Breast Cancer Cytogenetics: A Review and Proposal for Clinical Application*†

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ABSTRACT

Cytogenetic analyses of hematological neoplasms have proved to be relevant in the diagnosis, treatment, and prognosis of afflicted patients. Breast cancer (BC) cytogenetics is expected to contribute in the same fashion. Literature has shown that clone-specific chromosomal changes do occur in BC, yet, their clinical significance is unknown. Most of the studies have been conducted on patients with advanced stage tumor. Karyotypic analyses of the few reported cases from stage I BC tumors revealed a higher frequency of single clonal abnormalities. This work describes an ongoing BC cytogenic study on samples from stage I tumors to enhance and clarify this observation. Included as control are chromosomal analyses of peripheral blood and peritumoral normal tissue samples of these patients, which might provide information regarding predisposing cytogenetic aberrations. Non-random chromosomal abnormalities in BC include involvement of chromosomes 1, 3, 6, 11, 16, and 17. Three groups of non-random chromosomal alterations, ranging from specific abnormalities, partial monosomies, and secondary changes (eg, numerical loss of chromosomes) are described. Survival appears to be more favorable in patients without complex karyotypes. A better understanding of the clinical and etiologic implications of BC is expected to emerge from continued assessment of breast tumor cytogenetics.

Introduction

Breast cancer (BC) is the most common cancer and cause of death among women between 15 to 54 years of age.1 These epidemiological data reflect still inadequate understanding of the molecular biological changes leading to the development of BC.3 In the last 10 years, several cytogenetic and molecular alterations have been associated with the development and progression of this disease and several tumor-specific alterations have been identified.2,3,4,5 These alterations include quantitative and quali-
Cytogenetic analyses of hematological neoplasms have proved relevant not only in establishing evidence of clonality but also in the diagnosis, treatment, and prognosis of afflicted patients. Cytogenetic analysis of solid tumors, including BC, was expected to contribute similarly. Clonal specific chromosomal changes do occur in BC; yet, the majority of the studies have been conducted on advanced stage tumor samples which have made their clinical pathological correlation difficult. The purpose of this work is to review the findings of BC cytogenetics and to explore its role at earlier tumor stages, in which less genomic instability is expected, as an aid in understanding the pathogenesis of this disease.

Materials and Methods

Conventional cytogenetic studies for BC that included tumors up to 2 cm in size were primarily reviewed and compared with those performed in advanced tumors. The cytogenetic findings and culture techniques of these studies were compared. Only clonal non-random chromosomal abnormalities were included and are reported using the ISCN (International System for Human Cytogenetic Nomenclature) 1991 guidelines for Cancer Cytogenetics.

Results

Cytogenetic Techniques

The current culture types used for the cytogenetic analysis of solid tumors, including advantages and disadvantages, are summarized in table I. Recently, an improved short-term culture technique for BC samples has been shown to obtain a better yield of analyzable metaphases and a pure tumor cell population devoid of stromal, nonneoplastic cells. Most of the reported cytogenetic aberrations found in primary BC, prior to 1991, were obtained from tumor cell cultures that yielded a low percentage of cases with abnormal karyotypes, ranging from 4 percent to 34 percent.

Most of these BC cytogenetic studies were obtained from long-term and/or direct preparation techniques. In contrast, BC cytogenetic studies using the short-term cultures appear to have a much better rate ranging from 44 to 81 percent, of obtaining clonal chromosomal alterations (in table I). For comparison, two series of BC cytogenetic studies based on direct culture preparations are included in table II to illustrate the low yield of analyzable abnormal clonal metaphases recovered (~25 percent of the cases) with this methodology.

Besides culturing difficulties, another reason for delay in the clinical application of BC cytogenetics is that, in most cases, the analyzable abnormal karyotypes not only are complex, but also have multiple clones and marker chromosomes that make the cytogenetic interpretation difficult and laborious.

Recently, the use of fluorescence in situ hybridization (FISH) technology has expanded the conventional cytogenetic methodology into molecular cytogenetics. In the past few years, the use of centromeric, whole-painting chromosome and other gene- or region-specific DNA probes have contributed to the
**TABLE I**

**Type of Culture Used in the Cytogenetic Analysis of Solid Tumors**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-term</td>
<td>Most successful yield</td>
<td>Culture dependent, clonal selection</td>
</tr>
<tr>
<td>Long term</td>
<td>Established cell lines, good analysis</td>
<td>Stromal overgrowth, non–in vivo alterations</td>
</tr>
<tr>
<td>Direct preparations</td>
<td>Quick analysis</td>
<td>Analysis of only actively mitotic cells, inferior quality, poor yield</td>
</tr>
<tr>
<td>Xenografts (mice &amp; rats)</td>
<td>No stromal cells</td>
<td>Laborious and expensive, followed by 1 to 3 cultures</td>
</tr>
<tr>
<td>Prophasing (PCC)(^b)</td>
<td>Chromosomes in late G1 &amp; G2, fast chromosome breakage analysis</td>
<td>Difficulties in obtaining and interpreting results</td>
</tr>
</tbody>
</table>

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b Prematurely condensed chromosomes.

Identification and/or clarification of chromosomal regions not well characterized by the traditional cytogenetic method.\(^{22}\)

In addition, a "microFISH" technique has been described. This technique combines the microdissection of the specific chromosomal aberration with subsequent Alu-polymerase chain reaction to elaborate a DNA probe that can be used in FISH assay to disclose that specific cytogenetic abnormality.\(^{23}\) This microFISH technique has proved useful in clarifying some conventional cytogenetic findings in BC samples.\(^{24}\) In the future, the use of FISH and microFISH techniques together with the better chromosomal quality of the G-banded chromosomes obtained with the new short-term improved culture will aid in the interpretation and turnaround of the cytogenetic analysis of BC samples.\(^{13,16}\)

**Chromosomal Abnormalities**

Multiple cytogenetic abnormalities have been found in BC involving virtually all chromosomes with an extensive variety of numerical and structural alterations, including multiclonality.\(^2,3,4,5,15,16,18,19,20,21\) This abnormal clonal variation is found even within the same tumor. Most of these chromosomal changes are summarized in tables II and III. Flow cytometric studies have corroborated these observations by demonstrating intra- and intertumoral ploidy heterogeneity in BC tumors.\(^6,7,20\) Despite the extensive chromosomal aberrations observed in BC tumors, nonrandom alterations have been found and frequently involved chromosomes 1, 3, 6, 11, 16, and 17.\(^4,5,17,18,19,20,21\)

A better characterization of the most frequent chromosomal abnormalities was published this year and is summarized in table III. That report was based on a cytogenetic study of 103 primary BC tumors using the improved short-term technique.\(^{13}\) The specific chromosomal alterations included gains and losses of chromosomes 1p, 1q, 16p, 3p, and 6p specific regions as well as trisomy of chromosomes 7, 18, and 20, which were found in
Recent Cytogenetic Studies of Primary Breast Cancer

<table>
<thead>
<tr>
<th>Author</th>
<th>Culture Type</th>
<th>Total # Cases</th>
<th>Success Rate</th>
<th>Percent Culture</th>
<th>Total Cases</th>
<th>Cases with Clonal Changes (Single Abnormality)</th>
<th>Reported Stage I Tumor Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pandis$^b$</td>
<td>Short-term</td>
<td>97</td>
<td>100</td>
<td></td>
<td>79 (31)</td>
<td>(n.d.)</td>
<td>(n.d.)</td>
</tr>
<tr>
<td>Steinarsdottir$^c$</td>
<td>Direct</td>
<td>34</td>
<td>29</td>
<td></td>
<td>44 (3)</td>
<td>12</td>
<td>12 (3)</td>
</tr>
<tr>
<td></td>
<td>Short-term</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pandis$^d$</td>
<td>Short-term</td>
<td>20</td>
<td>100</td>
<td></td>
<td>16 (6)</td>
<td>12</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Lu$^e$</td>
<td>Short-term</td>
<td>50</td>
<td>44</td>
<td></td>
<td>22 (0)</td>
<td>4</td>
<td>4 (0)</td>
</tr>
<tr>
<td>Thompson$^f$</td>
<td>Short-term</td>
<td>91</td>
<td>61</td>
<td></td>
<td>28 (5)</td>
<td>(n.d.)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Hainsworth$^g$</td>
<td>Direct</td>
<td>143</td>
<td>21</td>
<td></td>
<td>26 (3)</td>
<td>9</td>
<td>7 (3)</td>
</tr>
<tr>
<td>Lopez–Gines$^h$</td>
<td>Short-term</td>
<td>22</td>
<td>50</td>
<td></td>
<td>12 (0)</td>
<td>1</td>
<td>(n.d.)</td>
</tr>
<tr>
<td>TOTAL$^i$</td>
<td>Direct</td>
<td>177–25</td>
<td></td>
<td></td>
<td>148 (17)</td>
<td>38</td>
<td>37 (11)</td>
</tr>
<tr>
<td></td>
<td>Short-term</td>
<td>228</td>
<td></td>
<td></td>
<td>~66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ (n.d.) = not determined or unknown.


$^i$ The first series (Pandis$^b$) was not included since Stage I tumors were not indicated in it.

over half of the cases studied. A second group of specific chromosomal alterations found in less than half of the cases included partial monosomies of chromosomes 1p, 8p, 11p, 11q, 15p, 17p, 19p, 19q regions translocations of chromosomes 3p12-13 and 4q21 regions, and inversions of chromosome 7p and 7q regions.

A third group of nonrandom alterations were considered as secondary changes and included numerical losses of chromosomes X, 8, 9, 13, 14, 17, and 22. One clinical implication of some of these cytogenetic abnormalities has been recently published regarding the survival in BC patients. This study showed a 92 percent survival in patients without complex karyotypes versus a 63 percent survival in those with complex karyotypes. However, most of these cytogenetic findings were found in advanced stage breast tumors, and an increased genetic instability is expected.

Few cytogenetic studies have been conducted on early stage breast tumors. In table II are summarized most of the primary BC cytogenetic series that
**TABLE III**

Most Connem Recurrent Chromosomal Abnormalities in 103 Primary Breast Cancers

<table>
<thead>
<tr>
<th>Clonal Change</th>
<th>Specific (55% of Cases)</th>
<th>Possible Specific Regions</th>
<th>Other Non-random Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single: ~ 55% cases</td>
<td>i(1)(q10)</td>
<td>Partial monosomies</td>
<td>Numerical losses</td>
</tr>
<tr>
<td>Polyclonal (up to 8 clones): ~ 45% cases</td>
<td>der(1;16)(q10;p10)</td>
<td>1p</td>
<td>X</td>
</tr>
<tr>
<td>Unrelated clones: ~ 35% cases</td>
<td>del(1)(q11–12)</td>
<td>8p</td>
<td>8</td>
</tr>
<tr>
<td>Single abnormality: ~ 40% cases</td>
<td>del(3)(p12–21)</td>
<td>11p</td>
<td>9</td>
</tr>
<tr>
<td>(Structural 8%, numerical 32%)</td>
<td>del(6)(q21–22)</td>
<td>11q</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>+7</td>
<td>15p</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>+18</td>
<td>17p</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>+20</td>
<td>19p</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>t(3p12–13) &amp; (4q21)</td>
<td>19q</td>
<td>Inv 7</td>
</tr>
</tbody>
</table>


include stage I tumor cases. Unfortunately, the most recent cytogenetic BC series study did not indicate the tumor stage of the cases. Nevertheless, as indicated in table II, the few reported cytogenetic studies of stage I tumors in other series have shown single chromosomal abnormalities in approximately 30 percent of the cases, a frequency up to 2.5 times higher than in advanced stage tumors. This observation could be biased owing to the reduced number of stage I tumor samples studied. Yet, this finding might indicate that earlier-stage BCs are more genetically stable. Further BC cytogenetic studies at early stages would help to clarify these observations.

In regards to BC chromosomal etiology, cytogenetic studies on peripheral blood lymphocytes for identification of fragile sites in familial BC patients at risk have also been conducted, but their results have been controversial. Another recent and related cytogenetic analysis of peripheral blood lymphocytes from familial and sporadic BC patients and predisposed family members revealed a significantly high frequency of chromosome 1q rearrangements, which were suggested as one of the primary lesions associated with the development of BC.

**Discussion**

Five additional studies may be useful in characterizing BC. These include: (1) cytogenetic analyses of samples from untreated patients with stage I tumors using the improved short-term culture methodology; (2) cytogenetic analyses of the corresponding nonneoplastic, peritumoral breast or skin tissue samples using the same short-term culture technique; (3) cytogenetic analyses of peripheral blood lymphocyte samples from each of these patients using a standard routine cytogenetic technique; (4) FISH analyses of samples from the cytogenetic studies noted previously when appropriate and
limited by the availability of the specific DNA probes needed; and (5) clinico-pathological correlation of the cytogenetic findings. These studies will provide unique information regarding BC cytogenetic chromosomal abnormalities at early tumor stages and could be used subsequently to design other approaches, such as production of DNA molecular probes to test the specificity and significance of these chromosomal changes. For example, in patients with breast atypical ductal hyperplasias and/or carcinomas in situ, the previously described probes could, by using interphase FISH techniques, provide information to identify those patients at risk to develop invasive carcinoma. Some of the expected chromosomal changes could also lead to novel undiscovered oncogenes or tumor suppressor genes that might be implicated in the etiology of breast cancer.

A variety of chromosomal alterations found in the cytogenetic analysis of hematological neoplasms has proved to be relevant not only in establishing evidence of clonality, but also in the diagnosis, treatment, and prognosis of afflicted patients. Despite technical difficulties, the cytogenetic analysis of solid tumors, including BC, is expected to contribute in the same fashion. A review of the literature has shown that clonal-specific chromosomal changes do occur in BC. However, the majority of the studies have been conducted on advanced-stage tumor samples. Cytogenetic analysis of the few reported tumor samples from stage I BC tumors revealed a frequency of single clonal abnormalities up to 2.5 times higher, than at advanced-stage tumors. This observation suggests that earlier-stage BC's are more genetically stable. It is proposed to expand BC cytogenetic studies of patients at earlier tumor stages to enhance and clarify this hypothesis.

In addition, cytogenetic analysis of peripheral blood lymphocytes as well as the peritumoral, normal breast, or skin tissues would be useful in these patients. These additional samples not only will serve as control samples, but also will provide information regarding predisposing cytogenetic chromosomal aberrations, if present. Finally, recent improvements in the methodology of cytogenetic cultures, chromosomal banding resolution of solid tumors, and the FISH and microfish technologies will help in the interpretation as well as turnaround time of the cytogenetic analysis of BC samples. Better BC cytogenetics can be expected to result in expanded understanding of the pathogenesis and hopefully provide effective tools for measuring prognosis and response to therapy.

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References


