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Breast Ductal Carcinoma in situ Theme Issue

REVIEW

Ductal Carcinoma *in Situ* Biomarkers in a Precision Medicine Era

Current and Future Molecular-Based Testing

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Historically, ductal carcinoma *in situ* (DCIS) of the breast has been managed aggressively with surgery and radiotherapy because of a risk of progression to invasive ductal carcinoma. However, this treatment paradigm has been challenged by overtreatment concerns and evidence that suggests that DCIS can be stratified according to risk of recurrence or risk of progression to invasive disease. Traditional methods of risk stratification include histologic grade and hormone receptor status. Recent technological advancements have enabled an era of precision medicine, where DCIS can be molecularly analyzed by tools, such as next-generation DNA and RNA sequencing, to identify molecular biomarkers for risk stratification. These findings have led to the development of tools such as the Oncotype DX Breast DCIS Score, a gene expression–based assay with the potential to prevent overtreatment in low-risk disease. *(Am J Pathol 2019, 189: 956–965; https://doi.org/10.1016/j.ajpath.2018.08.020)*

Human cancers of epithelial origin are thought to arise from a multistep process of tumorigenesis, where a normal stem cell acquires various insults to its genome and transforms into a premalignant cell.[1] These cells in turn will acquire additional alterations that in time will result in transformation into a tumor cell with a malignant phenotype, which may include the ability to invade surrounding tissue and metastasize. Currently, the exact biological drivers that govern the transformation from premalignancy to malignancy are not well understood. Identifying these drivers in patients and targeting them appropriately represent a major opportunity in the clinical management of cancer.

The rapid technological development of methods for characterizing disease, including genomics, proteomics, metabolomics, cellular and histologic assays, and bioinformatics analyses, have ushered in a new era of precision medicine. Precision medicine involves the tailoring of individualized treatment strategies based on variability among patients.[2,3] The availability of these tools has enabled large, highly collaborative research efforts, such as The Cancer Genome Atlas, to perform analyses on large collections of cancer samples and clinical data to define the genomic landscape of cancers, including breast cancer.[4] These groups, along with research from laboratories around the world, have been instrumental in advancing our understanding of the molecular drivers of various aspects of cancer biology, including initiation, metastasis, and response to treatment. Such discoveries have translated into biologic targets for the development of precision medicine therapy and combination therapy approaches for patients. To date, most large-scale efforts have been limited to invasive disease,[5,6] making the

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characterization and treatment of preinvasive disease an exciting research opportunity.

Ductal carcinoma *in situ* (DCIS), accounting for approximately 25% of all newly diagnosed breast cancers,[7] is defined as a clonal proliferation of breast epithelial cells confined to the lumen of a mammary duct. DCIS is a heterogeneous disease, ranging from indolent quiescent forms to more aggressive forms that may rapidly evolve to invasive ductal carcinoma (IDC), with clinical, morphologic, and genetic variability.[8–10] DCIS is treated with the goals of reducing the risks of recurrence and transformation to IDC. Currently, the standard of care for most DCIS is surgical excision by lumpectomy (breast-conserving surgery), with the addition of radiotherapy and appropriate systemic therapy (eg, tamoxifen) based on multiple histologic, biologic, and clinical factors. Adjuvant radiotherapy after breast-conserving surgery can reduce risk of local and invasive recurrence up to 48% and 42% at 10 years, respectively.[11] The addition of adjuvant tamoxifen reduces both recurrence and progression to invasive carcinoma by approximately 50%[12,13]; this reduction was largely driven by estrogen receptor (ER)–positive lesions.[14] Furthermore, clinicopathologic studies have elucidated important demographic and histologic risk factors for recurrence, including age at diagnosis, lesion size, histologic grade, and width of the excision margin.[15,16]

Even with the advances in disease control, the management of DCIS remains controversial. Several randomized trials have found that many DCIS lesions removed by surgical excision will not develop IDC or local recurrence, regardless of radiotherapy or systemic therapy.[17] There is also evidence that low-risk DCIS can be adequately treated with no more than active surveillance,[18–20] which is being further investigated in active clinical trials, such as the US Phase III Comparison of Operative to Monitoring and Endocrine Therapy trial for low-risk DCIS (Clinical Trial Identifier: NCT02926911) and the European Low Risk DCIS study (Clinical Trial Identifier: NCT02492607). In contrast, observations of the natural history of low-grade DCIS have also highlighted the continued risk for development of IDC. For example, in a small cohort of 45 women with low-grade DCIS treated by biopsy only and followed up for up to 42 years, 11 invasive breast cancers were diagnosed, and seven women developed distant metastases.[19] Because both overtreatment and cancer progression remain significant concerns in DCIS, discerning which patients are more likely to have progressive disease remains an area of intense research efforts.

Models of DCIS Progression

The models that govern progression of DCIS to IDC can be separated into intrinsic and extrinsic causes. Intrinsic causes involve changes that stem from genetic alterations in the DCIS cells, leading to an invasive phenotype. Extrinsic causes involve changes independent of genetic changes within the lesion, such as changes mediated by the surrounding tissue microenvironment. These models are summarized in Figure 1.

There are three proposed intrinsic models of genomic evolution from DCIS to IDC.[21] The first intrinsic model is the independent lineage model, in which DCIS and IDC arise from independent clonal cell populations. This model is also described as the field cancerization phenomenon, whereby regions of tissue that may be generally exposed to external mutagens can give rise to multiple, genetically distinct lesions. Support for this model involves single-marker studies that found a discordance between synchronous DCIS and IDC cases; for example, a study analyzing *PIK3CA* mutations in patients with matched IDC and DCIS reported only 30% concordance.[22] This model is further supported by mathematical modeling.[23] The second intrinsic model is the evolutionary bottleneck model, in which multiple clones are present in DCIS but only a single clone progresses to become IDC. This model is best supported by multiple phylogenetic studies that have identified truncal events concordant between DCIS and IDC, with additional copy number alterations (CNAs) and mutations that occurred later in evolution.[24,25] The third intrinsic model is the multiclonal invasion model, where multiple clones can escape from the duct and invade surrounding tissue. This model is supported by indirect evidence from extremely high levels of concordance between CNAs and mutations between DCIS and IDC (as high as 97%); in contrast, an evolutionary bottleneck model would likely have multiple divergent branches between its transition from DCIS to IDC, which would be expected to have lower levels of concordance.[26,27]

Extrinsic causes of DCIS progression to IDC involve the microenvironment, which consists of the cellular components (eg, fibroblasts, endothelial cells, immune cells, adipocytes) and noncellular components [eg, extracellular matrix (ECM), growth factors, cytokines, pH] that influence the cancer cells.[28,29] The microenvironment has been causally implicated in multiple aspects of cancer biology, including progression, metastasis, and drug resistance.[30–34] In DCIS, ECM remodeling, stromal cell interactions, myoepithelial disruption, and gene expression changes in stromal cells have been independently linked to DCIS progression. Studies have found that ECM remodeling mediated by lysyl oxidase–mediated collagen crosslinking are required for invasion in DCIS.[35] In addition, remodeling of ECM secondary to mammary gland involution after pregnancy has been implicated in progression to an invasive phenotype mediated by an up-regulation of fibrillar collagen and cyclooxygenase-2 (COX-2) in DCIS.[36] Stromal fibroblasts also increase COX-2 expression on interaction with DCIS epithelial cells, leading to up-regulation of vascular endothelial growth factor and matrix metalloproteinase (MMP)-14 and subsequent progression to an invasive phenotype.[37] Myoepithelial cells normally exert tumor suppressive effects on DCIS lesions by both acting as a

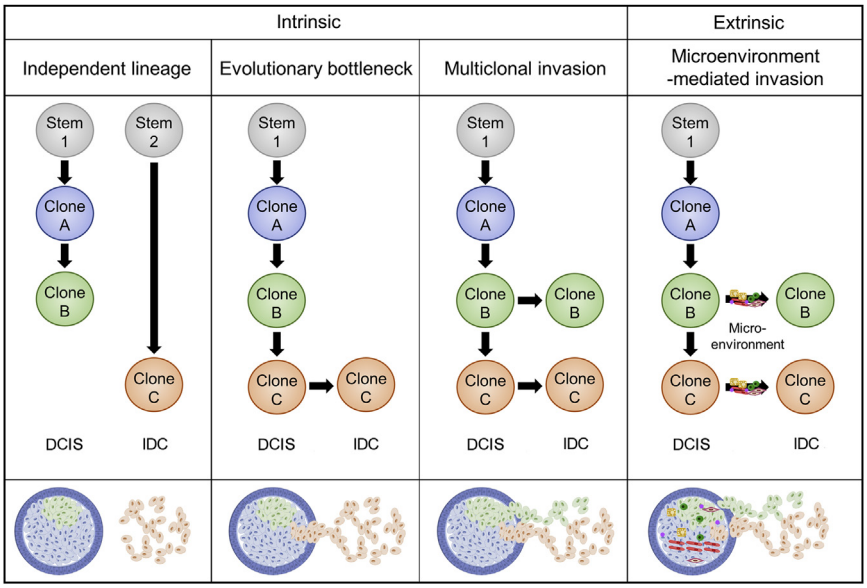

Figure 1 Existing models of ductal carcinoma *in situ* (DCIS) progression to invasive ductal carcinoma (IDC). The independent lineage, evolutionary bottleneck, and multiclonal invasion models involve cell-intrinsic mechanisms of DCIS progression to IDC. The microenvironment-mediated invasion model is cell extrinsic and involves cellular factors, such as immune cells, fibroblasts, and endothelial cells, and noncellular factors, such as hormones, growth factors, extracellular matrix, and more. There is varying evidence that supports each model of progression and the simultaneous occurrence of multiple models.

physical barrier to invasion and secreting ECM components and protease inhibitors[38]; these functions are disrupted in invasive disease.[39] Several studies have found significant differences in the gene expression among fibroblasts, myoepithelial cells, and leukocytes in DCIS versus IDC[40,41]; for example, transition to invasive growth was accompanied in one study by increased expression of several MMPs (MMP-2, MMP-11, and MMP-14), which are known effectors of cancer progression in multiple cancer types.[42] Other well-studied extrinsic breast cancer risk factors, such as host circulating hormone levels (eg, estrogen), prior radiotherapy, and lifestyle factors (eg, obesity and alcohol), may also directly affect DCIS progression but have yet to be characterized in detail and warrant further study.

These models represent a simplified explanation of progression in DCIS; in reality, they are likely not mutually exclusive. A recent study analyzing genome-wide CNAs for single cells in formalin-fixed, paraffin-embedded tissues in DCIS and IDC revealed evidence for both the bottleneck and multiclonal invasion models in different patients, suggesting that progression models may differ on a patient-by-patient basis.[43] Similarly, field cancerization and microenvironment-mediated effects could be direct drivers of transitions in the evolutionary bottleneck and multiclonal invasion models. The development of comprehensive mathematical models capable of incorporating the complexities of both intrinsic and extrinsic causes of DCIS progression represents an important area of future research.

Traditional Classification of DCIS

Traditionally, histopathologic features have been used as the standard for classifying DCIS, which have been reviewed extensively.[44] Although there is no universally accepted classification system for DCIS, the most commonly used systems typically classify DCIS into three grades (low, intermediate, or high) based largely on nuclear grade and the presence or absence of necrosis.[45] The association between nuclear grade and progression to IDC remains controversial; whereas some studies have reported evidence of an association between high-grade DCIS and progression to IDC,[46] others have not found such an association.[47] Future longitudinal studies are necessary to clarify these associations.

The most common immunohistochemical markers assessed in DCIS are ER and progesterone receptor (PR). Most DCIS lesions express ER (range, 49% to 96.6%) and PR (range, 40% to 83.3%), and expression of these receptors is highly correlated.[48] Multiple studies have also found that ER- and PR-negative DCIS is associated with increased grade and risk of local recurrence.[49,50] The role of routine testing for human epidermal factor 2 (HER2) in DCIS remains unclear. Although HER2 expression in DCIS is correlated with high nuclear grade and increased risk of recurrence and negatively correlated with ER and PR expression,[51–54] it is not currently recommended to routinely test DCIS for HER2. The effect of radiotherapy with or without trastuzumab in HER2-positive patients with DCIS who have had a lumpectomy is being evaluated in the National Surgical Adjuvant Breast and Bowel Project B43 study, which is expected to be completed in 2019 (Clinical Trial Identifier: NCT00769379). None of the above receptors can reliably predict for recurrence or progression to an invasive phenotype. Our ability to identify those DCIS lesions that are more likely to recur or progress to invasive cancer remains limited, and adequate studies investigating novel potential predictive or prognostic biomarkers in DCIS are lacking. Kerlikowske et al[47] reported an association between progression to invasive cancer and co-expression of Ki-67, p16, and COX-2. However, this

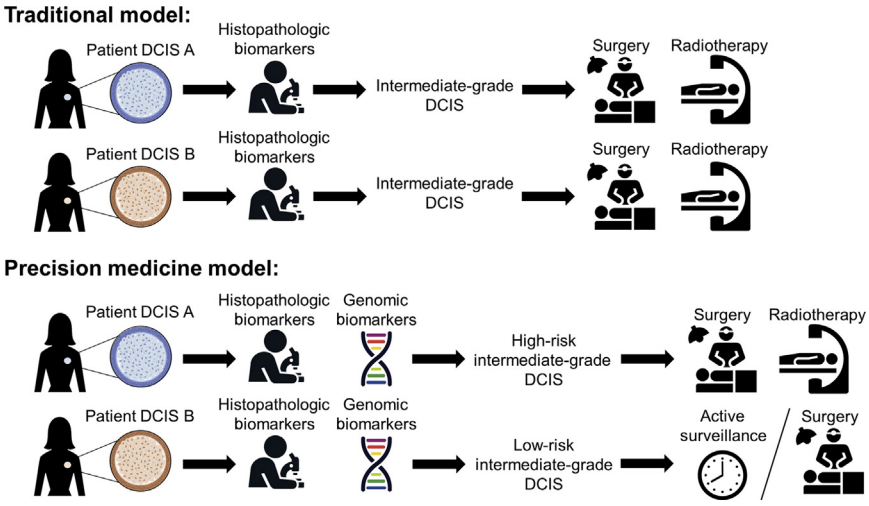

Figure 2 Comparing traditional treatment models to precision medicine—informed treatment models in ductal carcinoma *in situ* (DCIS). In a traditional paradigm of DCIS treatment, two patients (A and B) with an intermediate-grade DCIS would be treated with surgery and radiotherapy. In contrast, a precision medicine—based treatment approach would incorporate genomic biomarkers to stratify DCIS lesions according to risk of recurrence or invasion; in this example, one patient's DCIS (patient DCIS A) was determined to be high risk, which would be treated with the standard surgery and radiotherapy, whereas the other low-risk DCIS (patient DCIS B) would be treated with active surveillance or surgery only.

association has yet to be validated. A variety of factors, including small sample size, lesion heterogeneity, lack of significant clinical follow-up, and issues with standardization of scoring methods and reproducibility, are common limitations to studies investigating various biomarkers.

Classification of DCIS in a Precision Medicine Era

In the current precision medicine era, a large variety of molecular tools are available to fully characterize a patient's cancer. Single-nucleotide alterations, DNA CNAs, genomic structural rearrangements, gene expression, epigenetic alterations, and other features can be routinely tested using array-based and sequencing-based technologies.[55] These technologies have been used to comprehensively characterize invasive tumors, leading to the identification of new molecular targets and genetic loci associated with breast cancer risk and progression.[4,56–58] Recently, discovery of genomic biomarkers of progression from DCIS to IDC has been identified as a valuable precision medicine opportunity: clinically separating aggressive from indolent DCIS will allow for both prevention of progression to IDC and, ultimately, prevention of overtreatment in patients who would not likely benefit. For example, two patients with histologically identical intermediate-grade DCIS could both be recommended for treatment with surgery and radiotherapy; however, by using a precision medicine—based treatment approach that incorporates genomic biomarkers, the two lesions could be separated as high or low risk. The high-risk DCIS can be treated with surgery and radiotherapy, whereas, the low-risk DCIS can be treated with active surveillance or surgery alone (Figure 2). Although there are multiple areas being explored in the molecular characterization of DCIS, we restrict this review to two major areas of focus: genetic alterations and gene expression.

Genetic Alterations

Multiple studies have compared the genetics of DCIS and IDC within a given patient to identify genetic biomarkers that might contribute to disease progression, using a variety of array- and sequencing-based technologies. In general, many studies reported no association between genetic alterations and progression of DCIS to IDC. Burkhardt et al[59] performed fluorescence *in situ* hybridization (FISH) on a tissue microarray of 130 pure DCIS samples and 159 DCIS samples with concurrent invasive breast cancer. They found no significant differences in amplification rates of genes commonly amplified in breast cancer, including *ERBB2*, *ESR1*, *CCND1*, and *MYC*, and found high concordance in general gene amplification rates between the two groups. Pan et al[60] used quantitative multigene FISH to assess gene CNAs in 30 genes in 66 tumors with synchronous DCIS and IDC. They identified frequent amplification of genes such as *MDM4*, *CCNE2*, *ERBB2*, *IGF1R*, *CKS1BP7*, and *MYC* and frequent deletion of *TP53*, *CHEK1*, *RB1*, *CDH1*, *CHEK2*, *and NEK9*, each of which was observed in >20% of cases; however, no significant differences were detected between DCIS and IDC. Finally, Rane et al[61] conducted a meta-analysis based on results from 26 studies, comparing cases of atypical ductal hyperplasia, pure DCIS, synchronous DCIS with IDC, and pure IDC. Again, no significant differences were found between the numbers and types of CNAs identified between DCIS and IDC, which suggests that CNAs are early events in the development of breast cancer.

Some studies have suggested genetic differences between DCIS and IDC that may be implicated in DCIS progression. Johnson et al[26] found differences in CNAs between 21 cases of synchronous DCIS and IDC. Although CNA profiles between DCIS and IDC were highly synchronous (mean of 83% of the genome shared), IDC samples had regions of chromosomal gain that included the oncogenes *CCND1* and *MYC* when compared with DCIS samples. Furthermore, observational comparisons suggest that *TP53* mutations

may contribute to invasive progression because studies have found a lower prevalence of TP53 mutations in DCIS (approximately 17%)[62] compared with IDC (approximately 37%).[4] Finally, a recent study of 111 DCIS lesions by Pang et al[63] found that the frequency of *GATA3* mutations was increased in DCIS compared with levels found in multiple studies of IDC. This finding, coupled with the fact that *GATA3* mutations are associated with improved survival in invasive breast cancer,[64] suggests that these mutations are selected against in progression of IDC.

Despite the wealth of data published in the literature, limited evidence suggests that there are significant genetic differences between DCIS and IDC; the studies that suggest significant differences are limited by small sample size and lack of functional applicability. Larger longitudinal studies are warranted.

Gene Expression

Compared with genetic alteration–based biomarkers, gene expression–based biomarkers have had much greater clinical success in DCIS. The first and only commercially available multigene expression panel is the 12-gene Oncotype DX Breast DCIS assay, which was developed to stratify individual patients with DCIS into groups with different degrees of risk for local recurrence.[65] This assay uses a combination of seven cancer-related genes [*MKI67* (Ki-67), *STK15*, *BIRC5* (Survivin), *CCNB1* (Cyclin B1), *MYBL2*, *PGR* (PR), and *GSTM1*] and five reference genes [*ACTB* (β-actin), *GAPDH*, *RPLPO*, *GUS*, and TFRC] to predict the risk of breast cancer recurrence after breast-conserving therapy for DCIS. The test provides an individualized 10-year risk of local recurrence (DCIS and/or IDC), a prediction of benefit from radiotherapy, quantitative ER and PR gene expression values, and a numeric score that places patients into low-, intermediate-, or high-risk categories.

The Oncotype DX Breast DCIS assay predictions are supported by two clinical validation trials, the Eastern Cooperative Oncology Group–American College of Radiology Imaging Network E5194 study[66] and the Ontario DCIS Cohort study,[67] which used retrospective samples from patients with surgically treated DCIS that did not receive radiotherapy. A meta-analysis of these two validation studies also found that combining the Oncotype DCIS Score with lesion size and age identified more patients at the extremes of the risk spectrum: higher risk and very low risk of local recurrence.[68] These studies also endorse that the Oncotype DX Breast DCIS Score predicts 10-year local recurrence risk more accurately than traditional clinical and pathologic factors. Additional studies have found that conventional clinical and histopathologic characteristics correlate with Oncotype DX Breast DCIS Scores (ie, high nuclear grade DCIS with comedo necrosis correlates with higher Oncotype DCIS Scores; low nuclear grade, strongly ER-positive DCIS correlates with lower Oncotype DCIS Scores).[69,70] Two recent studies suggest that the Oncotype DX Breast DCIS assay has already led to a significant reduction in radiotherapy recommendations by surgeons and radiation oncologists.[71,72]

A few limitations of the Oncotype DX Breast DCIS Score should be noted. One major limitation is expense; a recent cost-benefit analysis revealed that although incorporation of the Oncotype DCIS Score decreases the proportion of women undergoing radiotherapy per recurrence event prevented, these strategies were not cost-effective.[17] Similarly, it is currently unknown whether Oncotype DCIS Score alone, clinicopathologic features alone, or a combination of the two should be used to predict recurrence and identify patients who may or may not benefit from radiotherapy. Finally, there is currently a lack of prospective evidence for ability of the Oncotype DCIS Score to significantly change patient outcomes, although this may change with the results of ongoing prospective clinical trials (eg, NCT02766881).

Additional gene expression studies have identified differences between patients with DCIS and patients with DCIS and synchronous IDC. In a matched case-control study with 24 patients by Doebar et al,[73] hierarchical clustering of gene expression data revealed distinct DCIS gene expression patterns of patients with and without synchronous IDC. Genes highly expressed in DCIS samples with IDC included *PLAU*, *COL1A1*, *KRT81*, *S100A7*, *SCGB1D2*, *KRT18*, and *NOTCH3*, whereas genes higher in DCIS only cases included *EGFR* and *CXCL14*; these findings were confirmed by immunohistochemical analyses. Another study of 53 DCIS and 51 IDC lesions by Lee et al[74] identified a 74-gene signature capable of predicting DCIS and IDC in the 104 lesions with 96% accuracy, as well as lesions from three independent patient cohorts with 94% accuracy. The authors additionally found that inhibition of four genes (*CSTA*, *FAT1*, *DST*, and *TMEM45A*) in a DCIS xenograft model (grown in mice) suppressed progression of DCIS. Recently, Sokol et al[75] found that *SMARCE1* expression is required for DCIS invasion by regulating the expression of secreted proteases targeting the basement membrane. Although these studies have identified many gene expression differences between indolent and aggressive disease, confirming a functional role of such changes in the transformation of DCIS remains a necessary next step.

One of the major drivers of gene expression changes is epigenetic modifications, which involve biological mechanisms of altering gene expression without changing the underlying DNA. The most well-studied epigenetic change in DCIS is DNA methylation, which typically acts to repress gene transcription. For example, studies have linked increased promoter methylation of a subset of genes (eg, *TWIST1*, *FOXC1*, and *HOXA10*) to invasive progression.[76–78] A study by Johnson et al[79] found that differentially methylated loci in DCIS that progressed to IDC were enriched for homeobox-containing genes and genes involved with limb morphogenesis (eg, *HOXB13* and *EN1*). In a study by Fleischer et al,[80] DNA methylation signatures were

Table 1 Biomarkers and Available Technologies for Future Ductal Carcinoma *in Situ* Studies

Category	Available technologies	Assayed biomarkers	Advantages	Disadvantages
DNA	Sanger sequencing	Mutations	Low cost, easy setup and analysis	Low throughput, primer dependent, large input requirement, high error rate
	Next-generation sequencing	Mutations, copy number alterations, rearrangements, epigenetics	High resolution, sensitive	High cost, difficult analysis pipeline
	Single-cell sequencing	Mutations, copy number alterations, rearrangements, epigenetics	Single-cell resolution, can assess heterogeneity	High cost, difficult setup and analysis pipeline
	PCR	Mutations, rearrangements	Low-input material requirement, sensitive	Low throughput, primer dependent, high error rate
	Microarray (SNP, CGH)	Mutations, copy number alterations, rearrangements	High throughput	Restricted to predetermined alterations, prone to batch effects
	Karyotyping	Rearrangements	Low cost	Time-consuming, difficult setup, low resolution
	FISH	Copy number alterations, rearrangements	Quantitative, specific	Probe dependent, sensitivity
RNA	RT-PCR	mRNA, miRNA, lncRNA	Easy setup and analysis, low-input material requirement, specific	Low throughput, primer dependent
	NanoString	mRNA, miRNA (DNA and protein assays also available)	Reproducibility, sensitivity	Proprietary technology, high cost
	Microarray	mRNA, miRNA, lncRNA	High throughput	Restricted to predetermined alterations
	Next-generation sequencing	mRNA, miRNA, lncRNA	High resolution, sensitive	High cost, difficult analysis pipeline
	Single-cell RNA sequencing	mRNA, miRNA, lncRNA	Single-cell resolution, can assess heterogeneity	High cost, difficult setup and analysis pipeline
Protein	Immunohistochemistry	Protein expression, posttranslational modifications, metabolites	Low cost, easy setup and analysis, preserves histologic information	Tissue-preparation variability, antibody dependent, semiquantitative
	ELISA	Protein expression, posttranslational modifications, metabolites	Easy setup and analysis, low cost	Antibody dependent
	Western blot	Protein expression, posttranslational modifications, metabolites	Easy setup and analysis, low cost	Antibody dependent, semiquantitative
	Flow cytometry	Protein expression, posttranslational modifications, metabolites	Live cell setting, single-cell resolution	High cost, difficult setup and analysis pipeline, cell surface proteins only
	Mass spectrometry	Protein expression, posttranslational modifications, metabolites	Antibody independent, sensitive, specific	High cost, difficult setup and analysis pipeline

CGH, comparative genomic hybridization; ELISA, enzyme-linked immunosorbent assay; FISH, fluorescence *in situ* hybridization; lncRNA, long noncoding RNA; SNP, single-nucleotide polymorphism.

prognostic of survival in patients with breast cancer and DCIS, mixed DCIS and IDC, and IDC lesions. However, this study was unable to use methylation data to separate DCIS, mixed DCIS and IDC, and IDC using unsupervised clustering methods, limiting the value of these findings for characterizing DCIS progression. In general, epigenetic biomarkers have lacked the validation of larger prospective trials but remain an important potential factor affecting disease progression.

Emerging Research in DCIS

Although gene expression-based subtyping has been successful in predicting indolent versus invasive disease and stratifying patients into risk groups, we have only begun to scratch the surface of precision medicine biomarker opportunities in DCIS: the precision medicine era has a wealth of assays at the DNA, RNA, and protein levels at its disposal for future study (Table 1). Most of the studies performed

thus far have used array-based gene expression technologies, FISH-based approaches for CNAs, and targeted DNA panels for mutation changes. In comparison, advances in whole-genome RNA and DNA sequencing now allow for much more powerful tools for analysis that are no longer cost-prohibitive.[81] There are many benefits of high-depth sequencing approaches in the identification of biomarkers of DCIS progression: i) increasing the depth of sequencing will allow for the ability to detect mutations and genetic alterations present in smaller fractions within a lesion and can also be used to more specifically track clonality as lesions evolve from DCIS to IDC[82,83]; ii) sequencing enables detection of chromosomal alterations, such as translocations, inversions, and deletions,[84] which have been described as putative oncogenic events in breast cancer[85]; iii) sequencing allows for detection of nonprotein coding regions of the genome, such as long noncoding RNAs,[86] which have recently been implicated in multiple aspects of cancer biology, including invasion and metastasis[87,88]; and iv) although it requires a different sample processing step, sequencing is also able to evaluate microRNAs, such as microRNA-155, which is regulated by transforming growth factor-β signaling to induce invasion and metastasis.[89] Furthermore, additional sequencing technologies, such as single-cell DNA and RNA sequencing, are now available, which will enable more detailed analysis of genetic alterations in subclonal populations.[90] Together, these technologies may be able to clarify mechanisms of progression from DCIS to IDC.

Additional avenues of research include moving beyond the DNA and mRNA levels and looking for biomarkers of progression at the protein and metabolite levels. For example, a study by Mao et al[91] found that mass spectrometry−based proteomics could predict both subtype and grade of DCIS and IDC. Furthermore, they found that subtypes and histologic grades of IDC and DCIS could be discriminated by lipid content: phospholipids were found to be more abundant in IDC compared with DCIS, whereas fatty acids were more abundant in DCIS than IDC. In addition, there are many posttranslational modifications that affect proteins that are known to contribute to cancer progression, including phosphorylation, acetylation, methylation, ubiquitination, sumoylation, and prenylation.[92] Incorporating such biomarkers into existing prognostic signatures of DCIS represents exciting future research directions.

Conclusion

More than 50% of DCIS will remain indolent and never progress to IDC.[20] Identifying which will remain indolent and which will progress is the subject of many ongoing research efforts. Newer technologies, such as next-generation DNA and RNA sequencing, have enabled the identification of potential genomic and transcriptomic biomarkers that have the potential to replace or enhance current histopathologic risk stratification methods. Certain risk classification systems, such as the Oncotype DX Breast DCIS assay, have been found in limited studies to successfully predict risk of recurrence in DCIS and have the potential to prevent overtreatment in lower-risk disease. Future studies using next-generation technology for genomics, proteomics, and other -omics approaches may identify novel biomarkers that may enhance or replace these existing methods, although they will need to be validated in large cohorts of patients. Biomarkers of DCIS risk have the potential to transform patient care by informing appropriate clinical management and remain an important focus of future research.

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