Flow Cytometric DNA Analysis of Breast Cancers with Predominance of Carcinoma in Situ: A Comparison of the Premalignant and Malignant Components

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ABSTRACT
Flow cytometric DNA analysis was performed on unfixed frozen tissue samples from 48 cases of invasive breast cancer (IC) with a predominance of ductal carcinoma in situ (DCIS). In 15 cases the samples contained only the DCIS component, in 17 cases only the IC component, whereas in 16 cases separate samples from the DCIS as well as the IC part within the individual lesion were available. In the latter 16 cases, complete or partial accordance in DNA ploidy between DCIS and IC was found in 12 cases, whereas no correspondence could be demonstrated in the remaining 4 cases, possibly due to intratumoral DNA heterogeneity. Comparison of the DNA index distribution in samples of DCIS and IC from the 48 cases showed concordant results except for the DNA hyperdiploid subclass, in which 6 clones were found in the DCIS portion compared to 18 clones in the IC portion. S-phase fractions were also comparable in the two groups. A comparison of the DCIS component from the present series of breast cancers to our previous study of pure DCIS showed similar results with respect to the DNA index distribution, DNA heterogeneity, and S-phase fraction. No differences could be demonstrated between DCIS with and without invasion. The results indicate that the DNA ploidy pattern of breast cancer, as detected by flow cytometric DNA analysis, is established at the preinvasive stage of carcinogenesis.

INTRODUCTION
Carcinogenesis is in general explained as a complicated, multistep set of genetic DNA changes in combination with epigenetic events causing the development from the normal cell to a neoplastic, malignant cell with the ability to invade the surrounding tissues and eventually to metastasize. The genetic events may cause changes in differentiation, function, and expression, and may be characterized by classical histopathology, immunohistochemistry, FCM, and chromosomal analysis as well as modern molecular biological methods.

In general, these methods are applied to malignant tumors, i.e., correlating to the later stages of the carcinogenic process. For a more profound understanding of the developmental stages, it may be relevant to study lesions at earlier stages of carcinogenesis. In this context, several types of epithelial cancers are suitable since they may go through a spectrum of premalignant stages before becoming invasive.

In breast cancer, an increasing number of cases, particularly related to mammography screening, are now diagnosed at the premalignant stage of DCIS. A more basic knowledge of the biological behavior might contribute to the selection for treatment of the most aggressive lesions. On the basis of histopathological classification, we found in a prospective, nationwide study of DCIS (1) that clinical DCIS cases did show the highest frequency of recurrence. A strong correlation to nuclear atypia was found. Recently we found by FCM DNA analysis of unfixed frozen tissue samples from 41 clinical cases of DCIS (2) that 85% of the cases disclosed abnormal nondiploid clones. These values correspond closely to those of node-negative breast cancers (3). The distribution of the DI was also similar. We therefore concluded that major genetic alterations have been established already at the premalignant stage of carcinogenesis.

The similarity in DNA ploidy pattern between DCIS and IC, however, represents overall results for the two groups and may not necessarily reflect the events in the individual case. To focus on this subject, we chose a material of breast cancers with a predominating component of carcinoma in situ, thus making it possible to compare the in situ and the invasive component within the individual case.

The goals of the present study were (a) to compare the DNA ploidy pattern of the carcinoma in situ and the invasive component of the individual case; (b) to investigate whether there are differences in the DNA ploidy pattern of DCIS lesions with and without invasion by comparing the results from our previous study of pure DCIS (2) to the present results for the DCIS component of the breast cancers; and (c) to correlate the DNA ploidy results to histopathology.

MATERIALS AND METHODS
The material consists of ductal breast carcinomas, in which the DCIS component constitutes >75% [B2b, according to the

1 The abbreviations used are: FCM, flow cytometry; DCIS, ductal carcinoma in situ; DI, DNA index; IC, invasive breast cancer; SPF, S-phase fraction; CV, coefficient of variation; TRBC, Trout Red Blood Cell.
1981 WHO classification (4)]. Only cases from which unfixed frozen tissue was available were included. A total of 48 cases were found from August 1985 to April 1994, all from one Department of Pathology (Odense University Hospital). The diagnosis was based on extensive histopathological examination of the formalin-fixed, paraffin-embedded tissue, the median number of paraffin blocks being 22 (range, 3–71).

All cases were histopathologically reviewed. The size, i.e., the largest diameter, was recorded for the entire lesion as well as the IC component. The IC component was classified by the grade of anaplasia and multifocality. Lymph node status was registered. The DCIS component was classified according to the dominating histological subtype (solid, cribriform, clinging, or papilliferous), nuclear size (small, intermediate, or large), and the presence of comedonecrosis, as previously described (1). Examples are shown in Fig. 1.

Only unfixed frozen tissue samples were used for FCM. From each sample, a frozen section was cut to make a diagnosis of IC or DCIS in the individual sample. Among the 48 cases, the unfixed frozen tissue contained only the DCIS component in 15 cases, only the IC component in 17 cases, whereas in the remaining 16 cases it was possible to obtain the DCIS as well as the IC component in separate samples. Thus, from the 48 cases, a total of 64 samples (31 DCIS and 33 IC samples) were obtained.

In 16 samples, the tissue was too thin (about 1 mm) to enable aspiration, and in these cases the tissue was minced with scalpels. In the remaining 48 samples, the tissue was aspirated for FCM. After fine-needle aspiration, the tissue remnants were formalin-fixed and processed for conventional histological examination to confirm the presence of the same type of lesion throughout the tissue sample.

FCM. The frozen tissue blocks were prepared for FCM, as previously described (2), according to the method of Vindeløv et al. (5). For the FCM measurements a Becton Dickinson FACSort was used.

The median cell concentration of the cell suspensions was 1.2 × 10⁶/ml (range, 0.1–34.0), being lower among the small, minced samples (median, 0.5 × 10⁶/ml), compared to the aspirated samples (median, 1.7 × 10⁶/ml).

Samples were analyzed on the flow cytometer randomly; however, corresponding samples of DCIS and IC were always analyzed consecutively. All samples with poor model fit to the histogram or other potential problems, in particular near diploid clones, were reanalyzed.

Interpretation of DNA Histograms and Statistics. The DNA fluorescence histograms were analyzed as previously described (2) using a model described in a previous study (6).

A clone is defined to be DNA diploid if the estimated DI is within the 95% confidence limit as calculated from the lymphocyte standards, ± 3.3%. This definition leads to the following arbitrary 7 subclasses: the DNA diploid interval was set as 0.967 < DI ≤ 1.033, and the DNA tetraploid interval consequently was 1.934 < DI ≤ 2.066. A DNA triploid class was defined as 1.451 < DI ≤ 1.550. Hypodiploid, hyperdiploid, hypotetraploid, and hypertriploid DI classes were then defined as being less than, between, or greater than these classes.

DNA indices in two samples are considered to be the same if the difference between them is within the 95% confidence interval using the variance of the DI estimated from the benign samples. Clusters of DIs from each case were found using this variance.

DNA ploidy heterogeneity is defined as the occurrence of two or more nondiploid clones within a sample. Because the samples, in particular the DCIS lesions, contain a varying, but often abundant number of benign cells (epithelial, stromal, endothelial, inflammatory), a diploid G1 peak will nearly always be present. Only in a purely diploid histogram was the DCIS/IC lesion classified as DNA diploid, whereas in a histogram with a nondiploid peak in addition to the diploid peak, it is not possible by single parameter analysis to determine the presence of a diploid DCIS/IC clone among the benign cells.

Evaluable SPFs were chosen from samples which (a) are purely DNA diploid, (b) are DNA aneuploid with DI > 1.4 comprising at least 25% of the total sample and with no other subpopulations confounding the S-phase distribution, or (c) are DNA aneuploid with only small (<15%) subpopulations interfering with the S-phase distribution.

Visual inspection of forward light scatter versus propidium iodide fluorescence was used to control for artifacts, in particular for cases with near-diploid peaks. In some cases the time sequence of the measurements was inspected to ensure that no shifts had occurred in the propidium iodide fluorescence as a function of time.

Tests for association were done using Fisher’s exact test where applicable or the χ² test.

RESULTS

FCM. The median CV of the TRBC reference peak was 1.2% (0.9–2.0), and of the DNA diploid G1 peak, 1.8% (1.2–4.0). No difference in CV was found for minced samples compared to the aspirated samples.

Of the 31 samples from the DCIS portion of the cancers, 7 (23%) were classified as exclusively DNA diploid, 3 (10%) had a single abnormal clone within the tetraploid area, and 21 (67%) disclosed at least one aneuploid peak. DNA heterogeneity was found in 8 (26%) samples. Of the 33 samples from the IC portion of the cancers, 4 (12%) were diploid, 2 (6%) were

Fig. 1. a, case 42, portion of breast carcinoma with predominance of DCIS. The DCIS component is seen at the top and to the left (thin arrows), while the invasive component is shown at the bottom and to the right (broad arrows). H & E, × 32. b, cases 42 (upper panel) and 32 (lower panel), same cases as c and d. Giemsa, × 320. Touch preparations from unfixed frozen samples. The DIs of the two cases were 1.02 and 2.66, respectively. c, case 42, the DCIS component (thin arrow) is of dominating cribriform type, with small and intermediate nuclear size and without comedonecrosis. The IC component (broad arrow) is histological grade 1 with some tubuli, rather small and uniform nuclei, and few mitoses. H & E, × 200. d, case 32, the DCIS component (thin arrow) is of solid type, with large nuclei and with comedonecrosis (C). The IC component (broad arrow) is histological grade III without tubular structures and with large, polymorphic and hyperchromatic nuclei. H & E, × 200.
DNA Analysis of Breast Cancer

Table 1  Distribution of DNA ploidy subclasses of 64 samples from 48 cases of breast cancer with predominance of carcinoma in situ

<table>
<thead>
<tr>
<th>Histological component</th>
<th>No. of samples</th>
<th>No. of clones</th>
<th>Hypodiploid</th>
<th>Diploid</th>
<th>Hyperdiploid</th>
<th>Tripliod</th>
<th>Hypotetraploid</th>
<th>Tetraploid</th>
<th>Hypertetraploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCIS</td>
<td>31</td>
<td>39</td>
<td>1</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>13</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>IC</td>
<td>33</td>
<td>46</td>
<td>1</td>
<td>4</td>
<td>18</td>
<td>2</td>
<td>14</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2  DIs of clones from 16 cases of breast cancer with predominance of DCIS, in which the DCIS and the IC component were represented in separate tissue samples

<table>
<thead>
<tr>
<th>Case</th>
<th>DCIS</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.02</td>
<td>1.03-1.13</td>
</tr>
<tr>
<td>8</td>
<td>1.01-1.81</td>
<td>1.02-1.82-3.68</td>
</tr>
<tr>
<td>9</td>
<td>1.00-1.99</td>
<td>1.02-1.96</td>
</tr>
<tr>
<td>10</td>
<td>1.02-1.90</td>
<td>1.04-1.93</td>
</tr>
<tr>
<td>11</td>
<td>1.01-1.14-2.04</td>
<td>1.01-1.12</td>
</tr>
<tr>
<td>18</td>
<td>1.00-2.71</td>
<td>1.03-2.71</td>
</tr>
<tr>
<td>19</td>
<td>0.99-1.92-1.99</td>
<td>1.00-2.01</td>
</tr>
<tr>
<td>22</td>
<td>0.96</td>
<td>0.96-1.00-1.05</td>
</tr>
<tr>
<td>23</td>
<td>0.99</td>
<td>1.01-1.05-1.09</td>
</tr>
<tr>
<td>26</td>
<td>1.00-1.04</td>
<td>1.01-1.04</td>
</tr>
<tr>
<td>38</td>
<td>1.01-1.90</td>
<td>1.09</td>
</tr>
<tr>
<td>41</td>
<td>1.00-1.62-1.79</td>
<td>1.02-1.72</td>
</tr>
<tr>
<td>42</td>
<td>1.02</td>
<td>1.02</td>
</tr>
<tr>
<td>45</td>
<td>1.02-1.09-1.20</td>
<td>1.02-1.11-1.23-1.33</td>
</tr>
<tr>
<td>47</td>
<td>1.01-1.62-1.70</td>
<td>1.01-1.61-1.72</td>
</tr>
<tr>
<td>48</td>
<td>1.01</td>
<td>1.00</td>
</tr>
</tbody>
</table>

a Identical tumor clones from corresponding DCIS and IC components.

tetraploid, 27 (82%) were aneuploid, and DNA heterogeneity was found in 11 (33%).

Due to the presence of two or three DNA nondiploid clones in several histograms, the number of clones detected is higher than the number of samples. A total of 85 clones was found in the 64 samples. Table 1 shows the DI distribution of the 85 clones into the 7 subclasses. No remarkable differences were found among the DCIS and IC groups, except for the hyperdiploid subclass, in which 18 clones were found in the IC portion compared to 6 in the DCIS portion ($P = 0.004$). Of these 24 clones, 13 had a DI of $<1.10$. In one case (case 43) a clone with a DI of 1.03 was classified as nondiploid due to the coincidence of a peak with a DI closer to 1.

The 16 cases in which histograms were obtained from both the DCIS and the IC component are shown in Table 2. An agreement between the DCIS and the IC component was demonstrated in 12 cases (cases 8, 9, 10, 11, 18, 19, 22, 26, 42, 45, 47, and 48) with complete or partial accordance in DNA ploidy pattern since in both samples identical clones were present, as defined by the narrow DI clusters. In cases 22 and 45 an extra hyperdiploid clone was found in the IC portion, and in case 8 an additional hypertetraploid clone was present in the IC portion. The clone with a DI of 3.68 might have formed by polyploidization from the clone with a DI of 1.82. In cases 11 and 19 an additional tetraploid and hypotetraploid clone, respectively, was found in the DCIS portion. In cases 45 and 47 two clones were rederived in corresponding samples of DCIS and IC. In the remaining 4 cases (cases 1, 23, 38, and 41), a relationship between the DCIS and IC components could not be demonstrated. Thus, in cases 1 and 23, the DCIS was classified as DNA diploid whereas the corresponding IC was DNA aneuploid with one and two hyperdiploid clones. In cases 38 and 41, both DCIS and IC were aneuploid but disclosed different DI clones, although in case 41 the rather broad IC clone with a DI of 1.72 may represent two clones.

The DIs of the 32 cases, from which only the DCIS or the IC component was present in the frozen tissue, are not shown in detail, but the DI distribution was similar to that of the 16 cases shown in Table 2.

A comparison of the diploid DIs for cases with both a DCIS and IC component (case 38 excluded) showed a significantly higher DI for the IC samples (mean difference = 0.01, paired t test, $P = 0.002$). A corresponding test on the CV normalized by the TRBC showed the same trend (paired t test, $P = 0.02$). For cases with only a DCIS or a IC component, the mean DIs of the DNA diploid clones were significantly different between DCIS and IC (t test, $P = 0.02$, IC being the larger). The CVs were not significantly different ($P = 0.14$); however, the CV of the IC samples was highest (1.63% versus 1.47%).

Examples of histograms are given in Figs. 2 and 3.

SFP. Evaluable SPFs were estimated in 17 samples from DCIS and 14 samples from IC. The median SPF in the DCIS samples was 8% (range, 2–38%) compared to 11% (range, 2–28%) in the IC samples. The median SPF of DNA diploid samples was 5% (range, 2–20%) and of DNA aneuploid subpopulations, 13% (range, 3–38%). The difference between DCIS and IC SPFs is not significant, whereas SPFs from subpopulations with aneuploid DNA content were significantly higher than for those with diploid DNA content (two-way ANOVA, $P = 0.01$).

Histopathology. The median size of the entire tumor from the 48 cases was 38 (range, 15–120) mm, and for the IC component alone it was 10 (range, 2–20) mm, the size referring to the diameter of the largest IC focus. Multifocality is a characteristic of this type of breast cancer and was found in 25 of the 48 cases.

In the DCIS component, a large nuclear size was found in 32 cases, while in 16 cases the nuclear size was intermediate. No lesions had small nuclei only. comedonecrosis was present in 32 cases. The dominating histological subtype was solid in 26 cases, cribriform in 17 cases, clinging in 4 cases, and papilliferous in 1 case. As in another study (1), a strong correlation was found between solid histological subtype, large nuclear size, and the occurrence of comedonecrosis.

In the IC component, 19 cases were grade I, 13 cases were grade II, and 12 cases were grade III. In the remaining four
lymph nodes were not removed. No association was found between the histological grade and lymph node status \( (P = 0.63) \) or tumor size \( (P = 0.3) \), respectively, whereas a correlation was found between the lymph node status and tumor size \( (P = 0.03) \) if the tumors were grouped into those that were \( \leq 10 \) mm and \( >10 \) mm.

The comparison of the parameters for DCIS and IC showed an association between the histological grade of the IC and nuclear size \( (P < 0.001) \) and comedonecrosis \( (P < 0.001) \), respectively.

Correlation of DNA Ploidy to Histopathology. In the DCIS component, comparison of the FCM results to the histopathological data (Table 3) indicated moderate association between DNA diploid versus DNA aneuploid to nuclear size and comedonecrosis. However, considering the small number of cases, these results must be validated on a larger population. There was no obvious correlation between DNA aneuploid subclasses and histopathological parameters but for the 5 clones with a DI >2 that were all found in the large nuclear, solid, comedotype of DCIS (data not shown). No correlation was found between the DI and size of the lesion.

In the IC component (Table 4), no conclusive correlation was found between the DI and histological grade, except for the diploid and hyperdiploid clones that were significantly related to grade 1 tumors. Thus, 14 of the 18 hyperdiploid clones were found in grade 1 tumors (data not shown). No relationship between the lymph node status and DI could be demonstrated. The four DNA diploid cases were node negative, while the occurrence of hyperdiploid clones was equally related to the presence or absence of lymph node metastases. No correlation was found between the DI and size.

There was no statistical difference between the DCIS lesions in this study and our previous study (2) with respect to the relevant parameters.

**DISCUSSION**

The present study is primarily addressed to biological issues related to pre-breast cancer and the progression to cancer, whereas the search for prognostic parameters is secondary.

In the literature, we have found no FCM DNA studies on this subject performed on unfixed tissue, whereas it has been investigated by image analysis of formalin-fixed, paraffin-embedded tissue from breast cancers (7-10) as well as tumors from other organs (11-13). In general, a considerable accordance in DNA ploidy has been demonstrated between the invasive component and the corresponding carcinoma in situ component.

The major disadvantages of image analysis on fixed tissue are the relatively poor measurement resolution (high CV), larger amounts of debris, limited number of cells analyzed, and lack of internal standardization, preventing the precise estimation of DI. We found it necessary to perform the FCM DNA analysis on unfixed frozen tissue to obtain high-resolution histograms with precise DI estimation and the ability to discriminate closely related cell clones in DNA heterogeneous lesions. The use of unfixed tissue was also essential for comparison of the results to our previous study of DCIS (2).

For the study of premalignant and malignant components within the individual case, two types of material were conceiv-
Fig. 3 DNA fluorescence histograms (A and B) and dot plots of forward light scatter versus fluorescence (C and D) from samples of breast cancer with predominance of DCIS. The two first peaks in each histogram are the Chicken Red Blood Cell (CRBC) and TRBC internal standards, respectively. The histograms are shown as step curves with the fitted model superimposed in the inserted histograms. A and C, case 40, the IC component, DNA hyperdiploid. Inset, diploid/hyperdiploid region from channels 190 to 250. The small peak has a DI of 1.00 and comprises 6% of the total sample (excluding standards). The larger peak has a DI of 1.06. CV = 1.3%. The small diploid peak has forward light scatter as would be expected from a normal peak representing intact nuclei and therefore is considered to be true (C). B and D, case 41, the IC component, DNA hypotetraploid. The large DNA diploid peak has a DI of 1.02 and the hypotetraploid peak has a DI of 1.72. CV = 2.2%. Inset, hypotetraploid region from channels 300 to 450. The skewed population to the right of the large hypotetraploid peak is not considered to be real; the forward light scatter is too low to represent intact nuclei as can be seen in the dot plot D as the tail with low light scatter starting from the major aneuploid peak.

One purpose of the study was to compare the DNA ploidy pattern of DCIS in cases with invasion, i.e., the present series, to cases of pure DCIS (2). Histopathologically, the two series were comparable. In both series there was an overrepresentation of cases of solid subtype, large nuclear size, and comedonecrosis compared to the whole spectrum of DCIS lesions. Also, FCM DNA analysis, including frequency of aneuploidy, heterogeneity, DI distribution, and SPF, showed concordant results. There-
whereas a discrepancy cannot in the individual case be taken as a counterevidence, but may as well be caused by intratumoral DNA heterogeneity. If the recognition of identical clones is strong evidence of a developmental relationship between the two components, except for the additional presence in the IC component, partly identical to that found in the corresponding DCIS component, extensive DNA ploidy changes are established already at the preinvasive stage and that no differences could be demonstrated in the DNA ploidy pattern between DCIS lesions with and without invasion. It was also shown that differences in the histograms from the same sets of histograms, partial on complete accordance in DNA ploidy and nuclear size and comedonecrosis in this study but not in the previous study reflects the small number of cases.

The second purpose of the present study was to compare the DNA pattern of the DCIS and the IC component within the individual case of breast cancer. Among the 16 corresponding sets of histograms, partial or complete accordance in DNA ploidy was found in 12 cases, with identical clones in both components, as defined by the narrow DI clusters. In 2 of the 16 cases (cases 45 and 47), two identical DNA aneuploid clones were found in both DCIS and IC. In the remaining four cases no interrelation could be shown. However, the recognition of identical clones is strong evidence of a developmental relationship whereas a discrepancy cannot in the individual case be taken as counterevidence, but may as well be caused by intratumoral DNA heterogeneity. If 5 or more samples from a DCIS lesion were analyzed, differences in the histograms from the same lesion were found in 8 of 10 cases (2). Therefore, related cases can show different DI patterns due to the stochastic nature of sampling.

Among all 48 cases, a comparison of the DI distribution among the 31 DCIS samples and the 33 IC samples (Table 1) showed similar results except for the hyperdiploid subclass, in which 6 (15%) of the total number of clones were found in the DCIS group compared to 18 (39%) clones in the IC group. This value of 39% is very high compared to the results from the literature. If the recognition of identical clones is strong evidence of a developmental relationship whereas a discrepancy cannot in the individual case be taken as counterevidence, but may as well be caused by intratumoral DNA heterogeneity. If 5 or more samples from a DCIS lesion were analyzed, differences in the histograms from the same lesion were found in 8 of 10 cases (2). Therefore, related cases can show different DI patterns due to the stochastic nature of sampling.

Table 3  FCM DNA analysis of 31 cases of DCIS from breast cancers with predominance of in situ carcinoma

<table>
<thead>
<tr>
<th>Histological parameters</th>
<th>No. of cases</th>
<th>DNA diploid</th>
<th>DNA tetraploid</th>
<th>DNA aneuploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>large</td>
<td>21</td>
<td>2</td>
<td>2</td>
<td>17 (7)</td>
</tr>
<tr>
<td>intermediate</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Comedonecrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ necrosis</td>
<td>19</td>
<td>1</td>
<td>2</td>
<td>16 (6)</td>
</tr>
<tr>
<td>- necrosis</td>
<td>12</td>
<td>6</td>
<td>1</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Histological subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>solid</td>
<td>17</td>
<td>2</td>
<td>2</td>
<td>13 (4)</td>
</tr>
<tr>
<td>cribriform</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>6 (2)</td>
</tr>
<tr>
<td>clinging</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2 (2)</td>
</tr>
<tr>
<td>papilliferous</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers in parentheses, number of cases with multiple nondiploid clones.

Another possibility is our selection of a subtype of cancer that may not be representative of breast cancer in general. It is of course important to exclude the possibility that the hyperdiploid clones, particularly the near-diploid peaks with a DI <1.10, are not true clones, but artifacts. Indications of true clones are that the clones are reproducible by reanalysis, they appear as symmetrical narrow peaks with a low CV, and an additional, strictly diploid peak is present in all histograms. This was fulfilled except for 2 cases (case 22, the DCIS component with a single peak with a DI of 0.96, and case 38 with a single, rather broad clone with a DI of 1.09). In all cases the forward light scatter (Fig. 3, C and D) was inspected, and in cases of doubt the time sequence of measurements in the list mode data files was used for supplementary evaluation of the histogram. On this technical basis we consider it probable that the DNA hyperdiploid peaks represent true clones, as supported also by the biological findings. Thus, the DNA hyperdiploid clones are mainly present in IC histograms and in particular in histological grade I tumors, whereas the frequency in the DCIS histograms corresponds to the data from our previous study of DCIS (2). The fact that DI and CV values of the DNA diploid clones in IC samples were higher than those of DCIS samples also suggests that IC samples may be more heterogeneous with respect to DNA content than DCIS samples. Finally, the slight DNA hyperploidy found by FCM may correspond to some of the subsets of breast carcinomas that by chromosome analysis have been found to be characterized by only minimal deviations from the normal 46,XX karyotype (17).

In a selected series of breast cancers with predominance of carcinoma in situ, high-quality FCM DNA analysis of unfixed frozen tissue from separate samples of the DCIS and the IC components showed that extensive DNA ploidy changes are established already at the preinvasive stage and that no differences could be demonstrated in the DNA ploidy pattern between DCIS lesions with and without invasion. It was also shown that the DNA ploidy profile of IC in most cases was completely or partly identical to that found in the corresponding DCIS component, except for the additional presence in the IC component of DNA hyperdiploid clones that may possibly be of importance for the process of invasion. These results might indicate that...
DNA changes, as detected by FCM DNA analysis, may not on their own be important for invasiveness but that this biologically crucial event may be caused by very limited genomic changes as, e.g., point mutations. Our point of view is in line with the comments from the DNA Cytometry Consensus Conference (18) that significant chromosomal aberrations that cannot be disclosed by FCM may be more relevant to biological aggression than the gross alterations in chromosome content associated with an abnormal histogram.

REFERENCES


Flow cytometric DNA analysis of breast cancers with predominance of carcinoma in situ: a comparison of the premalignant and malignant components.


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