DCIS: Pathology and Biological Features

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5.1 Pathological Definition of DCIS

Ductal in situ carcinoma (DCIS) or intraductal carcinoma refers to a group of lesions characterized by a neoplastic proliferation confined to the mammary duct. They are composed of epithelial cells with different grade of cytological and architectural atypia, surrounded by a layer of myoepithelial cells and by an intact basement membrane. This pathological definition excludes the invasion of the mammary stroma by cancer cells [1].

5.2 Histological Classification

In standard histologic sections, DCIS is confined within duct and lobules, and pathologists must identify myoepithelial cells around these neoplastic structures. The lack of myoepithelium is a marker of invasiveness. Several antibodies have been proposed to detect myoepithelial cells, such as p63, smooth muscle actin, calponin, CD10, cytocheratin 5/6, and, more recently, p40 [2]. In general, expression of more than one marker is tested based on cytoplasmic or nuclear staining; several recommendations suggested performing routinely both nuclear and cytoplasmic antibodies on the same samples [3].

Due to the current understanding of DCIS as a heterogeneous group of cancers, with different morphology, immunophenotype, and molecular biology, there is no agreement on their classification.

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Over the past 25 years, a number of histological and cytological criteria have been proposed to subdivide these lesions in groups with different prognosis.

Traditionally, DCIS was classified based on architectural growth pattern of the epithelial proliferation, into comedo, solid, cribriform, papillary, micropapillary, clinging, apocrine, and mixed subtypes [4].

However, due to: (1) the low reproducibility of these diagnoses, (2) the high rate of mixed lesions, and (3) the low predictive value of local recurrences, this classification was then replaced by a modern systems based on cyto-nuclear atypia [5]. In particular several international Consensus Conferences recommended that the classification of DCIS should be based primarily on nuclear grade and encouraged pathologists to secondarily include in their diagnoses additional information on necrosis, cell polarization, and architectural differentiation [5, 6].

Depending on the degree of nuclear atypia, DCIS is generally classified in low (small, monomorphic, well-polarized cells, with uniform size and regular chromatin pattern and rare mitotic figures, Fig. 5.1), intermediate (similar to those of low grade but with occasional nucleoli, mitotic figures, and coarse chromatin, Fig. 5.2), or high nuclear grade (large size, pleomorphic, and poorly polarized nuclei, with prominent nucleoli, numerous mitotic cells, and presence of necrosis, Fig. 5.3) [7].

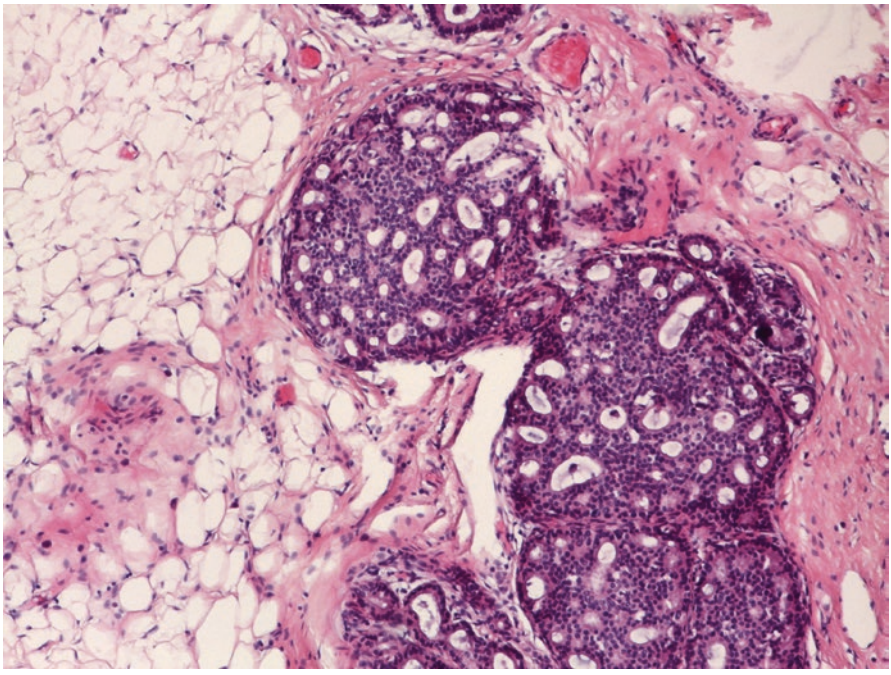

Fig. 5.1 Low nuclear grade DCIS with small, monomorphic cells, with uniform size; generally cribriform proliferation is the most common phenotype

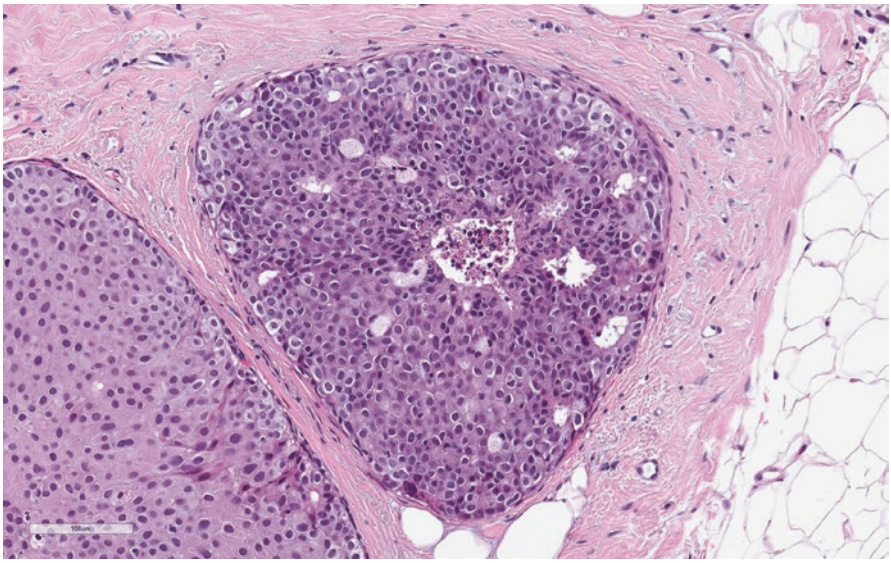

Fig. 5.2 Intermediate nuclear grade DCIS with moderate variation in nuclear size and nuclear pleomorfism. Necrosis may be present

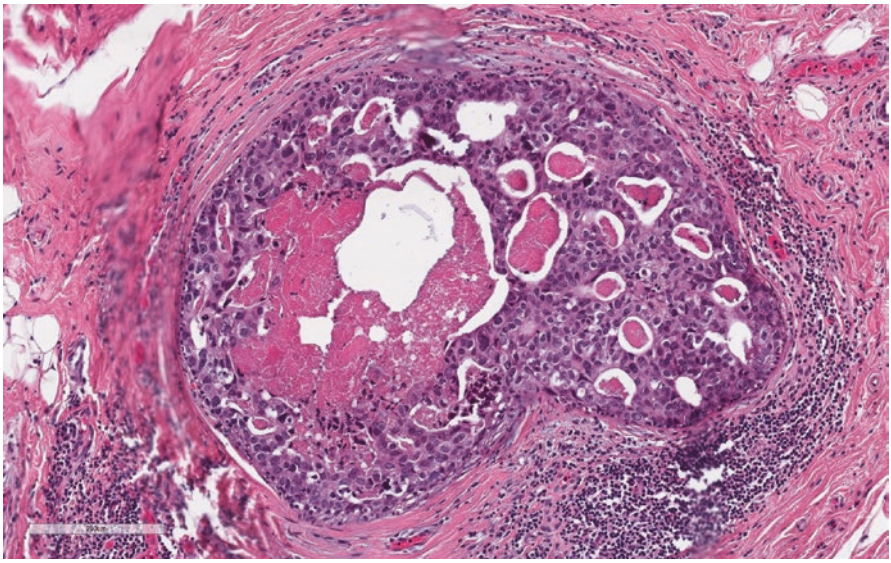

Fig. 5.3 High nuclear grade DCIS with cytological atypia, prominent nucleoli, presence of comedo-necrosis and mitotic cells

5.3 DCIS Carcinogenesis and Progression

In recent years, molecular studies suggested that the assessment of nuclear grade, as proposed by WHO, not only could better correlate with prognosis [8, 9], but it may highlight distinct genetic alternations [10] and distinct evolutionary pathways [11].

In fact, low-grade DCIS tends to be estrogen receptor (ER)/progesterone receptor (PR) positive (Fig. 5.4 a, b) and HER2 negative, and it is frequently characterized by the concurrent presence of deletion of 16q and gains of 1q and 16p. Otherwise, high-grade DCIS tends to be ER/PR negative and HER2 positive (Fig. 5.5 a, b), and it has complex karyotypes [12, 13], including frequent events in 1q+, 5p+, 8p−, 8q+, 11q−, 13−, 14q−, and 17q+ and focal amplifications on 6q22, 8q22, 11q13, 17q12, 17q22–24, and 20q13 [10, 14–16]. Thus, low- and high-grade DCIS may represent two distinct disorders, which may evolve in two distinct forms of invasive cancers (with low and high aggressiveness). In particular, genomic studies of synchronous and metachronous DCIS-invasive carcinoma have shown that there is a molecular continuum between low-grade DCIS and low-grade, well-differentiated invasive carcinoma (such as tubular carcinoma), as well as between high-grade DCIS and high-grade invasive carcinoma. The "low-grade arm" has similar gene expression profile, characterized by ER activation. On the contrary "high-grade arm" lacks ER in favor of the expression of genes related to cell proliferation and promoting invasive growth pattern [17].

Although the mechanisms underlying the progression from DCIS to invasive ductal carcinoma of the breast are yet to be fully elucidated, recent gene expression profile studies demonstrated that, inside specific molecular subtypes, DCIS and invasive carcinoma cells share similar genes and that the largest part of molecular changes occurs from normal epithelium to in situ carcinoma cells [18–21]. These mutations may include TP53 [22], PTEN [23], likewise amplifications of chromosome 20, 11, and 17 [24, 25]. In line with these findings, experimental data confirmed that precursor cells with ability to invade the stroma and with metastatic potential may be present in DCIS lesion and that treating breast cancer before it can become invasive may prevent the progression to infiltrating carcinoma [26]. Another important gene involved in the process of DCIS growth and progression is CDH1 (E-cadherin) that is expressed in normal and DCIS epithelial cells. CDH1 is a cell–cell adhesion protein with a role in epithelial differentiation. It has been shown that a partial or total loss of its expression may occur in the transition from DCIS to invasive breast cancer and in metastatic behavior and poor prognosis [27–29].

It is well established that the evolution of DCIS to invasive breast cancer is not only determined by molecular changes in epithelial cells, but may also strongly depend on stroma, cell-mediated immune mechanisms, and myoepithelial cells [20, 30–32]. In particular, myoepithelial cells seem to act as a tumor suppressor in DCIS [32], and several studies demonstrated that many of the genes that are specific for normal myoepithelial cells, such as CTK14, CTK17, and EGFR, are absent or downregulated in the myoepithelial cells of DCIS lesions. Hence, these changes may lead to breakdown of the ducts and release of the tumor epithelial cells into the surrounding stroma [32, 33]. Other genes involved in extracellular matrix

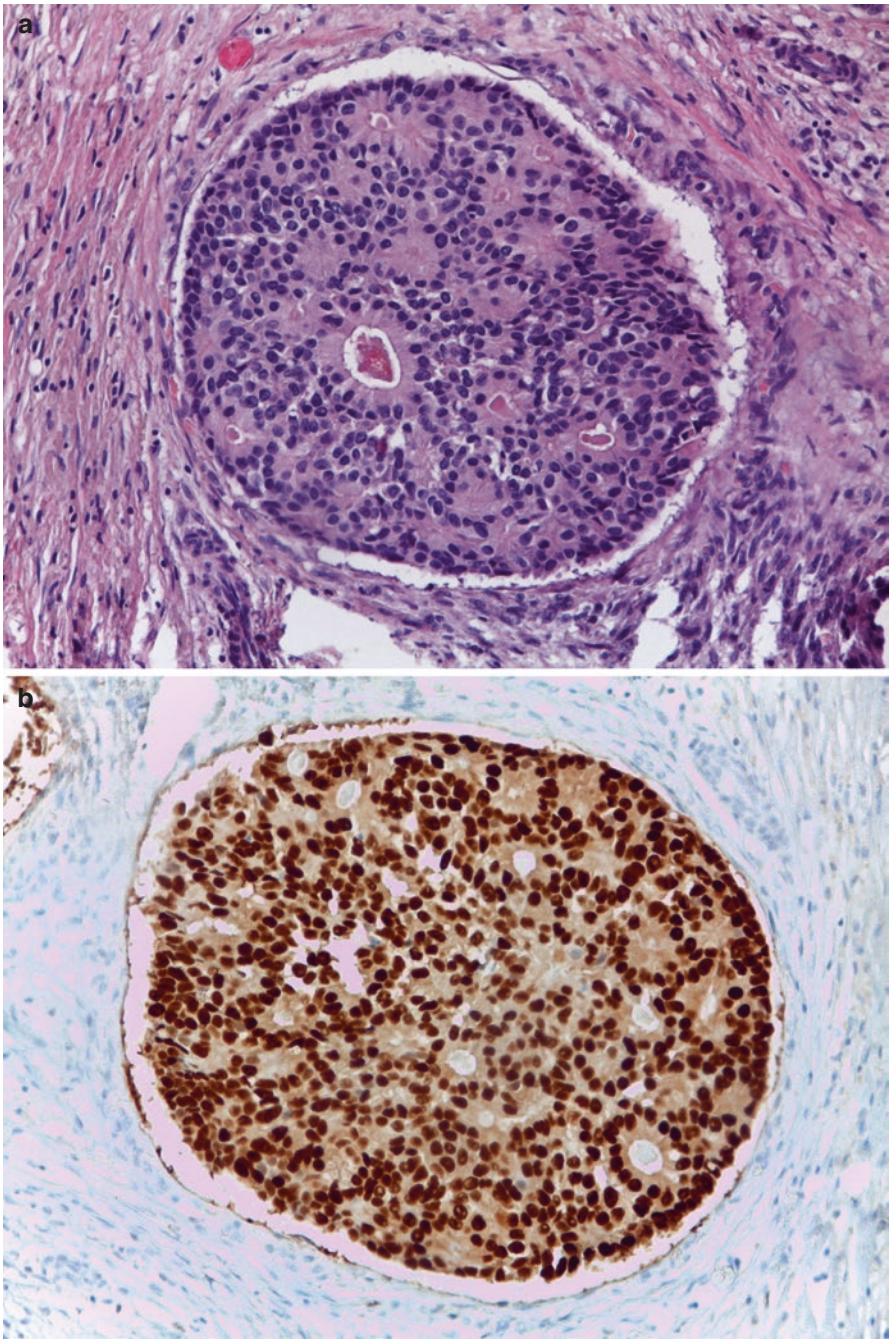

Fig. 5.4 Low nuclear grade DCIS (**a**) that shows uniform immunostain for estrogen receptor (**b**)

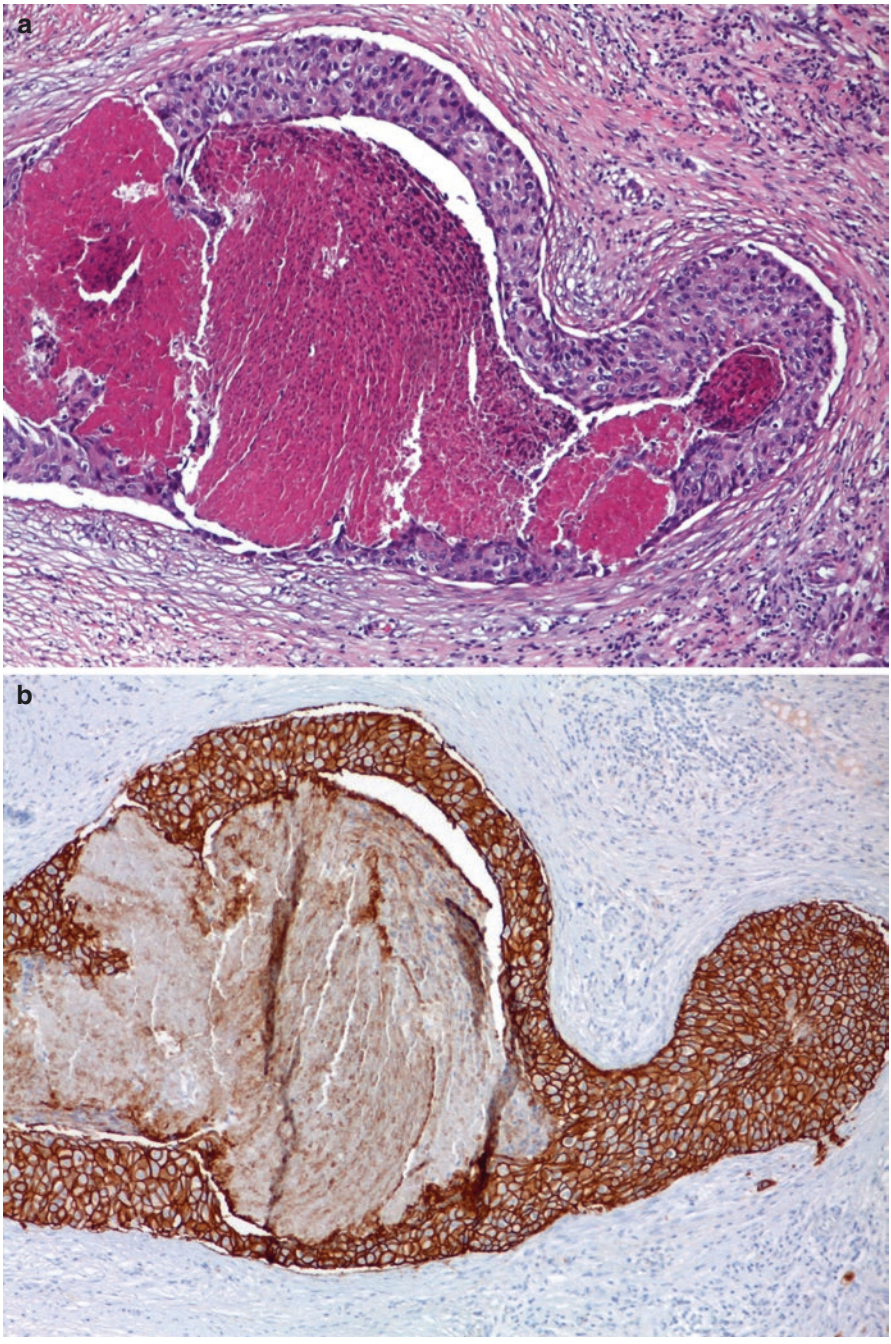

Fig. 5.5 High grade DCIS (**a**) with HER2 overexpression in immunohistochemistry (**b**)

remodeling, such as matrix metalloproteinase 2 (MMP2), are closely related to DCIS progression in invasive cancer. It has been shown that MMP2 overexpression can lead to a degradation of the basement membrane, a barrier that inhibits the migration of cells in the surrounding stroma [34].

5.4 The Concept of "DIN"

Due to these emerging genetic data and to the difficulties in distinguishing between low-grade DCIS and other proliferative intraductal lesions such as atypical ductal hyperplasia (ADH), a new classification system was proposed by Tavassoli et al. in 2003 (WHO. 2003) [35]. They suggested to replace the term DCIS in favor of ductal intraepithelial neoplasia (DIN), reserving the term "carcinoma" only for the invasive neoplasia. The subgroup of lesions classified as "DIN 1" encompassed a series of low-grade intraductal proliferations, such as flat epithelial atypia (DIN1a), ADH (DIN 1b), and low-grade DCIS (DIN1c), not only with similar morphological features but also with similar genetic alterations, typical of low-grade neoplasia. DIN 2 represented intermediate-grade DCIS with intermediate level of differentiation between low- and high-grade DCIS. This latter group was finally classified as DIN 3 lesions, with atypical and pleomorphic cells and genetic features typically observed in high-grade arm. Although several studies supported the DIN classification [36, 37], this terminology did not gain widespread acceptance, in part because it includes entities, such as DIN1a, in which neoplastic nature is not fully demonstrated, partly because, in specific subgroups, such as DIN 1B and DIN 1C, the morphological distinction remains subjective [38]. Thus the latest WHO classification in 2012 [39] abounded the term DIN in favor of the previous classifications based on the nuclear grade. However, this topic remains a matter of discussion, even for the psychological impact on patients. In fact, some works suggested that the term "DIN" may eliminate the anxiety produced by the term "carcinoma," contributing to reduced adverse psychological reactions and decreased confusion in healthcare settings [37, 40].

5.5 Pathological Prognostic Markers

Traditionally, size of lesion, nuclear grade, type and extension of comedo-necrosis, hormone receptor expression, and margin status have been described as prognostic markers [39, 41, 42].

Thus, when DCIS is diagnosed on surgical specimens, all of these variables should be cited in the pathological report. To reach this aim, the use of large histological tissue sections could help pathologists to better describe DCIS in terms of extension, heterogeneity and margin status.

Among prognostic factors, some studies have reported nuclear grade to be the most significant predictor of local recurrence on both univariate and multivariate analysis [43, 44].

Otherwise, the presence of comedo-necrosis, generally associated with high-nuclear-grade DCIS, is closely related to the risk of ipsilateral recurrences following lumpectomy, and a meta-analysis [44], based on 44 articles, confirmed these data, showing a risk of recurrences for DCIS with comedo-necrosis ranging from 1.3 to 5.0 [45–51].

The prognostic impact of histotype is still debated, mainly due to the low reproducibility of these diagnoses and to the presence of high rate of mixed lesions. Several studies reported that the "cribriform" growth pattern is related to indolent lesions with a low risk of subsequent invasive carcinoma, whereas solid DCIS are generally an aggressive neoplasia, especially if associated with comedo-necrosis [45, 52, 53].

In regards to micropapillary growth patterns, some studies suggested that low-grade micropapillary DCIS may be treated with excision without additional irradiation, for an exceptionally low risk of recurrences of these entities [52]. Otherwise, others reported that this phenotype is often multicentric [54, 55] and larger than other subtypes [56] and that it may remain clinically and radiologically silent, even if it is found to be extensive and of high grade [57]. In addition two studies reported that the micropapillary growth pattern is an independent high-risk factor for local recurrences [58, 59].

Traditionally, both ER and PR are frequently tested in DCIS; however, ER is the only one validated for routine clinical practice in DCIS (WHO 2012) [39]. National Comprehensive Cancer Network (NCCN) guidelines include its determination as part of the workup of DCIS [60].

The majority (80%) of DCIS cases are ER positive [20]; its expression is generally related to low- to intermediate-nuclear-grade DCIS cases. On the other hand, the predictive value of this marker remains a matter of discussion, and there are not enough data to make general recommendations for the use of ER in DCIS to decide about antihormonal treatment [61].

Very few data are available on PR, and there is disagreement regarding its routine determination (WHO 2012, 40) on DCIS samples. Among other immunohistochemical markers that are currently under investigation, HER2 is one of the most studied. Its role in DCIS is unclear. It is overexpressed/amplificated in 50% to 60% of DCIS cases, and its detection is generally associated with high-nuclear-grade DCIS with comedo-necrosis and presence of stroma microinvasion [62–64]. Several studies have suggested that HER2 may play a critical role in the progression to invasive carcinoma [65, 66] and its expression has been linked to recurrence after surgical excision, mainly in patients without radiation therapy [67, 68].

The identification of HER2 expression in DCIS may be useful even for a better radiological surveillance program: in a prospective observational study comparing mammography to magnetic resonance, the latter was more sensitive and specific in diagnosing high-grade DCIS [69]. Although several studies have been proposed with trastuzumab [70] or with lapatinib [71, 72] in HER2-positive DCIS patients, to date, there is no evidence of the clinical effect of anti-HER2 treatment. However, a first prospective, randomized phase III multi-institution clinical trial—National Surgical Adjuvant Breast Project (NSABP) B-43— is

currently ongoing. It compares whole breast irradiation alone with WBI given concurrently with trastuzumab in women with HER2-positive DCIS treated by lumpectomy [73].

The expression of ER, PR, and HER2 together with the rate of Ki67 may allow to classify DCIS, using "surrogate molecular subtypes," in Luminal A, B, HER2, and triple negative DCIS. However, the prognostic impact of molecular subtypes in DCIS, following St. Gallen surrogate definition (St. Gallen Consensus Conference—[74–76]), is yet to be clarified.

Lazzeroni et al. [77] found that immunohistochemically defined molecular subtypes in DCIS may be an indicator of prognosis, mainly due to the assessment of Ki67. Zhou et al. [78] demonstrated that combination of molecular markers ER−/HER2+ was statistically significantly associated with a high risk for a recurrence being in situ and that ER+/HER2−/EGFR− tumors were strongly associated with a subsequent recurrence being invasive. Otherwise, one study failed to demonstrate a prognostic value for the surrogate molecular subtyping of DCIS up to 10 years after diagnosis. However, it was shown that triple-negative DCIS had an elevated risk of recurrence [79].

Other immunohistochemical markers such as TP53, Bcl2, and androgen receptor have been investigated as potential prognostic markers. Presence of TP53 mutation together with an increased level of Ki67 in DCIS lesions are associated with high risk of recurrence [80].

In particular, mutations of TP53 occur more frequently in HG-DCIS and in HER2-positive tumors than in ER/PR-positive low-grade DCIS [81]. The expression of Bcl-2 that is present in the continuum of breast lesions from ADH to well-differentiated DCIS gradually decreases as lesions become more aggressive [82].

On the other hand, the role of AR expression in DCIS is not fully understood, and different results are present in literature [83, 84].

Very recently, to better stratify patients by prognosis, a multigene reverse transcriptase (RT)-PCR assay was recently proposed by Genomic Health. The test, called Oncotype DX Breast Cancer Assay for DCIS, is based on 12 genes from the Oncotype DX Invasive Recurrence Score (Genomic Health, Redwood City, CA, USA). The algorithm uses seven cancer-related genes (Ki67, STK15, Survivin, CCNB1, MYBL2, PR, and GSTM1) and five reference genes to create a score, designed to quantify the 10-year risk of local recurrence, both in situ and invasive, in patients with DCIS treated with breast-conserving surgery without radiation.

The results are reported as a numerical score called "DCIS Score," which classifies DCIS patients into low-, intermediate-, and high-risk groups with overall local recurrence rates of 10.6, 26.7, and 29.5%, respectively, at 10 years. Invasive recurrence rates are 3.7%, 12.3%, and 19.2% for these groups, respectively [85].

The application of this test, together with clinical, pathological, and immunohistochemical analyses, may result in a better definition of the risk profile of DCIS patients, allowed to avoid radiotherapy in low-risk categories. However, the Oncotype DX Breast Cancer Assay for DCIS is applicable only to patients with low-intermediate-grade DCIS with resection margins of at least 3 mm and to patients with high-grade DCIS with lesion of 1 cm or less in size [85].

In addition, a recent study suggested that incorporating the DCIS Score in routine clinical practice is cost-effective, even if DCIS Score lowered the proportion of women undergoing RT [86].

5.6 Molecular Assessment of DCIS and Future Approaches

In recent years, a number of studies have been proposed to assess the molecular and genetic features of DCIS cells, with the aim to discover biological features involved in growth and progression of these lesions. In particular, microRNA (miRNA) is a class of small RNA molecules that, through the control of mRNA expression, may regulate cellular processes such as stem cell division, cell growth, apoptosis, and carcinogenesis [87–90].

It has recently been discovered that some miRNAs are under- or overexpressed in DCIS in comparison with normal histological breast tissue [91]. For example, miR-132, which is frequently downregulated in DCIS, acts as inhibitor of cell proliferation [92]. The most significant miRNA deregulations seem to occur during the transition from normal to ADH, to DCIS epithelium, such as the loss of the tumor suppressor miR-125b and the gain of miR-182, miR-183, and miR-21 [91, 93]. Furthermore, although most miRNA changes in invasive carcinoma were already apparent in DCIS [94], nine-microRNA signature was identified as invasive carcinoma that progressed from in situ carcinoma, such as miR-210 and miR-221 that were downregulated in the in situ and upregulated in the transition to invasive lesion [95]. In the same study, authors reported that crucial genes in cancer development, such as BRCA1, FANCD, FANCF, PARP1, E-cadherin, and Rb1, are inversely related profiles to miR-210: they were all activated in the in situ and downregulated in invasive carcinoma. Another study found a consistent increase in the expression of miR-21 along with its targets (PTEN, PCCD4, and TMI) in breast cancer progression [96]. Together these findings underline the relevance for studying miRNAs as markers of risk of DCIS growth and progression.

Several molecular DCIS studies aimed to better define the role of DNA methylation in breast cancer differentiation and progression. In line with the above chapters, it has been shown that the number of methylated genes increased from normal breast to DCIS, whereas IDC did not differ from DCIS [20, 97–99]. Thus, DNA methylation seems not to play a role in the development of invasion, but it is very important in early breast carcinogenesis. Finally, a recent work [100] studying the molecular landscape of DCIS at the mutational, transcriptomic, and epigenetic levels, using DNA and RNA-Seq analysis, showed that important and complex epigenetic changes present in the invasive form are already operating at the in situ stage. In addition, they demonstrated that a subgroup of HG-DCIS lesions can be identified displaying more aggressive molecular profiles and that most high-grade DCIS lesions demonstrated profiles indistinguishable from invasive cancers.

Further studies of the genomic landscape of DCIS are needed to clarify the genomic and genetic alterations involved in DCIS progression and to discern the more aggressive phenotypes. Genomic technologies such as next-generation

sequencing (NGS) modalities, which are just beginning to be applied to DCIS [101], may offer in the future a depth molecular analysis of these lesions, revealing mutations, alternative splice variants, novel potential therapeutic targets, and promising biomarkers.

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