Progression of Ductal Carcinoma in Situ from the Pathological Perspective

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Key Words

DCIS · Linear progression · Parallel progression · Molecular classification · Microenvironment · Myoepithelial cells

Summary

Ductal carcinoma in situ (DCIS) now represents up to 20% of breast cancer cases, yet its behaviour is still poorly understood. Morphological classifications go some way to predicting prognosis, but more sophisticated approaches are required to better tailor therapy to the individual. A number of biological molecules have been identified that appear to relate to prognosis and, in model systems, promote progression to invasive disease. Some of these, such as COX-2, provide real therapeutic opportunities, whilst other marker combinations are showing promise in categorising women according to risk. Gene expression studies have led to an emerging molecular classification of invasive breast cancer, and it is now evident that at least some of these molecular subtypes can be identified at the pre-invasive stage. The difference in frequency of these subtypes between DCIS and invasive cancer may hold clues as to the biological mechanisms underpinning disease transition. It is increasingly clear that the host microenvironment can have a major impact on disease behaviour, and as well as acting as potential predictive factors, the altered microenvironment phenotype also offers novel therapeutic opportunities.

Schlüsselwörter

DCIS · Lineare Progression · Parallele Progression · Molekulare Klassifikation · Mikroumgebung · Myoepitheliale Zellen

Zusammenfassung

Bei bis zu 20% aller Mammakarzinome handelt es sich um ein duktales Carcinoma in situ (DCIS); dennoch ist das Verhalten dieser Erkrankung nur schlecht verstanden. Morphologische Klassifikationen sind für die Prognosestellung von großer Bedeutung, aber differenziertere Ansätze sind nötig, um eine bessere Anpassung der Therapie an das Individuum zu gewährleisten. Eine Anzahl biologischer Moleküle sind identifiziert worden, die sowohl von prognostischem Wert sind als auch in Modellsystemen die Progression zu invasiven Erkrankungsformen begünstigen. Einige dieser Moleküle, wie z.B. COX-2, bieten tatsächliche therapeutische Möglichkeiten, während andere Markerkombinationen in Hinsicht auf die Einteilung von Patientinnen in Risikogruppen vielversprechend sind. Genexpressionsstudien haben zu einer neuartigen molekularen Klassifikation des invasiven Mammakarzinoms geführt, und inzwischen ist ersichtlich, dass zumindest einige dieser molekularen Subtypen im präinvasiven Stadium identifiziert werden können. Unterschiede in der Häufigkeit dieser Subtypen beim DCIS und invasiven Mammakarzinom könnten biologische Hinweise auf die Mechanismen geben, die der Erkrankungsumwandlung zugrunde liegen. Es wird zunehmend deutlich, dass die Mikroumgebung des Wirtes einen bedeutenden Einfluss auf das Krankheitsverhalten haben kann. Außerdem kann der veränderte Mikroumgebung-Phänotyp neben potenziell prädiktiven Faktoren auch neue therapeutische Möglichkeiten bieten.

Introduction

Ductal carcinoma in situ (DCIS) is a neoplastic proliferation of cells within the ductal-lobular structures of the breast that has not penetrated the myoepithelial-basement membrane barrier into the surrounding stroma, and is widely considered at this stage to have no metastatic potential. Its incidence has been rising since the advent of screening mammography, and DCIS now represents 15–20% of all newly diagnosed cases of breast cancer [1]. Lesions that are diagnosed at screening but which would not have presented clinically during the individuals lifetime constitute over-diagnosis. The clinical challenge in the management of DCIS is how to tailor therapy more appropriately for a disease that is clearly heterogeneous in its behaviour. It is established that not all DCIS progress into invasive disease within the patient's lifetime. Certain subgroups are known to have a higher risk of progression [2], but even amongst the 'lower risk' subtypes, some studies suggest a similar proportion of cases will progress, though the time taken to do so may be much greater [3]. Quantifying the risk of progression for individual patients is the challenge, but the major limitation is that current pathological criteria do not sufficiently discriminate between those lesions that will or will not progress. For this reason, great efforts are being made to better understand the biology of DCIS. It has long been thought that disease progression occurs via a linear sequence of events from normal breast tissue, via precursor lesions to DCIS, and ultimately to invasive breast cancer [4]. Recently, this tenet has been challenged, with suggestions of a parallel development of tumour progression and metastasis [5], which may have important implications for how we view DCIS. Furthermore, there is evidence to suggest that biological diversity is generated at the DCIS stage [6]. These ideas, together with advances in molecular profiling and a greater understanding of the role of the microenvironment in DCIS, are gradually providing a clearer picture of the factors that may determine the behaviour of this disease. This review aims to examine the available data relating to progression of DCIS from the pathological point of view, and suggest approaches that could lead to a better understanding of this disease and arm the clinician with tools to improve the standards of care.

Morphological Classification of DCIS

One of the mainstays of pathological input to the management of DCIS is accurate classification of the disease based on its myriad morphological features. A number of classification systems have been proposed all of which are variously based on nuclear morphology, cytonuclear differentiation, growth pattern, and presence or absence of necrosis. To be of value, one system needs to have reproducibility and clinical relevance. The Van Nuys classification system, which is based on nuclear grade and necrosis, has achieved this. This system

yields 3 groups of DCIS – non-high grade without necrosis, non-high grade with necrosis, and high-grade with or without necrosis, and has demonstrated correlation with both recurrence and disease-free interval [7]. Another clinically useful system is that proposed by the National Coordinating Group for Breast Screening Pathology in the United Kingdom. This again classifies the disease into 3 groups – low, intermediate, and high grade DCIS. The differentiation is based on cytonuclear features, with low grade characterized by small regular cells showing a cribriform or micropapillary architecture, and high grade being characterized by large and pleomorphic cells with frequent mitotic figures, often organized in a solid architecture with associated necrosis. The intermediate grade shows characteristics intermediate between low and high grade DCIS. This system has proven clinical relevance with the demonstration that high grade DCIS is associated with higher incidence of recurrence than low grade DCIS [2].

In addition to grade, a number of other features have been shown to relate to higher frequency of recurrence, either as DCIS or invasive disease. These include young age at presentation, extent of disease and margin status, and symptomatic presentation rather than screen detection [3].

Although high grade DCIS is considered to have a greater propensity to recur or progress, several studies indicate that DCIS of all grades has a similar potential to progress to invasive disease, though high grade lesions are likely to progress more rapidly and lead to metastatic disease and death [2, 8, 9]. It is important to recognise that low and intermediate grade DCIS still have significant potential to progress as this underlines the limitation of histological grading alone to predict behaviour. Something long recognised by histopathologists and recently supported in a detailed study from Allred et al. [6] is that DCIS lesions exhibit considerable heterogeneity of grading within a single sample. They showed that almost 50% of samples exhibit diversity of grade, be it different grade in the same duct, different grade in different ducts in the same area, or different grades in different areas [6]. This recognition of diversity is not formally acknowledged in current pathological reporting schemes, but it may have clinical significance, although a study using the worst or the predominant grade in the Van Nuys system did not show association with recurrence or development of invasive disease in those patients with heterogeneity [10].

Pathways of Progression in DCIS

In order to define criteria that may better predict how individual DCIS lesions will behave, it is essential to try and understand the mechanisms by which progression to invasive disease occurs. There are different models of DCIS progression proposed, and though distinct, these are not totally incompatible.

The theory of linear progression maintains that the development of the malignancy from normal tissue happens in a linear stepwise way. Wellings and Jensen [4] first proposed that the terminal duct-lobular unit (TDLU) would progress through a series of increasingly abnormal stages over years and even decades until it becomes an invasive neoplasm. Using current terminology, they reasoned that the TDLU would first progress into hyperplastic lobular units, then into atypical ductal hyperplasia (ADH), DCIS, and finally into invasive cancer. However, this theory has recently been cited as an over-simplification of a highly complex process [6]. The latter study points out that, currently, ADH is considered as the non-obligate precursor of low grade but not high grade DCIS, and indeed, a number of genetic studies support the concept that there are several distinct pathways of progression of DCIS [11, 12]. It was shown that high grade DCIS and high grade invasive carcinoma share whole arm loss of 8p and 18q, as well as gain of 17q, and when DCIS and invasive disease co-exist, genomic changes are virtually identical [12]. Similarly, low grade DCIS and low grade invasive carcinoma have genomic similarities such as gain of 1q, loss of 16q, and distal loss of 8p [12]. These studies strongly suggest that low grade and high grade DCIS are distinct disease entities. However, there are other data that suggest that high grade DCIS evolves from low grade DCIS by random accumulation of genetic defects that are propagated to the invasive disease [6, 13, 14]. This also is supported by the finding that despite the increased frequency of detection of DCIS with the introduction of screening, there has been no increase in detection of high grade/comedo type DCIS [13], suggesting that screening leads to detection of low grade disease before it has progressed to higher grade disease. The concept that there is an intratumoral diversity of subtypes that would compete for dominance could explain the progression from low to high grade DCIS. For instance, a low grade DCIS would harbour high grade foci that would eventually be dominant, transforming the tumour into a high grade lesion. The progression from low to high grade lesions could mean not the transformation of one cell clone, but the dominance of a more aggressive clone in a tumour populated by a heterogeneity of clones. This intratumoral clonal heterogeneity has been reported in a wide range of solid malignancies, including breast cancer [14]. Such within-lesion competition for dominance would appear, ultimately, that a linear progression existed when, in fact, it did not. In truth, there is no unequivocal evidence supporting either of the theories, leading to the conclusion that both may co-exist and also that other still unknown progression pathways probably also exist.

One theory of tumour progression that is gaining momentum is the parallel progression model [5]. This suggests that tumour cells depart the primary lesion before the acquisition of the full malignant phenotype, and may then undergo somatic progression at the metastatic site [5]. Evidence from mouse models of breast cancer suggest that tumour cells

disseminate to bone marrow and lung from lesions as early as ADH and DCIS [15]. These experimental data were supported by human tissue studies, whereby disseminated tumour cells were detected in the bone marrow of 13% of patients diagnosed with DCIS [15]. Such findings profoundly challenge our current thinking of DCIS, and if validated, could have a major impact on patient management in the future. What regulates tumour dormancy at these distant sites is unknown, but interactions with the ectopic microenvironment may be implicated.

Biological Factors Relating to Disease Progression

Given the limitations of histopathology to accurately predict the potential for disease progression in DCIS, attention has turned to the identification of biological factors that may underlie, and therefore predict, the ability of DCIS to progress. There are numerous marker studies, therefore only those with the most promising clinical potential are reviewed here.

In invasive breast cancer, assessment of oestrogen receptor (ER), progesterone receptor (PR), and Her2/neu is used routinely to guide clinical management and predict outcome, and expression in DCIS may also be anticipated to reflect disease behaviour. In keeping with this, expression of ER is strongly associated with low grade DCIS [16, 17], whilst Her2 over-expression is strongly associated with high grade disease [16]. That the pattern of receptor expression may be important in predicting disease behaviour is suggested in a study by Kepple et al. [18] who found that ER-positive (ER+), PR-negative (PR-), Her2-positive (Her2+) DCIS was significantly more likely to recur than other DCIS types, and this receptor profile was associated with significantly reduced disease-free survival. The more frequent expression of Her2 in DCIS compared to invasive cancer is well established, and it has been suggested that this may be because Her2+ DCIS is a less aggressive disease [19]. In contrast, a recent study found over-expression of Her2 in DCIS to be the only significant predictor of invasive disease in a multivariate analysis [20], and suggested that Her2 may be important in promoting invasion. This has clear clinical implications, and further studies are needed to establish the prognostic value of Her2 in DCIS. Other growth factors implicated in invasive breast cancer, such as epidermal growth factor receptor (EGFR), are detected infrequently in DCIS [17, 20], which suggests they play a role in later stage disease.

The Notch pathway has been shown to act as a mediator of cell-cell communication in normal breast tissue and is thought to play a role in stem cell self-renewal, apoptosis, migration, and proliferation [21]. Dysregulated signalling pathways are frequent characteristics of cancerous cells, and aberrant Notch signalling appears to maintain the cells in a proliferative state that leads to an increased cancer risk [22]. The Notch receptor signals via its intracellular domain (NICD)

following cleavage by a protease and a second cleavage by γ -secretase [21]. Farnie et al. [23] showed that detection of NICD in DCIS predicted early recurrence 5 years after surgery, and using a model of primary DCIS mammospheres, they showed that inhibition of Notch signalling using a γ -secretase inhibitor could inhibit mammosphere growth [23]. The same group has recently shown that inhibition of Notch 4 signalling, in particular, in a stem-cell enriched population led to complete inhibition of tumour initiation in vivo [24]. This makes Notch an exciting potential predictive marker and therapeutic target.

Psoriasin, also known as S100A7, regulates pathways related to cell proliferation and survival via regulation of cytoskeleton [25]. What makes it particularly interesting is that it is one of the few known genes that is more highly and more frequently expressed in DCIS than in invasive breast cancer [26]. It has been detected more frequently in high grade DCIS and correlates with other negative prognostic markers [27], and probably not coincidentally, when expressed in invasive breast cancer it also associates with poor clinical outcome [26]. Psoriasin may have a very interesting role in DCIS progression to invasion. The protein negatively influences cell migration, invasion, and anchorage-free growth, and it has been suggested that in order to progress to invasion the cells must down-regulate Psoriasin expression [28]. The overexpression in a group of aggressive invasive breast cancers may indicate a requirement in this subgroup for the pro-angiogenic effects of Psoriasin [28].

A number of studies have demonstrated the impact of cell proliferative rate and expression of cell cycle regulatory proteins on the prognosis of both in situ and invasive breast cancer [29, 30]. A large review reported that high Ki-67 labelling indices correlated with high grade and with recurrence in DCIS [31]. Interestingly, a comparison of DCIS and DCIS with microinvasion also found higher Ki-67 indices in DCIS with microinvasive foci [32]. A recent exciting development has been reported by Kerlikowske's group [33], in which they report the ability to categorise patients with DCIS as low or high risk for the subsequent development of either recurrent DCIS or invasive disease. They showed that the risk for subsequent development of invasive breast cancer was significantly higher in women with DCIS detectable by palpation and positive for p16, COX-2, and Ki-67, compared to those detected by mammography and negative for p16, COX-2, and Ki-67 [33]. Interestingly, nuclear grade was not significantly associated with risk of subsequent invasive disease, echoing previous studies that suggest all grades of DCIS have the potential to progress with equal frequency [3]. The association of palpable DCIS with subsequent invasion is perhaps not surprising and is now a relatively uncommon clinical situation, so it will be particularly important to validate the predictive power of this algorithm in prospective studies where most DCIS is screen-detected. It is an exciting prospect that it may be possible to stratify women according to their risk of sub-

sequent invasive disease, but these factors could also provide potential therapeutic targets.

Molecular Classification of DCIS

The seminal work of Perou et al. [34] identified novel subtypes of invasive breast cancer based on an intrinsic gene signature established by cDNA microarray analysis. These subgroups have been refined by Sorlie et al. [35], classified as luminal A, B and C, Her2-positive, normal-like, and a basal-like category and shown to have prognostic significance. Many studies have aimed to identify an immunohistochemical profile that can act as a surrogate for gene array analysis, and it appears that a 5-marker panel of ER, PR, Her2, cytokeratin (CK) 5/6, and EGFR shows promise in its ability to categorise invasive cancers to their molecular subtype [36]. In invasive breast cancer, frequencies of 58–75% for luminal A, 11–16% for luminal B, 3–6% for Her2, and 11–20% for triple-negative (TN)/basal have been reported [37, 38], however, frequencies differ significantly in some studies on pure DCIS.

Meijnen et al. [17] carried out an immunohistochemical study on 163 cases of DCIS and showed no evidence of CK14 or EGFR expression, with CK5/6 being expressed in only 3 cases. In an unsupervised cluster analysis, they identified two major groups, an ER+/Bcl-2+ group designated as luminal, and an ER-/Bcl-2- group designated as non-luminal. The luminal subgroup was further subdivided into AR+, AR-, and mixed groups, whilst the non-luminal group subdivided into Her2+ and Her2- subsets. There was a significant relationship between grade and distribution between subsets, with significantly more well and intermediate grade DCIS clustering to the luminal groups compared to non-luminal groups. This is in contrast to a study by Livasy et al. [39] who, in an analysis of 245 pure DCIS cases, identified 8% as basal (defined as ER-, Her2-, EGFR+, and/or CK5/6+), and a further 6% fell into the TN category. This frequency of 14% correlates with the frequency of the basal/TN category in sporadic invasive breast cancers. Tamimi et al. [40] also reported significant differences between molecular phenotypes in DCIS and invasive breast cancer. In a large study comparing the immunoprofile of invasive breast cancer ($n = 2,249$) and their associated DCIS ($n = 272$), they showed that the basal subtype (defined as ER/PR/Her2-, EGFR+, CK5/6+) was less frequent in DCIS compared to invasive cancers (7.7% of 10.9%), and more so when restricted to invasive ductal carcinoma. They confirmed that the Her2+ subtype was significantly more frequent in DCIS compared to invasive disease (13.6% of 5.7%), as was the luminal B subtype (defined as ER/Her2+), reported as 13.2% frequency in DCIS compared to 5.2% in invasive cancers. The reasons for these discrepancies are unclear, but may be indicators of which types of DCIS progress more frequently. Considerably more work is required to clarify these relationships.

Role of the Microenvironment in DCIS Progression

The host microenvironment provides a complex milieu of cellular and non-cellular components that interact with neoplastic epithelial cells and modulate key cellular functions such as growth, differentiation, angiogenesis, and invasion [41, 42]. Attention has focused on the potential role of the microenvironment in determining the transition from DCIS to invasion, particularly in light of evidence that indicates few differences in gene expression between the neoplastic cells of DCIS and its invasive counterpart [43]. Major players of the microenvironment that have been implicated in promoting tumour progression include fibroblasts, inflammatory cells, blood vessels, extracellular matrix proteins, and a wide range of cytokines and chemokines [41–44]. One microenvironmental component specific to pre-invasive disease is the myoepithelial cell. Normal breast myoepithelial cells have been shown to exhibit a broad tumour suppressor function, maintaining epithelial cell polarity [45] and down-regulating matrix metalloproteinases and angiogenesis [46, 47], both key factors in mediating tumour invasion. However, there is growing evidence to indicate that myoepithelial cells adopt an altered phenotype in DCIS, with up-regulation of tumour-associated proteins such as tenascin-C isoforms, known to promote cell invasion [48], and a number of chemokines [49]. Indeed, in a detailed molecular analysis of microenvironment cell populations from normal and DCIS breast, the myoepithelial cell population exhibited the highest number of differentially expressed genes [49]. The functional consequences of this altered myoepithelial phenotype have not been established, though several of the up-regulated molecules, including CXCL12, have been shown to be tumour promoters in other systems [50].

The tumour-promoting role of stroma is well established in a number of systems, though less is known about its role in DCIS. The peri-ductal stroma is altered in DCIS [48, 49, 51], though again the functional significance has not been directly established. The interaction of tumour and stroma is bidirectional, and the importance of this dynamic relationship is well illustrated by COX-2. COX-2 expression in DCIS is associated with more aggressive features [52], and its expression in ADH has been correlated with risk of subsequent invasion [53]. In a DCIS xenograft model, tumour-derived COX-2 led to increased vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP) 9 and MMP14 expression in stromal cells, promoting tumour cell invasion [54]. Disappointingly, recent reports from a clinical trial examining the effectiveness of COX-2 inhibitors in DCIS showed no enhancement of anti-proliferative effects compared to exemestane alone [55], though this does not rule out potential longer term effects on disease progression that could not be examined in this type of study. Another molecule reported to play a role in stromal-mediated tumour regulation is caveolin-1. Caveolin-1 plays a role in membrane trafficking, gene regulation, and signal transduction, and recently, loss of caveolin-1

expression from DCIS-associated stroma was shown to be associated with early progression to invasive disease [56]. Although this was a small study, the role of caveolin-1 merits further study.

The immune response to tumours is complex, but inflammatory cells, particularly macrophages, have important tumour promoter functions. Gene expression profiling and immunohistochemistry have revealed a macrophage gene signature in the stroma of subsets of DCIS, and this is associated with a more aggressive phenotype [57]. It remains to be established if such a signature could help predict DCIS progression.

In summary, whilst the epithelial cells of DCIS display the dominant genetic aberrations associated with malignancy, significant alterations in a number of host cell populations are evident, and these changes appear to make a major contribution to the transition of DCIS to invasive disease.

New Therapeutic Approaches in DCIS

The mainstay of treatment for DCIS is surgical excision plus or minus radiotherapy and, in ER+ disease, adjuvant tamoxifen or aromatase inhibitor. Whilst a number of randomised control trials have indicated that radiotherapy after local excision of DCIS significantly reduces the incidence of local recurrence, no such trials have yet identified subgroups of patients in whom radiotherapy could safely be omitted (reviewed in [58]). In the International Breast Cancer Intervention Study (IBIS-I), tamoxifen has been shown to significantly reduce the risk of ER+ invasive breast cancer, and reduce the recurrence rate in DCIS by almost 50% [59]. A recent report has suggested that neoadjuvant endocrine therapy may be effective in ER+ DCIS. Chen et al. [60] treated premenopausal patients with tamoxifen and postmenopausal patients with letrozole during 3 months before surgery and compared pre-treatment and post-surgical tissues. They found that the Ki-67 labelling index was significantly reduced in both groups, but with a greater reduction in the postmenopausal group. They also showed that endocrine therapy was associated with a significant increase of macrophages within the lesional ducts. Similarly, another study examined the DCIS component of tumours in a prospective randomised window trial of letrozole versus anastrozole for ER+ postmenopausal breast cancer and showed a significant decrease in Ki67 in DCIS for both groups [61]. These data, though limited, support the rationale for neoadjuvant therapy in DCIS, though further studies are required. The IBIS-II trial aims to evaluate the potential role of third generation aromatase inhibitors in postmenopausal women with hormone-sensitive DCIS [62].

Recently, it has been proposed that trastuzumab may be effective in the treatment of DCIS, which would be of particular value since there are few effective medical treatments for ER– DCIS, and almost 50% of ER– DCIS overexpress Her2

[63]. The National Surgical Adjuvant Breast and Bowel Project (NSABP) is developing a phase III randomised trial of trastuzumab for patients with Her2-positive DCIS undergoing breast conserving surgery, which should help to establish the efficacy of targeting Her2 in pre-invasive disease [64].

Recommended Standards for Reporting of DCIS

It is established that grade and pattern of DCIS, presence of necrosis, disease extent, and margin status are important in helping to predict behaviour of disease [2, 3]. This was reinforced in a recent detailed histopathological review of >1,000 cases of DCIS entered into the UKCCR/ANZ DCIS trial which also identified the extent of necrosis (absent, focal, or extensive, the latter involving >50% of ducts) and presence of chronic inflammation as important disease predictors [65]. This study also identified a novel, very high grade of DCIS, based on cytonuclear grade, >50% ducts with necrosis and >50% solid growth pattern, which was associated with a significantly higher rate of recurrence [65]. Thus, this semi-quantitative analysis of morphological features may add prognostic value to histopathology reports.

At the current time, there is no agreed recommendation for the analysis of molecular markers in DCIS, however, the promising outcome of IBIS-I supports the value of adjuvant tamoxifen in DCIS and therefore the requirement for routine assessment of ER and PR. The system of quantitation of hormone receptors in DCIS is not well established, though a cut-off of 5% positivity was used for eligibility for treatment in IBIS-I [59]. If the new wave of studies assessing efficacy of trastuzumab are positive, then assessment of Her2 status may also be required.

It is probably premature to suggest that routine analysis of cell cycle proteins such as p16 and Ki67 should be undertaken, though it is entirely plausible that such an extended marker profile may form part of the pathology report in the not-so-distant future.

Conflict of Interest

The authors did not provide a conflict of interests statement.

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