Ploidy Analysis by In Situ Hybridization of Interphase Cell Nuclei in Fine-Needle Aspirates From Breast Carcinomas: Correlation With Cytologic Grading

Torill Sauer, M.D., F.I.A.C.,* Kahsai Beraki, Bsc.,1 Peter Wilhelm Jebsen, M.D.,1 Eli Ormerod, Bsc.,2 and Oddvar Naess, M.D.1

Fine-needle aspirates from 54 breast cancer patients were investigated for numeric aberrations in chromosomes 6, 7, 12, and 17 by in situ hybridization (ISH) of interphase cell nuclei. Ploidy findings were compared with cytologic grading of tumors. Aneuploidy was found in 73% of cases. Chromosomes 6 and 7 showed numeric abnormalities in 63% and 62% of cases, respectively, whereas chromosome 17 retained a disome pattern in 2/3 of the tumors. Thirteen cancers (28% of 47 with four analyzed probes) had a normal signal number in all four chromosomes. In 17 (36%), all four had signal gain. Another 17 showed a mixed disome/aneusome pattern. They presented a continuum of increasing numeric abnormalities, 82% disomy for chromosome 17, and 13 of them were grade 2, indicating intermediate biologic properties. Correlation between grading and ploidy was good, with 10 of 11 grade 1 carcinomas showing diploidy, whereas 33 of 36 grade 2 and 3 tumors had numeric aberrations. Diagn. Cytopathol. 1997; 17:267–271. © 1997 Wiley-Liss, Inc.

Key Words: fine-needle aspirates; in situ hybridization; cytologic grading; ploidy; DNA

Most breast cancers have abnormal DNA content. The frequency of aneuploidy in various series ranges from about 40–90%.1–3 Chromosomal aneuploidy reflects numeric and/or structural aberrations. In the cytogenetic progression of tumors, abnormalities will occur in an increasing number of chromosomes. Aberrations have been found in several chromosomes in breast cancer cells.4,6 Structural abnormalities, e.g., in chromosomes 1, 3, 6, and 17 have been found in 30–60% of breast carcinomas.6 Both numeric and structural aberrations are reflected in the biologic behavior of tumors. So far, prognosis has been correlated mainly with total DNA content, but an increasing number of structural abnormalities that are associated with the aggressiveness of breast cancer have been found.7–11

In situ hybridization (ISH) of interphase cell nuclei offers an opportunity to study both numeric and structural aberrations in target chromosomes in tumor cells. The aim of our study was to investigate numeric aberrations by ISH of interphase cell nuclei on fine-needle aspirates (FNA) in a chosen number of chromosomes that either harbor genes coding for known prognostic parameters (namely 6, 7, and 17) or that have often been found to be abnormal (e.g., 12) in breast carcinomas.12,13

DNA content is indirectly reflected in histologic grading, which is an important prognostic parameter in breast cancer.14–17 Several authors have described cytologic grading systems that correlate quite well with histologic grading18–23 and also with flow cytometric data.17,21 We therefore wanted to correlate cytologic grading to the ISH findings.24

Materials and Methods

The material consisted of FNA from 54 patients with palpable breast cancer. The aspirations were performed by two cytopathologists (P.W.J. and T.S.). For routine diagnostics, one alcohol-fixed Papanicolaou (Pap)-stained smear and one air-dried Giemsa-stained smear were used. Cytologic grading was done according to Robinson et al.23 (Table I). Both Pap- and Giemsa-stained slides were evaluated and without regard to tumor subtypes. Grading was done by one cytopathologist (T.S.).

In cases where additional air-dried smears were available, these were put aside for in situ hybridization and stored at −20°C until processing. ISH was performed using digoxigenin-labeled α-satellite probes for chromosomes 6, 7, 12,
tumors showed disomy, with >90% two-signal nuclei for all four chromosomes.

Chromosomal gain was the dominant event, occurring in all aneuploid cases. This was a tumor with monosomy for chromosome 6; numeric gain of chromosome 7, and a normal signal number in chromosomes 12 and 17 (Fig. C-1). The distribution of signals varied. In most cases, nuclei with 2–5 signals comprised the major part of the cell, whereas a few had a continuous distribution of signal number from 1–9. Distribution of disomy/aneusomy for each chromosome is given in Table IV.

Thirteen (28%) cancers had a normal signal number in all four chromosomes. In 17 (36%) all four had signal gain, while 17 (36%) showed a mixed pattern. Five lesions, lacking one or two chromosome counts, had one or more chromosomes with signal gain. One of these tumors also showed monosomy for chromosome 17. In total, 40 (73%) of 54 carcinomas had signal gain for some or all four analyzed chromosomes, consistent with aneuploidy.

Twelve tumors were cytologic grade 1, 31 were grade 2, and 11 were grade 3. Comparison of grading vs. ploidy is shown in Table V. Details of the mixed disome/aneusome lesions are shown in Table VI.

**Discussion**

Chromosomal abnormalities are reflected in DNA content, and the four probes provided a rough estimate of DNA ploidy. Essentially this gave us the minimal percentage of aneuploid cases, as some tumors, where all four analyzed chromosomes had a normal number, might still have had aberrations in nonanalyzed chromosomes. Seventy-three percent of the analyzed cancers showed a variable extent of numerical chromosomal aberrations. As we used additionally available smears only, our specimens did not represent the full range of breast cancer types; e.g., neither scirrhous nor tubular carcinomas were represented, and low-grade carcinomas were probably underrepresented.
Figs. C-1–C-2. Fig. C-1. A: ISH of carcinoma cells with monosomy of chromosome 6 (left) and disomy of chromosome 12 (right). B: Same tumor with disomy of chromosome 17 (left) and aneusomy of chromosome 7 (right). C: Same tumor, cytologic grade 2 (Papanicolaou and Giemsa stain).

Fig. C-2. A,B: ISH of carcinoma cells with disomy for all four chromosomes. C: Papanicolaou-stained smear of same tumor, cytologic grade 3 (left) and H&E stained histologic section (right) (Figs. C-1–C-2, ×500).
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Table IV. Distribution of Disomy/Aneusomy for Each Chromosome

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Disomy</th>
<th>Aneusomy</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>20 (37%)</td>
<td>34 (63%)</td>
<td>54</td>
</tr>
<tr>
<td>7</td>
<td>18 (38%)</td>
<td>29 (62%)</td>
<td>47</td>
</tr>
<tr>
<td>12</td>
<td>25 (49%)</td>
<td>26 (51%)</td>
<td>51</td>
</tr>
<tr>
<td>17</td>
<td>31 (64.5%)</td>
<td>17 (35.5%)</td>
<td>48</td>
</tr>
</tbody>
</table>

Table V. Cytologic Grading of Tumors vs. Ploidy

<table>
<thead>
<tr>
<th>Ploidy</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disomy ×4</td>
<td>10</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Mixed</td>
<td>1</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Aneusomy ×4</td>
<td>11</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Subtotal</td>
<td>11</td>
<td>26</td>
<td>10</td>
</tr>
</tbody>
</table>

Table VI. Cytologic Grading and Distribution of Disomy/Aneusomy in Mixed Tumors

<table>
<thead>
<tr>
<th>Distribution of disomy/aneusomy</th>
<th>Disomy of chromosome numbers</th>
<th>Aneusomy of chromosome numbers</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>D × 3; A × 1; no. 1</td>
<td>6, 7, 17</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>D × 3; A × 1; no. 2</td>
<td>6, 12, 17</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>D × 3; A × 1; no. 3</td>
<td>7, 17</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>D × 3; A × 1; no. 4</td>
<td>7, 12, 17</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>D × 3; A × 1; no. 5</td>
<td>6, 12, 17</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>D × 2; A × 2; no. 1</td>
<td>12, 17</td>
<td>6, 7</td>
<td>3</td>
</tr>
<tr>
<td>D × 2; A × 2; no. 2</td>
<td>12, 17</td>
<td>6, 7</td>
<td>2</td>
</tr>
<tr>
<td>D × 2; A × 2; no. 3</td>
<td>12, 17</td>
<td>6, 7</td>
<td>2</td>
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<tr>
<td>D × 2; A × 2; no. 4</td>
<td>12, 17</td>
<td>6, 7</td>
<td>2</td>
</tr>
<tr>
<td>D × 2; A × 2; no. 5</td>
<td>12, 17</td>
<td>6, 7</td>
<td>2</td>
</tr>
</tbody>
</table>

Chromosomes 6 and 7 showed aberrations in 88% and 72.5%, respectively, of aneuploid cases, and were the two most commonly affected chromosomes. In contrast, chromosome 17 retained a disome pattern in ⅓ of the tumors (Table IV). This is somewhat higher than in Ichikawa et al., who found disomy of chromosome 17 in 48.7% of their cases. They had a high incidence of monosomy (16.2%) in contrast to our single case (2%), but the same percentage of aneuploid tumors (35%). Differences in sampling and sensitivity of methods may account for these differences.

Devilee et al. found loss of heterozygosity (LOH) at 17p to be the most frequent abnormality in a quite extensive mapping of genetic aberrations in human breast cancers. The main finding was allelic loss, possibly representing deletions that would not be detected by our method. The probes used here (Fig. C-1) were directed towards the centromeric and pericentromeric region, and mutations and deletions outside this area could not be appreciated. A tumor with a normal number of chromosome 17 may still harbor abnormal genes.

As seen in Table V, grading correlated well with the four-chromosome-based ploidy measurement. Ten of 11 grade 1 carcinomas were diploid. In contrast, 33 of 36 grade 2 and 3 tumors had normal chromosome numbers (aberrant case shown in Fig. C-2). Cajulis et al. found the same when testing for chromosomes 8 and 12 with a similar method. In a series of 20 breast carcinomas, 4 of their 5 grade 1 tumors were diploid, whereas all grade 3 cancers were aneuploid (there were no grade 2 cases). The morphologic distinction of grade 1 vs. 2 and 3 may be seen as a biologic threshold of tumors that on the one hand have a normal chromosome number and are known to have good prognosis, with a survival of >80%, and those that on the other hand have distinct chromosomal aberrations, and a less favorable outcome.

Thirteen (76%) of 17 mixed disome/aneusome tumors were grade 2 (example in Fig. C-1). They present a continuum of increasing degrees of chromosomal abnormalities. As can be seen in Table VI, five had one aneusome chromosome, and another five had two, whereas seven harbored three abnormal chromosomes. From Table VI it is also evident that chromosome 17 retains a normal chromosome number until the last group, where 3 out of 7 tumors showed a gain of chromosome 17. This observation of disomy for chromosome 17 in 82% of the mixed carcinomas may indicate that numerical aberrations of chromosome 17 represent a late event in cancer progression that is found mainly in tumors with more advanced chromosome abnormalities.

Several prognostic marker genes, e.g., for p53, neu, and nm23 are located on chromosome 17. Chromosomal amplification will affect the expression of these markers. Expression of p53 protein and neu, as well as loss of nm23 expression, correlates with histologic grade and ploidy and is associated with an unfavorable prognosis. Chromosome 17 with its prognostic marker genes is therefore an especially interesting target for further studies. Such investigations might reveal patterns of expression capable of distinguishing prognostic subgroups of breast cancer patients.

In conclusion, ploidy analysis based on ISH of a limited number of chromosomes correlated well with cytologic grading. As signal counting is quite time-consuming, this method would not be practical for routine ploidy determination. ISH analysis of a few target chromosomes and genes, however, might well be applicable as a routine procedure. Cytologic grading confidently separates the grade 1 and diploid tumors from the mainly aneuploid grade 2 and 3 cancers.
Furthermore, mixed disome/aneusome tumors presented a continuum of increasing degrees of chromosomal abnormality and 76% were grade 2, consistent with intermediate biologic behavior. They retained disomy for chromosome 17 in 82% of cases, indicating that numeric aberrations of this chromosome are a late event in cancer progression.

References