

Patterns of Chromosomal Alterations in Breast Ductal Carcinoma *In situ*

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ABSTRACT

Purpose: Ductal carcinoma *in situ* (DCIS) is thought to be a nonobligate precursor of invasive cancer. Genomic changes specific to pure DCIS versus invasive cancer, as well as alterations unique to individual DCIS subtypes, have not been fully defined.

Experimental Design: Chromosomal copy number alterations were examined by comparative genomic hybridization in 34 cases of pure DCIS and compared with 12 cases of paired synchronous DCIS and invasive ductal cancer, as well as to 146 additional cases of invasive breast cancer of ductal or lobular histology. Genomic differences between high-grade and low/intermediate-grade DCIS, as well as between pure DCIS and invasive cancer, were identified.

Results: Pure DCIS showed almost the same degree of chromosomal instability as invasive ductal cancers. A higher proportion of low/intermediate-grade versus high-grade DCIS had loss of 16q (65 versus 12%, respectively; $P = 0.002$). When compared with lower grade DCIS, high-grade DCIS exhibited more frequent gain of 17q (65 versus 41%; $P = 0.15$) and higher frequency loss of 8p (77 versus 41%; $P = 0.04$). Chromosomal alterations in those cases with synchronous DCIS and invasive ductal cancer showed a high degree of shared changes within the two components.

Conclusions: DCIS is genetically advanced, showing a similar degree of chromosomal alterations as invasive ductal cancer. The pattern of alterations differed between high- and low/intermediate-grade DCIS, supporting a model in

which different histological grades of DCIS are associated with distinct genomic changes. These regions of chromosomal alterations may be potential targets for treatment and/or markers of prognosis.

INTRODUCTION

Breast cancer likely develops through a series of genetic events, with neoplastic conversion to invasive cancer occurring sometime during the preinvasive histological phases of usual hyperplasia, atypical hyperplasia, and ductal carcinoma *in situ* (DCIS). In the past 15 years, the diagnosis of DCIS has increased dramatically, largely due to the more widespread use of screening mammography (1). From 1983 to 1992, the incidence of DCIS had grown 500% and now accounts for >25% of all mammographically detected breast neoplasms (1). Because DCIS is thought to be a precursor for invasive cancer, current therapy consists of complete surgical extirpation to free margins by either lumpectomy or mastectomy. Surgical treatment is often combined with radiation to the ipsilateral breast and/or tamoxifen in those patients undergoing lumpectomy. Natural history studies suggest that <50% of all DCIS will progress to invasive cancer if treated with biopsy alone (2, 3). Nevertheless, because of limited prognostic utility of clinical and biological markers in DCIS, most patients are treated as if they would progress, often with treatments as aggressive as those offered to patients with early, localized invasive disease.

DCIS is thought to be a nonobligate precursor to invasive cancer. A number of studies have shown that DCIS and invasive ductal cancer (IDC) exhibit similar genetic abnormalities (4–6). These studies characterized genetic alterations at a small number of genomic loci and were able only to estimate the overall burden of genomic instability in these lesions. Furthermore, many previous articles characterized DCIS that were found in the presence of synchronous invasive cancer, rather than lesions occurring alone. Thus, genomic changes unique to DCIS, as well as analysis of changes unique to different subtypes of DCIS, have not been fully described.

To study chromosomal alterations occurring in pure DCIS, we used the technique of comparative genomic hybridization (CGH) to characterize genomic changes in 34 cases of DCIS occurring in the absence of invasive cancer. We compared these chromosomal alterations to 12 cases of synchronous DCIS and IDC, as well as to 146 cases of invasive ductal or lobular cancer. Genomic differences between high- and low/intermediate-grade DCIS were also analyzed to establish whether genomic differences between individual subtypes of DCIS could be determined.

MATERIALS AND METHODS

Clinical Material. Formalin-fixed, paraffin-embedded tissue blocks were identified from 46 patients from the University of California San Francisco, California Pacific Medical

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Center, University of Basel, or University of Vermont pathology archives. Institutional Review Board approval was obtained at all institutions participating in this study. Of these 46 samples, 34 patients were diagnosed with DCIS without invasion (pure DCIS), and 12 cases contained DCIS as well as synchronous invasive cancer either in the same or adjacent blocks. Seventeen of these cases have been described previously (7). The changes identified in the 34 cases of pure DCIS were compared with a combined set of 146 cases of invasive cancers of ductal, lobular, micropapillary, and tubular cancers reported previously (8–11). CGH profiles for these 146 cases were reviewed and rescored for the current study.

H&E-stained sections were reviewed to define areas of DCIS, invasive tumors (if present), and normal ducts or lobules. Nuclear grade of the DCIS was recorded as low, intermediate, or high and the histological pattern as comedo or noncomedo (solid, cribriform, and micropapillary) types (12). Comedo-type DCIS was defined as solid-type DCIS with high nuclear grade and moderate or extensive necrosis. Tumors exhibiting a mixture of histological grades were classified by the highest grade seen. DCIS were also classified based on estimated size of tumor and degree of necrosis. The size of the lesions was categorized as small (<1 cm in greatest diameter), medium (1–2 cm), and extensive (>2 cm). Necrosis was defined as negative when none was present, small when limited to an occasional duct or small amounts in multiple ducts, moderate when significant necrosis was present in more than two to three ducts, and extensive when present in the majority of ducts.

DNA Extraction and CGH Analysis. Tissue preparation, DNA extraction, and CGH were performed as described previously (7). Briefly, using the adjacent H&E-stained slide for orientation, two to three methyl-green-stained 5- μ m sections were microdissected to isolate DNA from DCIS, IDCs, and normal ducts. DNA was isolated by a 3-day proteinase K digestion. In five cases, regions of DCIS were sufficiently large (>2 cm, >70% tumor cells) so that CGH could be performed without prior PCR amplification. For the remaining cases, 1–2 μ l aliquots of microdissected DNA were amplified in duplicate by degenerate oligonucleotide primer PCR. PCR amplified normal reference DNA (Promega) was labeled by nick translation with Texas Red-5-dUTP or with Fluorescein-12-dUTP (DuPont). The MPE600 cell line was labeled with fluorescein or with digoxigenin-11-dUTP (Boehringer) and served as a positive control, with clearly defined chromosomal alterations, including gains on 1q, 11q13-14, 13q, and 17q and losses on 9p, 11qter, and 16q. Forty μ l of amplified DNA from paraffin sections were used per 50- μ l reaction, and DNA was labeled with fluorescein or digoxigenin. For the five larger tumors, 1 μ g of DNA was labeled by nick translation. Samples were labeled in duplicate with fluorescein and Texas Red. Samples were hybridized onto normal male metaphases. Each sample was hybridized in duplicate with different fluorochromes. Digoxigenin-labeled samples were stained with anti-digoxigenin rhodamine (Boehringer). Texas Red labeling was not used for microdissected PCR-amplified samples because of inconsistent results and lower intensities.

Successful hybridizations showed good intensity signals, with smooth, homogeneous staining over entire metaphases. At least five metaphase spreads were acquired for each case. Ac-

quisition was performed using our Quantitative Image Processing System (13). Two to three metaphases/sample were analyzed in each color. Gains and losses were defined if both the mean ratio and its SD were >1.2 or <0.85. Inverse CGH pairs were examined together, and all changes must have been seen in both hybridizations. Interpretations of changes at 1pter, 19, and 22 (GC-rich areas) were interpreted more stringently, requiring the threshold to be exceeded in both forward and reverse hybridizations for inclusion in the analysis. Overall, 94% of the PCR-amplified cases were successfully hybridized using these methods. When present in sufficient amounts, normal breast tissue was microdissected from the DCIS cases (both pure and synchronous) concurrent with microdissection of the DCIS. Of these normal samples, 20 contained sufficient DNA for evaluation. CGH of these specimens showed no chromosomal alterations compared with normal reference DNA.

Statistical Analysis. Genetic alterations were tabulated as a single change if they (a) consisted of a single regional change, (b) consisted of a whole arm change, or (c) were comprised of either two gains or two losses on one chromosomal arm. If both a gain and a loss were present on the same arm, two events were recorded. Frequencies of genetic changes between different tumor groups were compared with a χ^2 statistic calculated for each 2 \times 2 contingency table. No adjustment for multiple comparisons was done.

Percent concordance (7) was defined as

$$\frac{\text{number of changes in common}}{(\text{number in common}) + [1/2 \times (\text{number only in DCIS} + \text{number only in IDC])}$$

Concordance was used to determine the extent to which two specimens shared the same genomic changes, with higher concordance indicating a greater number of shared changes.

RESULTS

Clinical Characteristics. Patient and tumor characteristics for 34 cases of DCIS without invasive cancer (pure DCIS) are shown in Table 1. The mean age at diagnosis was 52 years. DCIS was 50% high grade, 44% intermediate grade, and 6% low grade. The extent of necrosis was categorized as extensive in 35%, moderate in 41%, and small or absent in the remainder. Four cases lacked necrosis. For the 12 cases of paired DCIS and concurrent invasive cancer (Table 2), the mean age at diagnosis was also 52 years. Only two patients in this group had an invasive tumor measuring <2 cm. Four patients had node-positive disease. Of the 34 patients with DCIS alone, 17 cases had no known recurrence within 5 years, whereas the remaining 17 cases had recurrences of DCIS (13 within 5 years and 4 additional cases with known recurrence in >5 years).

Chromosomal Alterations in Pure DCIS. Thirty-four cases of pure DCIS were characterized by chromosomal CGH (Fig. 1). The mean number of chromosomal alterations was 8.0 (average of 4.7 losses and 3.3 gains). All cases had at least one chromosomal CGH aberration (range, 1–20). The prevalence of specific CGH alterations within groups was analyzed by lesion type (Table 3); any genomic alteration exceeding a frequency > 25% for at least one lesion type is shown. The most common changes in pure DCIS were gains on chromosome arms 1q

Table 1 Histologic characteristics and chromosomal alterations in pure DCIS ($n = 34$)

Case no.	Age at diagnosis (yrs)	Histologic type	Nuclear grade	Necrosis	Size*	Number of CGH changes		
						Total	Losses	Gains
A	52	Comedo	High	Extensive	Extensive	2	0	2
1	66	Comedo	High	Extensive	Small	13	8	5
2	65	Comedo	High	Moderate	Medium	12	10	2
3	55	Comedo	High	Extensive	Medium	2	0	2
15	41	Comedo	High	Moderate	Extensive	4	1	3
18	36	Comedo	High	Extensive	Extensive	4	2	2
23	39	Comedo	High	Moderate	Medium	6	4	2
24	43	Comedo	High	Extensive	Medium	11	6	5
F3	60	Comedo	High	Extensive	Small	6	4	2
F10	57	Comedo	High	Moderate	Extensive	20	16	4
F11	69	Comedo	High	Extensive	Medium	20	12	8
F12	48	Comedo	High	Extensive	Medium	6	2	4
F23	51	Comedo	High	Moderate	Extensive	10	6	4
F25	71	Comedo	High	Moderate	Medium	1	1	0
F199	40	Comedo	High	Moderate	Small	15	7	8
27	43	Solid	High	Small	Extensive	7	4	3
F22	66	Solid	High	Small	Medium	2	0	2
19	84	Solid	Int†	Negative	Extensive	3	1	2
20	55	Solid	Int	Negative	Extensive	3	3	0
F29	39	Solid	Int	Moderate	Small	14	7	7
F1099	44	Solid	Int	Moderate	Medium	13	10	3
5	49	Solid	Int	Small	Extensive	13	7	6
25	59	Micropapillary	Int	Negative	Small	6	4	2
F33	69	Micropapillary	Int	Extensive	Small	8	4	4
14	52	Cribiform	Int	Small	Extensive	6	1	5
22	50	Cribiform	Int	Moderate	Extensive	7	6	1
F1	39	Cribiform	Int	Small	Medium	2	1	1
F8	45	Cribiform	Int	Small	Small	13	9	4
F14	45	Cribiform	Int	Small	Medium	3	2	1
F32	44	Cribiform	Int	Moderate	Medium	13	9	4
F41	58	Cribiform	Int	Small	Extensive	13	6	7
F46	45	Cribiform	Int	Small	Medium	10	2	8
F6316	61	Cribiform	Low	Negative	Small	2	2	0
F4	40	Cr/MP mix‡	Low	Negative	Medium	2	2	0

* Size of DCIS was determined as described in the methods.

† Intermediate grade.

‡ Equal mixture of cribriform and micropapillary.

(59%), 17q (53%), and 8q (38%), and losses on 8p (59%), 17p (44%), and 16q (38%). Loss of 8p occurred in two predominant patterns, consisting of either whole arm loss (38%) or regional loss (21%). For pure DCIS, all cases with partial loss of distal

8p21-pter also showed gain of the most proximal region of 8p (8p11-p12).

Separation of the pure DCIS cases into low/intermediate-grade ($n = 17$) and high-grade ($n = 17$) groups demonstrated a

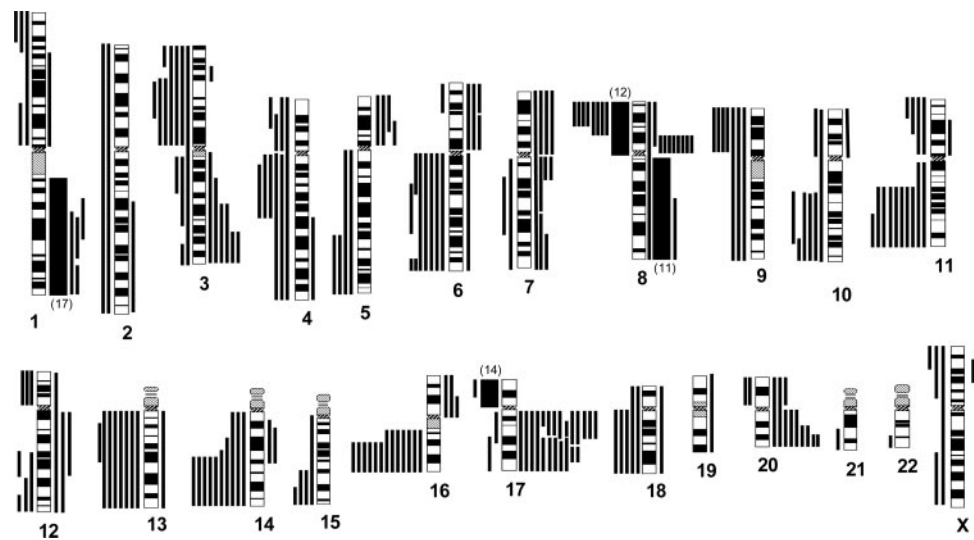
Table 2 Characteristics of concurrent DCIS and invasive ductal cancer ($n = 12$)

Case no.	DCIS histologic type	DCIS nuclear grade	DCIS size	IDC differentiation	IDC size (cm)	Lymph node status	Location of DCIS and IDC*	% concurrence between DCIS and IDC by CGH
17	Comedo	High	Medium	Poor	0.8	Negative	Differentblock	100%
30	Cribiform	Low	Medium	Moderate	2	Negative	Differentblock	100%
10	Micropapillary	Low	Small	Moderate	3	Negative	9 mm	100%
6	Solid	High	Small	Moderate	2	Negative	6 mm	94%
13	Cribiform	Int†	Medium	Moderate	2.4	Positive	2 mm	91%
31	Comedo	High	Extensive	Moderate	>5	Negative	Differentblock	90%
29	Cribiform	Low	Medium	Moderate	4	Positive	Differentblock	89%
7	Comedo	High	Extensive	Moderate	2	Negative	Differentblock	79%
8	Cribiform	Int	Small	Moderate	2.5	Positive	4 mm	75%
16	Solid	High	Extensive	Poor	2.5	Positive	Differentblock	57%
12	Solid	Low	Medium	Moderate	2.5	Negative	Differentblock	31%
9	Cribiform	Int	Extensive	Well	1	Negative	Differentblock	17%

* Distance between DCIS and IDC when both present in the same block.

† Intermediate.

Fig. 1 Copy number changes in 34 pure DCIS tumors. Losses are on the left of the chromosomes and gains are on the right. Solid bars represent multiple cases as indicated by the number in parentheses by these bars.



similar number of alterations (7.7 versus 8.2; $P = 0.39$) in the two groups. The low/intermediate-grade lesions showed an increased frequency of losses of 16q (65 versus 12%; $P = 0.002$) and 17p (53 versus 35%; $P = 0.25$) compared with high-grade DCIS but were less likely to have gains of 17q (41 versus 65%; $P = 0.15$) and losses of 8p (41 versus 76%; $P = 0.04$). In the high-grade DCIS, loss of 8p occurred predominantly as whole

arm loss (65%), whereas the low/intermediate-grade lesions showed a more common pattern of 8p loss as a partial arm loss combined with proximal gain (29%), rather than as whole arm loss (12%).

CGH alterations associated with comedo and noncomedo histologies were similar to high-grade and low/intermediate-grade lesions, respectively, because 15 of 17 cases of high-grade

Table 3 Prevalence (%) of chromosomal alterations in DCIS and invasive cancer by histologic type.

	Pure DCIS Low/Intermediate grade (n = 17)	Pure DCIS High grade (n = 17)	Pure DCIS All (n = 34)	IDC*,† (n = 94)	ILC† (n = 18)	Micropapillary‡ (n = 16)	Tubular§ (n = 18)	IDC Node negative (n = 63)	IDC Node positive (n = 29)
Mean number of alterations	7.7	8.2	8.0	8.8	6.9	7.4	3.6¶	7.7	11¶
1q+	59	59	59	57	72	31	44	57	55
3p-	18	29	24	22	6	6	0	16	35
6q-	24	24	24	20	28	0	17	14	35
8p-	41**	77**	59	33	11	100	28	29	45
8p- arm	12**	65**	38	18	11	38	0	19	17
8p- region	29	12	21	15	0	63	28	10	28
8p+	35**	6**	21	12	0	56	22	13	10
8q+	35	41	38	48	22	88	22	48	48
9p-	12	29	21	15	17	25	0	8	28
11q-	35	29	33	28	22	31	11	21	41
11q+	0	0	0	18	6	13	0	14	28
14q-	35	24	29	14	6	0**	0	14	14
16p+	12	6	9	17	17	25	39	10	35
16q-	65**	12**	38	34	67	44	78	33	38
17p-	53	35	44	38	33	56	6	40	35
17q+	41	65	53	29	17	38	6	19	48
18p-	6	6	6	11	11	19	0	2	31
18q-	6	24	15	22	28	19	0	13	45
20q+	18	29	24	23	6	0	6	22	24

* Includes two cases of IDC with unknown nodal status.
 † Samples have been reevaluated and most come from Nishizaki *et al.* (9).
 ‡ Samples are from Thor *et al.* (10).
 § Samples are from Waldman *et al.* (11).
 ¶ t test ($P < 0.05$) for histologic type versus 34 pure DCIS.
 || χ^2 ($P < 0.05$) for histologic type versus 34 pure DCIS.
 ** χ^2 ($P < 0.05$) for low/intermediate- versus high-grade DCIS comparison.

DCIS exhibited comedo necrosis. Comedo-type DCIS had fewer losses of 16q than the noncomedo DCIS (13 *versus* 57%; $P = 0.009$), as well as more 8p losses (80 *versus* 47%; $P = 0.055$), and 17q gains (67 *versus* 47%; $P = 0.219$).

Comparison of CGH Changes in DCIS and Invasive Cancers. Pure DCIS cases showed slightly fewer chromosomal changes than the invasive cancers, although this difference was not statistically significant (8.0 *versus* 8.8, $P = 0.26$). Many of the most common changes were similar in the pure DCIS and the invasive cancers, including gain of 1q (59% in DCIS *versus* 57% in invasive; $P = 0.53$), gain of 8q (38 *versus* 48%; $P = 0.22$), and loss of 16q (38 *versus* 34%; $P = 0.41$). Significant differences were seen in the frequency of 11q gain (0% in DCIS *versus* 18% in invasive cancer; $P = 0.003$), 17q gain (53 *versus* 29%; $P = 0.011$), and of 8p loss (59 *versus* 33%; $P = 0.008$). The concurrent loss of 8p21-pter with a gain of 8p11-p12 occurred in 21% of the DCIS but only in 6% of IDC ($P = 0.026$).

The degree of genomic alteration was similar between pure DCIS and node-negative IDC (8.0 *versus* 7.7, $P = 0.26$). However, node-positive cancers as a whole demonstrated a greater number of alterations (mean of alterations = 11.0; $P = 0.01$). Although 8p loss was seen in both pure DCIS and invasive cancer, the latter group was more likely to exhibit loss of the entire arm of 8p than either node-negative or node-positive invasive ductal cancers (59% in DCIS *versus* 19 and 17%, respectively, $P = 0.004$ and $P = 0.036$). Chromosome 14q loss was also seen more frequently in DCIS (29%) than either node-negative or node-positive invasive tumors (14% in both; $P = 0.07$ and $P = 0.12$, respectively).

Genomic changes most characteristic of node-positive *versus* node-negative invasive cancers was loss of 18p and 18q ($P = 0.001$ for both). However, other regions of genomic alteration significantly different between node-positive and node-negative cancers were gain of 17q (48 *versus* 19%; $P = 0.005$) and gain of 16p (35 *versus* 10%; $P = 0.005$). Other changes between node-positive and node-negative cancers approaching significance included losses of 1p ($P = 0.049$), 3p ($P = 0.043$), 4q ($P = 0.026$), 6q ($P = 0.028$), 9p ($P = 0.17$), 9q ($P = 0.026$), and 11q ($P = 0.036$), all showing greater frequency of loss in the node-positive group.

Among the invasive cancers, there were distinct genomic changes characterizing some individual histological subtypes. Invasive lobular cancers (ILCs) had a high prevalence of 1q gain (89%) and 16q loss (72%), as well as a low likelihood of 17q gain (17%). Interestingly, this pattern of 1q gain and 16q loss was shared with tubular cancers as well as with low/intermediate-grade DCIS. Very few cases of ILC showed 8p loss (17%), but all of these were characterized by loss of the entire arm of 8p. In contrast, all of the invasive micropapillary cancers ($n = 16$) had loss of 8p, with 63% of these cases showing regional, rather than whole arm loss. This IDC subtype also demonstrated a high prevalence of 8p gain and 8q gain, 56 and 88%, respectively.

DCIS with Synchronous Invasive Cancers. Twelve pairs of synchronous DCIS and invasive cancer were separately microdissected and characterized by chromosomal CGH (Table 4). The mean number of aberrations was 8.1 for the DCIS and 7.8 for their concurrent IDC ($P = 0.42$). Although the sample

size was small, this set of DCIS showed a significant difference in number of alterations between high-grade ($n = 5$) and low/intermediate-grade ($n = 7$) lesions (11.8 *versus* 5.4; $P = 0.002$).

The mean concordance (see "Materials and Methods") between the DCIS and the paired invasive cancers was 77% (range, 17–100%). The majority of the pairs (9 of 12 cases) showed a strong similarity between the DCIS and IDC with few differences (mean concordance of 91%; Table 2), whereas the remaining 3 cases appeared to be less genomically related (mean concordance of 35%). Four DCIS/IDC pairs were isolated from the same archival paraffin block, whereas eight were from different blocks in the same surgical tissue specimen. All three cases with lower concordance were from separate blocks.

DISCUSSION

This study clearly confirms that DCIS is a genetically advanced lesion, with almost the same degree of genomic instability as invasive cancer. The extent to which the genome is altered in DCIS indicates that genomic instability most likely precedes phenotypic evidence of invasion. Genomic data have supported several possible models of breast cancer progression. One hypothesis suggests that there exist genetically distinct subgroups of DCIS, only some of which ever have the potential to progress to invasion (14). An alternate dedifferentiation theory proposes that DCIS progresses from lower to higher grade and then to invasive cancer with progressive accumulation of genomic changes (15–17). Perhaps the most compelling model is one in which there are distinct genomic progression pathways leading from any DCIS to invasive cancer. Different histological grades are thus associated with specific genomic changes, and each DCIS pathway may carry a distinct invasive potential (18).

In this latter model, specific genomic changes are selected for from random genomic alterations arising from an unstable environment. Phenotype, particularly histological grade, is likely the result of distinct and different genomic changes. It has been shown that nuclear grade of DCIS is one of the most important clinical determinants of biological behavior, with high-grade tumors more likely to recur than low-grade tumors, even after optimal therapy (19–21). Prognosis is therefore likely to be a direct consequence of tumor genetics. In our study, when pure DCIS was divided into low/intermediate- *versus* high-grade groups, clear genetic differences between grades were apparent.

Lower grade DCIS was more likely than high-grade DCIS to show loss of 16q. Three small loss of heterozygosity studies have reported that loss of 16q is among the most frequent genomic changes seen in DCIS (6, 22, 23). CGH studies have confirmed and expanded on these results by analyzing gains as well as losses along the entire genome. Our data substantiates previous smaller studies that indicate loss of 16q is an early event most often found in lower grade DCIS lesions (24–26). We have previously found that loss of 16q can even be detected even at the stage of atypical ductal hyperplasia (27). Thus, loss of 16q appears to be a critical event in early cancer progression.

An important strength of our study is that the entire set of *in situ* and invasive cancers were evaluated under the same experimental conditions and with identical analytical techniques, thus allowing direct comparison between subsets of

Table 4 Chromosomal alterations in DCIS and synchronous invasive ductal cancer (n = 12)

Case no.	Changes in common	Changes only in DCIS	Changes only in IDC	% concurrence	CGH changes in common	CGH changes only in DCIS	CGH changes only in IDC
17	14	0	0	100	3q24-qter+; 5p+; 5q31+; 5q32-qter-; 6q25-qter-; 7p+; 8p-; 8q+; 9q22-qter-; 16q23-qter+; 17q11-q21+; 20q13+; Xp-; Xq11-q26-	None	none
30	2	0	0	100	1q+; 16q-	None	none
10	4	0	0	100	1p31-pter-; 1p12-p22+; 1q+; 2p23-p16-		
6	17	1	1	94	1q41-qtr+; 2q21-q24-; 2q32-q36+; 4p15-pter-; 6q15-q22+; 7p+; 8p-; 11p11-p14+; 11q11-q12+; 12q21-qtr-; 13q12-q21-; 14q11-q23+; 14q24-qtr-; 15q25-qtr+; 16q-; 17q23-qtr+; 18q12-q21-	6q23-qter+	12p+
13	5	0	1	91	1q+; 7q11-q21+; 7q22-q32-; 8p-; 16q21-qtr-	None	X-
31	9	2	0	90	6p+; 6q12-q16-; 6q21-q22+; 6q23-; 6q24+; 6q25-qtr-; 8p11-p12+; 8p21-pter-; 8q+; 11q14-qtr-; 17p-; 19+	7+; 17q+	none
29	4	0	1	89	1q+; 4q33-qtr-; 6q22-q23-; 8q22-qtr-	None	14q22-qter-
7	11	1	5	79	8p21-pter-; 8p11-p12+; 8q+; 11q13+; 11q14-qter-; 12q15+; 12q21-qtr-; 16q-; 17p11+; 17q11-q21+; 22q+	7+	6p+; 6q-; 13q-; 14q-; 17q22-qter+
8	6	2	2	75	1q+; 4p-; 5p+; 14q22-qtr-; 17p-; 17q11-q24+	8q21-qter+; 14q12-q21+	8p-; 11p-
16	2	3	0	57	17p-; 17q21-qtr+	6p21-pter+; 6q16-q22+; 20q13+	none
12	2	4	5	31	3p11-p23-; 20q+	1+; 13q+; 14q21-q31-; 22q13+	6p22+; 9q13-qtr-; 10q24-qtr-; 16q24-; 17p-
9	1	8	2	17	16q-	1p-; 1q+; 3-; 5-; 6+; 16p+; 20+; 22q-	8p-; 13q21-qter-

invasive cancer and DCIS. Comparison of genomic changes between preinvasive and invasive tumors showed that loss of 16q, typical of low/intermediate-grade DCIS, was also seen in some groups of invasive cancers. Chief among these were the tubular cancers, a subtype known to have a very favorable prognosis and characterized by low grade. Loss of 16q was seen in 78% of tubular cancers *versus* 34% in nontubular invasive ductal cancers. This corroborates observations by other groups who have also found that 16q alterations appear to be specific to low-grade invasive tumors (28).

Another subgroup of invasive cancers commonly demonstrating 16q loss were those of lobular histology. We have previously reported that 16q is also lost in most lobular carcinoma *in situ* (29). In invasive lesions, loss of 16q was highly prevalent in ILC when compared with IDC. In addition to increased prevalence, the pattern of 16q loss differed markedly between lobular and ductal cancers. Few IDCs had concomitant 16q loss and 1q gain, compared with over half of ILCs and tubular cancers. Fluorescence *in situ* hybridization analysis has shown that translocation of the long arms of chromosomes 1 and 16 often occurs in ILC and tumors of low grade (30–32). This is possibly a result of reciprocal translocation at centromeric heterochromatin. The lower frequency of 1q+/16q– translocation in IDCs than in lobular and tubular tumors suggests that the selective pressures resulting in 16q loss in tubular and lobular cancers (including lobular carcinoma *in situ*) may target differ-

ent genetic loci when compared with IDC. Furthermore, this implies that lobular carcinomas *in situ*, ILCs, and tubular cancers may share a common progression pathway, a suggestion that is supported by the frequent coexistence of tubular and lobular patterns (33).

Several candidate genes mapping to 16q may be important in cancer progression. Perhaps best studied is E-cadherin (CDH1), located on 16q22.1. This glycoprotein has been shown to be critical for cell adhesion and morphogenesis and is markedly absent by immunohistochemistry in ILCs and lobular carcinomas *in situ*, as well as in some tubular cancers. Perturbation of normal E-cadherin function either through promoter methylation or gene mutation has been implicated in lobular tumorigenesis (28). Buerger *et al.* (26) has correlated CGH findings in DCIS with mitotic index and has found that loss of 16q was associated with low tumor proliferation rate. Because regulation of cell turnover is not typically attributed to CDH1, it is possible that for ductal tumors, there may be other important regions of chromosome 16q subjected to selective pressure. Such candidate genes include E2F4 (16q22.1), a cell cycle transcription factor, and WWOX (16q23.3–24.2), which appears to be a potent tumor suppressor gene in breast cancer as well as in esophageal malignancies (34).

8p was another region frequently showing alterations in low-grade DCIS. Interestingly, we observed several distinct patterns of 8p alteration in DCIS. The loss of 8p involved either

the whole arm or was a partial loss in the 8p21-pter region often accompanied by a concomitant gain at 8p11-12, a pattern that may reflect a targeted translocation. Forty-one percent of low/intermediate-grade DCIS showed 8p loss. Of these, the majority had a partial loss of the distal arm of 8p, whereas almost all high-grade DCIS with 8p loss exhibited loss of the entire chromosomal arm. In our dataset of invasive cancers, the low-grade tubular cancers more often exhibited partial, rather than whole arm loss, whereas invasive cancers in general were equally likely to have either partial or whole arm loss.

The chromosomal region 8p21-23 is the site of the observed break point in these tumors and has previously been described in breast cancers (26, 35). Fibroblast growth factor receptor 1 (8p11.2-11.1) appears to be just proximal to the breakpoint region and codes for a tyrosine kinase, which is thought to lead to an increased cell proliferation and progression to invasion (36). Heregulin (NRG1; 8p22-11) is a glycoprotein which binds to ERBB4 to regulate cell growth and proliferation (37), and maps just distal to the breakpoint region. Our studies suggest that fibroblast growth factor receptor 1 is gained and NRG1 is lost in those cases with the partial chromosomal loss on 8p. Such distal loss just adjacent to a proximal gain (or amplification) has been noted in other chromosomal regions, and is postulated to be related to a breakage/fusion/bridge mechanism of amplification. With ongoing mapping studies, we hope to more clearly define this breakpoint region at higher resolution.

Some genomic alterations were seen at greater frequency in high-grade DCIS than in lower grade lesions. Most apparent among these was gain of 17q. Gain of 17q has been associated with amplification of ERBB2, located at 17q12 (38). Studies have shown that the erbB2/her2/neu protein product is overexpressed in 60–70% of DCIS by immunohistochemistry (39, 40), although in only 20–30% of invasive breast cancers. The higher prevalence of 17q gain in DCIS *versus* invasive ductal cancer is somewhat counterintuitive given the precursor-product relationship between them. It is unlikely that an acquired genomic alteration such as gain of 17q and amplification of ERBB2 would be consistently reversed during cancer progression. One possible explanation for this seeming paradox is detection bias, in that high-grade DCIS with ERBB2 overexpression may be more likely to have *in situ* necrosis and microcalcifications and thus be more apparent on mammography. The increased necrosis might be associated with a faster growth rate and greater hypoxia caused by the physical restraint of the intact basement membrane in DCIS. Alternatively, the higher prevalence of erbB2 positivity in DCIS when compared with invasive cancer could also be explained if not all DCIS progresses to invasive cancer. In addition to ERBB2, chromosome 17q also contains GRB7 (17q21-22), involved in cell cycle activation, and TBX2 (17q23), an immortalizing gene that has been identified in breast cancers (41). It is possible that selected gain of these genes also confers some survival advantage, particularly in high-grade DCIS.

17p loss was seen frequently in both low- and high-grade DCIS. This genetic alteration is seen in many subtypes of breast cancer and is detected in over a third of all DCIS and invasive cancers, although interestingly, it is uncommon in tubular cancers. It has been suggested that p53 (17p13.1) is the target for

this deletion, releasing cells from its checkpoint function controlling apoptosis, proliferation, cytoskeletal regulation, and DNA repair (42–44). Our findings suggest that loss of p53 may be important early in the development of many preinvasive cancers, conferring a survival benefit during this time of increased proliferation, and that 17p loss may be an important mechanism for cancer progression in DCIS regardless of grade.

The majority of cases in our small set of paired DCIS and synchronous invasive cancer were clonally related by CGH. Loss of heterozygosity studies have reported similar findings for DCIS and concurrent IDCs (4–6). The synchronous DCIS and IDCs in our study appear to be much more similar than different, as indicated by the high degree of concordance in 9 of 12 cases (mean concordance was 91%). The remaining three cases had a mean concordance of 35%, suggesting that in these cases, the IDC either had progressed from an entirely different tumor clone than the DCIS component or the invasive component separated from the DCIS at a very early stage of tumor evolution.

Given the heterogeneity of genomic alterations seen in both DCIS and invasive cancer, it is unlikely that there exists only a single pathway for breast cancer progression. Low/intermediate-grade DCIS manifests many of the same genetic changes as low-grade invasive cancers such as gain of 1q, loss of 16q, and distal loss of 8p. Similarly, high-grade DCIS and many invasive ductal cancers share whole arm loss of 8p and 18q, as well as gain of 17q. Our paired cases clearly show that when synchronous invasive and *in situ* cancer coexist, both components are likely to share virtually identical genomic changes. These findings are consistent with a model in which some lower grade DCIS may progress to low-grade invasive cancer, and some high-grade DCIS is likely to progress to high-grade invasive cancer. Thus, genetic alterations occurring early during *in situ* tumor development determines the phenotype of the DCIS as well as the subsequent invasive cancer. Tumor genetic signature therefore drives biological behavior, clinical behavior, and ultimately patient outcome. These divergent genetic characteristics of *in situ* tumors will help to establish molecular classification schemes, to serve as potential prognostic markers for behavior of preinvasive cancer, and eventually to identify targets for individualized therapy for this heterogeneous disease.

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