Detailed Deletion Mapping of Chromosome 17q in Ovarian and Breast Cancers: 2-cM Region on 17q21.3 Often and Commonly Deleted in Tumors

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ABSTRACT

Using 11 restriction fragment length polymorphism markers, we examined loss of heterozygosity on the long arm of chromosome 17, where one or more genes responsible for hereditary breast and ovarian cancers may be present, in sporadic forms of 94 ovarian and 246 breast cancers. Loss of heterozygosity was observed in 33 of 84 (39.3%) ovarian and in 88 of 214 (41.1%) breast cancers that were informative with at least one marker. Detailed deletion mapping of chromosome 17q in these cancers identified two distinct, commonly deleted regions. One was located between 17q12 and 17q21.3 and the other between 17q25.1 and 17q25.3. In breast cancers, the proximal commonly deleted region was between two loci defined by markers CI17-701 and CI17-730 at 17q21.3, which are 2.4 cM apart. This segment overlaps the region that includes the putative gene for hereditary breast and ovarian carcinomas. The results suggest that at least two tumor suppressor genes associated with sporadic ovarian and breast cancers are present on chromosome 17q and that one of them may be the same gene that is responsible for the hereditary form.

INTRODUCTION

The number of patients with breast and ovarian cancers, which are common malignancies in women, is increasing in many countries. We previously reported frequent allelic losses on chromosomes 4p, 6p, 7p, 8q, 12, 16q, 17q, and 19p in ovarian cancers (1), and chromosomes 3p, 11p, 13q, 16q, 17p, and 17q in primary breast cancers (2, 3). Because 17q alleles are lost frequently in both types of tumor, and this chromosomal arm appears to contain the gene responsible for hereditary forms of breast and ovarian cancers (4-13), 17q is considered to contain one or more genes that play a significant role during development and/or progression of primary cancer in both of these tissues.

As a first step toward identification of such gene(s), we have attempted to identify a region that is commonly deleted in a large number of tumors. To that end, we have screened loss of heterozygosity in sporadic forms of 96 ovarian and 246 breast carcinomas with 11 RFLP markers on the long arm of chromosome 17 and have constructed a deletion map of 17q. This map indicates that two distinct regions are commonly deleted in both types of cancer, and one of them overlaps the region already identified as containing a gene associated with hereditary forms.

MATERIALS AND METHODS

Materials. Tumor tissues were obtained at the time of surgery from 94 patients with ovarian cancer and 246 patients with primary breast cancer.

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The abbreviations used are: RFLP, restriction fragment length polymorphism; LOH, loss of heterozygosity.

Corresponding normal tissues or peripheral blood samples were obtained from each patient. The tumors were histopathologically classified according to the typing scheme of the Japanese Obstetrics and Gynecology Society (14), which is basically the same as the typing scheme for ovarian tumors recommended by the World Health Organization.

DNA Extraction from Tissues and Southern Blotting. DNA was extracted from tumors and from corresponding normal tissues or lymphocytes according to the method described previously (2). Each DNA preparation was digested with appropriate enzymes, electrophoresed in a 1.0% agarose gel, and then transferred to nylon membranes in 0.1 n NaOH-0.1 M NaCl, as described previously (2).

Probes. All probes used in this study are listed in Table 1. These markers had been physically localized to specific G-bands of chromosome 17 by fluorescent in situ hybridization (15). The linear order of CI17-701 and CI17-730 along the chromosome was determined by linkage analysis (16, 17).

Hybridization Conditions. After alkali transfer of the DNA, nylon membranes were neutralized in 2X standard saline citrate (0.15 M NaCl;0.015 M sodium citrate) and fixed by UV cross-linking at 120 mJ with Stratalinker (Stratagene, San Diego, CA) according to the instructions of the manufacturer. Prehybridization and hybridization were carried out in 7% polyethylene glycol 8000-10% sodium dodecyl sulfate with 200 μg/ml of human placental DNA or 100 μg/ml of salmon sperm DNA at 65°C overnight. The membranes were hybridized at 65°C for 16-24 h with 32P-labeled probes (specific activity >1 × 108 cpm/μg DNA) labeled using a random primer method. After hybridization, membranes were washed twice at 65°C with 0.1X standard saline citrate-0.1% sodium dodecyl sulfate and exposed for autoradiography to Kodak XAR film at -70°C. The membranes used were stripped in 0.1 n NaOH and were repeatedly rehybridized (2).

Definition of LOH. The signal intensity of the polymorphic alleles was quantified by a densitometer to ascertain loss of the hybridization signal. After DNA loading differences were corrected, the signal intensity of alleles of tumor tissue was compared with that of normal tissue. When signal reduction was >50%, it was counted as LOH.

RESULTS

LOH on Chromosome 17q in Ovarian Cancer. The frequencies of LOH at each of 11 RFLP loci are listed in Table 1. A total of 84 tumors were informative for at least one locus, and 33 (39.3%) of them showed LOH for at least one locus on chromosome 17q. Fig. 1a shows examples of Southern blot analyses that revealed partial deletions of chromosome 17q in three tumors. Tumors A40 and B6 lost one allele at the CI17-730 locus, while A40 retained both alleles at a more distal locus (CI17-507) and B6 retained alleles at a more proximal locus (CI17-316). On the other hand, tumor CI2 retained heterozygosity at the CI17-516 locus, although one allele at CI17-710 was lost. The results of LOH analyses of 17q in eight tumors showing partial or interstitial deletions are summarized schematically in Fig. 2a. Two distinct regions commonly deleted in ovarian carcinomas were identified; one was a region between CI17-316 (17q12-q21.1) and CI17-507 (17q21.3) and the other was distal to CI17-516 (q25.1). We compared the frequency of LOH with age of onset (postmenopausal or premenopausal) and with histopathological types. Although no difference in the frequency of LOH was observed among tumors with respect to the premenopausal or postmenopausal stage of the
patients (Table 2), the difference in the frequency of LOH among three histological types was significant (Table 3); 15 (60%) of 25 serous types showed LOH with at least one marker, while four of 14 mucinous types or one of 12 clear cell types revealed LOH (serous versus mucinous, \( P = 0.060 \); serous versus clear cell, \( P = 0.003 \) by Fisher's exact test). We have also examined the correlation between LOH of the 35 tumors show

**DISCUSSION**

We have described a detailed analysis of LOH at loci on chromosome 17q in ovarian and breast cancers, and have constructed deletion maps of tumors. Two distinct, commonly deleted regions were identified in primary tumors from either of these tissues, implying that two tumor suppressor genes for ovarian and breast cancers may be present on chromosome 17q. One of these genes lies between loci defined by markers CI17-701 and CI17-730; this segment corresponds to the region indicated by deletion mapping of ovarian cancers, but it is smaller.

Linkage analysis based on genotypes of 40 CEPH 3 generation families (16, 17) provided an estimated genetic distance of 2.4 cM between two markers CI17-701 and CI17-730 (lod score of 33.7), implying a physical distance of 2000–3000 kilobases. Because this region is suspected of containing a gene responsible for the early-onset type of familial breast cancer, we compared the frequencies of LOH among tumors developed in premenopausal versus postmenopausal patients. As shown in Table 2, no significant difference could be attributed to menopausal status.
DELETION ON CHROMOSOME 17 IN OVARIAN AND BREAST CANCERS

Table 2 Correlation of LOH at chromosome 17q21.3 with menopausal status

<table>
<thead>
<tr>
<th>Status</th>
<th>Frequency of LOH at chromosome 17q21.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian cancer</td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>7/20 (35.0)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>4/120 (35.3)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>6/17 (35.3)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>27/83 (32.5)</td>
</tr>
</tbody>
</table>

Table 3 LOH on chromosome 17q21.3 in serous versus nonserous ovarian carcinoma

<table>
<thead>
<tr>
<th>Histological type</th>
<th>LOH on 17q21.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss</td>
<td>Retained</td>
</tr>
<tr>
<td>Mucinous</td>
<td>4</td>
</tr>
<tr>
<td>Serous</td>
<td>15</td>
</tr>
<tr>
<td>Clear cell</td>
<td>1</td>
</tr>
</tbody>
</table>

* Calculated by Fisher's exact test.

CI17-516 and CI17-710 at 17q25.1–25.3. The other locus, lying between CI17-701 and CI17-730 at 17q21.3 in breast cancers, falls within a larger region commonly deleted in ovarian cancers (between CI17-316 and CI17-507 at 17q12–21.3). The two loci flanking this 2.4-cM proximal common region of deletion have shown close genetic linkage in 40 CEPH families to loci that are tightly linked to the putative tumor suppressor gene responsible for familial ovarian and breast cancers (4–13). Hence, it is likely that this region contains a gene responsible for both sporadic and familial forms of breast and ovarian cancers. However, although linkage has been demonstrated only in families with early-onset type of breast cancer, we saw no difference in the frequency of LOH in this region among sporadic tumors developed in premenopausal or postmenopausal patients.

In ovarian cancers, a significant difference in the frequency of LOH at 17q21.3 was observed among three histopathological groups; i.e., tumor of the serous type showed LOH more often than did mucinous or clear cell types. These results are similar to those we reported earlier for chromosome 6q, where LOH was frequently observed in the serous type but not in other histological types of ovarian cancers (1, 18). We suggest that these three types of tumor might have different etiological mechanisms and that tumor suppressor genes on 6q and 17q may be associated specifically with development or progression of the serous type of ovarian carcinoma.

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REFERENCES


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