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]**

The increasing use of mammography as a screening tool during the last 15 years has contributed to a dramatic increase in the diagnosis of ductal carcinoma *in situ* (DCIS). DCIS is considered to be a precursor to invasive breast carcinoma, although the likelihood of progression to invasive disease is uncertain *(1,2)*. Also, since DCIS is usually treated surgically, the natural history of the lesion has not been well studied. Before the 1980s, treatment of DCIS was primarily by mastectomy. More recently, breast-conserving surgery, often accompanied by radiotherapy, has been recommended *(3–6)*.

Breast-conserving surgery raises the possibility that DCIS may recur in the remaining breast tissue. The incidence of local recurrences following breast-conserving surgery for DCIS ranges from 5% to 25%, depending on the follow-up period and postoperative treatment *(3,7–12)*. The mechanism of recurrence is uncertain. Several investigators *(5,13,14)* have pointed out the importance of wide surgical excisions in preventing recurrent disease after lumpectomy. The recurrence of DCIS following mastectomy is only 1%–2% *(3,4,9)*, which suggests that many recurrent lesions are due to residual tumor cells rather than to growth of a different neoplasm.

If recurrent DCIS lesions reflect residual disease, we would expect to see a clonal relationship between the initial DCIS and the recurrent *in situ* tumor. One approach to assessing clonality is comparative genomic hybridization (CGH). CGH screens for losses and gains of DNA sequences along the entire genome by comparing the competitive hybridization of tumor and normal DNAs that are differentially labeled to normal metaphase chromosomes *(15–17)*. This approach allows the entire genome to be characterized in one analysis because tumor DNA is analyzed directly rather than with individual probes. To establish the extent to which DCIS recurrences arise from residual tumor cells as opposed to being new lesions, we have used CGH to compare genetic changes in 18 primary DCIS lesions and their ipsilateral recurrences.

Materials and Methods

Tumor Samples

Eighteen female patients from California Pacific Medical Center were identified who presented initially with DCIS and returned more than a year after the initial diagnosis with DCIS in the same breast. This study was approved by the Institutional Review Board of the University of California at San Francisco. All surgical slides were reviewed to confirm the DCIS diagnosis and to exclude the presence of microinvasion or more extensive invasive tumor. Initial DCIS cases were reviewed independently of the corresponding recurrence. Nuclear grade of the DCIS was recorded as low, intermediate, or high, and the histologic pattern was classified as comedo, solid, cribriform, or micropapillary type. Comedo-type DCIS was defined as solid-type DCIS with high nuclear grade and moderate or extensive necrosis. Tumors exhibiting a mixture of histologic types were classified by the predominant population. Tumor involvement of the surgical margin was also noted. Clear margins were defined if there was no tumor involvement within 1 mm of the surgical margin.

Tissue Dissection and DNA Extraction

Sections (5 μm) containing the DCIS were placed on slides for microdissection as previously described *(18)*. With the use of an adjacent hematoxylin–

Affiliations of authors: F. M. Waldman, S. DeVries, K. L. Chew, D. H. Moore (Cancer Center), K. Kerlikowske (Department of Epidemiology and Biostatistics and General Internal Medicine Section, Department of Veterans Affairs), B.-M. Ljung (Department of Pathology), University of California, San Francisco.

Correspondence to: Frederic Waldman, M.D., Ph.D., Cancer Genetics Program, UCSF Cancer Center, Rm. S436, University of California, San Francisco, San Francisco, CA 94143 (e-mail: waldman@cc.ucsf.edu).

See "Notes" following "References."

eosin-stained slide for orientation, one or two 5-μm deparaffinized, methyl green (0.1%)-stained sections were microdissected. Previously selected areas of DCIS were separated from surrounding lymphocytes and stroma. DNA was isolated with the use of a 3-day Proteinase K digestion *(18)*.

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:

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

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

$$S = \sum_j (-1)^{1+x_{1j}+x_{2j}}\{(x_{1j} + x_{2j})(\ln[p_j/(1-p_j)] + 2\ln(1-p_j)\}.$$

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 arms, 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*

Subject No.	Age at diagnosis, y	Time to tumor recurrence, y	Radiation treatment	Clear surgical margins	Histologic type, initial DCIS	Histologic type, recurrent tumor	Nuclear grade, initial DCIS	Nuclear grade, recurrent tumor
F1	39	2.8	No	No	Cribriform	Cribriform	Int	Int
F3	60	7.3	No	Ind†	Comedo	Comedo	High	High
F4	40	4.1	No	No	Cribriform/ micropapillary mix	Cribriform/ micropapillary mix	Low	Int
F10	57	4.8	Yes	No	Comedo	Micropapillary	High	High
F11	69	5.4	No	Yes	Comedo	Comedo	High	High
F12	48	1.3	No	Yes	Comedo	Solid	High	High
F14	45	4.3	No	Yes	Cribriform	Cribriform	Int	Low
F22	66	9.2	No	Yes	Solid	Solid	High	High
F23	51	1.8	Yes	Yes	Comedo	Comedo	High	High
F25	71	5.9	No	Ind†	Comedo	Comedo	High	High
F29	39	3.2	No	No	Solid	Solid	Int	High
F32	44	4.8	No	Yes	Cribriform	Solid	Int	Int
F33	69	9.3	No	No	Micropapillary	Solid	Int	High
F41	58	1.6	No	Yes	Cribriform	Solid	Int	High
F46	45	4.3	No	Ind†	Cribriform	Cribriform	Low	Int
F199	40	1.4	Unknown	No	Comedo	Comedo	High	High
F1099	44	1.4	No	Yes	Solid	Solid	Int	Int
F6316	61	2.3	No	Yes	Cribriform	Cribriform	Low	Low

*DCIS = ductal carcinoma *in situ;* Int = intermediate nuclear grade.
†Indeterminate, could not be assessed from material available.

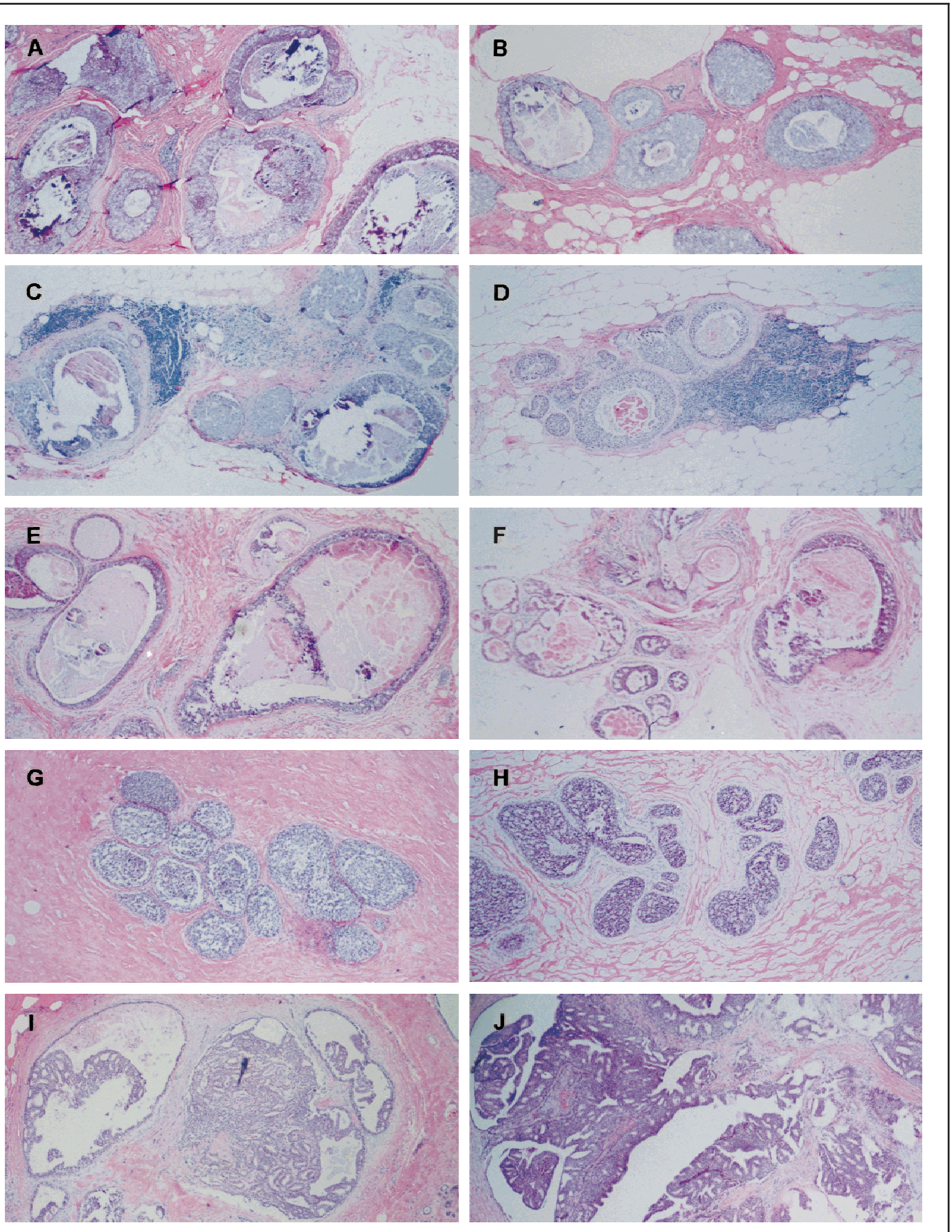

Fig. 1. Histopathology of paired initial and recurrent ductal carcinomas *in situ*. Photomicrographs represent paired primary tumor and recurrence for cases F25 (**A, B**), F3 (**C, D**), F32 (**E, F**), F46 (**G, H**), and F6316 (**I, J**). Tables 1 and 2 show clinical and comparative genomic hybridization 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 =

Table 2. Comparative genomic hybridization (CGH) aberrations in initial and recurrent ductal carcinoma *in situ* (DCIS) lesions*

Subject No.	% concordance†	CGH changes common to initial and recurrent tumors	CHG changes only in initial DCIS	CGH changes only in recurrent tumor
F1	100	1q+; 16q−	None	None
F3	80	1p12-p22−; 1q+; 3p11-p21−; 8p−; 17q11.2-q21+; Xp22−	None	17p−; 17q22-qter−; 22q−
F4	67	16q21-qter−; 17p−	None	17q23-qter+; 22q−
F10	100	1q+; 3q27-qter−; 4p−; 4q11-q27−; 5q−; 6q11-q16−; 6q26-qter−; 7p+; 7q11.2+; 7q21-q31−; 7q32-qter+; 8p−; 8q+; 9−; 11p−; 12p−; 12q21-q23−; 13q−; 15q24-qter−; 16q−; 18q−; X−	None	None
F11	92	1q+; 3q13.3-q25−; 3q26-qter+; 4−; 6q21-q25+; 8p−; 10q22-qter−; 12q12-q22+; 12q23-qter−; 13q−; 14q12-q22+; 14q23-qter−; 15q22-qter−; 17p−; 17q11-q21+; 18−; 20q13+; Xq22-qter−	8q+; 17q22–qter−	11p15−
F12	86	3q21-qter+; 6p22-pter+; 8p−; 17p−; 17q23-q24+; 20q13.2-qter+	None	18q−; 21q−
F14	86	12p−; 17p−; 17q22-qter+	None	20q13+
F22	0	None	8+; 20+	3q23-qter+; 6p11-p12+; 6q+; 8p−; 8q21-q23+; 9q22-qter−; 13q−; 16q−; 17p−; 18q−; 20p11-p12+; 21q−
F23	82	1p−; 1q+; 4q−; 8p21-pter–; 8p12-p13+; 11q14-qter−; 17q11.2-q23+	9−; 16p+; 18q−	None
F25	100	12p−	None	None
F29	79	1q+; 4p15-pter−; 4q12-q25−; 4q26-qter+; 5q−; 8p21-pter−; 8q+; 9p−; 11q−; 17q11.2-q21+; 17q23-qter+; 20q+	5p+; 8p11-p12−; 13q+; 17p−	18p−; 21q+
F32	90	1p34-pter−; 3p13-p21−; 3q26-qter+; 8p21-pter−; 8p11-p12+; 8q+; 9p−; 10p−; 11q14-qter−; 16q21-qter−; 17p−; 17q11-q21+; 20p−	None	3q12-q21−; 16p+; 20q12-q13.2+
F33	75	6p+; 8p21-pter−; 8p11-p12+; 8q+; 17p−; 17q+	6q−; 10−	14q24-qter−; X+
F41	65	6q21-q25+; 6q26-qter−; 8p11-p12+; 8p21-pter−; 10q22-qter−; 11p11-p14+; 14q22-qter−; 17p12-pter−; 17q+; 18−; 20p+	1q+; 15q25-qter+	1p12-p22−; 3p12-p21−; 3q26-q28+; 4p15-pter+; 5q−; 9p−; 13q−; 14q11-q21−; 15q−; 17p11−
F46	77	6p+; 7p+; 7q11+; 8p11-p12+; 8q+; 11q−; 12q+; 14q23-qter−; 16p11-p12+; 17q+	None	1q+; 8p21-pter−; 13q12-q14−; 15q+; 18p−; 18q22-qter−
F199	90	3p13-p21−; 3q24-qter+; 5p14-pter+; 5q31-qter−; 6p22-pter−; 6p11-p21+; 6q+; 8p−; 8q11-q22+; 10q22-qter−; 11q22-qter−; 14q23-qter−; 17q23-q24+; Xp21-p22.1+	17q11-q21+	5p12-p13+; 17p−
F1099	73	1q+; 3p24-pter−; 3p23+; 3p12-p22−; 3q+; 6q−; 8p−; 11q23-qter−; 14q23-qter−; 16q22-qter−; 17p−; 17q11-q22−	12q24−; 21q22−	4−; 9−; 11p11-p14+; 11q12-q21+; 13q−; 21q21+
F6316	80	7q−; 16q−	None	7p+
Median = 81% (95% confidence interval = 77%–90%)				

*+ = gain of chromosome arm; − = loss of chromosome arm; ter = chromosome terminal.

†% concordance = $\frac{\text{number of changes in common}}{(\text{number in common}) + [1/2 \times (\text{number only in initial tumor} + \text{number only in recurrent tumor})]}$.

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)

	Study subjects (n = 18)				
Alteration*	% initial DCIS with alteration	*P*†	% recurrent DCIS with alteration	*P*‡	Primary IDCs (n = 94), % with alteration§
1q+	44	.309	44	.309	57
3p−	22	.991	28	.617	22
3q+	28	.230	39	.025	16
6p+	22	.438	28	.182	15
8p−	61	.024	72	.002	33
8p−/p+‖	22	.031	28	.005	6
8q+	44	.790	33	.256	48
9p−	22	.438	28	.182	15
11q−	33	.625	33	.625	28
13q−	11	.101	33	.764	30
14q−	28	.140	33	.043	14
16q−	33	.954	39	.693	34
17p−	50	.354	61	.072	38
17q+	61	.008	67	.002	29
18q−	22	.991	33	.318	21
20q+	22	.913	28	.691	23

*+ = gain of chromosome arm; − = loss of chromosome arm. Chromosome arms included if aberration was present in initial or recurrences at greater than 20%.

†Two-sided *P* based on chi-squared test comparing frequency of alteration in initial DCIS tumors versus primary IDCs.

‡Two-sided *P* based on chi-squared comparing frequency of alteration in recurrent DCIS tumors versus primary IDCs.

§Values combined from Isola et al. *(15)* and Nishizaki et al. *(17)*.

‖Distal 18p showed relative loss while proximal 8p showed gain.

6.0–11.7) ($P = .019$, paired two-sided *t* 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.

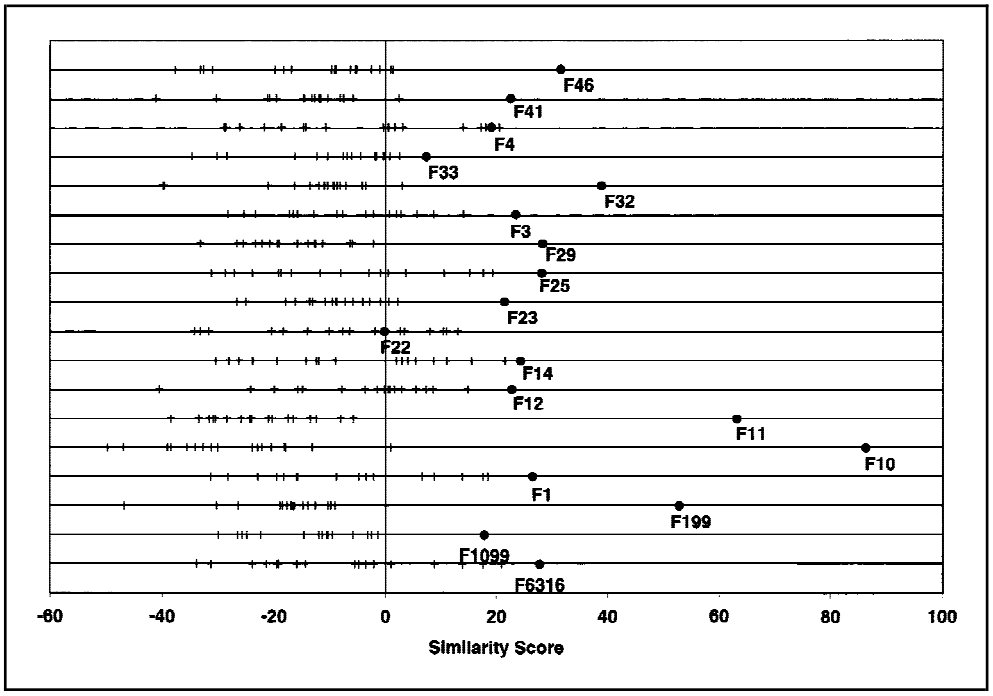

Fig. 2. Similarity scores for initial–recurrent pairs of ductal carcinoma *in situ*. Each line shows the distribution of similarity scores for a specific initial lesion (the label identifies which lesion) paired with each of the 18 recurrences in the dataset. **Solid circle** indicates that the recurrent tumor is from the same subject. **Vertical hatch marks** represent recurrences from different subjects.

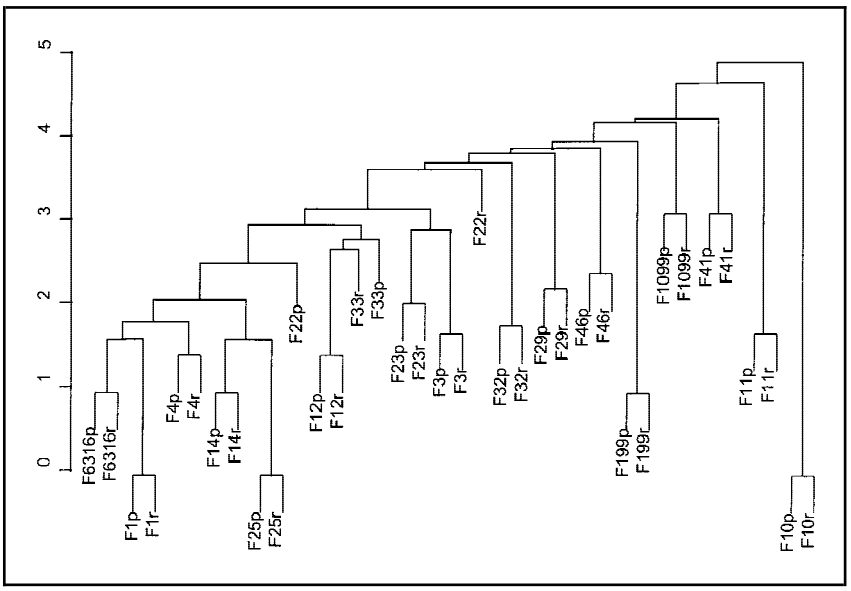

Fig. 3. Dendrogram showing results of clustering with Agnes from the S-PLUS statistical program. The **y-axis** shows the level of dissimilarity. **Horizontal lines** in the dendrogram indicate at what level of dissimilarity clusters are formed. The first pairs to cluster are initial–recurrent tumors for cases F1, F25, and F10; for each of these pairs, the dissimilarity is 0, indicating a perfect match between alterations on the initial lesion (p) and its recurrence (r).

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 associated with an agreement in grade, with only three cases showing a change from intermediate to high grade, as well as an agreement in architectural pattern, with 13 cases showing the same classification. A similarity in histologic type between DCIS lesions and associated invasive cancers has been described previously *(40).* These observations reinforce the conclusion that genetic alterations are the determinant of morphologic appearance for breast neoplasia.

The clonal relationship of initial DCIS lesions and recurrences suggests that DCIS recurrences arise from residual tumor cells that are not removed at the time of surgery. The importance of wide excision margins in the treatment of DCIS has been described in multiple studies *(5,9,13,14).* Silverstein et al. *(5)* recently reported that clear surgical margins of 10 mm or more lead to a very small recurrence rate that is unaffected by radiation 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 *(5,13).*

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 determination of preinvasive histology and biology will allow treatment tailored to the likelihood of clinically aggressive tumors.

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NOTES

Supported by Public Health Service grants CA44768 and CA58207 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; and by California Breast Cancer Research Program 2RB-0197.

Manuscript received August 12, 1999; revised November 18, 1999; accepted December 6, 1999.