Chromosomal Alterations in Ductal Carcinomas In Situ and Their In Situ Recurrences

Frederic M. Waldman, Sandy DeVries, Karen L. Chew, Dan H. Moore II, Karla Kerlikowske, Britt-Marie Ljung

Background: Ductal carcinoma in situ (DCIS) recurs in the same breast following breast-conserving surgery in 5%–25% of patients, with the rate influenced by the presence or absence of involved surgical margins, tumor size and nuclear grade, and whether or not radiation therapy was performed. A recurrent lesion arising soon after excision of an initial DCIS may reflect residual disease, whereas in situ tumors arising after longer periods are sometimes considered to be second independent events. The purpose of this study was to determine the clonal relationship between initial DCIS lesions and their recurrences. Methods: Comparative genomic hybridization (CGH) was used to compare chromosomal alterations in 18 initial DCIS lesions (presenting in the absence of invasive disease) and in their subsequent ipsilateral DCIS recurrences (detected from 16 months to 9.3 years later). Results: Of the 18 tumor pairs, 17 showed a high concordance in their chromosomal alterations (median = 81%; range = 65%–100%), while one case showed no agreement between the paired samples (having two and 20 alterations, respectively). Morphologic characterization of the DCIS pairs showed clear similarities. The mean number of CGH changes was greater in the recurrent tumors than in the initial lesions (10.7 versus 8.8; P = .019). The most common changes in both the initial and the recurrent in situ lesions were gains involving chromosome 17q and losses involving chromosomes 8p and 17p. The degree of concordance was independent of the time interval before recurrence and of the presence of positive surgical margins. Conclusions: In this study, DCIS recurrences were clonally related to their primary lesions in most cases. This finding is consistent with treatment paradigms requiring wide surgical margins and/or postoperative radiation therapy. [J Natl Cancer Inst 2000; 92:313–20]
Polymerase Chain Reaction Amplification

Amplification of the microdissected DNA was by degenerate oligonucleotide primer polymerase chain reaction (PCR) (18). Samples were amplified in duplicate but in separate PCR reactions, each containing a 1- to 2-μL aliquot of microdissected DNA. Each PCR run included samples of female genomic DNA from healthy donors (considered the reference and isolated from peripheral blood), MPE600 (breast cancer cell line with known CGH aberrations), and a PCR blank. Fifty nanograms of reference and MPE600 cell line DNA resulted in approximately 2–3 μg of amplified DNA, ranging in size from 200 base pairs (bp) to 6 kilobase pairs (kbp). Microdissected DNA yielded up to 1 μg of PCR product, averaging around 600 bp (range, 100 bp to 2 kbp).

Probe Labeling and CGH

PCR-amplified DNA from the initial DCIS as well as from the recurrent lesion was labeled in duplicate by nick translation. PCR-amplified normal reference DNA (25 μL) was labeled with fluorescein-12–deoxyuridine triphosphate (dUTP) (Du Pont NEN, Boston, MA) or indirectly with biotin–deoxyadenosine triphosphate (dATP) (Life Technologies, Inc. [GIBCO BRL], Gaithersburg, MD). The MPE600 cell line and PCR-amplified test DNA were labeled with digoxigenin-11–dUTP (Boehringer Mannheim Biochemicals, Indianapolis, IN). Nick-translation PCR products were close to the original product size, 100–1000 bp. Smaller probes tended to yield less than optimum CGH results and appeared to be granular or dim.

CGH was performed as previously described (18,19). Samples were hybridized onto normal male metaphase spreads. Digoxigenin-labeled test DNA samples were hybridized in duplicate against reference DNA labeled either with fluorescein-12-dUTP or with biotin–dATP. Digoxigenin-labeled samples were stained with anti-digoxigenin rhodamine (Boehringer Mannheim Biochemicals). Biotin-labeled samples were stained with fluorescein isothiocyanate-labeled avidin (Vector Laboratories, Inc., Burlingame, CA).

Successful hybridizations showed good intensity signals with smooth, homogeneous staining over the entire metaphases. At least five metaphase spreads were acquired for each case. Acquisition was performed with the use of our Quantitative Image Processing System [QUIPS (20)]. Two to three metaphases per sample were analyzed in each color. Tumor-to-reference fluorescence intensity ratios were calculated along chromosomal arms, and gains and losses were defined if the mean and standard deviation were above 1.2 or below 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 (and 4 and 13 in the opposite direction) were interpreted with caution. Definition of changes at these loci required the cut point to be exceeded in both hybridizations.

Statistical Analysis

For scoring of genetic alterations, whole chromosome changes were scored as one event. All other changes were scored by arm. A loss and a gain on one arm were scored as two changes, whereas two separate losses (or gains) on the same arm were scored as one change.

To compare frequencies of alterations in different groups of tumors, we calculated a chi-squared statistic for each 2 × 2 contingency table. All statistical tests were two-sided and were considered to be statistically significant at P<.05.

The following three methods were used to measure concordance between the initial and recurrent lesions for the CGH alterations: 1) percent concordance, 2) similarity score, and 3) hierarchical clustering.

The percent concordance was calculated in the following manner:

\[\frac{\text{number of changes in common}}{\text{number in common} + \frac{1}{2} \times \text{number only in initial tumor} + \text{number only in recurrent tumor}}\]

The similarity score was calculated for each pair as a weighted sum of the alterations for each chromosome arm. The weights were based on the overall probabilities of gains and losses for each chromosome arm, with greater weight being given to agreement when a gain or loss was rare than when it was common. The weights were proportional to the log of the probability for the observed CGH alterations in observed pairs.

The similarity score can be defined mathematically as follows: Let \(X_{ij}\) be an indicator variable equal to 1 if there is a gain (or loss) at the \(j^{th}\) chromosome arm in the \(i^{th}\) member of the pair (\(i = 1, 2; j = 1, \ldots, n\)) and define \(p_j\) to be the overall probability of gain or loss at the \(j^{th}\) chromosome arm (\(p_j\) are estimated from the 18 tumors in this study). The similarity score is defined by

\[S = \sum_{i=1}^{n} (-1)^{i+1} \frac{1}{2}[\ln(p_j/(1-p_j)) + 2\ln(1-p_j)].\]

The individual terms of the similarity score will be negative when alterations are discordant—i.e., when there is an alteration on a chromosome arm of one pair member but not on the corresponding arm of the other member of the pair. The similarity score will be positive when the pair members are alike—i.e., each has alterations of the same type on the same chromosome arm, or neither pair member has an alteration. The probability weighting ensures that agreements at rare alteration sites get more weight than alterations at common alteration sites. We calculated a similarity score for every possible pairing of initial–recurrent tumors. We then compared the distribution of similarity scores for initial–recurrent pairs from the same patient with that for initial–recurrent pairs where

### Table 1. Patient Information*

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age at diagnosis, y</th>
<th>Time to tumor recurrence, y</th>
<th>Radiation treatment</th>
<th>Clear surgical margins</th>
<th>Histologic type, initial DCIS</th>
<th>Histologic type, recurrent tumor</th>
<th>Nuclear grade, initial DCIS</th>
<th>Nuclear grade, recurrent tumor</th>
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<tr>
<td>F1</td>
<td>39</td>
<td>2.8</td>
<td>No</td>
<td>No</td>
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<td>Cribriform</td>
<td>Int</td>
<td>Int</td>
</tr>
<tr>
<td>F3</td>
<td>60</td>
<td>7.3</td>
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<td>Ind†</td>
<td>Comedo</td>
<td>Comedo</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>F4</td>
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<td>4.1</td>
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<td>No</td>
<td>Cribriform/ micropapillary mix</td>
<td>Cribriform/ micropapillary mix</td>
<td>Low</td>
<td>Int</td>
</tr>
<tr>
<td>F10</td>
<td>57</td>
<td>4.8</td>
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<td>No</td>
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</tr>
<tr>
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<td>Int</td>
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<td>4.8</td>
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<td>No</td>
<td>Micropapillary mix</td>
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<td>Low</td>
<td>Int</td>
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<td>1.6</td>
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<td>Int</td>
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</tr>
</tbody>
</table>

*DCIS = ductal carcinoma in situ; Int = intermediate nuclear grade.
†Indeterminate, could not be assessed from material available.
Fig. 1. Histopathology of paired initial and recurrent ductal carcinomas in situ. Photomicrographs represent paired primary tumor and recurrence for cases F25 (A, B), F3 (C, D), F32 (E, F), F46 (G, H), and F6316 (I, J). Tables 1 and 2 show clinical and comparative genomic hybridization findings for these cases. Original magnification ×2.5.
the initial lesion and the recurrence were from different patients. This latter distribution was used to define nonclonality.

For **hierarchical clustering**, the program “Agnes” from the S-PLUS statistical package was used (21). Agnes is based on pairwise similarities of the CGH data; similarities are not weighted by their likelihoods. Results from clustering are best displayed graphically as trees; interpretation tends to be subjective rather than to be based on objective measures. Nonparametric confidence intervals (CIs) for the median concordance and median similarity scores were obtained from the order statistics (22).

**RESULTS**

**Patients**

The mean age of the 18 patients at the time of diagnosis for the initial DCIS was 52.6 years (Table 1). Breast-conserving surgery was the only treatment for 15 of the cases, with two cases having both surgery and radiation therapy. (Radiation treatment was unknown for one case.) The mean time to recurrence for all 18 patients was 4.2 years (range, 16 months to 9.3 years).

**Histopathology**

Microscopic review of the initial and recurrent in situ tumor pairs revealed a striking similarity in histopathologic features (Fig. 1). Thirteen (72%) of the recurrent tumors had the same histologic type as their corresponding initial tumor (Table 1). Eight of the initial lesions were high grade, and 10 were low to intermediate grade. When low and intermediate grades were combined, 15 (83%) of the recurrent tumors had the same nuclear grade as their paired initial lesions, and the remaining three changed from intermediate to high grade.

Nine of the initial tumors showed clear surgical margins, as defined by no tumor involvement within 1 mm of the surgical margin. Six cases showed positive margins, even after re-excision. In three cases, the status of the surgical margins was uninterpretable as a result of artifact or inability to see the inked margins. In the nine cases with margins of at least 1 mm, the mean time to recurrence was 4.0 years (range, 16 months to 9.2 years). The mean time to recurrence for the six cases with positive margins was 4.3 years (range, 17 months to 9.3 years).

**Chromosomal Alterations by CGH**

All of the DCIS lesions showed at least one genetic aberration by CGH (Table 2). The total number of aberrations was higher in the recurrences (mean number = 10.7; 95% CI = 7.8–13.7) than in the initial lesions (mean number = 8.8; 95% CI = 7.8–10.7). The mean number of changes in common was 4.2 (95% CI = 2.6–6.8) by CGH (Table 2). The total number of aberrations was higher in the recurrences (mean number = 10.7; 95% CI = 7.8–13.7) than in the initial lesions (mean number = 8.8; 95% CI = 7.8–10.7). The mean number of changes in common was 4.2 (95% CI = 2.6–6.8) by CGH (Table 2).
6.0–11.7) \( (P = .019, \text{paired two-sided } t\text{ test}) \). The most common changes in the initial DCIS were gains involving 17q (61%) and losses involving 8p (61%) and 17p (50%). There were no statistically significant differences in the prevalence of individual CGH alterations between the initial and recurrent lesions (Table 3). However, there was an increased prevalence of a small number of alterations (gains involving 3q and 17q and losses involving 8p and 14q) in these DCIS lesions compared with our previously reported set of invasive ductal cancers (15,17).

### Comparison Between Initial DCIS and Recurrent DCIS

**Concordance.** The median percent concordance for the tumor pairs was 81% (range, 0%–100%; 95% CI = 77%–90%). One pair (F22) had a concordance of 0% (having two and 20 alterations in the initial and recurrent tumors), whereas the other 17 cases had a median concordance of 82% (range, 65%–100%). The concordance was similar whether the initial tumor showed clear surgical margins or margins that were involved by tumor (73% versus 85%).

**Similarity score.** When the initial lesion and the recurrence from the same subject were paired, the median similarity score was 24.3 (95% CI = 21.4–28.1) (Fig. 2). If the initial lesions were paired with recurrences from different subjects (306 possible pairs), the median similarity score was −11.9 (95% CI = −14.2 to −10.3). The difference in the distribution of similarity scores was statistically significant \( (P < .0001) \). Fig. 2 shows similarity scores plotted separately for each subject. In only two cases did initial lesions have a better match with recurrent tumors from other patients—one subject (F4) had a better match with a different recurrent tumor from another patient (F1) than with its initial lesion, whereas the other subject (F22) had better matches for six other recurrences than with its own recurrence. In the remaining cases, the initial tumor and the recurrent tumor from the same subject were more alike than matches with any other recurrences.

**Hierarchical clustering.** Clustering with the Agnes algorithm produced results similar to those of the other two methods (Fig. 3). Only one case (F22) failed to form a pair. This algorithm performed better than the similarity score, in that it was able to pair the initial DCIS for case F4 with its recurrence, since its closer match by similarity score (recurrence F1) had already been paired with its initial tumor.

![Fig. 2. Similarity scores for initial–recurrent pairs of ductal carcinoma in situ.](https://academic.oup.com/jnci/article-abstract/92/4/313/2624709)

![Table 3. Chromosomal alterations in initial and recurrent ductal carcinoma in situ (DCIS) lesions from the 18 study subjects compared with alterations observed in 94 invasive ductal cancers (IDCs)](https://academic.oup.com/jnci/article-abstract/92/4/313/2624709)
Association of Chromosomal Alterations With DCIS Grade

Losses involving 16q occurred more frequently in low/intermediate-grade DCIS lesions than in high-grade lesions, both for the initial (P = .094) and the recurrent (P = .024) lesions, although the difference was statistically significant only for the recurrent lesions. Conversely, losses involving 8p were statistically significantly associated with high grade in the recurrences (91% high grade versus 43% low/intermediate grade; P = .026) but not in the initial lesions (75% high grade versus 50% low/intermediate grade; P = .28).

DISCUSSION

These results describe a clonal genetic relationship between initial DCIS lesions and their subsequent local recurrences. Of the 18 cases, 17 showed a high degree of concordance in the genetic changes found in both lesions. Statistical clustering paired up 17 of the 18 pairs, and 16 of the 18 cases were classified as pairs on the basis of their similarity scores. In addition, we demonstrated a striking similarity in histologic architecture of the paired lesions.

Whether an initial DCIS lesion is related to its local recurrence may be difficult to determine. CGH is a powerful molecular tool that yields a genetic profile for each tumor, thus allowing a comparison of each lesion to its recurrence. Our analyses confirmed that DCIS recurrences are predominantly clonally related to their initial DCIS lesions, suggesting that the subsequent lesions are due to persistence of neoplastic cells rather than to newly arising lesions. These analyses used alterations involving chromosome arms as the unit for statistical analysis of genetic similarities. It is possible that differences between paired samples existed at the resolution of individual genes, since the resolution of CGH is limited in metaphase chromosomes and cannot routinely define alterations less than 10 megabase pairs in size. Our previous studies (17,23) support the use of CGH to define clonal relationships in other paired sets, including primary tumors and metastases from breast and bladder cancers. In the future, array-based CGH analyses will allow copy number alterations at gene resolution to be determined (24).

In this study, time to tumor recurrence was unrelated to both the number of genetic aberrations present in the initial lesion and the degree of concordance between the tumor pairs. In one case (F25), a single loss of chromosome 12p was seen in both the initial DCIS and the recurrent tumor after 5.9 years. In another case (F10), 20 genetic aberrations were seen both in the initial lesion and in the recurrence that was detected after 4.8 years. The one case (F22) without concordance by all three statistical methods showed two genetic changes in the initial DCIS lesion and 20 different changes in the recurrence. These data are most consistent with the second lesion being a new neoplasia rather than being a recurrence of the original lesion. The time to recurrence for this case was 9 years, one of the longer time intervals in our study.

Few studies have reported comparisons of genetic alterations in initial and recurrent breast lesions. Lininger et al. (25) showed a high concordance of loss of heterozygosity (LOH) in three ipsilateral DCIS primary/recurrence pairs. Similarly, a number of reports (26–32) have shown genetic similarities between DCIS lesions and their concurrently associated invasive tumors.

DCIS, especially of high grade, is a genetically advanced lesion despite the absence of invasion through the basement membrane. A mean of 8.8 chromosomal changes was seen in the 18 primary DCIS cases studied, similar to the 8.7 changes per tumor found in the combined set of 94 invasive breast carcinomas previously analyzed in our laboratory (15,17). These results confirm the presence of multiple genetic alterations in DCIS, previously shown by LOH (30,33,34), CGH (35,36), and other approaches (37–39).

The specific genetic changes seen in our set of DCIS lesions are similar to those seen by others and, for the most part, are present in invasive cancers as well (Table 3) (26,30–32). Our finding of a small, yet statistically significant, overall increase in the number of genetic alterations in the recurrences (8.8–10.7) suggests that genetic progression occurred, although no specific alterations appeared more likely than others to be increased. This observation is consistent with reports of clonal evolution detected by LOH in synchronous pairs of DCIS and invasive cancer (27,30,31).

In addition to genetic similarities, a striking similarity in histologic appearance was seen between the initial lesions and the
recurrences (Fig. 1). This overall histologic similarity was asso-
ciated with an agreement in grade, with only three cases showing a
change from intermediate to high grade, as well as an agree-
ment in architectural pattern, with 13 cases showing the same
overall histologic similarity. In our study, nine cases with margins of 1 mm or
more lead to a very small recurrence rate that is unaffected by radia-
tion treatment. In our study, nine cases with margins of 1 mm or
more still recurred; eight of these cases were clonally related to
the initial lesion. This result supports the conclusion that residual
DCIS may be left behind when surgical margins are less than 10
mm, as previously suggested.

We conclude that most DCIS recurrences result from growth of persistent neoplastic cells, which may remain indolent for
long periods. These data explain the importance of wide surgical
margins and/or radiation therapy during treatment of these
noninvasive neoplasias. Further insights into the genetic de-
termination of preinvasive histology and biology will allow
treatment tailored to the likelihood of clinically aggressive tu-
mors.

REFERENCES

(1) Kerlikowske K, Barclay J, Grady D, Sickles EA, Ernstner V. Comparison of
risk factors for ductal carcinoma in situ and invasive breast cancer. J Natl
Cancer Inst 1997;89:76–82.

(2) Lagois MD. Heterogeneity of duct carcinoma in situ (DCIS). Relationship
of grade and subtype analysis to local recurrence and risk of invasive

(3) Boyages J, Delaney G, Taylor R. Predictors of local recurrence after treat-

(4) Silverstein MJ, Barth A, Poller DN, Gierson ED, Colburn WJ, Waisman
JR, et al. Ten-year results comparing mastectomy to excision and radiation
treatment for ductal carcinoma in situ of the breast. Eur J Cancer 1995;31A:
1425–7.

(5) Silverstein MJ, Lagois MD, Groshen S, Waisman JR, Lewinsky BS, Mar-
tino S, et al. The influence of margin width on local control of ductal

Outcome and prognostic factors for local recurrence in mammographically
detected ductal carcinoma in situ of the breast treated with conservative

(7) Ernstner VL, Barclay J, Kerlikowske K, Grady D, Henderson C. Incidence
of and treatment for ductal carcinoma in situ of the breast. JAMA 1996;

(8) Fowble B, Hanlon AL, Fein DA, Hoffman JP, Sigurdson ER, Pateshky A,
et al. Results of conservative surgery and radiation for mammographically
detected ductal carcinoma in situ. Int J Radiat Oncol Biol Phys 1997;38:
949–57.

Influence of local treatment on the recurrence rate of ductal carcinoma

et al. Lumpectomy compared with lumpectomy and radiation therapy
1581–6.

Risk of recurrence after ductal carcinoma in situ of the breast. Cancer

Predictors of local recurrence following excision alone for ductal carci-

(13) Holland PA, Gandhi A, Know WF, Wilson M, Baildard AD, Bundred NJ.
The importance of complete excision in the prevention of local recurrence

(14) Ratanawichitrin A, Rybicki LA, Steiger E, Grundfest-Broniatowski S,
Hermann RE, Crowe JF. Predicting the likelihood of residual disease in

(15) Isola JJ, Kallioniemi OP, Chu LW, Fuqua SA, Hillsenbeck SG, Osborne
CK, et al. Genetic aberrations detected by comparative genomic hybrid-
ization predict outcome in node-negative breast cancer. Am J Pathol

F, et al. Comparative genomic hybridization for molecular cytogenetic

Genetic alterations in primary breast cancers and their metastases: direct
comparison using modified comparative genomic hybridization. Genes

(18) Willenbuchar RF, Zelman SJ, Ferrell LD, Moore DH 2nd, Waldman FM.
Chromosomal alterations in ulcerative colitis-related neoplastic progres-

(19) DeVries S, Gray JW, Pinkel D, Waldman FM. Comparative genomic hy-
bridization. In: Dracopoli NC, Haines JL, Korf BR, Moir DT, Morton CC,

(20) Piper J, Rutovitz D, Sudar D, Kallioniemi A, Kallioniemi OP, Waldman

(21) S-PLUS for Unix guide to statistics. Seattle (WA): Cluster analysis, chapt

(22) Hahn GJ, Meeker WQ. Table A15g. In: Statistical intervals. New York

alterations in primary bladder cancers and their metastases. Cancer Res

(24) Pollack JR, Perous CM, Alizadeh AA, Eisen MB, Pergamenschikov A,
Williams CF, et al. Genome wide analysis of DNA copy number changes

(25) Lininger RA, Fuji H, Man YG, Gabrielson E, Tavassoli FA. Comparison
of loss of heterozygosity in primary and recurrent ductal carcinoma

(26) Aldaz CM, Chen T, Sahin A, Cunningham J, Bondy M. Comparative
allelotype of in situ and invasive human breast cancer: high frequency of
microsatellite instability in lobular breast cancers. Cancer Res 1995;

(27) Fuji H, Marsh C, Cairns P, Sidranski D, Gabrielson E. Genetic divergence

(28) Marsh KL, Varley JM. Loss of heterozygosity at chromosome 9p in ductal
77:1439–47.

(29) O’Connell P, Pekkel V, Fuqua S, Osborne CK, Allred DC. Molecular
 genetic studies of early breast cancer evolution. Breast Cancer Res Treat

(30) O’Connell P, Pekkel V, Fuqua SA, Osborne CK, Clark GM, Allred DC.
Analysis of loss of heterozygosity in 399 premalignant breast lesions at 15

Allelic loss and the progression of breast cancer. Cancer Res 1995;55:
5180–3.

allelic loss on chromosome 11q13 in microdissected in situ and invasive

(33) Fuji H, Szunel R, Marsh C, Zhou W, Gabrielson E. Genetic progression,
histological grade, and allelic loss in ductal carcinoma in situ of the breast.


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