Allele Loss on Chromosome 16q24.2-qter Occurs Frequently in Breast Cancers Irrespective of Differences in Phenotype and Extent of Spread

Hitoshi Tsuda, David F. Callen, Takashi Fukutomi, Yusuke Nakamura, and Setsuo Hirohashi

INTRODUCTION

In human breast cancer, LOH3 on chromosomal arms has been detected frequently by RFLP analysis. LOH in a specific chromosomal region is generally considered to be involved in the genesis and progression of cancer, through inactivation of tumor-suppressor genes located there. Inactivation of tumor-suppressor genes occurs in a recessive manner; usually, one allele containing the gene is deleted, and the other allele contains point mutations or small deletions. Frequent LOH is reported for chromosomal arms 1p, 1q, 3p, 7q, 11p, 13q, 16q, 17p, 17q, and 18q in breast cancer (1–7). A gene associated with susceptibility to breast cancer is suggested to be located on chromosome 17q21 on the basis of linkage analysis of breast cancer families (8). Although it is impossible to apply such linkage to sporadic cases, several tumor-suppressor genes located on a commonly deleted chromosomal region in sporadic cancer have already been identified, e.g., p53 and prohibitin (9–10). For example, the p53 tumor-suppressor gene located on chromosome 17p13.1, which is a commonly deleted region in diverse types of human cancers, has also been shown to carry mutations frequently (9). The prohibitin gene mapped on 17q12-q21 also carries mutations in sporadic breast cancer (10); another gene that causes familial breast cancer is still suggested to exist around 17q21 (11).

Frequent LOH of chromosome 16q is reported not only in breast cancer (4, 5) but also in hepatocellular carcinoma (12) and prostatic cancer (13). We previously specified in hepatocellular carcinoma a commonly deleted region, where an unknown tumor-suppressor gene is considered to be located, on chromosome 16 between the HP locus on q22.1 and the CTRB locus on q23.2 (12). However, >60% of cases with the LOH were suggested to be due to monosomy of the entire chromosome 16. Sato et al. (5) also identified in breast cancer a commonly deleted region located between the HP and D16S157 loci on 16q24, using five polymorphic DNA markers. To further specify this region, a deletion map of a larger number of cases seemed to be necessary, employing a larger number of polymorphic DNA markers. Recently, physical and genetic chromosomal maps have been considerably refined using a larger number of novel polymorphic DNA markers (14–21). In the present study, we examined the incidence of LOH and tried to localize more accurately the region commonly deleted on chromosome 16, using polymorphic DNA markers at 27 different loci. Furthermore, association of the LOH with clinical and histological parameters was examined, to reveal its biological role in breast cancer development.

MATERIALS AND METHODS

DNA Probes and Their Chromosomal Locations. The polymorphic DNA markers used, all localized on chromosome 16, are shown in Table 1. To specify the most frequently deleted region, the loci of these markers were assigned by referring to both genetic and physical maps already published by scientists involved in chromosome 16 mapping (14–21). The order of DNA markers assigned on chromosome 16 according to the genetic map is as follows: pter→D16S83→pEKMDA2.1→D16S31→VK45C6→MT2→D16S151→CRI-089→HP→CTRB→D16S20→D16S157→D16S84→D16S44→qter (14–21). By the genetic map, the D16S155 (CRI2.199) locus is determined to be between D16S43 and D16S87.4 According to the physical map, the order is as follows: pter→(D16A1, D16S83)→D16S34 (16/55B)→D16S32/16/118→D16S31/16/5→(M2, CEP)→D16S151→D16S43→D16S152 (C25.1–21, HP, TAT→CTRB→D16S20→D16S157→D16S154)→D16S87→HP-APRT→D16S44-pter (19–21). D16S159 (CRI2.94), and D16S23 (p16–1) are localized in the same physical interval in 16p12.2, and D16S137 (KKA22) maps proximal to D16S159 on the genetic map.4 Westphal et al. (22) strongly suggested that the position of the TAT locus is proximal to CTRB but distal to HP from 16cen. When these data are combined, the most probable order of the DNA markers is as follows: pter→D16A1→D16S83→D16S34→D16S32→D16S131→D16S159→D16S23→D16S157, cen→D16S27→MT2, CEP)→D16S151→D16S43→D16S154–D16S87→HP-APRT→CTRB→D16S20→D16S157→D16S43→D16S155→D16S87 (14–21).

The D16S35 (16/32) and D16S30 (p16–8) loci are physically mapped to the region pter→p13 containing D16S32 and D16S131 and to the region q22.1–q24, respectively (14). Additional information on these two loci was not available.

Tissue Samples, DNA Isolation, and Southern Blot Analysis. We collected 234 paired samples of breast cancer and noncancerous tissue from 225 patients with primary disease. In nine patients, bilateral primary lesions were
Table 1 Incidence of loss of heterozygosity at 27 loci on chromosome 16 in breast cancer

<table>
<thead>
<tr>
<th>Name of locus* (Name of clone)</th>
<th>Chromosomal localization</th>
<th>Restriction enzyme</th>
<th>Total</th>
<th>Constitutional</th>
<th>Loss in tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HBA1 (1W101)</strong></td>
<td>p13.3</td>
<td>Rsal</td>
<td>64</td>
<td>22</td>
<td>5 (20)</td>
</tr>
<tr>
<td>D16S33 (p1EKMDA2.1)</td>
<td>p13.3</td>
<td>Rsal</td>
<td>62</td>
<td>31</td>
<td>6 (19)</td>
</tr>
<tr>
<td>D16S54 (16/51B)</td>
<td>p13.2</td>
<td>PstI</td>
<td>65</td>
<td>21</td>
<td>7 (33)</td>
</tr>
<tr>
<td>D16S32 (16/118)</td>
<td>p13.1</td>
<td>TaqI</td>
<td>48</td>
<td>21</td>
<td>3 (14)</td>
</tr>
<tr>
<td>D16S35 (16/23)</td>
<td>pter-p13</td>
<td>TaqI</td>
<td>77</td>
<td>26</td>
<td>4 (15)</td>
</tr>
<tr>
<td>D16S137 (pKV45C6)</td>
<td>p12.3</td>
<td>TaqI</td>
<td>49</td>
<td>8</td>
<td>1 (13)</td>
</tr>
<tr>
<td>D16S159 (C152.94)</td>
<td>p12.2</td>
<td>TaqI</td>
<td>61</td>
<td>34</td>
<td>6 (18)</td>
</tr>
<tr>
<td>D16S23 (p16-1)</td>
<td>p12.2</td>
<td>PvuII</td>
<td>70</td>
<td>36</td>
<td>5 (14)</td>
</tr>
<tr>
<td>D16S137 (pKV42A2)</td>
<td>16</td>
<td>PvuII</td>
<td>72</td>
<td>37</td>
<td>5 (14)</td>
</tr>
<tr>
<td>D16S27 (p16-56)</td>
<td>q12.1</td>
<td>PvuII</td>
<td>72</td>
<td>26</td>
<td>7 (27)</td>
</tr>
<tr>
<td>MT2 (hMT2)</td>
<td>q13</td>
<td>TaqI</td>
<td>76</td>
<td>29</td>
<td>9 (31)</td>
</tr>
<tr>
<td>CETP (CETP111)</td>
<td>q13</td>
<td>TaqI</td>
<td>77</td>
<td>36</td>
<td>9 (25)</td>
</tr>
<tr>
<td>D16S151 (C152.209M1)</td>
<td>q21</td>
<td>TaqI</td>
<td>78</td>
<td>28</td>
<td>16 (57)</td>
</tr>
<tr>
<td>D16S64 (ACR207)</td>
<td>q22.1</td>
<td>BglII</td>
<td>74</td>
<td>35</td>
<td>14 (49)</td>
</tr>
<tr>
<td>D16S38 (CRI-02)</td>
<td>q22.1</td>
<td>BamHI</td>
<td>75</td>
<td>21</td>
<td>4 (19)</td>
</tr>
<tr>
<td>D16S152 (C152.1)</td>
<td>q22.1</td>
<td>BamHI</td>
<td>74</td>
<td>27</td>
<td>11 (41)</td>
</tr>
<tr>
<td>HP (hp2 alpha)</td>
<td>q22.3</td>
<td>MspI</td>
<td>68</td>
<td>43</td>
<td>16 (37)</td>
</tr>
<tr>
<td>[TAT (hpo4) (BHO0.4)]</td>
<td>q22.3</td>
<td>BamHI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[BHO0.4]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT88 (pCXP33)</td>
<td>q23.2</td>
<td>TaqI</td>
<td>67</td>
<td>34</td>
<td>9 (26)</td>
</tr>
<tr>
<td>D16S20 (p16-17-6)</td>
<td>q24.1</td>
<td>BglII, PvuII</td>
<td>78</td>
<td>45</td>
<td>15 (33)</td>
</tr>
<tr>
<td>D16S59 (p16-8)</td>
<td>q22.1-2q4</td>
<td>PstI</td>
<td>71</td>
<td>6</td>
<td>3 (50)</td>
</tr>
<tr>
<td>D16S157 (C152.86)</td>
<td>q24.2-2q4.3</td>
<td>TaqI</td>
<td>78</td>
<td>21</td>
<td>9 (43)</td>
</tr>
<tr>
<td>D16S43 (CR1684)</td>
<td>q24.2-2q4.3</td>
<td>PvuII</td>
<td>71</td>
<td>20</td>
<td>8 (40)</td>
</tr>
<tr>
<td>D16S155 (C152.199)</td>
<td>q</td>
<td>MspI</td>
<td>37</td>
<td>11</td>
<td>2 (18)</td>
</tr>
<tr>
<td>D16S7 (P79-2-23)</td>
<td>q43.3</td>
<td>TaqI</td>
<td>76</td>
<td>68</td>
<td>32 (47)</td>
</tr>
<tr>
<td>[APRT (p12.3 apt)]</td>
<td>q43.3</td>
<td>TaqI</td>
<td>78</td>
<td>33</td>
<td>17 (52)</td>
</tr>
<tr>
<td>[D16S44 (CR1089)]</td>
<td>q43.3</td>
<td>BglII</td>
<td>74</td>
<td>10</td>
<td>4 (40)</td>
</tr>
</tbody>
</table>

*Brackets, DNA markers located within same physical interval defined by hybrid breakpoints.

For the other 156 tumors, RFLP analysis was performed using six DNA markers, i.e., CETP, D16S4, HP, TAT (BHO0.4), D16S7, and APRT. Constitutional heterozygosity for at least one locus was detected in 152 tumors. In total, information on LOH on chromosome 16q was available for 230 tumors.

Statistical analysis was performed by χ² test.

RESULTS

Region on Chromosome 16q Most Frequently Deleted in Breast Cancer. Table 1 shows the incidence of LOH in the tumors at each polymorphic DNA marker locus. Autoradiographs of six cases showing LOH are presented in Fig. 2. LOH on chromosome 16q was detected in 38 (49%) of 78 tumors for which deletion mapping was performed. No cases revealed rearrangement or homozygous deletion. For these 38 cases, the maximum extents of the deleted regions in one allele were deduced and are shown in Fig. 2. Fifteen tumors were suggested to have LOH on the entire long arm, whereas the other 23 were suggested to have partial allele loss on 16q. From the deletion map (Fig. 3), the incidence of LOH was suggested to be 36% (28 of 78) at the MT2 locus on 16q13 and 38% or higher (≥30 of 78) in the loci distal to MT2, although the deleted region differed among the cases with partial allele loss. In particular, LOH was most frequent in the 16q24.2-qter region between D16S43 or D16S155 and qter, containing the D16S7, APRT, and D16S44 loci. LOH on 16q24.2-qter was detected in 36 of the 38 cases, whereas in the exceptional two cases (cases 22 and 24) the commonly deleted regions were suggested to be located between the D16S32 locus at 16p13.1 and the D6S4 locus on q22.1 (Fig. 3).

LOH on the short arm of chromosome 16 was detected in 17 (23%) of 73 tumors in which constitutional heterozygosity was seen at one or more loci. Sixteen of the 17 tumors showing allele loss on the short arm also showed allele loss on the long arm.

Frequent Loss of Heterozygosity on Chromosome 16q24-qter Irrespective of Differences in Clinico pathological Parameters. In total, 127 (55%) of 230 tumors, including the cases for which mapping was performed, revealed LOH on chromosome 16q. Infor-
ALLELE LOSS ON CHROMOSOME 16q IN BREAST CANCER

In six tumors, LOH was detected not at the loci on 16q24.2-qter but at the CETP, D16S4, HP, and/or TAT loci in more proximal regions.

As shown in Table 2, the incidence of LOH on 16q24.2-qter was \( \geq 35\% \) in any group with respect to clinical stage, lymph node status, tumor size, histological grade and type, or estrogen receptor status. The incidence of LOH on 16q24.2-qter was relatively lower in the intraductal/predominantly intraductal group (8 of 27, 35%) than in the invasive group (110 of 198, 56%) \( (P < 0.005) \). In intraductal/predominantly intraductal carcinomas, the incidence of LOH was not significantly different among the grade 1 (5 of 16, 31%), grade 2 (1 of 6, 17%), and grade 3 (2 of 5, 40%) subgroups and, thus, between comedo and non-comedo types. The incidence was commonly high both in histological types with a favorable prognosis, i.e., mucinous, papillary, and tubular, and in those with an unfavorable prognosis, i.e., invasive ductal carcinomas of the “not otherwise specified” type and of the atypical medullary type (25) (Table 2). Among the variety of histological types, LOH on chromosome 16q24.2-qter was especially frequent in the papillary (77%) and invasive lobular (75%) types.

The eight cases in total, including cases 22 and 24, that showed LOH on 16q but not at 16q24.2-qter comprised six grade 3 cases and two grade 2 cases. This group did not contain cases of intraductal/predominantly intraductal carcinoma, at stage I, with a tumor size of \( \leq 2.0 \) cm, or of grade 1, but contained two cases without lymph node metastasis.

DISCUSSION

In the present study, we analyzed commonly deleted regions in 38 breast cancer tissue specimens which showed deletion of chromosome 16q in at least one locus, using 18 polymorphic DNA markers. The deletion map of chromosome 16 clarified that the region deleted most frequently in breast cancer was q24.2-qter (Fig. 3). This result was confirmed by the study of the second series of cases, in which 118 tumors in total carried LOH on 16q24.2-qter. An as yet undetermined tumor-suppressor gene was thus strongly suggested to be present in this region. To specify further the commonly deleted region and to identify accurately the unknown tumor-suppressor gene locus, it will be necessary to obtain much finer mapping data from the cancer cases showing the partial deletion.
This chromosomal region seemed to be somewhat different from those reported previously by us for hepatocellular carcinoma (12) and by Sato et al. (5) for breast cancer, in which the commonly deleted chromosomal region was shown to lie on 16q22-q23. The difference was not considered to reflect data inconsistency among the studies. The frequent occurrence (>36%) of LOH at any loci on the long arm distal to 16q12, including the region between the MT2 and D16S44 loci, suggests that multiple tumor-suppressor genes, other than the putative one located on 16q24.2-qter, may exist on 16q.

Table 2 Association of loss of heterozygosity on chromosome 16q24.2-qter with clinical and histological parameters of breast cancer

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>Total</th>
<th>16q24.2-qter</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>53</td>
<td>23 (43)</td>
<td>NS</td>
</tr>
<tr>
<td>II</td>
<td>122</td>
<td>68 (56)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>50</td>
<td>27 (54)</td>
<td></td>
</tr>
</tbody>
</table>

No. of metastatic lymph nodes

<table>
<thead>
<tr>
<th>No.</th>
<th>Total</th>
<th>LOH (16q24.2-qter)</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>127</td>
<td>64 (50)</td>
<td>NS</td>
</tr>
<tr>
<td>1-3</td>
<td>40</td>
<td>24 (60)</td>
<td></td>
</tr>
<tr>
<td>4-9</td>
<td>29</td>
<td>15 (52)</td>
<td></td>
</tr>
<tr>
<td>≥10</td>
<td>20</td>
<td>15 (56)</td>
<td></td>
</tr>
<tr>
<td>Not resected</td>
<td>3</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Tumor size on palpation

<table>
<thead>
<tr>
<th>Size (cm)</th>
<th>Total</th>
<th>LOH (16q24.2-qter)</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤2.0</td>
<td>59</td>
<td>26 (44)</td>
<td>NS</td>
</tr>
<tr>
<td>2.1-5.0</td>
<td>127</td>
<td>74 (58)</td>
<td></td>
</tr>
<tr>
<td>&gt;5.1</td>
<td>39</td>
<td>18 (46)</td>
<td></td>
</tr>
</tbody>
</table>

Histological grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>Total</th>
<th>LOH (16q24.2-qter)</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>23 (50)</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>83</td>
<td>42 (51)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>96</td>
<td>53 (55)</td>
<td></td>
</tr>
</tbody>
</table>

Histologic type

<table>
<thead>
<tr>
<th>Type</th>
<th>Total</th>
<th>LOH (16q24.2-qter)</th>
<th>&lt;0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraductal, predominantly</td>
<td>27</td>
<td>8 (35)</td>
<td></td>
</tr>
<tr>
<td>invasive ductal, NOS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>125</td>
<td>63 (50)</td>
<td></td>
</tr>
<tr>
<td>Invasive ductal, atypical</td>
<td>18</td>
<td>9 (50)</td>
<td></td>
</tr>
<tr>
<td>medullary</td>
<td>20</td>
<td>15 (75)</td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>13</td>
<td>10 (77)</td>
<td></td>
</tr>
<tr>
<td>Tubular</td>
<td>13</td>
<td>8 (62)</td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
<td>7</td>
<td>3 (43)</td>
<td></td>
</tr>
<tr>
<td>Medullary, pure</td>
<td>2</td>
<td>2 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Estrogen receptor

<table>
<thead>
<tr>
<th>Protein</th>
<th>LOH (16q24.2-qter)</th>
<th>&lt;0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥13</td>
<td>151</td>
<td>84 (57)</td>
</tr>
<tr>
<td>&lt;13</td>
<td>40</td>
<td>20 (50)</td>
</tr>
<tr>
<td>Not measured</td>
<td>34</td>
<td>14 (41)</td>
</tr>
</tbody>
</table>

* Statistical significance was calculated by χ² test. NS, not significant.
* NOS, not otherwise specified.

In hepato-cellular carcinoma, LOH on chromosome 16q has been shown to occur frequently in tumors in advanced clinical stages and of poorer differentiation, but not in those in early stages of progression (12). Furthermore, in hepato-cellular carcinoma the incidence is significantly higher in highly metastatic tumors (12). However, in breast cancer the incidence of LOH on chromosome 16q was high irrespectively of differences in clinical stage, lymph node status, tumor size, histological grade and type, or estrogen receptor status. LOH on chromosome 16q was frequent even in intraductal/predominantly intraductal carcinomas, in the group where histological grades and types indicated a favorable clinical outcome, and in cases without metastasis. The gene alterations reported previously, i.e., amplification of c-erbB-2, hst-1/int-2, and c-myc, genes, mutation of the p53 tumor-suppressor gene and its aberrant protein, and LOH on chromosomal arms 7q, 11p, and 17p, are all reported to be associated with aggressive clinical and/or histological characteristics in breast carcinoma (1, 3, 6, 24, 27, 29-32). We showed previously that alterations of the c-erbB-2 and p53 genes and their proteins were frequently detectable in breast cancers showing the highest histological grade of atypia (grade 3) but rare in those showing low or moderate histological grades of atypia (grades 1 or 2) (24, 27, 31). In contrast, LOH on chromosome 16q was shown to be frequent not only in grade 3 but also in grade 1 and 2 cancers. Therefore, LOH on 16q was strongly suggested to occur commonly in breast cancer at a very early developmental stage, not only among highly aggressive but also in relatively low-grade tumors. Alteration of chromosome 16q is suggested to play a special role in the genesis of breast cancer irrespectively of the grade of biological aggressiveness, whereas other gene alterations, e.g., oncogene amplification, p53 mutation, and LOH on other chromosomal arms, are suggested to be involved in the development of various aggressive biological and morphological characteristics, e.g., a high proliferation rate, marked nuclear atypia, and/or dedifferentiation.

Eight of the present cases composed a group which showed LOH on 16q but not on 16q24.2-qter. This group consisted mainly of tumors in relatively advanced stages, with aggressive phenotypic characteristics. The region commonly deleted in these eight cases was suggested to be 16cen-q22.1. This result and the consistently frequent occurrence of LOH on the long arm distal to 16q12 suggest that, in addition to the putative gene on 16q24.2-qter, additional multiple tumor-suppressor genes may exist within this region and their inactivation may be involved in the formation of biologically aggressive phenotypes of breast cancer. Particularly, on a percentage basis, the D16S4 locus was most frequently deleted, showing LOH in 57% of the cases and in 16 of 17 tumors with LOH on 16q, with the one tumor (case 24) showing...
retention at this locus having a breakpoint relatively near. In the area of the D16S4 locus, probably in a more proximal region, there may be a gene which is affected at the same or higher frequency, compared to the putative, more distal gene.

Linkage analyses have suggested the presence of a gene locus on chromosome 16q that confers susceptibility to breast cancer (8, 33). Therefore, alteration of the unknown gene on 16q is suggested to be involved not only in the genesis of sporadic breast cancers but also in susceptibility to and/or genesis of the familial form.

ACKNOWLEDGMENTS

We are grateful to Drs. T. Nanasawa and H. Yamamoto, Department of Surgery, National Cancer Center Hospital, for providing surgically resected tissue specimens and to Y. Tanaka for excellent technical assistance. The authors are grateful to Drs. G. Scherer, D. Drayna, J. Arrand, and G. I. Bell for kindly providing DNA probes. DNA probes were also provided by the American Type Culture Collection (Rockville, MD) and Collaborative Research Inc. (Bedford, MA).

REFERENCES

Allele Loss on Chromosome 16q24.2-qter Occurs Frequently in Breast Cancers Irrespectively of Differences in Phenotype and Extent of Spread

Hitoshi Tsuda, David F. Callen, Takashi Fukutomi, et al.


Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/54/2/513](http://cancerres.aacrjournals.org/content/54/2/513)

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.