Before the era of mammography, ductal carcinoma in situ (DCIS) comprised only less than 3% of newly diagnosed breast cancers, most as large palpable masses, and many with microscopic foci of invasion. With widespread use of mammography, DCIS now makes up about 25% of breast cancers diagnosed in the U.S., most as non-palpable lesions. There is also concurrent increased prevalence of atypical ductal hyperplasia (ADH) and flat epithelial atypia (FEA): ADH increases from 2% in premammographic biopsies up to 12% in mammographically screened women; while FEA (which was designated under various names) was rare and not recognized by many pathologists in premammographic biopsies, it is now being diagnosed in about 3.6% of the biopsies performed for microcalcifications.

This lecture will review (1) histological criteria for DCIS, ADH, FEA and usual ductal hyperplasia (UDH); (2) selected diagnostic issues in DCIS including special variants of DCIS and identification of invasion in association with DCIS; (3) morphologic mimics of FEA; (4) biology and breast cancer risk associated with FEA; and (5) management of FEA on core biopsy and excision specimens.

**Histologic criteria for DCIS, ADH, FEA and UDH**

**Distinction between UDH and low-grade DCIS**: Combined cytological and architectural features are used for evaluation and diagnosis of intraductal proliferative lesions of the breast. Usual ductal hyperplasia (UDH) is characterized by proliferation of a heterogeneous cell population with irregularly shaped and sized secondary lumens. The cells are haphazardly arranged with overlapping nuclei and indistinct cell borders. Low-grade DCIS is composed of mildly atypical, monotonous, uniform rounded cells growing in Roman bridges, rigid bars, micropapillae, cribriform or solid patterns. The cells are evenly spaced with distinct cell membranes. Many authors also require a minimal size for low-grade DCIS which includes either complete involvement of two duct spaces or an aggregate length exceeding 2mm.

**Boundary between ADH and minimal low-grade DCIS**: Atypical ductal hyperplasia has "some but not all" of the features of low-grade DCIS. In practice, ADH can be viewed as having two morphologic subtypes. The first subtype is when the epithelial proliferation is qualitatively insufficient for the diagnosis of DCIS. The ducts are only partially populated by the atypical cells as defined for low-grade DCIS, with a second nonatypical population also noted. Alternatively, the ducts are largely populated by the atypical monomorphic cells. However the
lesion falls short of DCIS because the architectural atypia is not fully developed with scattered arches, bars, micropapillae or cribriform spaces involving only part of the duct space or circumference. In the second subtype, which can also be viewed as “mini” DCIS, the atypical epithelial proliferation is quantitatively insufficient for the diagnosis of DCIS. The affected ducts demonstrate the architectural and cytologic atypia characteristic of low-grade DCIS. However, the aggregate length of the lesion measures ≤ 2 mm.

Using these criteria, distinction between ADH and low-grade DCIS can be made on morphological grounds alone in most cases. However, since the epithelial proliferative process is a morphologic continuum, sharp distinction in practice may not be possible in some cases. A conservative approach is generally recommended in that, when in doubt (between ADH and DCIS), the more benign diagnosis (ADH) is appropriate.

**Histologic features for FEA:** FEA is characterized by replacement of the native epithelial cells of the terminal duct and lobular units (TDLUs) by one and up to five cell layers of mildly atypical cuboidal to columnar cell population with apical snouts. The cells have monotonous, round to oval nuclei and variably prominent nucleoli. The nuclear chromatin may be either evenly dispersed or slightly clumping and margined and the nuclei may be enlarged with increased N/C ratio. Cytologic atypia in FEA resembles that seen in low-grade DCIS and tubular carcinoma. The growth pattern is flat; while small cellular tufts and small mounds may be present, complex architectural features are absent. The involved TDLUs are variably distended, often containing secretory or floccular material and microcalcifications.

**Distinction between ADH and FEA:** The distinguishing feature is the degree of architectural complexity or atypia. In FEA, the lesion lacks architectural atypia and forms flat growth patterns that may include simple cellular stratification, tufts, small mounds or short micropapillations. However, complex architectural patterns with well-formed micropapillations, rigid cellular bars, Roman arches, or cribriform spaces are absent. When architectural atypia is present in addition to cytologic atypia, the lesions warrant the diagnosis of either ADH or DCIS. The distinction between ADH and DCIS is based on the degree of architectural atypia and the extent (size) of TDLUs involved by the atypia.

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Diagnosis of High-grade DCIS: The cytologic and architectural features and the size criteria discussed above are applied to low-grade DCIS to help differentiation from UDH and also to avoid overdiagnosis of DCIS. Diagnosis of high-grade DCIS is based mainly on the presence of highly atypical cells with high-grade, markedly pleomorphic nuclei with irregular contour, coarse chromatin and prominent nucleoli. If unequivocal malignant cytology is present, even involvement of a single duct is sufficient for the diagnosis of DCIS. Necrosis, especially comedo pattern, is often present in association with high grade DCIS and therefore could be a helpful feature. It should be noted however that necrosis can also be encountered in benign breast lesions. In addition, duct ectasia with luminal secretion/cellular debris, macrophages and calcifications can mimic DCIS with comedo necrosis especially in a core biopsy specimen.

Ancillary tests in evaluation of ductal epithelial lesions: Using the combined cytologic and architectural criteria, most intraductal epithelial lesions can be appropriately categorized with excellent interobserver agreement. Differentiating DCIS from florid UDH can sometimes pose a diagnostic challenge, especially for those with limited extent of intermediate grade DCIS. For intermediate-grade DCIS, on one hand, the neoplastic cells are not as overtly malignant as those in high-grade DCIS, the diagnosis of which relies mainly on the cytologic features. On the other hand, the neoplastic cells are not as monotonous and the architecture not as rigid as those in low-grade DCIS. The combination of variation in cytology and irregularity in architecture could resemble florid UDH and present diagnostic difficulty. Immunohistochemical analysis with basal-type cytokeratins (CK5/6, CK14) could be a useful diagnostic adjunct in evaluating difficult ductal epithelial lesions. In UDH, a variable fraction of proliferating epithelial cells co-express basal-type cytokeratins. In contrast, virtually all low and intermediate-grade DCIS cells express only luminal-type cytokeratins (CK 8, CK18 and CK19) and lack basal-type cytokeratins. In a minority (~5-10%) of high-grade DCIS, the tumor cells are positive for CK5/6 and CK14. These lesions are also known as basal-type DCIS. The neoplastic cells however have clearly malignant cytologic features and correct diagnosis can be rendered on routine H&E histologic evaluation. In addition to CKs, ER has been noted to show distinct expression patterns between DCIS and UDH. While ER expression tends to be diffusely and strongly positive in low-grade and most of intermediate grade DCIS cells, its expression is usually focal and/or weak in UDH.

Diagnostic Challenges in DCIS

DCIS with spindle cells: A minority of DCIS lesions is composed of spindle cells. The spindle cells often grow in a swirling pattern, mimicking the streaming growth of florid UDH. Most DCIS lesions with spindle cells have low to intermediate grade nuclei, are positive for ER and PR, demonstrate
neuroendocrine differentiation and lack expression of basal CKs and SMA. Morphologic distinction of spindle cell DCIS from florid UDH may be problematic. Immunostaining with neuroendocrine markers and basal CKs could be helpful in evaluation of these lesions. The spindle cell morphology could also raise the possibility of a myoepithelial lesion and a negative SMA stain helps the differential diagnosis.

**Apocrine lesions:** Apocrine cytologic change is seen in a wide spectrum of breast lesions, ranging from simple apocrine cysts to papillary apocrine change, non-atypical apocrine proliferation, atypical apocrine proliferation, apocrine DCIS and invasive apocrine carcinoma. Normal apocrine cells have uniform, large round nuclei with prominent nucleoli. Trying to apply the same histologic criteria used in the normal ductal epithelial lesions can be confusing and may lead to overdiagnosis in some cases. Diagnosis of most apocrine DCIS is not difficult to make as the lesion apocrine cells often show marked nuclear pleomorphism with enlarged nuclei, multiple prominent nucleoli and irregular nuclear membrane, often accompanied by comedo necrosis. On the other hand, it can be quite challenging to distinguish lower-grade apocrine DCIS from atypical apocrine proliferation or even non-atypical apocrine proliferation. Size criteria have been suggested by some authors to help make this distinction. However, there is no agreement on the size cut off. Furthermore, there are no molecular markers that can reliably discriminate between benign and atypical apocrine lesions. Due to the lack of long-term clinical follow-up data, the clinical significance of atypical apocrine proliferation is unknown. Given these limitations, overdiagnosis of lower-grade apocrine DCIS should be avoided and a conservative approach is suggested for borderline apocrine lesions, similar to that recommended by non-apocrine ductal lesions.

The vast majority of apocrine lesions, regardless whether they are benign or malignant, are negative for ER and PR, but strongly positive for androgen receptor.

**Identification of invasion associated with DCIS:** Diagnosis of invasion in the setting of DCIS is one of the most challenging areas in breast pathology. It is fueled with problems of both over- and under-diagnosis. Some histologic patterns of DCIS or artifact can mimic invasion. These include extension of DCIS to the lobules or pre-existing radial sclerosing lesions; branching ducts containing DCIS particularly those with tangential sectioning; periductal inflammation and sclerosis obscuring the architecture of DCIS and causing individual neoplastic cells to appear floating freely in the stroma; and mechanical crush artifact and cautery effect.

Immunohistochemistry with myoepithelial cell (MEC) markers has become an essential diagnostic armamentarium for pathologists in evaluation of invasion. However, there are some limitations and pitfalls in using the MEC markers to confirm or exclude the diagnosis of invasion either due to the intrinsic biology of
MEC in various breast lesions or the property of individual MEC marker in its sensitivity and specificity. Recent studies have clearly demonstrated phenotypic alterations in MEC associated with both DCIS and benign sclerosing lesions. Compared to normal ducts and lobules, expression of MEC markers is significantly decreased and may even become undetectable in DCIS. Therefore, focal lack of MEC staining does not necessarily prove the rare clusters of tumor cells represent true invasion. MEC markers differ in their degree and extent of reduced expression in DCIS, and use of multiple rather than a single MEC marker is highly recommended in evaluation of invasion to prevent overdiagnosis. On the other hand, invasive carcinoma may express MEC markers (especially p63 and calponin), potentially leading to underdiagnosis of invasion. Another potential pitfall in evaluating invasion is epithelial displacement following prior core needle biopsy or fine needle aspiration procedure. The displaced neoplastic cell clusters are typically negative for MEC, thus immunostaining with MEC markers will be misleading and not helpful in evaluation of such foci. The presence of associated fat necrosis and granulation tissue is the key diagnostic clue for the displaced nature of the cells.

**Morphologic Mimics of FEA**

**Columnar cell change and hyperplasia (CCC/CCL):** FEA and CCC/CCL share similar architectural features with variably dilated TDLUs lined by columnar cells displaying prominent apical snouts. The distinguishing feature is the presence of cytologic atypia in FEA. The lining columnar cells in CCC and CCH exhibit no cytologic atypia and have ovoid to elongated nuclei that are oriented perpendicular to basement membrane and are often crowded and overlapping. Cytologic atypia in FEA resembles that seen in low-grade DCIS and is characterized by monomorphic cells with round to oval nuclei and variably prominent nucleoli. The nuclei lack polarity with respect to the basement membrane. The nuclear chromatin may be either evenly dispersed or slightly clumping and margined and the nuclei may be enlarged with increased N/C ratio. Therefore, the TDLUs have a more basophilic appearance.

**Apocrine metaplasia:** Apocrine metaplasia with its apical snouts and uniform round nuclei containing prominent nucleoli can occasionally be confused with FEA. The cytoplasm of apocrine metaplasia is more abundant, eosinophilic and granular. Furthermore, apocrine cells have significant lower N/C ratio and the nuclei have open chromatin, whereas FEA cells have hyperchromatic nuclei with chromatin that may be evenly dispersed or slightly marginated. Of note, apocrine cells consistently lack estrogen receptor (ER) and progesterone receptor (PR) and are negative for Bcl-2, an immunophenotype that is distinct from FEA cells (see below).

**ADH:** FEA is frequently associated with ADH and low-grade DCIS. In fact, in a given case, the affected TDLUs often show a spectrum of cytologic and architectural atypia ranging from CCC/CCH to FEA, ADH and low-grade DCIS.
with these lesions merging with each other. Both FEA and ADH are lined by epithelial cells with similar atypical cytology. The difference lies in the architecture with FEA showing flat growth and ADH exhibiting at least focal complex architectural features.

**High-grade clinging type DCIS:** The lesion now known as FEA was originally described by Azzopardi as “clinging carcinoma” of the monomorphic type. Although clinging carcinoma is no longer advocated to be used for FEA, FEA needs to be distinguished from high-grade DCIS with clinging architectural pattern (also known as “clinging carcinoma of the pleomorphic type.”) Both lesions have similar flat architectural pattern at low-power examination with dilated spaces lined by one to several cell layers lacking complex architectural features. However, on high power examination, FEA is lined by low-grade monomorphous epithelium whereas clinging pattern of high-grade DCIS is lined by severely atypical and pleomorphic epithelial cells with overt malignant cytologic features.

**Biomarkers and Breast Cancer Risk Associated with FEA**

**Immunophenotype and Genetic Alterations in FEA:** The cells in FEA are intensely and diffusely positive for ER and PR, show strong cytoplasmic expression of bcl-2 protein, and are negative for HER2 and p53. They are consistently positive for CK19 and negative for basal cytokeratins. The Ki67 proliferation index is similar to low-grade DCIS (mean 8.2% vs 8.9%) and higher than columnar cell lesion (CCL) without atypia (mean <1%). Molecular studies have revealed clonal chromosomal alternations, suggesting FEA as a neoplastic process. Overall, FEA exhibit low numbers of genetic alterations with recurrent 16q loss. Other common genetic changes are noted at chromosomes 3p, 11q, 15q, 16p, 17p and 19.

**FEA is a high risk factor for concomitant cancer:** Emerging data have shown an association of FEA with in situ and invasive carcinoma. Recent data from histologic evaluation of large cohort of patients have demonstrated frequent co-existence of FEA with ADH, DCIS, invasive carcinoma, and lobular neoplasia (atypical lobular hyperplasia and lobular carcinoma in situ). Of note, DCIS associated with FEA are most likely to be low nuclear grade with micropapillary and cribriform architectural pattern, and absence of comedo necrosis. The invasive carcinomas associated with FEA are frequently of tubular and lobular subtypes. FEA and these associated cancers not only have similar atypical cytologic features but also display similar biomarker profile including positive ER, PR and Bcl-2, and negative CK5/6 and HER2. Furthermore, molecular studies have revealed that FEA share same, although often less degree, genetic alterations when compared to the co-existent in situ and invasive carcinoma. Based on these morphologic and molecular analyses, FEA are currently considered by some investigators as a neoplastic proliferation that may represent
either a non-obligate precursor to or the earliest morphologic manifestation of low-grade DCIS and invasive carcinoma.

**Columnar cell lesion shows only mild increase in long-term breast cancer risk:** There are only limited clinical follow-up studies to address the long-term cancer risk associated with FEA and CCL. A recent case-control study from Nashville found a mild increase in the overall cancer risk associated with CCL with a relative risk of 1.47 at 17 years. In that study, there was no significant difference among the three categories of CCL (i.e., CCC, CCH and FEA) with regards to future cancer risk. Another case-control study from Nurses’ Health Studies also demonstrated a mildly increased risk associated with CCL (OR=1.44). However, this increase in risk was attenuated after adjustment for histologic category of concurrent proliferative breast lesions. Other studies have shown that the absolute risk of local recurrence and progression to invasive carcinoma after a diagnosis of FEA on excision is extremely low, ranging from 0-4% with follow-up time from 5 to 19 years.

Overall, the available data suggest that identification of FEA on a biopsy imply a possible concomitant more worrisome lesion, rather than a significantly increased long-term breast cancer risk.

**Management of Flat Epithelial Atypia**

How to best manage patients with FEA in their biopsies is still in debate because of the limited clinical follow-up data. Based on whether FEA is an isolated lesion or associated with more significant lesions and whether FEA is identified on a CBN or an excision specimen, following are the practical approaches suggested by some experts.

**FEA as the most advanced lesion on CNB:** If FEA is identified on the CNB, an excisional biopsy is recommended. Based on limited retrospective data, follow-up excision of FEA shows a more advanced lesion (DCIS +/- invasive carcinoma) in 20-30% of cases, with an upgrade rate similar to that reported for ADH.

**FEA as the most advanced lesion on excision specimen:** If FEA is found at an excisional biopsy, multiple levels should be obtained to look for features that are diagnostic for ADH or DCIS. Furthermore, if feasible, the remainder of the specimen or at least all the parenchymal breast tissue should be submitted for microscopic evaluation to search for more advanced lesion. Based on the limited data which indicate a very low risk of cancer progression, when FEA is the most advanced lesion on excision specimen, further treatment (re-excison or radiation) is not necessary. However, regular clinical and mammographic follow-up of patients would be prudent.
FEA in excision specimen containing DCIS: FEA is currently not taken into consideration for size evaluation of DCIS. Likewise, when FEA is found on the excision margin, no further surgery is necessary. It is unclear whether FEA at excision margin may contribute to future recurrence since they share similar genetic alterations as the adjacent DCIS. Future studies are required to address the optimal clinical management for patients who have FEA present at the margins of an excision specimen containing DCIS and admixed FEA.

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