Research Article

A Comparison of Tumor Biology in Primary Ductal Carcinoma In Situ Recurring as Invasive Carcinoma versus a New In Situ

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Introduction. About half of all new ipsilateral events after a primary ductal carcinoma in situ (DCIS) are invasive carcinoma. We studied tumor markers in the primary DCIS in relation to type of event (invasive versus in situ).

Methods. Two hundred and sixty-six women with a primary DCIS from two source populations, all with a known ipsilateral event, were included. All new events were regarded as recurrences. Patient and primary tumor characteristics (estrogen receptor (ER), progesterone receptor (PR), HER2, EGFR, and Ki67) were evaluated. Logistic regression was used to calculate odds ratios and 95% confidence intervals in univariate and multivariate analyses.

Results. One hundred and thirty-six of the recurrences were invasive carcinoma and 130 were in situ. The recurrence was more often invasive if the primary DCIS was ER+ (OR 2.5, 95% CI 1.2–5.1). Primary DCIS being HER2+ (OR 0.5, 95% CI 0.3–0.9), EGFR+ (OR 0.4, 95% CI 0.2–0.9), and ER−/HER2+ (OR 0.2, 95% CI 0.1–0.6) had a lower risk of a recurrence being invasive.

Conclusions. In this study, comparing type of recurrence after a DCIS showed that the ER−/HER2+ tumors were related to a recurrence being a new DCIS. And surprisingly, tumors being ER+, HER2−, and EGFR− were related to a recurrence being invasive cancer.

1. Introduction

Ductal carcinoma in situ (DCIS) of the breast is a clinically and molecularly heterogeneous disease with different malignant potentials [1, 2]. The risk of local recurrence after breast-conserving surgery only (BCS) is rather high and even higher than after surgery for invasive breast cancer [3, 4]. In DCIS, adding radiotherapy after BCS lowered the relative risk with approximately 50%, from 28.1% to 12.9% after ten years in a meta-analyses including four randomized studies [5, 6]. About half of the women with a local recurrence develop a new DCIS and the other half an invasive carcinoma [7–10]. Although women with a primary DCIS have a very good prognosis [6], those with a subsequent invasive carcinoma have an increased risk of dying from breast cancer. In a recently published study the 15-year breast cancer specific survival was just over 60% among those with an invasive recurrence after a primary DCIS [11, 12].

One of the major goals of the treatment of DCIS is to prevent invasive disease. There are surgical and biological risk factors for local recurrence, for example, young age, mode of detection (clinically detected as compared to screening detected), margins, and grade [13, 14]. Also, a recently published study using the Oncotype DX DCIS score showed that genomic-based data could predict local relapse risk independently from classical factors [15]. The expression of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor-2 (HER2) has been
shown to predict local recurrence [16–19]. However, little is known about factors associated with the risk of developing invasive breast cancer.

The aim of this study was to look for patient and primary tumor characteristics that might be associated with progression from in situ to invasive breast cancer. If we can predict who has a low risk for an invasive recurrence, this might help us to individualize type of surgery and adjuvant treatment, but also it is interesting for our knowledge in cancer progression in general. We studied a large number of women with a known local recurrence after a primary DCIS and compared the expression of specific biomarkers and other patient characteristics in those with an in situ and those with an invasive recurrence, respectively. This paper does not look at risk of recurrence, as we did not have the background information for women without a new ipsilateral event in all women.

2. Methods

2.1. Patients. Patients were recruited from two different source populations. One was a population-based cohort comprising all 458 women diagnosed with a primary DCIS between 1986 and 2004 in the Uppland and Västmanland regions of Sweden. All women with a recurrence (n = 100) up to December 31, 2008, were included. The other source was the SweDCIS trial which was a randomized multicentre trial consisting of 1,046 women diagnosed between 1987 and 1999 with a primary DCIS, administered through the Regional Oncological Centres in all six Swedish Health Care Regions. Patients included in the SweDCIS had a diagnosis of primary DCIS, occupying less than one-quadrant of the breast, and underwent surgery with breast conservation. After surgery, the women were randomized to receive postoperative radiotherapy of the breast or not. We included all women from the study with a registered local recurrence (n = 166) up to December 31, 2005. All new ipsilateral breast cancer events were considered a local recurrence and the earliest event considered a recurrence occurred after seven months.

2.2. Tissue Microarray (TMA) Construction. Prior to the TMA construction, H&E sections from all paraffin blocks from the primary DCIS cases were histopathologically reevaluated and graded by one pathologist (KJ) and appropriate tumor areas selected. Two cores with a diameter of 1.0 mm were mounted into the recipient TMA block using a manual arraying device (MTA-1, Beecher Inc., WI, USA). The concordance of immunohistochemical (IHC) staining between whole section slides and TMA slides has previously been reported. [20, 21]. The concordance was 80.4% for HER2, 84.2% for ER, and 81.5% for PR.

2.3. IHC and Silver-Enhanced In Situ Hybridization (SISH). We performed IHC for ER, PR, HER2, epidermal growth factor receptor (EGFR), cytokeratin 5/6 (CK5/6), and Ki67 on 4 μm paraffin sections cut from the TMAs. Immunostains for each marker were performed on a Dako Autostainer (Dako Corporation). IHC was conducted according to established protocols. Appropriate positive and negative controls were included in all staining runs. The antibodies used were c-erbB-2 poly rabbit, A0485, DAKO, USA, ER NCL-6F11, Novocastra, UK, and PgR NCL-1A6, Novocastra, UK, Ki-67 MIB-1, Immunotech, KEBO, CK5/6, D5/16B4, Zymed, USA, and EGFR 31G7, Zymed, USA.

HER2 SISH was also performed on TMA slides using an automated instrument, Ventana Benchmark (Ventana Medical Systems, Tucson, AZ, USA), as per the manufacturer’s protocols for the INFORM HER2 DNA probe and chromosome 17 probes. Testing for the HER2 gene and chromosome 17 was performed on sequential sections. Both probes were labeled with dinitrophenol. Denaturation occurred on the instrument with enzyme digestion in protease 3 for 8 minutes. The detection system used a multimer labeled with goat anti-rabbit antibody horseradish peroxidase as the linking step. Visualization occurred with the sequential addition of silver acetate as the source of ionic silver, hydroquinone, and hydrogen peroxide to give a black metallic silver precipitate at the probe site. Counterstaining was performed with hematoxylin II on the instrument. The time taken for the complete run was 6.5 hours. Both HER2 and chromosome 17 detection were performed on the same slide run. Gene amplification was assessed using the American Society of Clinical Oncology/College of American Pathologists guideline and Australian HER2 Advisory Board criteria for single HER2 probe testing (diploid 1 to 2.5 copies/nucleus; polysomy >2.5 to 4 copies/nucleus; equivocal >4 to 6 copies/nucleus; low-level amplification >6 to 10 copies/nucleus; and high-level amplification >10 copies/nucleus) and for dual HER2/CHR17 probe testing (nonamplified ratio <1.8; equivocal ratio 1.8 to 2.2; gene amplification >2.2). The status of HER2 protein expression was firstly assessed using SISH and secondly, for those cases on which SISH failed, the evaluation was based on IHC.

2.4. Scoring and Classification. Data on tumor size and multifocality was obtained from the original histopathological reports. Stained TMA slides were scanned (ScanScope XT, Aperio, USA) for evaluation of expression of ER, PR, HER2, EGFR, CK 5/6, and Ki67 by ImageScope (Aperio, USA). Tumors that showed nuclear staining more than 10% were considered ER or PR positive, as this was and still is the routinely used cutoff in Sweden. Using the HerceptTest classification system, tumors were considered HER2 positive if the score was 3+ Any degree of cytoplasmic staining for CK 5/6 and any degree of distinct membranous staining for EGFR were counted as positive, even if focal. CK 5/6 and EGFR were used to define an ER−/HER2− tumor as basal like according to the classification system by Livasy et al. [22]. Proliferation was considered high if immunostaining for Ki67 was seen in more than 10% of tumor nuclei. These latter IHC criteria are similar to those previously used for scoring these markers in invasive breast cancer [22–27]. If only one core included enough tumor tissue, this was used for classification but at least 200 cells had to be counted. Each marker was scored by one person blinded for outcome (WZ or CJ). HER2 SISH
Table 1: Baseline clinical and histopathological characteristics among women with a primary DCIS who later developed either an invasive cancer or an *in situ* recurrence. Women with a known recurrence were recruited from two source populations: a population based cohort (U/V cohort, *n* = 458) and a randomized study (SweDCIS, *n* = 1,046).

| Baseline characteristics at diagnosis of primary DCIS | All DCIS with a recurrence (*n* = 266) |
|--------------------------------------------------------|---------------------------------------|
|                                                      | U/V cohort (*n* = 100) Type of recurrence | SweDCIS (*n* = 166) Type of recurrence | All DCIS with a recurrence (*n* = 266) Type of recurrence |
|                                                      | Invasive (*n* = 55) | In situ (*n* = 45) | Invasive (*n* = 81) | In situ (*n* = 85) | Invasive (*n* = 136) | In situ (*n* = 130) |
| Time to recurrence, months (mean ± SD)                | 65 ± 44 | 54 ± 49 | 67 ± 43 | 37 ± 27 | 67 ± 44 | 44 ± 37 |
| Age at diagnose (*n* = 266)                           |                                                      |
| ≤45                                                    | 9        | 7       | 16      | 14      | 25 (18.4) | 21 (16.2) |
| 46–60                                                  | 22       | 22      | 36      | 39      | 58 (42.7) | 61 (46.9) |
| >60                                                    | 24       | 16      | 29      | 32      | 53 (38.9) | 48 (36.9) |
| Mode of detection (*n* = 265)                         |                                                      |
| Screening                                             | 37       | 36      | 53      | 65      | 90 (66.7) | 101 (77.7) |
| Clinically                                            | 18       | 9       | 27      | 20      | 45 (33.3) | 29 (22.3) |
| Tumor size (*n* = 234)                                |                                                      |
| ≤15 mm                                                | 34       | 16      | 40      | 41      | 74 (63.2) | 57 (48.7) |
| >15 mm or multifocal                                  | 17       | 24      | 26      | 36      | 43 (36.8) | 60 (51.3) |
| Type of surgery (*n* = 266)                           |                                                      |
| Breast conserving surgery                             | 50       | 41      | 81      | 85      | 131 (96.3) | 126 (96.9) |
| Mastectomy                                            | 5        | 4       | —       | —       | 5 (3.7) | 4 (3.1) |
| Postoperative radiotherapy (*n* = 266)                |                                                      |
| Yes                                                    | 14       | 12      | 27      | 20      | 41 (30.2) | 32 (24.6) |
| No                                                     | 41       | 33      | 54      | 65      | 95 (69.8) | 98 (75.4) |
| Free margins (*n* = 255)                              |                                                      |
| Yes                                                    | 47       | 34      | 61      | 65      | 108 (80.0) | 99 (76.2) |
| No or doubtful                                        | 8        | 11      | 19      | 20      | 27 (20.0) | 31 (23.9) |
| Nuclear grade (*n* = 241)                             |                                                      |
| I                                                      | 5        | 6       | 6       | 1       | 11 (9.2) | 7 (5.8) |
| II                                                     | 19       | 17      | 26      | 21      | 45 (37.5) | 38 (31.4) |
| III                                                    | 26       | 21      | 38      | 55      | 64 (53.3) | 76 (62.8) |

was scored by WZ with RMA as a reference. The recurrences were defined as invasive or *in situ* based on the original histopathological report. We did not have data on ER, PR, or HER2 for the new events.

2.5. Statistical Analyses. Among those primary DCIS with a recurrence, the associations between baseline characteristics and type of recurrence (*invasive* or *in situ*) were analyzed using logistic regression models. Odds ratio (OR) and 95% confidence interval (95% CI) were used to estimate the relative risks. In the multivariate models, we adjusted for age group, free margins, and type of surgery. Data analyses were conducted using the SAS System 9.2 (SAS Institute, NC, USA).

The guidelines for tumor marker prognostic studies (reporting of tumor MARKer studies (REMARK)) including relevant items about test evaluation were followed [28]. This study was approved by the Ethics Committee at Uppsala University Hospital (Dnr 2005:118) and Umeå University (Dnr 05-065 M).

3. Results

Of the 1,504 (458 + 1,046) women with a primary DCIS, 136 developed an invasive recurrence and 130 developed an *in situ* recurrence. Baseline clinical and histopathological characteristics for the two groups (DCIS with an invasive recurrence and DCIS with an *in situ* recurrence) are presented in Tables 1 and 2. Age at diagnosis was comparable between the groups. Time to an *in situ* recurrence was on average 44 months and to an invasive recurrence was 67 months. No women in this study received pre- or postoperative chemotherapy.
Table 2: Molecular characteristics among women with a primary DCIS who later developed either an invasive cancer or an in situ recurrence. Women with a known recurrence were recruited from two source populations: a population based cohort (U/V cohort, n = 458) and a randomized study (SweDCIS, n = 1,046).

| Molecular Characteristics of primary DCIS | All DCIS with a recurrence (n = 266) |
|-----------------------------------------|-------------------------------------|
|                                         | U/V cohort (n = 100) | All DCIS with a recurrence (n = 266) |
|                                         | Type of recurrence | Type of recurrence |
|                                         | Invasive (n = 55) | In situ (n = 45) | Invasive (n = 81) | In situ (n = 85) | Invasive (n = 136) | In situ (n = 130) |
| ER (n = 181) | Positive | 38 | 27 | 16 | 15 | 36 | 32 | 74 (81.3) | 59 (65.5) |
|             | Negative | 10 | 16 | 7 | 15 | 17 (18.7) | 31 (34.5) |
| PR (n = 183) | Positive | 25 | 20 | 28 | 24 | 53 (55.8) | 44 (50.0) |
|             | Negative | 26 | 22 | 16 | 22 | 42 (44.2) | 44 (50.0) |
| HER2 (n = 177) | Positive | 15 | 18 | 13 | 22 | 28 (30.4) | 40 (47.1) |
|             | Negative | 37 | 24 | 27 | 21 | 64 (69.6) | 45 (52.9) |
| EGFR (n = 143) | Positive | 10 | 16 | 14 | 19 | 24 (32.0) | 35 (51.5) |
|             | Negative | 33 | 18 | 18 | 15 | 51 (68.0) | 33 (48.5) |
| CK5/6 (n = 170) | Positive | 42 | 32 | 40 | 46 | 82 (94.3) | 78 (94.0) |
|             | Negative | 3 | 4 | 2 | 1 | 5 (5.7) | 5 (6.0) |
| KI67 (n = 146) | High | 15 | 11 | 13 | 16 | 28 (37.3) | 27 (38.0) |
|             | Low | 32 | 25 | 15 | 19 | 47 (62.7) | 44 (62.0) |

Subgroups based on IHC (n = 266)
| ER+/HER2− | 10 | 6 | 8 | 9 | 51 (37.5) | 36 (28.0) |
| ER+/HER2+ | 28 | 19 | 23 | 17 | 18 (13.2) | 15 (11.5) |
| ER−/HER2+ | 4 | 11 | 4 | 10 | 8 (5.9) | 21 (16.2) |
| **ER−/HER2−/CK+ or EGFR+** | 3 | 5 | 3 | 3 | 6 (4.4) | 8 (6.2) |
| Unknown | 10 | 4 | 43 | 46 | 53 (39.0) | 58 (44.6) |

**We used the classification for basal-like DCIS published by Livasy et al., 2007 [22], and also used in an earlier paper by us [37].

3.1. Results of Factors Associated with Subsequent Invasive Cancer versus DCIS. Clinically detected DCIS lesions with a known recurrence were associated with a higher risk of the recurrence being invasive (OR 1.80, 95% CI 1.02–3.19) compared to those DCIS detected by mammography screening (Table 3). Large size in the primary DCIS (>15 mm or multifocality) was associated with a lower risk of a recurrence being invasive (OR 0.54, 95% CI 0.32–0.92). Type of surgery, involvement of margins, and NG were not statistically significantly associated with type of recurrence.

ER+ primary DCIS with a known recurrence had a higher risk of the recurrence being invasive (OR 2.52, 95% CI 1.24–5.10). HER2+ and EGFR+ primary DCIS tumors with a known recurrence were associated with a halved risk of the recurrence being invasive OR 0.48, 95% CI 0.26–0.90 and...
| Table 3: Univariate and multivariate analysis of the associations between baseline clinical-, histopathological-, and molecular characteristics and the risk for a recurrence being invasive carcinoma compared to in situ carcinoma, among women with a primary DCIS and a known recurrence in the Uppsala/ Västerås cohort, in the SweDCIS randomized study and in the two groups pooled together (n = 266). |
|---|---|---|---|---|---|
| **Risk of a recurrence after DCIS being invasive compared to a new in situ** | **U/V cohort (n = 100)** | **SweDCIS (n = 166)** | **All DCIS with a recurrence (n = 266)** |
| **Mode of detection** | | | | |
| Screening | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Clinically | 1.82 (0.66–5.08) | 1.83 (0.61–5.5) | 1.64 (0.83–3.27) | 1.71 (0.85–3.46) | 1.72 (0.98–3.01) | 1.80 (1.02–3.19) |
| **Tumor size** | | | | |
| ≤15 mm | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| >15 mm or multifocal | 0.31 (0.12–0.84) | 0.32 (0.11–0.93) | 0.84 (0.41–1.63) | 0.84 (0.32–1.57) | 0.55 (0.33–0.93) | 0.54 (0.32–0.92) |
| **Type of surgery** | | | | |
| Breast conserving surgery | 1.0 | — | — | — | 1.0 | — |
| Mastectomy | 0.88 (0.20–3.88) | — | — | — | 1.13 (0.29–4.42) | — |
| **Postoperative radiotherapy** | | | | |
| No | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Yes | 0.95 (0.39–2.34) | 1.27 (0.46–3.56) | 1.59 (0.80–3.20) | 1.70 (0.83–3.46) | 1.32 (0.76–2.27) | 1.41 (0.80–2.48) |
| **Free margins** | | | | |
| Yes | 1.54 (0.28–8.36) | — | 0.93 (0.41–2.13) | — | 1.24 (0.69–2.22) | — |
| No or doubtful | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| **Nuclear grade** | | | | |
| I | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| II | 1.37 (0.35–5.4) | 1.23 (0.29–5.15) | 0.21 (0.02–1.87) | 0.21 (0.02–1.81) | 0.75 (0.26–2.12) | 0.70 (0.25–2.02) |
| III | 1.58 (0.41–6.06) | 1.55 (0.38–6.37) | 0.12 (0.02–1.02) | 0.11 (0.01–0.96) | 0.53 (0.19–1.45) | 0.49 (0.18–1.55) |
| **ER** | | | | |
| Negative | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Positive | 2.23 (0.86–5.78) | 2.29 (0.87–6.03) | 2.54 (0.91–7.10) | 3.34 (1.10–10.2) | 2.33 (1.17–4.65) | 2.52 (1.24–5.10) |
| **PR** | | | | |
| Negative | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Positive | 1.03 (0.45–2.38) | 1.03 (0.44–2.39) | 1.83 (0.76–4.42) | 2.20 (0.83–5.40) | 1.32 (0.73–2.38) | 1.36 (0.75–2.47) |
| **HER2** | | | | |
| Negative | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Positive | 0.55 (0.23–1.30) | 0.60 (0.24–1.47) | 0.46 (0.19–1.11) | 0.42 (0.17–1.07) | 0.50 (0.27–0.92) | 0.48 (0.26–0.90) |
| **EGFR** | | | | |
| Negative | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Positive | 0.35 (0.13–0.92) | 0.36 (0.13–0.98) | 0.62 (0.23–1.64) | 0.57 (0.21–1.58) | 0.45 (0.23–0.88) | 0.44 (0.22–0.88) |
| **Ki67** | | | | |
| Low | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| High | 1.10 (0.41–2.97) | 1.09 (0.38–3.12) | 1.03 (0.38–2.82) | 1.10 (0.38–3.19) | 0.98 (0.50–1.94) | 0.93 (0.46–1.85) |
| **Subgroups based on IHC** | | | | |
| ER+/HER2− | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| ER+/HER2+ | 1.15 (0.35–3.77) | 1.27 (0.37–4.39) | 0.64 (0.21–2.02) | 0.64 (0.20–2.02) | 0.84 (0.37–1.89) | 0.83 (0.37–1.88) |
| ER−/HER2− | 0.25 (0.07–0.93) | 0.26 (0.07–0.98) | 0.30 (0.08–1.12) | 0.22 (0.05–0.95) | 0.27 (0.11–0.68) | 0.24 (0.09–0.62) |
| **ER−/HER2−/CK5/6+ or EGFR+** | 0.42 (0.09–1.99) | 0.41 (0.08–0.98) | 0.75 (0.13–4.20) | 0.75 (0.13–4.30) | 0.54 (0.17–1.71) | 0.52 (0.16–1.65) |
| Unknown | 1.68 (0.46–6.17) | 1.62 (0.43–6.12) | 0.70 (0.33–1.48) | 0.70 (0.33–1.48) | 0.75 (0.42–1.34) | 0.76 (0.42–1.35) |

* Adjustments for age group. † Adjustments for age group, free margin, and type of surgery. ** We used the classification for basal-like DCIS published by Livasy et al., 2007 [22], and also used in an earlier paper by us [37].
OR 0.44, 95% CI 0.22–0.88, respectively. The ER−/HER2+ tumors, with the ER+/HER2− as a reference, were associated with a lower risk of the recurrence being invasive (OR 0.24, 95% CI 0.09–0.62), while the other subgroups were not statistically significantly associated with type of recurrence. Other molecular factors including PR, CK 5/6, and Ki67 were not statistically significantly associated with type of recurrence (Table 3).

4. Discussion

In this study, the purpose was to study patient and tumor characteristics in women with a primary DCIS followed by a recurrence and to compare primary tumors recurring as invasive carcinoma with those recurring as DCIS. Surprisingly, we found that ER+, HER2−, and EGFR− tumors were strongly associated with a subsequent recurrence being invasive.

Usually, studies are designed to evaluate the risk of recurrence in relation to different tumor markers, patient characteristics, or type of treatment. In this study setting we chose to only include DCIS with a known recurrence. Hypothetically, we had two comparable groups of DCIS that recurred either as invasive carcinoma or DCIS. Differences found regarding tumor biology related to type of recurrence can possibly reflect a true potential to progress from in situ to invasive carcinoma. Of course, we cannot rule out the possibility of some of the tumors being new cancers instead of true recurrences.

We collected a large number of primary DCIS with a documented recurrence and constructed TMAs. TMA construction from DCIS is somewhat challenging due to the often relatively small and scattered lesions, and there will always be a selection bias regarding tumor size. Additionally, the loss of representative tumor material for each subsequent section cut tends to increase. This resulted in missing IHC data in about 30% for ER that was stained for in the first section and in nearly 50% of the cases for EGFR and Ki67 that were stained for in the last sections.

Nuclear grade and age at diagnosis were not associated to type of recurrence in our study. Clinically detected DCIS showed a higher risk for a recurrence being invasive. This finding is consistent with the observations that clinically detected invasive breast cancers tend to be more aggressive than lesions detected by mammography screening [17, 29]. Large tumor size was associated to recurrences being of the in situ type after adjusting for age, margins, and type of surgery in the multivariate analysis. This might be due to a higher risk of residual in situ after surgery in larger lesions.

We found that a certain combination of molecular markers ER−/HER2+ was statistically significantly associated with a high risk for a recurrence being in situ. This finding is consistent with the results from one previous, nested case-control study, showing that ER−/HER2+ DCIS was associated with an increased risk of recurrent DCIS, but not with the risk of invasive recurrence [17].

We found that women with HER2+ tumors had a higher risk of a recurrence being of the in situ type. Recently, Rakovitch et al. [30] observed that women with a HER2+/Ki67+ DCIS had a higher risk of developing in situ local recurrence after breast-conserving surgery which is consistent with our results regarding HER2 status. HER2 is one of the most extensively studied biological prognostic factors in invasive breast cancer. However, its importance in DCIS has yet to be elucidated [31]. There are three published studies where no significant associations were found between a variety of biologic markers, including HER2, and the risk of recurrence after a DCIS [16, 31, 32]. In contrast to those studies, we used a stringent cutoff for HER2 positivity. We used SISH when possible (n = 162) and secondly IHC (3+) to determine HER2 positivity. Hence, it is still possible that high-level HER2 overexpression due to gene amplification does predict recurrence after a primary DCIS but the tumor biology explaining why recurrences after a HER2+ DCIS more often are of the in situ type remains to be explored.

EGFR, like HER2, is a potent stimulating factor of cell-growth-activating pathways and thus stimulates tumor growth when activated [33]. EGFR has been used as a surrogate marker for basal like invasive breast cancer and for DCIS [22, 26]. EGFR overexpression has been associated with a poor outcome in invasive breast cancer but very little is reported on DCIS [34–36]. In our study, EGFR positivity was associated with a higher risk for a recurrence being of the in situ type, similar to the recurrences after HER2+ DCIS.

In conclusion, given that a woman experienced a recurrence after a primary DCIS, tumor markers related to a recurrence being invasive compared to a new in situ were ER+, HER2−, and EGFR−. This marker profile might signal a potential for a DCIS to progress from in situ to invasive carcinoma.

Abbreviations

ER: Estrogen receptors
PR: Progesterone receptors
HER2: Human epidermal growth factor receptor 2
EGFR: Epidermal growth factor receptor
CK 5/6: Cytokeratin 5/6
TMA: Tissue microarray
IHC: Immunohistochemistry
SISH: Silver-enhanced in situ hybridization
OR: Odds ratio
CI: Confidence interval
NG: Nuclear grade.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors’ Contribution

Wenjing Zhou was responsible for data analyses, and paper preparation and editing. Karin Jirström performed IHC and SISH staining from the TMAs and helped provide expertise in breast cancer pathology. Wenjing Zhou, Christine Johansson, and Rose-Marie Amini were involved in pathology review and scoring of stains and contributed substantially to paper
editing. Carl Blomqvist and Anita Ringberg helped with the interpretation of the results and with drafting the paper. Fredrik Wärnberg designed the overall study, compiled and curated the datasets, coordinated the study, and helped draft and finalize the paper. All authors read and approved the final paper.

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References

[1] D. Porter, J. Lahti-Domenici, A. Keshaviah et al., “Molecular markers in ductal carcinoma in situ of the breast,” *Molecular Cancer Research*, vol. 1, no. 5, pp. 362–375, 2003.

[2] E. S. Wai, M. L. Lesperance, C. S. Alexander et al., “Predictors of local recurrence in a population-based cohort of women with ductal carcinoma in situ treated with breast conserving surgery alone,” *Annals of Surgical Oncology*, vol. 18, no. 1, pp. 119–124, 2011.

[3] S. Darby, P. McGale, C. Correa et al., “Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10 801 women in 17 randomised trials,” *The Lancet*, vol. 378, no. 9804, pp. 1707–1716, 2011.

[4] E. T. Siponen, H. Joensuu, and M. H. Leidenius, “Local recurrence of breast cancer after mastectomy and modern multidisciplinary treatment,” *Acta Oncologica*, vol. 52, no. 1, pp. 66–72, 2013.

[5] C. Correa, P. McGale, C. Taylor et al., “Overview of the randomised trials of radiotherapy in ductal carcinoma in situ of the breast,” *Journal of the National Cancer Institute Monographs*, vol. 2010, no. 41, pp. 162–177, 2010.

[6] N. Bijker, P. Meijnen, J. L. Peterse et al., “Breast-conserving treatment with or without radiotherapy in ductal carcinoma in situ: Ten-year results of European organisation for research and treatment of cancer randomized phase III trial 10853—a study by the EORTC breast cancer cooperative group and EORTC radiotherapy group,” *Journal of Clinical Oncology*, vol. 24, no. 21, pp. 3381–3387, 2006.

[7] C. Correa, P. McGale, C. Taylor et al., “Overview of the randomised trials of radiotherapy in ductal carcinoma in situ of the breast,” *Journal of the National Cancer Institute*, no. 41, pp. 162–177, 2010.

[8] S. Emdin, B. Granstrand, A. Ringberg et al., “SweDCIS: radiotherapy after sector resection for ductal carcinoma in situ of the breast. Results of a randomised trial in a population offered mammography screening,” *Acta Oncologica*, vol. 45, no. 5, pp. 536–543, 2006.

[9] B. Fisher, S. Land, E. Mamounas, J. Dignam, E. R. Fisher, and N. Wolmark, “Prevention of invasive breast cancer in women with ductal carcinoma in situ: an update of the National Surgical Adjuvant Breast and Bowel Project experience,” *Seminars in Oncology*, vol. 28, no. 4, pp. 400–418, 2001.

[10] J.-P. Julien, N. Bijker, I. S. Fentiman et al., “Radiotherapy in breast-conserving treatment for ductal carcinoma in situ: First results of the EORTC randomised phase III trial 10853,” *The Lancet*, vol. 355, no. 9203, pp. 528–533, 2000.

[11] L. A. Lee, M. J. Silverstein, C. T. Chung et al., “Breast cancerspecific mortality after invasive local recurrence in patients with ductal carcinoma-in-situ of the breast,” *American Journal of Surgery*, vol. 192, no. 4, pp. 416–419, 2006.

[12] M. Donker, S. Litière, G. Werutsky et al., “Breast-conserving treatment with or without radiotherapy in ductal carcinoma in situ: 15-year recurrence rates and outcome after a recurrence, from the EORTC, 10853 randomized phase III trial,” *Journal of Clinical Oncology*, 2013.

[13] A. Ringberg, H. Nordgren, S. Thorstensson et al., “Histopathological risk factors for ipsilateral breast events after breast conserving treatment for ductal carcinoma in situ of the breast—results from the Swedish randomised trial,” *European Journal of Cancer*, vol. 43, no. 2, pp. 291–298, 2007.

[14] G. F. Schwartz, L. J. Solin, I. A. Olivotto, V. L. Ernster, and P. I. Pressman, “Consensus conference on the treatment of in situ ductal carcinoma of the breast, April 22–25, 1999,” *Cancer*, vol. 88, no. 4, pp. 946–954, 2000.

[15] L. J. Solin, R. Gray, E. L. Baehner et al., “A multigene expression assay to predict local recurrence risk for ductal carcinoma in situ,” *Journal of the National Cancer Institute*, vol. 105, no. 10, pp. 701–710, 2013.

[16] D. B. Cornfield, J. P. Palazzo, G. F. Schwartz et al., “The prognostic significance of multiple morphologic features and biologic markers in ductal carcinoma in situ of the breast: a study of a large cohort of patients treated with surgery alone,” *Cancer*, vol. 100, no. 11, pp. 2317–2327, 2004.

[17] K. Kerlikowske, A. M. Molinaro, M. L. Gauthier et al., “Biomarker expression and risk of subsequent tumors after initial ductal carcinoma in situ diagnosis,” *Journal of the National Cancer Institute*, vol. 102, no. 9, pp. 627–637, 2010.

[18] E. Provenzano, J. L. Hopper, G. G. Giles, G. Marr, D. J. Venter, and J. E. Armes, “Biological markers that predict clinical recurrence in ductal carcinoma in situ of the breast,” *European Journal of Cancer*, vol. 39, no. 5, pp. 622–630, 2003.

[19] A. Ringberg, L. Anagnostaki, H. Anderson, I. Idvall, and M. Fernö, “Cell biological factors in ductal carcinoma in situ (DCIS) of the breast—relationship to ipsilateral local recurrence and histopathological characteristics,” *European Journal of Cancer*, vol. 37, no. 12, pp. 1514–1522, 2001.

[20] K. Jirström, A. Ringberg, M. Fernö, L. Anagnostaki, and G. Landberg, “Tissue microarray analyses of G1/S-regulatory proteins in ductal carcinoma in situ of the breast indicate that low cyclin D1 is associated with local recurrence,” *British Journal of Cancer*, vol. 89, no. 10, pp. 1920–1926, 2003.

[21] F. Wärnberg, R.-M. Amini, M. Goldman, and K. Jirström, “Quality aspects of the tissue microarray technique in a population-based cohort with ductal carcinoma in situ of the breast,” *Histopathology*, vol. 53, no. 6, pp. 642–649, 2008.

[22] C. A. Livasy, C. M. Perou, G. Karaca et al., “Identification of a basal-like subtype of breast ductal carcinoma in situ,” *Human Pathology*, vol. 38, no. 2, pp. 197–204, 2007.

[23] R. Diallo-Danebrock, E. Ting, O. Gluz et al., “Protein expression profiling in high-risk breast cancer patients treated with high-dose or conventional dose-dense chemotherapy,” *Clinical Cancer Research*, vol. 13, no. 2, pp. 488–497, 2007.

[24] C. U. Ihemelandu, L. D. Leffall Jr., R. L. Dewitty et al., “Molecular breast cancer subtypes in premenopausal African-American
women, tumor biologic factors and clinical outcome," *Annals of Surgical Oncology*, vol. 14, no. 10, pp. 2994–3003, 2007.

[25] M. Jumppanen, S. Gruvberger-Saal, P. Kauraniemi et al., "Basal-like phenotype is not associated with patient survival in estrogen-receptor-negative breast cancers," *Breast Cancer Research*, vol. 9, no. 1, article R16, 2007.

[26] C. A. Livasy, G. Karaca, R. Nanda et al., "Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma," *Modern Pathology*, vol. 19, no. 2, pp. 264–271, 2006.

[27] E. K. A. Millar, P. H. Graham, C. M. McNeil et al., "Prediction of outcome of early ER breast cancer is improved using a biomarker panel, which includes Ki-67 and p53," *British Journal of Cancer*, vol. 105, no. 2, pp. 272–280, 2011.

[28] L. M. McShane, D. G. Altman, W. Sauerbrei, S. E. Taube, M. Gion, and G. M. Clark, "REporting recommendations for tumor MARKer prognostic studies (REMARK)," *Breast Cancer Research and Treatment*, vol. 100, no. 2, pp. 229–235, 2006.

[29] M. J. Silverstein, K. A. Skinner, and T. J. Lomis, "Predicting axillary nodal positivity in 2282 patients with breast carcinoma," *World Journal of Surgery*, vol. 25, no. 6, pp. 767–772, 2001.

[30] E. Rakovitch, S. Nofech-Mozes, W. Hanna et al., "HER2/neu and Ki-67 expression predict non-invasive recurrence following breast-conserving therapy for ductal carcinoma in situ," *British Journal of Cancer*, vol. 106, no. 6, pp. 1160–1165, 2012.

[31] E. K. Latta, S. Tjan, R. K. Parkes, and F. P. O’Malley, "The role of HER2/neu overexpression/amplification in the progression of ductal carcinoma in situ to invasive carcinoma of the breast," *Modern Pathology*, vol. 15, no. 12, pp. 1318–1325, 2002.

[32] T. Perin, V. Canzonieri, S. Massarut et al., "Immunohistochemical evaluation of multiple biological markers in ductal carcinoma in situ of the breast," *European Journal of Cancer A*, vol. 32, no. 7, pp. 1148–1155, 1996.

[33] A. W. Burgess, "EGFR family: structure physiology signalling and therapeutic target," *Growth Factors*, vol. 26, no. 5, pp. 263–274, 2008.

[34] G. Viale, N. Rotmensz, P. Maisonneuve et al., "Invasive ductal carcinoma of the breast with the “triple-negative” phenotype: prognostic implications of EGFR immunoreactivity," *Breast Cancer Research and Treatment*, vol. 116, no. 2, pp. 317–328, 2009.

[35] H. Nogi, T. Kobayashi, M. Suzuki et al., "EGFR as paradoxical predictor of chemosensitivity and outcome among triple-negative breast cancer," *Oncology Reports*, vol. 21, no. 2, pp. 413–417, 2009.

[36] W. Hwangbo, J. H. Lee, S. Ahn et al., "EGFR gene amplification and protein expression in invasive ductal carcinoma of the breast," *The Korean Journal of Pathology*, vol. 47, no. 2, pp. 107–115, 2013.

[37] W. Zhou, K. Jirström, C. Johansson et al., "Long-term survival of women with basal-like ductal carcinoma in situ of the breast: a population-based cohort study," *BMC Cancer*, vol. 10, article 653, 2010.