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Molecular Markers for the Diagnosis and Management of Ductal Carcinoma In Situ

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Ductal carcinoma in situ (DCIS) is a heterogeneous group of lesions reflecting the proliferation of malignant cells within the ducts of the breast without invasion through the basement membrane. Numerous studies analyzing the molecular profiles of DCIS using genome-wide unbiased and candidate gene approaches have been conducted with the aim of identifying clinically useful markers that would predict the risk of progression to invasion. Results of these investigations defined the heterogeneity of DCIS at the molecular level, but a gene signature predictive of invasive progression has not been identified. Major diagnostic criteria that differentiate DCIS from invasive cancer are the presence of intact basement membrane and myoepithelial cell layer. Based on this, perturbation of normal myoepithelial cell differentiation has been proposed to explain progression to invasion. Comprehensive molecular studies analyzing large cohorts of DCIS with long-term clinical follow-up are necessary to resolve the many remaining questions.

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Cancer is an evolution of a population of cancer cells with diverse hereditary characteristics. With rare exceptions, tumors are derived from a single initiated cell and the progressive accumulation of genetic, epigenetic, and phenotypic features combined with selection drives cancer progression. The currently accepted stepwise model of breast tumorigenesis assumes a gradual transition from epithelial hyperproliferation to ductal carcinoma in situ (DCIS), to invasive and metastatic carcinomas (1,2). This progression model is strongly supported by human clinical and epidemiological data and by molecular clonality studies addressing the relationships between in situ and invasive areas of the same tumor and between DCIS and its local invasive recurrence. Until 1980, DCIS was diagnosed very rarely and represented less than 1% of all breast cancer cases. All of this changed dramatically with the increased utilization of mammography during the 1980s, and DCIS became the most rapidly increasing subset of breast cancers. Currently, DCIS accounts for 15%–25% of newly diagnosed breast cancer cases in the United States (3). In contrast to the dramatic improvement in our ability to detect DCIS, our understanding of the pathophysiology of this disease and factors involved in its progression to invasive carcinoma are still poorly defined. Numerous studies compared the gene expression, genetic, and epigenetic profiles of DCIS and invasive breast carcinomas, but a molecular alteration differentiating in situ and invasive tumors has not been identified (2). A dramatic change occurs during the normal to DCIS transition, but surprisingly in situ and invasive breast carcinomas of the same histological subtype essentially share the same genetic and epigenetic alterations and expression patterns. In contrast, the molecular profiles of breast tumors of distinct subtypes (ie, luminal, HER2+, and basal-like) are dramatically different. Emerging data also suggest a critical role for microenvironmental changes in the regulation of tumor progression, particularly that of the in situ to invasive breast cancer transition (4). Here, I summarize the results of molecular studies aiming to identify diagnostic markers that differentiate in situ and invasive tumors and predictive markers that correlate with the risk of invasive progression.

Candidate Molecular Markers in DCIS

The expression and mutation status of numerous tumor suppressor and oncogenes have been analyzed in DCIS and invasive ductal carcinoma (IDC) including TP53, PTEN, PIK3CA, ERBB2, MYC, and differences in the frequency of these changes have been found according to the tumor subtype but not histological stage (2). Thus, mutations in TP53 are more frequent in basal-like and HER2+ compared with luminal tumors; in basal-like cases, PIK3CA is rarely mutated but PTEN is frequently lost, and amplification of ERBB2 is specific for the HER2+ subtype.

The expression of several candidate genes selected based on their biological function has also been analyzed in DCIS (5). Two recent studies identified a set of promising markers that may correlate with the risk of recurrence of DCIS (6,7). Gauthier et al. (6) demonstrated that high expression of COX-2 and Ki67 in DCIS correlates with higher risk of local (both in situ and invasive) recurrence and also implicated abnormalities in the Rb pathway as potential contributors to invasive progression. Lu et al. (7) identified a functional cooperation between ERBB2 and 14-3-3ζ that may increase the risk of invasive progression via promoting epithelial to mesenchymal transition. A major limitation of both of these studies was the use of small cohorts of patients that increases the

probability of findings associations that may not hold up in larger populations. However, large cohorts of patients diagnosed with pure DCIS, treated uniformly, and followed up for a long (eg, >10 years) time are rare, and tumor tissue samples may not be available for all cases. Examples of such cohorts include the National Surgical Adjuvant Breast and Bowel Project B-17 and B-24 trials analyzing the recurrence of DCIS treated with lumpectomy alone vs lumpectomy + radiation (B-17) and comparing lumpectomy + radiation vs lumpectomy + radiation + tamoxifen (B-24). Neither of these cohorts has been subject to molecular studies and none of the histopathologic features analyzed predicted the risk of invasive recurrence (8,9). However, in the B-24 trial, tamoxifen treatment significantly (by 40%–50%) reduced the risk of subsequent breast cancer (both ipsilateral and contralateral) in patients with estrogen receptor–positive DCIS (D. C. Allred, S. J. Anderson, S. Paik, D. L. Wickerham, I. Nagtegaal, J. P. Costantino, et al., submitted). Despite the fairly large size of these cohorts (>1400 cases in each trial) because of the low rate of invasive recurrence (<10%) even after 10–15 years of follow-up, the identification of markers predictive of invasive recurrence is a challenging task.

In addition to protein-coding genes, a few recent studies also examined the expression patterns of microRNAs at different stages of breast tumor progression, including DCIS. One study analyzed the expression of miR-21 and its targets (PTEN, PDCD4, and TMI) in normal breast and in DCIS and IDC and found a gradual increase in miR-21 expression with tumor progression, although very few cases were analyzed in each histological group (10). An independent study confirmed the increased expression of miR-21 during tumorigenesis and also found an increase in miR-145 levels in DCIS compared with atypical hyperplasia implying that microRNAs may be used as novel biomarkers for early cancer diagnosis (11).

Comprehensive Molecular Profiles of DCIS

Major progress has been made in the molecular classification of invasive breast cancer by applying a combination of high throughput genomic technologies. Gene signatures differentiating tumors into clinically relevant subtypes and prognostic groups have been described, and some of these such as OncotypeDX and Mammaprint are already used in clinical practice (12). Several genome-wide unbiased studies have also been conducted investigating the gene expression and genetic profiles of pure DCIS and in situ and invasive areas of the same tumor. Gene expression profiling of bulk tissue samples or dissected epithelial cells of pure DCIS and DCIS adjacent to IDC using different platforms (ie, nucleotide arrays and sequence-based methods) and dissection approaches (ie, immunomagnetic bead sorting and laser capture microdissection) failed to identify progression stage–specific expression patterns (13–17). However, gene expression profiles of DCIS demonstrated the same subtype-specific patterns as that observed in IDC implying distinct progression pathways for luminal, basal-like, and HER2+ tumors.

Similarly, analysis of chromosomal alterations in DCIS and IDC using multiple different comprehensive approaches including array comparative genomic hybridization and single-nucleotide polymorphism arrays demonstrated no qualitative differences according to progression stage, although IDC and DCIS adjacent to IDC overall tended to have more chromosomal changes than pure DCIS (16,18,19). In contrast, distinct genetic changes were detected in luminal, HER2+, and basal-like tumors (Table 1), emphasizing subtype-specific tumor evolution.

Table 1. Breast tumor subtype-specific chromosomal alterations*

Tumor subtype	Genetic change	Candidate genes targeted
Luminal A	1q gain	Multiple, S100 proteins
	8q24 gain	*MYC*
	11q13 gain	*CCND1, PAK1, EMSY*
	16p gain 16q loss	Multiple, *CDH1*
	20q13 gain	*AIB1*
HER2+	1q gain	Multiple, S100 proteins
	8q24 gain	*MYC*
	17q12 gain	*ERBB2*
Basal-like	8q24 gain	*MYC*
	10p13 gain	*ITGA8, MEIG1*
	12p13 gain	*RAS, ETV6, H2AFJ*

* Several of these changes involve whole chromosome arms likely targeting multiple genes.

Intratumor Diversity in DCIS

In addition to the dramatic intertumor differences according to the tumor subtype, a significant fraction of DCIS display pronounced intratumor heterogeneity for the expression of multiple markers and for genetic alterations such as chromosomal gains and mutation in *TP53* (17,20). Allred et al. (17) demonstrated this by the immunohistochemical analysis estrogen receptor, progesterone receptor, HER2, and p53 expression in a large panel of DCIS and found that heterogeneity was associated with positivity for p53 reflecting mutant *TP53* (17). This result correlates with the findings in Barrett esophagus, a precursor of esophageal carcinoma, where intratumor clonal diversity was associated with mutant p53 (21).

In a more recent study, Park et al. (20) analyzed intratumor diversity for chromosomal gains and for the expression of markers associated with stem cell-like (eg, CD44) and more differentiated luminal (eg, CD24) epithelial cell phenotypes in both in situ and invasive areas of the same tumors at the single-cell level using immuno–fluorescence in situ hybridization. Intratumor heterogeneity was quantified using ecological and evolutionary models, and significant heterogeneity was found in cell populations uniformly positive for the expression of CD44 or CD24 within the same histological type as well as between in situ and invasive areas. Interestingly, basal-like tumors uniformly displayed high diversity, whereas a subset of HER2+ and luminal tumors had low diversity. These results implicate intratumor diversity as a potential predictor of invasive progression, as in Barrett esophagus, a precursor of esophageal carcinoma, higher intratumor clonal diversity was associated with increased risk of invasive progression. Thus, it would be worthwhile to conduct similar studies in DCIS with clinical follow-up.

Although the molecular mechanisms underlying intratumor diversity are poorly defined, genomic instability because of telomere shortening and subsequent telomere crisis that is observed at the DCIS stage is one of the potential explanations (22). However, alternative mechanisms include epigenetic instability, malignant stem cells, and nonhereditary cell-to-cell variability (23).

Experimental Models of Human DCIS

Our relative lack of understanding of the in situ to invasive breast carcinoma progression is in part because of the fact that there are no good experimental models for DCIS that would faithfully reproduce the human disease. Carcinogen-induced mammary gland tumors in rats reproduce certain aspects of human DCIS, such as ovarian hormone dependence and gradual progression to invasive disease (24). However, the carcinogen used for the initiation of these tumors may have caused numerous genetic changes that are not easy to identify making this model unattractive for molecular studies. The same limitation applies to the use of DCIS xenografts formed by subcutaneous injection of pieces of human DCIS tumors into nude mice (25) or the intraductal injection of DCIS tumor epithelial cells (26). Although no model is ideal, they allow for the functional testing of genes implicated in breast cancer and the evaluation of novel cancer preventative and therapeutic interventions. A good model of DCIS would have to resemble the histology of high-risk human premalignant breast lesion that with time progress to invasive carcinomas. The MCF10AT human breast cell line is one of the most well-characterized human models of breast tumor progression that fulfills these criteria (27,28). These cells were derived from the immortalized MCF-10A cells via transformation with T24 mutant c-Ha-ras (27,28). Interestingly, the MCF10AT cells appear to contain multipotent (or bipotential) breast stem cells because both luminal epithelial and myoepithelial cells can be derived from these cells in vivo (29). A derivative of the MCF10AT premalignant human cell line model was established MCF10DCIS.com that reproducibly forms comedo DCIS-like lesions that spontaneously progress to invasive tumors (27,28). This xenograft model has successfully been used for delineating the role of myoepithelial cells and the microenvironment in DCIS progression (30,31). However, as MCF10DCIS cells represent only basal-like breast cancer, additional DCIS models are needed for other tumor subtypes.

The Role of Myoepithelial Cells and the Microenvironment in DCIS Progression

The role of the microenvironment in the regulation of normal mammary gland function and the alterations of these in breast cancer has been increasingly recognized. Due to this and to the failure to find markers differentiating DCIS and IDC based on the analysis of bulk tumors and epithelial cells, Allinen et al. (32) analyzed the genome-wide gene expression and genetic profiles of all major cell types (eg, epithelial, myoepithelial, and endothelial cells; fibroblasts; myofibroblasts; and leukocytes) composing normal breast, DCIS, and IDC tissues. Dramatic changes were found in gene expression profiles in all cell types during tumor progression, whereas clonal genetic alterations were limited to tumor epithelial cells. Both these findings have been confirmed by multiple different groups using different technologies (33,34). Particularly, myoepithelial cells showed significant differences and these changes implicated loss of differentiated and gain of angiogenic and invasive features in DCIS-associated compared with normal myoepithelium. Interestingly, a large fraction of the differentially expressed genes encoded secreted proteins, including basement membrane components, and receptors indicating a key role for myoepithelial cells in the synthesis of the basement membrane and in paracrine interactions.

Despite the lack of clonal genetic alterations in tumor-associated myoepithelial and stromal cells, changes in gene expression patterns and phenotypes appear to be hereditary because they are maintained even after prolonged cell culture and in xenograft studies (4). Thus, Hu et al. (35) hypothesized the presence of epigenetic alterations and analyzed the global DNA methylation profiles of epithelial and myoepithelial cells, and stromal fibroblasts from normal and in situ and invasive breast carcinomas using methylation-specific digital karyotyping. Using this approach, several genomic loci were identified that showed significant and consistent differences in DNA methylation between normal and neoplastic breast tissue in each cell type analyzed. Thus, aberrant epigenetic programs of DCIS-associated myoepithelial cells and stromal fibroblasts may underlie their tumor growth and progression-promoting effects.

To experimentally test this hypothesis, in a follow-up study, Hu et al. (30) analyzed the role of myoepithelial cells and fibroblasts in DCIS to IDC transition using a xenograft model of human DCIS and validated these findings in primary human tissue samples. Normal myoepithelial cells exerted pronounced tumor growth and progression-suppressive effects, whereas stromal fibroblasts promoted progression to invasion, and tumor-associated and inflammatory fibroblasts even increased tumor growth via enhancing angiogenesis. These growth- and progression-promoting effects of fibroblasts were at least in part due to the upregulation of COX-2 in tumor epithelial cells because treatment with a COX-2 inhibitor (celecoxib) was able to suppress them (36). Several pathways including TGFβ and Hh signaling were identified to be required for normal myoepithelial cell differentiation as their inhibition using small hairpin RNA lead to the disappearance of these cells and progression to invasion (30).

The importance of myoepithelial cells in DCIS progression is supported by findings from several different groups. Man et al. (37) have demonstrated in multiple publications the presence of focally disrupted myoepithelial cell layer in DCIS and implicated this in invasive progression. Hilson et al. (38) described phenotypic alterations in DCIS-associated myoepithelial cells determined by immunohistochemistry. More recently, Sotiriou and colleagues analyzed the expression profiles of DCIS with clinical follow-up and demonstrated that decreased expression of CD10, a differentiated myoepithelial cell–specific marker, was associated with decreased disease-free survival (39). All these data support the hypothesis that loss of normal myoepithelial cell function in DCIS may be the key determinant of invasive progression. Thus, characterizing the regulation of normal myoepithelial cell differentiation and the perturbations of these in DCIS may identify predictors of invasion and targets for therapeutic interventions.

Conclusions and Future Directions

In contrast to the significant progress made in the molecular-based classification of invasive breast cancer and the use of this information for the design of individualized therapy, the clinical management of DCIS patients is still largely based on histopathologic findings and molecular markers guiding treatment decisions are lacking. Progress

is mainly limited by the availability of pure DCIS tissue samples from large cohorts of uniformly treated patients with long-term clinical follow-up. In addition, the type (ie, formalin-fixed paraffin-embedded) and limited amount of tissue available because of the small size of DCIS lesions poses a technical challenge. However, technologies are continuously improving and several methods are already suitable for the comprehensive molecular analysis of DCIS, which is favored over candidate gene approaches as larger amount of information can be gained from the same amount of tissue. Multi-institutional collaborative efforts by multidisciplinary research teams would be required to overcome these hurdles, and this is likely to be facilitated by prioritizing the "DCIS problem" by the National Cancer Institute and providing long-term funding for this difficult task.

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Note

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