TUMORIGENESIS AND NEOPLASTIC PROGRESSION

Gene Expression Differences between Ductal Carcinoma in Situ with and without Progression to Invasive Breast Cancer

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To understand the molecular alterations driving the progression of ductal carcinoma in situ (DCIS), we compared patients with pure DCIS and patients with DCIS and synchronous invasive breast cancer (IBC). Twelve patients with extensive pure DCIS were included as a representation of indolent lesions with limited invasive capacity. These cases were matched with 12 patients with a limited DCIS component and IBC, representing lesions with a high invasive potential. Matching included age and surrogate DCIS subtypes. Gene expression profiling was performed on DCIS cells to identify transcriptional differences between these two groups. The identified genes were validated by immunohistochemistry. Nine genes showed significantly different expression. Most of these genes were highly expressed in DCIS samples with IBC, including PLAU (P = 0.002), COL1A1 (P = 0.006), KRT81 (P = 0.009), S100A7 (P = 0.015), SCGB1D2 (P = 0.023), KRT18 (P = 0.029), and NOTCH3 (P = 0.044), whereas EGFR and CXCL14 showed a higher expression in cases with pure DCIS (P = 0.015 and P = 0.028, respectively). This difference was only significant for SCGB1D2 (P = 0.009). Hierarchical clustering revealed distinct clustering of patients with and without invasion. Patients with pure DCIS have a different gene expression pattern as compared to patients with DCIS and synchronous IBC. These genes may pinpoint to driver pathway(s) that play an important role in DCIS progression. (Am J Pathol 2017, 187: 1648–1655; http://dx.doi.org/10.1016/j.ajpath.2017.03.012)

Ductal carcinoma in situ (DCIS) is a nonobligate precursor lesion of invasive breast cancer (IBC).1 In the past decades, the detection rate of DCIS increased dramatically as a result of the increased use and improved resolution of mammographic screening.2 Nowadays, DCIS accounts for 15% to 30% of all new breast cancer cases detected in a well-screened population.1,2

The mechanism behind progression of DCIS to IBC remains to be elucidated. In daily practice, most patients with pure DCIS are treated with local resection with or without radiation. Therefore, data are limited regarding the biological behavior of DCIS. Only a few small retrospective studies reported on the frequency of progression of untreated patients with a biopsy diagnosis of pure DCIS.1,3,4 In these series, approximately 40% to 50% of cases progressed to IBC after a follow-up of 20 to 30 years, whereas the other cases remained indolent.

There is much debate regarding the optimal treatment of DCIS. Because DCIS is a noninvasive disease, current local treatment protocols result in overtreatment for many patients, which is associated with increased costs and morbidity without clinical benefit. On the other hand, a substantial proportion of DCIS cases progress to IBC and, obviously, these patients may benefit from prevention and early treatment.

Paired comparative genomic assays have widely been performed on cases with DCIS and synchronous IBC, showing a high genomic resemblance.5–7 However, comparative genomic assays of pure DCIS versus DCIS with progression to IBC are sparse and partly biased by the inclusion of different DCIS subtypes.8–10 Recent studies

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reported differences in the behavior of DCIS according to DCIS subtypes, based on immunohistochemistry or gene expression patterns. These reported features allow some recurrence risk prediction, but they are not widely used to select individual patients who can avoid adjuvant therapy. The identification of novel genetic alterations and molecular pathways underlying the transformation from DCIS to IBC may help to establish biomarkers that have the potential to distinguish low-risk patients who do not require aggressive treatment and high-risk patients who are likely to progress to IBC.

In daily practice, a proportion of patients presents with extensive involvement of the breast with DCIS without any signs of invasion. Although one cannot exclude that these cases would progress over time, the DCIS growth pattern suggests that these cases have a limited invasive potential. On the other hand, other patients have a limited amount of DCIS adjacent to an invasive component or multiple foci of invasion, suggesting a high invasive potential. These differences in biological behavior of DCIS imply different alterations at the molecular level. On the basis of these observations, we attempted to identify molecular differences at the transcriptional level with robust quantitative RT-PCR assays. For this purpose, we compared breast tissues of patients with extensive DCIS (representing a group with limited invasive potential) with breast tissues of patients who presented with a limited DCIS component and synchronous IBC (as a surrogate for a DCIS subtype with a high invasive potential). On the basis of these data, we aimed to increase our understanding regarding molecular alterations driving DCIS progression and, consequently, facilitate the identification of novel, potential therapeutic targets.

Materials and Methods

Patients

In this retrospective study, two groups of patients were selected from the histopathology files of the Erasmus MC Cancer Institute (Rotterdam, the Netherlands). The first group included patients with extensive pure DCIS, which was defined as DCIS with a diameter of ≥5 cm, to represent a group of DCIS with a biologically indolent behavior with limited invasive capacity. The second group included patients with a limited amount of DCIS (defined as DCIS with a diameter of ≤1 cm) with adjacent IBC. This latter group was selected as a representation of a biologically aggressive type of DCIS with high invasive capacity. Patients from the first group were matched with patients from the second group to correct for potential confounders. Matching included age (categorized from 30 to 40, 40 to 50, 50 to 60, and 70 to 80 years) and surrogate DCIS subtypes, as described below. Patients with a history of breast cancer, ipsilateral breast irradiation, or a BRCA mutation were excluded.

We used coded leftover patient material in accordance with the Code of Conduct of the Federation of Medical Scientific Societies in the Netherlands (http://www.federa.org/codes-conduct, last accessed January 10, 2017). According to institutional and national guidelines, no informed consent was needed for this study.

Pathological Evaluation

Formalin-fixed, paraffin-embedded (FFPE) hematoxylin and eosin—stained whole sections of excision specimens were collected and reviewed by two pathologists (C.H.D. and S.C.D.). Cases of pure DCIS were extensively sampled, according to the national Dutch guidelines, with a minimum of 10 tissue blocks of the lesion (The Dutch Guidelines Database, https://richtlijndatabase.nl/en/richtlijn/breast_cancer/pathology/criteria_for_dcis.html, last accessed January 10, 2017). Histopathological features of DCIS included grade according The Dutch Guidelines Database and surrogate subtyping based on immunohistochemistry, as originally described for IBC. According to these criteria, DCIS was categorized as luminal A (estrogen receptor (ER)+, progesterone receptor (PR) high, Her2−, Ki-67 low), luminal B Her2− (ER+, Her2−, PR−, or low and/or Ki-67 high), luminal B Her2+ (ER+, Her2+, any PR, any Ki-67), or nonluminal Her2 positive (ER−, PR−, Her2+). A cutoff of 20% Ki-67 (MB-1; Dako, Glostrup, Denmark) positive cells was used to distinguish cases with a low versus high proliferative index. Low progesterone (PR 1E2; Ventana, Tucson, AZ) expression was defined as ≤20.

Immunohistochemical evaluation was performed on FFPE whole slides (4 μm thick) using the Ventana Benchmark Ultra automatic stainer. ER (ER SP1; Ventana) was considered positive when at least 10% of the DCIS cells were positive, irrespective of intensity (https://www.gov.uk/government/collections/breast-screening-professional-guidance, last accessed January 10, 2017). Immunohistochemical HER2 expression (Her2 4B5; Ventana) was scored on all cases, according to international guidelines. Equivocal cases were evaluated by silver in situ hybridization.

We also stained for P53 (BP53-11; Ventana), which was considered aberrant in case of a confluent negative staining or a strong diffuse positive staining. An intermediate expression of any intensity was considered to be normal.

RNA Extraction, cDNA Synthesis, Preamplification, and Gene Expression Evaluation (Quantitative RT-PCR)

Areas composed of at least 50% DCIS cells were micro-dissected from 10 to 15 hematoxylin and eosin—stained sections (6 μm thick) of FFPE tissue. Microdissection was performed with a sterile needle under a stereomicroscope (Zeiss, Oberkochen, Germany). On the basis of this method, contamination of other cell types (myoepithelial cells, stroma, lymphocytes) cannot completely be avoided. However, the estimated tumor cell percentage in our series
was high (75% to 90% in the group of patients with pure DCIS and 70% to 85% in the group of patients with DCIS and synchronous IBC). RNA was extracted from these cells using the Qiagen (Hamburg, Germany) AllPrep DNA/RNA FFPE Kit, according to the manufacturer’s instructions. Concentrations were measured with a Nanodrop 1000 system (Thermo Fisher Scientific, Waltham, MA). cDNA was generated from a total of 100 ng RNA for 30 minutes at 48°C with RevertAid H minus (Thermo Fisher Scientific). Gene-specific preamplification was performed for 96 genes (93 tumor-specific genes and 3 reference genes, including GUSB, HMBS, and HPRT1), using the TaqMan PreAmp Master mix (ThermoFisher Scientific) for 15 cycles. This was followed by TaqMan probe—based real-time PCRs, according to the manufacturer’s instructions, in an MX3000P Real-Time PCR System (Agilent, Santa Clara, CA).

These 93 tumor-specific target genes were selected based on their reported involvement in tumorigenesis and/or mutagenesis. Gene expression levels were quantified relative to the average expression of GUSB, HMBS, and HPRT1 using the 2^−ΔΔCq target method. Samples with an average reference gene expression of Cq >25 were considered to be of insufficient RNA quality and were excluded from further analysis. In one of our previous studies, we compared the expression levels of 55 of our 93-gene panel between paired freshly frozen and FFPE samples and reported high levels of concordance (data not shown).

Immunohistochemistry and Gene Function

Genes with a significantly different expression level between pure DCIS and DCIS with synchronous IBC were validated by immunohistochemistry. Antibodies and scoring methods are described below.

We used the DAVID Gene Functional Classification tool to evaluate the gene function of the differently expressed genes, according to Gene Ontology.

Statistical Analysis

We used a paired-samples t-test (IBM SPSS statistics 23) to compare the expression levels of the 93 genes in matched pure DCIS and DCIS with synchronous IBC.

To evaluate whether pure DCIS cases could be distinguished from cases with DCIS and synchronous IBC, a DCIS index score was calculated to evaluate the impact of significant differences between the matched samples. For this DCIS index score, the paired-samples t-test was used in the following equation:

\[ \text{SUM}_{19} = \frac{t\text{-test value gene } X_1 \times \Delta Cq \text{ of gene transcript } X_1 + \text{t\text{-test value gene } X_2 \times \Delta Cq \text{ of gene transcript } X_2 + \ldots \text{ t\text{-test value gene } X_9 \times \Delta Cq \text{ of gene transcript } X_9} }{9} \]  

The \( \chi^2 \) test was used to analyze immunohistochemical differences between pure DCIS lesions and DCIS with synchronous IBC. \( P < 0.05 \) was considered to be statistically significant.

Results

Patients

In total, 24 patients were included, divided into two matched groups of 12 patients each. The overall median age was 56 years (range, 31 to 80 years). The median age in the group of patients with pure DCIS was 55 years (range, 31 to 76 years); in the group with an adjacent invasive component, it was 58 years (range, 32 to 80 years). The median follow-up of patients with pure DCIS was 37 months (range, 24 to 76 months). No invasive recurrences or distant metastases were reported. In the pure DCIS group, the median DCIS size was 7 cm (range, 5 to 13 cm). The DCIS lesions of both groups were graded as grade 2 or grade 3.

On the basis of immunohistochemical subtyping, nine matched pairs were categorized as luminal A subtype and three matched pairs as luminal B subtype. None of the cases showed an aberrant P53-staining pattern. Table 1 provides an overview of the clinicopathological features of the 12 matched pairs.

Gene Expression Profiles of Matched Cases with Pure DCIS and DCIS with Synchronous IBC

On the basis of the 93 selected genes, 9 showed a significant different expression between patients with pure DCIS and patients with DCIS and synchronous IBC. In total, 4 of 93 genes (AURKA, CD133, MAGEA3, and SNAPC2) were not expressed at all. Supplemental Table S1 provides an overview of the remaining 89 genes. The differently expressed genes included COL1A1, CXCL14, EGFR, KRT81, KRT18, NOTCH3, PLAU, S100A7, and SCGB1D2 (Table 2). Most of these nine genes were significantly highly expressed in DCIS samples with synchronous IBC as compared to pure DCIS cases: PLAU (\( P = 0.002 \)), COL1A1 (\( P = 0.006 \)), KRT81 (\( P = 0.009 \)), S100A7 (\( P = 0.015 \)), SCGB1D2 (\( P = 0.023 \)), KRT18 (\( P = 0.029 \)), and NOTCH3 (\( P = 0.044 \)). The remaining two genes, EGFR and CXCL14, showed a significantly higher expression in cases with pure DCIS as compared to cases with DCIS and synchronous IBC (\( P = 0.015 \) and \( P = 0.028 \), respectively).

On the basis of these nine genes with a significantly different expression between both groups, a DCIS index score was calculated (Figure 1). The DCIS index value ranged from −2.31 to 86.84. The optimal cutoff value to discriminate pure DCIS samples from DCIS samples with synchronous IBC was 65.16 (Figure 1A). Supervised hierarchical clustering analysis based on these nine genes separated the most pure DCIS lesions from DCIS lesions with synchronous IBC (Figure 1B). However, three samples of pure DCIS clustered within the group of DCIS cases with synchronous IBC as a result of a high DCIS index score.
Notably, two of these three samples were PR negative and/or HER2 positive and, therefore, categorized as luminal B.

**Immunohistochemistry**

The identified nine genes with a significantly different gene expression between pure DCIS and DCIS with synchronous IBC were evaluated by immunohistochemistry. Figure 2 provides an overview of the immunohistochemical staining pattern. For each antibody, a representative case is shown. As described above, seven of these nine genes showed a higher gene expression in cases with DCIS and synchronous IBC. Immunohistochemically, CK81 (catalog number H00003887-M01; Abnova, Taipei, Taiwan) and NOTCH3 (catalog number ab 23426; Abcam, Cambridge, UK) were only expressed in the myoepithelial cells, whereas the luminal cells were negative in both groups (Figure 2A and B). COL1A1 (catalog number NB600-408; Novus Biologicals, Littleton, CO) showed periductal stromal staining (Figure 2C) in six cases, but no significant difference (P = 0.317) was observed between both groups.

**Table 1** Clinicopathological Features of Patients with Pure DCIS and Matched Patients with DCIS and Synchronous IBC

<table>
<thead>
<tr>
<th>Matched pairs</th>
<th>Pure DCIS or DCIS and synchronous IBC</th>
<th>Age, years</th>
<th>Histological grade DCIS</th>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
<th>Ki-67</th>
<th>DCIS subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pure DCIS</td>
<td>56</td>
<td>3</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>Low</td>
<td>Luminal B</td>
</tr>
<tr>
<td>2</td>
<td>DCIS + IBC</td>
<td>51</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>High</td>
<td>Luminal B</td>
</tr>
<tr>
<td>3</td>
<td>Pure DCIS</td>
<td>56</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Low</td>
<td>Luminal A</td>
</tr>
<tr>
<td>4</td>
<td>DCIS + IBC</td>
<td>58</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Low</td>
<td>Luminal A</td>
</tr>
<tr>
<td>5</td>
<td>Pure DCIS</td>
<td>61</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Low</td>
<td>Luminal A</td>
</tr>
<tr>
<td>6</td>
<td>DCIS + IBC</td>
<td>67</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Low</td>
<td>Luminal A</td>
</tr>
<tr>
<td>7</td>
<td>Pure DCIS</td>
<td>76</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Low</td>
<td>Luminal A</td>
</tr>
<tr>
<td>8</td>
<td>DCIS + IBC</td>
<td>76</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Low</td>
<td>Luminal A</td>
</tr>
<tr>
<td>9</td>
<td>Pure DCIS</td>
<td>80</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Low</td>
<td>Luminal A</td>
</tr>
<tr>
<td>10</td>
<td>DCIS + IBC</td>
<td>61</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Low</td>
<td>Luminal A</td>
</tr>
<tr>
<td>11</td>
<td>Pure DCIS</td>
<td>59</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Low</td>
<td>Luminal A</td>
</tr>
<tr>
<td>12</td>
<td>DCIS + IBC</td>
<td>58</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Low</td>
<td>Luminal A</td>
</tr>
</tbody>
</table>

n = 12 pairs.

+, positive; −, negative; ER, estrogen receptor; IBC, invasive breast cancer; PR, progesterone receptor.

**Table 2** Overview of Genes with a Significantly Different Expression between Pure DCIS and DCIS with Synchronous IBC

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene name</th>
<th>Paired differences</th>
<th>P value (two-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>PLAU</td>
<td>Plasminogen activator, urokinase</td>
<td>1.178</td>
<td>1.020</td>
</tr>
<tr>
<td>COL1A1</td>
<td>Collagen, type I, α 1</td>
<td>2.164</td>
<td>2.179</td>
</tr>
<tr>
<td>KRT81</td>
<td>Keratin 81</td>
<td>2.346</td>
<td>2.563</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor [erythroblastosis leukemia viral (v-erb-b) oncogene homolog, avian]</td>
<td>−1.075</td>
<td>1.289</td>
</tr>
<tr>
<td>S100A7</td>
<td>S100 calcium binding protein A7</td>
<td>2.918</td>
<td>3.519</td>
</tr>
<tr>
<td>SCGB1D2</td>
<td>Secretoglobin, family 1D, member 2</td>
<td>3.227</td>
<td>4.224</td>
</tr>
<tr>
<td>CXCL14</td>
<td>Chemokine (C-X-C motif) ligand 14</td>
<td>−1.481</td>
<td>2.025</td>
</tr>
<tr>
<td>KRT18</td>
<td>Keratin 18; keratin 18 pseudogene 26; keratin 18 pseudogene 19</td>
<td>0.499</td>
<td>0.688</td>
</tr>
<tr>
<td>NOTCH3</td>
<td>Notch homolog 3 (Drosophila)</td>
<td>0.867</td>
<td>1.317</td>
</tr>
</tbody>
</table>

IBC, Invasive breast cancer.

*Upper and Lower refer to 95% confidence interval of the difference.
SCGB1D2 (lipophilin B; catalog number NBP1-81304; Novus Biologicals) was positive in the cytoplasm of neoplastic DCIS cells (Figure 2D) and was scored dichotomous (negative/weak or moderate/strong). There was a significantly higher expression in cases with DCIS and synchronous IBC as compared to the pure DCIS cases ($P < 0.009$), which was in line with the gene expression pattern of SCGB1D2.

S100A7 (catalog number NB100-56559; Novus Biologicals) was positive in the nucleus of the neoplastic DCIS cells (Figure 2E) and was also scored dichotomous (negative/weak or moderate/strong). Although no significantly different expression was seen between both groups, there was a trend toward a higher expression in those cases with DCIS and synchronous IBC as compared to the pure DCIS cases ($P = 0.150$). CK18 (catalog number HPA001605; Sigma-Aldrich, Darmstadt, Germany) and urokinase plasminogen activator (clone 150; Grünenthal, Stolberg, Germany) were expressed cytoplasmatically in the neoplastic DCIS cells (Figure 2, F and G). No difference was seen between both groups ($P = 0.48$ and $P = 0.572$, respectively).

Epidermal growth factor receptor (EGFR) and CXCL14 showed a higher gene expression in the group of patients with pure DCIS. Immunohistochemically, EGFR (3C6 790-2988; Ventana) was only expressed in myoepithelial cells (Figure 2H). None of the cases showed expression in the luminal cells. Immunohistochemical evaluation of CXCL14 was not feasible because of a non-specific staining pattern.

**Functional Annotation of Differently Expressed Genes according to Gene Ontology**

According to Gene Ontology, a total of five genes (of the nine differently expressed genes) were annotated. These...
genes are involved in several biological processes, including signal transduction (EGFR, CXCL14, and PLAU), chemotaxis (CXCL14 and PLAU), angiogenesis (S100A7 and PLAU), cellular response to epidermal growth factor stimulus (EGFR and COL1A1), positive regulation of extracellular signal regulated kinase 1 and 2 cascade (EGFR and S100A7), response to hyperoxia (COL1A1 and PLAU), and cellular response to amino acid stimulus (EGFR and COL1A1). None of these functions were statistically significantly enriched ($P > 0.05$) compared to the functions annotated to the total list of 93 measured genes.

Discussion

To achieve optimal individualized treatment for patients with DCIS, it is necessary to unravel the molecular events that contribute to DCIS progression. In this study, we identified significantly different gene expression profiles between patients with extensive pure DCIS (representing a group with a biologically indolent behavior) and patients with a limited amount of DCIS and synchronous IBC (representing a group with a biologically aggressive behavior). Most of these differently expressed genes (7/9) showed a higher expression in the DCIS group with synchronous IBC, including PLAU, COL1A1, KRT81, S100A7, SCGB1D2, KRT18, and NOTCH3. At the protein level, this could only be confirmed for SCGB1D2. The remaining two genes, EGFR and CXCL14, were up-regulated in pure DCIS lesions at the transcriptional level. These findings are in line with previous studies that reported differences between pure DCIS lesions and DCIS with synchronous IBC based on gene copy number changes and whole exome sequencing.10,27

On the basis of these identified genes, supervised hierarchical cluster analysis showed distinct clustering for patients with pure DCIS (characterized by lower expression levels) and patients with DCIS and synchronous IBC (characterized by higher expression levels). However, three patients with pure DCIS clustered within the group of DCIS cases with synchronous IBC. Two of these three cases were classified as luminal B, which might explain a gene expression profile that is more similar to DCIS with synchronous IBC.15,28

The progression of in situ to invasive carcinoma is a multistep process that includes several biological processes (ie, regulation of transcription, cell adhesion, immune response, chemotaxis, apoptosis, and cell proliferation).8,29,30 According to Gene Ontology, the differently expressed genes in our study are involved in several of these processes, mainly signal transduction. This suggests that signal transduction might play an important role in the progression of DCIS. In our series, COL1A1 and NOTCH3 were up-regulated in cases with DCIS with synchronous IBC as compared to pure DCIS cases. This is in line with previous studies that reported that these genes have an important role in cell adhesion and migration.31,32 Furthermore, NOTCH3 plays an important role in cell growth by the inhibition of apoptosis and induction of cell proliferation, although the exact mechanism in breast cancer remains unknown.32 Another gene with an important role in cell growth is CXCL14, although in contrast to NOTCH3, overexpression of this gene inhibits cell proliferation and invasion.33 In line with this, a recent study reported that this gene is a negative regulator of growth and metastases in breast cancer. This anticancer effect correlates with the up-regulation of CXCL14 in pure DCIS samples in our study, where it might have contributed to the indolent behavior.33

Several previous studies reported that disruption of the myoepithelial cell layer is one of the critical events in DCIS progression.3,34 This is in line with the results of our study, in which several of the up-regulated genes (EGFR, COL1A1, KRT81, and NOTCH3) are involved in the
myoepithelial cell layer. Our finding that up-regulation of these genes was reported in both groups suggests that they could have both proinvasive and anti-invasive effects. Besides, it makes it unlikely that this finding was influenced by the amount of myoepithelial cells in the analyses. EGFR has been described as a specific marker in myoepithelial cells of the breast. Col1a1, together with other members of the collagenase family, was recently characterized as a myoepithelial-type gene in ER-positive breast cancer. Regarding Krt81 and Notch3, we could not find such data in the literature, although we identified this protein in the myoepithelial compartment by immunohistochemistry.

The strength of our study is that we matched for surrogate DCIS subtypes. This was assumed to be more reliable than matching on grade, which is known to be a subjective feature. Ideally, matching should also be based on other factors (including grade, PR status, and Ki-67 index) because these factors could affect biological behavior, but this was not feasible. Our study also has several other limitations, in particular the sample size, and as such, our work should be considered as a hypothesis-generating study. In addition, the presence of an invasive component cannot be ruled out in those cases classified as pure DCIS. However, because these specimens were examined extensively, it is unlikely that a large invasive component was missed.

The immunohistochemical staining provided important information regarding the localization of the expression (neoplastic cells, myoepithelial cells, or periductal stroma), but the ability to detect different expression levels between pure DCIS and DCIS with synchronous IBC was limited by group size. Besides, we only evaluated a subset of luminal-like cases. Although this is the most common subtype, our data cannot be extrapolated to other DCIS subtypes (eg, ER-negative/HER2-positive cases and triple-negative cases). Furthermore, because we evaluated only a selected number of tumor-specific target genes, it is likely that some cancer genes and functional pathways have been missed.

In conclusion, we reported distinct gene expression profiles in cases with pure DCIS and cases with DCIS and synchronous IBC. If these results can be validated in independent and larger cohorts, these differently expressed genes could be used to predict progression in individual patients diagnosed with DCIS to facilitate individualized treatment. Besides, these genes may pinpoint potentially targetable driver pathway(s) that play an important role in the progression of DCIS to IBC, which could ultimately result in the prevention of progression.

Acknowledgments

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Supplemental Data

Supplemental material for this article can be found at http://dx.doi.org/10.1016/j.ajpath.2017.03.012.

References


