REVIEW

Morphologic and Molecular Features of Breast Ductal Carcinoma *in Situ*

Souzan Sanati

From the Department of Pathology and Immunology, Washington University in St Louis, St. Louis, Missouri

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Address correspondence to
Souzan Sanati, M.D.,
Department of Pathology and
Immunology, Washington
University in St. Louis, 660 S
Euclid Ave., Campus Box
8118, St. Louis,
MO 63110. E-mail: ssanati@wustl.edu.

Ductal carcinoma *in situ* (DCIS) encompasses a highly heterogeneous group of lesions that differ with regard to their clinical presentations, histologic features, biomarker profiles, genetic abnormalities, and potential for progression. DCIS is a nonobligatory precursor for invasive carcinoma. With the advent of screening mammography, the incidence of DCIS has significantly increased. There is an argument that many of these lesions will not progress to invasive carcinoma within the lifetime of a patient. In addition, many studies have found enormous heterogeneity within DCIS. There is a need for biomarkers that can stratify patients with DCIS into different prognostic groups based on the biology of the disease. Estrogen and progesterone receptors are the established biomarkers that are used for clinical decision making. In addition, a number of biomarkers, such as human epidermal growth factor receptor 2 protein, *p53* tumor suppressor gene, Ki-67 proliferation marker, and tumor-infiltrating lymphocytes, carry prognostic significance, although their use is considered investigational and has not been transferred to clinical practice. On the molecular level, low-grade and high-grade DCIS have different molecular alterations, and the intrinsic molecular subtypes that exist in invasive carcinoma also exist in DCIS with prognostic implications. In this article, we review the morphologic features, prognostic biomarkers, and molecular features of DCIS. (*Am J Pathol* 2019, 189: 946–955; <https://doi.org/10.1016/j.ajpath.2018.07.031>)

The term ductal carcinoma *in situ* (DCIS) encompasses a highly heterogeneous group of lesions that differ with regard to their clinical presentations, histologic features, biomarker profiles, genetic abnormalities, and potential for progression. They have in common a monoclonal proliferation of neoplastic epithelial cells confined to the terminal ductal-lobular units (TDLUs; the smallest terminal branches of the ductal system) and typically grow in a segmental distribution without extension through the basement membrane.¹ DCIS may grow into larger ducts or into adjacent lobules, giving an appearance referred to as cancerization of lobules.^{1,2} DCIS is a nonobligatory precursor for invasive carcinoma. Diagnosis of DCIS indicates a high risk of subsequent breast cancer at the index breast or near the site of prior DCIS.³ The evidence that DCIS is a precursor of invasive carcinoma is indirect but convincing: invasive carcinoma is rarely seen without DCIS, women who are diagnosed with DCIS have a 10 times higher risk of development of ipsilateral invasive carcinoma if left

untreated, DCIS and invasive carcinoma share the same genetic and molecular features, and microscopic examination reveals continuity between DCIS and invasive carcinoma.⁴

Before widespread use of screening mammography, DCIS was an incidental pathologic finding in more advanced, palpable tumors. With the advent of screening mammography, DCIS is now diagnosed mostly through screening-detected calcifications or architectural abnormalities, and its incidence has increased from 5% in the premammographic era to 20% to 25% of breast cancer.^{2,5} The main concern in the management of DCIS is prevention of disease recurrence, mainly as invasive carcinoma, which constitutes 50% of disease recurrences.⁵ It has been argued that higher prevalence of diagnosed DCIS has

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resulted in diagnosing indolent lesions that would not result in an invasive recurrence during the lifetime of the patient. This theory is also supported by the high prevalence of DCIS in autopsy cases of patients who died of other causes.^{6,7} Although this may be true because only 30% of DCIS will have an invasive recurrence,⁸ there are no known biomarkers that can reliably identify DCIS with potential to progression. The result is a potential overdiagnosis and overtreatment in a large number of patients with DCIS. A consensus conference at the National Institute of Health State of the Science conference in 2009 has recommended eliminating the term *carcinoma* for these lesions.⁹ Other experts have proposed using the term *borderline breast lesions* to avoid overdiagnosis and more importantly overtreatment in these lesions with indolent biology.¹⁰ These terms also take into consideration the high interobserver variability in diagnosing these lesions. The overdiagnosis rate is approximately 61% for low-grade (LG), 57% for intermediate grade (IG), and 45% for high-grade (HG) lesions.⁸ In this article, we review morphologic features of DCIS with a discussion of its common differential diagnoses and molecular features.

Morphologic Features of DCIS

Most cases of DCIS are not grossly visible. If palpable, DCIS may form a mass with cords of tissue extruding a pastelike material from their cut surfaces (a gross correlation to the material seen on histologic sections as comedo necrosis). DCIS is morphologically seen as proliferation of neoplastic cells with variable nuclear cytologic features and in a variety of growth patterns that usually grow over and obliterate the luminal spaces of the ducts and TDLUs. HG-DCIS can incite a desmoplastic stromal response mixed with chronic inflammation, which may raise the concern for invasive carcinoma (Figure 1). Myoepithelial markers may be useful in such cases because they highlight the myoepithelial layer, thus providing evidence that proliferation has not yet breached the TDLUs. Overall, it is recommended not to rely on only one myoepithelial marker. Combination of a nuclear (such as p63) and a cytoplasmic (such as smooth muscle myosin heavy chain or calponin) marker is recommended for the best results.¹¹ DCIS

Figure 1 High-grade ductal carcinoma *in situ* with a desmoplastic stromal response and mixed chronic inflammation. Notice the single cells and small clusters of atypical cells within the dense inflammatory background, suggestive of microinvasion. Hematoxylin and eosin stain was used. Original magnification, $\times 20$.

Figure 2 **A:** Ductal carcinoma *in situ* with clear cell features. **B:** High-grade ductal carcinoma *in situ* with apocrine features. Hematoxylin and eosin stain was used (**A** and **B**). Original magnification, $\times 20$ (**A** and **B**).

may become displaced during previous needle biopsy, leading to carcinoma within a biopsy tract that may be confused with invasive breast carcinoma (IBC) in the subsequent excisional specimen. DCIS may have a variety of morphologic features, including clear cell and apocrine differentiation (Figure 2). Although familiarity with these morphologic patterns is helpful in recognizing the lesion, most of these morphologic variants do not bear clinical significance independent of their nuclear grade.

For clinical purposes, a few prognostic and/or diagnostic characteristics of *in situ* carcinomas should be reported in a pathology report. This information may affect the clinical decision making.

Estimate of Size and Extent

If the lesion is only present on one slide, microscopic measurement of the focus of DCIS will be the most accurate measurement. If the lesion is present on multiple slides, the most accurate measurement is achieved by correlating the microscopic sections that contain DCIS, with a gross diagram of the breast specimen indicating location and relationship of different sections within the specimen. By correlating the two, an estimate size of the lesion can be provided. In rare cases where an accurate size estimate cannot be given, extent of the lesion can be reported as the fraction of slides involved by DCIS.

Nuclear Grade

Nuclear grade has emerged as a strong predictor of local recurrence for DCIS.⁵ On the basis of nuclear features, including nuclear size, polarization, presence and size of nucleoli, and mitotic figures, DCIS is classified into 3 grades: LG, IG, or HG.^{1,6} LG-DCIS consists of a proliferation of small, monomorphic, and evenly spaced luminal epithelial cells with homogeneous chromatin distribution and inconspicuous nucleoli. The neoplastic cell nuclei approximate that of normal ductal epithelial cells. Mitotic counts are usually low. Small laminated calcifications are common.⁴ In contrast, HG-DCIS consists of a proliferation of large, highly pleomorphic, variably spaced, less organized epithelial cells with vesicular or coarse chromatin, irregular nuclear contours, and prominent or multiple nucleoli. The neoplastic cells

can have up to three times the variation in nuclear size. Mitotic counts are high, apoptotic cells are frequent, and comedo necrosis (necrosis in the center of the epithelial proliferation) is common. Amorphous calcifications associated with necrosis are common.⁴ IG carcinomas are lesions at the overlapping ends of LG- and HG-DCIS with features that fall between the two categories.^{1,5} Studies have found that the IG lesions have the highest interobserver variability.¹² As with invasive carcinomas, DCIS commonly has significant heterogeneity with areas of multiple histologic grades.¹³

Growth Pattern

DCIS is extremely diverse histologically, with respect to both nuclear grade and architecture. A variety of different growth patterns can be recognized, although commonly more than one growth pattern is present within a given lesion. Familiarity with these growth patterns is necessary for recognition of DCIS. The most common growth patterns include solid, papillary, micropapillary, and cribriform (sieve-like) architectural patterns (Figure 3).

In the cribriform growth pattern, the neoplastic cells form punched-out spaces with round and rigid extracellular lumens formed by neoplastic cells polarizing around the spaces. LG-DCIS with cribriform growth pattern should be differentiated from collagenous spherulosis, particularly when collagenous spherulosis is involved by lobular carcinoma *in situ*. The spaces in collagenous spherulosis are lined by myoepithelial cells with spindled nuclei (which can be highlighted by myoepithelial markers), and the round spaces are filled by basement membrane material. In cases in which collagenous spherulosis is associated with lobular carcinoma *in situ*, an E-cadherin stain can highlight the lobular nature of neoplastic cells.¹⁴ Adenoid cystic carcinoma occasionally can be mistaken for cribriform DCIS, but adenoid cystic carcinoma is composed of combined epithelial and myoepithelial cells

with basement membrane material and has an infiltrative growth pattern, whereas cribriform DCIS has a monomorphic appearance with epithelial cells polarized surrounding the cribriform spaces. A panel of stains, including myoepithelial markers, estrogen receptor (ER), c-kit, and MYB, can aid in the differential diagnosis.¹⁴ Invasive cribriform carcinoma has sieve-like irregular contours and an infiltrative growth pattern. Myoepithelial markers can confirm the absence of myoepithelial cells surrounding the cellular proliferations.

The micropapillary growth pattern is manifested by a proliferation of tufts of neoplastic cells that project into the lumen of TDLUs. These tufts are characterized by a lack of fibrovascular cores, a club-shaped end, and proliferation of a monomorphic, evenly distributed cell population.¹ Usual ductal hyperplasia can have a micropapillary growth pattern. The micropapillations of usual ductal hyperplasia have a broad base and a narrow pinched tip and are composed of cells that show larger nuclei at the base and appear smaller at the tip. This is in contrast with micropapillary DCIS, which has a narrow base and a broad bulbous tip and is composed of nuclei that are enlarged throughout the micropapillae.¹⁴

The solid growth pattern is characterized by a proliferation of sheets of monomorphic, cohesive cells that fill the lumen of TDLUs. These cells may form pseudorosettes or microacini with polarization of surrounding cells.

Papillary DCIS consists of a papillary proliferation characterized by branching fibrovascular cores covered by a monotonous epithelial proliferation with mild to moderate nuclear atypia. In contrast to benign papillomas, papillary DCIS has no invested myoepithelial layer, as indicated by a lack of staining with myoepithelial markers, such as p63 and calponin. Some markers, such as smooth muscle actin can highlight the myoepithelial cells, but they can also stain the vascular walls within the fibrovascular cores of papillary DCIS, which should not be overinterpreted as evidence of a myoepithelial layer.

The clinging growth pattern is recognized by some experts as a distinct growth pattern. It is characterized by a duct lined by one or a few layers of highly atypical neoplastic cells at the periphery and filled by abundant comedonecrosis. Some experts consider this growth pattern a variation of the solid growth pattern with extensive necrosis.

Extent of Comedo Necrosis

Comedo necrosis is usually seen in IG- and HG-DCIS. It is usually calcified necrosis within the center of a duct that is associated with DCIS. Some studies have found that abundant comedo necrosis is associated with increased local recurrence after lumpectomy for DCIS.¹

Paget Disease of the Nipple

Skin involvement (Paget disease) of the nipple can be seen when neoplastic cells percolate through the underlying lactiferous ducts to the epithelium without breaching the basement membrane. This condition is usually associated with

Figure 3 A: Ductal carcinoma *in situ* with solid growth pattern. B: Ductal carcinoma *in situ* with cribriform growth pattern and central comedo necrosis. C: Ductal carcinoma *in situ* with papillary growth pattern. D: Ductal carcinoma *in situ* with micropapillary growth pattern. Hematoxylin and eosin stain was used (A–D). Original magnification, $\times 20$ (A–D).

underlying HG-DCIS. It is important to distinguish the Paget cells within nipple squamous epithelium from other clear cells that occasionally can be seen within the nipple, mainly Toker cells. Toker cells are incidentally found clear cells within the nipple, which are smaller compared with Paget cells and do not have significant atypia. A human epidermal growth factor receptor 2 (HER2) immunohistochemical stain can be useful in difficult cases because the cells of Paget disease are positive for HER2, whereas Toker cells are negative.¹⁵

Surgical Margins

Surgical margin status is of particular importance and has an inverse association with the incidence of local recurrence. Because DCIS usually is not grossly visible, margins should be microscopically examined, and the location of the closest margin and its distance from DCIS should be mentioned in the report. A recent meta-analysis and consensus statement indicated that for patients with pure DCIS who will undergo whole breast radiation, a margin of ≥ 2 mm will be considered adequate. Patients with foci of DCIS within 2 mm from an inked margin may require additional excision, but multiple other patient- and tumor-related factors may affect the decision for further surgery.^{14,16} The 2-mm distance from the margin was chosen to account for skip lesions and discontinuity of DCIS in the ductal system.¹⁷ Other studies have questioned the validity of the 2-mm margin requirement.¹⁸ The extent of involved margin (focal, multifocal, extensive) should be reported. The presence of associated microcalcifications should be noted because DCIS that is first detected by microcalcifications will frequently recur with microcalcifications.

Diagnosis on Core Biopsy Material

Atypical ductal hyperplasia has significant morphologic overlap with LG-DCIS. The distinction between DCIS and atypical ductal hyperplasia is usually based on quantitative (≥ 2 mm or >2 duct spaces) or qualitative criteria. The widespread use of screening mammography has resulted in targeting smaller lesions on core biopsies. It is recommended that only lesions with unequivocal features of DCIS be diagnosed as such on core biopsy and DCIS classification of limited volume of disease with borderline morphologic findings be deferred to full examination of the extent of the disease on surgical specimen.

Diagnosis of DCIS on core biopsy should prompt a surgical excision with clear margins. Excision is usually performed to preserve breast tissue. A mastectomy is performed only when achieving clear margins cannot be obtained by conservative surgery, for example, in patients with extensive, multifocal DCIS or in patients who cannot receive radiotherapy.⁵ Sentinel lymph node biopsy generally is not performed unless there is a high risk of invasive cancer (large lesions, HG-DCIS) or in patients who will undergo mastectomy. An excision in most patients will be followed by adjuvant radiotherapy. Adjuvant radiotherapy reduces the recurrence rate of DCIS by 50%,

regardless of nuclear grade.¹⁹ There are ongoing efforts to identify a group of patients who have such low risk of recurrence or progression who will not benefit substantially from radiotherapy; however, efforts in identifying biomarkers for such a low-risk group of patients have not yet been successful.¹⁴ Patients with hormone receptor–positive DCIS may further benefit from administration of tamoxifen.^{3,20} The National Surgical Adjuvant Breast and Bowel Project B-24 trial evaluated 732 patients with DCIS without any knowledge of their biomarker status. Tamoxifen was administered to all patients. ER was retrospectively evaluated, and response to treatment was correlated with the degree of ER expression. The study found significant risk reduction in subsequent breast cancer recurrence in 10 years of follow-up in patients who had ER-positive DCIS.²⁰ The hormone receptor–negative patients did not benefit from tamoxifen.

A variety of clinical and histopathologic factors have been used as prognostic markers in DCIS. These factors include young patient age,²¹ mode of presentation (mass versus mammographic abnormality), extent of DCIS, nuclear grade, comedo necrosis, multifocality, and margin status. Nuclear grade and comedo necrosis are the strongest pathologic prognostic factors.^{5,22} Long-term follow-up of >800 patients with DCIS in UK National Health Service Breast Screening Programme shows a shorter median interval to invasive recurrence in HG-DCIS (76 months) compared with LG-DCIS (131 months).²³

A combination of the above-mentioned prognostic factors has been used to stratify patients with DCIS into different risk groups. The University of Southern California/Van Nuys Prognostic Index was developed using nuclear grade, tumor size, margin width, comedo necrosis, and patient age.⁵ The prognostic utility of the Van Nuys system was validated by some groups²⁴ but not others.¹² Other groups have developed nomograms based on a combination of clinicopathologic parameters (eg, Memorial-Sloan-Kettering Cancer Center nomogram)²⁵ with imperfect results.²⁶ Inclusion of endocrine and molecular biomarkers in this nomogram has been suggested to improve its predictive value.²⁷

Although these parameters are able to provide prognostic information, they are not able to accurately predict which patients will have disease recurrence.²⁸ In addition, there is significant lack of reproducibility among pathologists; for example, assessment of nuclear features has found that up to 30% of cases in multicenter clinical trials required reclassification on central review.² There is an unmet need for more objective methods for classification and risk stratification of DCIS. The main challenge in the clinical management of DCIS continues to be tailoring of treatment to individual risk to avoid overtreatment of LG lesions or undertreatment of HG lesions.²⁹

Prognostic and Predictive Biomarkers

The role of biomarkers in establishing the prognosis and management of patients with breast cancer cannot be

overemphasized. Established biomarkers, such as ER and progesterone receptors (PRs), are expressed in 70% to 80% of DCIS and have been used both as prognostic factors and predictors of response to endocrine therapy.¹²

Currently, evaluation of ER in patients with DCIS is considered standard of care and is mandated by National Comprehensive Cancer Network guidelines.²⁸ The shift toward an earlier diagnosis of breast cancer because of improved imaging methods and screening programs highlights the need for further biomarker discovery to quantify the risk for individual patients.³⁰

ERs and PRs

The ER belongs to a family of nuclear hormone receptors that function as transcription factors when they are bound to their respective ligands. ERs and PRs are parts of complex signaling pathways that interact with multiple survival and proliferation pathways in the cell and play a critical role in the development and progression of breast cancer. They have proven useful as prognostic factors, and more importantly as predictive factors, in the clinical management of breast cancer.

Assessment of ER expression by immunohistochemistry is an essential step in the evaluation of DCIS. Immunohistochemistry can be highly affected by a variety of preanalytic factors, including time of tissue fixation and the antigen retrieval method.³¹ The expression of PR is strongly dependent on the presence of ER and is reflective of a functioning ER pathway.

Several studies have found an excellent concordance rate between evaluation of biomarkers on needle core tissue and excisional specimen.^{32–34} To minimize the effect of preanalytic variables on test results, the American Society of Clinical Oncology/College of American Pathologists recommended using only 10% neutral buffered formalin as a fixative and controlling the formalin fixation time between 6 and 72 hours.

To standardize immunohistochemical evaluation, several scoring systems that incorporate both intensity and percentage of staining have been established. In general, similar to invasive carcinoma, to obtain benefit from hormonal treatment, a sample should demonstrate nuclear staining in at least 1% of tumor cells.^{20,31} For patients with a diagnosis of DCIS, ER can be assessed on core biopsy or excision; however, given that approximately 13% to 30% of cases diagnosed as DCIS on core biopsy will upgrade to invasive carcinoma on surgical excision, there is an economical argument that it is better to assess ER on excisional biopsy once it is known whether there is an invasive component.¹⁴ If biomarkers are performed on core biopsy and an invasive carcinoma is identified on excisional biopsy, additional evaluation of biomarkers on the excisional specimen with invasive carcinoma is warranted.

ER expression in DCIS is inversely related to the nuclear grade,^{12,35} with higher expression in LG-DCIS and lower expression in HG-DCIS. LG- and IG-DCIS are almost always ER positive, whereas HG-DCIS has ER positivity in 69% of cases.¹¹

HER2

Receptor tyrosine-protein kinase erbB-2 is a member of the epidermal growth factor receptor (EGFR) family of tyrosine kinase proteins. It is a membranous protein that is expressed in all epithelial cells at low levels. The *HER2/neu* oncogene is involved in the regulation of cell proliferation, survival, motility, and invasion. HER2 expression has also been associated with promotion of carcinogenesis by maintaining and increasing cancer stem cells. In invasive carcinoma, *HER2* amplification is an independent prognostic marker of poor outcome in the absence of adjuvant treatment and has been associated with an increased rate of metastasis, decreased time to recurrence, and decreased overall survival. HER2 is also a predictor of responsiveness to anthracycline-based chemotherapy and trastuzumab. Immunohistochemistry (to evaluate protein overexpression) and *in situ* hybridization (to evaluate gene amplification) are the most common methods to evaluate HER2 status.

In DCIS, expression of HER2 occurs in a higher proportion of cases compared with invasive carcinoma (approximately 35% versus 20%).³⁶ HER2 expression in DCIS is associated with high nuclear grade, comedo necrosis, myxoid stromal change, and increased risk of *in situ* and invasive recurrence, especially if the lesion has an elevated Ki-67 index.^{12,28,37,38}

HER2 expression seems to be an early event in DCIS and is not necessarily related to its progression to IBC.³⁶ In the United States, HER2 evaluation in DCIS is not recommended for clinical decision making.

Tumor-Infiltrating Lymphocytes

Assessment of tumor infiltrating lymphocytes (TILs) can provide important prognostic information in solid tumors. TILs are also a robust prognostic marker in IBC.³⁹ Assessment of TILs in DCIS has a strong correlation with ER-negative, HER2-overexpressing HG-DCIS.^{40,41} HG-DCIS, especially if associated with comedo necrosis, has higher TILs. The comedo necrosis is related to the increased growth of the neoplastic cells with clonal expansion that surpasses the available blood supply.^{41,42} Stromal TILs also mirror the mutational load, fraction of genome altered, and the number of telomeric imbalances, which all cause neoantigen formation and evoke an immune response.^{41–43} TILs are significantly higher in DCIS with *p53* mutation. This could be confounded by the association of *p53* mutation with other features associated with TILs, such as high nuclear grade, ER negativity, and HER2 positivity.^{41,43} Stromal inflammation in DCIS diagnosed on core biopsy is a predictor of invasive cancer on subsequent excision.⁴⁴

The method for TIL assessment has been previously published for solid tumors.³⁹ Toss et al⁴³ compared different methods for TIL assessment in DCIS and correlated the results with outcome. They concluded that TILs that touch

neoplastic epithelial cells or at a distance of one lymphocyte from the epithelial cells had the highest correlation with outcome. Dense TILs were associated with shorter recurrence-free survival irrespective of ER status.

TILs in DCIS are dominated by CD3⁺ T cells, with CD4⁺ helper T cells slightly outnumbering cytotoxic CD8⁺ cells. If DCIS-associated TILs have lower T-cell to B-cell ratio, with a higher proportion of FOXP3⁺ regulatory T cells, the patient will have a higher recurrence rate.^{41,45,46} Because the data with respect to the clinical significance of the DCIS-associated immune response are rapidly expanding, pathologists may be challenged by providing this information in their reports in the future.⁴⁷

Molecular Features of DCIS

There are three main models for the development of breast cancer. The first model was described by Wellings and Jensen⁴⁸ >30 years ago. This model is a linear model of tumor evolution that describes progressive accumulation of random mutations from normal epithelium to atypical hyperplasia, carcinoma *in situ*, and ultimately invasive carcinoma. Wellings and Jensen⁴⁸ proposed that the origin of most breast carcinomas is in the TDLUs. They described a continuous but nonobligatory evolution to invasive carcinoma through a series of increasingly abnormal phases during long periods.²⁶ On the basis of this model, the transition from one stage to the next is primarily dependent on the accumulation of random genetic mutations.

The second model for breast cancer evolution, known as the branched model, is based on the evidence that LG- and HG-DCIS and invasive carcinomas have distinct molecular profiles; therefore, it is hypothesized that they evolve through distinct evolutionary pathways.² LG-DCIS has lower number of alterations⁴⁹; they are usually diploid or near diploid, express ER/PR, have *bcl2* mutation, and have recurrent loss of chromosome 16q and gain of chromosome 1q.² In contrast, HG-DCIS is usually aneuploid with complex genetic profiles.² *HER2* amplification and *EGFR* mutation are common in HG-DCIS.^{49–53} Copy number gains in 1q, 5p, 8q, 17q, and 20q; amplifications of 11q13, 17q12, and 17q22-24; and loss of 8p, 11q, 13q, and 14q are characteristics of HG-DCIS. The *p53* gene mutation, which is correlated with genomic instability, is present in approximately 40% of HG-DCIS.^{28,50,54} This model was later refined to distinguish the two pathways based on ER expression rather than grade and acknowledged the progression of some DCIS and invasive carcinomas from LG to HG.¹²

A third hypothesis is based on parallel progression of different grades of DCIS from a common progenitor stem cell diverging and progressing through different grades in parallel. These theories are all based on compelling observations and they may not be incompatible.¹³

The genomic events that determine DCIS occur long before morphologic evidence of disease.⁵⁵ Recent studies

have found that histologically normal-appearing breast epithelium is not always normal at the molecular level, and some of these morphologically silent genetic abnormalities may predispose the cells to premalignant or malignant transformation.⁵⁶ The most significant alterations appear during transition from normal epithelium to DCIS.^{26,28} However, DCIS and invasive carcinoma are similar at the high resolution of global gene expression evaluated by microarrays, gene copy numbers, and promoter gene hypermethylation.^{3,28}

Carcinoma *in situ* is a late stage of tumor progression and the immediate precursor of invasive disease.^{28,56} DCIS has a continuum of histologically diverse proliferations that range from very well to very poorly differentiated, although, in clinical practice, they are often simply divided into two (eg, noncomedo versus comedo) or three (eg, LG versus IG versus HG) categories. This classification may be an oversimplification that fails to adequately convey their true diversity.⁵⁶

A number of studies have found extensive heterogeneity and diversity in DCIS by assessment of morphologic features, protein expression, gene expression, and molecular alterations in a number of studies. Allred et al¹³ reported the extensive diversity in DCIS by a combination of morphologic evaluation, biomarker studies using immunohistochemistry, and molecular studies. They found broad distribution of different nuclear grades and biomarker profiles within a lesion. They also found that the magnitude of diversity in DCIS correlated with *p53* mutation, which was generally higher in HG-DCIS. King et al⁵⁷ reported that most of their LG-DCIS developed HG invasive carcinoma and a substantial proportion of HG ER-positive cancers harbor genomic changes characteristic of LG-DCIS. Vincent-Salomon et al,⁵⁴ in a detailed morphologic and gene expression profiling study, found molecular heterogeneity in DCIS and a high degree of phenotypic, genomic, and transcriptomic similarities between DCIS and invasive ductal carcinoma. Using multiplex immunofluorescence, Gerdes et al⁵⁸ reported that within DCIS there is significant heterogeneity among cells and cell clusters, different ducts, and patients.

These findings support that although earlier stages of carcinogenesis, such as flat epithelial atypia and atypical ductal hyperplasia, lack diversity and have LG morphologic and biological features, extensive diversity and heterogeneity occur at the stage of DCIS. They also support that at least a subgroup of HG ER-positive DCIS evolve from LG lesions because of accumulation of additional mutations.^{13,57} There is considerable plasticity within DCIS, and it is likely that regions with different characteristics compete for dominance, and eventually the most aggressive areas prevail.¹³

Interestingly, among clinicopathologic characteristics of DCIS, nuclear grade, more than any other pathologic feature, is correlated with number and pattern of molecular genetic abnormalities.⁵¹ The use of nuclear grade, however, is limited because of high interobserver variability among pathologists, which is particularly true for IG-DCIS.^{12,50}

Intrinsic Subtypes

Gene expression profiling of IBC has categorized IBCs into luminal (luminal A and B) and nonluminal groups based on ER expression. The nonluminal group has been further classified into HER2 overexpressing and basal-like categories. Luminal A tumors differ from luminal B by higher ER expression and lower expression of proliferation-associated genes.⁵⁹ This classification has major prognostic implications for IBC. Makretsov et al⁶⁰ reported that it is possible to make the same categorization by IHC using markers that are aimed for luminal, HER2, and basal-like features. A number of studies have found that the same intrinsic molecular subtypes that exist in IBC are also identifiable in DCIS.^{13,54} Allred et al¹³ reported the enormous heterogeneity within every DCIS lesion, not only by nuclear grade and biomarker immunophenotype, but also by demonstrating multiple intrinsic molecular subtypes that coexisted in different regions of the same tumor. Others have applied immunohistochemistry surrogate markers to classify DCIS into intrinsic molecular subtypes.⁶¹ The luminal A group had high expression of ER, PR, and bcl2 and lacked HER2 expression, and the HER2-overexpressing group was negative for ER, PR, and bcl2 and overexpressed HER2.⁶¹ The distribution of molecular subtypes in DCIS using surrogate IHC markers has been reported as follows: luminal A, 55% to 60%; luminal B, 13%; HER2 overexpressing, 14%; and basal-like, 6% to 7%.^{54,62} The luminal A subgroup had the lowest recurrence rate (7.6%) in 5 years of follow-up.^{36,63} The HER2-overexpressing subtype had a higher prevalence in DCIS when compared with IBC, and the basal-like subtype (ER, PR, and HER2 negative and EGFR or CK5/6 positive) was relatively rare.^{28,63–65} Patients with luminal B (ER, PR, and HER2 positive) and HER2-overexpressing phenotypes had significantly higher risk of recurrence.^{17,29,63,66} There was a high correlation between HG-DCIS, HER2 overexpression, high Ki-67, and lack of ER and PR expression.²⁴

The discovery of intrinsic molecular subtypes in DCIS has further validated DCIS as a precursor of different subtypes of invasive carcinoma, although these subtypes occur at different frequencies; for example, the frequency of the luminal B and HER2 subtypes is much higher in DCIS compared with IBC, and the frequency of the luminal A subtype is much higher in IBC compared with DCIS.²⁸ In addition, the molecular subtypes correlate with grade; LG-DCIS is mostly composed of luminal phenotype (ER positive), and HG-DCIS has lower expression of ER, while expressing HER2 or basal-like phenotypes. Many studies have found prognostic value for classification of DCIS using surrogate markers by predicting overall and invasive recurrence.⁶⁷

Other Molecular Biomarkers

In addition to the above-mentioned biomarkers, there is an increasing list of biomarkers that have the ability to provide

prognostic information for DCIS. Ki-67 is a well-established marker of proliferating cells. It is a nuclear antigen and is expressed in all active phases of cell cycle but not in the resting cells (G0 phase). A meta-analysis of published literature found an association between high Ki-67 expression and risk of recurrence in women with DCIS regardless of the nuclear grade, type of treatment, age, and the cutoff value chosen.^{68,69} Rakovitch et al⁷⁰ found that HER2-overexpressing and Ki-67 high HG-DCIS was associated with an increased risk of recurrence regardless of nuclear grade and age. Balleine et al⁷¹ found that a combination of nuclear grade and Ki-67 was able to correctly classify DCIS into prognostic groups in 95.7% of cases. Unfortunately, lack of standardized methods and specific cut-off values limit the utility of Ki-67 in clinical practice.¹²

The *TP53* tumor suppressor gene inactivating mutation is seen in 40% of HG-DCIS and has been associated with genomic instability and a worse outcome.⁷² Cyclooxygenase 2 (COX-2) is overexpressed in many human malignant tumors and has been linked to the process of carcinogenesis, cell survival, invasion, and metastasis.^{73,74} COX-2 catalyzes the synthesis of prostaglandins from arachidonic acid and is induced through inflammatory and mitogenic stimuli.⁷⁴ de la Torre et al⁷³ found increased COX-2 expression to be associated with higher Van Nuys prognostic grade. COX-2 overexpression in DCIS is also strongly correlated with HER2 overexpression, high proliferation rate, high nuclear grade, ER-negative status, and risk of recurrence.^{28,74,75} Bcl-2 is an apoptosis regulator and an independent prognostic marker in early phases of breast carcinoma. It is highly expressed in LG-DCIS; however, its expression gradually decreases as lesions become more aggressive.⁷² Androgen receptor is expressed in 30% to 40% of DCIS regardless of the grade.⁷⁶ Most androgen receptor–positive cases are HG with apocrine features. *GATA-3* mutations are more commonly seen in ER-positive DCIS.⁵³ C-met increased levels are associated with vascular endothelial growth factor A and fibroblast growth factor receptor 1 expression, which contribute to angiogenesis. High expression of vascular endothelial growth factor A is correlated with biological aggressiveness of DCIS, whereas fibroblast growth factor receptor 1 amplification is correlated with progression to invasive cancer.⁷²

The molecular signature of ER, PR, and bcl2 negativity and high expression of *p53* and Ki-67 correlate with local recurrence. The p16, COX-2, and Ki-67 high expression is correlated with risk of progression to IBC.⁷²

Conclusions

We reviewed the morphologic features of DCIS, major differential diagnoses, and pathologic information that need to be included in pathology reports to assist clinical decision making. With widespread use of mammography, the number of cases diagnosed as DCIS has significantly increased.

Many of these lesions will never progress to invasive carcinoma. There is a need for identification of biomarkers that can stratify DCIS into different risk groups based on biology of the lesion because morphologic findings alone are not sufficient to separate lesions with potential to progression from lesions that do not progress. DCIS is extremely heterogeneous, further suggesting that classification based on morphologic findings may be an oversimplification. LG- and HG-DCIS have different patterns of molecular alterations. Characterization of DCIS on the molecular level may be able to better explain the variability in patient outcome and response to therapy. Unfortunately, despite intensive research, robust biomarkers that can predict risk of recurrence or progression of DCIS have not been discovered. In the future, most likely DCIS classification will include combining histologic findings with molecular profiling to improve risk stratification and to provide a personalized approach so patient management can be tailored to biological nature and malignant potential of the disease.

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