5.3 IDP with atypia. Intraductal papilloma with ductal hyperplasia (**a**) and focally atypical with cribriform pattern (**b**). CK5/6 stain, low-power view (**c**) and high-power view for the ADH area (**d**)

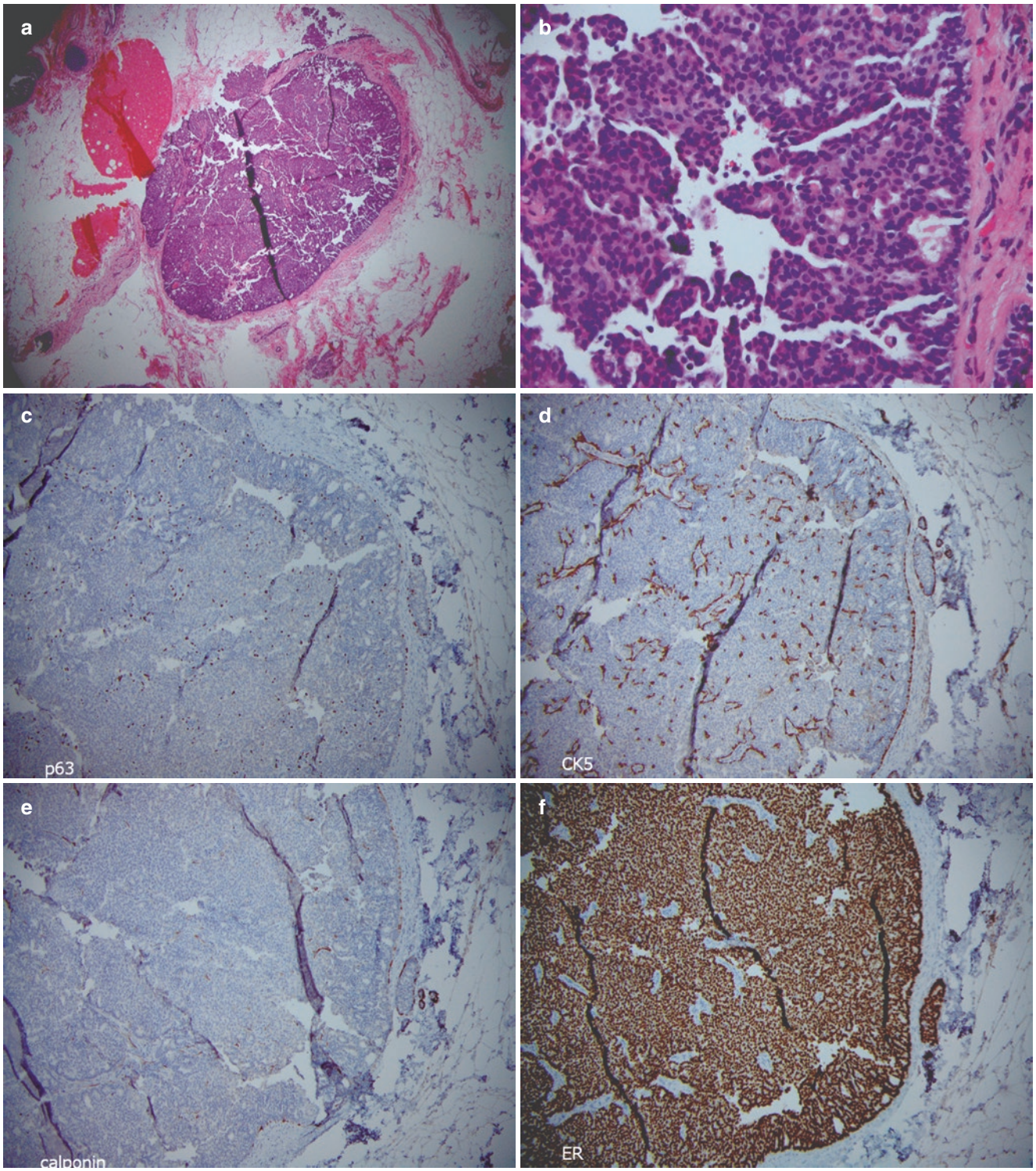


Fig. 5.4 IDPC. Intraductal papillary carcinoma low power (**a**). High-power view shows low-grade monotonous tumor cells with cribriform pattern (**b**). IHC stains for p63 (**c**), CK5 (**d**), calponin (**e**), and ER (**f**)

the spaces between papillary fronds. The complete or near complete (90%) absence of myoepithelial cells within the intraluminal papillary fronds is one of the histologic features to distinguish IDPC from IDP. While myoepithelial cells are mostly absent in the papillary processes, they may be present in attenuated form at the periphery of ducts. Nonetheless, the presence of myoepithelial cells, even extensively, does not necessarily exclude the possibility of an IDPC.

Immunohistochemistry can assist in the differentiation of IDP and IDPC. Myoepithelial markers – such as p63, smooth muscle actin, CD10, calponin, or high molecular weight cytokeratins (HMWCK) – can be used. The absence of myoepithelial markers along the papillary fronds and the presence of myoepithelial cells at the periphery of the involved ducts will identify IDPC. Of note, some IDPCs may show a dimorphic cell population, with some of the neoplastic cells showing clear cytoplasm adjacent to the basement membrane, and these may be mistaken for myoepithelial cells. In these cases, staining of myoepithelial marker will be particularly essential to illustrate the absence of myoepithelial cells. Since in IDPCs the neoplastic epithelial cells show clonal changes, they show diffuse and strong epithelial expression of ER/PR and absence of epithelial HMWCK expression. Conversely, IDP will show positivity of epithelial HMWCK and heterogeneous epithelial staining of ER/PR. As no single marker demonstrates entirely satisfactory performance in differentiating between these entities, variable marker combinations have been proposed to increase specificity and sensitivity. ER and MUC3 were shown to specifically identify IDPC, but CK5/6 and p63 were better suited for IDP. Other combinations that have been proposed to differentiate between benign and malignant papillary lesions in general include: CK5/6 and ER for atypical papillary lesions; CK5/6, p63, and neuroendocrine markers for SPC; a CK5/CK8/18/p63 cocktail; or a combination of HMWCK, namely, CK5/6, CK14, and 34βE12, for IDPC and EPC.

10. **How can one differentiate IDPC, EPC, and SPC [7, 22, 23]?** (Figs. 5.5a, b and 5.6a–f)

Compared to IDPC, EPC tends to show more extensive involvement by the neoplastic epithelial cells, and the lesion is typically larger. The most important feature for their differentiation is the presence of a thick fibrous capsule around the tumor in EPC, and the peripheral layer of myoepithelial cells may also be absent. In IDPC, a peripheral layer of myoepithelial cells is almost always present, and a thick fibrous capsule is lacking. SPC has a distinct morphology compared to IDPC and EPC. The predominant architecture of SPC is solid, with the neoplastic epithelial cells filling up the intervening spaces between the fibrovascular cores within the lesion, resulting in a solid overall appearance. Cribriform or discrete papillary architectures are not apparent in low power. The delicate underlying fibrovascular stromal network devoid of ME layer is typically discernible only at higher magnification, and, even so, the fibrovascular cores tend to be fewer than in IDPC and EPC. In SPC, the lesional neoplastic cells may show streaming and occasionally spindled appearance which can be mistaken for intraductal papilloma with florid ductal hyperplasia. Indeed, many SPCs display neuroendocrine differentiation, often have at least focal granular eosinophilic cytoplasm, and are immunoreactive for neuroendocrine markers. They may be associated with intracellular and extracellular mucin. As with EPC, myoepithelial cells may be lost both inside and at the periphery of SPC.

11. **What are the different IHC myoepithelial staining characteristics of IDPC, EPC, SPC, and DCIS involving papilloma?**

Papillary lesions can be differentiated by evaluation of myoepithelial markers staining in the two different compartments of the lesions, i.e., myoepithelial cells at the periphery of the tumor, and around the fibrovascular cores within the tumor. All papillary lesions (IDP and IDPC) are derived from ducts, and myoepithelial cells are present at the periphery. They are absent or markedly attenuated around the

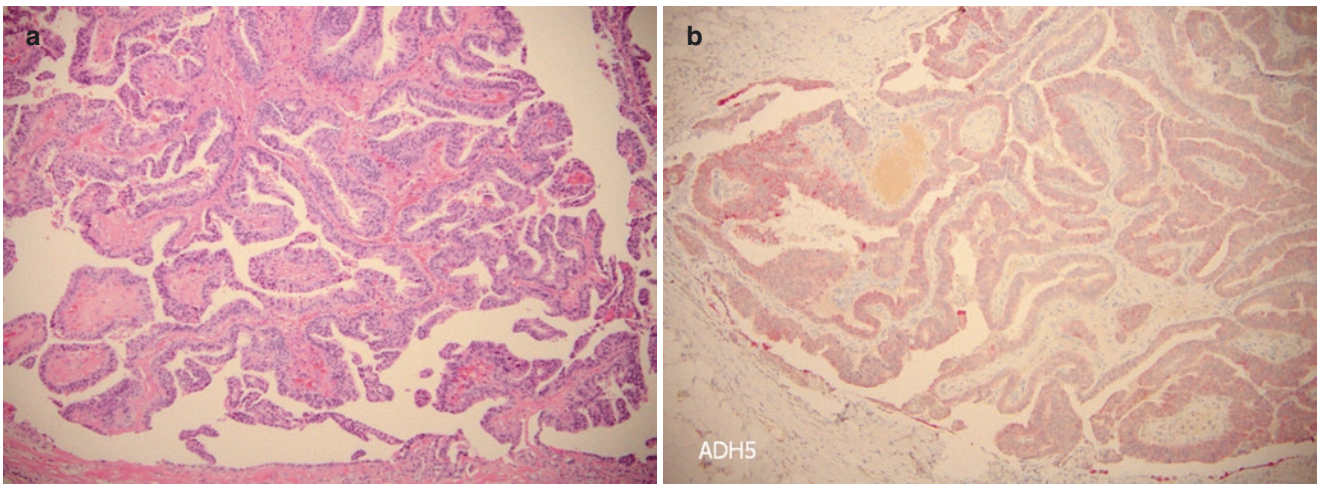


Fig. 5.5 EPC. Encapsulated papillary carcinoma (**a**) and ADH5 stains showing lack of myoepithelial cells both at the peripheral and fibrovascular cores (**b**)

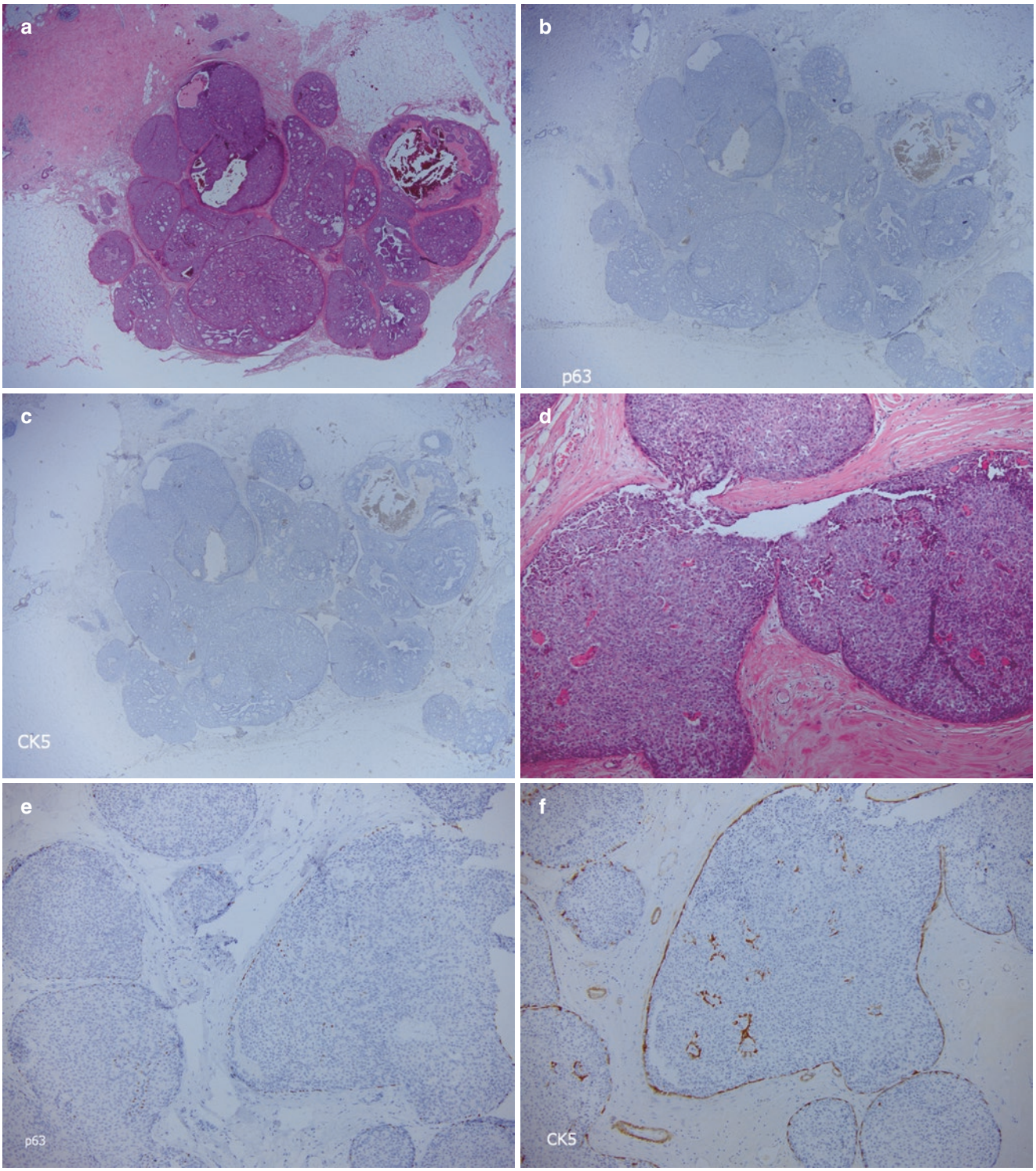


Fig. 5.6 SPC. Solid papillary carcinoma (**a**, **d**) with myoepithelial marker p63 (**b**, **e**) and CK5 (**c**, **f**) IHC stains

fibrovascular cores within IDPC. In DCIS involving papilloma, myoepithelial cells can be found throughout the lesion. In EPC and SPC, there may be a lack of an outer myoepithelial cell layer, and myoepithelial cells can be lost, attenuated, or retained around the fibrovascular core within the lesion. See Table 5.1.

12. **How is invasion defined in IDPC, EPC, and SPC** [7, 14, 17, 24–31]**?**

IDPC is generally considered a carcinoma in situ, as evidenced by the presence of myoepithelial cells in the periphery of the ducts containing the IDPC. Occasionally, the

Table 5.1 ME marker staining on different papillary compartments

ME marker staining	IDPC	EPC	SPC	DCIS involving papilloma
Periphery	Present	Absent	Absent/present	Present in papilloma
Around fibrovascular cores	Absent/scant	Absent/attenuated/retained	Absent/attenuated/retained	Present in papilloma

presence of glandular entrapment within a hyalinized or fibrotic stroma and mechanical dislodgement during a previous biopsy may mimic invasion. In addition to IDPC, these may also occur in other types of papillary carcinomas.

To differentiate benign entrapment and true invasive foci, identification of myoepithelial cells (albeit attenuated), the bland morphology of the "infiltrative" epithelial cells, a hyalinized/altered stroma, and the general directional alignment between the epithelium and the fibrosis of the stroma are useful to confirm glandular entrapment rather than genuine invasion.

The lack of a myoepithelial cell layer in EPC and SPC has led to the postulation that these lesions represent a minimally invasive low-grade carcinoma rather than an in situ carcinoma. This postulation for EPC is further supported by evidence of occasional lymph node and distant metastases. Other evidence suggests EPC may represent an intermediate form between DCIS and invasive duct carcinoma. EPCs with high-grade nuclear morphologic features and high mitotic indexes are more often associated with an invasive component. However, there are reports of continuous envelopment of basement membrane in EPC, arguing for an in situ rather than invasive carcinoma. So far, there is a lack of consensus as to whether EPCs are invasive or in situ; thus, they are best considered as in situ carcinomas to avoid overtreatment.

In SPC, there may also be a lack of myoepithelial cells in the periphery of the tumor. In such cases, particularly when a rounded configuration is maintained, the current thinking is to consider such as in situ carcinoma. SPC has a low but definite risk of lymph node metastasis. Nevertheless, cases that are purely "in situ" (without invasive component) have significantly better outcomes than other pure DCIS. Thus, most solid papillary carcinomas, when stringently defined and excluding invasive component, represent carcinoma in situ with favorable prognosis.

In all papillary carcinomas (IDPC, EPC, and SPC), unequivocal invasion occurs when neoplastic elements infiltrate beyond the fibrous capsule, and the invasive component tends to show a geographic jigsaw pattern with ragged and irregular margin. The invasive component of SPC may show mucinous tumor with neuroendocrine differentiation.

13. What are the underlying molecular changes associated with IDP and papillary carcinomas [32–37]?

Both IDP and papillary carcinomas show LOH on chromosome 16p13 in the TSC2 gene region, with similar frequencies (60–63%), while LOH at locus 16q23 is limited to papillary carcinomas. Comparing changes in chromosomes, one study revealed alterations in chromosomes 3, 7, 17, and X in 15–21% of the papillary carcinomas, but not in any of the IDPs. Also, IDPs with/without florid hyperplasia show higher frequency in PIK3CA or AKT mutation compared to papillary carcinomas, suggesting a potentially divergent molecular pathway in pathogenesis.

Genomic profiling of papillary carcinomas (including EPCs and SPCs) shows a similar pattern of gene copy number aberrations as grade- and ER-matched infiltrating duct carcinomas, no special type (IDC-NSTs). However, papillary carcinomas display less genomic aberrations than IDC-NSTs and a higher mutation rate in PI3KCA. The genomic profiles of the three morphologic papillary carcinomas (EPC, SPC, and IPC) are remarkably similar. Regarding gene profiling, most papillary carcinomas are classified as luminal subtype, with rare basal-like subtype. In a study with limited cases, most EPCs and IDPCs are classified as luminal A cancers, while SPCs are classified as luminal B cancers. Compared with grade- and ER-matched IDC-NSTs, papillary carcinomas show distinct gene expression profiles with downregulation of proliferation-related, cell assembly and organization, cellular movement and migration genes, and overexpression of genes related to homeostasis and angiogenesis, thus suggestive of a less invasive phenotype. Apart from PI3KCA and TP53, highly recurrent gene fusions and recurrent mutations are unlikely to be involved in papillary carcinomas.

14. What are the further recommendations for management of papillary lesion in biopsy? [38, 39]

The standard recommendations for patients with atypical/malignant papillary lesions are surgical consultation and excision. However, for IDP the surgical management is more controversial, as on the one hand there exists a small possibility of malignant upgrade in excision for core needle biopsy (CNB)-diagnosed benign papillary cases and, on the other hand, excising all benign IDP may constitute overtreatment, as a majority of CNB-diagnosed benign cases do not show any upgrade at excision. Some authors suggested surgical excision for cases with segmental abnormalities observed radiologically because this may suggest coexisting DCIS. In the absence of other indications for excision, observation with close imaging follow-up has been suggested for radiologically concordant IDP and incidental micropapillomas.

15. Papillary lesion in biopsy – upgrade in excision [11, 17, 38, 40–43]

A meta-analysis of 34 studies involving over 2000 patients demonstrated that the pooled estimate for underestimation

was 15.7%. Other studies reported an upgrade rate for benign papillary lesions on CNB diagnosis to atypia/malignancy in excision to range from 3% to 33%. Of note, most of the malignancies found in excision were low-grade DCIS rather than invasive cancer.

The upgrade rate appears to be associated with tumor topography. Up to 25% of DCIS associated with papilloma had demonstrable eccentric distribution after careful histologic assessment and topographic reconstruction, and up to 83% of cases showed segmental abnormalities on radiology. These constitute high-risk features for a small-volume CNB and may result in sampling error, missing the malignant part of the lesions. Furthermore, by definition, underestimation of DCIS involving papilloma in CNB is unavoidable. As the diagnosis of DCIS involving papilloma is defined by the extent of the lesion (3 mm or more), in a small-volume CNB, sampling of only part of the atypical focus would mean sampling only a smaller focus, and then an atypical diagnosis would be rendered as the size criteria for DCIS may not be met. Higher upgrade rates have also been associated with small core biopsies, with fewer cores being taken, when the pathologist is not a subspecialty expert in breast pathology and when an immunohistochemical workup of the biopsy has not been performed. Adequate sampling is a prerequisite in preventing upgrade. Larger tissue samples significantly improved the predictive value of benign histology on CNB.

Case Presentations

Case 1 Misdiagnosed Sclerotic IDP as IDC (Fig. 5.7a–d)

History: A 45-year-old female with a mass lesion on screening mammogram, core needle biopsy, with a diagnosis of IDC NOS, histologic grade 1. You received this for review; the patient is scheduled for surgery next Monday.
IHC: ER+, PR+, HER2 – (0), Ki67 <5%.
DDx: IDC vs. sclerotic IP

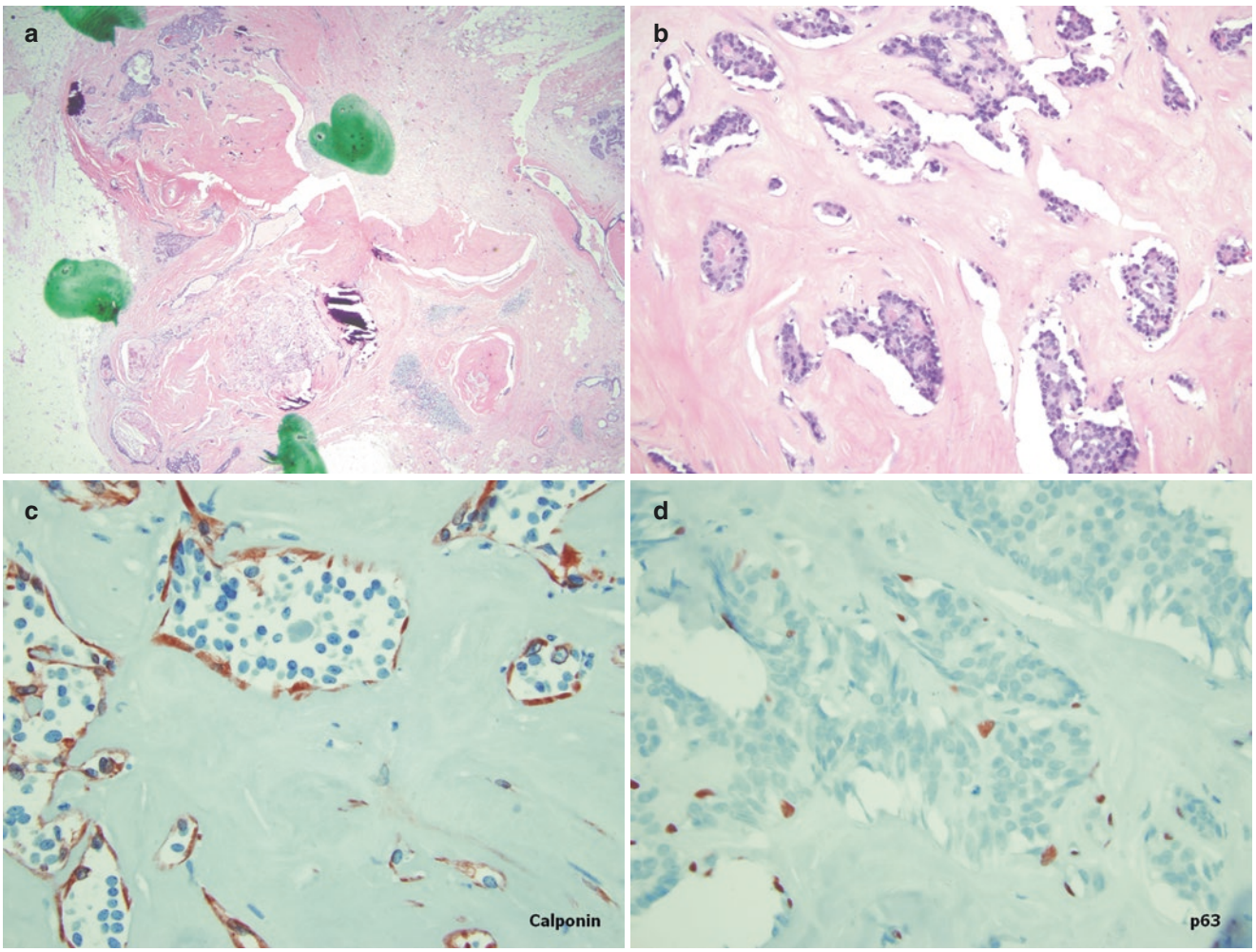


Fig. 5.7 Case 1. IP with sclerosis. Sclerotic lesions in irregular border (**a**) and small infiltrative glands (**b**) can mimic invasive ductal carcinoma; however, the well-demarcated border and hyalinized stroma can give a hint of a papillary lesion. Myoepithelial markers calponin (**c**) and p63 (**d**) can help prove the noninvasive process

Next steps:
- Call the surgeon to hold off on the for definitive diagnosis or only do local resection without SLNB.
- Call the original lab for blocks to confirm your suspicion.
- IHC results: Myoepithelial cells were highlighted around the infiltrating glands.

Final diagnosis: sclerotic IDP
Take-home messages:
- Be very cautious on core biopsy due to limited tissue sampled.
- Be suspicious when seeing infiltrative glands within hyalinized stroma in a well-demarcated lesion.
- Be aware of clinical symptoms: near nipple, bloody discharge, older patient.

Case 2 Sclerotic IDP with Florid Ductal Hyperplasia (Fig. 5.8a–d)

History: A 50-year-old female with a breast mass, core biopsy, diagnosed with atypical IDP. Surgical excision recommended. You have received the surgical specimen.
Histology: Solitary papillary lesion, with marked epithelial proliferation and sclerotic changes at the peripheral
IHC: CK5 – positive for most of the cells, no area with complete lack of it
P63 – only positive at the peripheral of the lesion
ER – weakly positive in some cells
Final diagnosis: Sclerotic IDP with florid ductal hyperplasia
Take-home messages:
- IDP with ADH has a specific diagnostic criteria, i.e., <3 mm ADH within an IDP.
- Sclerotic changes at the peripheral are often difficult to differentiate from IDC; a panel of IHC is recommended.
- Myoepithelial stains can be weak and attenuated.

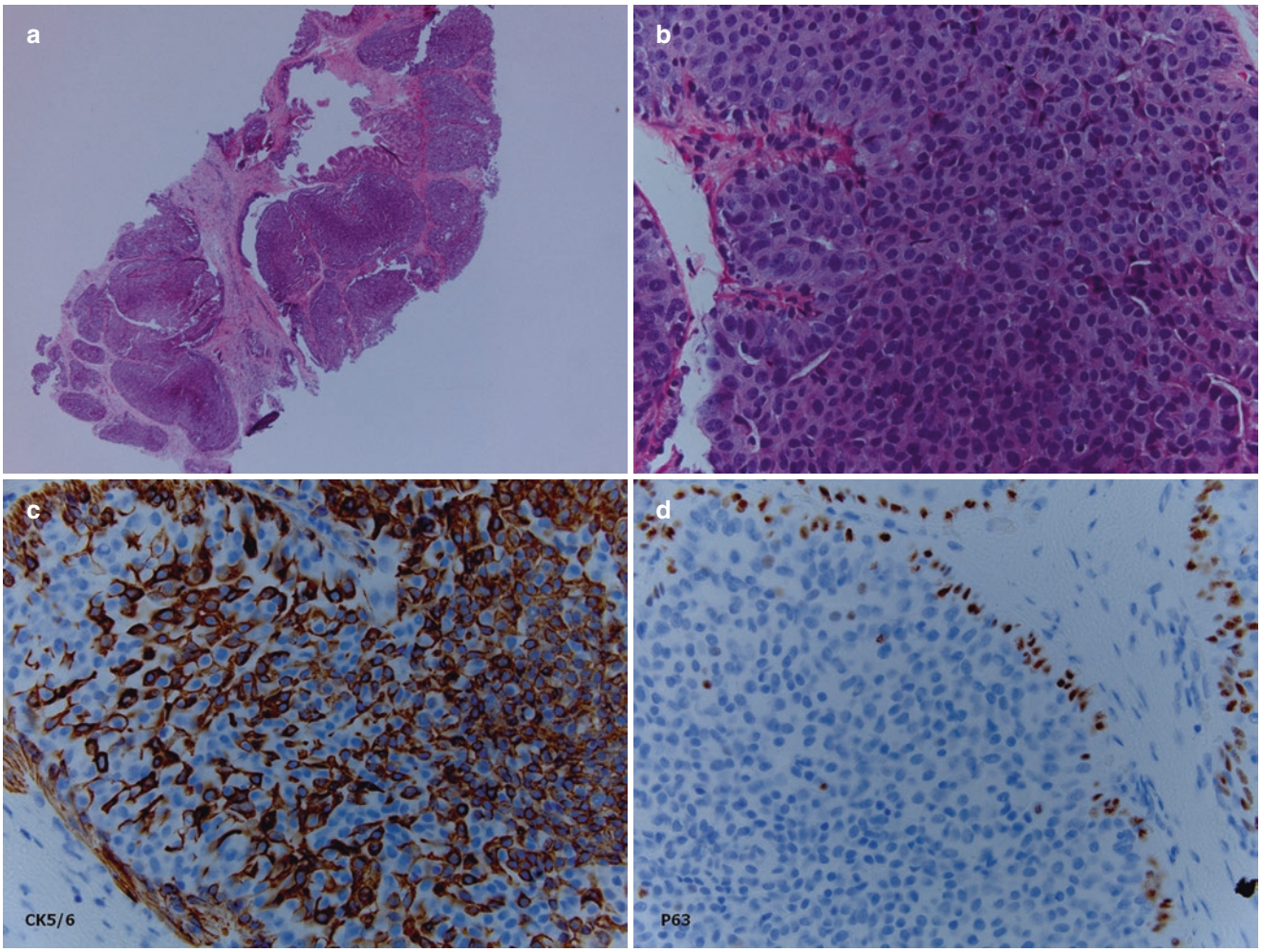


Fig. 5.8 Case 2. IDP with florid ductal hyperplasia. Marked florid ductal hyperplasia in an IDP can be easily confused with SPC at both low-power (**a**) and high-power (**b**) views. IHC staining for CK5/6 is the key for correct diagnosis, showing a nice mosaic staining pattern (**c**). Here, p63 is not as useful, as it only stains the peripheral myoepithelial cells as it does for DCIS (**d**)

Case 3 SPC In Situ Lesion - Without Peripheral Myoepithelial Cells but with Prior Core Biopsy (Fig. 5.9a–e)

History: A 50-year-old female with a mass lesion and core needle biopsy
Histology: DCIS, solid type
IHC: ER+, PR+
Surgical specimen, histology:
- Solid nests of tumor cells, low to intermediate grade.
- One area shows smaller nest with irregular borders.
- Myoepithalial markers negative for the entire tumor.
- Most nests contain hidden fibrovascular cores.

Final diagnosis: SPC with biopsy site changes
Take-home messages:
- Neuroendocrine markers only positive in 50–60% of SPC, thus not good for diagnosis.
- Morphologic assessment and identification of fibrovascular cores are most useful, but the latter may not be obvious.
- Glands trapped inside biopsy tract can be small, irregular, mimic IDC

Case 4 SPC with Invasion - Estimation of Size of the Invasion (Fig. 5.10a–d)

History: A 50-year-old female with a breast mass
Histology:
- Large nests of solid papillary lesions with focal irregular nests nearby.
- The borders are largely smooth.
- No necrosis present.
- Few mitoses present.

IHC: *ER*+, PR+, HER2 (0) negative, Ki67 10%
Next steps:
- IHC for p63, calponin, and myosin are absent in all areas with tumor.

Final diagnosis: Focal IDC associated with a large SPC
Take-home messages:
- SPC may or may not have the peripheral myoepithelial cells; and is considered an in situ lesion.
- True invasion is only diagnosed if the tumor cell clusters:
  - Possess an irregular border.
  - Are not located within prior biopsy site.
  - Lack myoepithelial cells.

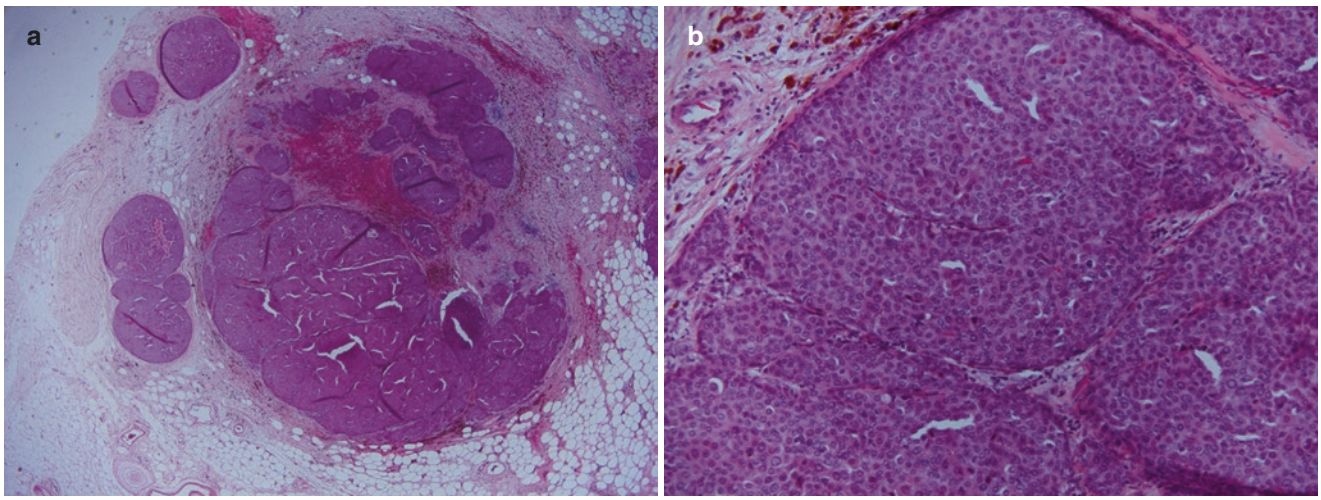
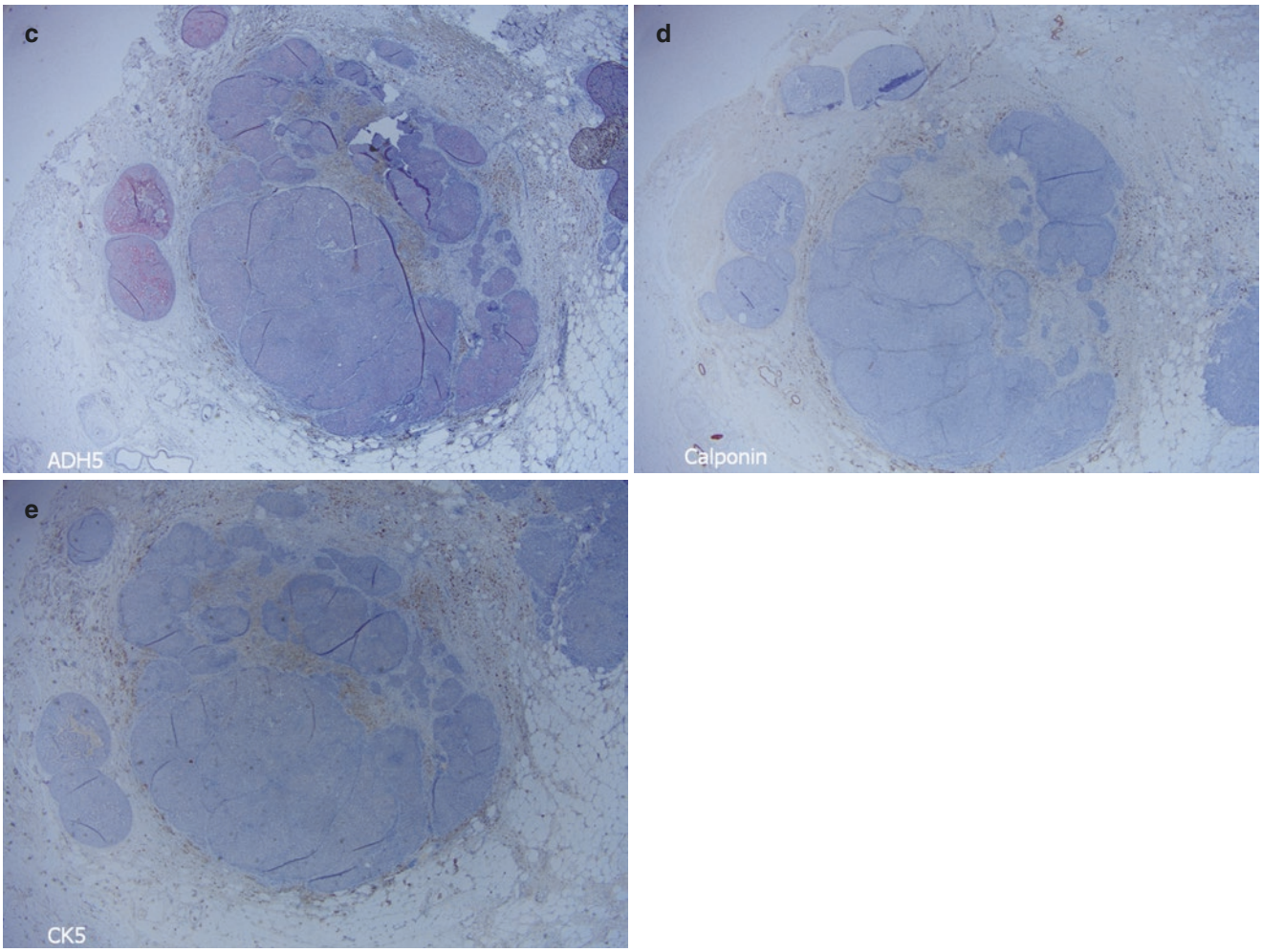


Fig. 5.9 Case 3. SPC with biopsy site changes. Roughly half of SPC lacks the peripheral myoepithelial cells, which makes the diagnosis of SPC with invasion very difficult purely based on morphology. A low-power view shows a very well-demarcated lesion, suggesting a SPC without invasion. The center of the lesion has a prior needle tract with smaller irregular and somewhat infiltrative glands and hemosiderin-laden macrophages, mimicking IDC (**a**); a higher-power view shows the smooth peripheral border of the tumor and confirms a diagnosis of SPC without invasion (**b**). Although this tumor lacks myoepithelial cells by ADH5 (**c**), calponin (**d**), and CK5 (**e**), it is still considered an SPC without invasion



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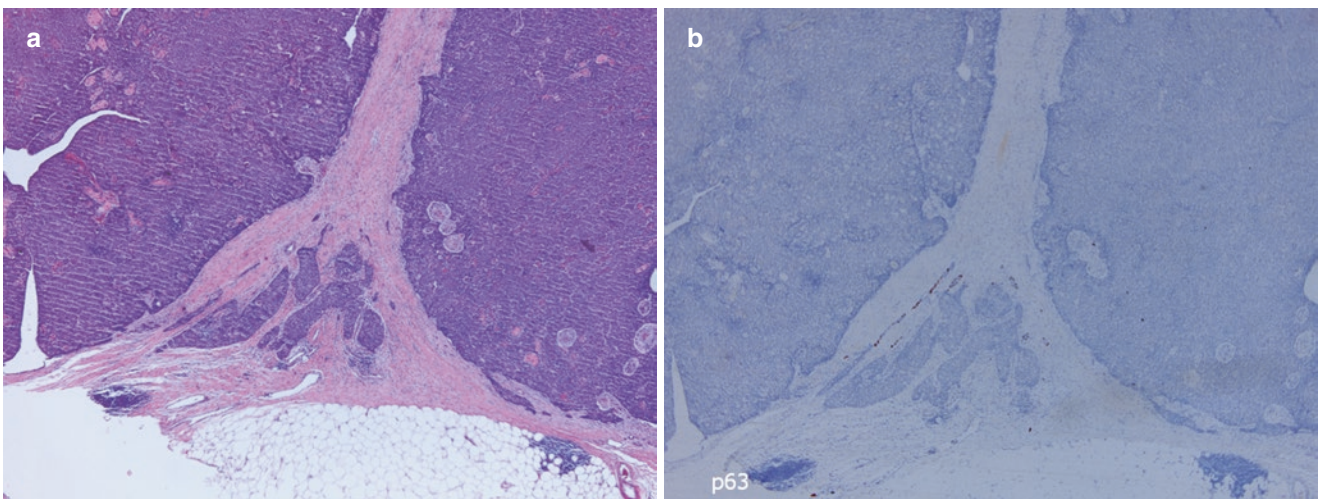

Fig. 5.10 Case 4. SPC with invasion. Although the larger tumor nests have smooth borders, the smaller ones in-between do not, suggesting invasion (**a**); lack of p63 staining confirms the invasion (**b**) for the small tumor nests. Another area of this tumor with focal invasion is shown in (**c**, **d**)

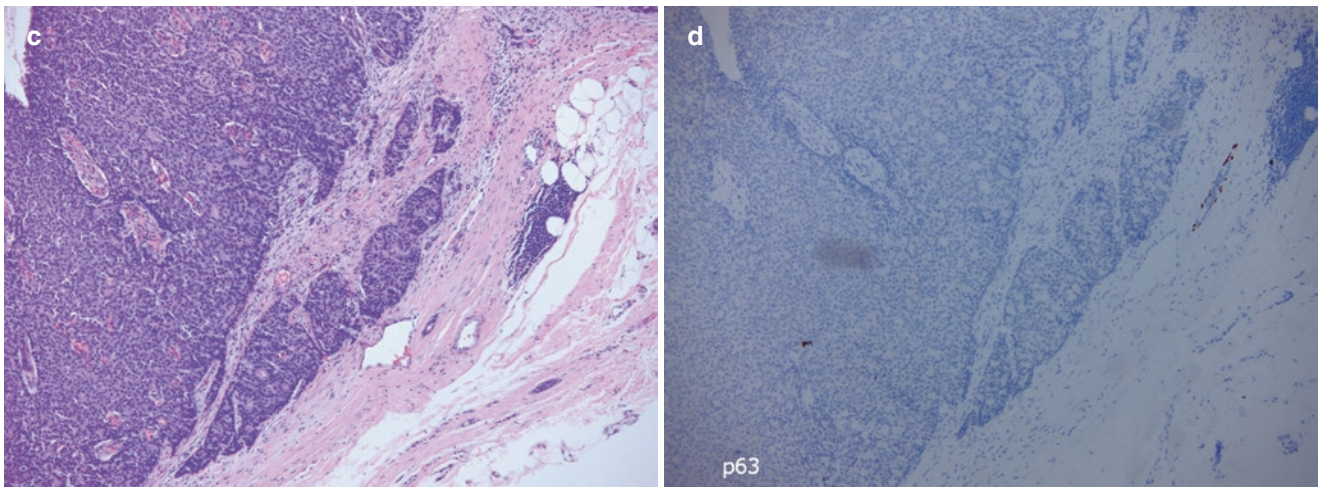

Fig. 5.10 (continued)

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Fibroepithelial Lesions (Phyllodes Tumor and Fibroadenoma) of the Breast

6

Julia Y. Tsang and Gary M. Tse

List of Frequently Asked Questions

Fibroadenoma: Morphological Variants

1. **What is the morphological spectrum of fibroadenoma** [1]**?**

Fibroadenomas (FAs) are well-circumscribed biphasic lesions showing proliferation of stroma and epithelium arising from the terminal duct lobular unit. In general, the stromal component may show focal or diffuse hypercellularity (especially in young women <20 years), bizarre, multinucleated giant cells, extensive myxoid changes, or hyalinization with dystrophic calcification (popcorn calcification) and rarely ossification (especially in postmenopausal women).

There are two distinct growth patterns: pericanalicular and intracanalicular patterns, which are of no clinical significance. In pericanalicular pattern, stromal cells proliferate around ducts or sometimes lobules in a circumferential fashion without epithelial compression. Open lumens can be observed. This pattern is more frequent in women in their second or third decades. In intracanalicular pattern, stromal proliferation compresses ducts into clefts. Any FAs may show either or both patterns.

Apart from the growth pattern, the stromal composition of FA may show variations. The typical FA has even distribution of epithelium and stroma which is consistent throughout the lesion. Prominent cellular stroma, either diffusely or focally, may be encountered, especially for FA in younger women. These FAs are called cellular FAs. Cellular FAs may have overlapping histologic features with benign phyllodes tumors (PTs).

An FA with prominent stromal myxoid changes throughout is described as a myxoid FA. Myxoid FAs can be associated with Carney syndrome, as such they are commonly multicentric or bilateral while sporadic cases are more often solitary. Hyalinized FAs may show prominent stromal sclerosis and hyalinization, with or without associated calcifications. These FAs tend to be distinctly hypocellular and are especially common in older women, presenting as long-standing static or regressing breast masses. Popcorn calcifications can be seen in this type of FA.

2. **What are the diagnostic criteria of complex fibroadenomas and the clinical implications** [2–4]**?**

Complex FAs contain one or more of the followings: cyst of or greater than 3 mm, sclerosing adenosis, papillary apocrine hyperplasia, or epithelial calcification. Among these, the presence of cysts and sclerosing adenosis are the most common. Proliferative epithelial changes may occur more often adjacent to complex rather than noncomplex FAs. Complex FAs are associated with older age of patients and tend to be smaller than noncomplex FAs. A minimally higher risk of subsequent invasive cancers had been found in patients with complex FA compared to those with noncomplex FA. Such risk remained elevated for decades after diagnosis.

3. **What are the criteria for juvenile fibroadenoma?**

Juvenile FAs are characterized microscopically by mildly increased stromal cellularity with a fascicular stromal arrangement and epithelial hyperplasia. The architecture is often pericanalicular rather than intracanalicular or mixed. Usual ductal hyperplasia in the epithelium often features delicate micropapillary epithelial projections with epithelial hyperplasia with bulbous tips, referred to as "gynecomastoid"-like due to its resemblance to epithelial changes in

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Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5_6

gynecomastia. Stromal cellularity is typically evenly distributed throughout the lesion. However, mild intralesional heterogeneity may occasionally be observed. There is no stromal cytologic atypia. Mitotic activity can be seen in some adolescents but is mostly rare.

4. **How many mitoses are allowed in fibroadenoma** [5–8]**?**

Stromal mitoses are mostly absent in the majority of common FAs. However, widely scattered mitotic figures may be seen in some cases, especially in young patients or during pregnancy. Mitotic activity (up to 7 mitoses/10 HPF) has been described in juvenile FA. A diagnostic threshold for mitoses in FA has not been established. In general, less than 3 per 10 HPF is applied in most studies. Nonetheless, a benign diagnosis also requires support from other histologic and clinicopathologic features, such as a circumscribed border, lack of cytologic atypia, stromal overgrowth, or necrosis.

5. **What are the pregnancy changes in fibroadenomas** [9]**?**

During pregnancy, the high concentration of estrogen, progesterone, and prolactin promotes the growth of ducts, and the formation of tubuloalveolar structures could lead to significant enlargement of an FA. The microscopic changes correspond to the parenchymal hyperplasia seen in the surrounding normal breast tissue. Secretory hyperplasia sometimes occurs diffusely in FA during pregnancy, or a preexisting FA may be unaltered. Epithelial proliferation may be extensive enough to obscure the original morphology or it may be almost or completely absent. Usually the distribution of the change is very irregular, and large areas of the FA may be apparently unmodified by the pregnancy-driven hyperplasia. Multiple FAs in the same breast may have different degrees of response. In general, unless the FAs are hyalinized or of long standing, some degree of change is always seen during pregnancy.

Phyllodes Tumor: Morphological Variants

6. **What are the morphological variants in phyllodes tumor** [10–15]**?**

There are uncommon morphologic variants in phyllodes tumors (PTs). The stromal component may show cartilaginous, osteoid, or lipomatous metaplasia. The epithelial component may show apocrine or squamous metaplasia. Squamous metaplasia may occur in PTs of different grades, with a reported incidence of 3.6–10%. Rarely, ductal or lobular carcinoma in situ or invasive carcinoma may arise in the epithelial component.

7. **What are the diagnostic criteria for benign phyllodes tumor** [12, 16–18]**?**

Benign PTs are well circumscribed and show mildly increased stromal cellularity as compared with an FA with no to mild nuclear atypia. Mitoses are rare, ranging from 0 to 4 mitotic figures/10 HPF. Stromal overgrowth (defined as the presence of stroma without epithelium in at least one low-power field as observed with a × 4 microscope objective) is absent. The margins are usually well delimited and pushing, although very small tumor buds may protrude into the surrounding tissues. Such protrusive expansion may be left behind after surgical removal and is a source of local recurrence. Heterologous elements are absent. Benign lipomatous, cartilaginous, and osseous metaplasia have been reported. Benign PTs are more likely to demonstrate pseudoangiomatous stromal hyperplasia (PASH), multinucleated stromal giant cells, and epithelial hyperplasia. See Figs. 6.1, 6.2, and 6.3.

8. **What are the diagnostic criteria for malignant phyllodes tumor** [16]**?**

Malignant PTs showed marked stromal cellularity and nuclear atypia. Mitotic activity is usually abundant (at least 10 per 10 HPF). The tumor border is at least focally infiltrative. Stromal overgrowth is often present. Owing to overgrowth of sarcomatous components, the epithelial component may only be identified after examining multiple sections with diligent sampling of the tumors. Malignant tumors are also diagnosed when malignant heterologous elements (pleomorpgic liposarcoma, chondrosarcoma, and osteosarcoma) are present even in the absence of other features.

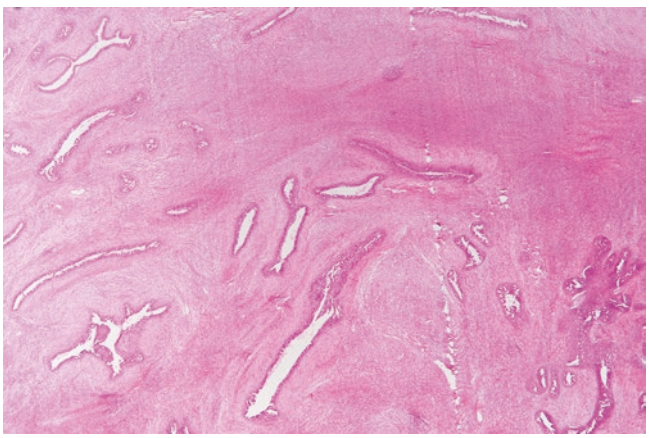

Fig. 6.1 Benign phyllodes tumor showing expansion of the stroma, with a variability of the stromal cellularity, and condensation of the stromal cells in a subepithelial location, whereas the intervening areas retain a low cellularity. The ductal element may be tubular

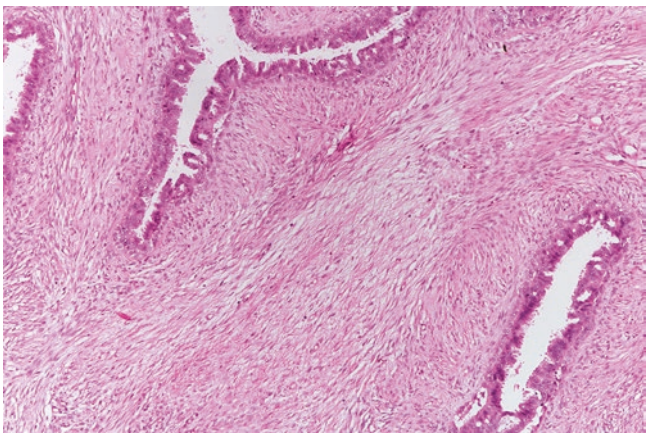

Fig. 6.2 Higher magnification of a benign phyllodes tumor showing increased stromal cellularity, and the stromal cells exhibit bland morphological features devoid of significant atypism. The epithelial component shows hyperplasia with stratification of the nuclei. There is again no atypism

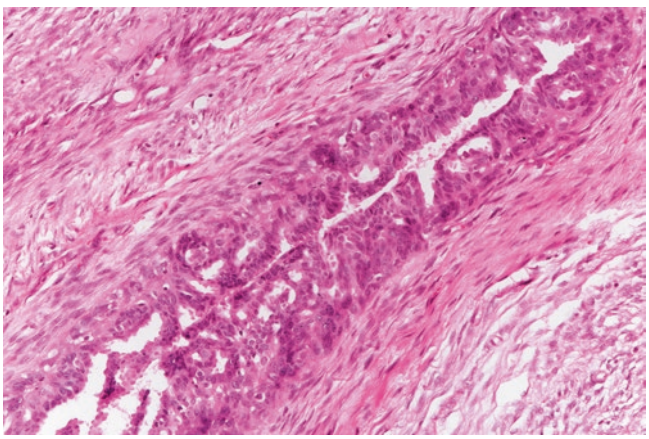

Fig. 6.3 Epithelial hyperplasia is common in all grades of phyllodes tumors. In this example, there is prominent epithelial hyperplasia in a benign phyllodes tumor. The hyperplastic epithelium shows typical florid epithelial hyperplasia changes, devoid of atypical architecture

9. **What are the diagnostic criteria for borderline phyllodes tumor** [16]**?**

Borderline PTs are diagnosed when the tumors possess some but not all features of malignancy. The classification is arbitrary, and borderline PTs have varied morphological appearances, overlapping with malignant and benign PTs. In general, they show well-defined or focally invasive border, frequent mitoses (5–9/10 HPF), moderate stromal cellularity, and mild to moderate stromal atypia. Stromal overgrowth is usually absent or very focal.

10. **Can fibroadenomas or phyllodes tumors be associated with other breast diseases** [4, 12, 14, 19–23]**?**

FA and PT may display a wide spectrum of proliferative changes in their epithelium. Usual ductal hyperplasia was reported in 43.7% of FAs, with most demonstrating a moderate degree of hyperplasia. Atypical epithelial proliferations, including atypical ductal or lobular hyperplasia and ductal or lobular carcinoma in situ, have also been observed infrequently in FA. The rate of carcinoma associated with FA ranges from 0.01% to 0.3% in screened populations. There is a predominance of carcinomas in situ (over 95%) than invasive carcinomas. Similar to FA, usual ductal hyperplasia is also commonly seen in PT (up to 74%). Benign PTs showed more hyperplasia than borderline or malignant tumors. Other atypical epithelial proliferations may also be found rarely. In a large series, there was 1.5% of PT with ADH and 0.03% each with DCIS, ALH, and LCIS. There are case reports showing association of PT with invasive cancers.

Phyllodes Tumor: Core Needle Biopsy

11. **Can we diagnose phyllodes tumor on needle core biopsy** [24–27]**?**

Diagnosis of PT on core needle biopsy (CNB) can be difficult. The false-negative rate ranged from 8% to 39% and false-positive rate ranged from 0% to 17%, although a sensitivity of 83% and a specificity of 92% were reported by others. With increasing degree of malignancy, a definitive diagnosis of PT on CNB can be made with more confidence. In a reported series with PTs in excision, a diagnosis of PT in the corresponding CNB was made in 44%, 67%, and 83% of benign, borderline, and malignant PT, respectively. None of the malignant PTs were diagnosed incorrectly. Only one case of malignant PT was put in the less specific diagnosis of FEL and all others were correctly diagnosed in CNB.

12. **How should we work up a core needle biopsy with hypercellular and pleomorphic spindle cells based on H&E morphology?**

The main differential diagnoses for core needle biopsy with hypercellular and pleomorphic spindle cells are malignant PT, metaplastic CA, and sarcoma. As PT and some metaplastic carcinoma possess a biphasic pattern, the presence of both malignant spindle cells and epithelial cells indicates metaplastic carcinoma. Benign epithelial component points to a diagnosis of malignant PT, and a malignant epithelial component (carcinoma, carcinoma in situ, squamous cell carcinoma) suggests metaplastic carcinoma. Sometimes the

epithelial component is much attenuated and requires extensive sampling. The presence of single malignant spindle cell component indicates sarcoma or some metaplastic carcinoma. If the spindle cells exhibit cytokeratin expression or basal marker expression, the diagnosis should be in favor of metaplastic carcinoma. The presence of malignant heterologous elements is NOT useful for the differential diagnosis, as all the lesions considered (malignant PT, metaplastic carcinoma, and sarcoma) may possess such heterologous element. (For more details, see the section on differential malignant PT, questions 25 and 26.)

13. **What are the features in core needle biopsy that help to differentiate between phyllodes tumor and fibroadenoma** [6, 8, 27–29]**?**

Many studies identified increased stromal cellularity and/or mitotic activity as useful for the differential diagnosis of PT and FA. Marked stromal cellularity is found in PTs, but FAs have lower cellularity. Likewise, higher mitotic counts are observed in PTs than FAs. However, in PT, there may be areas of variable stromal cellularity; in the low stromal cellularity area, there may be no histomorphologic difference from FA. Hence, there may be broad overlap of these features between PT and FA, and diagnosis based on these features solely is difficult. In addition, some studies also suggested tissue fragmentation and adipose tissue within lesional stroma in CNB of PT. However, one should note that FA with prominent intracanalicular pattern may also fragment with possible frond-like features in CNB; also FA rarely may show adipose metaplasia. Others also cited stromal hypercellularity, stromal nuclear atypia, stromal overgrowth, the presence of >2 mitotic figures per 10 HPF, and the presence of pseudoangiomatous stromal hyperplasia (PASH), to be predictive of PT over FA. Given the overlapping histologic features between PT and FA, a combination of these histologic features could be helpful for diagnosing PT over FA, but no hard and fast rules can be laid down.

14. **How accurate is core needle biopsy in diagnosing benign phyllodes tumor** [8, 29, 30]**?**

Benign PT can usually be differentiated from FA with typical features, such as absence of cellular stroma and mitotic activity on CNB. However, for lesions showing atypical features, when a clear differentiation of benign PT and FA is not possible, a generic diagnosis of fibroepithelial lesion is prudent. Several studies evaluated features in CNB to predict PT on excision. One study showed features including stromal cellularity, stromal overgrowth, fragmentation, and adipose tissue within the lesion could correctly identify 90% of PTs, but 31% of FAs were incorrectly diagnosed. Another approach took into account the number of histologic features present for PT diagnosis on CNB, including stromal overgrowth, increased stromal cellularity, mitotic activity, stromal fragmentation, adipose tissue infiltration or fat entrapment, stromal heterogeneity, and stromal cell pleomorphism. The presence of any three or more of these features showed a sensitivity of PT detection of 85.2%.

15. **Can one diagnose phyllodes tumor in fine needle aspiration cytology** [31–38]**?**

PTs are characterized by heterogeneous distribution of stromal cellularity, atypia, secondary epithelial component, and frond-like architecture. In the absence of the architectural details and limited sampling in fine needle aspiration cytology (FNAC), it is difficult to adopt universally accepted cytologic criteria for accurate diagnosis of PT. A wide range of accuracy rate (25–70%) was reported, and FA was the most common erroneous diagnosis. Occasionally, the presence of epithelial hyperplastic changes led to a misdiagnosis of carcinoma. The cytologic differentiation of PT from FA is often not possible. Some features, when present, may be more suggestive of PT; these include cellular stromal fragments; enrichment of elongated spindled nuclei in over 30% of the sample; large (>1 mm), elongated, and wavy epithelial clusters (compared to smaller tubular or blunt branching clusters in FA); and increased stromal epithelial area ratio. Accuracy of FNAC depends on an adequate and representative sample.

Phyllodes Tumor Grading

16. **In general, what are the relative proportions of benign, borderline, and malignant phyllodes tumors** [16, 39]**?**

In general, benign, borderline, and malignant PTs comprise 60–70%, 15–20%, and 10–20%, respectively, of all PTs. However, there is wide range of variation in the relative proportion of each grade in different series, indicating the current practice of using a combination of histologic features in PT grading, resulting in a large "gray zone" where the PTs do not exhibit all features of benign or malignant PT and are thus relegated to the borderline category.

17. **What are the histologic criteria for grading of phyllodes tumor** [13, 16, 40, 41]**?**

Grading of PT is based on semiquantitative assessment of stromal cellularity, cellular pleomorphism, mitotic activity, margin/border, and stromal overgrowth, rather than any isolated feature. The presence of malignant heterologous element is also relevant and, if present, will lead to diagnosis of malignant PT irrespective of other parameters. The histologic grading of PT is subjective. The assessment of most of

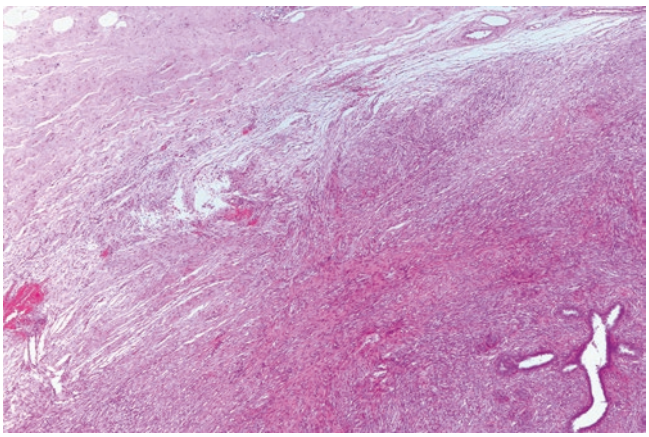

Fig. 6.4 Phyllodes tumor of borderline malignancy showing expansion of the stroma with spacing out of the epithelial component. The stromal component shows high degree of stromal cellularity, but the degree of nuclear pleomorphism is mild to moderate. The margin is infiltrative with seepage of irregular tongues of stromal cells into the adjacent normal breast stroma

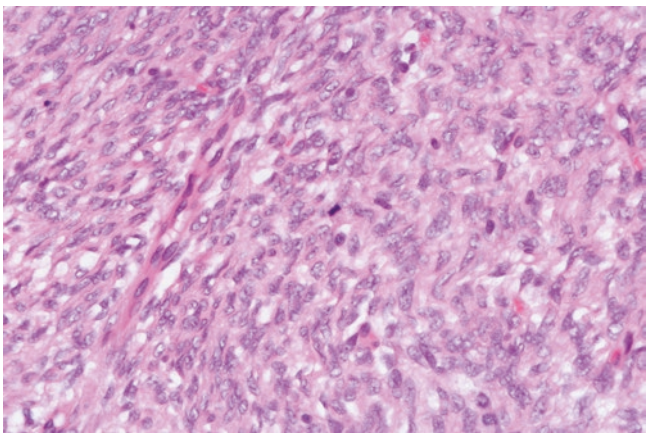

Fig. 6.5 Malignant phyllodes tumor showing very high stromal cellularity with crowding of stromal cells exhibiting moderate to high degree of nuclear pleomorphism. Mitotic figure can be seen

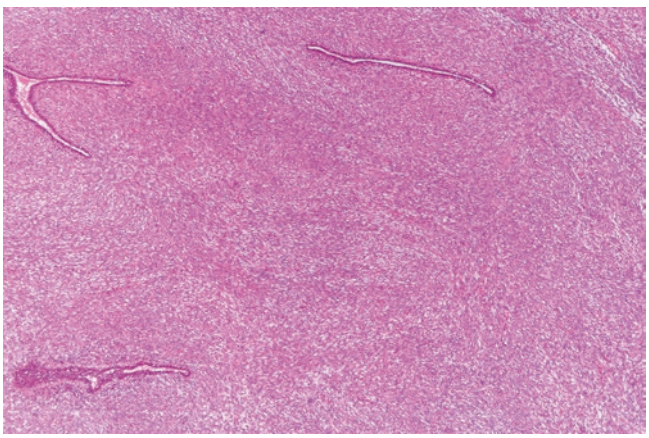

Fig. 6.6 Overgrowth of the stroma is characteristic in borderline or malignant phyllodes tumors. There is expansion of the stroma to the exclusion of the epithelial component

the criteria is into three grades; the separation from minimal/mild to moderate and moderate to marked changes are not clearly defined, in particular cellularity and atypia. It has been suggested that mild hypercellularity is characterized by a slight increase in stromal cells as compared with normal perilobular stroma, with evenly spaced nuclei that are not touching or overlapping. Marked stromal cellularity shows confluent areas of densely overlapping nuclei, whereas moderate stromal cellularity has findings that are intermediate, with some overlapping stromal nuclei. Mild stromal atypia shows nuclei with little variation in size, with smooth nuclear contours. Moderate atypia shows some variation in nuclear size, with wrinkled nuclear membranes, exceeding that in mild atypia but less than that in marked atypia. Marked atypia shows marked variation in nuclear size, coarse chromatin, and irregular nuclear membranes with discernible nucleoli. Furthermore, there is intralesional heterogeneity within the PT. PT could harbor features that typify benign lesion in some areas and borderline and malignant features in other foci. In such situation, the diagnosis should be based on the worse area. Lastly, there has not been any consensus as to whether any criteria are more important than others. See Figs. 6.4, 6.5, 6.6, and 6.7.

18. **What is the clinical relevance of grading phyllodes tumor** [42]**?**

The grading of PT allows prediction of prognosis and clinical behavior. Benign PTs have potential for local recurrence while borderline, and malignant PTs have the potential to locally recur and to metastasize to distant organs, and the risk is higher in malignant PT. While there is correlation between grade and recurrence, grading of PT is subject to inter-observer variation, and not all histologic parameters for PT grading are actually relevant in predicting PT outcome. In fact, a recently developed predictive nomogram only included three histologic criteria (atypia, mitosis, and stromal overgrowth) and surgical margin status for risk assessment of PT recurrence.

19. **Use of a two-tier or three-tier grading system: which one is more popular** [16, 19, 43]**?**

Earlier grading scheme with a two-tier system classified PT as benign (low grade) and malignant (high grade). A three-tier system grading PT into benign, borderline, and malignant has been used by most investigators and is adopted by WHO. It is suggested to be more clinically relevant as this approach leads to greater certainty at the ends of the PT spectrum (i.e., benign and malignant PT) (WHO classification).

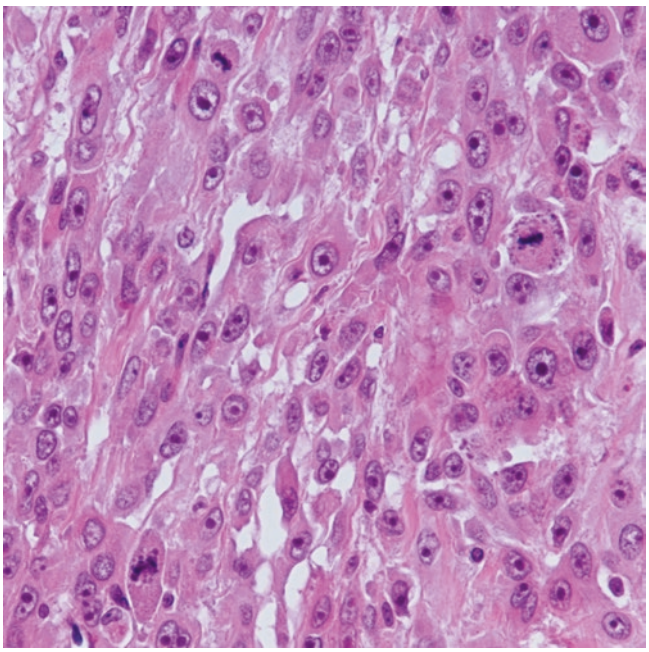

Fig. 6.7 Another example of malignant phyllodes tumor showing high-grade sarcoma-like features, composing of poorly cohesive malignant stromal cells with brisk mitotic activity

20. **Are there any biomarkers and genetic changes that are useful for grading of phyllodes tumor** [44–49]**?**

Correlations of immunohistochemical (IHC) markers with PT grading have been extensively studied. Some markers showed promise but have not been proven clinically; see question 28 for more details. Genetic changes in PT also correlated with increased malignancy. Benign PT showed few chromosomal changes, compared to numerous chromosomal imbalances in malignant and borderline PT. Chromosomal changes, namely, a high number of copy number variations (gains of 7p and 8q, losses of 3q and 10, losses in 9p21.3) and the presence of amplifications, especially involving *EGFR*, could be potential markers for malignant PT. IGF1R gene amplification was also reported in malignant PT only and could be another potential marker. Mutations in cancer genes (e.g., p53, EGFR, RB1, etc.) have been found exclusively in borderline/malignant PT and may also be useful in aiding of PT grading.

21. **In a biopsy report of phyllodes tumor, can one grade the phyllodes tumor** [27, 29, 50]**?**

In CNB reporting of PT, there exists up to 40% of overgrading (from benign to atypical) or under-grading (from atypical to benign). Among the different parameters used in PT grading, overall stromal cellularity, stromal cell pleomorphism, and mitotic count in CNB show good correlation with excisions. On the other hand, stromal architectural changes are more variable. The certainty increases with increasing degree of malignancy. Grading of PT in CNB could be problematic unless overtly malignant features are seen. A more accurate assessment can only be made in the excision specimens.

Differentials of Benign PT and FA

22. **What are the differentiating features between benign phyllodes tumor and fibroadenoma** [7, 51]**?**

The features to differentiate benign PT from FA include a pronounced intracanalicular growth pattern, the presence of increased stromal cellularity along with well-developed stromal fronds. In the absence of well-developed stromal fronds, the presence of elongated, branching, and cleft-like ductal spaces meandering through a cellular stroma, giving a staghorn appearance, may be a histologic clue to the diagnosis of PT. In benign PT, the increased stromal cellularity should be mostly present throughout the lesion, with subepithelial accentuation (cambium layer). At times, it may be difficult to distinguish benign PT from cellular FA because of overlapping histologic features. Definitive differentiation may not be possible. Stromal fronds typical of PT are not seen in cellular fibroadenoma, but, if present, are focal and not well developed. Cellular FA should not demonstrate significant nuclear overlap or areas of sheetlike stromal growth; the presence of these features favors PT.

23. **Should we differentiate between fibroadenoma and benign phyllodes tumors in all cases** [12, 16, 42, 52, 53]**?**

The differentiation between FA and benign PT appears important because of their purported differences in treatment and prognosis. A second surgical procedure may be considered to achieve negative margins for a benign PT incompletely excised initially. FA shares with benign PT a similar recurrence rate (15–17%) if incompletely excised. However, due to the similar clinical outcomes, a differentiation between the two may not be significant. Although some reports suggested that a minuscule proportion of benign PT may recur as malignant lesions, there is yet no identifiable feature/marker to enable prediction of such recurrence. Thus, when there is histologic ambiguity, a diagnosis of fibroadenoma will be preferred over a benign PT to avoid overtreatment.

24. **In a biopsy, how should we report if we are not sure whether the lesion is a fibroadenoma or a phyllodes tumor** [54]**?**

The term "benign fibroepithelial neoplasm" with the explanation of the diagnostic difficulties as needed could be employed in equivocal cases, in order to avoid overtreatment. This term, however, should be used sparingly, as it does not represent a new diagnostic category. More definitive characterization can be performed on the subsequent excision specimen.

Differentials of Malignant PT

25. **What are** the **differential diagnoses for malignant phyllodes tumor? How can one differentiate on H&E** [41, 55]**?**

The differential diagnoses for malignant PT mainly are spindle cell carcinoma, metaplastic carcinoma, and sarcomas (primary or metastatic). The diagnosis depends on the presence of residual epithelial structures. Malignant PT show frond-like epithelium with typically intracanalicular growth pattern associated with the malignant spindle cell proliferation. However, stromal overgrowth can be prominent and the biphasic nature (benign epithelial component) may not be readily appreciated, necessitating extensive sampling with many sections. The stroma of a malignant PT may, on occasion, show heterologous sarcomatous differentiation, including liposarcoma, rhabdomyosarcoma, leiomyosarcoma, angiosarcoma, chondrosarcoma, and osteosarcoma. A spindle cell metaplastic carcinoma contains varying proportion of malignant epithelial components, which may be of squamous, adenocarcinoma (ductal), or adenosquamous types. Metaplastic carcinoma can also be entirely devoid of frank epithelial element or show similar heterologous mesenchymal differentiation, although liposarcomatous element is unusual. The presence of DCIS adjacent to a malignant spindle cell tumor greatly favors metaplastic carcinoma. Primary and metastatic sarcomas of the breast are rare. There is significant overlap with malignant PT and metaplastic carcinoma, and differentiating them based on H&E staining is difficult. A history of previous or metastatic sarcoma, imaging, and clinical correlation may be helpful.

26. **What are common IHC markers to differentiate malignant phyllodes tumors from other spindle cell lesions** [56–64]**?**

Spindle cell carcinoma (metaplastic carcinoma) expresses p63 and cytokeratins (CK) (AE1/3, CAM5.2), in particularly high molecular weight cytokeratins (HMWCK) (34βE12, CK5/6, CK14). Positive staining with any of these markers supports a diagnosis of metaplastic carcinoma. However, such interpretation could be tempered with focal CK and p63 staining reported in both metaplastic carcinomas and PT. p40, postulated to be a more specific marker for squamous differentiation, may also be expressed by the stromal cells in malignant PT. Nevertheless it was considered more specific, albeit less sensitive than p63 for metaplastic carcinoma. It is important to note that not all CK markers are expressed in all metaplastic carcinoma. A CK panel is required in the differential diagnosis of malignant spindle cell lesions. CD34 conversely is variably expressed in PT but not in spindle cell metaplastic carcinoma. The expression of CD34 was reported to be negatively correlated with adverse histologic features. Another differential diagnosis is fibromatosis, and IHC nuclear staining for β-catenin is routinely used. However, aberrant nuclear expression of β-catenin has been reported in up to 94% benign PT and less in higher-grade PT, compared to essentially all cases in fibromatosis, and 23% in metaplastic carcinoma. Other markers, which are more frequently expressed in PT and which could be used as diagnostic adjuncts, include bcl-2 and CD117.

Biomarkers

27. **Are there any IHC markers that help with the diagnosis of phyllodes tumors** [28, 61, 65]**?**

For diagnosis of PT, IHC markers are mainly used for the differentiation of PT from metaplastic carcinomas or sarcomas. On IHC, PT and FA show similar IHC profiles and thus are considered as a whole; the reported useful markers include CD34 and proliferative indices (Ki67 and topoisomerase IIα). In normal breast, stromal fibroblasts express CD34 which becomes diminished in malignant disease. In FA, nearly all stromal cells express CD34 while patchy staining is seen in PT. A lower proportion of cells shows CD34 positivity in the malignant PT, compared to borderline and benign PT. One of the discriminatory features of PT from FA is the presence of mitotic activity. Thus proliferation indices correlate significantly with key histologic features of PT and may be discriminatory for PT and FA. IHC staining for proliferation indices in CNB was higher in those cases that diagnosed as PT on the subsequent excision than those diagnosed as FA. However, there are no established threshold and methodology for its assessment in this setting. For the diagnosis of PT from spindle cell carcinoma, CD34, CK, and p63 are useful. (Please refer to question 26 for details.)

28. **Are there any IHC markers that help with the grading of phyllodes tumors** [46, 64, 66–78]**?**

Biological markers that showed positive correlation with PT grade, such as p53, Ki67, C-kit, CD10, and EGFR, may

potentially be useful for PT grading. Several studies showed p53 staining correlated positively with grade of PT. Moderate to strong nuclear staining of stromal cells was noted in malignant PTs while no staining was noted in benign PTs. p53 staining also correlated positively with stromal cellularity and overgrowth. As mitosis is one of the criteria for PT grading, stromal proliferation index as measured by Ki67 staining is suggested for PT grading. A positive correlation of Ki67 staining was found with PT grade. Immunostaining of Ki67 was reported as 5% and 15% for benign and malignant PT, respectively. The stromal cells of PT express membrane tyrosine kinase receptor, c-kit. c-kit expression was found in 17% of benign, 24% of borderline, and 46% of malignant PTs. The overall rate of EGFR expression was 16.2%, 30.6%, and 56% for benign, borderline, and malignant PTs, respectively. EGFR staining not only correlated positively with PT grade but also with other markers implicated for PT grading such as p53, Ki67, and c-kit. CD10 (CALLA) expression in stromal cells of PT also positively correlated with PT grade. A positive staining in 5.9% of benign, 31.4% of borderline, and 50% of malignant PTs was reported, especially in the periductal zone with increased stromal cellularity and mitotic activity. Caution should be taken for CD10 staining in malignant PT as CD10 is also expressed in some high-grade sarcomas of the breast.

29. **Are there any IHC markers that help with the prediction of recurrences and metastases in phyllodes tumors** [64, 66, 73, 78–85]**?**

Despite the number of marker studies in PT, many showed only an association with grade but not prognostic value in multiple studies. p53 expression and Ki67 index were reported in some studies to be significantly associated with either disease-free and/or overall survivals. However, others showed no association with recurrence or clinical behavior. c-kit expression is more consistently correlated with poor clinical outcome. Cases with stromal c-kit expression are more prone to recur, and a worse overall survival was noted. Other markers reported to have prognostic implications in PT include six 1, Yap, and α-catenin; yet, these are mostly single studies and require further validation.

30. **Should we do ER, PR, and HER2 testing in malignant phyllodes tumor** [79, 86–88]**?**

In malignant PT, the neoplastic stromal cells do not usually express ERα, PR, and HER2. Only ERβ expression has been reported in these stromal cells. ERα and PR expression is confined to the epithelial compartment, but shows inverse association to grade. Epithelial HER2 expression has been observed but there was no correlation with PT grade and prognosis. The practical significance of these observations is unclear. The current data indicate a limited role for hormonal therapy in treatment of PTs. Thus, ER, PR, and HER2 testing in PT may not have clear clinical relevance and are not routinely tested in most centers.

31. **Are there genetic changes in fibroadenoma** [89–97]**?**

Abnormal karyotypes have been identified in 20–30% of FAs, usually with structural aberrations. However, there are no consistent chromosomal alterations in FA within or across studies. Genetic changes are reported to be polyclonal. A lower level of nonsynonymous somatic mutations in FA than breast cancer (a median of five mutations in FA compared to 33 in breast cancers) has been demonstrated. Among these, MED12 somatic mutation has been reported in FA, with a frequency ranging from 47 to 62%. The mutation mostly occurs at a hotspot of exon 2 in codon 44, although the average allele frequency was around 14%.

32. **Are there genetic changes indicating that fibroadenoma and phyllodes tumor are distinct** [44, 46–49, 85, 98–104]**?**

PTs are genetically more advanced than FA and displayed more genetic changes. SNP arrays demonstrated rare LOH in FA with fractional LOH rates ranging from 0 to 1.5%, compared to an average of 9.4% for PT. Contrasting the lack of recurrent chromosomal aberrations in FA, consistent 1q gain and 13q loss in PT was observed in a number of studies. One study showed these genomic alterations mainly occur in PT with higher grade; others showed no association of 1q gain with grade or 1q gain in benign PT. These findings are not conclusive at this stage. In addition, regions of amplification and homozygous deletion are observed in PT, particularly high-grade PT, but rarely in FA. Homozygous deletion at 9p21 accompanied by loss of p16 expression has been reported in high-grade PT. Recurrent EGFR amplifications associated with malignant PTs have been reported in several studies. Other amplifications reported in PTs include MDM4, RAF1, TERT, and MET. More recently, with massively parallel sequencing, a higher mutational burden was shown to be displayed by PT (median non-silent somatic mutation per case: 5 in FA vs 13 in PT). In addition to MED12 mutation which is also commonly found in FA, PT harbors other recurrent mutations. Recurrent mutations in FLNA, SETD2, and KMT2D were found across the spectrum of PT while mutations in bona fide cancer genes (such as NF1, RB1, TP53, PIK3CA, and EGFR) were only detected in borderline and malignant PT. Furthermore, PT may harbor TERT promoter mutations which are absent or exceedingly rare in FA. TERT mutations have been detected in the majority of high-grade PTs and in a small subset of benign PTs.

33. **Are there any potential targets in the genetic changes of phyllodes tumor** [46–48, 49, 75, 85, 102, 103, 105–112]**?**

Several potential targetable genetic changes have been found in PT. Recent NGS studies revealed hotspot mutations in PT including *AKT1* (E17K), *ERBB2* (V777 L), *ERBB3* (V104 L), *NRAS* (Q61K), and *PIK3CA* (H1047R). Some of these mutations have been shown to be predictors for response to specific therapeutic agents in other malignancies. AKT inhibitor AZD5363 induced partial responses in patients with breast and ovarian cancer with tumors containing $AKT1^{E17K}$ mutations. Neratinib, an irreversible dual ERBB2/EGFR tyrosine kinase inhibitor, has been found to be active in breast cancers with activating *ERBB2* mutations. In addition, c-MET amplification, the major mechanism of constitutive c-MET activation in human cancers, was also reported in malignant PTs. There is evidence showing responsiveness to Crizotinib in a subset of esophagogastric adenocarcinoma with MET amplification. Across different studies, the most frequently altered gene in PT with potential therapeutic relevance is EGFR. *EGFR* amplifications and overexpression have been reported in many studies. Its pathogenic missense mutations, namely, L62R, L62R, G63R, E84V, and V774 M, have been revealed in PT. Although the therapeutic relevance of these alterations remains to be investigated, these genetic changes could represent potential therapeutic target. The driver role of EGFR amplification and V774 M mutation has been shown in other cancers. The other mutations located at the site target by cetuximab and panitumumab have also been identified to be new functional variants of EGFR.

34. **What is the outcome for phyllodes tumor** [13, 16, 40, 42, 113–119]**?**

Most PTs behave in a benign fashion without recurrence and metastasis following excision. The local recurrence rate is 21%, with ranges of 10–17%, 14–25%, and 23–30% for benign, borderline, and malignant PTs, respectively. Most local recurrences occur within the first two years after diagnosis. It appears that Asian patients experienced a higher recurrence rate than non-Asians. The recurrences mostly mirror the microscopic pattern of the original tumor, but grade progression has been reported in 25–75% of cases. Very rarely, PT (less than 2%) metastasizes to distant organs. It occurs essentially only in malignant PT. Metastases usually occur within 5 years of initial diagnosis. The most common sites are lung and pleura, but all organ sites can be affected. PT tends to spread via the hematogenous rather than lymphatic route. Metastases in PT invariably indicate a dismal prognosis with ensuing death.

35. **What is the difference in outcome between fibroadenoma and phyllodes tumor** [3, 2, 42, 52, 53, 120–123]**?**

In general, FAs rarely recur, even complex FAs, and some may regress spontaneously. A study has shown that one-half of FAs followed clinically without excision disappeared on imaging after 5 years. Several studies reported, however, a recurrence rate of 15–17% for FAs after excision, occurring exclusively in FAs measuring more than 2 mm at initial diagnosis. Thus, FA and benign PT may have similar recurrence rate. However, benign PT may recur as malignant PT. There are rare cases of benign PT showing metastasis.

Case Presentations

Case 1

A 50-year-old woman has a fast-growing mass in her right breast. No axillary lymph node enlargement was noted. Incisional biopsy of the mass was performed, and the histology was reviewed.

Highly pleomorphic malignant cells with no tubule formation are noted, but areas of necrosis are seen. Mitotic account was very brisk, counting to 30 to 40 per 10 high-power fields. No definite benign ductal and carcinoma components are identified.

What are the differential diagnoses? What is the recommended treatment? The differential diagnoses include spindle cell carcinoma, sarcoma, and malignant phyllodes tumor:

1. To identify the presence of epithelial component or to determine if it is not present, one needs to consider sarcoma or spindle cell carcinoma (metaplastic carcinoma). If benign epithelial component is present, a diagnosis of malignant phyllodes tumor has to be considered. If the epithelial component is malignant (either glandular or squamous), then the diagnosis will be metaplastic carcinoma.
2. If malignant heterologous element is identified, the diagnosis can also be malignant phyllodes tumor, sarcoma, or metaplastic carcinoma; thus, the identification of malignant heterologous element is not useful in the diagnosis.

In this case, no epithelial component is identified and no malignant heterologous element is seen:

1. Additional sampling may be undertaken, and, in this case, a small area of benign epithelial component is seen.
2. Immunohistochemistry can be done to characterize the spindle cells.

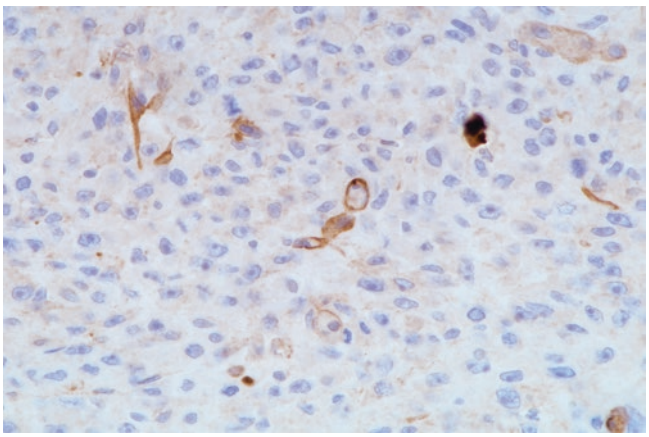

Fig. 6.8 Staining for CK14, highlighting the staining in single cells in a focal manner in this case of malignant phyllodes tumor

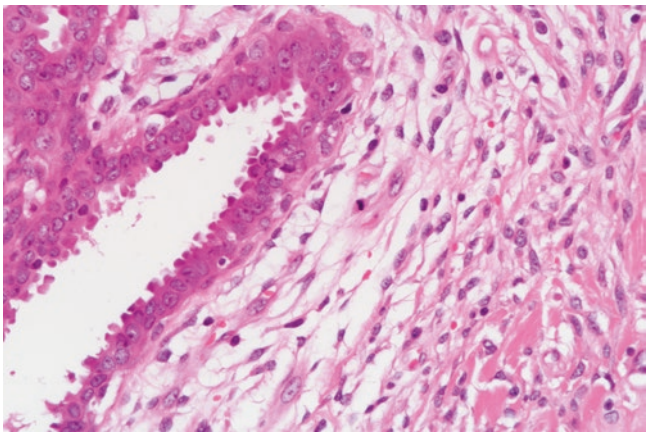

Fig. 6.9 Rare mitotic figure can be seen in a fibroadenoma, particularly in a juvenile variant

3. AE1/3 will be positive in the spindle cells in spindle cell carcinoma, but not usually in phyllodes tumor and seldom in sarcoma. In phyllodes tumor, AE1/3 and other cytokeratins (high molecular weight cytokeratins) have been reported to be more likely positive in malignant than benign phyllodes tumors and usually focally and patchy (Fig. 6.8).
4. p63 will be positive in the spindle cells in spindle cell carcinoma, but not usually in phyllodes tumor and seldom in sarcoma. In phyllodes tumor, p63 has been reported to be more likely positive in malignant than benign phyllodes tumors and usually focally and patchy.

The final diagnosis is malignant phyllodes tumor, as benign epithelial component is identified, despite focal cytokeratin positivity.

Recommended further treatment is complete excision. There is no need for axillary sampling, as axillary lymph node metastasis is uncommon.

Case 2

A 35-year-old woman presented with an asymptomatic breast mass. The mass has been rapidly growing for the past 2–3 months, and it now measures 3 cm. Physical examination shows a mobile firm mass not fixed to underlying tissue. Imaging (USG) shows a lobulated mass that is wider than tall, with posterior shadowing. Cleft-like spaces are seen.

A needle core biopsy was performed. The needle core biopsy shows a fibroepithelial lesion with fragmentation. There is prominent intracanalicular/phyllodal architectural pattern, with slightly increased stromal cellularity, and occasional mitotic figures can be seen.

What are the differential diagnoses? The differential diagnoses include fibroadenoma and benign phyllodes tumor:

1. The history of rapid growth, the mild increased stromal cellularity, and mitotic figures seem to suggest a diagnosis of phyllodes tumor.
2. Immunohistochemistry is not helpful.
3. The relatively young age is in favor of fibroadenoma and variants.
4. Absence of areas of variable stromal cellularity favors a diagnosis of fibroadenoma, and the juvenile fibroadenoma may sometimes show mitotic activity (Fig. 6.9).

The diagnosis is a juvenile fibroadenoma.

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Immunohistochemistry in Breast Cancer

7

Ping Tang, Marilyn M. Bui, and Yan Peng

List of Frequently Asked Questions

1. **Why does ductal carcinoma in situ (DCIS) need only ER and PR testing, while invasive breast carcinoma needs ER, PR, and HER2 testing?**

Breast cancer is the most common cancer in women. Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status not only provide prognostic information but also are critical predictive markers for currently available anti-hormonal and anti-HER2 therapies. DCIS patients are treated with hormonal therapy; thus, knowing the ER and PR status is critical. Although up to 40% of DCIS can be positive for HER2, there is no evidence that DCIS will benefit from Herceptin (Genentech, San Francisco, CA, USA); that is why HER2 testing is not needed for DCIS currently. It is the standard of care that invasive breast carcinoma that are HER2 positive are treated with HER2-targeted therapy. HER2 positivity can be HER2 protein overexpressed and/or HER2 gene amplified. Therefore, HER2 testing is required for all primary and recurrent invasive breast cancers.

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2. **Why is ER/PR/HER2 testing called a companion test?**

A companion test is a diagnostic test used to determine if a specific patient is applicable for a therapeutic drug. One of the most common testing methods is by immunohistochemistry (IHC). These IHCs are not ordinary, because the results have prognostic and predictive implications. For example, ER- and PR-positive patients have better outcome and significantly benefit from hormonal therapy. In contrast, HER2-positive patients have poor outcome and significantly benefit from HER2-targeted therapy.

IHC studies of biomarkers ER, PR, and HER2 are commonly used companion tests for breast cancer treatment. These tests are typically performed on formalin-fixed paraffin-embedded tissue. Unlike other conventional IHC tests that serve solely as an adjunct to a diagnosis, cancer biomarker testing by IHC evaluates tumor biology and molecular pathways driving disease progression that can potentially be targeted for therapy. Unlike other conventional IHC tests, in which the score is typically binary—that is, positive or negative—the scoring of breast cancer biomarkers is quantitative and more involved. Accurate breast cancer biomarker testing is critically important for patient care. However, 20% of ER, PR, and HER2 testing results worldwide are inaccurate. Thus, one should not assume that all breast cancer biomarker testing results are accurate, unless the laboratories performing IHC assays for breast cancer biomarkers closely follow the updated quality control and quality assurance measures outlined in published guidelines recommended by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP).

3. **How does one evaluate ER/PR testing, and what is the cut-off for ER/PR positivity?**

ER and PR expression are routinely tested in breast cancers as prognostic and predictive markers. These receptors are expressed in about 75–80% and 65% of all breast tumors,

Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5_7

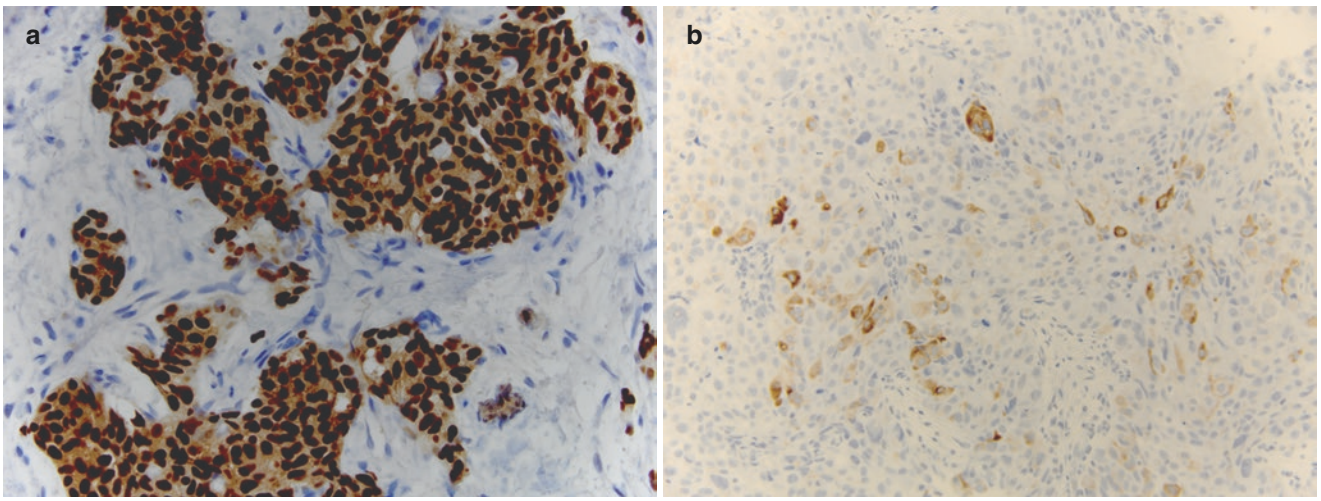

Fig. 7.1 (**a**, **b**) ER in breast carcinoma cells shows both nuclear and cytoplasmic staining (**a**) and only cytoplasmic staining (**b**)

respectively. The ASCO/CAP guidelines recommend that ER and PR should be considered positive if ≥1% of tumor cells shows nuclear staining of any intensity [1]. The intensity of ER and PR stains should be included in the pathology report as weak (1+), moderate (2+), or strong (3+). The evaluation of normal breast ductal epithelium as an internal positive control is an integral part of the IHC evaluation for ER and PR expression in breast cancer specimens. Cytoplasmic staining should not be considered as positive staining. See Fig. 7.1a, b. These guidelines also emphasize standardization and quality assurance that must be followed to help ensure testing accuracy. Allred scoring and H-scoring are also two commonly used systems for ER and PR reporting [2, 3].

4. **How does one correlate ER/PR results with clinicopathological characteristics of breast cancer?**

Expression of ER/PR plays a major role in tumor development in hormonal receptor-positive tumors and drives disease progression in these tumors; thus, ER/PR-positive breast cancer is eligible for endocrine therapy. Clinically, ER/PR-expressing invasive breast cancers are usually better differentiated and have a more indolent course and favorable prognosis compared to ER/PR-negative tumors. However, sometimes hormonal receptor-positive breast cancers can be poorly differentiated and can have aggressive clinical behavior. There is a positive correlation between the likelihood of tumor response to hormonal therapy and the levels of ER/PR expression. However, on some occasions, tumors with very low levels of ER/PR expression can show significant response to hormonal therapy that is above that of entirely receptor-negative tumors.

5. **How does one troubleshoot if ER/PR results are in question?**

Normal breast elements should show ER expression in 10–20% or higher of epithelial cells, and this serves as positive internal controls for evaluation of hormone receptor status. The presence of the positive internal controls indicates that the tissue is adequate for the evaluation, which is particularly important in ER- and PR-negative tumor cases. If the internal normal breast epithelial cells are not stained properly with ER and/or PR, the test should be repeated. If the absence of staining in normal breast epithelial cells persists in a repeated test, the test should be signed out as indeterminate. The cold ischemic time and formalin fixation time for each test should be documented as recommended by the ASCO/CAP guidelines, which are critical for troubleshooting for pre-analytical variables.

One slide with external positive controls is highly recommended for ER, PR, and HER2 tumor biomarker testing, which helps troubleshooting issues at the analytical stage. Also, due to the marked intratumoral heterogeneity of breast cancer, a negative ER and PR result in a core biopsy specimen should prompt a repeat test in the subsequent resection specimen if the status of biomarker expression does not fit in the clinical pictures and pathological features of the tumor, for example, in the case of a negative ER and PR result in a grade 1 invasive lobular carcinoma, classic type.

6. **What subtypes of breast cancer are usually positive for ER?**

Low-grade invasive ductal carcinoma (IDC) NOS, classic invasive lobular carcinoma, tubular carcinoma, and muci-

nous carcinoma are almost always strongly positive for ER and PR. Of note, apocrine carcinoma can be ER positive or negative. Using unsupervised clustering analysis, five intrinsic subtypes of breast cancer were identified: luminal A, luminal B, normal breast-like, HER2-enriched, and basal-like subtypes. Each is unique in incidence, patterns of recurrence and survival, and response to therapy. Among them, luminal-type breast cancers are largely ER/PR positive.

7. **Can low-grade (grade 1) invasive breast carcinoma be ER/PR negative?**

Yes, some special types of low-grade breast cancers can be ER/PR negative. Low-grade invasive ductal and lobular carcinomas are usually ER/PR positive; but rarely, when these tumors demonstrate some degree of apocrine features, ER/PR can be negative. See Fig. 7.2a, b. Other low-grade invasive carcinomas that are ER/PR negative include but not limited to breast cancer resembling the tall cell variant of papillary thyroid carcinoma, secretory carcinoma, adenoid cystic carcinoma, acinic cell carcinoma, malignant adenomyoepithelioma, low-grade adenosquamous carcinoma, and carcinoma arising from microglandular adenosis.

8. **When should one communicate with clinicians regarding ER/PR results?**

Direct communication with the clinician is critical in the era of multidisciplinary approach in treating breast cancer. If the status of ER/PR expression does not fit in with a patient's clinical and pathological pictures, such as low-grade invasive ductal carcinoma NOS or classic invasive lobular carcinoma with negative ER/PR, communicating with the clinician directly is warranted for reconciliation.

9. **Is PR result important for treatment of breast cancer?**

Both ER and PR are a transcription factor. PR is largely regulated by ER [4] and to some degree by growth factors. Given that PR is regulated by an active ER pathway, PR is co-expressed with ER in most of luminal-type breast cancer cases. PR is expressed in 55–65% of invasive breast carcinomas. The loss of PR expression in ER-positive tumors is associated with a worse prognosis and decreased response to anti-estrogen therapy [5]. Therefore, PR is considered as an important prognostic marker for breast cancer patients, and routine PR testing is necessary in assisting endocrine treatment decision making.

Of note, both ER and PR can show immunophenotypic, intratumoral heterogeneity; thus, obtaining an adequate sample from a tumor tissue for their testing is very important. See Fig. 7.3a, b.

10. **Do ER-negative/PR-positive tumors exist?**

Although it has long been controversial, recent studies revealed that ER-negative/PR-positive (ER-/PR+) tumors do exist as a distinct subtype of breast cancer and have a reported incidence of 1–4% [6, 7]. A study showed that no significant survival advantage was found between the ER+/PR- and ER-/PR+ tumors; furthermore, a higher PR expression was associated with a favorable relapse-free survival and disease-specific survival in patients with ER-/PR+ tumors [6].

11. **Why use HER2 testing** for **breast cancer patients?**

The human epidermal growth factor receptor 2 (HER2) is a member of a family of transmembrane tyrosine kinase recep-

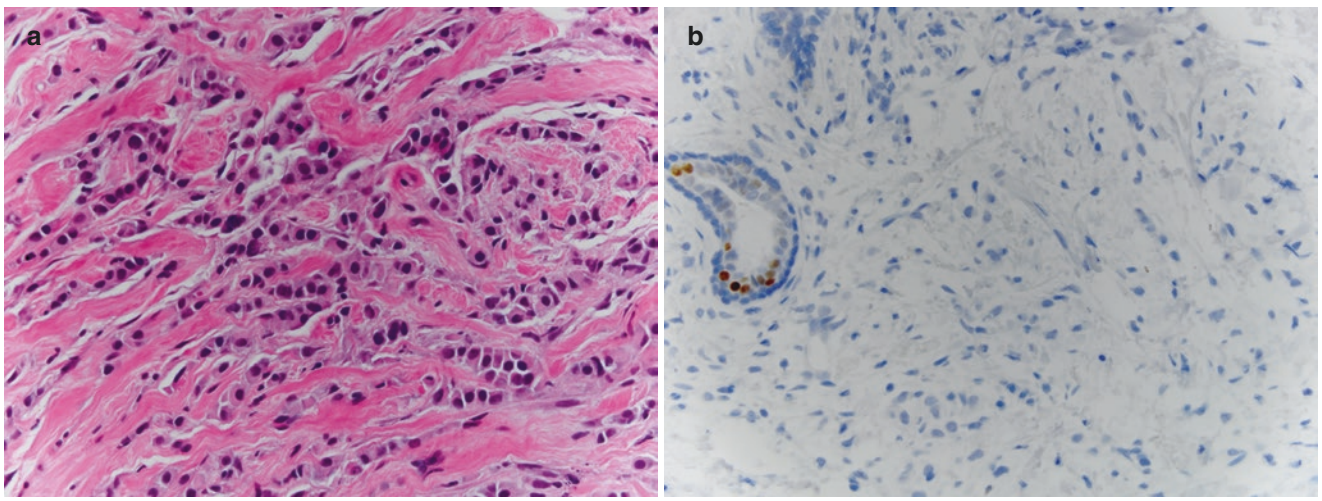

Fig. 7.2 (**a**, **b**) Low-grade invasive lobular carcinoma with apocrine features (**a**) shows negative ER immunostain (**b**)

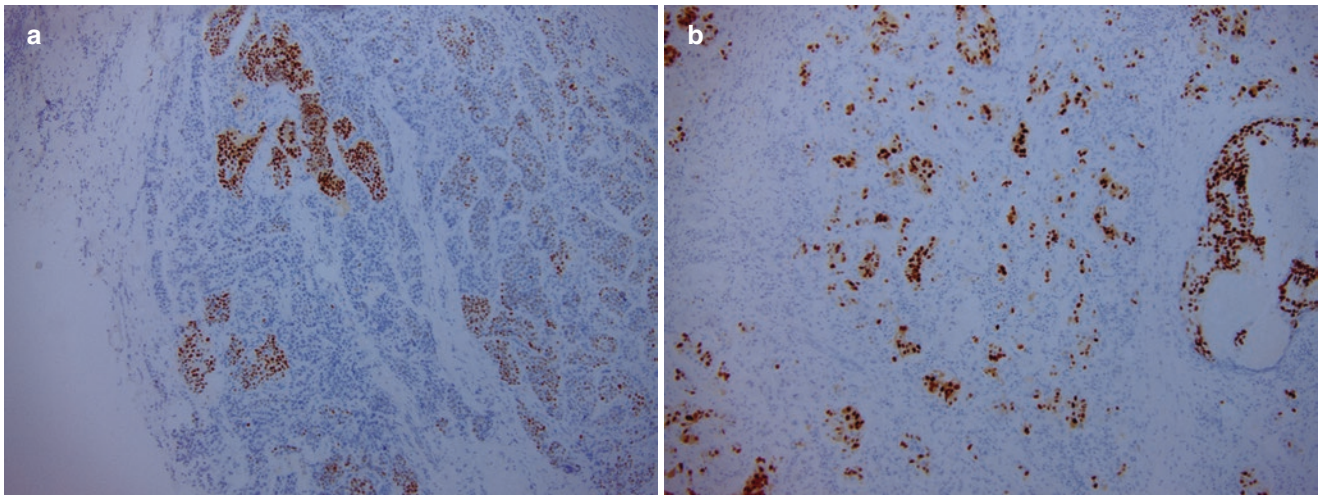

Fig. 7.3 (**a**, **b**) Different immunostaining patterns of intratumoral heterogeneity of PR (**a** and **b**)

tors that plays an important role in the regulation of cellular signaling that affects cell growth, differentiation, and survival. HER2 protein overexpression and HER2 gene amplification are in 10–20% of invasive breast cancers and have an important bearing on prognosis, as HER2-positive breast cancer is associated with an aggressive clinical course and poor outcome. HER2 status of a breast cancer provides both prognostic and predictive information for clinicians, guiding their treatment planning [8]. Trastuzumab (Herceptin, Genentech, San Francisco, CA, USA) is well known to be used for treatment of Her2-amplified/overexpressing invasive breast carcinoma. Since the approval of trastuzumab for HER2-positive metastatic breast cancer in 1998, outcomes for patients diagnosed with this aggressive cancer have vastly improved. However, trastuzumab has some serious side effects including severe headache, fast or pounding heartbeat, and easy bruising or bleeding. Pertuzumab (Perjeta, Genentech, San Francisco, CA, USA) is a new anti-HER2 drug binding to a different domain of HER2 gene. It is approved for use in combination with trastuzumab and chemotherapy for use prior to surgery (neoadjuvant treatment) in patients with HER2-positive locally advanced, inflammatory, or early-stage breast cancer (tumor is greater than 2 cm in diameter or node positive) and use after surgery (adjuvant treatment) in patients with HER2-positive early breast cancer that has a high likelihood of recurrence.

Rate of pathologic complete response (pCR) in HER2-positive breast cancer after the neoadjuvant therapy can reach greater than 60% [9].

Accurate determination of HER2 status is critical to guide anti-HER2 therapy for improving breast cancer outcomes and avoiding overtreatment.

Given the fact of continued expansion of options for targeting the HER2 pathway in breast cancer, accurate and reliable HER2 testing to help ensure that the right patients receive the right treatment is now more critical than ever.

12. How does one evaluate HER2 IHC testing based on the ASCO/CAP guidelines?

In 2007, a Joint Expert Panel assembled by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) met to develop and publish guidelines with the aim of improving the quality, consistency, and reliability of HER2 testing in clinical samples from breast cancer patients [10]. According to the guidelines for HER2 testing in breast cancer, indicators for anti-HER2 therapy are HER2 protein overexpression by IHC (score 3+) and HER2 gene amplification by in situ hybridization (ISH). HER2 positivity can be seen in both invasive and in situ breast carcinoma components in tumor samples; only positive HER2 result in invasive carcinoma portion should be reported and used for treatment decision making. See Fig. 7.4a, b.

The 2013 HER2 guideline recommended changes to the testing algorithm and pathologist interpretation criteria and added new language on reflex and/or repeat testing when there is an apparent histopathologic discordance with the test result [11]. The 2013 guideline update also advocates interpreting the HER2 results in the context of the clinical and morphologic features of the patient's breast cancer, and the guideline further recommends that pathologists and oncologists should exercise clinical judgment with respect to which patients will require additional testing before the HER2 status can be assuredly determined [11]. In the 2018 guideline focused update, uncommon clinical scenarios that are of uncertain tumor biologic or clinical significance are addressed [12].

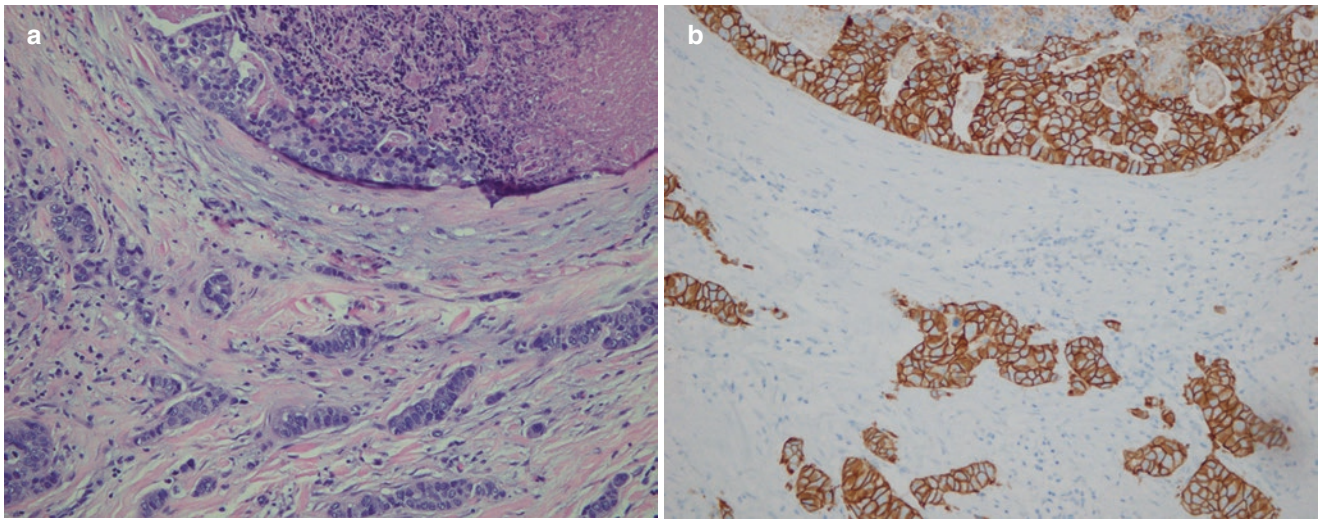

Fig. 7.4 (**a**, **b**) One tumor case shows both invasive ductal carcinoma and DCIS (**a**) and HER2 protein overexpression (score 3+) in both components (**b**)

13. **What are the main updates on HER2 testing in the 2018 HER2 guideline recommended by the ASCO/CAP?**

The 2013 guideline [11] indicated either the HER2/CEP17 ratio or HER2 copy number can be used for reporting HER2 FISH results:

- Ratio ≥2.0 or HER2 copy number ≥6 shall be considered HER2 positive/amplified.
- Ratio <2 and HER2 copy number <4 shall be considered HER2 negative.
- Ratio <2 and HER2 copy number ≥4.0 and <6.0 shall be considered HER2 equivocal.

Please note the minimal cell count required for HER2 FISH analysis is 20 invasive tumor cells.

In the 2018 guideline focused update [12], recommendations include the following:

1. The revised definition of 2+ HER2 IHC (equivocal) is weak to moderate complete membrane staining observed in >10% invasive breast cancer cells.
2. Instead of "must," a new HER2 test "may be" ordered on the excision specimen based on specific clinical criteria, if the initial core needle biopsy specimen of a primary invasive breast cancer is negative.
3. In less than 5% of the cases, dual-probe ISH assay may result in some uncommon scenarios:
   - HER2/CEP17 ratio ≥2.0, average HER2 copy <4.0 signals per cell
   - HER2/CEP17 ratio <2.0, average HER2 copy ≥6.0 signals per cell
   - HER2/CEP17 ratio <2, average HER2 copy ≥4.0 and <6.0

     In these situations, concomitant IHC review is required to achieve the most accurate HER2 interpretation.
4. The expert panel recommends all single-probe ISH assays to be reviewed with concomitant IHC.

Compared to the 2013 guideline, the 2018 HER2 guideline includes more rigorous interpretation criteria for dual-probe in situ hybridization (ISH) testing and requires concomitant IHC review to arrive at the most accurate HER2 status designation (positive or negative); equivocal ISH cases no longer exist, which would better guide anti-HER2 therapy. IHC seems to play a more important role in determining final HER2 status compared to ISH; furthermore, HER2 protein overexpression appears to be more important for targeted therapy than HER2 gene amplification. In conjunction with the 2018 guideline [12], we recommend the HER2 testing clinical practice in invasive breast cancer as follows (Tables 7.1 and 7.2):

- Perform HER2 IHC first for all invasive carcinomas as a "screening" tool.
- If IHC 3+ (Fig. 7.5a) = HER2 positive (no ISH), that is defined as circumferential membrane staining that is complete and intense in more than 10% of tumor cells.
- If IHC 2+ (Fig. 7.5b) = HER2 equivocal, per the 2018 updated guideline [12], that is defined as tumor cells with weak to moderate complete membrane staining observed in greater than 10% of tumor cells; the HER2 IHC equivocal result requires reflex ISH test (with or without concomitant IHC review) to determine final HER2 status (positive or negative).

Table 7.1 Suggested clinical practice on HER2 Testing in conjunction with the 2018 Guideline. Starting with Her2 IHC as the "screening" tool

IHC	ISH	HER2 status
3+	No	Positive
2+ (equivocal)	*Reflex ISH*	Pending
0 or 1+	No	Negative

Table 7.2 Suggested clinical practice on HER2 testing in conjunction with the 2018 guideline for IHC 2+ cases requesting reflex ISH only

HER2/CEP17 ratio	Average HER2 copy number/cell	Further work-up	HER2 status ± comment
≥2.0	>4.0	No	Positive
≥2.0	<4.0	Observer blinded to previous results recounts ISH in at least 20 cells = *same result*	*Negative* because of low copy number and lack of protein overexpression. Evidence is limited on the efficacy of HER2-targeted therapy
<2.0	≥6.0	Recounts ISH = *same result*	Positive
<2.0	≥4.0 and < 6.0	Recounts ISH = *same result*	*Negative* because of absence of protein overexpression. It is uncertain that this group of patients benefit from HER2-targeted therapy
<2.0	<4.0	No	Negative

- IHC 0 or 1+ (Fig. 7.5c) = HER2 negative (no ISH); the IHC 0 is defined as no staining observed or membrane staining that is incomplete, faint/barely perceptible, and within ≤10% of tumor cells; the IHC 1+ is defined as incomplete membrane staining that is faint/barely perceptible and within >10% of tumor cells.

14. Are there invasive breast carcinoma cases with negative HER2 IHC and positive HER2 ISH?

Although it is rare, breast cancers showing HER2 gene amplification but no HER2 protein overexpression have been reported, particularly in high-grade invasive ductal carcinoma [13]. See Fig. 7.6a, b.

15. What are key elements for HER2 IHC quality control and quality assurance?

HER2 IHC quality control and quality assurance are critical for accuracy of HER2 testing and should be conducted in pathology laboratories on a regular basis. The key elements for the process include but not limited to the following:

- Use an FDA-approved IHC antibody, such as Ventana (Oro Valley, AZ, USA) HER2 (4B5) or Genentech (San Francisco, CA, USA) HercepTest.

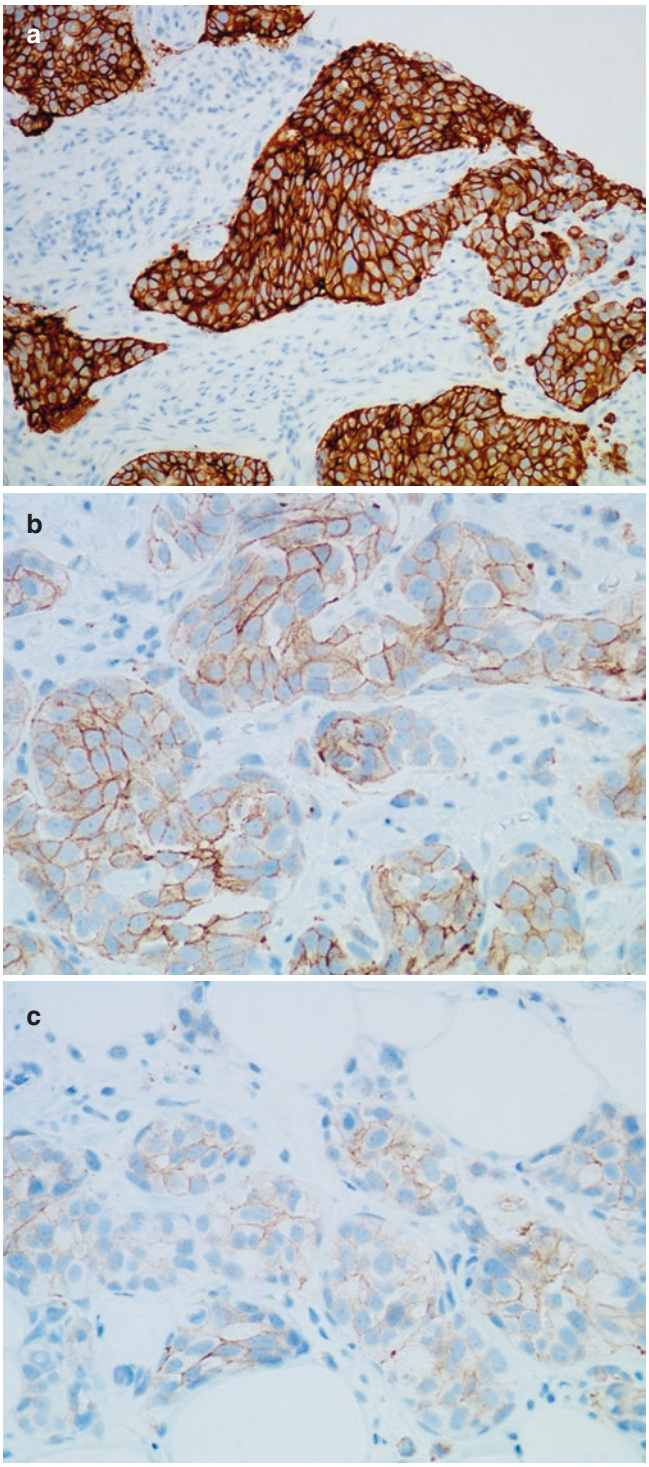

Fig. 7.5 (**a**–**c**) Positive HER2 IHC 3+ (**a**), equivocal HER2 IHC 2+ (**b**), and negative HER2 IHC 1+ (**c**)

- Meet optimal tissue handling requirements (pre-analytical factors) recommended by the ASCO/CAP (See question 23).
- Be aware that decalcification treatment for bone samples may affect Her2 IHC expression in bone metastatic breast cancer. If there is a discrepancy in Her2 IHC results

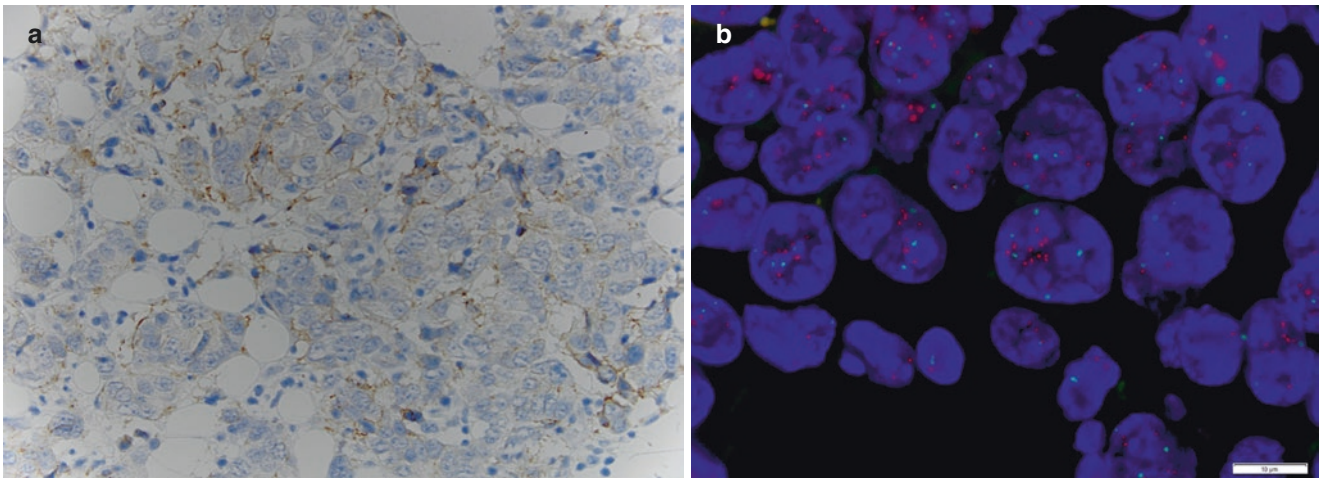

Fig. 7.6 (**a**, **b**) Invasive carcinoma shows HER2 IHC negative (score +1) (**a**) and FISH positive (**b**)

between primary breast tumor and bone metastasis, a diagnostic note with possible explanation should be in the report.
- Review and document external and internal controls with each test and each batch of tests.
- Perform ongoing competency assessment and document the actions taken as a part of the laboratory record. Per the CAP recommendation and guideline, the labs should conduct an annual evaluation of interobserver variability among pathologists who practice Her2 IHC interpretation.

16. When should a reflex HER2 FISH testing be ordered?

The HER2 guideline recommended reflex HER2 FISH testing is only requested in HER2 IHC equivocal (2+) cases. However, pathologists shall take other clinical and pathological information into consideration when making a decision on ordering HER2 FISH test. For example, invasive breast micropapillary carcinoma cells often show U-shape, weak, membranous HER2 staining; in the 2018 HER2 guideline, it has been suggested to reflex a HER2 FISH test in such cases, especially when PR is low and Ki67 is high in the tumor cells [12].

17. What are the pros and cons of HER2 tests by IHC and FISH?

Both HER2 IHC and FISH tests have their pros and cons, respectively. One of the major cons of the HER2 IHC is lack of internal positive controls. In this situation, it is not certain if the tumor tissues have any immune reaction in a setting of negative HER2 result. However, if HER2 IHC test runs together with ER/PR IHC and internal positive controls for ER/PR are present, this shall not be an issue. In terms of pros of the IHC, the entire tumor on a whole slide stained by IHC can be reviewed to ensure invasive carcinoma areas are evaluated and to identify HER2 tumor heterogeneity immunophenotypically. For FISH test, the key element is to identify areas containing invasive tumor cells to count average copy number for HER2 gene and chromosome 17-centromere, respectively. However, the identification can be challenging under the fluorescent microscope, because in situ carcinoma and others can resemble invasive carcinoma. This is the major issue for FISH test. Thus, selecting tissue blocks with invasive tumor cells and marking the tumor areas for FISH test are critical for getting correct results.

18. What are chromosomal monosomy and chromosomal polysomy?

Monosomy of chromosome 17 can make HER2/CEP17 ratio artificially high, and polysomy of chromosome 17 can make the ratio artificially low. Thus, interpretation of HER2 FISH results in these types of cases should be undertaken with caution. For these situations, the 2018 HER2 guideline [12] recommended assessing IHC using sections from the same tissue sample used for FISH.

19. How does one correlate HER2 result with clinicopathological characteristics of breast cancer?

HER2-positive breast cancers are usually high-grade invasive carcinomas, including but not limited to tumors with apocrine features, invasive micropapillary carcinoma, and pleomorphic invasive lobular carcinoma. The levels of Ki67 expression in these tumors are usually greater than 15% but less than 50–80% that are commonly seen in triple-negative breast cancer (TNBC) cases. If the results between HER2 and Ki67 are discordant, further investigation is warranted before releasing the pathology report.

20. **What are the most common causes for a discrepancy in HER2 IHC and FISH results?**

While IHC evaluates the entire tumor tissues present on a whole slide for the status of HER2 protein overexpression, FISH usually only counts 20–40 invasive tumor cells for the status of HER2 gene amplification. Due to the inherent issues of tumor heterogeneity in breast cancer, these two test methods may provide different results even though they are done in the same tissue block. Thus, it has been recommended to use HER2 IHC slide as the guide map for FISH test to determine which areas to count, rather than blindly counting.

21. **How does one troubleshoot if HER2 IHC and FISH results are discordant? How does one communicate with clinicians on cases with HER2 discrepancy?**

If HER2 results do not fit the clinical pictures, investigation should be conducted for all possible variables: pre-analytic, analytic, and post-analytic factors. Repeat IHC and/or FISH tests on the same block, increase the number of cells counted for FISH analysis, or send the block to a reference lab for testing: all these steps should be considered, and then appropriate actions should be taken. If HER2 result discrepancy persists after repeating, the discrepancy should be clearly documented in the pathology report. Direct communication with the clinician on the results via the phone is highly recommended, as it shall be a multidisciplinary decision on what is the most appropriate management approach for this group of patients.

22. **Can low-grade (grade 1) invasive breast carcinoma be HER2 positive?**

Yes. Yu and colleagues [14] have shown that classic-type grade 1 invasive lobular carcinomas can be rarely positive for HER2, especially when they have apocrine or histiocytic features or they are PR negative. ER is still expressed in these HER2-positive low-grade invasive carcinomas, although the level of expression is significantly lower than that of HER2-negative low-grade tumors.

23. **What are pre-analytical factors affecting breast cancer biomarker results?**

The ASCO/CAP guidelines on ER, PR, and HER2 testing [15] recommended optimal breast tissue handling, including the following:

1. Time from tissue acquisition to fixation should be as short as possible (less than 1 hour).
2. Tissue fixation in 10% neutral buffered formalin (NBF) with minimum fixation time of 6 hours and maximum fixation time 72 hours.
3. Core needle biopsy specimen is preferred for testing to avoid prolonged tissue fixation.
4. Most importantly, the cold ischemic time and fixation time must be documented in the pathology report for reporting breast cancer biomarker results, which are subjected to lab accreditation inspection.
5. Any exceptions on the handling must be mentioned in the pathology report.

24. **What is Ki67 testing?**

Ki67 has been used as a proliferation marker. In general, high Ki67 expression is associated with poor differentiation and prognosis, and low Ki67 expression is associated with better differentiation and prognosis for breast cancer. Ki67 expression level in breast cancer is prognostically and predictively important. The most widely used Ki67 testing involves an IHC assessment of Ki67 antigen, also known as MKI67, which is a nuclear protein expressing in all cells except those in G0 phase.

25. **Is Ki67 a useful marker for breast cancer, and when should it be tested?**

Although numerous data have shown that Ki67 is a useful prognostic marker for breast cancer, it has not been recommended by the ASCO/CAP as a required biomarker for breast cancer, like ER, PR, and HER2. However, Ki67 has been routinely tested together with ER, PR, and HER2 for breast cancer cases in many institutions at the request of clinicians. Ki67 has also been included in many predictive models for breast cancer, such as Magee Equations [16] proposed by Klein and colleagues in 2013 and IHC4 scores [17] proposed by Cuzick and colleagues in 2011. The PEPI score originally reported in 2008 [18] and recently modified in 2017 [19] included Ki67 evaluation to better predict the tumor response to neoadjuvant endocrine therapy in ER-positive breast cancer. Interestingly, it is frequently observed that Ki67 expression is higher in invasive carcinomas without co-existing in situ carcinoma compared to those with in situ carcinoma component. In cases with both invasive and in-situ carcinoma components, Ki67 expression in the invasive carcinoma is usually higher than that in the in-situ carcinoma. See Fig. 7.7a–d.

26. **Why is Ki67 not recommended for routine testing for breast cancer?**

Per the ASCO Practice and Guidelines in 2016 [20], IHC for Ki67 analysis lacks reproducibility across laboratories and, therefore, cannot be consistently interpreted when performed in a broad range of laboratories. IHC for Ki67 is not recommended for broad clinical use to determine whether a patient should receive chemotherapy.

At present, the enormous variation in analytical practice markedly limits the value of Ki67 as a prognostic marker for

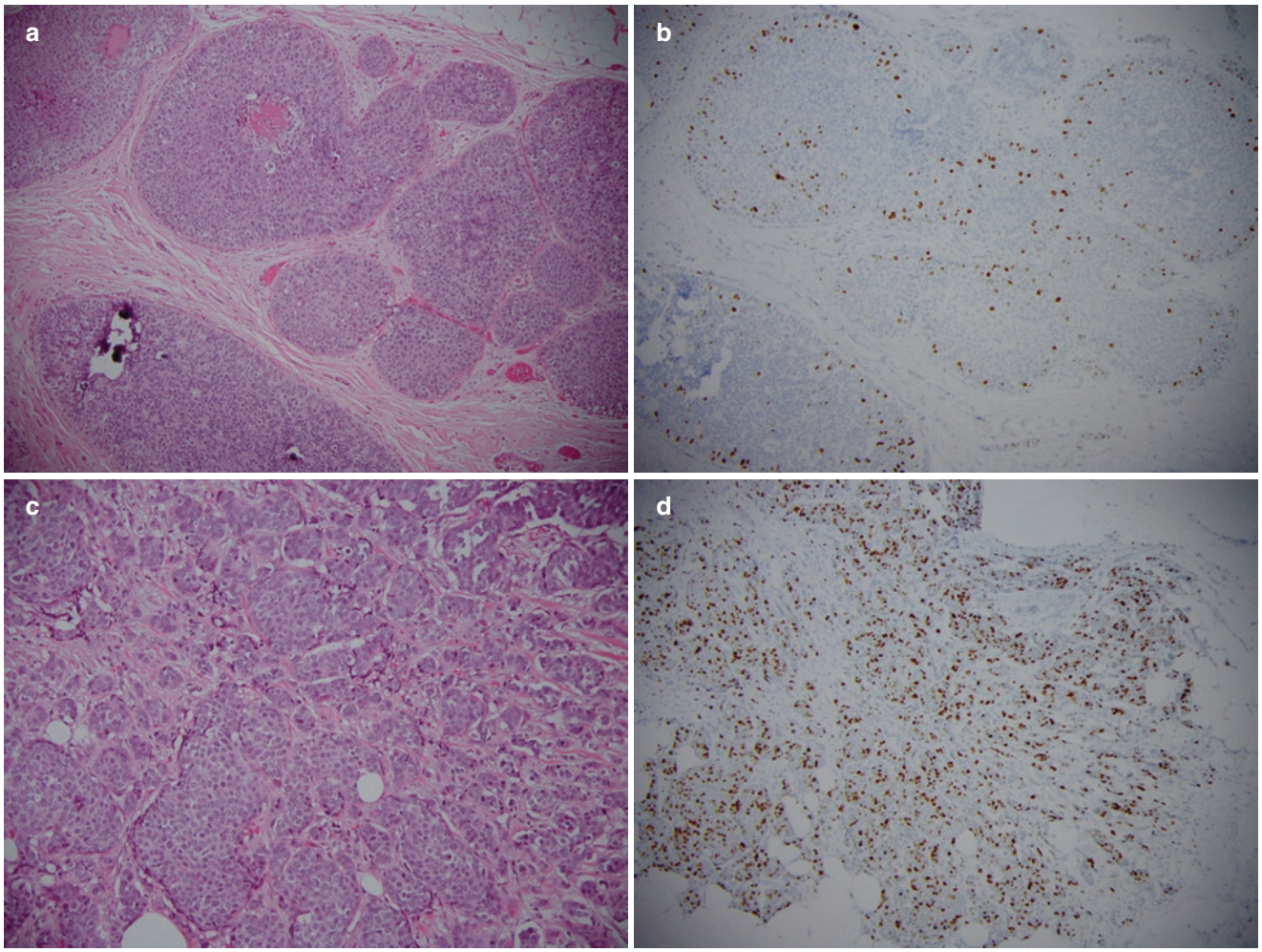

Fig. 7.7 (**a**–**d**) Ki67 expression in DCIS (**a**, **b**) and invasive ductal carcinoma (**c**, **d**) from the same tissue specimen, respectively

breast cancer. There are two major issues that need to be solved before Ki67 can be recommended for routine use: First is testing variability between laboratories. Thus, at the St. Gallen International Expert Consensus meeting in 2015, experts suggested that each lab use its own cut-off for Ki67 [21]; for example, if 20% is the mean value for Ki67 for ER-positive tumors in a lab, then 30% is considered high Ki67 and 10% is low Ki67. Second is interpretation variability of Ki67 scoring by manual assessment or via automated, quantitative image analysis (QIA) system [22], as stromal cells, inflammatory cells, and sometimes even adipocytes stain Ki67. See Fig. 7.8a, b.

27. **Is there any difference on Ki67 expression in breast cancers before and after chemotherapy or endocrine therapy?**

Generally speaking, in most cases with neoadjuvant chemotherapy, Ki67 expression levels are decreased in residual tumors. See Fig. 7.9a, b. Also, high Ki67 after endocrine therapy is considered a poor prognostic factor for ER-positive breast cancer by the modified PEPI score [19].

28. **How does one evaluate Ki67 testing manually and by image analysis, respectively?**

In 2011, Dowsett and coworkers [23] proposed an international working group recommendation for Ki67 evaluation. MIB1 antibody for Ki67 testing is recommended. Three random fields of a tumor section, 500–1000 tumor cells shall be counted and scored, using average across the section, not edge or center, including cells stained with all levels of intensity. For image analysis, pathologists' confirmation for tumor areas scored is critical; it is important to avoid areas with stromal cells and inflammatory cells, areas with prior biopsy site changes, and areas of necrosis for scoring. Since the recommendation was published, numerous studies have investigated the ways to increase concordance in Ki67 scoring between laboratories [24].

29. **How does one correlate Ki67 result with clinicopathological characteristics of breast cancer?**

Low-grade carcinoma usually has low Ki67 expression, while high-grade carcinoma tends to have high Ki67.

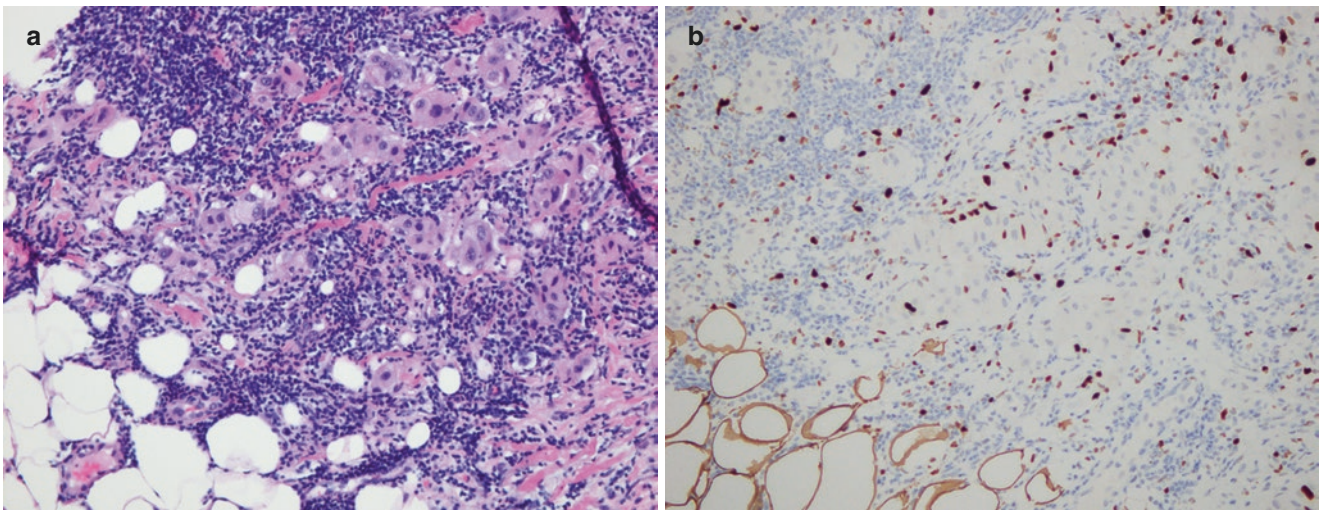

Fig. 7.8 (**a**, **b**) High-grade invasive ductal carcinoma with prominent tumor-infiltrating lymphocytes (**a**), making assessment of Ki67 expression in tumor cells difficult (**b**)

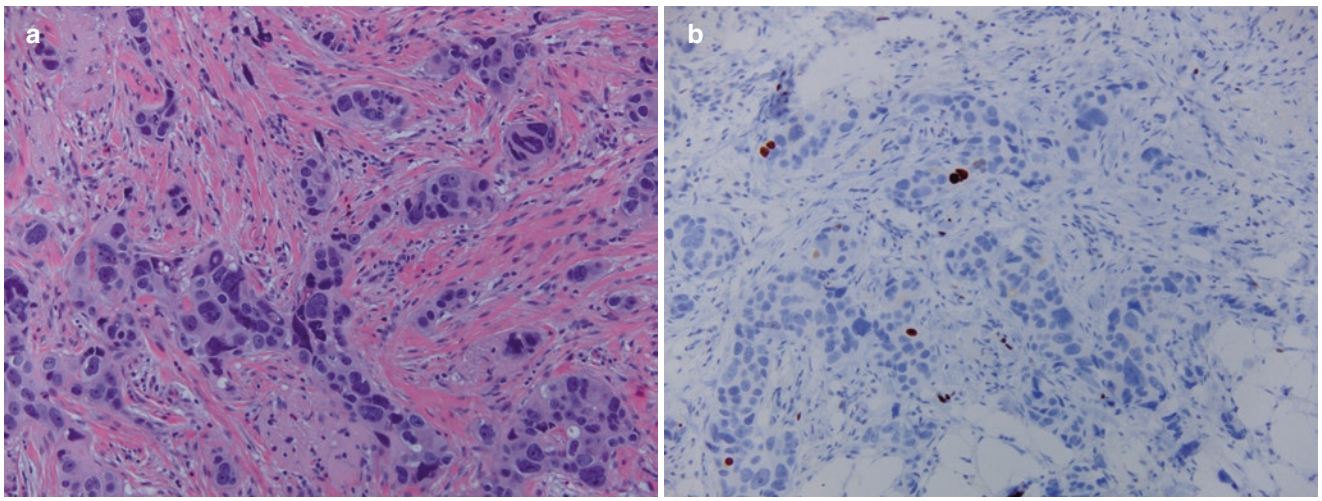

Fig. 7.9 (**a**, **b**) High-grade invasive ductal carcinoma (**a**) with low Ki67 expression (**b**) post-neoadjuvant chemotherapy

However, apocrine carcinoma tends to have low Ki67 even when the tumor is high grade. 15–20% Ki67 has been used as a cut-off value for favorable vs. poor prognosis for invasive ductal carcinoma, largely because this is the cut-off value used to separate luminal B subtype breast cancer from luminal A subtype [25]. Recently, Carbognin and coworkers have proposed that 5% is a biologically meaningful cut-off for Ki67 for invasive lobular carcinoma [26].

30. **How does one do troubleshooting if Ki67 result is in question?**

Ki67 is very sensitive with pre-analytic variables; thus, the presence of adequate internal positive controls is the key to determine if this test works properly in a given case. Normal breast tissue (both epithelium and stroma) shows about 5% Ki67 labeling. Lack of internal Ki67 labeling indicates the tissue sample is suboptimal for evaluation of the test. Also, a proper Ki67 staining in different tissue samples serving as external positive controls is important for the assessment.

31. **Why use quantitative image analysis to score breast cancer biomarker IHC testing?**

The goal of quantitative image analysis (QIA) is to achieve accurate, precise, and reproducible results of breast cancer biomarker testing. Validation of an FDA-approved algorithm is an important step to ensure the algorithm delivers the intended result. The current guideline in validation is general

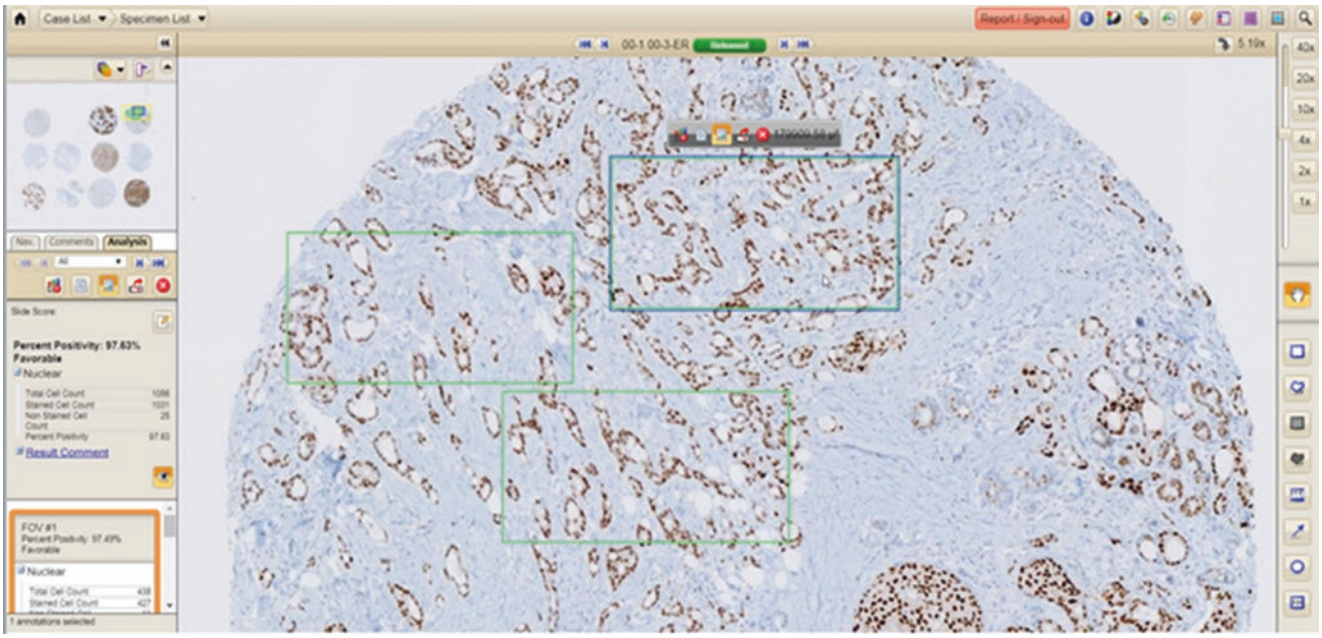

Fig. 7.10 The illustration shows this breast cancer sample is positive for ER in 97.63% cells

and non-specific. It is the medical director's responsibility to establish the lab's own standard operation procedure adhering to the CAP guideline. The parameters to consider validating include system, test, pathologist, and result. Using HER2 as an example, the gold standard for the validation is an alternate validated method including consensus scoring of HER2 IHC, fluorescence in situ hybridization (FISH) result, bright-field chromogenic in situ hybridization (CISH) result, and previously validated QIA result. Histotechnologists are also critical team members in the delivery of accurate and reliable breast cancer biomarker quantitative results to the clinical team for effective management of the patients. The billing code for QIA is CPT 88381 which is different from that for manual reading (CPT 88380). An example of an FDA-approved quantitative image analysis platform is illustrated in Fig. 7.10.

32. What percent of ER-positive breast carcinomas are GATA3 positive?

GATA3, a zinc-binding transcription factor, is a novel, sensitive, and relatively specific marker for primary breast carcinomas. Some studies revealed that GATA3 is expressed in up to 94% of ER-positive breast cancers [27, 28]. Due to the high sensitivity and specificity of GATA3 for breast origin, currently GATA3 has been widely used as a breast marker in routine clinical practice to confirm a breast primary tumor and to identify a metastatic carcinoma of breast origin.

33. What percent of triple-negative breast cancer cases are GATA3 positive?

The sensitivity of GATA3 in triple-negative breast cancers (TNBCs) is significantly lower than that in ER-positive breast cancers, and the sensitivity ranges from 43% to 66% [29–31]. Recent studies demonstrated that GATA3 is a much more sensitive marker for TNBC as compared with mammaglobin and GCDFP-15, which were commonly used breast markers previously. Of note, different clones of GATA3 antibodies have different sensitivities on breast ductal epithelium. A study reported that GATA3-L (L50-823) was diagnostically more sensitive than GATA3-H (HG3-31) in TNBC [30].

GATA3 is particularly useful in identifying metastatic TNBCs as ER, PR, or HER2 by IHC cannot serve as markers for the detection and other breast markers have a lower sensitivity.

34. What non-breast tumors are also GATA3 positive?

According to a recent comprehensive immunohistochemistry (IHC) study on GATA3 [32], in non-breast epithelial neoplasms, GATA3 was expressed in urothelial carcinomas (>90%), skin adnexal tumors (100%), mesotheliomas (58%), chromophobe renal cell carcinomas (51%), and salivary gland (43%) and pancreatic (37%) ductal carcinomas, whereas frequency of expression in adenocarcinomas of the lung, stomach, colon, endometrium, ovary, and prostate was

<10%. Among mesenchymal and neuroectodermal tumors, paragangliomas were usually positive, which differentiates these tumors from epithelial neuroendocrine tumors. GATA3 is a useful marker in characterization of not only mammary but also urothelial and renal tumors, mesotheliomas, paragangliomas, and other origins of tumors.

35. **Can a TNBC be ruled out if GATA3 is negative in an unknown primary immunohistochemistry work-up?**

Since only up to around 70% of TNBC tumors are GATA3 positive, GATA3 negativity result in an unknown primary carcinoma cannot exclude the possibility of a metastatic TNBC. Clinical and radiographic correlation is essential to accurately determine the primary site.

36. **Can breast cancers express androgen receptor (AR)? If so, is there any difference on frequency of androgen receptor expression between ER-positive breast cancer and TNBC?**

The androgen receptor (AR) is widely expressed in breast cancers. AR expression has prognostic implications in breast cancers; higher AR expression levels have been associated with higher expression of ER or PR, lower nuclear grade, and smaller tumor size with lower risk of recurrence and death [33, 34]. Significant differences in AR protein expression have been found in different molecular subtypes of breast cancer. However, the role of AR in breast cancers remains uncertain, particularly in ER-positive tumors. Enzalutamide, an AR inhibitor that impairs nuclear localization of AR, was used to elucidate the role of AR in women with ER-positive and ER-negative breast cancers [35].

37. **What is AR expression status in ER-positive breast cancer?**

AR is expressed in up to 90% of ERα-positive tumors. Although multiple studies suggest that AR is associated with a favorable prognosis, AR overexpression and, in particular, high AR/ER ratio seem to be involved in resistance to hormonal treatment [36].

38. **What is AR expression status in TNBC?**

TNBCs that are by nature ER negative characteristically have a much lower frequency of AR expression compared to ER-positive breast cancers [33, 34, 37, 38]. A recent study revealed that only 31% of TNBC patients expressed AR by IHC [37], which is similar to other studies reporting 25–35% positivity [33, 39–41].

Recent studies by molecular analysis have demonstrated that TNBCs are a heterogeneous group of tumors. Six subtypes of TNBCs were identified using gene expression profiling; they were basal-like (BL1 and BL2) which is the major subtype, immunomodulatory, mesenchymal, mesenchymal stem-like, luminal androgen receptor (LAR), and unstable subtypes [38]. The AR-expressing TNBCs were defined as the LAR subtype [38], which may have a better prognosis compared to other molecular subtypes of TNBC.

Previous studies on the AR expression in TNBCs have shown that AR negativity is associated with a shorter disease-free interval and overall survival compared to AR-positive TNBCs [40, 42–44]. Recently, a group reported that, among AR-positive TNBCs, cases with distant metastases (pathologic staging: pM1) were significantly associated with a lower intratumoral expression of AR protein as compared to cases without distant metastasis or only having loco-regional disease (pathologic staging: pM0) [37]. The study also demonstrated that the AR expression inversely correlated with Ki67. This result suggests that TNBCs with higher AR expression may be associated with a better prognosis, partly due to decreased tumor cell proliferation caused by the increased anti-proliferative effect of androgen receptor stimulation. These findings indicate AR could be used as a prognostic marker for TNBCs.

39. **Is there any therapeutic value of AR expression for patients with TNBCs, particularly those with metastatic/recurrent TNBCs or chemotherapy-resistant TNBCs?**

The AR is a clinical target for targeted therapy for treating prostate cancer. Recent research indicates that it is an emerging hormonal target in breast cancer as well, with potential clinical benefit in both ER-positive and ER-negative tumors [45]. The AR signaling pathway may represent a molecular driver that can be therapeutically targeted in AR-expressing TNBCs by utilizing AR inhibitors. Several studies have shown promising results for AR antagonists. One such study demonstrated that the LAR cell lines of TNBC were uniquely sensitive to bicalutamide, an AR antagonist [38]. In mouse models of TNBC with a low percentage of AR-positive tumor cells, an anti-androgen drug, enzalutamide, was significantly effective in reducing proliferation, growth, migration, and invasion of the cancer cells. This study also showed that only a mere 1% of TNBC cells in a tumor must be AR positive to show benefit from AR-targeted therapies [46]. Anti-androgen therapy has been proposed as a targeted therapy in women with TNBC. This may be of particular interest for those who do not respond to conventional chemotherapy or even as an addition to first-line therapy. Therefore, knowing the AR expression status of a TNBC tumor is necessary for the selection of patients who may benefit from anti-androgen therapy. Multiple ongoing clinical trials have shown promising preliminary results of AR-targeted thera-

pies against TNBC with a higher percentage of AR-positive cells [47]. We therefore propose testing for AR expression in all metastatic/recurrent TNBCs and primary TNBCs that do not completely respond to chemotherapy in order to help guide management decisions.

40. What is p53 expression status in TNBC and the potential prognostic value of p53?

p53 acts to induce apoptosis and arrest the cell cycle in response to cellular stresses such as DNA damage. p53 is a tumor suppressor protein, and its overexpression has been reported in the majority of TNBC tumors [48]. TNBCs can be divided into two subtypes based on their p53 expression status: p53-positive and p53-negative tumors [49]. From the molecular perspective, TP53 gene mutations have been reported in the vast majority of TNBCs in recent publications, and as such is a target of particular interest [48].

A recent study demonstrated that p53 expression in TNBCs with nodal metastasis was significantly higher than that in non-TNBCs with nodal metastasis [50]. It was revealed that p53 expression had the strongest prognostic significance in TNBC patients in a multivariate analysis [51]. It has also been demonstrated that TP53 mutations were strongly predictive for relapse-free and overall survival in TNBC patients treated with adjuvant anthracycline-based chemotherapy regimen [52]. p53-positive TNBCs have worse overall and disease-free survival compared with p53-negative tumors [48, 53]. Since p53 overexpression is associated with a poorer survival, it has been suggested that p53 can be used as a prognostic marker for TNBC.

41. What is p16 expression status in TNBC? What is the diagnostic utility of p16 in metastatic TNBC?

p16 normally acts as a cyclin-dependent kinase (CDK) inhibitor by inactivating CDK4/6 and preventing the phosphorylation of retinoblastoma (Rb). p16 is a tumor suppressor protein, which plays an important role in cell cycle regulation.

p16 by IHC is widely used as a surrogate marker for high-risk HPV infection in diagnosing high-grade dysplasia and squamous cell carcinoma of the cervix and the oropharynx. Recent studies demonstrated that a strong and diffuse p16 expression was seen in the majority of TNBCs [54, 55]. A case series showed that 75% of TNBC tumors overexpressed p16 protein and the expression was significantly higher than that in paired normal breast ducts (Fig. 7.11a–d) [49]. While there are very limited reports in the literature, this finding is especially important for the pathologist when attempting to distinguish metastatic TNBCs from p16-positive carcinomas originating from non-breast sites, such as the fallopian tube/ovary/peritoneum since the majority of high-grade serous carcinomas arising from these locations show p16 overexpression.

42. What IHC makers can be used to distinguish metastatic p53+ p16+ TNBC to the gynecologic sites from high-grade serous carcinoma of the fallopian tube/ovary/peritoneum that is commonly p53 and p16 positive?

From a clinical practice standpoint, when dealing with an ER/PR/Her2-negative high-grade carcinoma presenting in the female reproductive tract in a patient with a history of TNBC, the differential diagnosis should include metastatic TNBC to the gynecologic site or a serous carcinoma presenting as a second primary tumor. Conversely, metastatic high-grade serous carcinoma to the breast or axillary lymph nodes is not uncommon. However, the differential diagnosis can be problematic when these two different primary tumors have an identical immunophenotypic profile, ER-/PR-/Her2−/p53+/p16+, and similar morphologic features. For such cases, we recommend using a differential IHC panel that includes, but is not limited to, GATA3, PAX8, and WT-1. Breast primary tumors are commonly GATA3+/PAX8-/WT-1-, while serous carcinomas are GATA3-/PAX8+/WT-1+. Correlation with clinical and radiographic information is essential for making a correct diagnosis.

Of note, both TNBC and high-grade serous carcinoma can occur in patients with BRCA mutations, and both types of carcinomas can co-overexpress p16 and p53. Making a distinction between these two primary tumors has important prognostic and therapeutic implications.

43. What is the potential predictive value of p16 expression in TNBC?

To better understand TNBC tumor biology as related to the Rb/p16 pathway, a group of researchers investigated the relationship of p16 expression with Ki67 in TNBC cases [49]. Ki67 is widely used as a prognostic and predictive tumor biomarker for breast cancer, and a higher expression of Ki67 is associated with a more aggressive clinical behavior. They found that the mean value of Ki67 expression was high (>60%) in both p53-positive and p53-negative TNBC tumors regardless of the status of p16 expression; there was a significant positive correlation between p16 and Ki67 only in p53-negative tumors, but not in p53-positive tumors. These findings suggest that p16 may play a role in the poor outcomes of p53-negative tumors that provide insights into the potential prognostic value of p16 for TNBC. The p16-related cell cycle pathway might be potentially targetable for treatment of TNBCs, particularly for p53-negative tumors. Another study reported that TNBC patients whose tumors showed strong p16 immunoreactivity had complete pathologic response and that decreasing response correlated with decreasing expression of p16 [56]. This finding suggests that the status of p16 expression may have a predictive value for tumor response to chemotherapy.

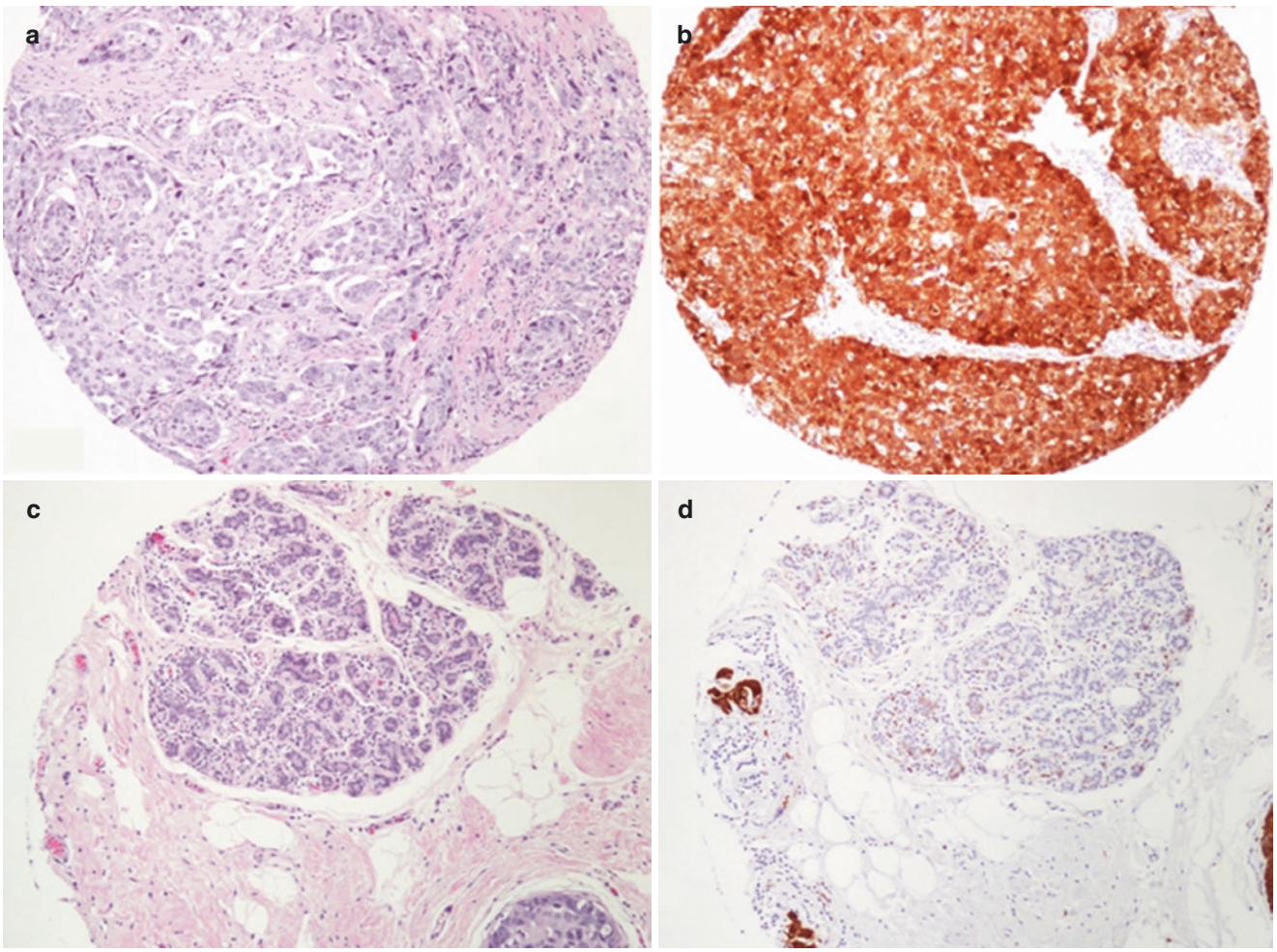

Fig. 7.11 (**a**–**d**) p16 expression in a TNBC tumor and paired normal tissue. TNBC shows high-grade ductal carcinoma morphology (**a**) and diffuse, strong p16 immunostaining (**b**); paired normal breast tissue shows benign ducts (**c**) and negative p16 staining (**d**)

44. What is basal cytokeratin expression status in TNBC?

Basal cytokeratins include CK5/6, CK5, CK14, and CK17; and they can express in TNBC tumors.

TNBCs can be divided into basal-like and non-basal-like subtypes according to basal cytokeratin expression status, with the majority falling into the basal-like subtype [57, 58]. CK5/6 is expressed in around 62% of TNBCs [59]. However, a recent study reported CK5 positivity in 97% of TNBC tumors and suggested that CK5 is more sensitive than CK5/6 in identifying the basal-like tumors [60].

From a diagnostic standpoint, immunoreactivity of basal cytokeratins can be helpful in diagnosing ER-/PR-/Her2- poorly differentiated invasive carcinomas in the breast as basal-like TNBCs, particularly in the core biopsy specimens where in situ lesions may be absent.

45. What are potential prognostic and predictive values of basal cytokeratins in TNBC?

Recent studies have demonstrated that, among the histologic subtypes of breast cancer, basal-like TNBCs have significantly worse outcomes [57, 61–63]. A group of researchers reported that approximately one-third of basal-like TNBCs developed distant metastasis [59]. They found that TNBCs with metastases had significantly higher expression of CK5/6 as compared to cases without metastases. Their findings suggest that high-level expression of CK5/6 may be predictive of metastatic disease in TNBC patients. While some reports have demonstrated that TNBCs have a good response to an adjuvant anthracycline-based chemotherapy, it has been shown that patients with a basal-like phenotype of TNBC had a significantly poorer response to the chemotherapy [64].

46. What cancer stem cell markers are enriched in triple-negative breast cancer?

Recent studies suggest that cancer stem cells play an important role in tumorigenesis and tumor biology of TNBC. Both $CD44^{+}/CD24^{-}$ and $ALDH1^{+}$ breast cancer stem cells are enriched in TNBC compared to non-TNBC tumors and may contribute to the propensity of TNBC for chemotherapy resistance and tumor metastasis. It has been proposed that targeting cancer stem cells may be a promising, novel strategy for treatment of TNBC patients [65].

47. Can breast carcinomas be positive for TTF-1 and Napsin A that commonly express in adenocarcinoma of the lung?

It has been well documented that the majority of lung adenocarcinomas are TTF-1 (75%) and Napsin A (87%) positive [66, 67].

Recently published studies showed that both the sensitive lung markers can express in a small portion of breast ductal carcinomas; TTF-1 was positive in 2.4% from focal and weak to diffuse and strong [68] and Napsin A in 3% [66].

48. Can lung adenocarcinomas be positive for GATA3, a sensitive breast marker?

GATA3 expresses in more than 90% of ER-positive luminal-type breast carcinomas and up to around 70% of TNBCs [27, 31]. Currently, GATA3 is widely used as one of the most sensitive breast markers in routine clinical practice. However, a recent study reported that GATA3 expressed in 8% of lung adenocarcinomas [69].

49. Can lung adenocarcinomas be positive for ER?

Recent studies demonstrated that a subset of lung adenocarcinomas express breast tumor biomarkers by IHC. ER positivity was identified in approximately 5–27% of lung adenocarcinomas, and most of ER-expressing lung tumors are positive for TTF-1 [70–72].

50. Can lung adenocarcinomas be positive for HER2 by IHC?

Her2 protein overexpression or gene amplification was found in primary lung adenocarcinomas [73, 74], although this phenomenon is uncommon.

51. How does one distinguish breast primary tumor from lung adenocarcinoma despite the presence of overlapping immunohistochemistry between these two tumors?

Incidence of metastatic extramammary carcinomas to the breast is up to 2%, and the lung is one of the most common primary sites [75–77]. Distinguishing metastatic carcinoma of lung origin from primary breast carcinoma has significant prognostic and therapeutic implications. However, sometimes making the distinction can be diagnostically challenging due to the following reasons:

- No or insufficient clinical and radiographic information available
- Absence of in situ carcinoma of the breast, such as DCIS, particularly in core biopsy specimens
- Morphologic similarity of both adenocarcinomas, especially in high-grade tumors
- Overlapping immunoprofile between these two primary tumors

Morphologically, identification of in situ carcinoma of the breast helps make a diagnosis of primary breast carcinoma. The majority of primary breast carcinomas express ER and/or PR by IHC, and some overexpress HER2 protein. GATA3 is a recently recognized sensitive and specific maker for breast primary tumors. TTF-1 and Napsin A are sensitive and specific markers for lung adenocarcinomas. In general, an IHC panel including TTF-1, Napsin A, GATA3, and ER serves as a useful ancillary tool in the differential diagnosis between these two primary tumors in the majority of cases. Lung adenocarcinomas are commonly TTF-1+/Napsin A+/GATA3-, and breast carcinomas are GATA3+/ TTF-1-/Napsin A-. A recent study reported that a simple IHC panel of GATA3 and TTF-1 correctly differentiated breast carcinoma from lung adenocarcinoma in 93% of cases in their series [78].

However, a small portion of breast and lung carcinomas have overlapping immunohistochemical expression on ER, Her2, GATA3, TTF-1, and Napsin A [66–68, 70–72], which makes a distinction between these two primaries diagnostically challenging. Moreover, the presence of ER, Her2, or GATA3 immunoreactivity in a carcinoma cannot by itself be used to exclude the possibility of a lung origin. On the other hand, TTF-1 and/or Napsin A expression cannot rule out a primary breast cancer.

For diagnostically challenging cases—such as metastatic TTF-1+ and/or Napsin A+ breast carcinoma to the lung vs. primary lung adenocarcinoma or metastatic ER+ and/or Her2+ lung adenocarcinoma to the breast vs. primary breast ductal carcinoma—comprehensive analysis with clinical history and radiographic finding and comparison of morphology between primary and metastatic tumors are essential to distinguish breast primary from lung origin [79]. Furthermore, comparison of primary breast cancer's tumor profile (extent and intensity of immunoreactivity) with secondary tumor's profile is one of the important clues to identify ER+ extramammary metastasis in the breast in patients with a prior history of ER- and/or HER2-positive breast carcinomas [80].

52. **What ER-positive carcinomas from Müllerian origin can metastasize to the breast?**

Gynecologic cancers can metastasize to the breast and axillary lymph nodes, although this is uncommon. The majority of metastatic carcinomas from the fallopian tube/ovary/peritoneum are high-grade serous carcinomas, and 64% of those tumors are ER positive [81, 82]. Metastatic endometrial carcinoma to the breast is extremely rare, and there are only a few case reports available in the literature [83, 84].

In an appropriate clinical, radiographic, and morphologic context, a limited IHC panel can help distinguish breast primary from gynecologic origin: GATA3 and PAX8. Breast tumors are usually GATA3+/PAX8-, and GYN tumors are GATA3-/PAX8+.

53. **Can hormone receptor and Her2 status be different between primary breast cancer and paired metastatic tumor?**

A group of researchers from the Netherlands studied receptor conversion for ERα, PR, and HER2 [85]. They found that receptor conversion by immunohistochemistry in (non-bone) distant breast cancer metastases does occur, and the conversion was mainly from positive in the primary tumor to negative in the metastases for ERα and PR, while HER2 conversion occurred equally both ways. The conversion rates on ERα and PR are 15.1% and 32.6%, respectively. The conversion is relatively uncommon for ERα and HER2 and is more frequent for PR, especially in brain, liver, and gastro-intestinal metastases [85].

We suggest taking the possibility of receptor conversion into consideration while dealing with a discrepancy of hormone receptor results between primary and metastatic/recurrent breast cancers to accurately interpret biomarker results to guide optimal patient management.

Case Presentations

Case 1. ER heterogeneity in breast cancer (Fig. 7.12a–d)

- *History*: A 65-year-old female with a mass lesion on screening mammogram underwent a core needle biopsy
- *Histology on the biopsy*: Invasive ductal carcinoma, not otherwise specified (NOS), histologic grade 2

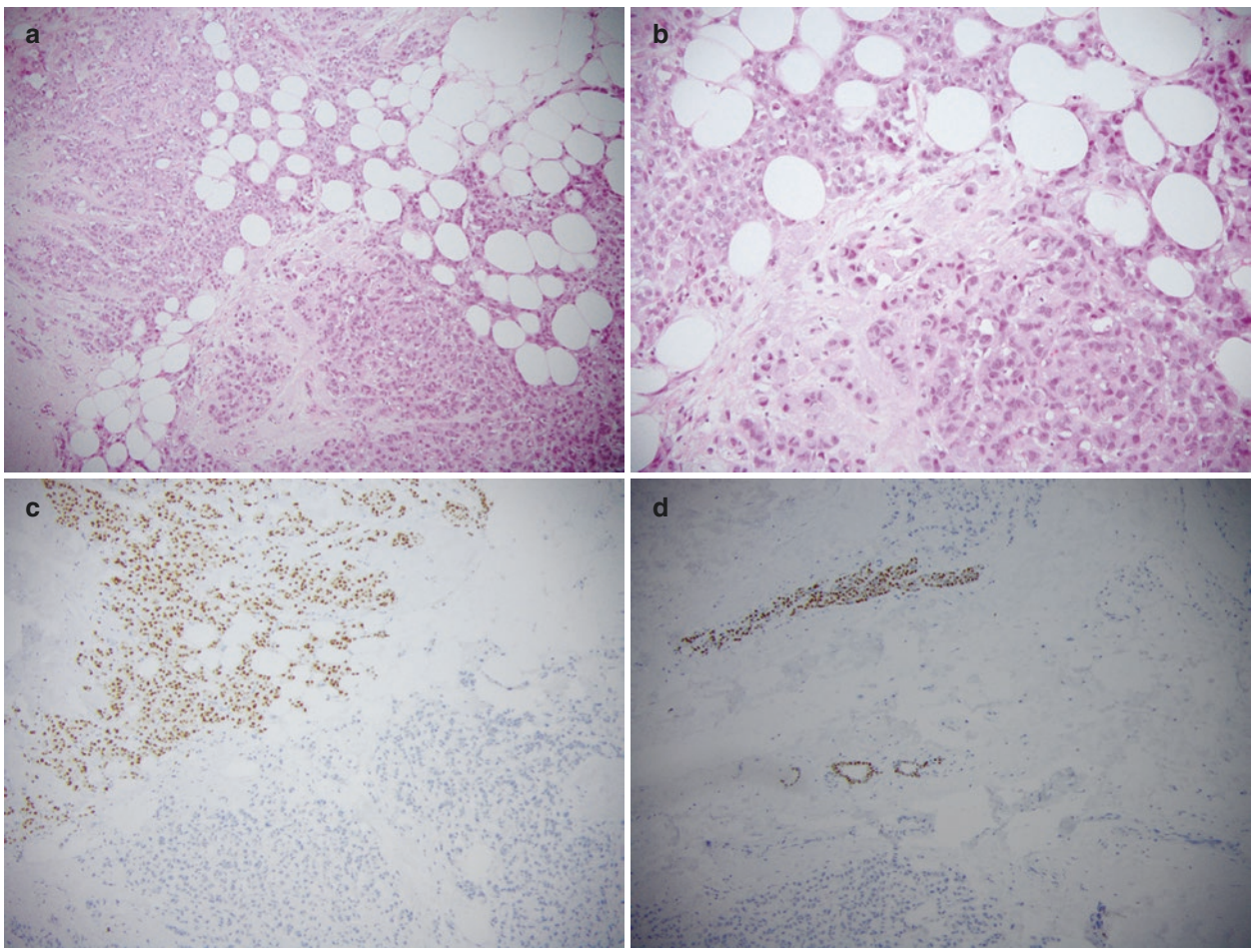

Fig. 7.12 (**a**–**d**) Case 1. ER heterogeneity. Low power (**a**) and high power (**b**) view of the invasive carcinoma, partial positivity of ER immunostain of this tumor (**c**), and positive internal controls on the same slide (**d**)

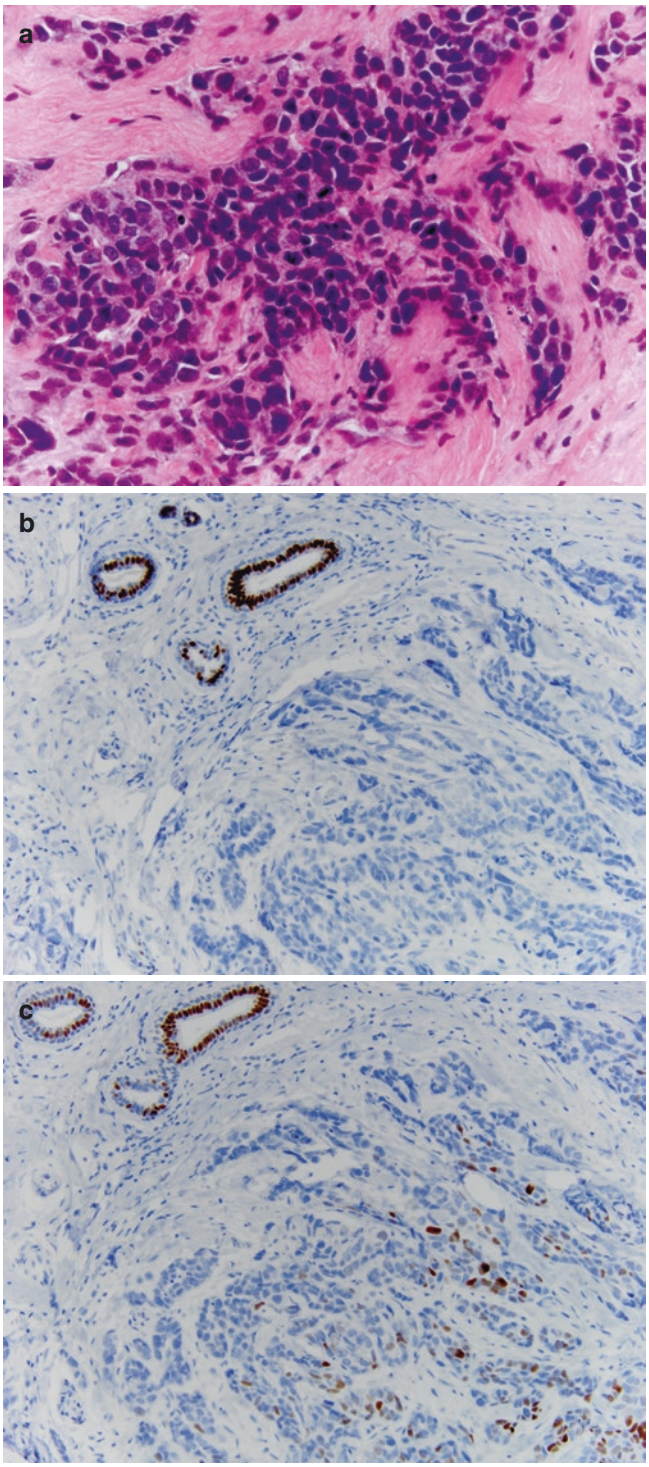

Fig. 7.13 (**a**–**c**) Case 2. ER-negative/PR-positive breast cancer. High-power view (**a**) of the invasive ductal carcinoma, with negative ER immunostain (**b**) and scattered positive PR immunostain (**c**). Both (**b**) and (**c**) show adequate positive internal controls adjacent to the tumor

- *Immunohistochemistry (IHC) on the biopsy*: ER-negative, PR-negative, HER2-negative (0), Ki67 10%
- *Next Steps*:
  - Report this tumor as triple-negative breast cancer.
  - Repeat ER and PR testing on the biopsy specimen.
  - Discuss the results with the patient's clinician.
  - Recommend repeating the testing on subsequent surgical specimen.
- *Final Diagnosis on the resection specimen*: ER-positive, PR-positive, HER2-negative invasive ductal carcinoma, NOS
- *Take-Home Messages:*
  - Heterogeneity of intra-tumoral ER immunostain does exist; further investigation is needed if result of biomarkers does not fit in the clinical/pathological features.
  - Triple-negative breast cancers are usually high grade tumors with a high proliferative index (Ki67 > 50%), however, there are some exceptions (see Question 7.)

Case 2. ER-negative/PR-positive breast cancer
(Fig. 7.13a–c)

- *History*: A 50-year-old female with a breast mass that was biopsied
- *Histology on the biopsy*: Invasive ductal carcinoma, NOS, histologic grade 3, no necrosis present
- *IHC on the biopsy*: ER-negative, PR-positive (10%), HER2-negative (1+), Ki67 30%
- *Next Steps*:
  - Report this tumor as ER-negative/PR-positive/HER2-negative breast cancer.
  - Repeat ER and PR testing on the biopsy specimen.
  - Discuss the results with the patient's clinician.
  - Recommend repeating the testing on subsequent surgical specimen.
- *Final Diagnosis* on the resection specimen: ER-negative/PR-positive/HER2-negative invasive ductal carcinoma, NOS
- *Take-Home Messages:* Up to 5% of breast cancers have been reported as ER-negative/PR-positive breast cancers. Since this group of tumor is rare and still controversial, many pathologists would repeat the testing to confirm the results before rendering final interpretation on the ER/PR results.

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Breast Cancer with Hereditary Cancer Predisposition Syndromes

8

Roshni Rao, Caitlin B. Mauer, Margaret Chen-Seetoo, and Yan Peng

List of Frequently Asked Questions

1. **What percentage of breast cancers are associated with hereditary syndromes?**

At least 10% of breast cancers occur in patients with hereditary mutations. Mutations in high-penetrance genes, such as *BRCA1, BRCA2, PTEN, TP53, CDH1*, and *STK11*, account for up to 25–50% of the hereditary breast cancers [1, 2]. Mutations in moderate-penetrance genes (*CHEK2, ATM,* and *PALB2*) are thought to be as common as mutations in the *BRCA1* and *BRCA2* genes [3]. Low-penetrance gene mutations, such as mutations in *BARD1* and *RAD51D*, have also been identified to confer elevated breast cancer risks [4].

2. **What is the most common age of presentation of breast cancer in hereditary syndromes?**

The age of presentation of breast cancer in patients with a hereditary breast cancer predisposition varies depending on the particular gene. High-penetrance genes, such as *BRCA1* and *BRCA2*, are known to have earlier ages of onset. The average age of onset of breast cancer in a woman with a *BRCA1* mutation is between 35 and 55 years, while the average age of onset in a woman with a *BRCA2* mutation is between 41 and 57 years [5]. More data are needed to determine the average age of onset of breast cancer in moderate-penetrance genes, such as *ATM, PALB2,* or *CHEK2*.

3. **What clinical presentations or indications in patients with breast cancer should prompt genetic counseling and testing?**

The current National Comprehensive Cancer Network (NCCN) guidelines (version 2.2019) recommend a referral for genetic counseling for patients with personal or family histories of any of the following [6]:

- Breast cancer diagnosed at or before age 50
- Two breast cancer primaries
- Ovarian cancer
- Triple-negative breast cancer diagnosed at or before age 60
- Pancreatic cancer
- Male breast cancer
- Metastatic prostate cancer
- Ashkenazi Jewish ancestry with a diagnosis of breast, ovarian, pancreatic, or high-grade (Gleason score ≥7) prostate cancer
- Three or more of the following cancers on the same side of the family:
  - Breast cancer
  - Breast cancer, sarcoma, adrenocortical cancer, brain tumor, leukemia (consider Li-Fraumeni syndrome)
  - Lobular breast cancer, diffuse gastric cancer (consider Hereditary Diffuse Gastric Cancer syndrome)
  - Colon cancer, endometrial cancer, thyroid cancer, kidney cancer, dermatologic manifestations, macrocephaly,

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Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5_8

hamartomatous polyps of the gastrointestinal tract (consider Cowden syndrome)
  - Breast cancer, gastrointestinal cancer or hamartomatous polyps, pancreatic cancer, ovarian sex cord-stromal tumors, testicular Sertoli cell tumors, or childhood skin pigmentation (consider Peutz-Jeghers syndrome)
- A known cancer gene mutation in the family

4. **What is the risk for patients with BRCA gene mutations to develop breast cancer?**

BRCA1 and *BRCA2* are considered high-penetrance genes, although there is considerable variation among specific patterns of cancer in families. Meta-analyses have demonstrated a lifetime risk (defined as age 70) of breast cancer to be 57% for women with a *BRCA1* gene mutation and 49% for women with a *BRCA2* gene mutation [7].

5. **What is the risk for patients with *BRCA* gene mutations to develop ovarian cancer?**

Given the very large number of actual mutations identified in the *BRCA1* and *BRCA2* genes, there is a broad range of ovarian cancer risk estimates. Most contemporary series demonstrate a 40% lifetime risk of ovarian cancer in *BRCA1* mutation carriers and an 11–17% lifetime risk in *BRCA2* mutation carriers [8]. In addition, *BRCA1* and *BRCA2* mutation carriers have a 1.3% lifetime risk of developing primary peritoneal serous carcinoma [9].

6. **On which chromosomes are the *BRCA1* gene and the *BRCA2* gene located in humans?**

Mary-Claire King and colleagues were the first to identify the genetic susceptibility to breast cancer conferred by mutations in the *BRCA1* gene in 1994. This gene is located on the long arm of chromosome 17 [10] and encodes a protein of 1863 amino acids. Shortly after Mary-Claire King's discovery, in 1995, a research group led by Sir Michael Stratton at the Institute of Cancer Research, United Kingdom, identified the *BRCA2* gene. The *BRCA2* gene is located on the long arm of chromosome 13; it is larger than the *BRCA1* gene as it encodes a protein of 3418 amino acids [10]. Both genes function as tumor suppressor genes by mediating double-stranded break repairs in genes through homologous recombination [11].

7. **Is the *BRCA1* gene phosphorylated in response to cell damage?**

It was the initial recognition that the *BRCA1* gene is hyperphosphorylated in response to DNA damage that prompted its identification as a tumor suppressor gene. It is this phosphorylation that helps activate the repair of double-stranded breaks [12].

8. **How is a founder mutation in *BRCA1/BRCA2* carriers defined?**

The concept of a founder effect is predicated on the fact that new populations can be formed from a numerically smaller group of individuals. Subsequently, only a small fraction of the genetic variability from the original group will be expressed in the new population [13]. When a recurrent mutation is identified within a specific geographic area, this variant may have originated from a single mutation event and is referred to as a founder mutation. Haplogroup analysis allows for differentiation between a founder effect and independent mutations. The Ashkenazi Jewish population in particular has three well-studied founder mutations in the *BRCA* genes [13]. *BRCA1* and *BRCA2* founder mutations exist in many other populations, including but not limited to the Icelandic, Finnish, Dutch, Norwegian, French Canadian, Italian, Chinese, and Pakistani populations [14]. Ongoing research among Latino [15], Middle Eastern [16], and Asian [17] populations reveals the importance of identifying these founder mutations to more accurately stratify cancer risks in particular ethnic populations [18].

9. **Do lifestyle modifications in *BRCA1* and *BRCA2* mutation carriers convey risk reduction?**

Differences of cancer presentation among families reflect the varying degrees of penetrance, suggesting that environmental and behavioral factors may modify breast cancer risks in hereditary breast cancer. Among *BRCA* mutation carriers, higher body weight increased breast cancer risk; and smoking, nulliparity, and oral contraceptive use were associated with earlier disease onset [19, 20]. The effects of lifestyle intervention on risk modification in hereditary breast cancer are unknown. A multicenter trial is currently underway to assess potential risk reduction benefits [21].

10. **What is the lifetime risk of developing breast cancer after prophylactic mastectomy for *BRCA1* and *BRCA2* mutation carriers?**

Multiple studies demonstrated prophylactic mastectomy in women with *BRCA1* and *BRCA2* mutations reduced the risk of subsequent breast cancer by 90% or more [22].

11. **What are management recommendations for patients with *BRCA1* and *BRCA2* mutations in regard to their ovarian cancer risks?**

Despite significant advances in therapy, survival from ovarian cancer continues to be poor, with 5-year survival in advanced

disease to be ~20%. Approximately 80% of patients are diagnosed at an advanced stage, primarily due to a lack of effective screening modalities. Nonetheless, secondary to the known high risk of ovarian cancer in *BRCA* mutation carriers, screening can be considered beginning at the age of 30 and continuing until prophylactic bilateral salpingo-oophorectomy (BSO) is complete [23]. Screening techniques for ovarian cancer in these patients include annual serum antigen to evaluate for CA-125 levels and transvaginal ultrasound. Combined, these continue to have low sensitivities of ~60% [24, 25]; currently, no proven alternatives for ovarian cancer screening have been validated. To help with risk reduction, a meta-analysis demonstrated that the use of oral contraceptives for 1 year in *BRCA1* and *BRCA2* mutation carriers can decrease the risk of ovarian cancer by 33–80% and 58–63%, respectively [26]. Regardless, the National Comprehensive Cancer Network (NCCN) and the Society of Gynecologic Oncology (SGO) recommend *BRCA1* mutation carriers consider a prophylactic bilateral salpingo-oophorectomy (BSO) between ages 35 and 40 or after childbearing is complete. Because the average age of onset of ovarian cancer is slightly later in *BRCA2* mutation carriers, these women can consider prophylactic BSO between ages 40 and 45 or after completion of childbearing [6, 23]. Given that newer data suggest ovarian cancer may originate in the fallopian tubes [27], current trials are being conducted to determine if a salpingectomy followed by delayed oophorectomy may reduce the incidence of ovarian cancer in *BRCA* mutation carriers.

12. **How does oophorectomy change survival in the treatment of early-stage breast cancer in *BRCA*-positive patients?**

Several studies have attempted to determine the impact of oophorectomy on mortality from breast cancer in *BRCA* mutation carriers. Of specific interest is whether oophorectomy is helpful after a diagnosis of breast cancer, particularly given that *BRCA1* carriers have a predilection for triple-negative breast cancers where endocrine manipulation and HER2 targeted therapy would not be helpful. Retrospective cohort studies have demonstrated a 70–80% [28, 29] reduction in mortality when oophorectomy is performed after a diagnosis of breast cancer in *BRCA* mutation carriers. A multicenter cohort study of 2482 *BRCA1* and *BRCA2* carriers by Domchek and colleagues [30] evaluated breast cancer-specific mortality with oophorectomy and demonstrated an odds ratio of 0.35 (95% CI, 0.19–0.67). A similar analysis performed by Metcalfe and colleagues [31] confirmed this mortality benefit for *BRCA1* carriers only.

13. **Should known deleterious gene mutation carriers with a breast lesion on imaging undergo needle biopsy?**

Both *BRCA* and non-*BRCA* mutation carriers, as well as other breast cancer predisposition mutation carriers, with any suspicious breast lesion should undergo needle biopsy (if technically feasible) for tissue diagnosis [32]. Biopsy can be performed under palpation or image guidance.

14. **What is the incidence of metachronous, contralateral primary breast cancer in *BRCA1* and *BRCA2* mutation carriers?**

Compared to non-*BRCA* mutation carriers, the risk of a contralateral primary breast cancer for *BRCA* carriers is higher. Quantifying a specific risk for these patients is important so informed choices regarding prophylactic surgery vs. appropriate surveillance can be made. A large meta-analysis [33] revealed the cumulative risk of contralateral breast cancer increases as a function of time from the initial cancer diagnosis. In *BRCA1* carriers, pooled risk estimates reached 33% at 15 years. In contrast, for *BRCA2* carriers, similar analysis revealed only a 23% contralateral breast cancer risk at 15 years.

15. **Do patterns of metastatic disease differ between patients with *BRCA1*-associated breast cancers and breast cancer patients with no mutations?**

In a study comparing *BRCA1* mutation carriers with triple-negative breast cancer to a group with sporadic triple-negative breast cancer, overall survival and prognosis were not impacted by *BRCA1* mutation carrier status [34]. In addition, patients developed metastases in a similar time frame (median was ~20 months after diagnosis), with the most common site of metastatic disease being the lungs in both groups, followed by liver metastases and then bone metastases.

16. **Can endocrine therapy reduce breast cancer risk in known *BRCA1* mutation carriers?**

There are very limited data regarding the use of tamoxifen for breast cancer prevention in *BRCA* mutation carriers. The only prospective study was the National Surgical Adjuvant Breast and Bowel Project P1 trial, which identified eight *BRCA1* and eleven *BRCA2* carriers. In contrast to *BRCA2* carriers whose breast cancers are estrogen receptor positive in 77% of cases, 75–80% of cancers in *BRCA1* carriers are estrogen receptor negative [22, 35]. The effectiveness of tamoxifen in the prevention of breast cancer among *BRCA1* carriers is currently unknown and warrants investigation.

17. **Is a nipple-sparing mastectomy (NSM) for known mutation carriers an appropriate management strategy?**

Nipple-sparing mastectomy (NSM) is increasingly performed for women with breast cancer, with no difference

found in disease recurrence or survival compared with skin-sparing and total mastectomies [36]. Data examining the safety of NSM in *BRCA* mutation carriers are limited. Yao and colleagues found that among 150 patients who underwent NSM for prophylaxis and 51 cancer patients, only 3 of the cancer patients (5.8%) had cancer in the nipple-areolar complex. Four cancer events were found with a mean follow-up of 32.6 months, three in cancer patients and one in a risk reduction patient, but none of the cancers were in the nipple-areolar complex. These findings suggested NSM in *BRCA* carriers appears to be safe, but long-term studies are needed to assess its use [37].

18. **Is axillary staging utilizing sentinel node biopsy accurate in *BRCA*-associated breast cancers?**

Sentinel lymph node biopsy utilizes the known, orderly patterns of breast lymphatic drainage to identify the initial lymph nodes that breast cancer would spread to if there were lymph node metastases. The nodes are typically identified by utilizing a combination of a radioactive tracer and a blue dye that allows visual confirmation of the sentinel node(s). In the setting of breast cancer, surgical axillary staging with a sentinel node biopsy is appropriate regardless of gene mutation carrier status [38].

19. **How often is a clinically occult breast cancer identified on prophylactic mastectomy specimens?**

Occult breast cancer is identified in 0–3% of *BRCA* mutation carriers who undergo prophylactic mastectomy and 0.1–5.6% of non-*BRCA* mutation patients [37].

20. **Is survival from genetic mutation-associated breast cancers worse than that from sporadic breast cancers?**

Breast cancers associated with *BRCA1* mutations are more likely to be high grade and triple negative. *BRCA2*-positive patients also have been identified to present with higher-grade tumors. The largest meta-analysis [39] reveals that, compared to patients with sporadic breast cancers, *BRCA1*-associated breast cancer patients have worse overall survival and worse breast cancer-specific survival, resulting in a 30% higher risk of dying from breast cancer for patients who are *BRCA1* mutation carriers [39]. In contrast, *BRCA2*-associated breast cancers do not demonstrate lower rates of overall survival, but do demonstrate worse breast cancer-specific survival [39]. For *BRCA*-associated triple-negative breast cancers, overall survival is better when compared to sporadic cases of triple-negative breast cancer [39].

21. **Are there any targeted therapies for breast cancer patients with hereditary cancer syndromes?**

Poly-ADP ribose polymerases (PARPs) are a group of enzymes that aid in DNA repair. PARP inhibitors block this DNA repair mechanism and can result in cell death in patients who already have DNA repair deficiencies, such as individuals with a *BRCA* mutation. As such, PARP inhibitors have been approved by the Food and Drug Administration (FDA) to be used to treat breast cancer in *BRCA*-positive patients [40].

22. **Which genetic mutations are contraindications for breast-conserving therapy in the setting of a breast cancer diagnosis?**

Li-Fraumeni syndrome is caused by a germline mutation in the TP53 tumor suppressor gene. It is associated with soft tissue sarcoma, osteosarcoma, brain tumor, adrenocortical carcinoma, and premenopausal breast cancer. Breast-conserving therapy is contraindicated in patients who carry TP53 gene mutations due to the concern for radiation-induced secondary cancers in this specific patient population [41].

In addition to long-term, high-risk breast cancer surveillance, the current NCCN guidelines for the management of *BRCA*-associated breast cancer include the consideration of bilateral total mastectomy for risk reduction and treatment [6]. While breast-conserving therapy has been performed, a large meta-analysis [42] that included ten studies specifically evaluating breast conservation in *BRCA* gene mutation carriers demonstrated higher rates of in-breast recurrence with long-term follow-up. When median follow-up was at least 7 years, the in-breast recurrence rate was 23.7% in *BRCA*-associated breast cancers versus only 15.9% in sporadic breast cancers.

23. **What are the most common cancers associated with Li-Fraumeni syndrome?**

Li-Fraumeni syndrome is a rare, inherited hereditary cancer disorder caused by mutations in a tumor suppressor gene known as the *TP53* gene. The *TP53* gene is known as the "guardian of the genome," and germline mutations in it can lead to the development of multiple cancers during an individual's lifetime. Patients with germline *TP53* mutations have nearly a 100% chance of cancer in their lifetime, with 50% of patients developing their first cancer before age 30 [43]. The most common tumors seen in patients with Li-Fraumeni syndrome include sarcomas (both soft tissue and osteosarcoma), premenopausal breast cancer, tumors of the central nervous system, adrenocortical carcinomas, and myeloid leukemias. Patients with Li-Fraumeni syndrome are

thought to be sensitive to ionizing radiation, which may induce secondary malignancies [44].

24. What are the mutations in which genes are associated with male breast cancer?

Male breast cancer accounts for about 1% of all breast cancer cases diagnosed in the United States annually; about 1/1000 (0.01%) of men will develop breast cancer in their lifetime [45]. Male carriers of a *BRCA1* mutation are estimated to have a 1.2% lifetime (defined as age 70) risk of breast cancer, while male *BRCA2* mutation carriers are estimated to have a 6.8% lifetime risk of male breast cancer [7]. The *CHEK2* c.1100delC mutation, a common European founder mutation, is suspected to increase male breast cancer risk by tenfold in certain populations (e.g., Finnish, Dutch) and may also confer an earlier average age of onset of male breast cancer when compared to the general population [46, 47]. Further studies are needed to determine if other *CHEK2* mutations also confer a risk of male breast cancer. Mutations in the *PALB2* gene may increase the risk of male breast cancer by 6.6-fold, although larger population studies are needed to more precisely define this risk [47]. To date, large studies have not shown a statistically significant increased risk of male breast cancer in *PTEN*, *CDH1*, or *TP53* mutation carriers; further analyses of these populations are warranted [47–49].

25. The diagnosis of a triple-negative breast cancer in a young patient should prompt concern for which genetic mutations?

Triple-negative breast cancer makes up about 12–15% of all female breast cancer diagnoses and is more prevalent in African American women than in Caucasian women [50, 51]. Triple-negative breast cancer occurs in about 60% of women with *BRCA1* gene mutations and about 25% of women with *BRCA2* gene mutations [52]. Other hereditary breast cancer gene mutations, including *PALB2*, *BARD1*, *RAD51D*, *RAD51C*, and *BRIP1*, have been identified in patients with triple-negative breast cancer; but further studies are needed to clarify the association [53].

26. What germline mutations should be of concern in a patient who presents with synchronous breast and colon cancers?

Mutations in the *CHEK2* gene increase the risks of both female and male breast cancer and colon cancer. Mutations in this gene (specifically the common founder mutation c.1100delC) have also been associated with an increased risk of prostate, kidney, and thyroid cancers [54]. Additionally, mutations in the *STK11* gene are associated with Peutz-Jeghers syndrome and increase the risks of colon, breast, gastric, small bowel, pancreatic, gynecological, and testicular cancers, as well as mucocutaneous staining most vivid during childhood [55]. *CDH1* gene mutations, causative of hereditary diffuse gastric cancer syndrome, increase the risks of lobular breast cancer, diffuse gastric cancer, and colon cancer [56].

27. Is breast cancer associated with *CHEK2* mutation? If so, what are the most common subtypes of breast cancer?

The *CHEK2* c.1100delC founder mutation occurs in 1–2% of the European/North American population and is associated with increased breast cancer risk [57]. This mutation is one of the best studied and increases breast cancer risk by twofold in women and tenfold in men [54]. The breast cancers most commonly seen in female *CHEK2* mutation carriers are luminal type (estrogen receptor positive) and tend to occur in the ducts [58–60].

28. In addition to breast cancer, *CHEK2* mutation carriers are at greater risk of what other types of cancers?

In addition to breast cancer, truncating *CHEK2* mutations increase the risks of prostate cancer, renal cancer, colon cancer, and thyroid cancer [54, 61]. Exact risks of the development of these cancers are unknown at this time.

29. What is the risk for patients with Cowden syndrome to develop breast cancer?

Cowden syndrome results from mutations in the tumor suppressor gene, *PTEN*. Meta-analysis suggests that mutations in *PTEN* confer a 67–85% lifetime risk of breast cancer [62]. Additionally, women with *PTEN* mutations who have already had a diagnosis of breast cancer are at a 29% risk to develop a second primary breast cancer in the subsequent 10 years after diagnosis [63]. Given these risks, the current NCCN guidelines recommend women with *PTEN* gene mutations begin breast cancer screening (annual mammogram alternating every 6 months with breast MRI) beginning at age 30–35 or 10 years younger than the earliest diagnosis of breast cancer in the family. Women with *PTEN* gene mutations should also be counseled on the consideration of prophylactic bilateral risk-reducing mastectomy [6].

Females breast cancer is the most common malignancy observed in Cowden's patients. Up to 75% demonstrate benign breast lesions such as intraductal papillomatosis, fibroadenomas and others.

30. What are clinical manifestations of Cowden syndrome?

In addition to the known predisposition to breast cancer, *PTEN* mutations confer a 25–38% lifetime risk of follicular thyroid cancer, a 21–28% lifetime risk of endometrial

cancer, and a 2–34% lifetime risk of renal cancer. *PTEN* mutations also increase the risk of hamartomatous/ganglioneuromatous gastrointestinal polyps and colon cancer, oral mucosal papillomatosis, macrocephaly, penile freckling, benign dermatological findings such as lipomas or trichilemmomas, autism/developmental delay, and Lhermitte-Duclos disease (dysplastic gangliocytoma of the cerebellum) [62].

31. What are management and surveillance options for patients with Cowden syndrome?

Given the spectrum of cancers in known *PTEN* mutation carriers, risk-reducing management and surveillance recommendations, per the current NCCN guidelines [6], include the following:

- Annual thyroid ultrasound beginning at the time of diagnosis, as childhood thyroid cancers have been observed.
- Mammography alternating with breast MRI beginning at age 30–35 (or 10 years younger than the earliest diagnosis of breast cancer in the family), consideration of prophylactic bilateral mastectomy.
- Awareness of abnormal menstrual/post-menopausal bleeding as a sign of uterine cancer. Endometrial screening with transvaginal ultrasound or endometrial sampling has not been proven effective in patients with Cowden syndrome, but can be considered. Patients should consider a prophylactic hysterectomy after the completion of childbearing.
- Colonoscopy starting at the age of 35 (or 5–10 years younger than the earliest colon cancer diagnosis in the family, if diagnosed at or before age 40), repeating every 5 years.
- Consideration of renal ultrasound beginning at age 40, repeating every 1–2 years.
- Consideration of dermatological exam, psychomotor exam, or brain MRI in patients presenting with symptoms.

32. What are the cancer risks associated with *ATM* gene mutations?

The ATM protein has multiple functions, including a central role in DNA double-stranded break repair. Mutations in the *ATM* gene increase a woman's lifetime risk to develop breast cancer to be between 28% and 50% [64, 65]. *ATM* gene mutations also increase the risk of prostate cancer in men [66, 67] and elevate the lifetime risks of pancreatic cancer in men and women [68].

33. What are polygenic risk scores, and how are they used in the assessment of breast cancer risks?

Polygenic risk scores are the results of algorithms used to compile genetic data, personal, and/or family risk factors that may be associated with risk of specific disease. Polygenic risk scores may be clinically useful in helping to determine breast cancer risk management or in making lifestyle/behavioral recommendations. However, current breast cancer screening/management guidelines do not take polygenic risk scores into account when making these recommendations [6]. Historically, polygenic risk tools have been developed based on data from primarily Caucasian populations; thus, further research is needed to better refine the risk scores to be applicable across multiple demographics [69].

34. How has hereditary cancer predisposition testing changed over time?

Historically, genetic testing consisted of single-gene sequencing and exonic deletion/duplication analysis to analyze for mutations in disease-associated genes. In 2013, with the advent of next-generation sequencing technology, larger genetic panels became available to analyze for mutations in multiple genes at one time. Currently, targeted panels focusing on particular cancers, such as breast cancer, are available, as are pan-cancer panels [70].

35. What types of test results can be received from genetic testing for breast cancer?

Disease-causing mutations, deemed "pathogenic" or "deleterious," identified through genetic testing are classified as positive results. When no disease-causing mutations are identified, this is considered a negative result. Variants of uncertain significance (VUSs) can also be identified through genetic testing. VUSs are alterations in the DNA in which not enough information is known to classify them as positive or negative. It is recommended that these VUSs are not used to alter medical management until additional information is learned [71].

36. Do all labs classify genetic variants that predispose to cancer in the same way?

The American College of Medical Genetics (ACMG) and the Association for Molecular Pathology (AMP) published guidelines in 2015 to help laboratories classify DNA variations [71]. However, these guidelines are subject to interpretation by independent laboratories, and thus variants may not be classified the same between labs. ClinVar is a public data-

base available upon which laboratories can contribute their variant classifications to help unify classifications among labs [72].

37. What is direct-to-consumer (DTC) testing, and how should it be used in patients with personal or family histories of breast cancer?

Direct-to-consumer (DTC) testing is genetic testing that is marketed toward the patient without the direct involvement of a medical provider. DTC testing can provide information such as ancestral background, phenotypic characteristics, or predilection to certain disease (e.g., Alzheimer's, breast cancer). Not all DTC companies are certified by CLIA or CAP regulatory bodies; as such, clinicians need to be aware of the quality of genetic test results from DTC testing. It is critical to assess the quality of genetic testing from DTC companies before using it to determine medical management [73, 74].

38. How is tumor sequencing of breast cancer different from germline testing for breast cancer?

Recent advancements in next-generation sequencing technologies allow for the sequencing of tumors to identify somatic mutations that may be driving the growth of the cancer. These mutations may be targetable by certain therapies, and thus somatic tumor testing can aid in cancer treatment [75]. Genetic testing for hereditary cancer predisposition syndromes evaluates for germline mutations, or mutations that an individual inherits. Germline mutations can be identified via somatic tumor testing; thus, clinicians ordering tumor testing should counsel their patients accordingly [76]. However, germline mutations can also be filtered out through somatic testing reporting pipelines. Thus, individuals undergoing somatic tumor testing may still need a hereditary cancer work-up. As such, consideration of paired tumor and germline testing is preferred as a means to avoid missed mutations [77].

39. What happens if someone inherits two genetic mutations in the same gene that predisposes to breast cancer?

Individuals who inherit two *BRCA2* or two *PALB2* gene mutations (one from each parent) have significant genomic instability. As such, they have a rare, recessive condition known as Fanconi anemia that results in physical abnormalities and bone marrow failure that frequently occurs in childhood/young adulthood [78]. Individuals who inherit mutations in both alleles of the *ATM* gene mutation are known to have ataxia-telangiectasia, a recessive syndrome that significantly affects childhood development, particularly motor control, and greatly increases the risk for multiple types of cancer [79].

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Mesenchymal and Lymphoid Lesions in the Breast

9

Xi Wang and Andrew G. Evans

List of Frequently Asked Questions

1. **How does one approach breast mesenchymal lesions?**

Histologically, the breast stroma could be divided into intralobular and interlobular stroma [1]. Tumors, such as fibroadenomas and phyllodes tumors, are considered as the neoplasms arising from the intralobular stroma of the terminal ductal-lobular unit (TDLU). They are virtually breast-specific neoplasms and technically are not considered as breast mesenchymal lesions, even though they could share morphological similarities with other breast mesenchymal tumors. The interlobular stroma, on the other hand, may not be breast specified; and the lesions/tumors arising in this location are generally the same as the soft tissue lesions/tumors in other anatomy locations, in terms of types of lesion and morphology. One could appreciate this outlook from the 2012 WHO classification of breast mesenchymal tumors wherein pseudoangiomatous stromal hyperplasia (PASH) is probably the only lesion relatively unique to the breast parenchyma. Myofibroblastoma might be primarily a breast tumor. However, the extramammary version does exist and is not always along the anatomical "milk line."

Nevertheless, the approach to breast mesenchymal lesions is unique, compared with the other organ systems. Non-mesenchymal malignancies in the breast, such as metaplastic spindle cell carcinoma (MSC) and malignant phyllodes (MP) tumor which could be even more common tumors in the breast, could greatly mimic the mesenchymal lesions, making the differential diagnosis difficult. Meanwhile, the post-treatment changes – such as fat necrosis, scarring, vascular proliferation, etc. – are very common in this location, given that breast cancer is the most common malignancy in women and breast conservation is the common practice nowadays. These changes could be sometimes exuberant and impose diagnostic challenges for mesenchymal tumors. Furthermore, post-radiation sarcomas, especially angiosarcomas, and possible post-implant tumors have added to the complexity of breast mesenchymal lesions and should always be in the differential diagnosis in a patient with history of breast cancer and treatments.

As in the general soft tissue tumor classifications, breast mesenchymal tumors have been classified based on the types of cell differentiation in the 2012 WHO classifications of tumors of the breast, such as fibroblastic/myofibroblastic lesions, lipocytic tumors, vascular lesions, peripheral nerve sheath tumors, muscular (including smooth muscle and skeletal muscle) differentiation lesions, and bone/cartilage matrix-forming lesions. However, in daily practice, the growth pattern recognition might be more practical for initial differentiation, such as spindle cell vs. vascular vs. lipocytic lesions. Giving the breast as the specific location, this chapter will focus on the mesenchymal lesions and their mimics relatively unique to the breast.

2. **What are the entities that should be considered in the differential diagnoses of spindle cell lesions in the breast?**

In lesions with spindle cell growth pattern, some authors prefer the approach of dividing the lesions into bland-appearing and malignant-appearing lesions [2]. However, there might be lesions that will fall in between these simplified groupings.

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Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5_9

In principle, metaplastic spindle cell carcinoma (MSC) should always be the number one on the list of differential diagnoses for spindle cell lesions in the breast, regardless of bland- or malignant-appearing morphology. Even though a carcinoma, MSC could be lacking of epithelial component, thus appearing as a stromal lesion [3]. This distinction is very important, as the biology and clinical behavior of MSC might be significantly different from other spindle cell lesions.

The bland-appearing spindle cell lesions are relatively more common in the breast parenchyma, compared with malignant-appearing tumors. They include previous procedure site scar, nodular fasciitis, pseudoangiomatous stromal hyperplasia, primary mammary fibromatosis, and mammary myofibroblastoma. They are the tumors of fibroblast/myofibroblast proliferation and often with morphological and immunohistochemical overlap. The other types of low-grade spindle cell lesions, not by any means particular to the breast, but having been reported in the breast parenchyma, include spindle cell lipoma, solitary fibrous tumor, leiomyoma, and peripheral nerve sheath tumor. Benign and borderline phyllodes tumor usually will not enter as a differential diagnosis in this group, as the benign ductal epithelium will usually present and associate with spindle cells in a distinct pattern in phyllodes tumor.

The morphology of inflammatory myofibroblastic tumor (IMT) could be variable. The myxoid vascular pattern or scar-like pattern could appear bland, while the compact fascicular spindle cell pattern could mimic malignancy morphologically.

The list of differential diagnoses for malignant-appearing spindle cell lesions, other than metaplastic spindle cell carcinoma and malignant phyllodes tumor, is very short. Angiosarcoma, primary or secondary, is the most common sarcoma in the breast. It could appear as a spindle cell lesion, when the vascular differentiation is not obvious (so-called poorly differentiated). Other spindle cell sarcomas are very rare in the breast, with leiomyosarcoma, synovial sarcoma, rhabdomyosarcoma, malignant peripheral nerve sheath tumor (MPNST), and others being reported. A metastasis to the breast from other anatomy locations should be ruled out first, before the diagnosis of a breast primary sarcoma can be rendered. It is very important to rule out melanoma, primary from breast skin or metastatic from other locations, in this setting, as it could assume spindle cell morphology and its clinical significance could be completely different from other high-grade sarcomas (Figs. 9.1 and 9.2).

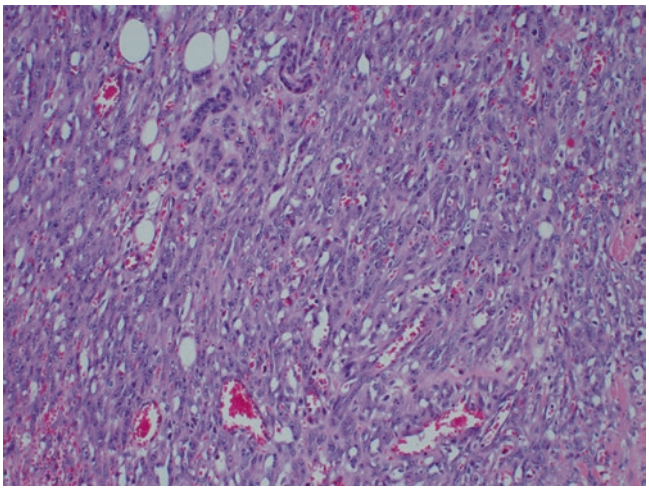

Fig. 9.1 The so-called poorly differentiated angiosarcoma with predominantly spindle cell appearance (100×)

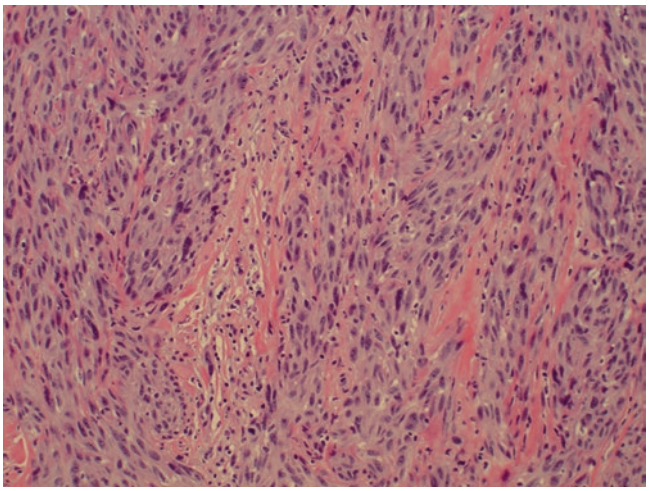

Fig. 9.2 Malignant melanoma with spindle cell appearance (100×)

3. **How does one differentiate mammary fibromatosis from fibromatosis-like metaplastic carcinoma (FLMC)?**

Fibromatosis is an uncommon neoplasm in the breast, with the incidence rate of around 0.2% in breast tumors. It is the proliferation of fibroblasts and myofibroblasts. Typically, the lesional cells are elongated, bland, and uniform spindle cells, forming long, sweeping fascicles and infiltrating into the surrounding breast parenchyma. The diagnostic challenge is to differentiate it, most importantly from fibromatosis-like metaplastic carcinoma (FLMC), as they share many morphological similarities: (1) They are both infiltrative. (2) Both are rich in collagen. (3) Tumor cellularity is similarly modest. (4) Cells are not as pleomorphic as other high-grade tumors (Fig. 9.3a, b).

There are a few morphological features which could be helpful in this regard. Firstly, FLMC could be associated with ductal carcinoma in situ (DCIS), or clusters of epithelioid cells could be identified in between the spindle cell bundles, while fibromatosis will not have this association. Secondly, the cells in fibromatosis are uniformly bland with pale nuclei and inconspicuous nucleoli, while the cells in

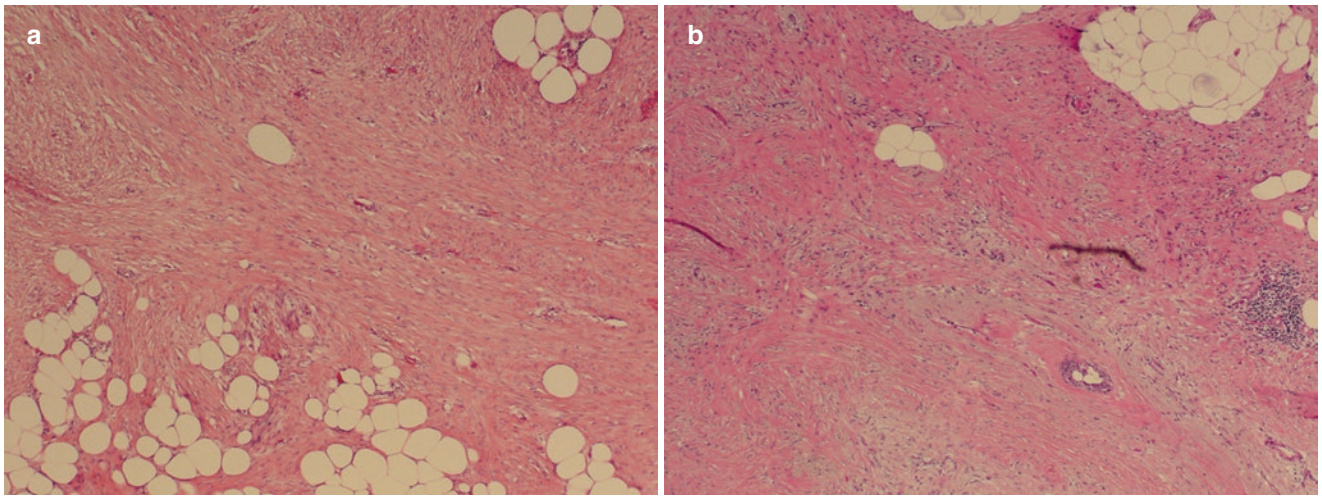

Fig. 9.3 (**a**) Fibromatosis with infiltrative long sweeping fascicles (40×). (**b**) FLMC morphologically resembles fibromatosis (40×)

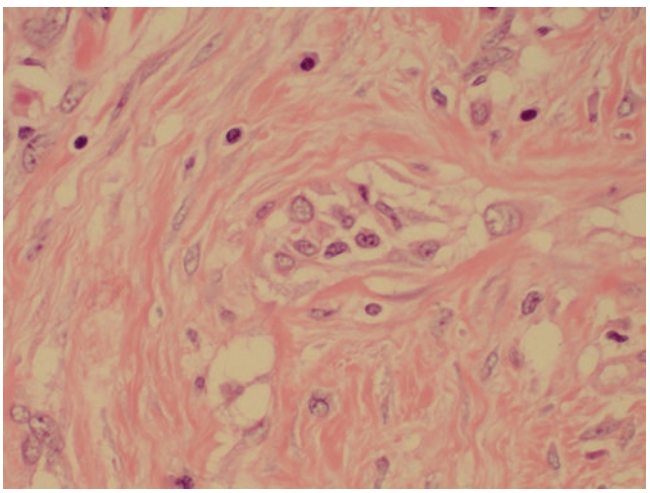

Fig. 9.4 Epithelioid cell clusters in between the spindle cells in FLMC (400×)

FLMC will at least have certain pleomorphism and hyperchromasia. One could appreciate this feature by referencing the nearby endothelial cells. The vasculature in fibromatosis is usually prominent, and the endothelial cells are generally more prominent than the lesional cells, while in FLMC, the tumor cells are more prominent and pleomorphic compared with endothelial cells. Thirdly, lymphoid aggregates or even lymphoid follicles are often identified at the periphery of fibromatosis, while FLMC could have some chronic inflammatory cells mixed with the spindle cells (Figs. 9.4 and 9.5a, b).

A panel of immuno markers will be very helpful. It could not be emphasized more that a group of epithelial markers – such as pan-cytokeratin, p63, and high-molecular-weight cytokeratin – should be applied, as the FLMC could be only focally positive or even negative for one of these markers [4, 5]. Beta-Catenin may not be helpful in this regard, as it could be positive in FLMC [6, 7].

4. **How does one differentiate nodular fasciitis from metaplastic carcinoma?**

Nodular fasciitis is a rare spindle cell lesion in the breast. It consists of plump but uniform fibroblasts/myofibroblasts forming irregular short bundles/fascicles in a myxoid background. The features such as focally infiltrative border, irregular spindle cell bundles/fascicles, active mitosis, and mixed inflammatory cells make it often morphologically overlap with metaplastic carcinoma, especially fibromatosis-like metaplastic carcinoma (FLMC). There are case reports showing that nodular fasciitis could be positive for cytokeratin cocktail, which further complicates the issue [8]. However, the cells in nodular fasciitis are, even though plump, uniform with pale-staining nuclei. There are often numerous small vessels resembling granulation tissue. The mitotic activity could be brisk but not atypical, and p63 and high-molecular-weight cytokeratin should be negative. On the other hand, metaplastic spindle cell carcinoma, even the FLMC type, should have certain cellular pleomorphism, such as hyperchromasia. It will usually have scattered clusters of epithelioid cells mixed with spindle cells. Atypical mitosis could be identified, and at least some of the epithelial markers in a panel including p63 and high-molecular-weight cytokeratin will be positive, especially for those epithelioid cells (Fig. 9.6a, b).

5. **How does one differentiate mammary myofibroblastoma from its mimics?**

Mammary myofibroblastoma is a usually bland-appearing spindle cell neoplasm composed of fibroblasts/myofibro-

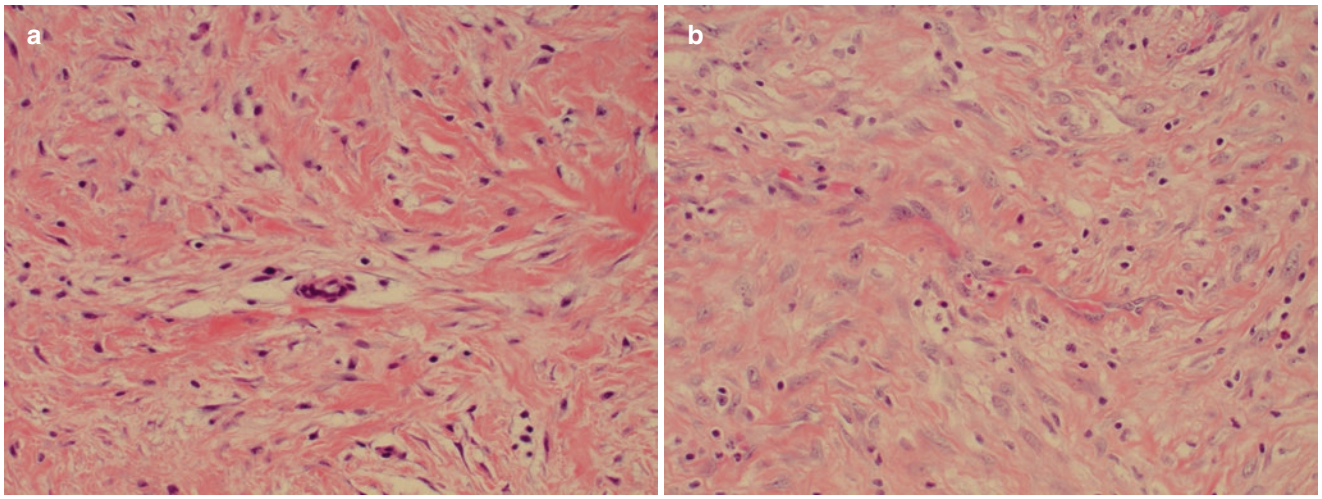

Fig. 9.5 (**a**) The spindle cells in fibromatosis are less prominent than the adjacent endothelial cells (200×). (**b**) The spindle cells in FLMC are more prominent than the adjacent endothelial cells (200×)

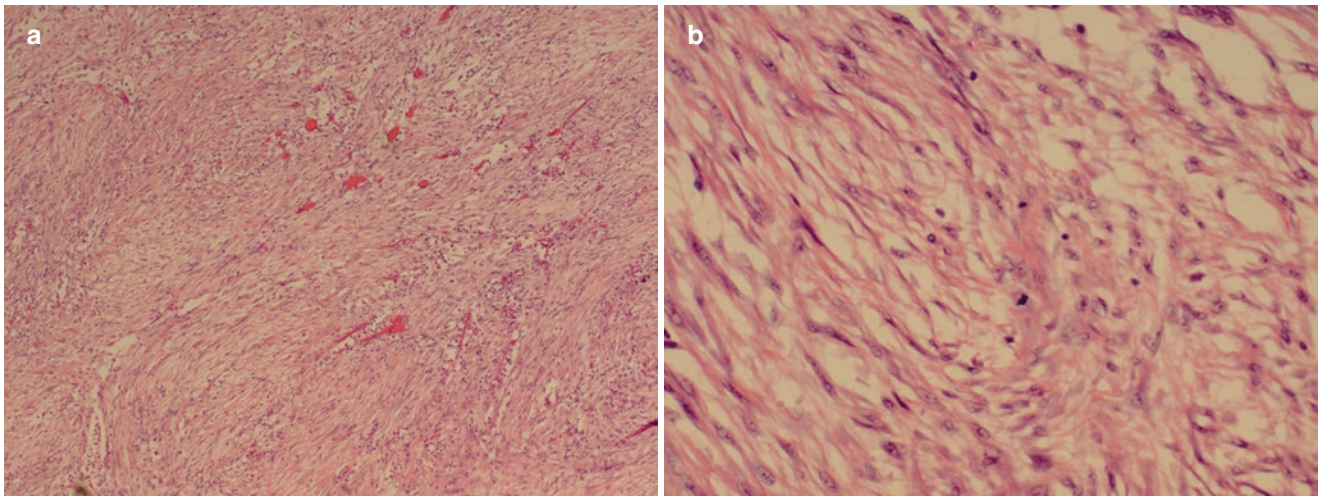

Fig. 9.6 (**a**) Nodular fasciitis with short irregular spindle cell fascicles in a myxoid background. The cells are uniform with pale-staining nuclei (40×). (**b**) Mitosis is easy to find in nodular fasciitis (200×)

blasts. It is normally circumscribed, with adipocytes as the tumor component, without breast glandular structure in the tumor mass. The spindle cells are plump and form short fascicles intersecting with each other and often interrupted by the thick deeply eosinophilic collagen bands. Mitosis could be identified, but the rate is very low (<2 mitosis per 10 high-power fields (HPFs)). The classical myofibroblastoma usually will not pose diagnostic challenge. However, this is the tumor with a wide spectrum of morphological variants, such as cellular, epithelioid, infiltrative, lipomatous, collagenized/fibrous, and myxoid variants. A differential diagnosis from metaplastic spindle cell carcinoma is always required and could be achieved through the appreciation of the general tumor features such as demarcation, no epithelial components, and low mitotic count, as well as the immunohistochemical stain. Myofibroblastoma is variably positive for desmin, CD34, ER, PR, and AR, but negative for cytokeratin markers. The differential from spindle cell lipoma might be more academic, instead of any practical value, as they are in the same histological spectrum and share the same genetic changes (a deletion of 13q14, which includes RB1 and FOXO1A genes) [9]. Fibromatosis may enter as a differential diagnosis, especially for the infiltrative variant of myofibroblastoma. However, fibromatosis usually has only modest cellularity, with broad bands of long fascicles, and a different immunohistochemical staining panel. The myxoid variant of myofibroblastoma could share some morphological similarities with nodular fasciitis. It is very rare and has only been reported in male patients [10]. The circumscription, low mitotic rate, and CD34 positivity could be helpful features in

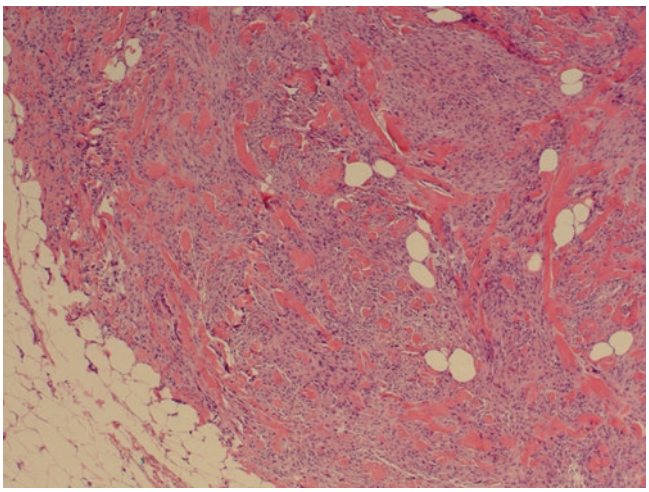

Fig. 9.7 Myofibroblastoma with thick deeply eosinophilic collagen bands (40×)

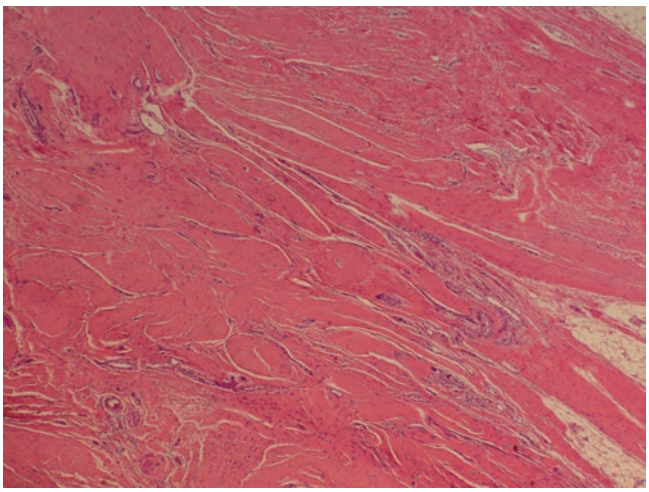

Fig. 9.8 Scar tissue radiating out from the center (40×)

distinction. Myofibroblastoma could exhibit smooth muscle differentiation, which may complicate the diagnosis of leiomyoma in the breast. It is speculated that some of the reported "parenchymal leiomyoma" may represent the myofibroblastoma with smooth muscle differentiation. It appears that this variant could be negative for CD34 – the immunostain hallmarker of myofibroblastoma – but with the deletion of 13q14, the molecular signature for myofibroblastoma (Fig. 9.7) [11].

6. **What are the breast mesenchymal lesions which could have smooth muscle differentiation?**

Breast leiomyoma mostly arises from the skin or nipple/areolar complex, while breast parenchymal leiomyoma is rare. Other benign tumors, which could have smooth muscle differentiation, include myoid hamartoma, fibromatosis, fibroadenoma, benign phyllodes tumors, and myofibroblastoma. Usually, these tumors will have their own characteristic features, other than smooth muscle differentiation. Primary breast parenchymal leiomyosarcoma is even more rare, with about 50 cases being reported in the literature. Leiomyosarcoma could also be a heterologous component of metaplastic carcinoma or malignant phyllodes tumor. Extensive sampling and immunohistochemical staining should help the distinction.

7. **How does one differentiate previous procedure site scar from fibromatosis and other lesions?**

Previous procedure site scar could very much resemble mammary fibromatosis microscopically and sometimes enter as the differential diagnosis of desmoplastic-like metaplastic carcinoma. Clinical history of previous surgery or trauma could be the first clue for scar tissue. However, this history may not always be provided. Microscopically, compared with fibromatosis and other lesions, the spindle cell proliferation in scar tissue is less cellular and denser with abundant collagen; the cells are usually stellate shaped, less organized, not forming a long fascicular pattern, and radiating from the center of the lesion. Even though with irregular borders, scar tissue is usually locally restricted, not as destructively infiltrative as fibromatosis. Further, depending on the age of the scar tissue, there might be associated hemosiderin-laden macrophages, foreign body-type granulomatous changes, and fat necrosis. Beta-Catenin is generally negative, while it is nuclear positive in approximately 80% of fibromatosis (Figs. 9.8 and 9.9a, b) [12].

8. **How does one differentiate inflammatory myofibroblastic tumor from spindle cell metaplastic carcinoma?**

Inflammatory myofibroblastic tumor (IMT) is a distinct neoplasm of myofibroblast/fibroblast spindle cell proliferation mixed with prominent inflammatory cells. It can virtually occur in any anatomy locations throughout the body, including the breast. The age for IMT in the breast ranges from 33 to 76 years according to the case reports. Its morphological features can be variable among cases, depending on the cellularity of spindle cells, collagenous background, and density of inflammatory cells. The most important issue in breast pathology is to differentiate IMT from metaplastic spindle cell carcinoma (MSC), as they do share some morphological similarities: (1) They both consist of spindle cells with variable cellularity. (2) They both commonly have inflammatory cell infiltration. (3) Same as metaplastic carcinoma, osseous metaplasia can occasionally be seen in IMT. However, the

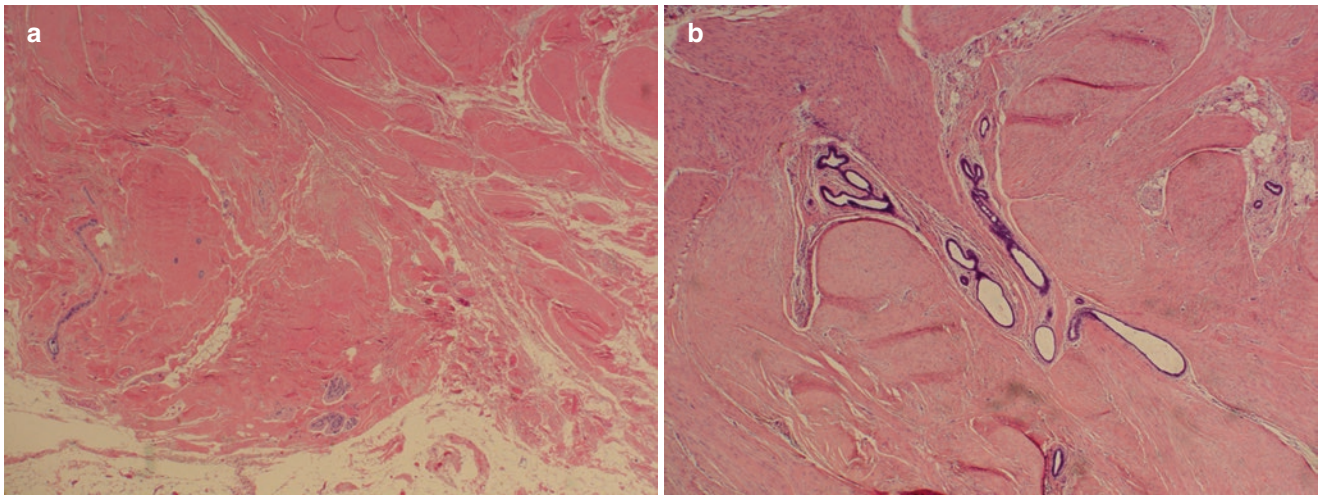

Fig. 9.9 (**a**) Scar tissue is locally restricted (40×). (**b**) Fibromatosis destructively infiltrating in the breast parenchyma (40×)

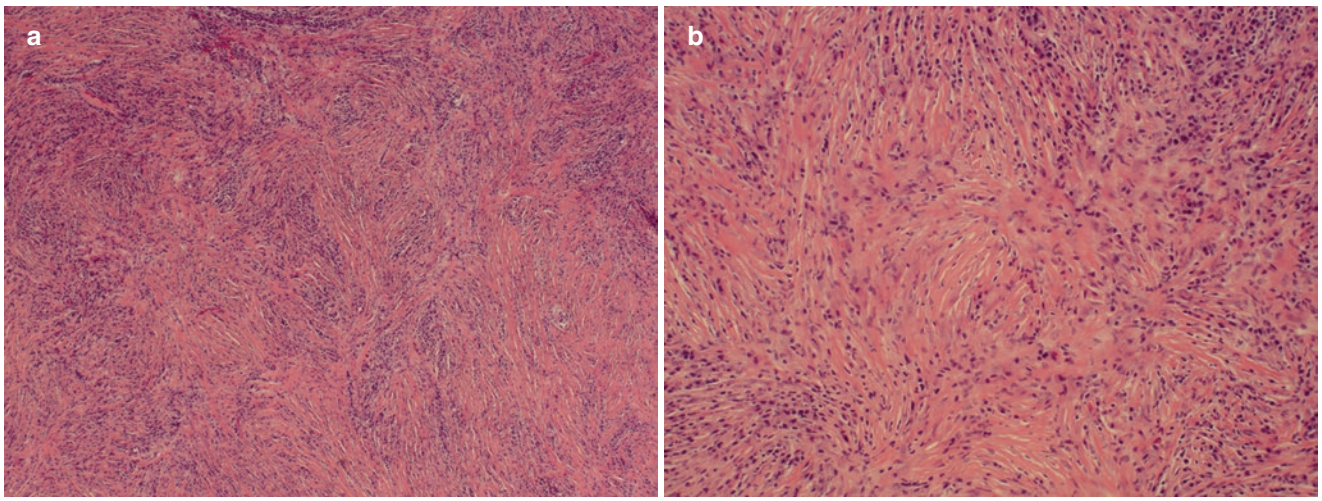

Fig. 9.10 (**a**, **b**) Inflammatory myofibroblastoma with spindle cell proliferation and inflammatory cell infiltration (40×, 20×)

presence of epithelial carcinoma – invasive or in situ, in or around the lesional spindle cells – will be the key feature to recognize metaplastic carcinoma. The spindle cells in MSC will usually show certain pleomorphism, mitotic activity, and even necrosis, while these are not common features in IMT. Immunohistochemically, 50–60% of IMT will be ALK positive, while MSC usually will be at least focally positive for one of the cytokeratin markers. Nevertheless, caution should be taken to differentiate IMT from MSC based on immunohistochemical stain only. As reported, about one-third of the IMT will be focally weakly positive for cytokeratin [13], while MSC could be only cytokeratin focally positive or, under rare circumstance, negative [4]. Also, ALK stain is usually negative in IMTs in patients at an older age [14], when the differential from breast carcinoma is even more pertinent. A panel of immune markers including p63 and high-molecular-weight cytokeratin will be necessary in this regard (Fig. 9.10a, b).

9. **How does one distinguish lipoma from normal adipose tissue in the breast?**

Lipoma is a neoplasm consisting of mature adipocytes with a thin fibrous capsule. The adipocytes in a lipoma are morphologically not much different from the adipose tissue in the breast parenchyma. The distinction between lipoma and localized overgrowth of fat in the breast, especially on a core biopsy, if it is by any means necessary, could be impossible. A demarcation by a thin fibrous membrane somewhere in the core could be the only hint for an intramammary lipoma. Fatty cores deprived of any breast glandular elements could be the feature helping this distinction. The variant types of

lipoma, such as spindle cell lipoma and angiolipoma, usually should not be a problem for diagnosis, as they will have their own characteristic components to be distinguished from the fat in the breast parenchyma.

10. What are the morphological mimics of liposarcoma in the breast parenchyma?

Giving the breast as the specific location, the most important differential diagnosis in this regard includes silicone granuloma from the leaking of breast implant, fat necrosis due to trauma or previous surgery, and fat atrophy. These are the lesions more often encountered in the breast parenchyma. Regular lipoma in the breast parenchyma or chest subcutaneous tissue could present with focal fat necrosis, which could impose a diagnostic challenge. The variants of lipoma, such as pleomorphic lipoma/spindle cell lipoma and angiolipoma, could also mimic liposarcoma sometimes.

11. How does one differentiate lipoatrophy from liposarcoma?

Breast lipoatrophy could occur in malnutrition associated with eating disorders such as anorexia nervosa or with chronic disease. Localized breast lipoatrophy could be the result of pressure from the breast transplant or local trauma/injury to the breast parenchyma. The intracellular lipid is markedly reduced in lipoatrophy, so that the lipocytes are shrieked, which makes the nuclei much more prominent, superficially resembling the morphology of lipoblasts. However, there is no mass formation clinically, and histologically it is rather a diffuse process, instead of a localized neoplasm. No clear demarcation could be appreciated. The lobular architecture of normal fat is maintained. There will be normal breast glandular tissue among the "liposarcoma"-like fatty tissue. A true liposarcoma is usually a well-demarcated mass often with a pushing border. It will not "infiltrate" the breast parenchyma in such a manner. The lipocytes in lipoatrophy are morphologically uniform in size, and the nuclei are pale in color, unlike the variable sizes of fat vacuoles and hyperchromatic nuclei of lipoblasts (Figs. 9.11a, b and 9.12a).

12. How does one differentiate fat necrosis from liposarcoma?

Fat necrosis is a relatively common event in the breast parenchyma. The main etiology could be grouped as (1) trauma, which includes all kinds of surgical procedures, injections, and incidental injuries; (2) radiation therapy; (3) fibrocystic changes, such as ductal ectasia; (4) breast infection; and (5) anticoagulative treatment. These events could result in the disruption of the oxygen supply to the fat cells and cause fat necrosis.

Clinically, fat necrosis often presents as a palpable lump. Sometimes it may show up on a screening mammogram as a mass. A core biopsy is usually conducted with a suspicion for malignancy, mostly carcinoma.

Fat necrosis is basically a non-suppurative inflammatory process. In the early stage, the disrupted fat will present as irregular fatty space with lipid-laden macrophages scattered in between. This picture could be confused with an atypical lipomatous tumor/well-differentiated liposarcoma, especially in the thick sections when the nuclei of the macrophages become somewhat hyperchromatic, mimicking atypical stromal cells. However, the macrophages in fat necrosis are with rounded nuclei and usually with faint staining in adequate sections. The fat vacuoles in these cells are

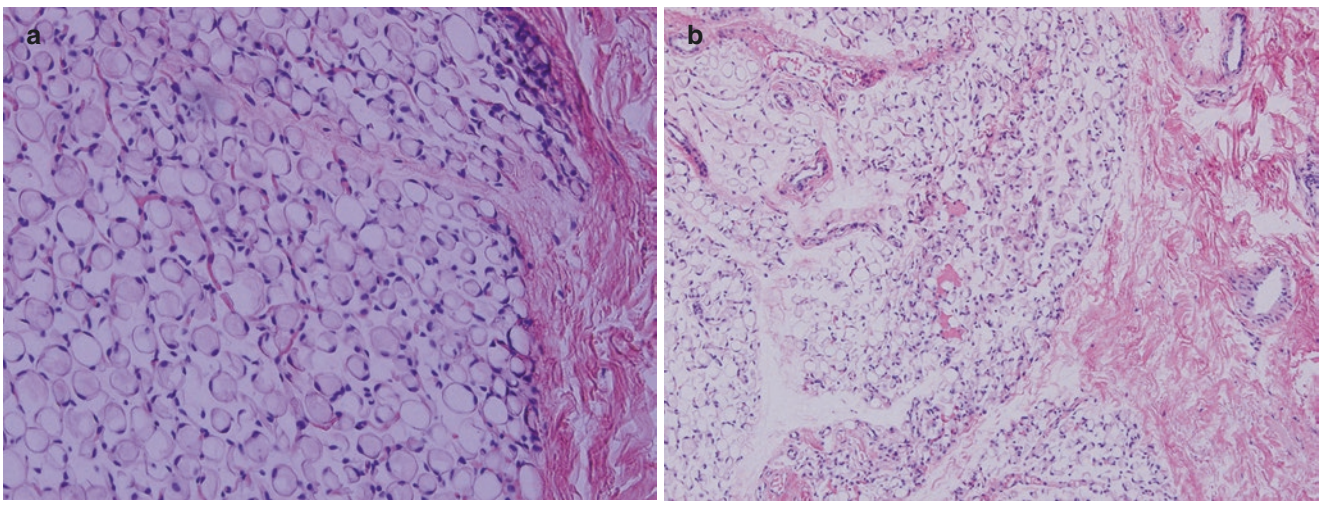

Fig. 9.11 (**a**) Lipocytes in lipoatrophy with uniform nuclear size and fat vacuoles (200×). (**b**) Lobulation of lipoatrophy (40×)

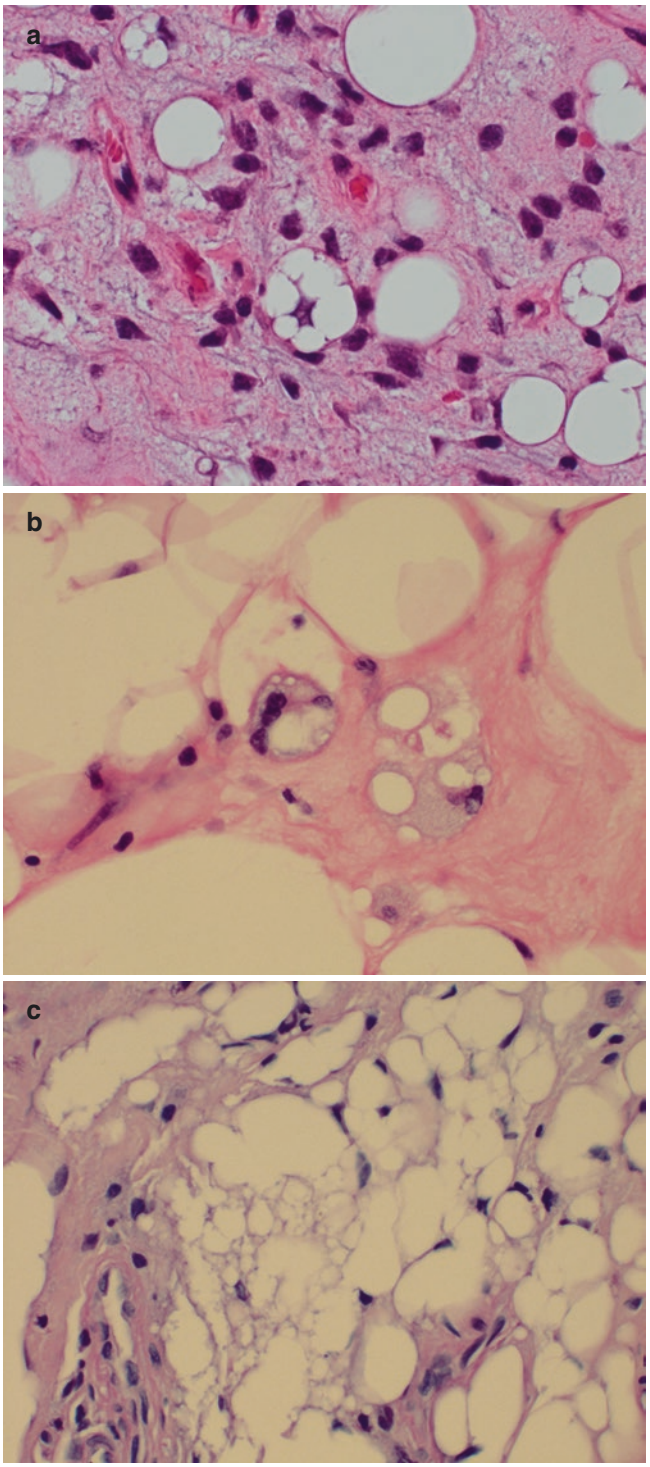

Fig. 9.12 (**a**) Lipoblasts with hyperchromatic indented nuclei (400×). (**b**) Macrophages in fat necrosis with paler and not indented nuclei (400×). (**c**) Macrophages in silicone granuloma with multivacuoles and centrally located small nuclei (400)

multiple and small, without nuclear indentation. More importantly, the other changes will serve as an appropriate background to indicate a fat necrosis process. These changes include multinucleated giant cells engulfing lipid, chronic inflammatory cells such as plasma cells, hemosiderin deposition, and even central cavity formation due to liquefaction. In the later stage, fibrosis will gradually replace the fatty tissue, with dystrophic calcification and sometimes squamous metaplasia. The whole process of fat necrosis could last for several years (Fig. 9.12b).

13. How does one differentiate silicone granuloma from liposarcoma?

Understandably, this differential has its significance in breast pathology. Silicone granuloma, as a foreign body inflammatory reaction, is associated with breast implants for postmastectomy reconstruction or for breast augmentation. Microscopically, the vacuolated macrophages could resemble lipoblasts. Liposarcoma could present as a primary breast sarcoma or as a component of a malignant neoplasm, such as malignant phyllodes tumor and metaplastic carcinoma. Different types of breast primary liposarcomas have been reported in the literature.

In silicone granuloma, the markedly distended macrophages become multivacuolated with different sizes when the silicone is lost during the tissue process. The vacuoles will distort the nuclei, resembling lipoblasts. However, the lipoblast-like macrophages usually contain small, vague to poorly delimited "bubbles" with small bland centrally located nuclei, while the real lipoblasts are with sharply outlined, often large cytoplasmic vacuoles and indented hyperchromatic nuclei. The vacuolated macrophages in silicone granuloma will be immunohistochemically positive for CD68 and lysozyme and negative for S-100, in contrast to lipoblasts. In general, lipoblasts in a well-differentiated liposarcoma are usually few, difficult to find, and never as numerous as the lipoblast-like macrophages in silicone granuloma. More importantly, the background of silicone granuloma is an inflammatory process, with fibrosis, chronic inflammation, and foreign body giant cells, which will not be an appropriate background for true "lipoblasts." Of course, clinical history of breast implant would be the key (Fig. 9.12c).

14. What lesions in the breast will have chondroid matrix formation?

It is well known that breast carcinoma could have chondroid matrix formation, a type of the metaplastic carcinoma. It usually has a poorer prognosis compared with other types of metaplastic carcinomas [15]. Chondrosarcoma could also be one of the heterologous components of a metaplastic carcinoma [16], so as in malignant phyllodes tumor. Mammary hamartoma, fibroadenoma, and myofibroblastoma are the benign breast tumors which could have chondroid matrix formation. Breast de novo chondroma and chondrosarcoma have been reported [17–19], even though not common. Other

lesions, such as intraductal papilloma [20], sclerosing adenosis, and even microglandular adenosis, have been reported to have chondroid or chondromyxoid changes.

15. How does one differentiate chondroid matrix from myxoid changes?

Myxoid changes are commonly recognized in both benign and malignant neoplasms as well as non-neoplastic reactive lesions. They share the bluish appearance with the chondroid matrix on the H&E-stained sections, sometimes overlapping with each other (so-called chondromyxoid changes). In fact, both myxoid extracellular matrix and chondroid matrix mainly consist of proteoglycans and collages, but with differences in the types of collages and relative proportions of the constituent macromolecules. The interplay of various macromolecules determines their texture and consistency. Under the light microscope, the chondroid matrix is more finely textured and assembled with lacunae and round to oval nucleated chondrocytes, while the myxoid changes are thinner, faint blue to clear, less organized, and resembling edematous changes (Fig. 9.13a, b).

16. How does one differentiate chondrosarcoma from chondroid matrix-forming tumors in the breast?

Breast primary sarcoma is very rare, with a prevalence of 0.5% of all breast tumors. Primary chondrosarcoma is even rarer. Approximately less than 20 cases have been reported in the literature. The differential of chondrosarcoma from malignant phyllodes (MP) tumor or metaplastic carcinoma (MC) with chondrosarcomatous component could be difficult. Immunohistochemical staining is not helpful in differentiating the chondroid area of MP tumor and MC from primary chondrosarcoma. Extensive sampling to identify the leaf-like structure and benign ductal epithelium in phyllodes tumor or the carcinomatous component (with the help of cytokeratin stain) in the metaplastic carcinoma will be the more definitive way to distinguish these tumors. This distinction may have clinical relevance, as chondrosarcoma could behave differently from metaplastic carcinoma. Other benign conditions with chondroid component usually should not cause confusions with chondrosarcoma.

Breast primary chondrosarcoma, same as breast primary osteosarcoma, is a diagnosis of exclusion, when other possibilities have been excluded. These other possibilities, other than the abovementioned MC and MP tumor, include metastasis to the breast from other locations and primary tumor of the adjacent bone (rib or sternum) involving the breast.

17. What breast lesions will have osteoid matrix formation?

Osteoid matrix formation or osseous metaplasia could be identified in a variety of lesions in the breast parenchyma, including reactive/non-neoplastic lesions and benign or malignant tumors. It can also be present idiopathically in the breast without any associated lesions [21, 22]. The non-neoplastic lesions reported with osseous metaplasia include cholesterol granuloma, lipogranuloma, fasciitis ossificans, implant capsules (saline or silicone implants), amyloid tumor [23], and post-radiation changes. Fibroadenoma, pleomorphic adenoma, and papilloma are the common benign neoplasms which could have osseous metaplasia as a degenerative change. Primary osteosarcoma of the breast is extremely rare, but has been reported, representing 12.5% of mammary sarcomas. Metaplastic carcinoma and malignant phyllodes tumor are the more common malignancies that could harbor osteosarcomatous component.

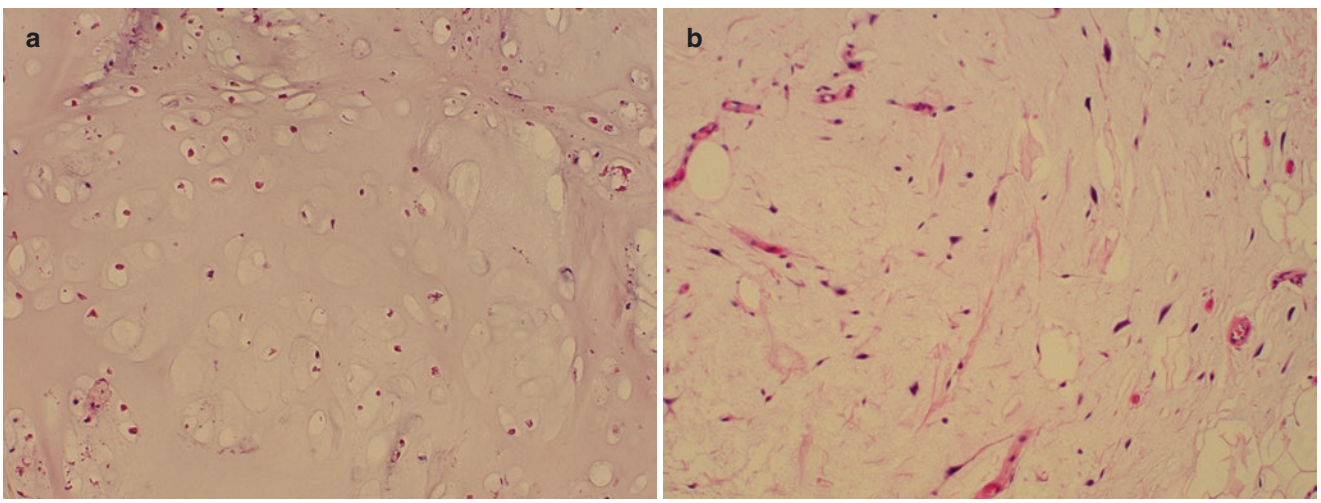

Fig. 9.13 (**a**) Chondroid matrix (100×). (**b**) Myxoid changes (100×)

18. **What are the subtypes of osteosarcoma reported in the breast?**

Breast primary osteosarcoma is part of the extraskeletal osteosarcoma. It typically arises in the late adulthood. Approximately 150 cases of breast primary osteosarcoma have been reported in the literature. It has the similar morphology as the conventional osteosarcomas of the bone or other extraskeletal osteosarcomas. The subtypes of osteosarcoma that have been reported in the breast include osteoblastic [24, 25], fibroblastic, and chondroblastic osteosarcomas [26], with the osteoblastic type as the major subtype reported. Telangiectatic osteosarcoma has been reported in a case associated with recurrent phyllodes tumor [27]. Small cell and well-differentiated subtypes have not been reported in the breast.

As mentioned in the diagnosis of breast primary chondrosarcoma, breast primary osteosarcoma is also a diagnosis of exclusion. Interestingly, breast primary osteosarcomas have been reported to be associated with pre-existing ossified fibroadenoma [28]. As with other sarcomas, metastasis is usually hematogenous. However, axillary lymph node metastasis was reported [29].

19. **How does one differentiate osteosarcoma from osteoid matrix-forming tumors in the breast?**

Osteosarcoma by definition is the sarcomatous tumor cells producing osteoid matrix. The pleomorphic tumor cells are embedded in the lace-like or sheet osteoid matrix. It will usually not be a problem to differentiate it from other reactive/repair processes or benign tumors with osteometaplasia, such as fibroadenoma. In these benign lesions, the typical original lesional tissue usually can be discerned. However, fasciitis ossificans may sometimes cause confusion. It is a bone-forming subtype of nodular fasciitis. It shares the same features as the myositis ossificans, but is more superficially located (fascia or tendon). It consists of fibroblast and myofibroblast proliferation with osteoid and/or trabecular bone formation. The zonal distribution pattern may not be obvious as in myositis ossificans. In the cellular area, it could resemble osteosarcoma. However, the spindle cells are usually not pleomorphic, with no atypical mitosis. More importantly, the woven bone trabeculae are lined with osteoblasts, indicating a reactive process (Fig. 9.14a, b).

20. **What are the differential diagnoses of granular cell tumor in the breast?**

Approximately 8.5% of granular cell tumors arise in the breast. Breast granular cell tumor shares the same morphology with the granular cell tumor in other locations. The tumor cells are polygonal to slightly spindle shape, with round to oval nuclei, distinct nucleoli, and abundant eosinophilic granular cytoplasm. The cells are arranged in cords, nests, or sheets, in an infiltrative pattern, in between the fibrous stroma (sometimes could be collagenous) and breast lobules, with the tendency to grow into the terminal ductal and lobular units. The cells in several breast lesions could resemble the granular cell tumor. Breast primary carcinomas, such as invasive apocrine carcinoma and histiocytic-type lobular carcinoma, could have abundant granular-like cytoplasm and are with an infiltrative growth pattern. However, these carcinomas are usually associated with in situ carcinomas, with pleomorphism, and will be cytokeratin positive. When the granular cell tumor presents as a breast skin lesion involving dermis/subcutaneous soft tissue, melanoma could enter as a differential diagnosis. Again, an in situ

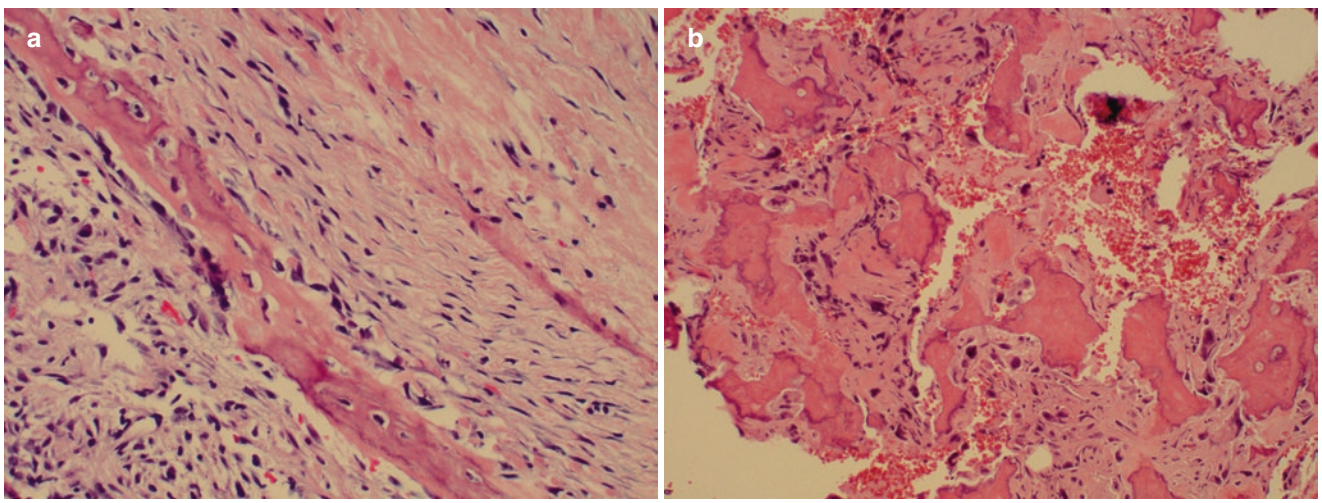

Fig. 9.14 (**a**) Osteoblasts rimming the ossification in ossifying fasciitis (100×). (**b**) Osteoblastic osteosarcoma with pleomorphic tumor cells forming bone (100×)

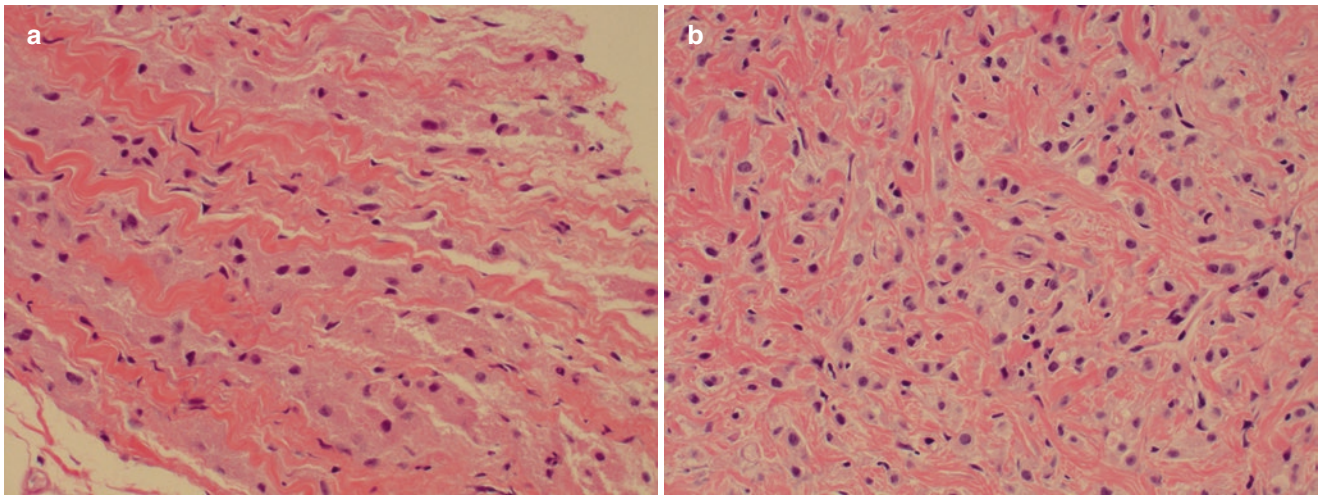

Fig. 9.15 (**a**) Granular cell tumor infiltrating the dense stroma (200×). (**b**) Invasive lobular carcinoma with histiocytic features, resembling granular cell tumor (200×)

process or junctional activity could usually be identified in melanoma. Other than S-100 positivity, melanoma cells will also be melan A and HMB45 positive. Histiocytes in a reactive inflammatory process could superficially resemble granular cells. However, the histiocytes will not be organized as the granular cells, will have other inflammatory or reactive changes, and will be alpha1-antitrypsin and alpha1-antichymotrypsin positive, even though the histiocytes and granular cells could be both S-100 and CD68 positive. Metastatic disease, such as renal cell carcinoma, due to its clearing and sometimes eosinophilic cytoplasm, should be considered, especially for the patient with appropriate history. RCC is usually rich in vasculature, with the tumor cells arranged in a sinusoidal pattern along the vessels and immunohistochemical stain positive for cytokeratin and PAX8 (Fig. 9.15a, b).

Granular cell tumor of the breast is usually benign and usually will not recur even with positive margin [30]. The criteria for malignant granular cell tumor have been a debate. Cellular pleomorphism, mitosis, and tumor necrosis are the well-accepted features for malignancy [30].

21. What are the lesions that could be induced by radiation therapy in the breast?

Breast parenchyma atrophic changes, fibrosis, dystrophic calcification, and ossification are the common benign post-radiation changes. Radiotherapy-induced neoplasms are usually sarcomas. Post-radiation lymphoma is also reported. The latency of radiotherapy-induced sarcoma is usually more than 10 years [31]. However, post-radiation cutaneous angiosarcoma could have a shorter latency period even less than 4 years. To be classified as radiotherapy-induced sarcoma, the proposed criteria are as follows: The initial tumor has a different histology. The secondary sarcoma is in the irradiated field. There is a prolonged latency period between the two malignancies. A wide range of different types of radiation-induced sarcoma have been reported, including angiosarcoma, leiomyosarcoma, liposarcoma, fibrosarcoma, chondrosarcoma, and osteosarcoma [32–34]. These sarcomas share the same morphological pattern as their sporadic counterparts. More often, radiation-induced sarcoma lacks distinct differentiation and is classified as pleomorphic sarcoma (previously MFH).

22. Is breast implant associated with increased risk of mesenchymal neoplasm?

The long-term safety of silicone implant has been a particular concern, since it was first introduced in 1962. Experimental studies did show its carcinogenicity in rodents. After a certain latent period, solid silicone compound could elicit mesenchymal sarcoma at the implantation site in susceptible rodents through the so-called solid-state carcinogenesis, with an incidence of approximately 29–40%. However, well-designed epidemiologic studies have not proven an association between silicone implants and malignant solid tumors in human breasts. Nevertheless, it is well documented that silicone implants are associated with anaplastic large cell lymphoma. Implant-associated breast fibromatosis has been reported in more than 30 cases, with the mean interval time between implant placement and tumor occurrence of 3 years [35]. There have been three case reports on silicone implant-associated breast stromal sarcomas. The first case was a 55-year-old female who developed “malignant fibrous histiocytoma” after receiving silicone injection augmentation mammoplasty 19 years ago [36]. The second case was a 69-year-old woman who was diagnosed with a high-grade

angiosarcoma after 35 years of silicone implantation [37]. The third case was a 35-year-old-woman with Li-Fraumeni syndrome who had bilateral mastectomy and implantation for the treatment of her right breast triple-positive invasive ductal carcinoma. She developed bilateral sarcomas (not otherwise specified) a year and half after the insertion of the implants [38].

The speculation on the difference in propensity toward sarcomas with silicone implantation between humans and rodents could be because of their contrasting genetic stabilities [39]. Much evidence exists that rodents' cells are less efficient in DNA repair and maintenance of DNA methylation. Therefore, it is understandable that the risk of malignant transformation could be much increased in the patients with genetic instability syndromes, such as Li-Fraumeni syndrome.

23. What are the vascular lesions in the breast?

As in other anatomy sites, breast vascular lesions have benign vs. malignant versions. Benign vascular lesions include different types of hemangiomas, namely, capillary, cavernous, and venous hemangiomas, and angiomatosis. Perilobular hemangioma is a type of capillary hemangioma unique to the breast stroma. Even though named as perilobular, it is not limited to this location and could be intralobular as well. Perilobular hemangioma is usually an incidental finding, identified microscopically in the specimens excised for other reasons. Other types of benign hemangiomas are usually bigger in sizes and could be identified by palpation or mammogram.

Primary angiosarcoma in the breast is uncommon. It is usually in the breast parenchyma of younger patients, whereas radiation-induced secondary angiosarcoma is more frequently diagnosed in this location. It is commonly associated with the skin and infiltrating into the breast parenchyma. Atypical vascular lesion is another type of vascular proliferation associated with radiation exposure. It is considered by many as a precursor lesion of secondary angiosarcoma, even though it may not be supported by others at this stage.

24. How does one differentiate hemangioma from angiosarcoma in the breast?

Hemangioma in general is demarcated vascular proliferation, with a lobular architecture. Anastomosis could be present, but not prominent. It is usually lined by a single layer of endothelial cells with occasional cell tufts, but without cytological atypia or mitosis. The Ki67 labeling index should be very low. In contrast, angiosarcoma has an infiltrative growth pattern and is usually not lobulated. It could consist of well-formed vascular channels with prominent anastomosis (morphologically well differentiated) or be with solid growth pattern (morphologically poorly differentiated). The lining endothelial cells are multi-layered, with cell tufts and pleomorphism, especially hyperchromasia. Mitosis is present, and Ki67 labeling index is high (Figs. 9.16a, b and 9.17a, b).

The most problematic differential diagnosis is between the atypical vascular lesion and the angiosarcoma with well-differentiated morphology. They are both post-radiation vascular lesions with certain degrees of atypia. However, the vascular channel for atypical vascular lesion is usually simpler, has no apparent anastomosis, is typically more superficial, and is not dissecting through dermis to subcutaneous soft tissue, with only mild cellular pleomorphism and no mitosis; and Ki67 labeling index is low. In recent years, immunohistochemical stain for MYC has been applied to this distinction, and the result seems to be convincing. The atypical vascular

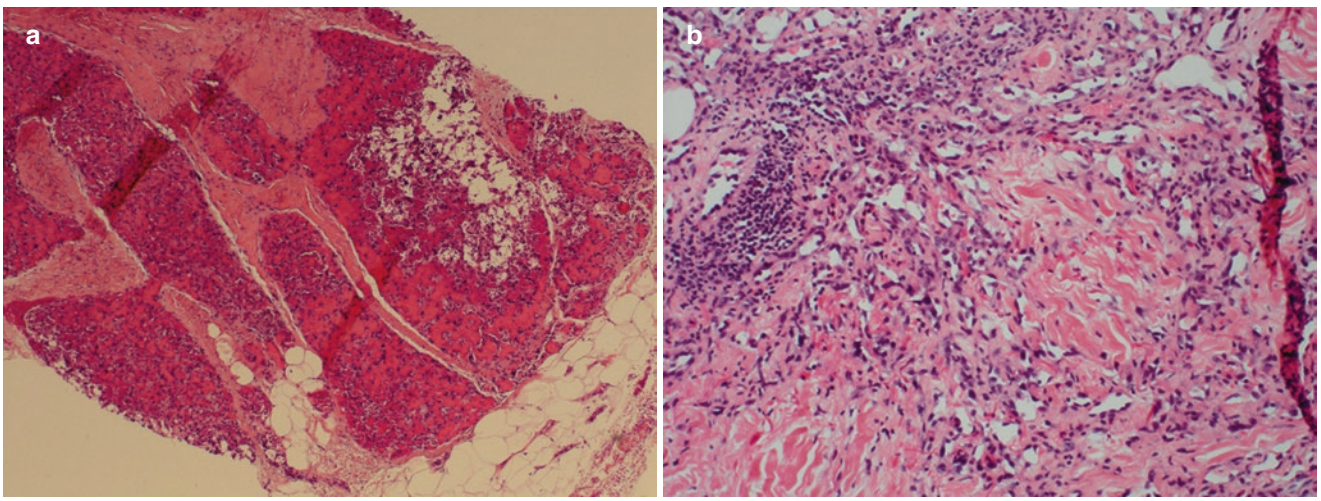

Fig. 9.16 (**a**) Well-demarcated hemangioma with lobulation formed by fibrous septa (40×). (**b**) Angiosarcoma with an infiltrative pattern (100×)

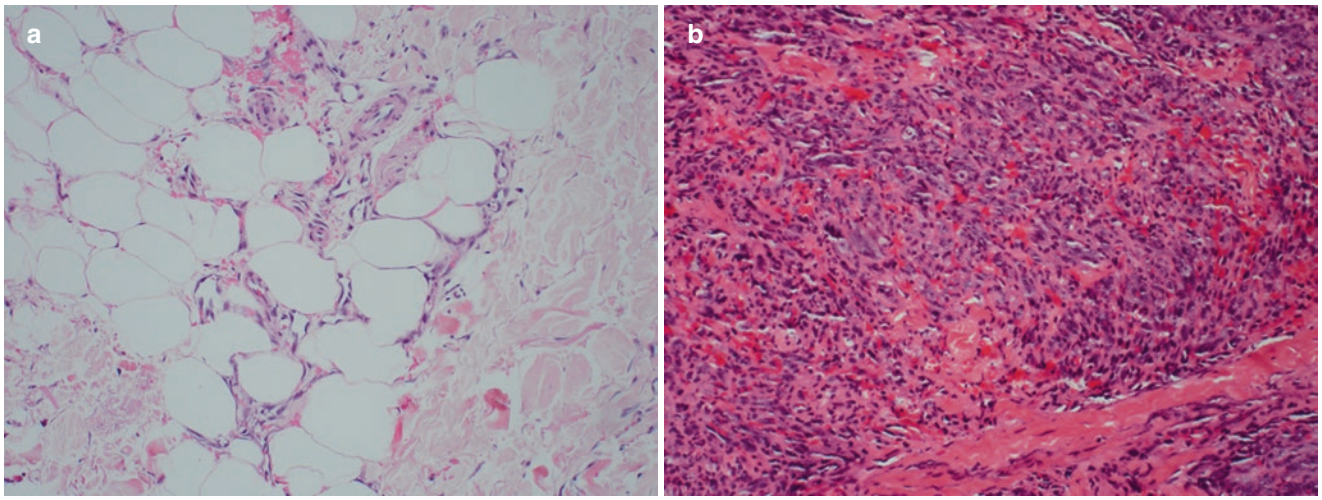

Fig. 9.17 (**a**) The so-called well-differentiated angiosarcoma with well-formed lumens (100×). (**b**) The so-called poorly differentiated angiosarcoma with solid growth pattern (100×)

lesion is usually MYC negative, while radiation-induced angiosarcoma is positive (Fig. 9.18a–c) [40–42].

Another differential diagnosis which could potentially be a problem is the angiomatosis vs. breast primary angiosarcoma. Other than the general features mentioned above for benign and malignant vascular lesions, the vasculature in angiomatosis is, even though infiltrative, regularly and evenly distributed throughout. It could replace the glandular units of the breast parenchyma, but will not dissect or destroy them, whereas angiosarcoma is irregularly infiltrating the breast parenchyma and could dissect though the terminal ductal-lobular units.

25. What are the other mimics of angiosarcoma in the breast?

Pseudoangiomatous stromal hyperplasia (PASH) is a myofibroblast proliferation of the breast stroma. It forms slit-like spaces in a hyalinized stroma, with spindle cells lining the spaces, sometimes mimicking a structural variant of angiosarcoma. Architecturally, even though PASH appears infiltrative and could involve intralobular stroma, it does not disturb the epithelium in the lobules. The space is empty, and the anastomoses are not as complex as angiosarcoma. The lining spindle cells are usually discontinuous, flat, and bland. In the so-called atypical PASH, the cells could have some atypia and even rare mitosis, but immunohistochemical stain will show that they are negative for endothelial markers (Fig. 9.19).

Cellular angiolipoma could be another mimic of the angiosarcoma variant, when the angiosarcoma is composed predominantly of spindle cells with a diffuse infiltrating pattern, especially on a core biopsy. Cellular angiolipoma is an encapsulated lesion with clear circumference. No breast terminal ductal-lobular units should be identified in the tumor mass. The capillaries are more prominent at periphery. The endothelial cells are bland and with no mitosis. Fibrin thrombi are usually identified in the capillary lumen (Fig. 9.20a, b).

26. What is the definition of primary breast lymphoma (PBL)?

The definition of primary breast lymphoma (PBL) is somewhat controversial, as lymphomas by definition are systemic diseases. However, owing to several distinct features and the predilection of certain lymphoma to present in the breast, much study has been devoted to the spectrum of lymphoma that can present primarily as breast disease [43–54].

- The working definition(s) of PBL was proposed by Wiseman and Liao in 1972 and updated by Hugh and colleagues in 1990.
- It includes a variety of lymphoma histologies that occur within the breast parenchyma or in close proximity to breast tissue.
- Requires no antecedent diagnosis of lymphoma.
- Limited to cases with no extramammary disease other than ipsilateral axillary lymph node involvement (although some studies allow a slightly broader definition to include regional lymph nodes, i.e., internal mammary and supraclavicular).
- It comprises 0.5% of breast malignancies, ~1% of all non-Hodgkin lymphomas (NHLs), and <3% of extranodal lymphomas.

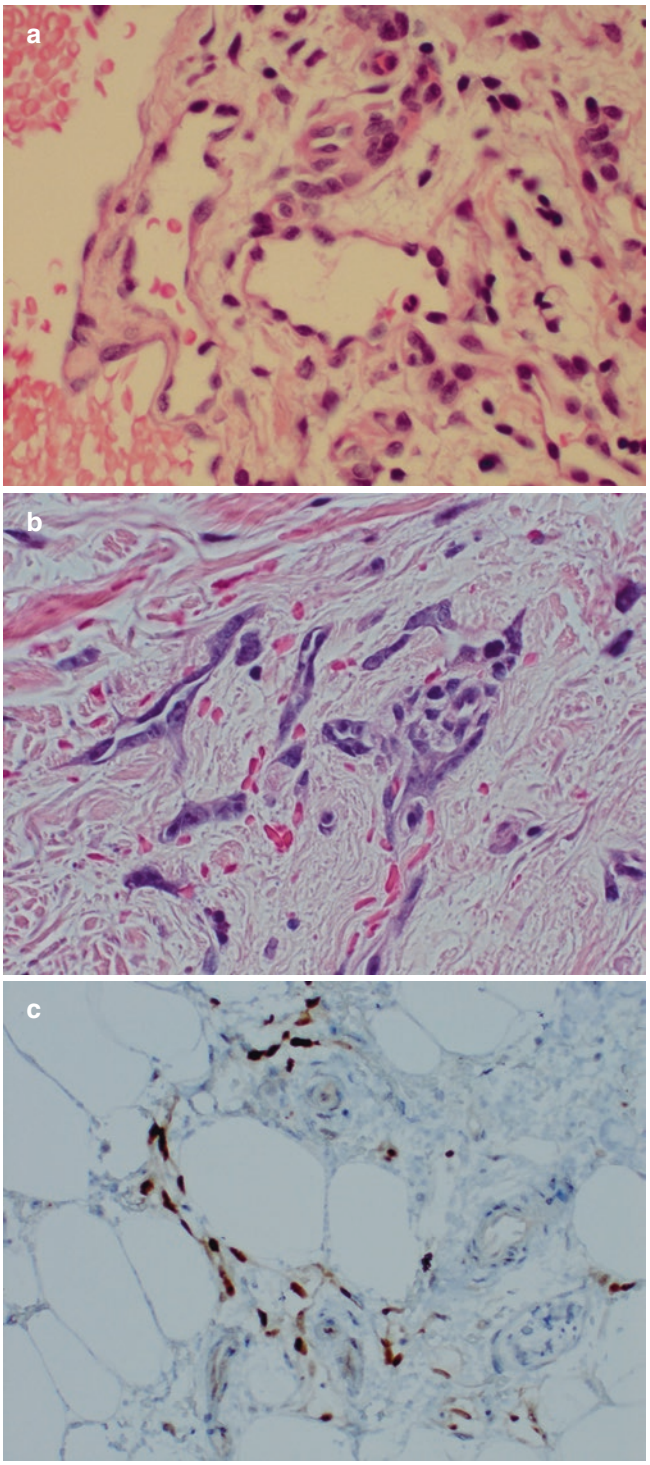

Fig. 9.18 (**a**) Post-radiation atypical vascular proliferation (400×). (**b**) Post-radiation angiosarcoma with hyperchromatic cells lining the vascular channels (400×). (**c**) Immunohistochemical stain for MYC in post-radiation angiosarcoma (400×)

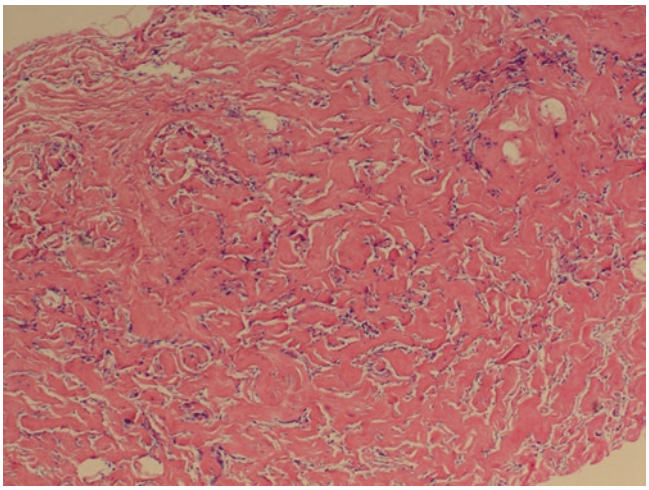

Fig. 9.19 Pseudoangiomatous stromal hyperplasia with bland spindle cells lining the spaces (100×)

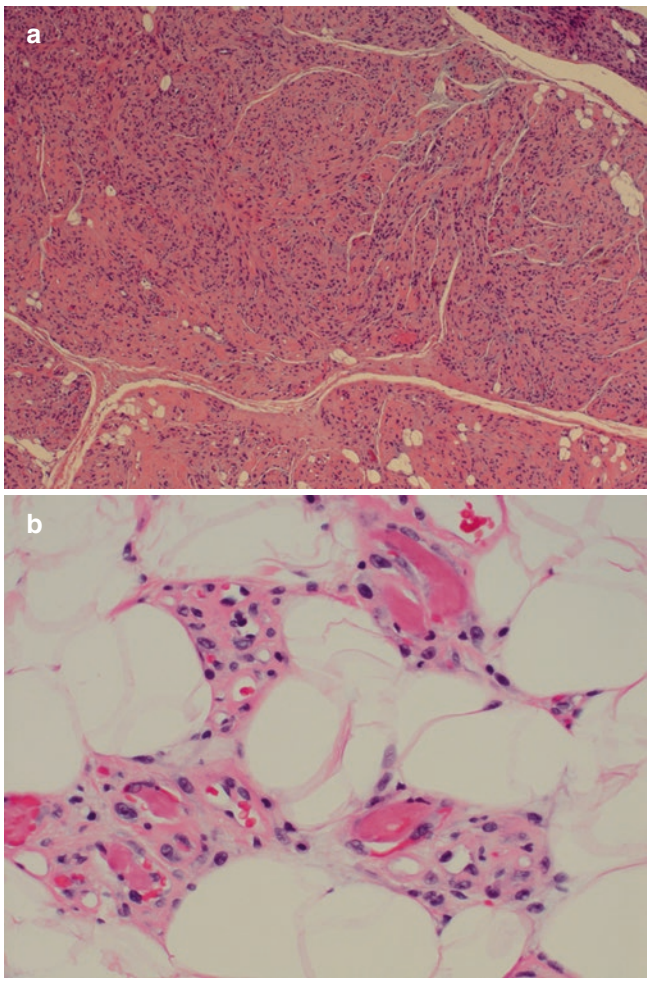

Fig. 9.20 (**a**) Cellular angiolipoma with bland endothelial cells (100×). (**b**) Fibrin thrombi in the capillary lumen in angiolipoma (400×)

- Secondary involvement of the breast by systemic lymphoma appears to be more common than primary disease. Lymphoma is thought to be the most common malignancy to involve the breast secondarily.

27. How does lymphoma clinically present in the breast?

Presentation of PBL can be similar to other more common primary breast tumors, including carcinoma. A constellation of signs and symptoms may be associated. Importantly, constitutional (a.k.a. "B-type") symptoms (i.e., fever, night sweats, fatigue, and weight loss) are relatively uncommon in PBL, being reported in only 4% of patients and more often occurring in those with breast involvement by disseminated disease [54–60].

- Age distribution: The median age at presentation ranges from 60 to 65 years in Western countries (reportedly lower in East Asian countries, at 45–53 years), with a wide age distribution (teens thru 90s).
- Gender: Women are much more commonly affected than men. Cases among males have been anecdotally reported.
- Bilateral breast involvement occurs in 5–11% of cases and may occur more often during pregnancy or postpartum. Along with the strong female predilection, this pattern suggests that hormone stimulation may promote tumor growth.
- Symptoms: Most PBLs present as painless masses (median tumor diameter 4 cm, up to 20 cm reported). Right > left, for unknown reasons. Skin changes (nipple retraction, color change, or discharge) are rare and more likely to be found with T cell lymphoma.
- Radiologic imaging. (See question 28.)

28. What radiographic features are most typical of lymphoma in the breast?

Up to 20% of patients with PBLs are detected by screening mammography [61–63].

- Features that are more common in lymphoma than carcinoma include larger tumor size and lack of the following: spiculated appearance, calcification, or architectural tissue distortion.
- Typically solitary lesions, oval to round, circumscribed, or without distinct delineation.
- Ultrasound may be more informative than mammography for detecting infiltrating or small lymphoma tumors, which appear as hypoechoic solid masses.
- No radiographic features are definitively diagnostic, and biopsy is required.

Table 9.1 Benign lymphoid proliferations in the breast

Condition	Histologic finding
Lymphocytic mastitis/diabetic mastopathy (auto-immune associated)	B cell-predominant perivasculitis, lobulitis, and ductitis (including lymphoepithelial lesions), surrounded by dense keloid-like stromal fibrosis
Cutaneous lymphoid hyperplasia	Cutaneous proliferation of B cells organized as coalescing follicles with non-polarized germinal centers lacking mantle zones and infiltrates of lymphoid cells spreading into collagen, smooth muscle, vessel walls, and nerve sheaths
Intramammary lymph node with reactive follicular hyperplasia	Intact nodal architecture with patent sinuses and B cell-rich polarized germinal centers, containing tangible body macrophages and surrounding polarized mantle zones

29. What types of benign proliferations or inflammatory lesions can mimic lymphoma in the breast?

A few specific benign lymphoid proliferations or inflammatory breast lesions can mimic lymphoma in the breast, as well as generalized reactive lymph node hyperplasia of intramammary lymph nodes. The primary histologic differential is with low-grade B cell NHL, as these malignant tumor cells most closely resemble benign lymphocytes [64–69]. See Table 9.1.

30. What are the most common types of primary lymphoma in the breast?

The most common form of PBL is diffuse large B cell lymphoma (DLBCL), followed by marginal zone lymphoma (MZL), follicular lymphoma (FL), and Burkitt lymphoma (BL). DLBCL presenting as PBL is not a distinct pathology entity according to current WHO classification schemes (unlike other anatomic DLBCL variants such as primary cutaneous, CNS, intravascular, etc.) [48, 49, 54, 61, 70–74].

Table 9.2 outlines the relative proportion and salient histologic and immunophenotypic features of the most common type of PBL. For more details, see additional sections on each distinct entity (questions 34–38).

31. What types of lymphoma occur more rarely, but are also known to occur as primary within the breast?

The following entities each comprise ≤1% of lymphomas presenting within the breast: anaplastic large cell lymphoma (ALCL), peripheral T cell lymphoma (PTCL), small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), lymphoplasmacytic lymphoma (LPL), mantle cell lymphoma (MCL), and classic Hodgkin lymphoma (CHL). [48, 49, 54, 71, 73, 75] See Table 9.3.

Table 9.2 Features of common histologic subtypes of primary breast lymphoma

	DLBCL	MZL	FL	BL
PBL (%)	56–84%	9–28%	10–19%	<6%
Cell size/ morphology	Large/pleomorphic, centroblastic, or immunoblastic features	Small, mature monocytoid or plasmacytoid	Predominantly small with mature cleaved nuclei (low grade), centroblastic (high grade)	Intermediate in size, high nuclear grade
CD20	+	+	+	+
CD10	Variable	–	+	+
BCL6	Variable	–	+	+
BCL2	+/–	+	+	–

Table 9.3 Immunohistochemical features of rare breast lymphoma subtypes

	ALCL	PTCL	SLL/CLL	LPL	MCL	CHL
Cell size/morphology						
CD3	–/+	+	–	–	–	–
CD20	–	–	+	+	+	–
CD30	++	+/–	–	–	–	+
CD15	–	–	–	–	–	+
CD5	–	+/–	+	–	+	–
CD23	–	–	+	–	–	–
Additional	ALK +/–			CD138+	Cyclin-D1+	

32. Which types of lymphoma are associated with breast implants?

No definitive increase in risk of lymphoma among women with breast implants has been found among several large epidemiologic studies. Regardless, a distinct form of breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) does occur [76–88], and is now considered a provisional entity by WHO diagnostic criteria. BIA-ALCL is an extremely rare malignancy, with an estimated incidence of 1 per 0.5–3 million women with implants [77, 82]. While the etiology and pathogenesis is unknown, an etiologic association between implants and BIA-ALCL has at least been suggested.

Most patients present with involvement of a breast implant-associated seroma and infrequently with a breast mass. Pathologic evidence of lymphoma may be limited to the seroma fluid, or histologic evidence of infiltration within the pericapsular fibrotic tissue may also be seen if capsulectomy is performed. Regional lymph nodes may also be involved.

Morphologically, BIA-ALCL cells are typical of other anaplastic lymphomas, with large pleomorphic features and abundant cytoplasm, and usually include prototypical "hallmark cells" (with horseshoe-shaped, reniform, or ring-like nuclear contours). Tumor cells express strong CD30, are negative for ALK, and exhibit variable T cell antigen expression [76, 84, 86].

A wide time interval has been reported between implant placement and lymphoma diagnosis, averaging about 10 years. Clinical outcome has also generally been good, with median survival of 12 years. Treatments have often employed excision alone, and chemotherapy does not appear to affect outcome in cases without tumoral involvement. Presentation as a solid tumor mass appears to be the worst prognostic factor.

Even more rarely, occasional and anecdotal case reports of other NHLs have been reported in association with breast implants, including T cell lymphoma, primary effusion lymphoma, follicular lymphoma, marginal zone lymphoma, and lymphoplasmacytic lymphoma.

33. How does one distinguish low-grade from various high-grade B cell lymphomas (HGBLs), and what is the clinical significance?

Low-grade B cell NHLs are generally indolent diseases. They do not constitute medical emergencies and are routinely treated in the outpatient setting.

- The major categories include FL, MZL, SLL/CLL, and LPL, with the first two being the only ones encountered frequently as primary diagnoses within the breast [90]. (See question 30.)
- Management of low-grade B cell NHL is highly variable and may include expectant "watch and wait" or active surveillance approach in low-stage non-symptomatic patients or alternatively combined modalities including single-agent or combined chemotherapy, antibody treatment, or radiation. While active surveillance is still indicated in certain early-stage diseases among some asymptomatic

patients, more sophisticated clinical and genetic risk stratification schemes (and the increasing availability of targeted small molecular inhibitors) are changing treatment paradigms.

High-grade B cell NHLs present with variable disease severity, from incidental detection to severely ill patients in near-critical condition. Although PBL is less likely to present as severe illness (as by definition unilateral disease is early Ann Arbor stage I_E [breast alone] or II_E [breast plus ipsilateral lymph nodes]), some presentations of high-grade lymphoma constitute medical emergencies and/or require immediate medical attention with inpatient management. Therefore, such diagnoses should be communicated immediately to providing clinicians according to guidelines for reporting urgent medical information or critical values [89].

- BL and particularly aggressive subtypes of DLBCL now identified as "high-grade B cell lymphoma" (HGBL) may constitute medical emergencies when patients have concomitant metabolic derangement (e.g., tumor lysis syndrome with incipient acute renal failure, attributable to the rapid turnover and apoptosis of highly proliferative tumor cells). (See question 34.)
- Standard chemotherapy for BL (CODOX-M/IVAC) typically necessitates inpatient admission. By comparison, standard therapy for DLBCL (R-CHOP) is routinely administered outpatient, while more intensive regimens that have been recently studied (e.g., R-EPOCH) may also necessitate inpatient admission for a subset of patients.
- Although not counted among the typical forms of PBL, T cell or B cell acute lymphoblastic leukemia/lymphoma (T- or B-ALL) is a medical urgency/emergency that necessitates inpatient admission for standard treatment (Hyper-CVAD). See Table 9.4.

34. **How does one distinguish and subclassify diffuse large B cell lymphoma** [91–95]**?**

- Morphology: DLBCL-NOS (not otherwise specified) are morphologically diverse tumors. Distinguishing features include diffuse sheet-like infiltration of tissue by large-sized cells (2–3 times the size of normal lymphocytes). Cytoplasm is moderate to abundant, with relatively low nuclear-to-cytoplasmic ratio for lymphoma, and variably amphophilic to basophilic, with sometimes even clear appearance. Nuclei have irregular contours, with course-to-vesicular chromatin, and are prominent. The so-called centroblastic (multiple nucleoli) vs. immunoblastic (single central nucleoli) variants are commonly described. Anaplastic variants, and even those with myxoid or fibrillary stroma, have rarely been reported.

Table 9.4 Histologic features distinguishing between low-grade and high-grade B cell non-Hodgkin lymphoma

	Low grade	High grade
Cell size	Small with scant cytoplasm	Medium to large, with variable cytoplasm
Nuclear contours	Round to slightly irregular, may have cleaved or angulated contours	Round to highly pleomorphic, with prominent nuclear membrane
Chromatin	Condensed, hyperchromatic, either lacking nucleoli or with small indistinct forms	Vesicular, open, or finely dispersed chromatin, with prominent nucleoli (single or multiple)
Architecture and associated findings	Variable architecture, from nodular to sheet-like, depending on subtype	Diffuse replacement, with abundant mitoses, apoptosis, and sometime "starry sky" appearance (i.e., tangible body macrophages)
Ki67 index	Low, typically 10–20%, should not exceed 50–60%	High, 90–100% in BL and ALL, 80–100% in HGBL, >50% in DLBCL-NOS

- Subclassification: When diagnosed in the breast, DLBCL-NOS should be subclassified similar to other nodal or extranodal presentations. The first subcategories of DLBCL-NOS that have penetrated clinical practice are based on gene expression profiling studies of primary lymphomas. The so-called "cell-of-origin" (COO) classification was developed by using gene expression profile (GEP) patterns that are typical of benign B cells and applying them to malignant lymphoma. Among the patterns that emerged were two groups which most closely resembled germinal center B cells (GCBs) and post-germinal center B cells that are transitioning toward memory or plasma cells. DLBCLs with GEPs that more closely match either of these two patterns have come to be known as germinal center B cell (GCB) or activated B cell (ABC) type, respectively. Then, a simplified algorithm of immunohistochemical stains was proposed by Hans and colleagues in 2004 [95] to more readily classify GCB vs. ABC (a.k.a. *non*-germinal center type) DLBCL using routine formalin-fixed paraffin-embedded tissue. This COO classification scheme (summarized below), which corresponds to GEP patterns, is now routinely reported for de novo DLBCL, as recommended by the updated WHO classification system in 2016.
- CD10 and BCL6 are both germinal center B cell markers, but BCL6 positivity *alone* is insufficient to qualify as GCB-type DLBCL, so MUM1 is needed to discriminate. See Table 9.5.

Apart from the COO classification, routine evaluation of additional cytogenetic and morphologic features is now indi-

Table 9.5 Cell-of-origin classification for DLBCL by Han's algorithm

CD10	BCL6	MUM1	COO subtype
+	n/a	n/a	GCB
–	+	–	GCB
–	+	+	ABC
–	–	n/a	ABC

n/a not applicable
Data from Ref. [95]

cated to determine other risk factors, particularly those chromosomal rearrangements which identify an aggressive subtype of B cell NLH, commonly referred to as double- or triple-hit lymphoma. These tumors are defined by the WHO as high-grade B cell lymphoma (HGBL) with *MYC*, *BCL2*, and/or *BCL6* rearrangements or HGBL-NOS when the morphology is suggestive (i.e., high nuclear grade, appropriate cytomorphology and architectural features, and nearly 100% Ki67 proliferation index), in the absence of these genetic findings.

- **FISH for the following markers is indicated:**
  - ***MYC* (8q24) break apart:** Alternatively *MYC-IGH* fusion, t(8;14), may be considered, but is less sensitive owing to alternative *MYC* gene translocation/rearrangements (e.g., kappa or lambda light chain on chromosome 2 or 22, respectively).
  - **IGH-BCL2 fusion, t(14;18):** Alternatively BCL2 (18q21) break apart may be used, but is less clinically widely available and considered less susceptible to false negative due to lack of alternative common translocation partners.
  - **BCL6 (3q27) break apart**
- **IHC evaluation for MYC and BCL2 is also recommended**: Some studies have suggested an independently poor prognosis for DLBCL co-expressing MYC and BCL2 protein (a.k.a. "double expressers").
  - MYC IHC (≥40% scored as positive)
  - BCL2 IHC (≥70% scored as positive)

35. How does one distinguish Burkitt lymphoma?

Precise recognition of BL is critical due to the aggressive nature of the disease, the often high clinical acuity of the patients, and the high curability rate when appropriately treated with distinct chemotherapy in advanced health-care settings.

- Morphology: BL is characterized by diffuse infiltration of monomorphic medium-sized cells with a high nuclear-to-cytoplasmic ratio, round nuclei, multiple nucleoli, and basophilic cytoplasm that is often vacuolated. Nuclear contours are round to oval without cleaves or folds, a key feature in the distinction from DLBCL. Nuclear chromatin is relatively fine or immature, and distinct nucleoli are typically multiple while small to intermediate in size. This tumor exhibits a characteristic "starry sky" appearance from low power, owing to a very high proliferation rate (≥95% as determined by Ki67 staining) and frequent interspersed apoptotic cells engulfed by tingible body macrophages. Frequent mitoses are also present.
- Immunophenotype: BL cells are mature B cells, positive for CD20, CD79a, and other markers routinely evaluated by flow cytometry (e.g., CD19, CD22, with monotypic surface immunoglobulin light-chain expression, the latter being an important feature which helps distinguish it from the majority of B-ALL). Additional markers are similar to germinal center B cells, including CD10 and BCL6; but importantly BCL2 is negative in the vast majority of cases, a feature which distinguishes BL from many DLBCLs or other high-grade B cell NHLs. TdT is also negative. Epstein-Barr virus (EBV), best evaluated by EBV-encoded RNA (EBER) in situ hybridization, is only seen in 20% of sporadic BL in Western countries and in 30–40% of HIV-associated cases, although the vast majority (98%) are positive in endemic cases in sub-Saharan Africa.
- Genetics: Virtually all cases of BL have a translocation between the long arm of chromosome 8, involving the protooncogene *MYC* (8q24), and one of the three immunoglobulin translocation partners. Translocation t(14;18), involving the immunoglobulin heavy-chain (*IGH*) region on chromosome 14, is the most common and can routinely be tested by FISH for *IGH-MYC* fusion. Much less commonly, the kappa light-chain locus on chromosome 2, or the lambda light-chain locus on chromosome 22, may alternatively be involved; and FISH using the so-called *MYC* "break apart" probes can be employed to confirm a *MYC* gene rearrangement. Importantly, however, *MYC* gene rearrangement alone should not be used as a surrogate for BL, as up to 10% of other aggressive high-grade B cell NHLs can harbor a conventional or non-conventional MYC aberration. (See question 39.)

36. What is the association between Burkitt lymphoma and bilateral breast masses?

Presentation of Burkitt lymphoma (BL) with bilateral breast involvement, often resulting in massive breast enlargement, appears to be particularly associated with pregnancy and/or lactation. (See also question 27.) Bilateral involvement is not exclusive to BL; but since the 1960s, numerous case reports have described similar findings in women of child-bearing age, usually occurring during pregnancy, postpartum, or in association with lactation, leading to speculation that BL is a particularly hormonally responsive tumor, at least under

these conditions. In the setting of high-intensity chemotherapy, outcomes appear similar as reported with conventional sporadic BL [96–104].

37. How does one distinguish extranodal marginal zone lymphoma?

Extranodal marginal zone lymphoma (MZL) (a.k.a. mucosa-associated lymphoid tissue (MALT) lymphoma) is a low-grade B cell NHL with distinct, but sometimes subtle, morphologic characteristics [90]. While the breast is a relatively uncommon site for organ of involvement by MZL/MALT lymphoma (only ~3% of total, after gastric, ocular, pulmonary, cutaneous, and salivary gland), it is the second most common subtype of all PBLs (after DLBCL). (See also question 34.) Although chronic inflammation, infection, and antigenic stimulation have been tightly linked to MZL/MALT lymphoma pathogenesis at other sites (e.g., *H. pylori* in the stomach, *Chlamydia psittaci* in the eye, *Borrelia burgdorferi* in the skin, Sjogren syndrome in the salivary gland, and Hashimoto disease in the thyroid), no such association has yet been identified in the breast.

- Morphology: MZL cells may resemble small lymphocytes, with plasmacytic differentiation in up to one-third of cases. The lymphocytes are small-to-medium-sized cells, with only slightly irregular nuclei, condensed chromatin, and indistinct nucleoli. Slightly more abundant pale cytoplasm may be seen, responsible for the so-called "monocytoid" appearance of these cells. Large centroblast- or immunoblast-type cells are frequently present, but should be scattered. Plasma cells may also be rare to scattered, but rarely predominant so as to mimic plasmacytoma. Amyloid deposition may also be seen. Tissue infiltration may be diffuse and effacing or infiltrative around existing structure and benign lymphoid follicles. Colonization of intact lymphoid follicles by neoplastic MZL B cells may produce a subtle and misleading nodular or follicular architecture in some cases. The so-called lymphoepithelial lesion, in which MZL cells infiltrate, distort, and/or destroy local epithelial structures, is a helpful diagnostic clue.
- Immunophenotype: MZL expresses mature B cell antigens CD19, CD20, and CD79a, with surface kappa or lambda light-chain restriction (reliable among lymphocytes usually only by flow cytometry) while typically being negative for CD5, CD10, and BCL6. When tissue biopsy alone is available (i.e., no flow), in situ hybridization for immunoglobulin light-chain restriction (i.e., kappa or lambda) among plasma cells may provide definitive evidence of B cell clonality (capable of substituting for flow or B cell gene rearrangements in some cases). Infrequent cases are CD5 positive and extremely rarely express CD10; thus, the presence of either of these should not be used alone to exclude or overrule a diagnosis of MZL.

38. How does one distinguish follicular lymphoma?

Follicular lymphoma (FL) is the most common indolent lymphoma and second most common B cell NHL outside of the breast [90, 105]. It has a highly variable cytologic appearance, which is reflected in different histologic grades and which partly explains the highly variable clinical outcome. FL has a natural rate of progression, with about 3% per year undergoing "transformation" to DLBCL.

- Morphology: FL is defined by its nodular and follicular architecture, in which neoplastic FL cells are intimately (but not exclusively) associated with intact follicular dendritic cell (FDC) networks, analogous to the architecture exhibited by benign germinal centers. By comparison, however, the FDC structures in FL are often enlarged, expanded, and organized into a back-to-back pattern. Critical features which distinguish these neoplastic follicles from benign follicles (or reactive follicular hyperplasia) are the lack of polarized mantle zones surrounding the follicle, the lack of polarized light and dark zones within the FDC network, and the absence of tingible body macrophages. The neoplastic B cells consist of two varieties: smaller centrocytes, with scant cytoplasm, irregular-to-cleaved nuclei, condensed chromatin, and indistinct nucleoli, and larger centroblasts with round-to-oval nuclei (i.e., not cleaved), vesicular chromatin, distinct nucleoli, and moderate amounts of cytoplasm. Distinguishing grade 1 thru grade 3 FL is based on the absolute number of centroblasts per high-power field (HPF) averaged across ten neoplastic follicles. Although clinically grade 1 FL and grade 2 FL are currently treated almost identically, the following criteria are still routinely applied on morphologic evaluation: Grade 1 is defined as 0-5 centroblasts/HPF; grade 2 is 6-15 centroblasts/HPF, and grade 3 is >15 centroblasts/HPF. Importantly, grade 3 is further subdivided based on the relative distribution of small centrocytes and large centroblasts, with grade 3A containing an admixture of each and grade 3B consisting entirely of large centroblast-like cells confined by FDC networks. Any distinct areas of diffuse large cell lymphoma should be separately diagnosed as concurrent DLBCL.
- Immunophenotype: FL is positive for mature B cell markers CD19 and CD20, with co-expression of germinal center markers CD10 and BCL6 and an aberrant expression of BCL2 (attributable to oncogenic prototypical immunoglobulin heavy-chain *(IGH)-BCL2* translocation). Negative markers include CD5 and typically CD43. Of note, in grade 3 FL, CD10 is occasionally lost. Staining

for FDC markers (e.g., CD21 and CD23) may aid in documenting follicular architecture in cases which are not overtly nodular.
- Genetics: For routine clinical testing, confirmation of the t(14;18)(q32;q21) translocation involving *IGH-BCL2* (most commonly by employing FISH) is recommended when differentiating among other low-grade B cell NHLs. *IGH-BCL2* translocation *cannot* be used to distinguish FL form DLBCL. Furthermore, up to 10% of FL may lack this translocation, so negative FISH studies or lack of BCL2 expression does not preclude the diagnosis if definitive morphologic features are present.

39. How does one distinguish lymphoma from triple-negative breast carcinoma?

Mistaking PBL for invasive breast carcinoma is a critical pitfall to avoid. Given the prevalence of DLBCL within the breast, this type of PBL may be most likely to mimic poorly differentiated, high nuclear grade, invasive ductal carcinoma (IDC). Most other varieties of B cell NHL have a more distinctive and normal lymphoid appearance and are less likely to be mistaken for breast carcinoma, with the notable exception of anaplastic large cell lymphoma (ALCL). (See question 40.) The pathologist's impression of an aggressive IDC may be reinforced by triple-negative staining for estrogen receptor (ER), progesterone receptor (PR), and HER2 in PBL. Importantly, treatment modalities are entirely different for the two diseases. Routine surgical excision or mastectomy is not indicated for PBL and may even be detrimental. (See question 43.)

- Morphology: As described in question 34, DLBCL is a heterogeneous disease that may morphologically mimic other primary cancers. These malignant lymphoma cells are large in cell size for lymphocytes (averaging 17–20 microns in diameter, 2–4 times the size of normal cells), but in many cases, these are not substantially larger than poorly differentiated carcinoma cells. Nuclear features in DLBCL are also highly variable, with chromatin pattern and nucleoli that may mimic high-nuclear-grade breast carcinoma. Furthermore, cytoplasm may be abundant, imparting an epithelioid appearance. Mitoses are also often readily identified. Histologic evidence of mucin or positive mucicarmine staining can help exclude DLBCL. Since the many cytologic features of DLBCL and IDC can be overlapping, often the most important morphologic features can be found in tissue architecture. While DLBCL may infiltrate in single-cell or even nested patterns, distinct glandular architecture is never present.
- Immunohistochemistry: In challenging cases, the most important distinction between lymphoma and IDC is the immunohistochemical profile. CD45 may be used to screen for hematopoietic malignancies, and reliable B lymphoid markers that should be used include CD20 and CD79a. Rarely, poorly differentiated DLBCL may be negative for one or more of these, and secondary B cell-specific markers (e.g., Pax5/BSAP, OCT-2, or BOB.1) must be employed to prove B cell histogenesis. Conversely, IDC should be confirmed by broad-spectrum cytokeratin immunoreactivity, and markers of breast tissue origin may be used for confirmation (e.g., mammaglobin, GCDFP-15, or GATA3). Importantly, ER is expressed in a subset of B cell lymphoma and should not be used to distinguish this differential.

40. How does one distinguish lymphoma (particularly anaplastic large cell lymphoma) from medullary (anaplastic) breast carcinoma (MBC)?

Anaplastic large cell lymphoma (ALCL) is T cell lymphoma that occurs in various forms, including systemic disease, primary cutaneous lymphoma, and a rare form of primary breast implant-associated lymphoma. (See question 32.) Similar to other anaplastic malignancies, poor histologic differentiation and limited antigen expression profile can make it difficult to determine ALCL histogenesis.

Of all the variants of epithelial breast cancer, medullary (anaplastic) breast carcinoma (MBC) shares the most overlapping features with lymphoma.

- Morphology: Shared features of MBC and ALCL which may contribute to misdiagnosis include presentation as well-circumscribed tumor mass, diffuse growth pattern, lack of glandular differentiation, lack of mucin production, and absence of associated ductal carcinoma in situ (DCIS). Cytologic features include numerous mitoses, pleomorphic nuclei, large nucleoli, indistinct cell borders, and prominent intratumoral lymphoid; or lymphoplasmacytic infiltration may be seen. Even bizarre features such as spindle cell morphology and pleomorphic giant cell formation can be seen in both.
- Immunophenotype: As with distinguishing DLBCL in the breast, immunohistochemical markers are frequently essential to distinguish ALCL from MBC (Table 9.6). (See question 39.) Importantly, CD45 is variable and may be negative in ALCL. Thus, CD45 cannot be used alone to completely exclude lymphoma. Not only does ALCL lack B cell markers (i.e., CD20, CD79a), but up to 70% are also negative for CD3. Other pan-T cell markers (CD2, CD5, and CD4) are more useful. CD30 is strongly and diffusely positive. CD43 and CD25 are also usually positive. Both ALK-positive and ALK-negative variants of ALCL exist, so absence of ALK staining does not rule out the diagnosis either.

Table 9.6 Immunohistochemical markers to differentiate poorly differentiated lymphoma from medullar breast carcinoma

Marker	DLBCL	ALCL	MBC
CD45	+	+/–	–
CD20	+	–	–
CD79a	+	–	–
CD3	–	–/+	–
ALK	–	+/–	–
CD30	–	++	–
CD43	–/+	+/–	–
CD2/CD5	–	+/–	–
Cytokeratin	–	–	+
ER	–/+	–	–
PR	–	–	–
HER2	–	–	–

Table 9.7 Immunohistochemical markers to distinguish myeloid and monocytic differentiation from B/T cell NHL

Marker	MS	HS	B-NHL	T-NHL
CD45	+ (weak)	+/–	+	+
CD34	+/–	–	–	–
CD117	+/–	–	–	–
MPO	+	–	–	–
Lysozyme	–/+	+/–	–	–
CD68	–	+	–	–
CD163	–	+	–	–
CD20/CD79a	–	–	+	–
CD3	–	–	–	+
CD2/CD5/CD7	–	–	–	+/–

41. What other hematopoietic malignancies besides lymphoma can occur in the breast?

Although exceedingly rare, initial presentation of hematologic malignancies other than lymphoma can occur in the breast. Myeloid sarcoma (MS) (a.k.a. granulocytic sarcoma or chloroma) refers to the extramedullary (i.e., outside of the bone marrow) presentation of acute myeloid leukemia (AML) and is the most commonly reported non-lymphoid hematopoietic malignancy to involve the breast. Such "aleukemic" presentations of AML can occur anywhere in the body and do not appear to have any particular predilection for breast tissue. Genetic features, cellular phenotypes, and morphology are all comparable to the bone marrow or circulating forms of the disease [106–112].

Other hematopoietic tumors of non-lymphoid histogenesis that have sporadically been noted to occur primarily or secondarily within the breast include histiocytic sarcoma (HS) and Langerhans cell histiocytosis (LCH) [109].

T or B cell acute lymphoblastic leukemia (T-/B-ALL) can also present primarily or secondarily within the breast, with or without peripheral blood involvement. Although these are invariably systemic leukemias, current classification schemes call for designation as T or B lymphoblastic lymphoma when presenting as a solid tumor mass.

42. How does one distinguish myeloid or histiocytic sarcoma from lymphoma?

The morphologic distinction of a myeloid sarcoma (MS) from poorly differentiated lymphoma can be challenging. Many of the diagnostic features of malignant "blasts" that distinguish AML cytologically cannot be easily appreciated on the histologic section. Myeloid sarcoma/AML cells are intermediate to large in size, with round-to-irregular nuclei and moderate amounts of cytoplasm, often eosinophilic to amphophilic, reflective of their oftentimes granular cytoplasmic content. Nuclear chromatin appears finer than is typical of mature lymphoid malignancies, but sometimes vesicular chromatin patterns following formalin fixation obscure the characteristic nuclear details of these malignant blasts [113–115].

Histiocytic sarcoma (HS) is an often more well-differentiated, but highly pleomorphic malignancy that exhibits variable degrees of monocytic/histiocytic maturation [108]. Cases range from overtly aggressive tumors with sarcomatoid features, including abundant cytoplasm, indistinct cell borders, round nuclei, and open chromatin, as well as histiocytic giant cell formation, to more immature and monotonous monocytoid tumors with smaller cells and more condensed nuclear chromatin.

Immunohistochemical (or cytochemical) staining is critical to demonstrate myeloid or monocytic differentiation. Myeloperoxidase (MPO) is a defining feature of myeloid malignancies with the exception of monocytic tumors which more often display specific cell surface markers, as shown in Table 9.7.

43. What is the role of mastectomy in primary breast lymphoma?

Mastectomy offers no benefit in the treatment of PBL; it provides neither survival benefit nor protection from recurrence. In fact, data suggest that surgical resection results in *inferior* local disease control and is associated with greater disease-specific and overall mortality. Accordingly, mastectomy or surgical intervention is strongly *contra*indicated [46, 70, 71, 75, 116].

In instances where surgical excision does occur, attributable to misdiagnosis of breast carcinoma or other reasons, chemo-immunotherapy and radiotherapy should be administered as soon as adequate wound healing has occurred. (See questions 39 and 40.)

44. Is there a role for axillary/sentinel lymph node biopsy in evaluating breast lymphoma?

Sentinel lymph node biopsy and mapping, in the traditional surgical sense (utilizing radioactive colloid and/or blue dye

injection at the site of primary breast disease), is not indicated. Although some studies indicate that adjacent lymph node involvement is a strong predictor of survival, this result has not been universally reproduced, and other conventional risk stratification schemes are typically employed (e.g., Ann Arbor staging, International Prognostic Index (IPI), and Eastern Cooperative Oncology Group (ECOG) performance status) [116–118].

As with all systemic lymphomas, adequate clinical staging typically involves whole-body positron emission tomography with computed tomography (PET-CT), with confirmatory biopsy of peripheral lymph nodes performed only as clinically indicated to exclude other causes of lymphadenopathy.

45. What are the most common patterns of relapse in primary breast lymphoma?

Relapse rates and patterns in PBL are most widely reported for DLBCL, as other less common low-grade histologic forms (i.e., MZL, FL, etc.) have less extensive data available and may behave similar to nodal disease [46, 49, 51, 75, 119–122].

Primary breast DLBCL relapses predominantly at extranodal sites. Recurrence within either the ipsilateral or contralateral breast is most common (12–44%). Outside of the breast, the central nervous system (CNS) is the next most common site (5–16%). Other extranodal sites do occur (including bone marrow, lung, skin, and gastrointestinal tract), but are much less common; and interestingly these are involved more often with PB-DLBCL than is typical of primary nodal disease. In this regard, PB-DLBCL is similar to some other tissue-specific types of extranodal DLBCL (e.g., primary testicular or primary CNS disease) in that it displays distinct extranodal tropism both at presentation and recurrence.

Recurrence in the ipsilateral breast presents typically within 3–5 years, but contralateral breast relapse can be much delayed, occurring up to 13 years after initial presentation. The frequency of CNS relapse (a high-risk feature with poor prognostic implications) appears to be greater in PBL than in comparable limited-stage (I_E/II_E) nodal DLBCL, but with similar average time to recurrence (<2 years). Limited data suggest that outcomes with relapsed PB-DLBCL are poor. This is particularly true with CNS involvement, where median overall survival averages less than a year.

Case Presentations

Case 1 (Fig. 9.21a–d)

Learning Objectives

1. To be aware of the association of the lesion with breast implant
2. To be familiar with the morphological characteristics of this lesion
3. To be able to generate appropriate differential diagnosis for this type of lesion

History

- A 35-year-old female with bilateral breast augmentation implants for 3 years presents with chest wall mass adjacent to the implant, 2.5 cm in size clinically.

Gross Examination

- Several cores of soft tissue

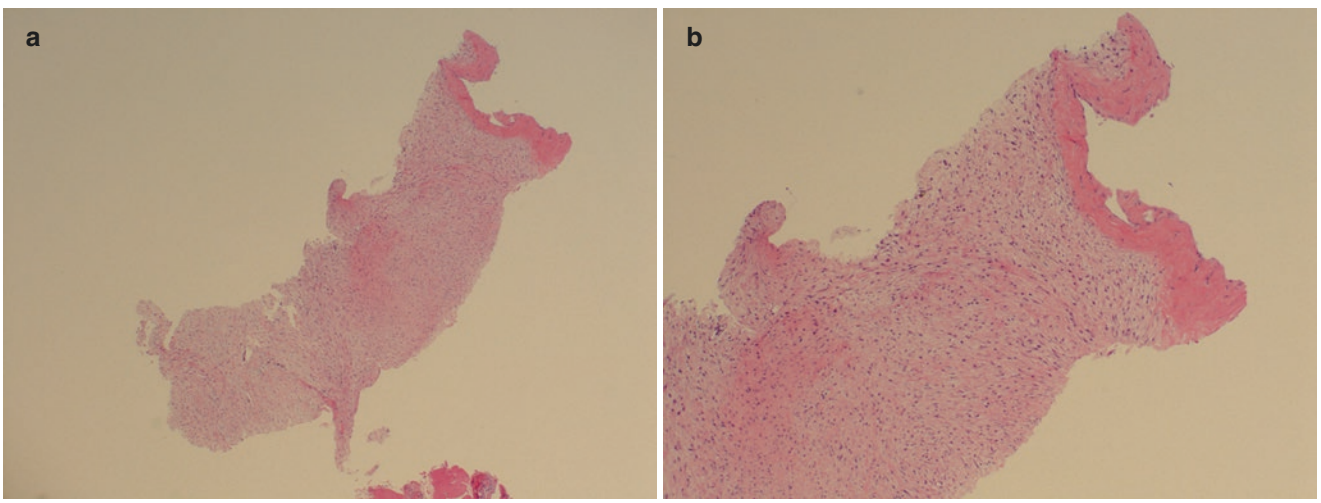

Fig. 9.21 Case 1. (**a**) Low-power view of spindle cell lesion with modest cellularity (20×). (**b**) Lesional cells which appear directly associated with thick fibrous capsule (40×). (**c**) Bland spindle cells, less prominent than endothelial cells (100×). (**d**) Nuclear positivity for beta-catenin by immunohistochemical stain (100×)

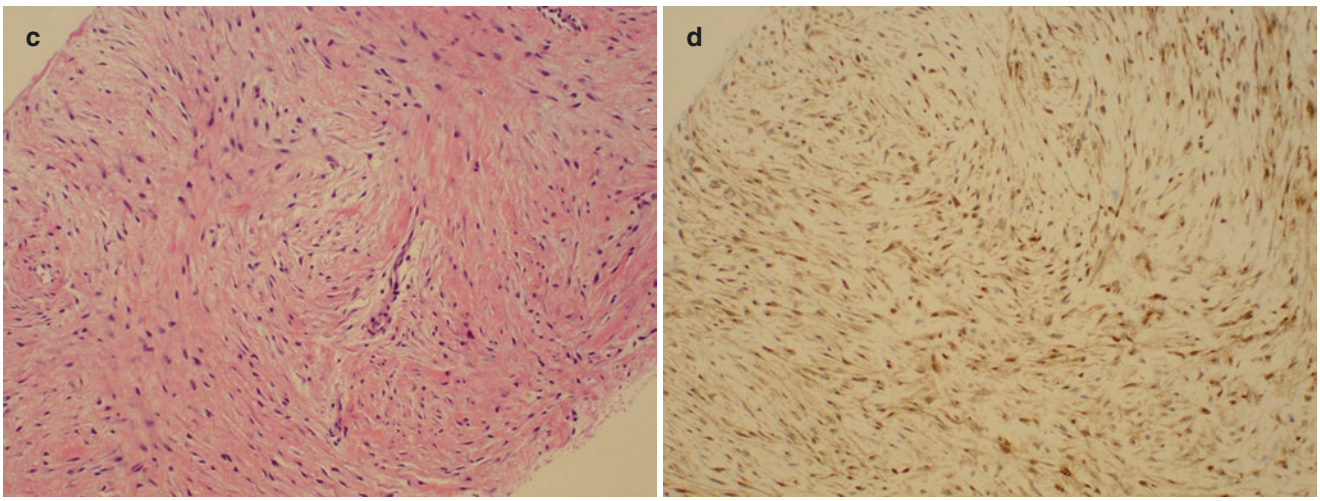

Fig. 9.21 (continued)

Histologic Findings
1. Fragments of spindle cell lesion with modest cellularity, tightly associated with a thick fibrous capsule.
2. The cells are less prominent than the endothelial cells; no mitosis is identified.

Immunohistochemical Stain
1. Positive: Beta-catenin (nuclear), focally positive for SMA
2. Negative: Cytokeratin cocktail, p63, CK5, desmin, CD34

Differential Diagnosis
1. Metaplastic carcinoma
2. Nodular fasciitis
3. Fibromatosis
4. Solitary fibrous tumor
5. Smooth muscle tumor

Final Diagnosis
- Fibromatosis associated with the capsule of implant

Take-Home Messages
1. Fibromatosis could be associated with breast implant.
2. It is with modest cellularity and bland spindle cells; mitosis is uncommon.
3. Beta-Catenin is nuclear positive.

Case 2 (Fig. 9.22a–d)

Learning Objectives
1. To be able to generate a list of differential diagnoses for hypercellular spindle cell tumor in the breast
2. To be familiar with the diagnostic characteristics of this tumor

History
- A 19-year-old female patient presents with breast mass. A core biopsy and then subsequent lumpectomy were performed.

Gross Examination
- An ill-defined, tan-white, lobulated mass, 2.6 cm in greatest dimension

Histologic Findings
1. Hypercellular spindle cell neoplasm; cells are plump and hyperchromatic.
2. Focal tumor necrosis.
3. Focal cells with pink cytoplasm.

Differential Diagnosis
1. Rhabdomyosarcoma, primary or metastatic
2. Heterologous component of malignant phyllodes tumor
3. Heterologous component of metaplastic carcinoma
4. Other hypercellular spindle cell sarcomas, primary or metastatic: leiomyosarcoma, synovial sarcoma, and MPNST
5. Melanoma

Immunohistochemical Stain
1. Positive: Myogenin, desmin
2. Negative: Cytokeratin cocktail, EMA, SMA, S-100, CD34

Diagnosis
- Rhabdomyosarcoma, embryonal type, breast primary

Take-Home Messages
1. Age could be a hint for the diagnosis.
2. It will be very productive to carefully look for rhabdomyoblasts under high-power view.

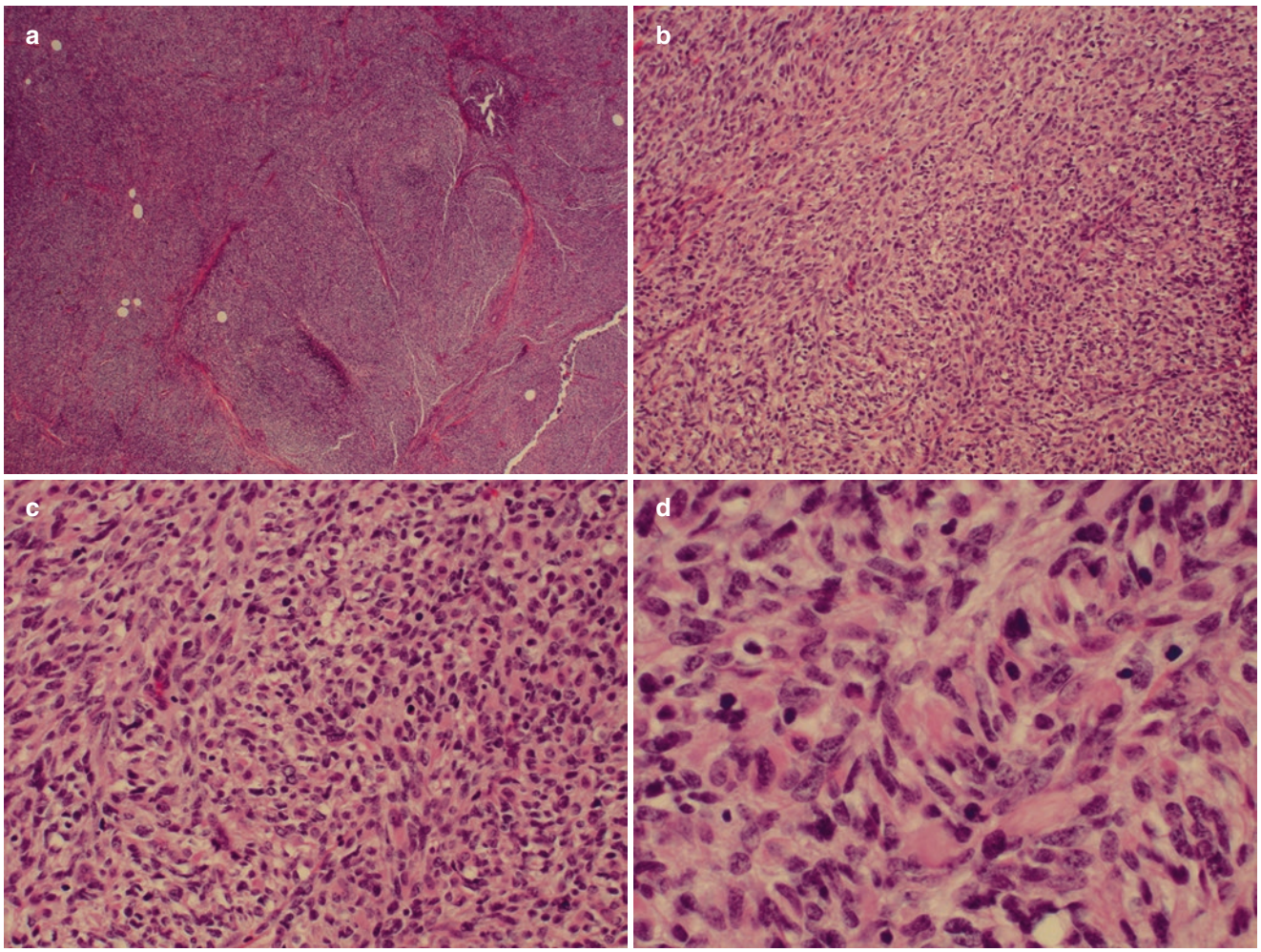

Fig. 9.22 Case 2. (**a**) Low-power view of hypercellular spindle cell lesion with trapped-in fat vacuoles and focal tumor necrosis (20×). (**b**). Spindle cells forming vague fascicles (40×). (**c**) Eccentric pink cytoplasm in many tumor cells (strap cells or tadpole cells) (100×). (**d**) Higher-power view (400×)

Case 3 (Fig. 9.23a–d)

Learning Objectives

1. To know the morphological characteristics of this vascular lesion
2. To be able to differentiate it from other reactive vascular lesions

Clinical History

- A 78-year-old female with 9-year history of left breast cancer, S/P mastectomy, and radiation now presents with skin lesion.

Gross Examination

- Skin punch biopsy

Histologic Findings

1. Scattered vasculature with well-formed lumen, dissecting through dermis to subcutaneous soft tissue.
2. The lining endothelial cells are hyperchromatic with enlarged nuclei.

Differential Diagnosis

1. Normal vasculature
2. Post-radiation atypical vascular proliferation
3. Post-radiation angiosarcoma

Immunohistochemical Stain

1. Positive: ERG and MYC, with Ki67 labeling index of 10% in endothelial cells
2. Negative:

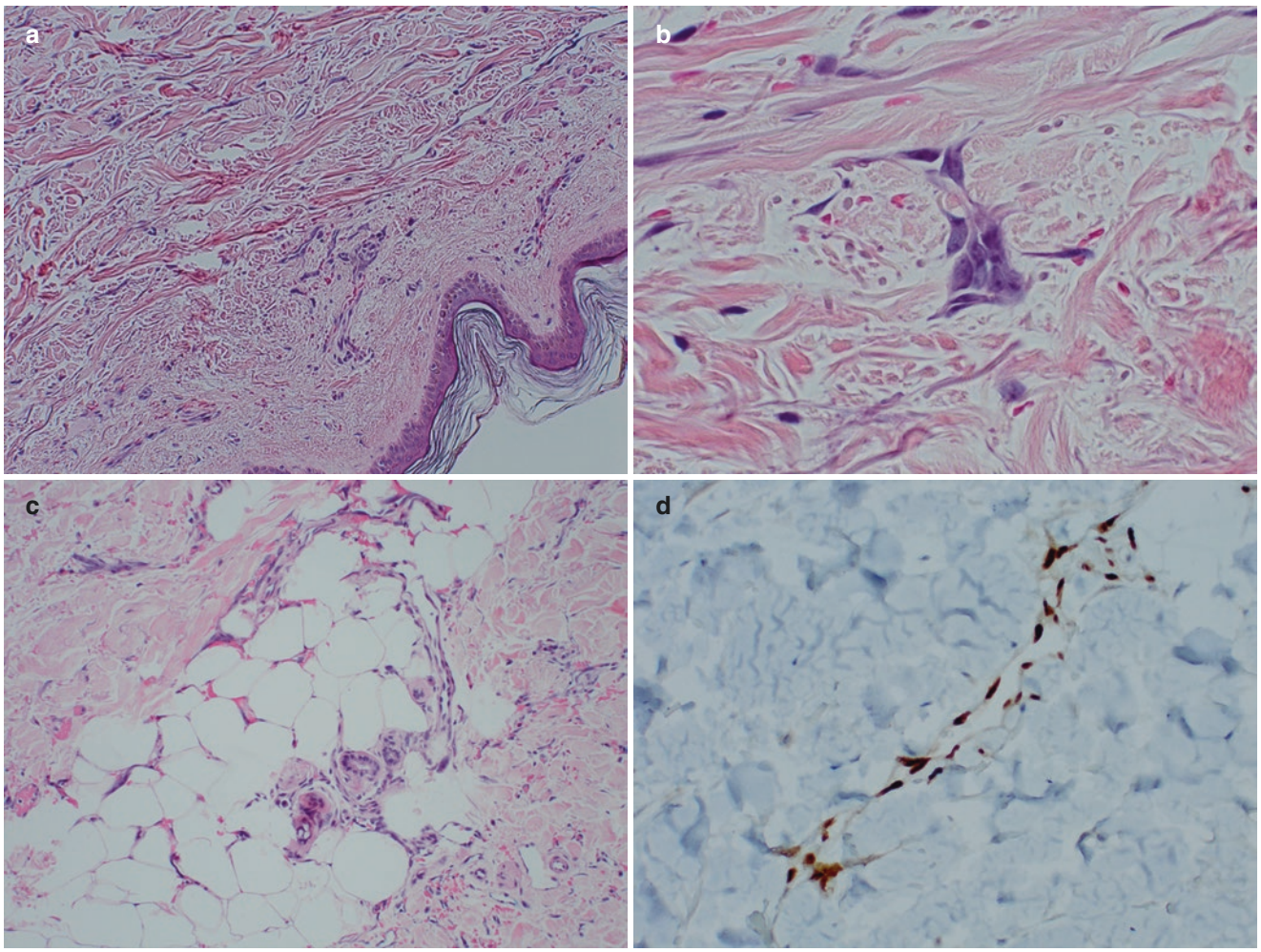

Fig. 9.23 Case 3. (**a**) Skin punch biopsy with scattered vasculature dissecting dermis (40×). (**b**) Large hyperchromatic cells lining the vascular channel (400×). (**c**) The same vasculature in subcutaneous soft tissue (40×). (**d**) Immunohistochemical stain for MYC highlighting the hyperchromatic nuclei (400×)

Final Diagnosis

- Post-radiation angiosarcoma

Take-Home Messages

1. Nuclear hyperchromasia and invasion to subcutaneous soft tissue are diagnostic features of this entity.
2. Immunohistochemical stain for C-MYC and Ki67 has distinct value in helping the diagnosis.

Case 4 (Fig. 9.24a–h)

Learning Objectives

1. To generate the differential diagnosis for lymphocyte rich lesions of the breast
2. To become familiar with the immunohistochemical workup to evaluate for lymphoma
3. To recognize the histologic features of the correct final diagnosis

History

- A 69-year-old female with history of hypothyroidism and Sjogren syndrome is found to have a 2.0 cm indeterminate mass in the left upper outer quadrant on routine screening mammogram. Mammography report indicates bi-rads 4c, "probable invasive carcinoma." Core needle biopsy was performed.

Histologic Findings

- Infiltration of the sclerotic parenchyma by a diffuse proliferation of highly atypical cells, moderate to large in cell size, with abundant intermixed small mature lymphocytes containing hyperchromatic nuclei (Fig. 9.24a).
- No evidence of ductal or lobular infiltration pattern.
- Large atypical cells have irregular nuclei with open vesicular chromatin, prominent centrally placed nucleoli, and

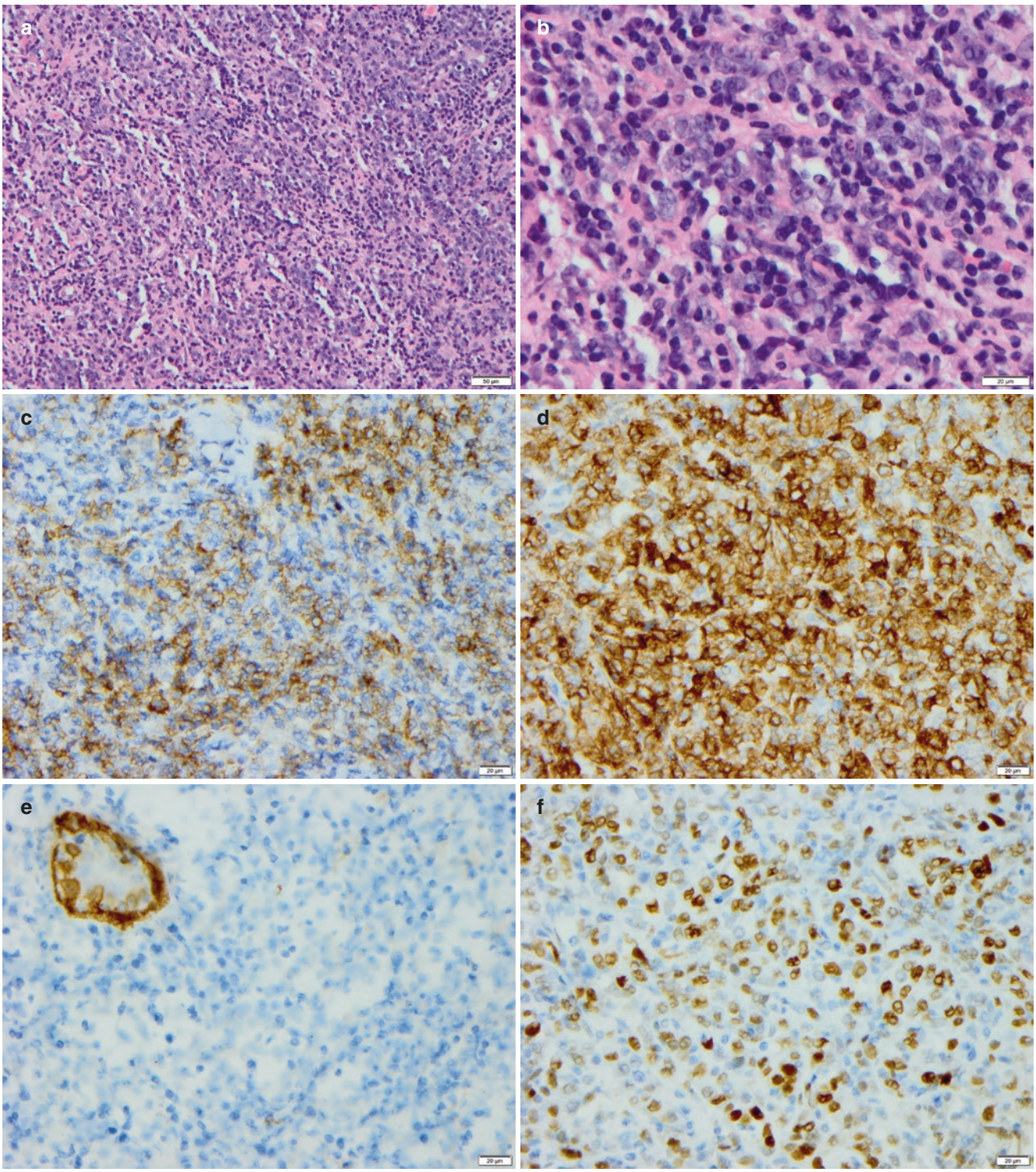

Fig. 9.24 Case 4. (**a**) Medium-power view of diffuse mixed atypical inflammatory infiltrate (200×). (**b**) High-power view of infiltrating cell populations, with focus on large cells, irregular nuclei, vesicular chromatin, prominent central nucleoli, and moderate cytoplasm (600×). (**c**) CD20 (400×). (**d**) CD79a (400×). (**e**) CD10 (400×). (**f**) BCL6 (400×). (**g**) MUM1 (400×). (**h**) Ki67 (400×)

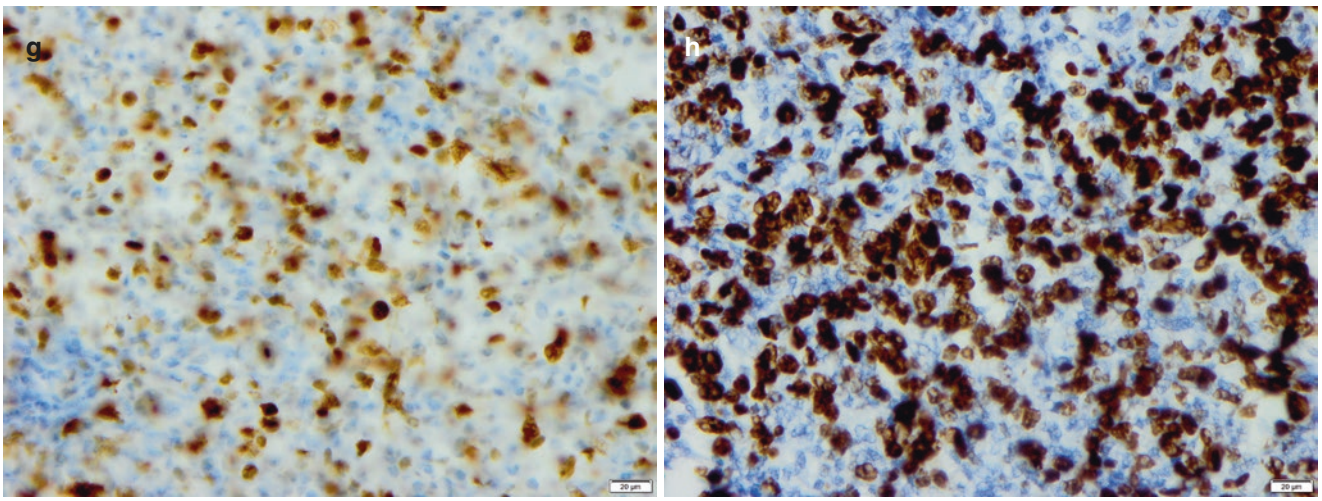

Fig. 9.24 (continued)

moderate amounts of amphophilic cytoplasm with indistinct cell borders (Fig. 9.24b).

Differential Diagnosis
- Lymphocytic mastitis
- Marginal zone lymphoma
- Triple-negative invasive ductal carcinoma, high nuclear grade
- Diffuse large B cell lymphoma
- Burkitt lymphoma

IHC and Other Ancillary Studies (Fig. 9.24c–h)
- Pancytokeratin negative
- ER, PR, Her2 negative
- CD20 weak/partial positive
- CD3 scattered positive on small cells
- CD79a strong positive
- CD10 negative
- BCL6 positive
- MUM1 positive
- Ki67: 80%
- FISH: Negative for *MYC*, *BCL2*, or *BCL6* rearrangement

Final Diagnosis
- Diffuse large B cell lymphoma, activated B cell (ABC) type

Take-Home Messages
1. In the absence of definitive architectural features of invasive carcinoma, lymphoid markers are indicated to exclude large cell, high-grade, or poorly differentiated lymphoma, while cytokeratin is also recommended to rule in triple-negative breast carcinoma.
2. CD20 expression may be weak to negative in DLBCL. Secondary B cell markers (e.g., CD79a or Pax5) are often needed.
3. Large cell size, marked nuclear pleomorphism, and intermediate Ki67 proliferation index in this case exclude low-grade B cell NHL and are inconsistent with Burkitt lymphoma.

Case 5 (Fig. 9.25a–e)

Learning Objectives
1. To generate a differential diagnosis for infiltrating lymphoid lesions of the breast
2. To become familiar with the immunohistochemical features that may distinguish a clonal B cell disorder from a reactive one
3. To become familiar with the gross and histologic features that distinguish teh correct final diagnosis

History
- An 89-year-old female presents with focal right breast asymmetry noted on screening mammography. No suspicious calcifications or dominant mass was noted in heterogeneously dense breast tissue.

Histologic Findings
- Infiltration of the breast parenchyma by small lymphoid cells, focally surrounding breast ductules (Fig. 9.25)
- Mostly small mature lymphoid cells surrounding residual follicular structures, with predominantly round nuclei, hyperchromatic condensed chromatin, and scant cytoplasm (Fig. 9.25b)
- Focal areas, including residual areas of adiposity, with prominent plasmacytic infiltration

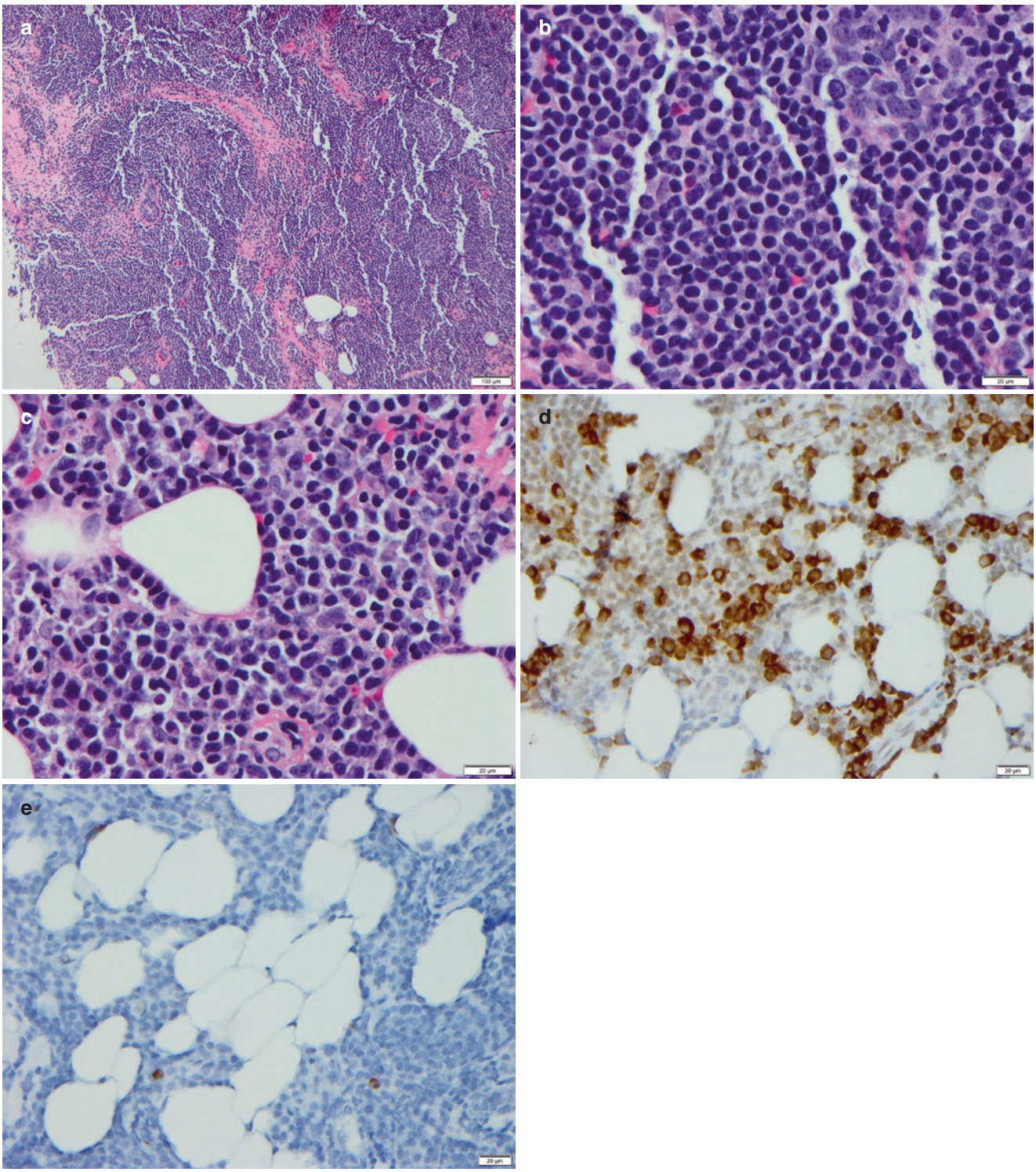

Fig. 9.25 Case 5. (**a**) Medium-power view of diffuse lymphocytic infiltrate, noting single breast ductile at the left (100×). (**b**) High-power view of small cell lymphoid infiltrate with hyperchromatic nuclei (600×). (**c**) High-power view of extensive plasmacytic component (600×). (**d**) Lambda light-chain in situ hybridization among plasma cells (400×). (**e**) Kappa light-chain in situ hybridization (400×)

Differential Diagnosis
- Diabetic mastopathy
- Follicular hyperplasia
- Follicular lymphoma, grade 1
- Marginal zone lymphoma
- Diffuse large B cell lymphoma

IHC and Other Ancillary Studies (Fig. 9.25d, e)
- In situ hybridization kappa/lambda ratio among plasma cells, <1:10 (kappa negative, lambda positive)
- CD5 negative
- CD10 negative
- PCR studies positive for clonal B cell population for immunoglobulin light-chain analysis

Final Diagnosis
- Marginal zone lymphoma

Take-Home Messages
1. Low-grade lymphoma may mimic benign lymphocytic inflammation, both cytologically (i.e., hyperchromatic nuclei, indistinct nucleoli, and minimally irregular nuclei) and architecturally (with increased plasma cells and residual benign or colonized lymphoid follicles present within the lesion).
2. Marginal zone lymphoma, in particular, frequently presents with clonally derived plasma cells as part of the malignant proliferation.
3. Documentation of B cell clonality by alternative methods is critical in extranodal low-grade B cell NHL, particularly with MZLs which frequently lack any other specific aberrant pattern of B cell antigen expression.
4. Given the likelihood that most non-lymphoid tissues are not available for flow cytometry evaluation, kappa vs. lambda immunohistochemical or in situ hybridization staining is indicated, frequently only reliable among plasma cells when performed by most routine laboratories. Therefore, alternative methods such as PCR for clonal immunoglobulin gene rearrangements are strongly recommended.

Case 6 (Fig. 9.26a–h)

Learning Objectives
1. To generate the differential diagnosis for nodular lymphoid proliferations
2. To become familiar with the immunohistochemical approach to evaluating this differential
3. To become familiar with the gross and histologic features that distinguish the correct final diagnosis

History
- A 49-year-old female noted a lump in her left breast, with mammographic evidence of a 0.9 cm mass in the left inner quadrant.

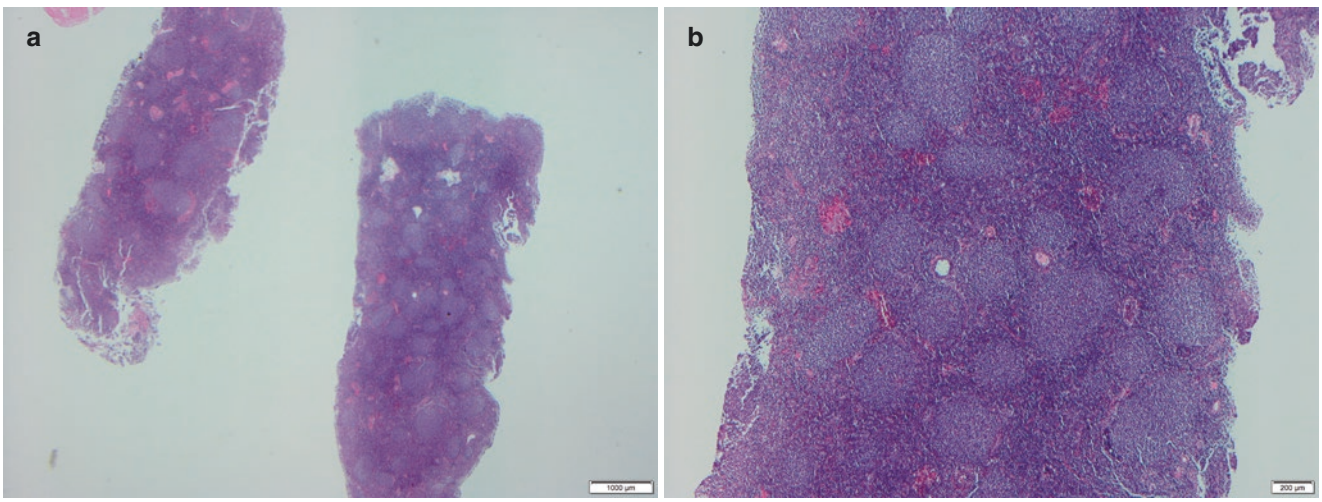

Fig. 9.26 Case 6. (**a**) Low-power view of nodular lymphoid proliferation (12.5×). (**b**) Medium-power view of follicular organization (40×). (**c**) High-power view of small irregular lymphoid cells with cleaved and hyperchromatic nuclei within the follicular proliferation (400×). (**d**) CD20 (4×). (**e**) CD10 (40×). (**f**) BCL6 (40×). (**g**) BCL2 (40×). (**h**) Ki67 (40×)

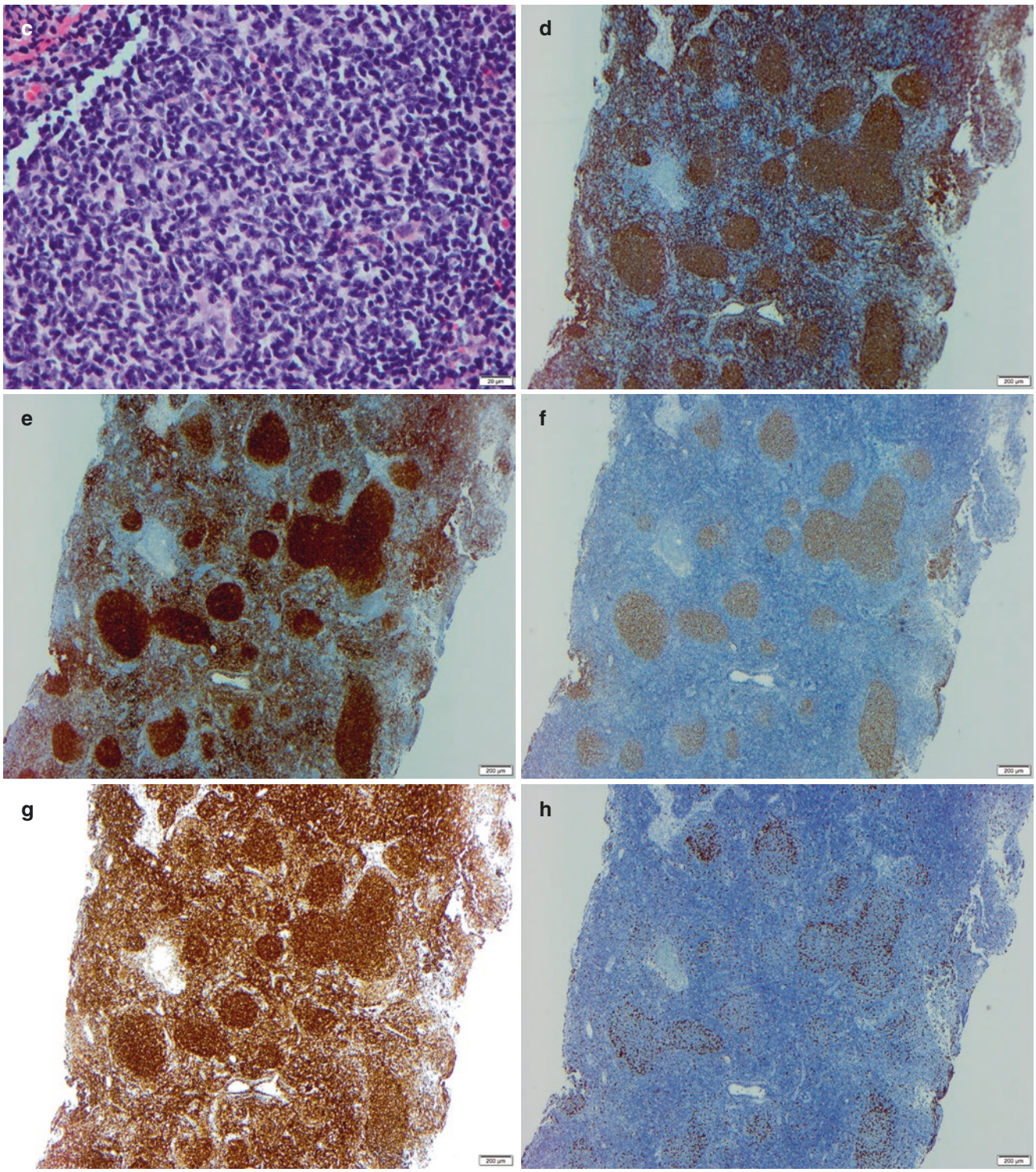

Fig. 9.26 (continued)

Histologic Findings

- Nodular proliferation of small lymphoid follicles. Some follicles are back-to-back, while others are more widely spaced, all with attenuated mantle zones (Fig. 9.26a, b).
- Nodules contain small bland irregular lymphoid cells. Mitoses are rare, and large cells (centroblasts) are lacking within proliferating nodules (Fig. 9.26c).

Differential Diagnosis

- Follicular hyperplasia
- Follicular lymphoma, grade 1
- Marginal zone lymphoma
- Diffuse large B cell lymphoma

IHC and Other Ancillary Studies (Fig. 9.26d–h)

- CD20 positive
- CD10 positive
- BCL6 positive
- BCL2 positive
- Ki67 low, 10–20% within nodules

Final Diagnosis

- Follicular lymphoma, WHO grade 1 (of 3)

Take-Home Messages

1. The primary differential for a nodular lymphoid proliferation (particularly in the breast) is reactive follicular hyperplasia, follicular lymphoma, and follicular colonization by marginal zone lymphoma.
2. Low-power architectural features are important to distinguish benign from neoplastic follicles, but neoplastic follicles may be variably and even widely spaced, without back-to-back organization throughout.
3. In low-grade neoplastic follicles, the cytologic atypia is less, and the rate of proliferation (i.e., Ki67) is lower than in benign reactive follicles where marked cellular pleomorphism and high proliferation rates are physiologically normal.
4. Lymphoid follicles co-expressing germinal center B cell markers (CD20, CD10, and BCL6), along with aberrant BCL2, are a hallmark of follicular lymphoma.

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Metastatic Cancer in the Breast

10

Bradley M. Turner

List of Frequently Asked Questions

1. **What is the frequency of metastatic cancer to the breast**?

The most common metastatic cancers in the breast are from a contralateral primary breast carcinoma [1–7]. Contralateral primary breast metastases are excluded in most series discussing metastatic disease to the breast [2] and will not be discussed further in this chapter. Metastases to the breast and axilla from extramammary locations are extremely uncommon. The frequency of metastases to the breast from extramammary locations varies depending on the clinical study, with published reports between 0.2% and 3% [1, 2, 4, 5, 7–9]. Higher frequencies of 2–7% have been reported in postmortem studies [2, 6, 10].

2. **Other than frequency, what is the epidemiology of metastatic cancer to the breast?**

A review of the English-language literature suggests that fewer than 750 cases of metastases to the breast from an extramammary location have been reported [1–3, 9, 11]. The first case of metastases to the breast from an extramammary location may have been reported as early as 1855 [11], and the first documented case in the peer-reviewed English literature was reported in 1903 [12].

In approximately 70% of patients with metastatic disease to the breast who have a breast lump, there is a known primary carcinoma in a known location [5, 13]; however, in approximately 30% of patients with metastatic disease to the breast, the metastases is the first sign of malignancy [2–5, 7, 8, 14]. In these cases, the occult primary tumor is often misinterpreted as a primary breast malignancy [13]. The male-to-female ratio has been reported as anywhere from one male for every six to eleven females [1, 3, 5, 13]. The age range for metastases to the breast from an extramammary location has been reported from as young as 12 years of age to as old as 90 years of age [1, 3, 4]. The most common tumor metastatic to the breast in the pediatric population is a rhabdomyosarcoma [1, 13, 15, 16], and the proportion of metastases in children and young adults is significantly higher compared to older adults [2, 13, 15]. Only a single study (the largest series found to date which addresses the topic) reports on ethnicity [3]. In this study of 169 patients with metastases to the breast from an extramammary location, 85.2% ($n = 144$) were Caucasian, 7.7% ($n = 13$) were African–American, and 7.1% ($n = 12$) were of Latino origin.

3. **Why is it important to recognize a metastatic cancer from a primary breast carcinoma?**

It is critical to consider metastatic disease as a possibility because the treatment options are different. Delays in diagnosis contribute to the poor prognosis associated with most metastases to the breast from an extramammary location. Patients with metastatic disease to the breast do not usually benefit from mastectomy [13], so a correct diagnosis is crucial in avoiding unnecessary procedures. In particular, radical breast surgery and axillary nodal dissection may not be appropriate in patients with systemic disease [15]. Systemic chemotherapy options are vastly different depending on the type of metastatic disease, and although most patients die within a year of diagnosis [2, 17–19], longer survival is well recognized if there is effective systemic treatment [2, 13, 14, 18].

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Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5_10

4. **What is the differential diagnosis for metastatic cancer to the breast?**

The first consideration of a primary location for metastatic disease to the breast should be a primary breast carcinoma [1–7, 16]. Some authors have suggested that the rarity of metastatic disease from an extramammary location is due to the characteristics of breast tissue [20–22]. When metastatic disease from an extramammary location does occur, it has been suggested that hormonal status may play a role, based on the increased occurrence of breast metastasis in pubescent, lactating, and pregnant females [20, 23–25]. Other hypotheses include a transfection phenomenon of the cancer genome and the dissemination of stem cells that spread by systemic, lymphatic, or transcoelomic migration [20, 26, 27]. This latter hypothesis may offer some insight into the second most common cause of metastatic disease to the breast, hematopoietic neoplasms [16, 28, 29]. Because breast lymphomas occur as a result of systemic involvement of the lymphoid tissue, some authors propose exclusion of lymphomas under the heading of "metastasis in the breast" [28], and most reviews and case series exclude hematopoietic disease when discussing metastatic disease to the breast from an extramammary location. Excluding hematopoietic tumors, a review of the literature [1–4, 11, 15, 20] suggests that the most common site of metastatic origin is the skin (Table 10.1), the most common classification of metastatic disease is a carcinoma (Table 10.2), and the most common type of metastatic neoplasm is melanoma (Table 10.2). Discordance in prevalence in individual studies is likely due to study location and disease prevalence. Many of the neuroendocrine tumors arise from the lung, so a neuroendocrine carcinoma of the breast should prompt an evaluation for possible pulmonary origin. Other less common sites and types of tumors that have been reported to be metastatic to the breast include non-melanoma skin (squamous cell carcinoma, Merkel cell [1]) and non-skin melanoma (typically ocular) [1, 4]. Other even more unusual sites and types of metastases have been reported, including the thymus [20], heart [20], mesothelioma [4], tongue [1], choriocarcinoma [1, 15], adenoid cystic carcinoma [1], neuroblastoma [15], and malignant fibrous histiocytoma [1, 28].

Table 10.1 Frequency of extramammary metastasis to the breast by site of origin[a]

Site of metastatic origin[a] (n = 678) [1–4, 11, 15, 20]	% frequency
Skin	28
Pulmonary	24
Gynecological	17
Genitourinary	12
Gastrointestinal	10
Soft tissue	5
Head and neck	4
Other	<1

[a]Excluding hematopoietic tumors

Table 10.2 Frequency of extramammary metastasis to the breast by tumor type[a]

Type of metastatic disease[a] (n = 707) [1–4, 11, 15, 20]	% frequency (carcinoma)	% frequency (all metastasis)
Carcinoma (n = 398)		56
Pulmonary	28	16
Gynecological	28	15
Genitourinary	21	12
Gastrointestinal	16	9
Head and Neck	7	4
Other carcinoma	<1	<1
Melanoma		27
Neuroendocrine		10
Sarcoma		7
Other non-carcinoma		<1

[a]Excluding hematopoietic tumors

5. **Are there clinical differences in patients with metastatic cancer to the breast as opposed to a cancer of primary breast origin?**

A thorough history may reveal the possibility of a metastatic origin in a patient diagnosed with breast carcinoma, as the majority of patients with metastatic breast disease from extramammary locations will have a history of extramammary malignancy [15]. It is critical that any history of prior carcinoma be provided to the pathologist in a patient diagnosed with breast carcinoma. In one series [1], the failure of the pathologist to recognize the metastatic nature of the lesion resulted most often because the clinician failed to provide history regarding a previous cancer. Most patients with primary breast carcinoma are asymptomatic, presenting after an abnormal screening examination. In contrast, patients with metastatic breast carcinoma may be more likely to present with a rapidly growing painless firm palpable breast mass [2, 4, 8, 17, 18, 23, 30–32], and the diagnosis is typically made by physical exam [2, 3]. Diffuse skin involvement is rare [2, 3]. The metastatic tumor is most frequently unilateral and solitary [1, 3, 13] but may present as multiple and/or bilateral lesions [1–3, 13]. Axillary and/or regional lymphadenopathy may or may not be present; if present, suspicion of metastatic disease should be aroused in correlation with other clinical, radiographic, and pathologic information [2, 16]. Suspicion should be particularly high in cases of an axillary tumor without any evidence of a primary breast carcinoma on clinical exam or radiographic imaging. Cases with metastatic disease beyond the axilla at presentation should also raise suspicion. In one series, the majority of metastatic cases to the breast presented with widespread metastatic disease, often at multiple sites, including the liver, bone, subcutaneous sites, and lymph nodes [15].

6. **Are there radiographic differences in patients with metastatic cancer to the breast as opposed to a cancer of primary breast origin?**

Although there are no specific features to distinguish primary breast carcinoma and metastatic breast disease from an extramammary location, imaging studies may be helpful in correlation with clinical history and pathologic information. The most common mammographic appearance is a rounded mass with well-defined or slightly irregular margins [2, 8, 23, 33, 34]. The majority of metastases are solitary and unilateral, although multiple and bilateral metastatic tumors do occur [16]. Unlike primary breast carcinoma, mammographic spiculations are rare [2, 23, 35]. Most lesions present in the upper outer quadrant [17, 20, 23, 35]. Calcifications are rarely seen [3, 14, 33, 35]; however, if present and metastatic disease is being considered, calcifications raise the suspicion for metastatic ovarian serous carcinoma [1–4, 14, 17, 19, 33, 35–37]. An ultrasound will often show a hypoechoic mass, which may be heterogeneous or poorly defined [33]. Multiple lesions or well-circumscribed masses felt to be radiographically benign, but with histology consistent with carcinoma, should raise suspicion for a metastatic disease [15, 38]. Radiographically, the metastasis may be present in the adipose tissue near the chest wall [16].

7. **Are there histologic differences in patients with metastatic cancer to the breast as opposed to a cancer of primary breast origin?**

Metastatic breast disease from an extramammary location often has histologic features similar to primary breast carcinoma; however, histology that is atypical for a primary breast carcinoma – such as pigment, intranuclear inclusions, neuroendocrine features, pure squamous features, well-formed papillae, tall columnar cells with mucin, high-grade dyscohesive cells, or clear cell features – may be present and should prompt consideration for metastatic breast disease from an extramammary location. A well-circumscribed lesion sharply demarcated from adjacent normal breast tissue, in the absence of in situ carcinoma, should also prompt consideration for metastatic breast disease from an extramammary location [1, 2, 13, 23, 39]. Elastosis, which is common in primary breast carcinoma (Fig. 10.1), is rare in extramammary tumors [2, 13, 40].

It is likely that metastatic breast disease from an extramammary location occurs in the setting of advanced disease and widespread systemic metastasis [3], and the presence of extensive lymphovascular invasion and the absence of microcalcifications should raise suspicion that another primary focus might be present [13]. The absence of lymphovascular invasion and the presence of microcalcifications should be viewed with caution, however, as lymphovascular invasion

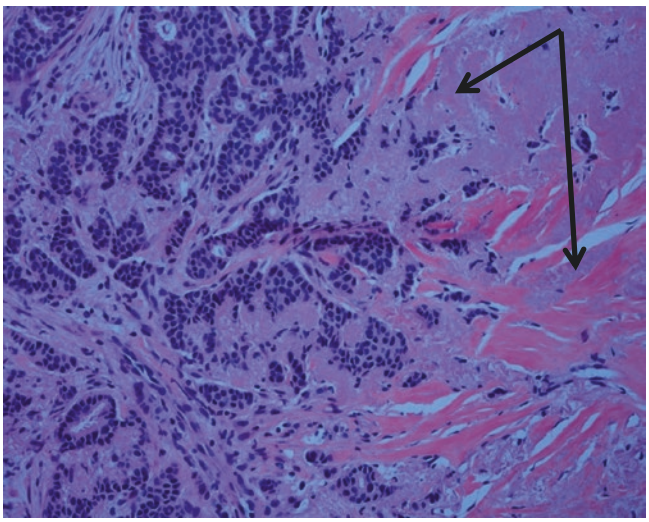

Fig. 10.1 Elastosis in breast carcinoma (20×). Note the clumps of elastic fibers (arrows), or elastosis, present associated with the invasive breast carcinoma. On hematoxylin and eosin staining, these clumps of elastic fibers often have a distinct grayish to bluish hue, well demarcated from the intervening eosinophilic stroma. The presence of elastosis, which is common in primary breast carcinoma, would suggest a primary breast origin, as elastosis is rare in extramammary tumors metastatic to the breast

was notably absent in 87% (40/46) of metastatic tumors to the breast in one report [1], and reports of metastatic hepatocellular [20], gastric [20], and ovarian carcinomas [1–3] with associated microcalcifications have all been previously reported in the literature. Additionally, the absence of estrogen receptor (ER) and progesterone receptor (PR) in a well-differentiated carcinoma, and all triple-negative (ER/PR/HER-2-negative) tumors should prompt consideration for metastatic disease.

8. **How might a metastatic melanoma present differently from primary breast carcinoma or a primary breast melanoma**?

A clinical history of melanoma should immediately raise suspicion for metastatic disease. A thorough skin examination (including a thorough examination of the breast skin to rule out the rare primary breast melanoma) should be performed on any new cancer diagnosis, and breast cancer is no exception. Although uncommon, 4–5% of melanomas arise from non-cutaneous sites [41], typically the eye. As such, a thorough ophthalmologic examination should be considered if the clinical presentation is suspicious for metastatic melanoma.

9. **How might metastatic pulmonary carcinoma present differently from primary breast carcinoma?**

A clinical history of pulmonary carcinoma should immediately raise suspicion for a metastatic disease. A thorough

pulmonary examination should be performed on any new cancer diagnosis, and breast cancer is no exception. A cough that does not go away or gets worse, coughing up blood or rust-colored sputum, hoarseness, shortness of breath, decreased exercise intolerance, new onset of wheezing, recurrent cough, recurrent bronchitis, recurrent pneumonia, and chest pain that is often worse with deep breathing, coughing, or laughing may all be signs and symptoms of lung involvement.

10. **How might a metastatic gynecological tract tumor present differently from primary breast carcinoma?**

The most common type of metastatic gynecological tract tumor to the breast is ovarian serous papillary carcinoma [1, 2, 16, 42–44]. If there is metastasis to the breast, it would be unusual for gynecologic malignancy not to be present clinically [16]. A clinical history of a gynecologic tract tumor should immediately raise suspicion for metastatic disease. A clinical history of vaginal bleeding, back or pelvic discomfort, or bowel obstruction may be present and might prompt evaluation for a metastatic gynecologic tract tumor.

11. **How might a metastatic genitourinary tract tumor present differently from primary breast carcinoma?**

Metastatic cancer from the genitourinary tract to the breast is most likely to arise from the kidney, bladder, or prostate (men) [1, 2, 4, 11, 15, 16, 20]. Physical examination may reveal a mass on the side or lower back in renal carcinoma. Blood in the urine and low back pain (not caused by injury) should raise suspicion for renal, bladder, or prostate involvement. Changes in urinary habits (i.e., urgency, increased frequency, or frank inability to urinate) should prompt consideration for bladder or prostate involvement. Prostate cancer is much more common in men than breast cancer and should always be considered in any breast cancer diagnosis in males.

12. **How might a metastatic gastrointestinal tract tumor present differently from primary breast carcinoma?**

Metastatic cancer from the gastrointestinal tract to the breast is most likely to arise from the stomach [4, 11, 15, 20], colon (including carcinoid) [1, 5, 20], and small bowel (including carcinoid and melanoma) [2, 11], although cases arising from the pancreas [1, 4], esophagus [2], liver (including carcinoid) [1, 20], appendix (carcinoid) [2, 23], and biliary tract [20] have also been reported. Patients that have a new breast cancer diagnosis with abdominal complaints, difficulty swallowing (particularly with esophageal carcinoma), jaundice (biliary tract or liver), abdominal ascites, or blood in the stool should raise consideration for gastrointestinal involvement.

13. **How might a metastatic neuroendocrine tumor present differently from primary breast carcinoma or a primary breast neuroendocrine tumor?**

Any neuroendocrine carcinoma in the breast should raise consideration for metastatic tumor to the breast, particularly from a pulmonary origin. A thorough pulmonary history and exam should be done, with consideration for radiographic imaging. Symptoms of carcinoid syndrome (facial flushing, severe diarrhea, wheezing, and tachycardia) should draw suspicion to the possibility of metastatic disease, as the gastrointestinal tract (including colon, liver, small bowel, and appendix) has been documented as an origin of metastatic neuroendocrine disease to the breast [1, 2, 11, 16, 23, 32]. Neuroendocrine tumor has also been reported to have metastasized to the breast from the cervix [1].

14. **How might a metastatic head and neck carcinomas present differently from primary breast carcinoma?**

The most common metastatic head and neck tumor reported to metastasize to the breast is thyroid carcinoma [1, 2, 4, 11, 20]. Submandibular gland (adenoid cystic and salivary duct carcinomas) [1, 45] and tongue (squamous cell carcinoma) [1] origins have also been reported. A rapidly growing lump in the neck, generalized neck swelling, pain in the front of the neck (sometimes going up to the ears), hoarseness, trouble swallowing or breathing, or a chronic cough may all be signs of head and neck involvement.

15. **How might a metastatic sarcoma present differently from primary breast carcinoma or a primary breast sarcoma?**

Any sarcoma in the adult breast is likely a malignant phyllodes tumor, sarcoma arising in the post-irradiation breast, or other primary breast sarcoma. Still, any sarcoma in the breast should raise consideration for metastatic tumor to the breast, as primary breast sarcomas are rare [1] (although metastatic sarcoma is even less common [46]). Clinical history and exam are essential. If the sarcoma is present in a pediatric patient, the literature would support a metastatic origin until proven otherwise, as the proportion of metastatic tumor to the breast in children and young adults is significantly higher [2, 13, 15].

16. **How might a metastatic hematopoietic tumor present differently from primary breast carcinoma or a primary breast hematopoietic tumor?**

Any diagnosis of lymphoma in the breast should raise consideration for secondary involvement. Primary breast lymphoma is rare, accounting for less than 1% of all patients

with non-Hodgkin lymphoma and approximately 1.7% of all patients with extralymphatic non-Hodgkin lymphoma [47]. A primary diagnosis is even less common in men [48, 49]. The clinical criteria for primary breast lymphoma have been previously defined by Wiseman and Liao [50]. The absence of mammary tissue in close association with lymphomatous infiltrates after adequate pathologic evaluation, evidence of disseminated lymphoma other than simultaneous ipsilateral lymph node involvement, or any prior diagnosis of lymphoma would all suggest secondary involvement.

17. **What are the typical gross characteristics of metastatic cancer to the breast versus a cancer of primary breast origin?**

Metastatic cancer to the breast is typically diagnosed by core needle biopsy and not excised, and therefore, gross examination is often not done. Even still, there are no specific gross characteristics that would raise suspicion for a metastatic cancer to the breast, except for the black pigmentation that might be present in a metastatic melanoma [16].

18. **How do metastatic melanomas histologically differ from primary breast carcinoma or a primary breast melanoma?**

The wide range of morphologic appearances in malignant melanoma makes it particularly difficult to recognize secondary breast melanoma as a metastatic disease. Histologic clues that might raise suspicion for a melanoma as opposed to a carcinoma include high-grade dyscohesive epithelioid cells (although lobular carcinoma can similarly present; see Case 4 at the end of this chapter), intranuclear inclusions, spindled cells, and cytoplasmic pigment. Clinical examination would then be of primary importance in differentiating primary from secondary disease, as histological differentiation between the two would be unlikely, unless there is an in situ component (Fig. 10.2), which would support a primary breast origin.

19. **How do metastatic pulmonary carcinomas histologically differ from primary breast carcinoma?**

If a breast cancer patient presents with a neuroendocrine histology, consideration should be given for a pulmonary primary origin. A review of the literature suggests that anywhere from 30% to 50% of primary lung tumors that metastasize to the breast have neuroendocrine features, either atypical carcinoid, small cell neuroendocrine, or large cell neuroendocrine [1, 2, 4, 15]. Other presentations include squamous cell, which should also bring up consideration for a primary pulmonary origin particularly

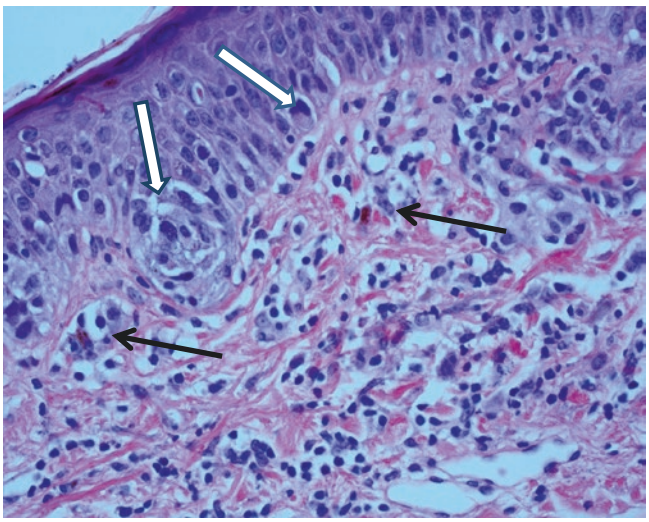

Fig. 10.2 Primary breast melanoma (40×). This patient presented with a breast lesion without any prior history of melanoma. Melanoma is present in the dermis (black arrow). The presence of an in situ component (white arrow) supports a melanoma of primary breast origin

if there is keratinization, and adenocarcinoma. Although a distinctive histology may not be evident with an adenocarcinoma (Fig. 10.3a, b), morphologic clues such as an acinar growth pattern or mucin-secreting columnar cells may be present [2].

20. **How do metastatic gynecological tract carcinomas histologically differ from primary breast carcinoma**?

Serous carcinoma, the most common type of metastatic gynecological tract tumor to the breast [1, 2, 16, 42–44], will often present with a papillary architecture, thus providing a histologic clue for the consideration of metastatic disease. A solid growth pattern may, however, be the dominant histology, making the diagnosis less evident (Fig. 10.4; Case 2 at the end of this chapter). Similar to breast carcinoma, serous carcinoma may present with calcifications [1, 2]; however, the calcifications are typically larger and of the psammomatous type [1, 3], and that histology should prompt consideration for a metastatic gynecological tract carcinoma. Metastatic endometrial serous [15], endometrial endometrioid adenocarcinoma [1, 4, 15], cervical (includes a carcinoid) [1, 11, 15], and choriocarcinoma [1, 15] have also been reported. These may have "unusual" histologic patterns such as well-differentiated glands with pseudostratified tall columnar cells, possibly with mucin (endometrioid adenocarcinoma), a neuroendocrine pattern (carcinoid), or a biphasic syncytial growth pattern resembling multinucleated and mononucleated trophoblastic cells, such as might be seen in a choriocarcinoma.

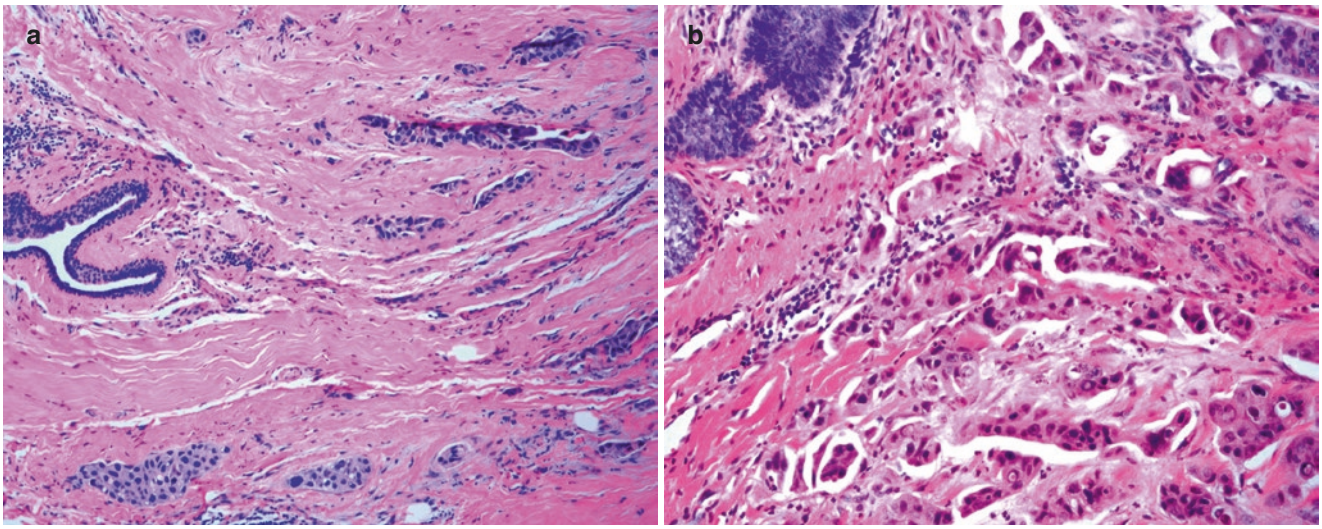

Fig. 10.3 (**a**) Primary breast carcinoma involving the dermis (20×). (**b**) Metastatic lung adenocarcinoma involving the dermis of the breast (40×). Note the similar histology in (**a** and **b**), including squamoid nests with high-grade nuclei present in the dermis of the breast. No clinical history was given on the requisition form. A high degree of suspicion would be necessary for consideration of metastatic disease in this case

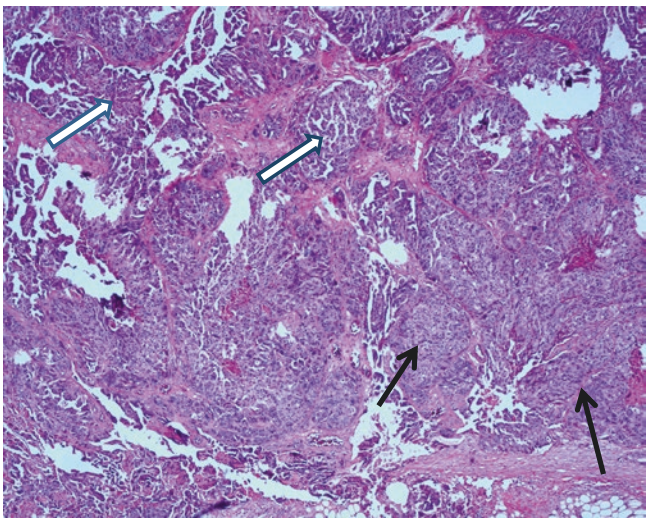

Fig. 10.4 Metastatic ovarian carcinoma involving the breast (10×). This tumor has varying morphology, including both solid and papillary architectures. The papillary architecture (white arrows) is classic for serous ovarian carcinoma; however, a solid growth pattern may be the dominant histology, as in this case (black arrows). Note that no calcifications were evident on microscopic review

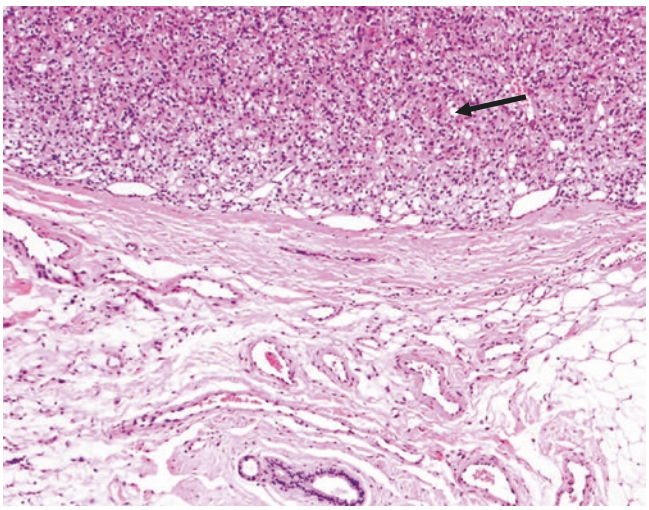

Fig. 10.5 Metastatic renal cell carcinoma to the breast (10×). The clear cell morphology (arrow) should prompt consideration for metastatic disease; however, focal clear cell change can also be seen in breast carcinoma, so clinical history is of vital importance. (Courtesy of Dr. David Hicks, University of Rochester, Rochester, NY)

21. How do metastatic genitourinary carcinomas histologically differ from primary breast carcinoma?

Metastatic cancer from the kidney, bladder, or prostate (men) may have distinctive histologic patterns, creating suspicion for a metastatic disease. Conventional renal cell carcinoma is the most common renal malignancy likely to metastasize to a wide range of sites [2, 51]. Diffuse clear cell change (Fig. 10.5), unlikely to be seen in primary breast carcinoma (likely to be more patchy, if present at all), should prompt consideration for a metastatic disease. Both bladder and prostate cancers have histology that overlaps with primary breast carcinoma [2, 15]. A diffuse transitional cell pattern may signal the possibility of bladder cancer and should prompt consideration for a thorough evaluation of the clinical history. A metastatic bladder cancer will likely present with symptoms or history consistent with primary bladder carcinoma. Prostate cancer may have low-grade morphology with columnar cells containing a nucleolus [2, 16]. Any male

with breast cancer should be considered to have metastatic disease until proven otherwise.

22. **How do metastatic gastrointestinal tract carcinomas histologically differ from primary breast carcinoma?**

Metastatic cancer from the gastrointestinal tract may have an intestinal or signet ring pattern (particularly stomach), causing confusion with either ductal or lobular carcinoma, respectively. Columnar mucin–secreting cells favor a gastrointestinal origin [2] as opposed to primary breast ductal carcinoma (Fig. 10.6a, b). Signet ring cells with diffuse foamy cytoplasm are more common in gastrointestinal carcinoma, while signet ring cells with distinct vacuoles with a central mucin dot would be more common in primary lobular carcinoma [16]. A hepatoid pattern consisting of sheets and cords of polygonal cells with abundant eosinophilic cytoplasm might be a useful clue in considering a hepatic metastasis. A thorough clinical history and exam would be particularly critical in detecting a metastatic tumor from the pancreas, esophagus, or biliary tract.

23. **How do metastatic neuroendocrine tumors histologically differ from primary breast carcinoma or a primary breast neuroendocrine tumor**?

Although neuroendocrine differentiation can be seen in up to 30% of invasive breast carcinomas (most commonly associated with mucinous and solid papillary carcinomas), primary neuroendocrine tumors of the breast are at best rare [52], and their existence is controversial [16]. The most common metastases occur from gastrointestinal (including colon, liver, small bowel, and appendix) and lung primaries [1, 2, 4, 11, 16, 23, 29, 32]. Recognizing the characteristic neuroendocrine histologic features of mitotically active sheets of cells with scant cytoplasm and speckled chromatin, lack of prominent nucleoli, and the possibly associated crush artifact or necrosis are the first steps in considering the presence of a neuroendocrine tumor as opposed to a carcinoma (Figs. 10.7, and 10.8). Histologic clues differentiating a primary and metastatic neuroendocrine tumor are not likely to be apparent, with the exception of recognizing the presence of ductal

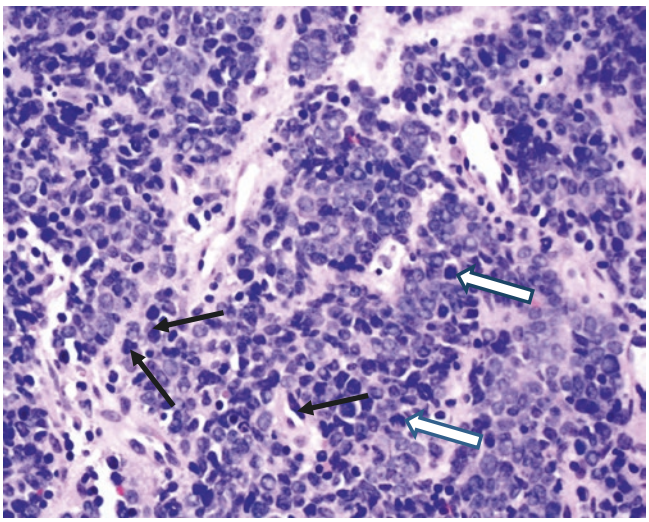

Fig. 10.7 Metastatic neuroendocrine carcinoma (40×). Classic high-grade neuroendocrine features are present including cohesive nests of tumor cells with a high nuclear–cytoplasmic ratio, nuclear molding (black arrows), and numerous mitoses (white arrows). The nuclear chromatin is finely stippled, with inconspicuous nucleoli

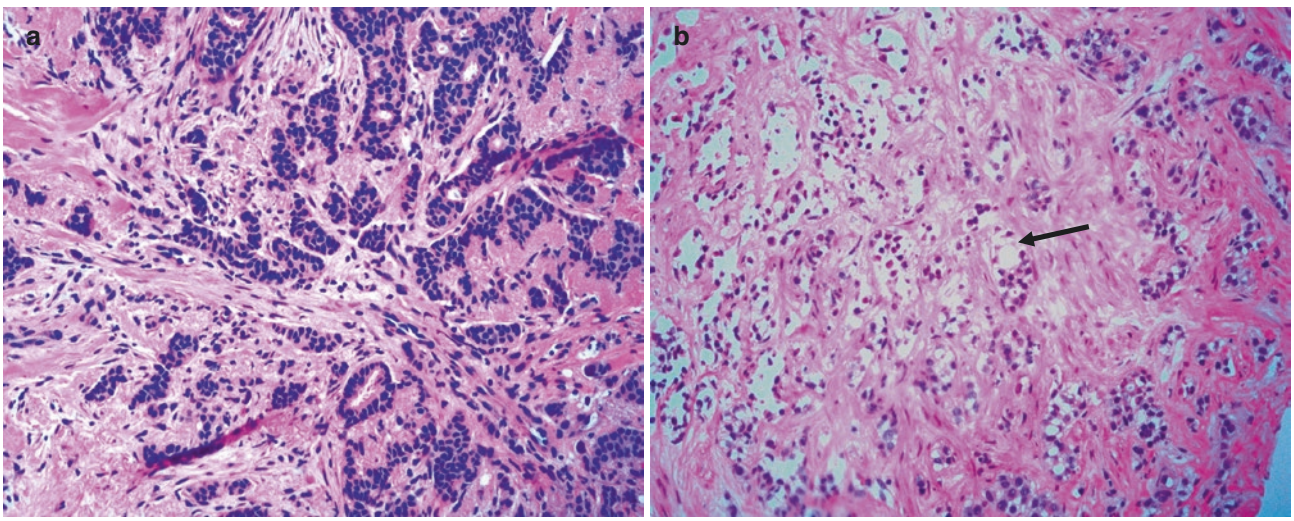

Fig. 10.6 (**a**) Primary breast carcinoma (20×). (**b**) Metastatic gastric adenocarcinoma involving the breast (20×). Both carcinomas are relatively well differentiated. The mucin secreting cells (**b**, arrow) are a clue to the possibility of metastatic disease. Also see Fig. 10.20.

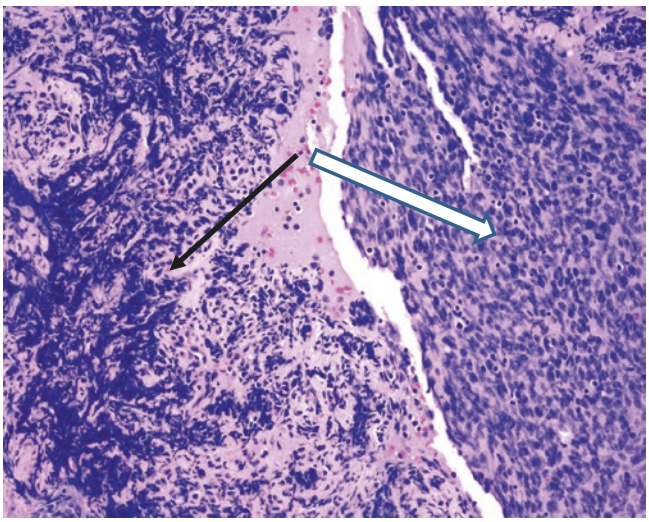

Fig. 10.8 Metastatic neuroendocrine carcinoma with crush artifact (20×). Crush artifact (black arrow), which results in artificial elongation and distortion of cells with subsequent spillage of cytoplasmic contents into the stroma, is present. Crush artifact in association with more classic neuroendocrine morphology (white arrow) should prompt consideration for a neuroendocrine tumor

carcinoma in situ (DCIS), which would favor a primary breast origin.

24. **How do metastatic head and neck carcinomas histologically differ from primary breast carcinoma**?

Thyroid carcinoma may have a variety of patterns including papillary, follicular, insular (medullary), tall cell, diffuse sclerosing type, and papillary with Hashimoto's thyroiditis. All of these patterns are unusual for primary breast carcinoma and should raise suspicion for the possibility of a metastatic focus. The presence of colloid may also be a useful clue. Salivary duct carcinoma may present a challenge as there is significant histological and immunophenotype overlap. A diffuse invasive (i.e., lack of myoepithelial cells) "DCIS pattern" with associated necrosis should prompt an evaluation of any abnormalities on the head and neck examination.

25. **How do metastatic sarcomas histologically differ from primary breast carcinoma or a primary breast sarcoma?**

The most common pitfall would be mistaking a sarcoma for a carcinoma, so recognition of characteristic morphology is critical. A spindled cell or mesenchymal morphology should prompt consideration for a sarcoma. Differentiating a primary from a secondary sarcoma is more challenging. Sarcoma in the breast is most commonly associated with a component of metaplastic carcinoma or phyllodes tumor, so a thorough sampling looking for areas of conventional carcinoma or classic phyllodes morphology (i.e., leaf-like areas) is critical. The most common sarcoma metastatic to the breast in adults is a uterine leiomyosarcoma [1]. A number of other subtypes have been reported, including rhabdomyosarcoma [1, 13, 15] (the most common pediatric sarcoma metastatic to the breast), liposarcoma [1, 15], non-uterine sarcoma [1, 2], Ewing sarcoma [1], malignant fibrous histiocytoma (pleomorphic sarcoma) [1, 15], angiosarcoma [1, 15], synovial sarcoma [1], dendritic cell sarcoma [1], and myxofibrosarcoma [1].

26. **How do metastatic hematopoietic tumors histologically differ from primary breast carcinoma or primary breast hematopoietic tumors**?

As with sarcomas, the most common pitfall would be mistaking a hematopoietic tumor for a carcinoma, so recognition of characteristic morphology is again critical (Fig. 10.9). The most common type of hematopoietic tumors in the breast, primary or secondary, is a lymphoma, most often a non-Hodgkin diffuse large B-cell lymphoma [2, 28, 47, 53]. Non-Hodgkin T cell lymphoma, Hodgkin lymphoma, and Sézary syndrome have also been reported [15, 46, 54]. These tumors are typically high grade. In non-Hodgkin lymphoma, the malignant cells are most commonly dyscohesive and centroblastic, less often immunoblastic. Hodgkin lymphoma may offer histologic clues, including Reed–Sternberg cells with abundant basophilic or amphophilic cytoplasm and binucleate or bilobed nucleus, with multilobate or large inclusion-like eosinophilic nucleoli. A rich inflammatory background may be present. Leukemia and multiple myeloma have also been reported in the breast [47]. Leukemia may present with blasts, and myeloma may present with lobulocentric cells that have plasmacytic morphology, which should prompt consideration for a hematopoietic origin.

27. **Can immunohistochemistry be helpful in distinguishing primary breast carcinoma from an extramammary malignancy metastatic to the breast?**

The use of a broad panel of antibodies can be helpful in distinguishing primary breast carcinoma from an extramammary malignancy metastatic to the breast. Table 10.3 outlines immunohistochemical panels that may be helpful in characterizing a primary breast carcinoma from a metastatic tumor to the breast. Breast carcinomas are typically cytokeratin positive. Exceptions include CK20 and CK5, although positive staining for CK5 can be seen in the basal subtype, myoepithelial carcinoma, and sarcomatoid carcinoma (carcinosarcoma, spindle cell carcinoma, and metaplastic carcinoma) [55]. p63 will be negative with rare exceptions, including myoepithelial carcinoma (occasionally) and sarcomatoid carcinoma (Fig. 10.10a, b) [55]. Gross cystic disease fluid protein fraction-15 (GCDFP-15) has a fairly high speci-

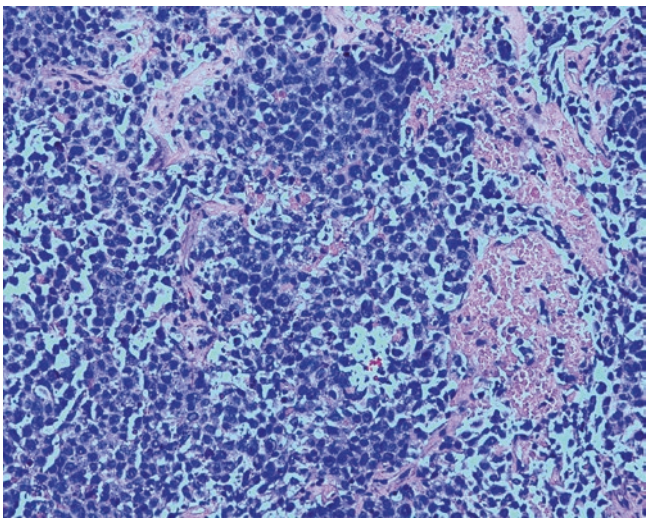

Fig. 10.9 B-cell lymphoma involving the breast (20×). This tumor presented in the right axilla as a mass and was initially thought to be a metastatic breast or uterine primary (a history of uterine carcinoma was given.) Note the "nests" of cells with epithelioid morphology. A carcinoma workup revealed negative immunohistochemistry for GATA-3 and PAX-8. Flow cytometry was negative (as it can be in 20–30% of lymphomas); however, a closer review of the histomorphology reveals the somewhat dyscohesive nature of these "nests" of epithelioid cells. Immunohistochemistry for CD45 was strongly positive, supporting the diagnosis of lymphoma. Also see Fig. 10.21

ficity (although low sensitivity) for breast carcinoma, although it should be remembered that salivary duct carcinoma will also stain positive for this marker. Mammaglobin A may also be useful, although the sensitivity is variable and the specificity for breast has not been firmly established [55]. Positive staining for ER and PR can be extremely helpful in making a diagnosis of breast carcinoma, because with the exception of gynecological (ovary and endometrial) carcinomas, most malignancies will be negative for ER and PR. It must be remembered, however, that ER and rarely PR staining has been reported in carcinomas of the lung, stomach, and thyroid [55]. GATA-3 is sensitive but not entirely specific for breast carcinoma, as it will often stain positive for urothelial carcinoma, and has also been reported to have more than infrequent positive staining in squamous cell carcinomas, mesotheliomas, salivary gland carcinomas, choriocarcinomas, chromophobe renal cell carcinomas, and pancreatic adenocarcinomas [56]. However, GATA-3 has infrequent labeling (<10%) in other common types of adenocarcinomas including those of pulmonary, gastrointestinal, and gynecologic origin [56]. A more detailed discussion of the immunohistochemical profile of the more common extramammary malignancies metastasizing to the breast can be found in questions 28–36.

Table 10.3 Immunohistochemical profiles of breast carcinoma and the most common non-breast tumors metastasizing to the breast

	Immunohistochemistry									
Type of malignancy	CK7	CK5	CK20	p63	GATA-3	TTF-1	CDX-2	CD45	S-100	Melan-A
Breast	*L*[a]	*U*[b,d]	*U*	*U*	*L*	*U*	*U*	*U*	*O*[e]	*U*
Melanoma	*U*	*U*	*U*	*U*	*U*	*U*	*U*	*U*	*L*[f]	*L*
Pulmonary										
Adenocarcinoma	*L*	*U*	*U*	*U*	*U*	*L*	*U*	*U*	*U*	*U*
Squamous	*U*	*L*	*U*	*L*	*O*[c]	*U*	*U*	*U*	*U*	*U*
Gynecological	*L*	*U*	*U*	*U*	*U*[g]	*U*	*U*	*U*	*U*	*U*
Genitourinary										
Urothelial	*L*	*U*	*L*	*U*	*L*	*U*	*U*	*U*	*U*	*U*
Chromophobe RCC	*L*	*U*	*U*	*U*	*L*	*U*	*U*	*U*	*U*	*U*
Clear cell RCC	*U*	*U*	*U*	*U*	*U*	*U*	*U*	*U*	*U*	*U*
Prostate	*U*	*U*	*U*	*U*	*U*	*U*	*U*	*U*	*U*	*U*
Gastrointestinal	*U*[h]	*U*	*L*[i]	*U*	*U*	*U*	*L*	*U*	*U*	*U*
Neuroendocrine	*U*	*U*	*U*	*U*	*U*	*L*[j]	*L*[k]	*U*	*U*	*U*
Head and neck										
Thyroid	*U*	*U*	*U*	*U*	*U*	*L*	*U*	*U*	*U*	*U*
Salivary duct	*L*	*U*	*U*	*U*	*L*	*U*	*U*	*U*	*L*	*U*
Sarcoma	*U*	*U*	*U*	*U*	*O*	*U*	*U*	*U*	*L*	*U*
Hematopoietic	*U*	*U*	*U*	*U*	*U*[l]	*U*	*U*	*L*	*U*	*U*

[a]*L* Likely positive
[b]*U* Unlikely to be positive
[c]*O* Occasionally positive
[d]Likely weak scattered
[e]Strong diffuse
[f]May be positive in the basal subtype, myoepithelial carcinoma, and sarcomatoid carcinoma (carcinosarcoma, spindle cell carcinoma, and metaplastic carcinoma)
[g]Expressed in a majority of trophoblastic (choriocarcinoma) and yolk sac tumors
[h]Variable patterns in gastric carcinoma; occasionally will stain positive in pancreatic carcinoma
[i]Variable patterns with gastric carcinoma; negative in hepatocellular carcinoma
[j]Positive staining observed in neuroendocrine tumors of pulmonary origins
[k]Positive staining observed in neuroendocrine tumors of gastrointestinal origins
[l]Positive staining has been reported in Hodgkin lymphoma

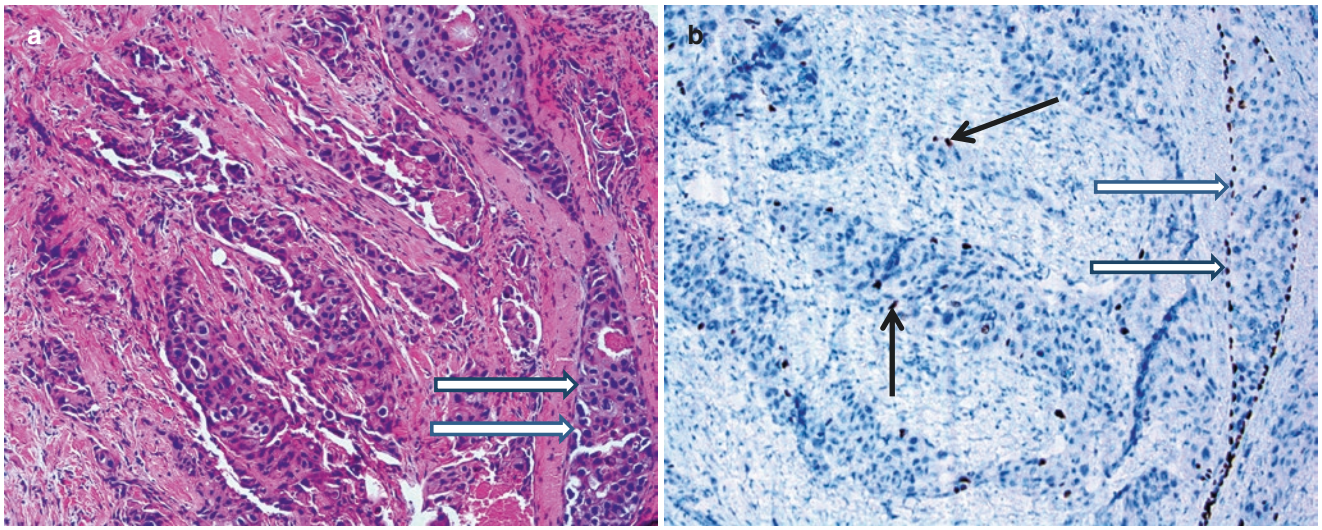

Fig. 10.10 (**a**) High-grade primary breast carcinoma with metaplastic features (20×). (**b**) p63 immunohistochemistry in high-grade primary breast carcinoma with metaplastic features (20×). Although p63 will likely be negative in invasive breast carcinoma, focal positive staining can be seen in some high-grade invasive carcinomas, myoepithelial carcinoma, and sarcomatoid carcinoma (carcinosarcoma, spindle cell carcinoma, and metaplastic carcinoma). This high-grade tumor had metaplastic features with scattered p63 staining in the invasive component (**b**, black arrow). Note the adjacent DCIS, with the expected positive p63 staining in the myoepithelial cell layer (white arrow in **a** and **b**). See also Fig. 10.22a, b

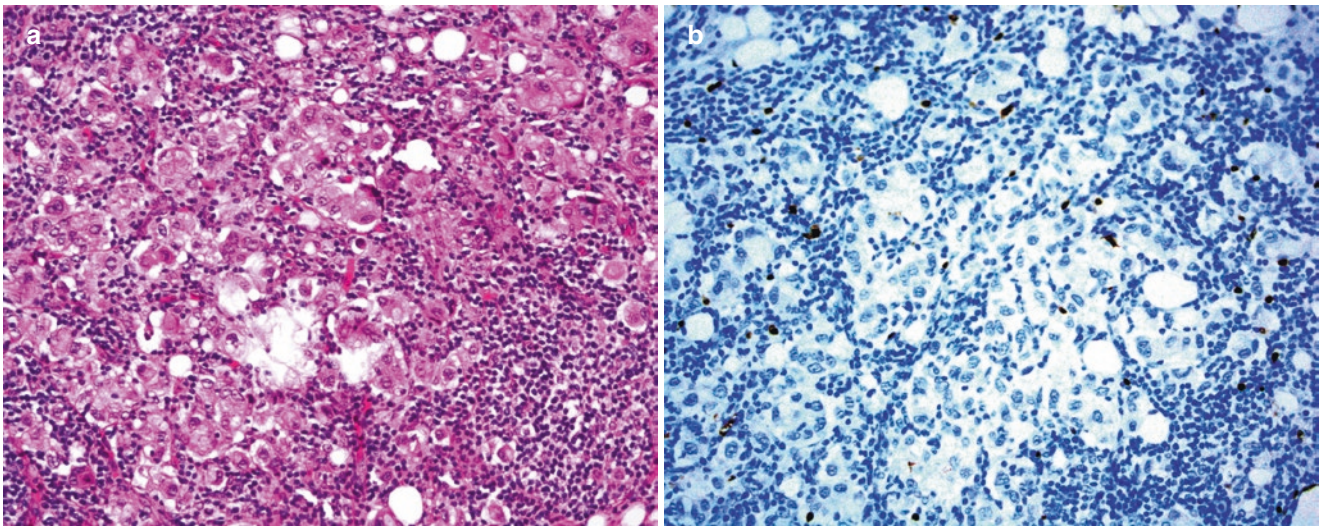

Fig. 10.11 (**a**) Pleomorphic lobular primary breast carcinoma (40×). (**b**) S-100 immunohistochemistry of pleomorphic lobular primary breast carcinoma (40×). Note the similar high-grade appearance of this tumor (**a**) in comparison to the metastatic melanoma in Fig. 10.12a. S-100 staining (**b**), which will likely be more focal and weak in primary breast carcinoma, supports a diagnosis of a primary breast origin

28. **How is metastatic melanoma immunophenotypically differentiated from primary breast carcinoma or a primary breast melanoma?**

Expression of GATA-3, ER, PR, GCDFP-15, and mammaglobin would favor a primary breast carcinoma. Breast carcinomas are not likely to stain for melanoma markers (Melan-A, HMB-45, microopthalmia transcription factor), with the exception of S-100, although S-100 will likely be more focal and weak in breast carcinoma (Fig. 10.11a, b, Case 4 at the end of this chapter), as opposed to more diffuse stronger staining in melanoma (Fig. 10.12a, b, Case 4 at the end of this chapter). Melanoma can show aberrant expression for cytokeratins, particularly CAM 5.2, and epithelial membrane antigen (EMA) [2, 57], both of which are also typically positive in breast carcinoma (Case 5 at the end of this chapter). As such, these markers should not be used if melanoma is being considered. CK7, which is typically positive in breast carcinoma and unlikely to be positive in melanoma, would be the cytokeratin of choice. Clinical history

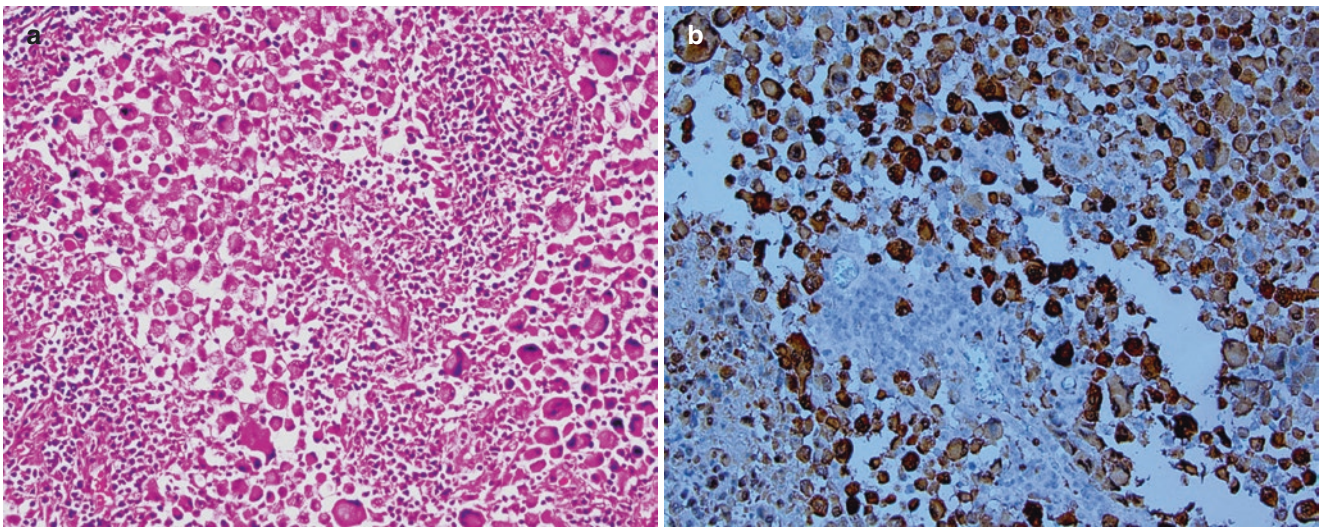

Fig. 10.12 (**a**) Metastatic melanoma to the breast (40×). (**b**) S-100 immunohistochemistry of metastatic melanoma to the breast (40×). Compare (**a**) to Fig. 10.11a. Metastatic melanomas may have high-grade dyscohesive epithelioid cells (**a**) with similar characteristics to a high-grade pleomorphic lobular carcinoma (Fig. 10.11a). Strong diffuse staining with S-100 (**b**) supports a diagnosis of metastatic melanoma

would be essential for differentiating a primary breast melanoma from a metastatic melanoma.

29. **How is metastatic pulmonary carcinoma immunophenotypically differentiated from primary breast carcinoma?**

Expression of GATA-3, ER, PR, GCDFP-15, and mammaglobin would favor a primary breast carcinoma. Less than 5% of breast carcinomas are positive for TTF-1 (Fig. 10.13a, b) or Napsin-A (Fig. 10.13a, c) [57, 58]. TTF-1 (Fig. 10.14a, b) and Napsin-A (Fig. 10.14a, c) are positive in approximately 75% [2] and 85% [57, 58] of pulmonary adenocarcinomas, respectively. Primary pulmonary large cell carcinoma may be less likely to express TTF-1 [59] and may be difficult to distinguish from a poorly differential breast carcinoma. A primary squamous carcinoma of the lung would be difficult to differentiate from a primary breast squamous cell carcinoma, as both will stain positive for p63 and CK5, and possibly GATA-3 [60]. Primary squamous cell carcinoma of the breast is much less common, making the clinical history essential. Similar difficulty is experienced in differentiating primary breast small cell carcinoma from a metastasis. About 80% of small cell carcinomas are positive for TTF-1, regardless of the primary location, again making the clinical history essential. GATA-3-positive staining has also been reported in mesothelioma [60].

30. **How is metastatic gynecological tract carcinoma immunophenotypically differentiated from primary breast carcinoma?**

Expression of GATA-3, GCDFP-15, and mammaglobin would favor a primary breast carcinoma, although GATA-3 staining may be seen in approximately 5% of gynecologic carcinomas (endometrial) [16] and in a majority of trophoblastic (choriocarcinoma) and yolk sac tumors [60]. Most cytokeratins have a similar expression in breast and gynecological tract adenocarcinomas, although EMA has been reported to have a different expression pattern in breast micropapillary carcinoma (Fig. 10.15a, b; expression only on the outside of the papillary clusters) compared to serous carcinoma of the ovary (Fig. 10.16a, b; expression on the outside and in central spaces [2]; Case 2 at the end of this chapter). PAX-8 has been found to be a useful marker, as it will show positive staining in most gynecologic cancers and has not been found to be positive in breast carcinoma [61–63]. WT-1 may be positive in up to 85% of ovarian serous carcinomas (Figs. 10.16a and 10.18, Case 2 at the end of this chapter), but is rarely positive in breast carcinoma (Fig. 10.17a, b), although more than frequent focal weak staining has been reported in mucinous breast carcinoma [1, 2, 16, 62–65]. ER and PR are expressed in both breast and gynecologic tumors of the ovary and endometrium, and are of limited value in differentiating a primary breast carcinoma from a metastatic gynecological tract carcinoma (Fig. 10.18).

31. **How is metastatic genitourinary tract carcinoma immunophenotypically differentiated from primary breast carcinoma?**

Expression of ER, PR, GCDFP-15, and mammaglobin would favor a primary breast carcinoma. GATA-3 expression can be seen in a majority of breast, urothelial, and chromophobe renal cell carcinomas [56, 60], so these genitourinary tract carcinomas must be considered in any GATA-3-positive staining breast carcinoma with unusual morphology (i.e.,

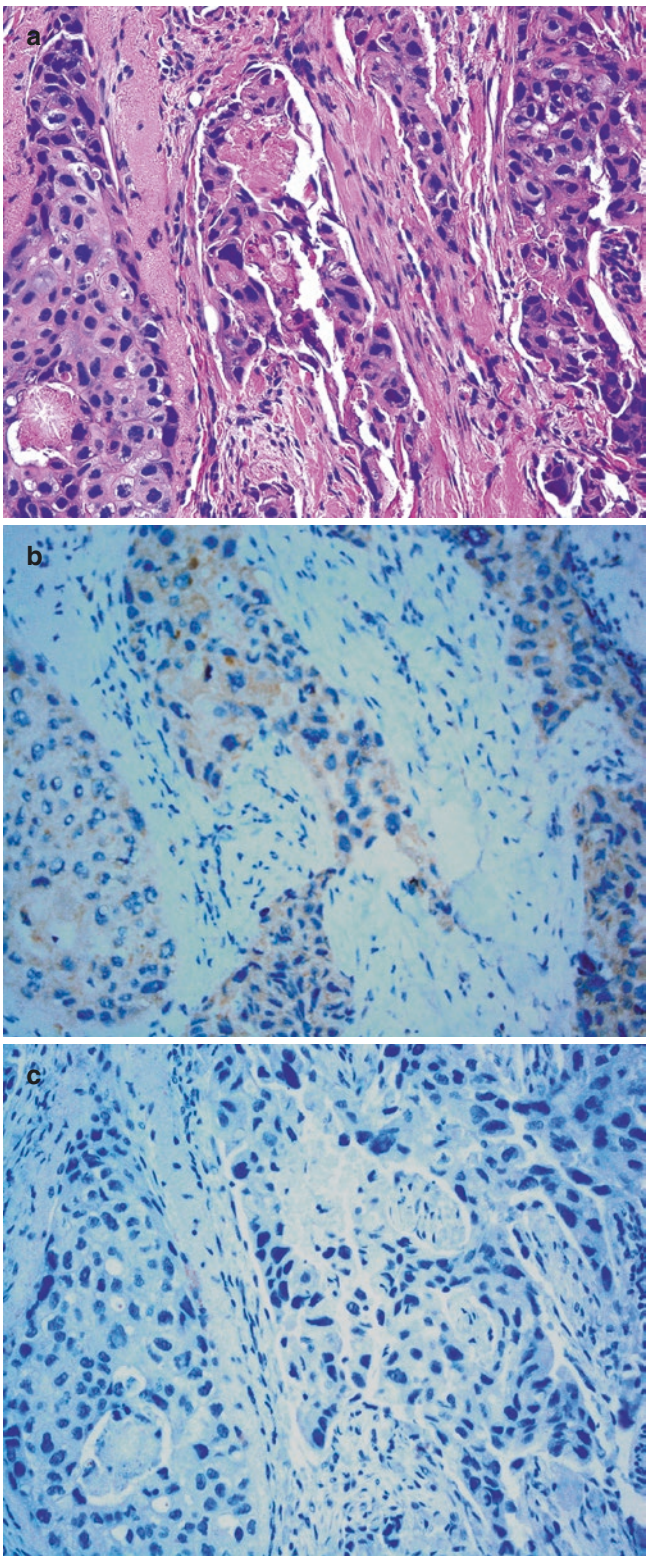

Fig. 10.13 (**a**) Primary breast carcinoma (20×). (**b**) TTF-1 immunohistochemistry of primary breast carcinoma (20×). Negative staining for TTF-1 in primary breast carcinoma. Note the background cytoplasmic blush. Do not mistake this for a positive TTF-1, which must show positive *nuclear* staining. See also Fig. 10.14b, c. Napsin-A immunohistochemistry of primary breast carcinoma (20×). (**c**) Negative staining for Napsin-A in primary breast carcinoma

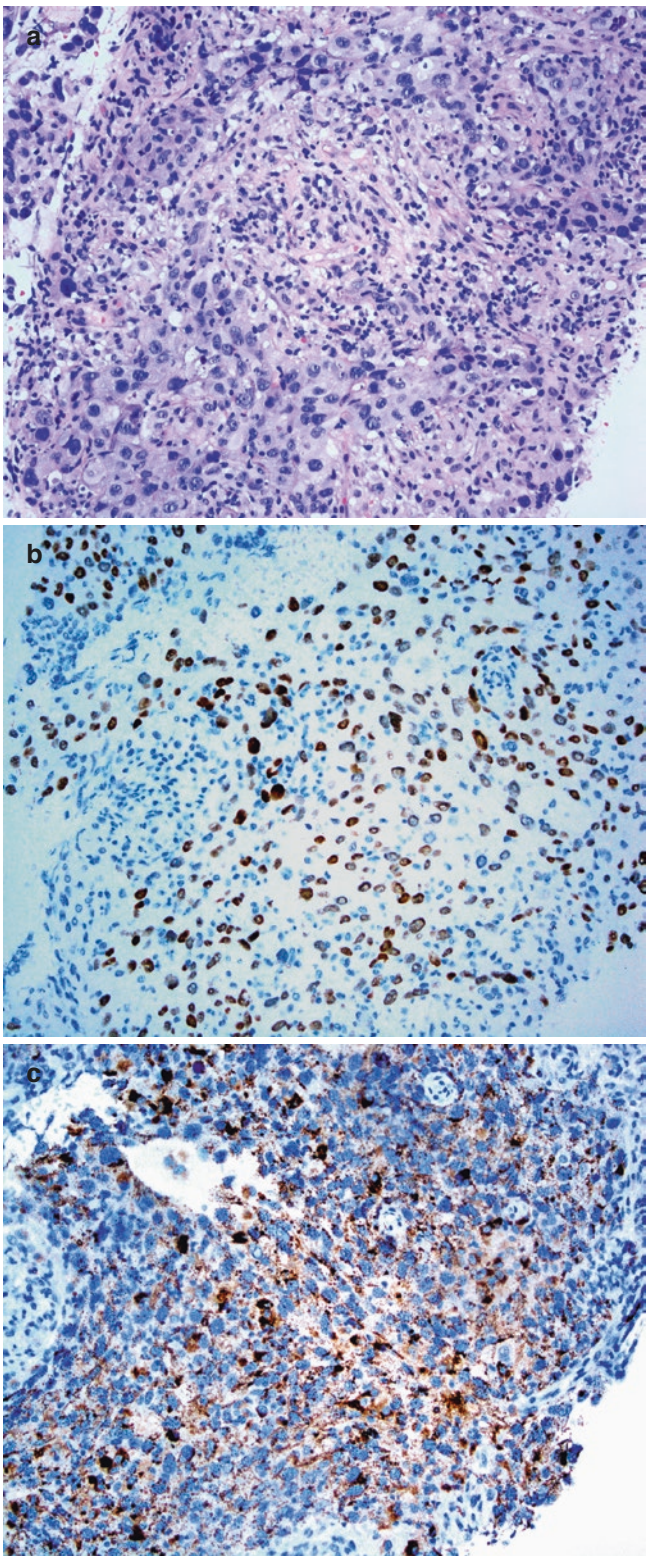

Fig. 10.14 (**a**) Metastatic lung adenocarcinoma involving the breast (20×). (**b**) TTF-1 immunohistochemistry of metastatic lung adenocarcinoma involving the breast (20×). Note the positive nuclear TTF-1 staining. (**c**) Napsin-A immunohistochemistry of metastatic lung adenocarcinoma involving the breast (20×). Note the diffuse strong cytoplasmic and membranous staining with Napsin-A

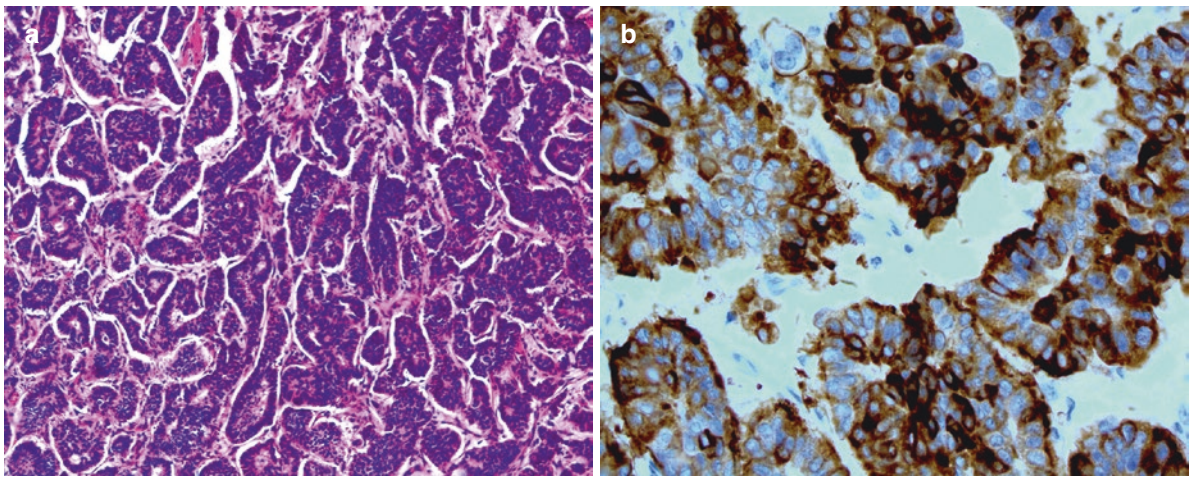

Fig. 10.15 (**a**) Primary breast carcinoma, micropapillary type (20×). (**b**) EMA immunohistochemistry of breast carcinoma, micropapillary type (40×). Note the more distinct expression of EMA (**b**) on the outside of the papillary clusters. Compare to Fig. 10.16b

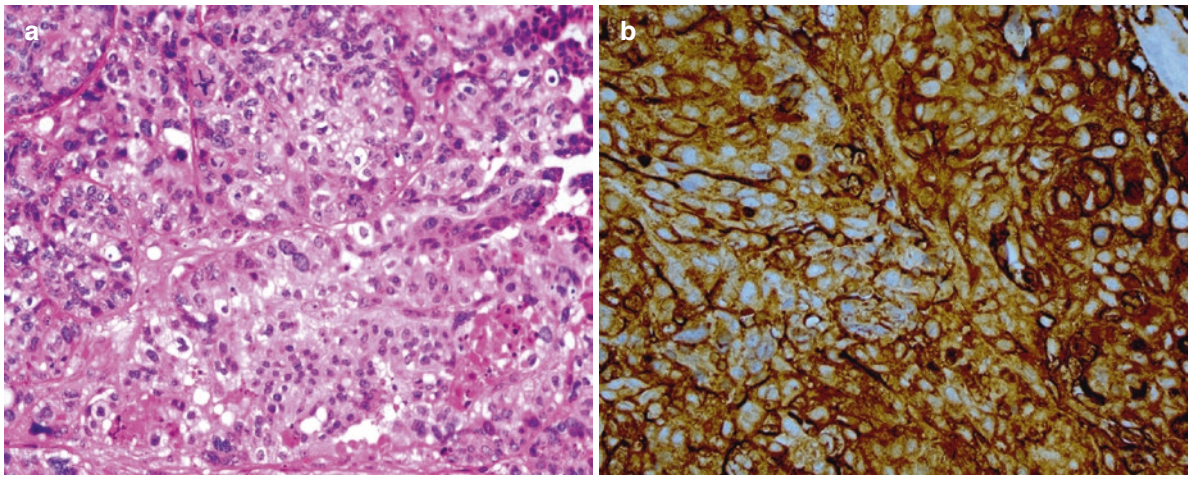

Fig. 10.16 (**a**) Metastatic ovarian serous carcinoma involving the breast (20×). (**b**) EMA immunohistochemistry of metastatic ovarian serous carcinoma involving the breast (20×). Note the strong EMA expression on the outside and in central spaces. Compare to Fig. 10.15b

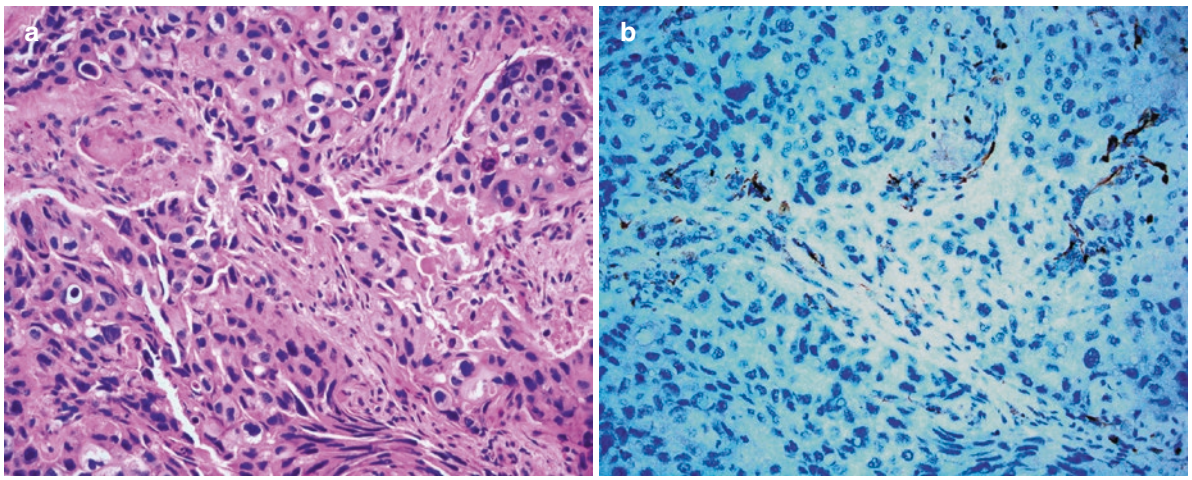

Fig. 10.17 (**a**) Primary breast carcinoma (20×). (**b**) WT-1 immunohistochemistry of primary breast carcinoma (20×). Negative staining for WT-1 in primary breast carcinoma. Do not be fooled by the positive staining in the associated vascular structures

transitional, large polygonal cells with transparent or slightly reticulated cytoplasm with a prominent cell membrane). CK20 will likely be positive in urothelial carcinoma, and a diffuse cytoplasmic staining reaction with Hale's colloidal iron will likely be seen with chromophobe renal cell carcinoma [55]. Clear cell renal carcinoma may express positive staining for CD10, RCC antibody, and vimentin [2, 55], which are unlikely to be expressed in a primary breast carcinoma [2]. Prostate carcinoma will express both prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) in nearly 100% of tumors [1]. Although PAP is not expressed in breast carcinoma, there have been reports of male breast cancer that express PSA [1, 16, 66–68], so both markers should be done if ruling out metastatic prostate carcinoma. Prostate carcinoma is CK7 and CK20 negative.

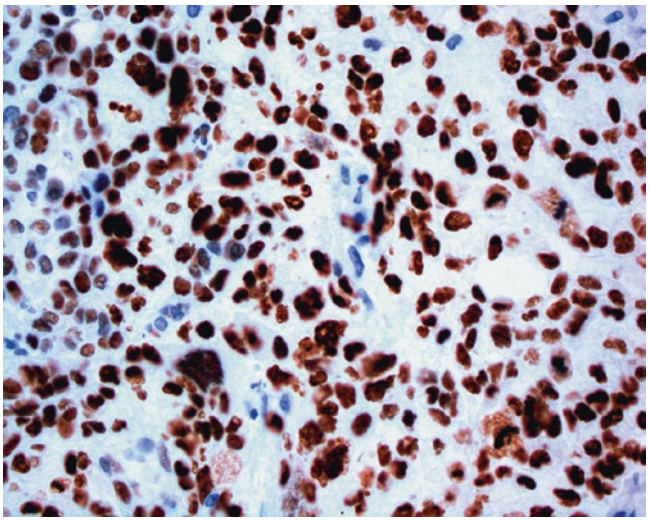

Fig. 10.18 WT-1 immunohistochemistry of metastatic ovarian serous carcinoma involving the breast (40×). Strong WT-1 expression supporting a diagnosis of metastatic ovarian serous carcinoma

32. **How is metastatic gastrointestinal tract carcinoma immunophenotypically differentiated from primary breast carcinoma?**

Expression of GATA-3, ER, PR, GCDFP-15, and mammaglobin would favor a primary breast carcinoma. CDX-2 and CK20 are not typically expressed in breast cancer (Fig. 10.19a, b) but they are expressed in a majority of gastrointestinal carcinomas (Fig. 10.20) [2, 16, 69]. Colorectal carcinoma is typically CK7 negative. A CK7-positive/CK20-negative pattern can be seen in 25% of gastric carcinomas [55], so additional immunohistochemistry should be done in cases of suspected metastatic gastric carcinoma. CK17 stains the majority of pancreatobiliary adenocarcinomas diffusely, but only rarely stains breast carcinoma, and in those breast carcinomas that do stain positive, staining usually only occurs within the centers of solid high-grade breast carcinoma nests [55]. Hepatocellular carcinoma is typically CK7 negative, CK20 negative, and Hepar-1 positive (negative in breast carcinoma).

33. **How is a metastatic neuroendocrine tumor immunophenotypically differentiated from primary breast carcinoma or a primary breast neuroendocrine tumor?**

Expression of GATA-3, ER, PR, GCDFP-15, and mammaglobin would favor a primary breast carcinoma. ER, PR, and

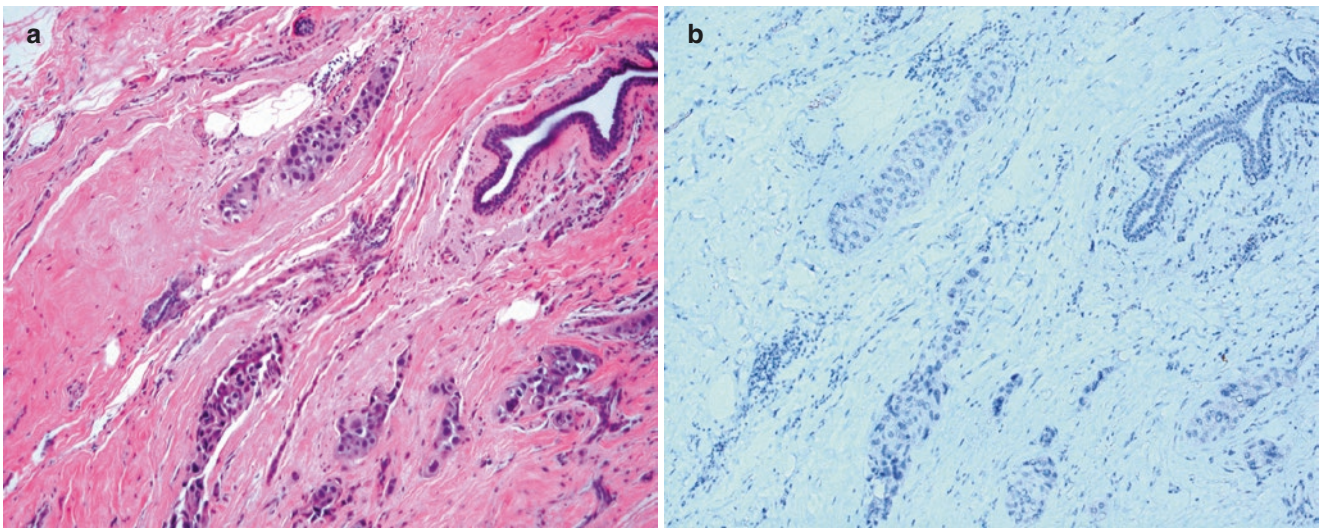

Fig. 10.19 (**a**) Primary breast carcinoma (10×). (**b**) CDX-2 immunohistochemistry of primary breast carcinoma (10×). Negative staining for CDX-2 in primary breast carcinoma. A background cytoplasmic blush may be seen; however, do not mistake this for a positive CDX-2, which must show positive *nuclear* staining. Also see Figs. 10.20 and 10.22a, b

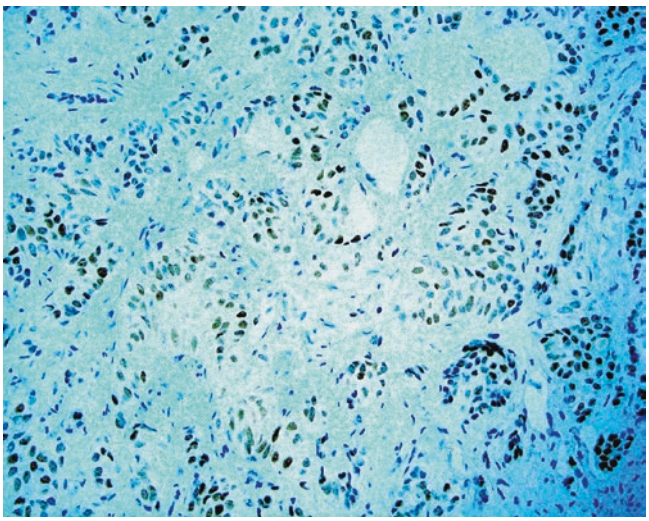

Fig. 10.20 CDX-2 immunohistochemistry of Fig. 10.6b: metastatic gastric adenocarcinoma to the breast (20×). Positive nuclear staining for CDX-2 in metastatic gastric adenocarcinoma involving the breast. See also Fig. 10.19a, b

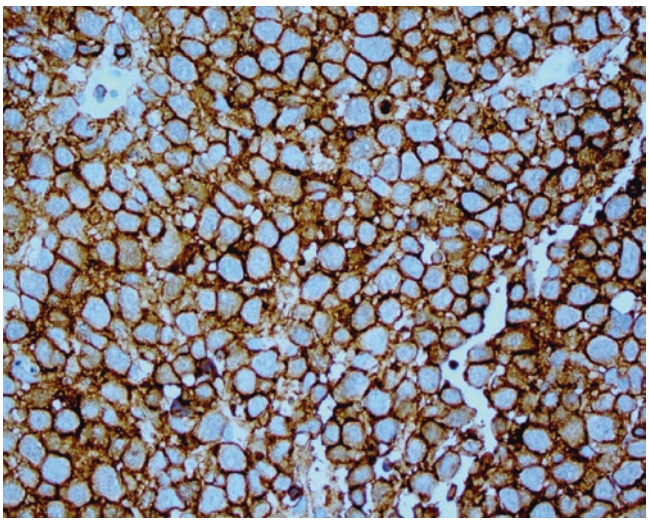

Fig. 10.21 CD45 immunohistochemistry of B-cell lymphoma involving the breast (40×). Strong positive staining for CD45 supports the diagnosis of lymphoma. See also Fig. 10.9

GCDFP-15 are often expressed by breast neuroendocrine carcinomas [2]. Of note, PR is expressed in some pancreatic endocrine tumors [2]. CK7 is typically negative in neuroendocrine tumors but is positive in breast carcinoma. Positive staining for CDX-2 and CK20 would favor a gastrointestinal origin (both carcinoma and neuroendocrine tumor) [2]. Positive staining for TTF-1 would favor a pulmonary origin (both carcinoma and neuroendocrine tumor) [2]. The clinical history would be essential if CDX-2, CK20, TTF-1, ER, PR, and GCDFP-15 were negative.

34. **How is metastatic head and neck carcinoma immunophenotypically differentiated from primary breast carcinoma?**

Expression of GATA-3, ER, PR, GCDFP-15, and mammaglobin would favor a primary breast carcinoma; however, caution must be used when suspicion arises for a metastatic salivary duct carcinoma, which can be positive for Her-2, GCDFP-15, and GATA-3 [45, 60]. Salivary duct carcinoma usually expresses androgen receptor (AR); however, AR can also be present in primary breast carcinoma, although in a lesser proportion of cases [70]. TTF-1 and thyroglobulin can be helpful in distinguishing metastatic thyroid carcinoma (typically positive) from primary breast carcinoma (typically negative).

35. **How is metastatic sarcoma immunophenotypically differentiated from primary breast carcinoma or primary breast sarcoma?**

Sarcoma will typically be negative for cytokeratin stains and carcinomas will typically be negative for vimentin, which will stain positive in sarcomas; however, a metastatic sarcoma will have a similar immunophenotype as a primary breast sarcoma. ER and PR may show scattered positive staining in primary breast sarcomas, but should still prompt a thorough search for a gynecological origin. GATA-3-positive staining has also been reported in sarcomas [60]. Clinical history is most essential in differentiating a primary breast sarcoma from a metastatic sarcoma.

36. **How are metastatic hematopoietic tumors immunophenotypically differentiated from primary breast carcinoma or a primary breast hematopoietic tumor?**

Although GATA-3 staining has been reported in Hodgkin lymphoma [71], the immunophenotype of hematopoietic tumors is otherwise distinctly different from breast carcinoma. A CD45-positive tumor essentially rules out a breast carcinoma (Fig. 10.21). Identifying light chain restriction in the neoplastic plasmacytic cells of myeloma can be helpful; however, clinical history and criteria [50] are most essential in further differentiating a primary breast hematopoietic tumor from a metastatic hematopoietic tumor.

37. **Can molecular profiling be helpful in distinguishing primary breast carcinoma from an extramammary malignancy metastatic to the breast?**

Although the diagnostic workup of any cancer of unknown primary strongly depends on clinical history, gross and morphologic examination, as well as immunohistochemical phenotype, molecular profiling offers a promising diagnostic technique to determine the tissue of origin in patients with carcinoma of unknown primary site [72]. The clinical history

and presentation may bias the diagnostic workup and may influence the choice of immunohistochemistry panel. Immunohistochemistry interpretation is subject to interobserver and intraobserver variability, and in 30% of the cases, the immunohistochemical staining pattern does not result in a conclusive diagnosis [73, 74].

At least three molecular profiling tests are commercially available: (1) Tissue of Origin® (TOO) test (Cancer Genetics, Inc., Rutherford, NJ, USA) [74, 75], (2) bioTheranostics Cancer Type ID (CTID) (Biotheranostics, San Diego, CA, USA) [75, 76], and (3) miRview® mets2 (Rosetta Genomics, Princeton, NJ, USA) [73, 75]. The principle underlying these molecular profiling tests is that different tissue types have distinct RNA profiles. Each test was developed using gene expression profiles [74, 75] from hundreds of different tumors. For each test, subsets of discriminatory genes were identified, and diagnostic algorithms were built for cancer classification. All three tests can use formalin-fixed, paraffin-embedded (FFPE) tissue or cytology specimens [73, 75, 76]. The cost is approximately $3000–$4000 per test [75].

The TOO test was initially validated using a 2000-gene classification model on 462 metastatic, poorly differentiated, or undifferentiated FFPE tumor specimens [74], grouped into 15 classifications [74, 75] in 9 different sites, all of which have been reported to metastasize to the breast. These 9 sites include breast, skin (melanoma), pulmonary (non-small-cell lung), gynecological (ovarian), genitourinary (bladder, kidney, prostate, testis [germ cell]), gastrointestinal (colorectal, gastric, hepatocellular, pancreatic), head and neck (thyroid), soft tissue sarcoma, and hematopoietic (non-Hodgkin lymphoma) [74, 75]. A similarity score (SS) is reported. The higher the SS, the more likely the diagnosis is. As the SS falls, agreement declines until a SS less than 5 rules out that tumor classification with >99% confidence [74, 75]. Most reports provide one highly likely diagnosis and rule out at least 12 tumor classifications [75].

CTID was initially validated using a 92-gene assay on a reference tumor database containing 2206 tumors [75, 76] of over 100 different histological subtypes [76], grouped into 12 different sites, most of which have been reported to metastasize to the breast. These 12 sites include breast, skin (including melanoma, squamous cell, basal cell, and Merkel cell), pulmonary (non-small cell and neuroendocrine), gynecological (ovary, cervix, endometrium, germ cell), gastrointestinal (biliary, esophageal, gastric, colon, small intestine, hepatocellular, neuroendocrine, pancreatic), genitourinary (kidney, prostate, bladder, testis[germ cell]), head and neck (thyroid, salivary gland), soft tissue sarcoma (various subtypes), hematopoietic (Hodgkin lymphoma and non-Hodgkin lymphoma), thymus, adrenal, and brain (various subtypes) [76]. CTID reports one main cancer class (with its probability), any main cancer class with >5% probability, and any other main cancer class with <5% probability, which has been ruled out [75, 76].

miRview mets2 was initially validated using a 64-microarray assay on a reference database of 1282 primary and metastatic tumor samples of 42 tumor types, grouped into 12 different sites [73, 75], most of which have been reported to metastasize to the breast. These 12 sites include breast, skin (including melanoma, squamous cell), pulmonary (non-small cell, neuroendocrine, and mesothelioma), gynecological (ovary, cervix,), gastrointestinal (biliary, esophageal, gastric, hepatocellular, neuroendocrine, pancreatic), genitourinary (kidney, prostate, bladder, testis [germ cell]), head and neck (thyroid), soft tissue sarcoma (various subtypes) hematopoietic (non-Hodgkin lymphoma), thymus, adrenal, and brain (various subtypes) [73]. Each test miRNA profile is subjected to two classification algorithms, which assign a tissue of origin based on the normalized expression of the 64 microRNAs to determine the tissue of origin [73, 75]. The two classification algorithms predict one of the 42 tumor types, and predict one of the following seven tumor classifications [73, 75]: (1) pulmonary (small-cell carcinoma or carcinoid); (2) gastrointestinal (adenocarcinoma of biliary tract or pancreas); (3) genitourinary (testicular germ cell tumor, seminomatous or nonseminomatous); (4) genitourinary (renal cell carcinoma); (5) head and neck (thyroid carcinoma, follicular or papillary); (6) soft tissue sarcoma (Ewing sarcoma, chondrosarcoma, malignant fibrous histiocytoma or fibrosarcoma, osteosarcoma, rhabdomyosarcoma, synovial sarcoma, liposarcoma); and (7) brain (astrocytic or oligodendroglial tumor) [73]. The two classifier predictions are then combined into a single predicted tissue of origin or two different predictions [73]. If the two classifier predictions agree with a high level of confidence, a single predicted tissue of origin is reported. When the two classifier predictions have high degree of certainty regarding the tumor class (e.g., sarcoma), but a low degree of certainty regarding the specific tumor type (e.g., which type of sarcoma), a single predicted tissue of origin is reported in addition to one of the additional seven tumor classifications [73]. When two predictions are reported, they are ordered by the likelihood as estimated by the positive predictive value of each of the answers. When both classifiers exhibit very low confidence in their result, the assay does not generate a result and reports that the microRNA expression pattern of the sample does not match any of the expression patterns in the panel closely enough [73].

Overall, all three tests have a high specificity of ≥99%, and their sensitivity in tumors of known origin ranges from 72% (CTID) to 95% (TOO) [75]. Of significance, sensitivity is often but not always lower in metastases than in primary tumors [75, 77], and profiling may not be helpful in necrotic tumors [75]. Other molecular profiling approaches to identifying cancers of unknown primary have been described.

Some are broad-based assays, classifying all likely tumors, while others are more specific, classifying tumor subsets. Like the previously discussed commercial tests, most of these assays involve pre-specified gene, and have sensitivities of 78–90% [75]. There is a clear and promising role for molecular profiling in the diagnostic workup of extramammary tumors metastatic to the breast. The three tests discussed all have the ability to diagnose the most common origins of metastatic tumors to the breast; however, molecular profiling requires further validation including comparing the performance of molecular profiling with immunohistochemistry in independent tumor sets containing metastases from both known primaries and cancers of unknown primaries. The cost and logistics of integrating molecular profiling must also be considered relative to available therapies and patient outcomes.

38. **What is the prognosis for metastatic breast carcinoma from an extramammary location?**

For patients with a known previous primary origin, the time from initial diagnosis to metastasis to the breast varies from between 1 month and 22 years [1–3, 5, 13, 23, 30, 31], with most patients dying within a year of diagnosis [2, 17–19]. Longer intervals are more likely to be associated with malignant melanoma and ovarian carcinoma [2, 13]. Prognosis for patients with metastases to the breast from an unknown extramammary location is poor, with a median time having been reported as 10–15 months in the two largest series found to date [1, 3]. Other studies have shown similar outcomes [1, 5, 18, 20]. In the largest series found to date, a significantly better survival was observed in patients who had no evidence of other disease at the time of diagnosis, patients with neuroendocrine tumors, and patients who underwent surgical resection for the metastatic tumor. The average interval between an extramammary tumor diagnosis and development of metastatic breast disease is approximately 2–3 years [4, 13, 39].

Case Presentations

Case 1

An 88-year-old woman with a history of bilateral mastectomy for bilateral invasive ductal carcinoma diagnosed 3 years prior and a history of high-grade urothelial carcinoma diagnosed 1 year prior presented with a left axillary tail mass confirmed on ultrasound. A core biopsy was done. *The history of high-grade urothelial carcinoma was provided to the pathologist.* The core biopsy found extensively necrotic poorly differentiated carcinoma (Fig. 10.22a), suggestive of metastasis from urinary bladder, with positive immunohistochemistry for p63 (Fig. 10.22b). The tumor was additionally negative for AE1/AE3, cytokeratin 7, and ER (the previous breast carcinoma was ER positive). Given this immunophenotype and the important provided history of urothelial carcinoma, a diagnosis of metastatic urothelial carcinoma is supported. The patient died 3 months after the diagnosis of breast metastasis.

Case 2

A 47-year-old woman presented with a symptomatic cutaneous lesion on the right breast. The patient had a history of

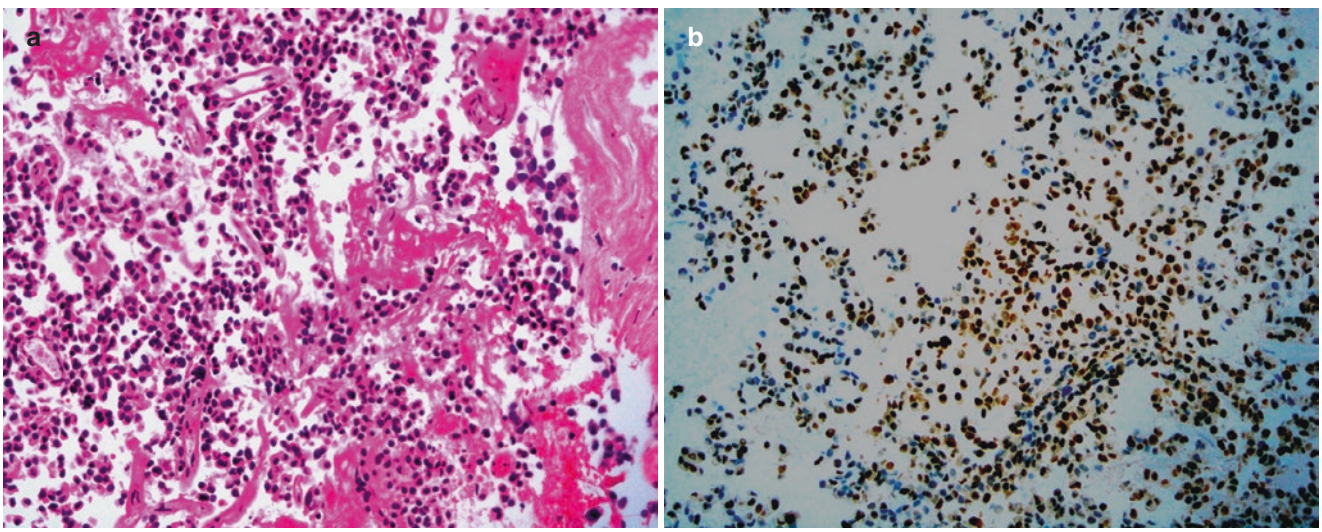

Fig. 10.22 Case 1. (**a**) Metastatic urothelial carcinoma involving the breast (10×). The tumor has extensive necrosis with only focal areas of viable appearing tumor, making it difficult to assess the morphology. (**b**) p63 immunohistochemistry of metastatic urothelial carcinoma involving the breast (10×). The tumor was positive for p63. Focal p63 can be rarely seen in high-grade breast carcinomas (Fig. 10.10b). The diffuse p63 staining in this case should prompt consideration for a metastatic focus. The tumor was negative for CK7 and AE1/AE3, supporting a primary urothelial metastasis

ovarian carcinoma diagnosed 2 years prior. *The history of ovarian carcinoma was provided to the pathologist*. The breast mass was excised and pathology showed a high-grade tumor with both solid and papillary architectures (Figs. 10.4 and 10.16a), suggestive of metastatic ovarian carcinoma. The tumor was positive for WT-1 (Fig. 10.18), with strong EMA expression on the outside and in central spaces (Fig. 10.16b), supporting metastatic ovarian carcinoma. The patient had widespread disease at the time of presentation and died 3 months after the diagnosis of breast metastasis.

Case 3

An 86-year-old man with a history of stage IV right lung adenocarcinoma diagnosed 2 years prior presented with a right axillary mass diagnosed on PET scan. A core biopsy was done. *The history of stage IV lung adenocarcinoma was provided to the pathologist*. The core biopsy was reported as metastatic adenocarcinoma consistent with lung primary (Fig. 10.14), supported by positive immunohistochemistry for TTF-1 (Fig. 10.14b) and Napsin-A (Fig. 10.14c). Although the patient has widespread bone metastasis with persistent malignant pleural effusion, the patient is still alive seven months after the diagnosis of breast metastasis, at the time of this writing.

Case 4

A 68-year-old woman with a history of excised melanoma 3 years prior presented to her physician with a left breast lump. A non-tender lymph node was also palpated by her physician in the right axilla. The patient had overall felt well over the past few months with no current symptoms, except an occasional cough, which she attributed to postnasal drip. The breast lump was verified mammographically and on ultrasound. The axillary lymph node was verified on ultrasound. A core biopsy was done. *The history of melanoma was provided to the pathologist*. The core biopsy found invasive pleomorphic lobular carcinoma in the left breast (Fig. 10.11a), with negative immunohistochemistry for S-100 (Fig. 10.11b). The lymph node was biopsied and verified as melanoma (Fig. 10.12a), after consultation with dermatopathology and positive immunohistochemistry for S-100 (Fig. 10.12b). The patient underwent partial left mastectomy and left sentinel lymph node biopsy with adequate margins, and a right axillary dissection with metastatic melanoma in one of the eleven lymph nodes. The patient was treated with radiation and briefly took hormone therapy. The patient is still alive 5 years after the diagnosis of breast metastasis, with no evidence of recurrent breast cancer or melanoma at the time of this writing.

Case 5

An 82-year-old woman with a history of excised melanoma three and a half years prior presented for a screening mammogram, which identified new right axillary lymphadenopathy confirmed by ultrasound, which also confirmed a breast mass at 9:00 o'clock. *The history of melanoma was not provided to the pathologist.* The core biopsy showed predominantly necrosis with a rim of viable tumor (Fig. 10.23a).

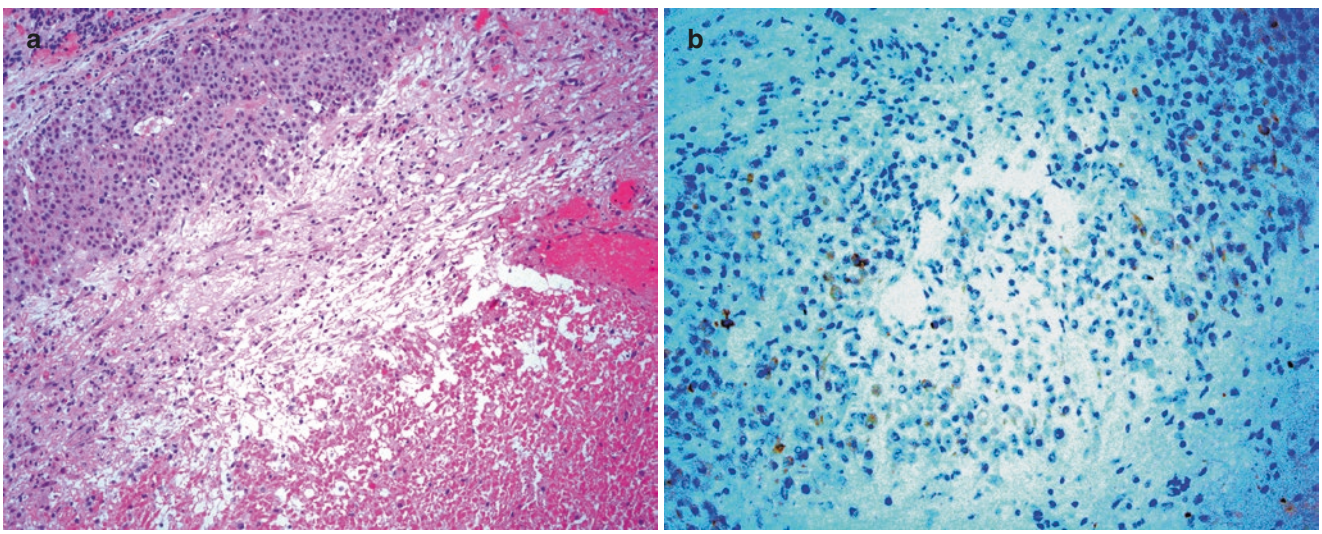

Fig. 10.23 Case 5. (**a**) Metastatic melanoma to the breast (10×). (**b**) "Keratin cocktail" immunohistochemistry of metastatic melanoma to the breast (20×). Note the weak staining with a "keratin cocktail". (**c**) S-100 immunohistochemistry of metastatic melanoma to the breast (20×). (**d**) Melan-A immunohistochemistry of metastatic melanoma to the breast (20×). Melanoma can show aberrant expression for cytokeratins, particularly CAM 5.2, and epithelial membrane antigen (EMA). As such, these markers should not be used if melanoma is being considered, and "keratin cocktails" should also be avoided. Strong diffuse staining with S-100 (**c**) and Melan-A (**d**) supports a diagnosis of metastatic melanoma

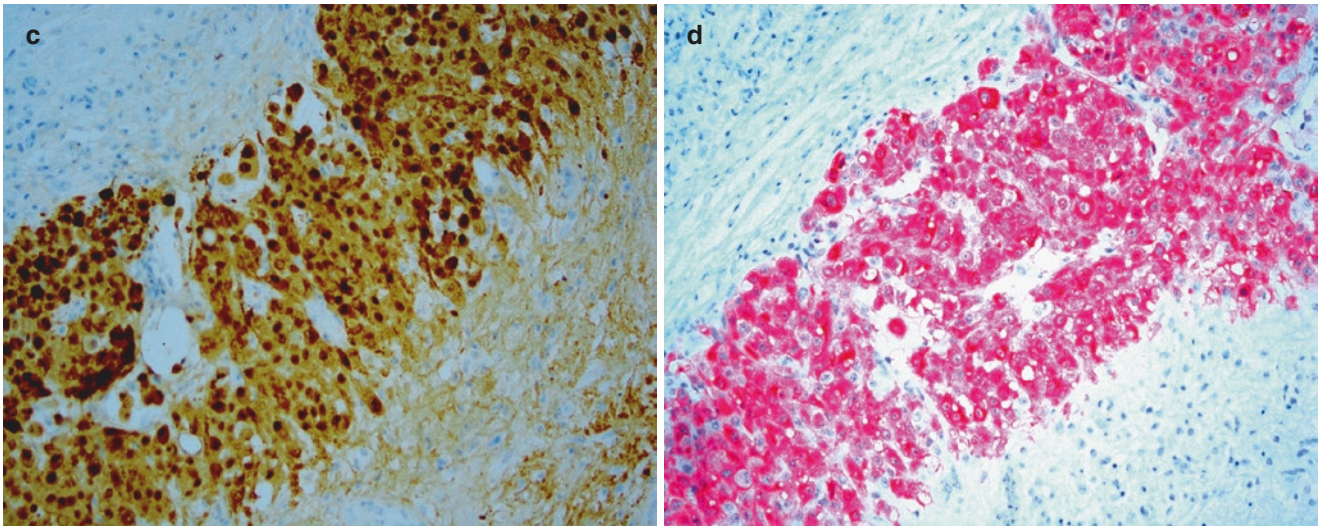

Fig. 10.23 (continued)

Immunohistochemistry for a "keratin cocktail" was positive; however, the pathologist astutely noted that it did not show the diffuse strong staining pattern expected for a breast carcinoma (Fig. 10.23b). A review of the medical records revealed a history of malignant melanoma. Suspicion for metastasis led to an immunohistochemical workup which was positive for S-100 (Fig. 10.23c) and Melan-A (Fig. 10.23d), supporting a diagnosis of metastatic melanoma. The patient developed shortness of breath and a pleural effusion 2 months after the diagnosis of breast metastasis, was placed on hospice, and died within 9 months after the diagnosis of breast metastasis.

Take-Home Messages

These case presentations highlight the importance of the clinical history (Cases 1–4), the variations in outcomes after a diagnosis of metastatic carcinoma to the breast (Case 4 versus Case 5), and the importance of a high suspicion for metastatic disease (Case 5).

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Y. Peng, P. Tang (eds.), *Practical Breast Pathology*, Practical Anatomic Pathology,
https://doi.org/10.1007/978-3-030-16518-5