Identification of Three Regions on Chromosome 17q in Primary Human Breast Carcinomas Which Are Frequently Deleted

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Abstract

We have examined the long arm of chromosome 17 in sporadic breast carcinomas for the loss of heterozygosity (LOH) at 18 polymorphic loci. At least three distinct regions could be identified by the frequency of LOH and confirmed by high density deletion maps of individual tumor DNAs. A proximal region affected by LOH is located in a 22-cM region defined by D17S73 and NME1 and thus is similar in location to the region thought to contain the BRCA1 gene associated with familial breast and breast/ovarian cancer. The central region affected by LOH is bordered by the D17S86 and D17S21 loci and is estimated to be 28 cM in size. The third region is bordered by the D17S50 and D17S77 loci which are 11 cM apart. These results define three independent regions of chromosome 17q which are likely to contain tumor suppressor genes relevant to the etiology of sporadic breast carcinoma.

Introduction

Several studies confirm the importance of genetic alterations to the development of breast carcinoma (1). We and others have been searching for tumor suppressor genes relevant to breast cancer by screening primary breast tumor DNA samples for LOH. According to Knudson’s “two-hit” hypothesis for the inactivation of a tumor suppressor gene, one allele contains a point mutation or a small deletion, while the second allele is lost by an interstitial deletion, chromosome loss, or an aberrant mitotic recombination event (2). Chromosome 17 contains several genes important to the development and/or progression of human breast cancer. AlREADY identified are the TP53 tumor suppressor gene on chromosome 17p13.1 (3, 4) and the ERBB2 gene on 17q (5, 6). We have previously shown a high frequency of LOH on chromosome 17q that was associated with estrogen receptor-negative tumors (7). Recently, several groups have published evidence for a gene on 17q linked to familial breast and breast/ovarian cancers (8–10). This gene, named BRCA1, maps to 17q21. An initial candidate tumor suppressor gene in this region was NME2 or NME1 (11). We found the NME1 locus deleted in 64% of sporadic breast cancers (12). However, no mutations were found in the coding sequence and thus excluded it as the BRCA1 gene. Others (13) have also excluded NME1 as a candidate for BRCA1. We have continued to further define the regions of chromosome 17q which might contain tumor suppressor genes relevant to breast cancer by screening tumor DNA samples for LOH. Here we describe our results with 18 polymorphic loci on chromosome 17q. A high density deletion map has been constructed, and the results are consistent with at least three independent regions which are affected by LOH.

Materials and Methods

Primary breast carcinomas and matching peripheral lymphocytes were collected at the Centre Rene Huguenin in Saint Cloud, France, from patients who had received no prior therapy. The distribution of clinical and pathological parameters associated with the tumors and patients has been published (14).

Genomic DNA was extracted, and 10 μg was digested with the restriction enzyme of choice. The digested DNA was fractionated by agarose gel electrophoresis, transferred to Genatran 45 nylon membranes (Plasco, Woburn, MA), and baked for 2–3 h at 80°C. The membranes were prehybridized and then hybridized with 32P-labeled DNA probes made by the nick-translation or random-primer system. The DNA probes and the polymorphic restriction sites used are listed in Table 1 in their published chromosomal order (15, 16). After hybridization, the membranes were washed under stringent conditions (15 mm NaCl-1.5 mm sodium citrate, pH 7, at 65°C for 20 min) and autoradiographed.

Results and Discussion

The frequencies of LOH detected at 18 different loci on chromosome 17q in our panel of sporadic breast carcinoma DNAs is summarized in Table 1. Based on the frequency of LOH at individual loci, chromosome 17q may be divided into at least three independent regions which are affected by interstitial deletions. The borders of these regions can be defined by the genotypes of individual tumor DNAs. Fig. 1 shows examples of Southern analysis that depict partial deletions of chromosome 17q. Panel A shows examples of interstitial deletions in the proximal region. Tumor DNAs 132 and 134 are informative and unaffected at D17S73 (LEW207) but have deletions of the lower allele for D17S41 (LEW102) and NME1, respectively. Tumor 132 is informative at D17S86 (CMM86) while tumor 134 is not. Tumor DNA 303 lost the upper allele at EPB3 but retained both alleles of the NME1 and D17S41 (LEW102) loci. Tumor 307 is deleted for the lower allele of NME1 but informative for D17S86 (CMM86) and uninformative for D17S73 (LEW207). Based on these data and data summarized in Fig. 2, we conclude that a target gene(s) for LOH is located in the 22-cM region defined by D17S73 and NME1 (15, 16). It is pertinent that recent genetic linkage studies also indicate that the familial breast and breast/ovarian cancer locus (BRCA1) is located centromeric to NME1 on chromosome 17q21, raising the possibility that this locus is also affected in sporadic breast cancer (13).

A second region of chromosome 17 that is affected by LOH is centered around the D17S40 locus. Fig. 1B illustrates two examples of interstitial deletions in this region (tumor DNAs 385 and 600). Both tumors are informative at D17S86 (CMM86) and TK1. Tumor 385 is deleted for the lower alleles of both D17S40 (LEW101) and D17S21 (c63), whereas tumor 600 is deleted for the lower allele at GH1 and the upper allele of D17S40 (LEW101). Taken together, these deletions point to the presence of a target gene within the 28-cM region lying between D17S21 and D17S86 (Refs. 15 and 16; summarized in Fig. 2).

In Fig. 1C are selections of interstitial deletions in the third or distal region of chromosome 17q. Tumors 70 and 106 each have LOH for the lower allele at D17S4 (THH59) but are informative and unaffected.

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3 The abbreviation used is: LOH, loss of heterozygosity.

4 Unpublished results.
at D17S20 (c1–26), D17S77 (p128E1), D17S79 (AC256), and D17S24 (RMU3). Tumor 271 lost the upper allele of D17S4 (THH59) but retained heterozygosity at D17S24 (RMU3). These data and the data summarized in Fig. 2 suggest that a third potential target gene could be located in the region between D17S20 and D17S77 which are estimated to be 11 cM apart (15, 16).

Other more complex patterns of LOH were also observed in the tumor DNAs such as tumors 540, 307, 385, and 448 in which more than one region appears to be affected by LOH (Fig. 2). Moreover, it is possible there is yet another target region of deletions more telomeric located around RMU3 (tumor DNA 448; Fig. 2). However, most of the tumor DNAs deleted for D17S24 (RMU3) were also deleted for D17S4 (THH59). Thus it is not clear whether some of these deletions are nonspecific.

There were 12 tumors that may have lost the entire long arm of chromosome 17. An average of 5.5 (range, 3–10) 17q probes were tested on these 12 tumors, and all were either deleted or not informative. Of these 12 tumors, 11 had been tested for one or more markers on chromosome 17p. All but 3 of these 11 tumors were also deleted for the 17p probes, thus suggesting that if the long arm is deleted the entire chromosome may be lost.

Our findings of at least three independent regions on 17q affected by LOH in primary breast tumor DNA is consistent with other reports. Comparative genome fluorescent in situ hybridization shows a complex pattern of 17q deletions in breast tumors. In addition, two groups recently published evidence of separate regions on chromosome 17q affected by LOH in breast tumor DNAs. Cornelis et al. (17) examined 11 loci on 17q and divided it into two regions with the border at the GH1 locus, which is in our central region of LOH. The difference between our results and theirs may be explained by their use of only three markers distal to the growth hormone locus. Similarly, Saito et al. (18) have also shown two regions of LOH on 17q in breast cancers.

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**Table 1** Frequencies of LOH detected at 18 loci on chromosome 17q in sporadic breast carcinoma DNAs

<table>
<thead>
<tr>
<th>Probe name</th>
<th>Locus symbol</th>
<th>Enzyme</th>
<th>Total no. tumors</th>
<th>Allelic loss/informative case (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAW212</td>
<td>D17S117</td>
<td>PSTI</td>
<td>49</td>
<td>6/15 (40)</td>
</tr>
<tr>
<td>LEW207</td>
<td>D17S73</td>
<td>PSTI, HINDIII</td>
<td>82</td>
<td>10/27 (37)</td>
</tr>
<tr>
<td>PPY</td>
<td>D17S73</td>
<td>PSTI, HINDIII</td>
<td>35</td>
<td>5/20 (25)</td>
</tr>
<tr>
<td>EPB3</td>
<td>D17S73</td>
<td>PSTI, HINDIII</td>
<td>60</td>
<td>6/15 (40)</td>
</tr>
<tr>
<td>NME1</td>
<td>D17S73</td>
<td>PSTI, HINDIII</td>
<td>140</td>
<td>25/55 (45)</td>
</tr>
<tr>
<td>LEW102</td>
<td>D17S41</td>
<td>PSTI, TAQI</td>
<td>70</td>
<td>21/43 (49)</td>
</tr>
<tr>
<td>CMM86</td>
<td>D17S86</td>
<td>MSPI, BGLII</td>
<td>93</td>
<td>9/44 (20)</td>
</tr>
<tr>
<td>GH1</td>
<td>D17S40</td>
<td>BGLII</td>
<td>79</td>
<td>13/59 (22)</td>
</tr>
<tr>
<td>LEW101</td>
<td>D17S40</td>
<td>BGLII</td>
<td>38</td>
<td>10/23 (43)</td>
</tr>
<tr>
<td>c63</td>
<td>D17S40</td>
<td>BGLII</td>
<td>38</td>
<td>7/20 (35)</td>
</tr>
<tr>
<td>TK1</td>
<td>D17S40</td>
<td>BGLII</td>
<td>23</td>
<td>1/21 (5)</td>
</tr>
<tr>
<td>Cl–26</td>
<td>D17S40</td>
<td>BGLII</td>
<td>92</td>
<td>5/72 (7)</td>
</tr>
<tr>
<td>THH59</td>
<td>D17S41</td>
<td>TAQI, ECORI</td>
<td>83</td>
<td>24/63 (38)</td>
</tr>
<tr>
<td>p128E1</td>
<td>D17S41</td>
<td>TAQI, ECORI</td>
<td>54</td>
<td>11/30 (37)</td>
</tr>
<tr>
<td>TH17.26</td>
<td>D17S41</td>
<td>TAQI, ECORI</td>
<td>20</td>
<td>2/9 (22)</td>
</tr>
<tr>
<td>EFDS2</td>
<td>D17S41</td>
<td>TAQI, ECORI</td>
<td>11</td>
<td>1/2 (5)</td>
</tr>
<tr>
<td>AC256</td>
<td>D17S41</td>
<td>TAQI, ECORI</td>
<td>21</td>
<td>2/9 (22)</td>
</tr>
<tr>
<td>RMU3</td>
<td>D17S41</td>
<td>TAQI, ECORI</td>
<td>69</td>
<td>27/59 (46)</td>
</tr>
</tbody>
</table>
Figure 2. Deletion map of representative tumors. The DNA probes are listed in the same chromosomal order as in Table 1 which is based on recent linkage analysis. Relevant tumors and their deletion patterns are shown which provide a summary of the three interstitial deletion regions. C retention of heterozygosity (i.e., an informative tumor).

The work described here expands our understanding of the genetic pathology of breast cancer by clearly defining the boundaries of three distinct regions on chromosome 17q. Because of the active research to identify and clone the hereditary BRCA1 gene, it may soon be possible to determine whether or not it is the same target gene as in our proximal region. However, the other two regions in the central and distal parts of chromosome 17q are newly characterized. The identification of their boundaries to a 28- and 11-cM region, respectively, enables the next step in their characterization. The availability of high density polymorphic microsatellite loci on chromosome 17q should help further localize and ultimately clone the putative target tumor suppressor genes.

References

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