Detection of Frequent Allelic Loss on Proximal Chromosome 17q in Sporadic Breast Carcinoma Using Microsatellite Length Polymorphisms

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Abstract

Analyses of losses of heterozygosity and linkage studies have implicated a gene(s) on chromosome 17q in the genesis of sporadic and early-onset familial breast carcinomas, respectively. To define the critical region of 17q, we examined DNAs from a series of 20 sporadic breast carcinomas and corresponding blood samples for allelic losses of chromosome 17q using microsatellite length polymorphisms. With these highly informative markers (average heterozygosity, 0.73), we observed frequent deletions of 17q at several loci. We found that D17S250 was deleted in 50% (7 of 14), THRA1 in 79% (11 of 14), D17S579 in 59% (11 of 19), NME1 in 29% (5 of 17), MPO in 36% (4 of 11), and GH in 25% (4 of 16) in the tumor set examined. A common region of deletion was found that was flanked by D17S250 to D17S579. These markers have recently been localized to a 6-CM interval of proximal chromosome 17q in bands 17q11.2-q21 and map within the region of the early-onset familial breast cancer locus, implying that the same gene or genes may be involved in both sporadic and familial breast tumors. Thyroid hormone receptor α and retinoic acid receptor α are two potential candidate genes in this region.

Introduction

The molecular basis of breast cancer is rapidly being defined. Although certain oncogenes are amplified and/or overexpressed, the involvement of multiple tumor suppressor genes seems to be important in these cancers (1). Several studies have demonstrated alterations in the tumor suppressor genes RB1 and TP53 (1, 2). Other regions of the genome have been implicated by cytogenetics and loss of heterozygosity studies including chromosomes 1, 6q, 11p, 16q, 17q, and 18q (3–7). Nonrandom chromosomal deletions and losses of heterozygosity of a segment of the genome are considered indicative of the presence of a tumor suppressor gene in that region. Recent reports have demonstrated the presence of a locus predisposing to early-onset familial breast cancer (BRCA1)3 and familial breast-ovarian cancer on 17q by linkage analysis of affected families (8–10). Loss of heterozygosity on 17q in breast carcinomas has also been reported, with the percentage of informative cases showing loss ranging from 20 to 64% (3–7, 11). We have initiated a detailed study of allelic loss in sporadic breast carcinomas with markers on chromosome 17q in order to map the critical region deleted and to determine the possible overlap of this region with the BRCA1 locus. The assessment of allelic loss has been limited by the position and frequency of heterozygosity when traditional restriction length polymorphisms are used. Given this, we chose a novel approach based on DNA amplification of microsatellite length polymorphisms using the PCR because of their high informativity, wide distribution, speed of typing, and minimal sample requirements.

Materials and Methods

Tissue Samples. Primary breast carcinoma tissue was obtained at surgery, immediately flash frozen, and stored at −120°C. Peripheral blood leukocytes were obtained from each patient as a source of normal DNA. DNA was prepared according to standard protocols or by using an ABI automated DNA extractor (Applied Biosystems, Inc.).

PCR Analysis of Microsatellites. Primers used for the analysis of microsatellite polymorphisms are given in Table 1 and were used in assays at a concentration of 1.0 µM. PCRs were done with 50 ng of genomic DNA in 20-µl volumes using Cetus buffers and AmpliTag DNA polymerase (Perkin Elmer Cetus) at 0.5 unit/reaction with dTTP, dCTP, and dGTP at 200 µM; dATP at 2.5 µM; and [α-32P]dATP (3000 Ci/mmol; Amersham) at 1.00 µCi/reaction. Reaction conditions consisted of 1 min at 94°C, 2 min at 55°C, and 1 min at 72°C for 27 cycles, followed by a final extension for 7 min at 72°C. An (AAAAT)n repeat in the TP53 gene was analyzed as described previously (12). Reaction products were diluted 1:2 in loading buffer (90% formamide, 10 mM EDTA 0.3% bromophenol blue 0.3% xylene cyanol), heated at 90°C for 5 min, and loaded (2 to 5 µl) onto 5% polyacrylamide sequencing gels. After electrophoresis, gels were dried at 80°C and exposed to X-ray film from 4 h to overnight.

Gene Amplification. Amplification of both the ERBB2 and THRA1 genes was determined as described previously (13). The probes used were pMAC117 (ERBB2), pH-EA1 (THRA1), and pHM2A (c-mos, used as a loading control) that has been mapped to 8q11. Probes were obtained from the American Type Culture Collection.

Results

Using a panel of 6 microsatellites (Table 1) that span a 30-cM interval of chromosome 17q (10), we examined a set of 20 matched blood/breast carcinoma pairs for losses of heterozygosity. Allelic losses were scored as decreases in intensity of one allele relative to the other, determined from visual comparison of tumor to normal allele intensities (3, 5). Incomplete loss was commonly observed and may reflect either normal tissue contamination or tumor heterogeneity (3). Representative results for three microsatellites are shown in Fig. 1. The proximal region of 17q showed the highest frequency of deletion (Fig. 2), with losses at THRA1 peaking at 79% (11 of 14 informative cases).

Several tumors had losses suggestive of nondisjunction, although not being informative at every marker typed (tumors 5, 13, 14, 17, and 20; see Fig. 2). One tumor was deleted for every
informative marker on the q arm examined while remaining heterozygous for TP53 (tumor 19). Other tumors clearly showed interstitial losses due to deletion or somatic recombination (tumors 2, 3, 4, 6, 7, 8, 9, 10, 15, and 16). A common region of deletion, flanked by D17S250 and D17S579, was observed.

The p53 gene was examined for allelic loss using a related PCR assay based on a (AAAAT)_n repeat at the 3′ end of an Alu element in the first intron (12). Fifty-five % of informative cases (6 of 11; tumors 3, 6, 7, 10, 11, 14) showed loss of one p53 allele, while tumors 1, 8, 9, 12, and 19 retained heterozygosity for the gene (Fig. 2).

The ERBB2 gene was found to be amplified in 30% (6 of 20, 2- to 32-fold amplification) of the cases examined, which is consistent with previous studies (13). Coamplification of ERBB2 and THRA1 has been reported previously (14) and was found in 50% (3 of 6) of the cases with amplification. Gene amplification, although a potential problem in allelic loss analysis using microsatellites (see "Discussion"), did not appear to explain the losses observed in this study because the majority of 17q losses observed, consistent with a tumor suppressor gene in this region. Chromosome 17 linkage maps place THRA1 distal to D17S250 and proximal to D17S74/GH with the gene being mapped in somatic cell hybrids to q11.2-q12 (15). THRA1 has been placed between the t(1;17) NF1 translocation breakpoint and the t(15;17) APL breakpoint by somatic cell hybrid analysis (15). Our analysis defined a minimum deletion unit flanked by D17S250 on the centromeric side and D17S579 on the telomeric side that Hall et al. (10) have shown spans a 6-cM interval (Fig. 3). The placement of THRA1 in this smallest region of overlap is supported by the linkage mapping of ERBB2 2 cM distal to D17S250 and 4 cM proximal to D17S579 (10). Since THRA1 and ERBB2 are sometimes coamplified, these genes are likely to lie in relatively close proximity physically, placing THRA1 between D17S250 and D17S579. ERBB2 has also been mapped between the NF1 and APL translocations (15), lending further support for the placement of THRA1. These data define a relatively small region of distal band 17q11.2 to band q12 as the location of THRA1 and, therefore, the likely region of the breast cancer tumor suppressor gene we have mapped by analysis of allelic losses.

The TP53 gene showed allelic loss in 55% of the informative tumors. Analysis of which tumors lost heterozygosity on the p and q arms demonstrated that simple nondisjunction could not account for the majority of 17q losses observed, consistent with the original findings of Cropp et al. (3). Likewise, the patterns of ERBB2 amplification and coamplification of THRA1 could not account for the observed losses. Gene amplification has the potential to perturb microsatellite-based analysis of allele loss in tumors where incomplete losses are frequently observed. Increased copy number of only one allele of a polymorphic marker would mimic partial loss at that locus. Only two cases (tumors 3 and 9) could be explained by gene amplification at the THRA1 locus, but this seems unlikely because multiple markers showed allele loss in these tumors. Although the size

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Table 1 Microsatellite loci used in this study

<table>
<thead>
<tr>
<th>Locus</th>
<th>Position</th>
<th>Primer sequences</th>
<th>Ref.</th>
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<td>TP53</td>
<td>17p13.1</td>
<td>GCACTTTTCTCTAAACACATCA AAGCGTCCCTTAAATGGCCAG AL3:CCA(C/T)TGCATCCAGGCTGGG</td>
<td>12</td>
</tr>
<tr>
<td>D17S250</td>
<td>17q11.2-q12</td>
<td>GGAAGATCAAATAGCAAT GCTGGCATTATATAATATTTAACC</td>
<td>20</td>
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<td>THRA1</td>
<td>17q11.2-q12</td>
<td>CTGGCGTTGGCACTATTGGG CGGGCAGCATACGTGGCCT</td>
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<tr>
<td>D17S579</td>
<td>17q12-q21</td>
<td>AGTCTCTAGACAAAAACCT CAGTTTCAACTACAGTCTTC</td>
<td>10</td>
</tr>
<tr>
<td>NME1</td>
<td>17q21.3-q22</td>
<td>TTGGACCGGGGTAGAAACTCT TCTCAGTACTCCCGTGACC</td>
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<tr>
<td>MPO</td>
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<td>TCCAGAGTCCGCTCTACAGA CACAGCTCAGAAGTGACAGCAG</td>
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<tr>
<td>GH</td>
<td>17q23</td>
<td>TCCAGCCTCGGAGAAGCAAT AGTCTTTTCTCCAGAAGCAGT</td>
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</table>
larger set of tumors will need to be examined to fully investigate any correlation of these parameters or other clinical factors.

Recently a locus for early onset familial breast cancer has been linkage mapped to 17q (8, 10). Likewise, a gene for familial breast-ovarian cancer has also been mapped to this region (9). The BRCA1 locus (17q12-q21) overlaps with the common deletion region we have mapped in sporadic tumors. This suggests the possibility that the same gene or genes may be involved in the genesis of both sporadic and familial tumors. Our results argue against NME1 as a candidate sporadic breast cancer gene and are likely to exclude the HOX2 cluster, the prohibitin gene, and WNT3 based on current gene assignments (10, 16). Further fine mapping of these genes may definitively rule them out as potential candidates. Based on the hypothesis of ERBB2 amplification has not been determined, amplification would have to extend at least 6 cM to account for our findings in tumor 3. A pulse field gel electrophoresis study is currently under way to determine the physical distance separating THRA1 and ERBB2.

No obvious correlation between either TP53 loss or ERBB2 amplification and 17q loss was observed, although a much

![Fig. 1](image1.png)
Fig. 1. Representative autoradiographs showing allele loss on 17q. DNA pairs from normal (N) and tumor (T) tissue were assayed as described in “Materials and Methods.” Allele losses were scored for a reduction in intensity of one allele relative to the other in tumor versus normal samples. A, D17S250. Loss of the upper allele in tumor 6 and loss of the lower allele in tumor 9. B, THRA1. Loss of upper allele in tumors 6, 7, 9, and 10. C, D17S579. Loss of upper allele in tumors 7 and 10.

![Fig. 2](image2.png)
Fig. 2. Summary of allele loss data in the tumor set. Open circles, informative, no loss; closed circles, informative, loss; hatched circles, not informative. ERBB2 and THRA1 amplification status are denoted by Footnotes a and b.

![Fig. 3](image3.png)
Fig. 3. Map of chromosome 17q showing placement of markers used in the study (10, 15, 16). The smallest region of overlap (SRO) of the deletions is shown. THRA1 maps close to but has not been ordered with respect to ERBB2 (see “Discussion”).
that the sporadic breast cancer gene is allelic with the \textit{BRCA1} locus, the \textit{THRA1} gene becomes a strong candidate tumor suppressor gene. The potential role of \textit{THRA1} as a tumor suppressor gene was recently reviewed by Weinberg (17). Intriguingly, Ali \textit{et al.} (18) described a common deletion region for breast carcinoma on chromosome 3p21-p25 that includes \textit{THRB}, a second thyroid hormone receptor gene. Retinoic acid receptor \(\alpha\), previously shown to be involved in neoplasia in the (t15;17) acute promyelocytic leukemia translocation (19), has also been mapped in this region of 17q (15) and is another good candidate gene. We are currently examining these genes for mutations in sporadic tumors and breast carcinoma cell lines.

In any event, the \textit{THRA1} microsatellite polymorphism will provide a very useful marker for familial breast cancer linkage analyses, as well as searching for critical recombinants in breast cancer pedigrees to further localize the \textit{BRCA1} locus. It may also be informative to examine tumors from affected family members for allelic losses in this region, using \textit{THRA1} and other markers to determine if the \textit{BRCA1} locus behaves as a genetic recessive as in the retinoblastoma paradigm.

Our results have shown that microsatellites are an extremely useful resource for investigating losses of heterozygosity and should be applicable to allelotyping as well as regional mapping of deletions in specific chromosomal regions. Our experience with breast cancer clearly indicates the general applicability of microsatellite typing to allele loss studies in a wide variety of tumor types.

Acknowledgments

The authors gratefully acknowledge Mary-Claire King for kindly providing us with information on marker order and distance prior to publication. We thank James Weber and Donny Black for providing \textit{D17S579} and \textit{NME1} primer sequences, respectively. We also thank Ian Jacobs for critical review and helpful discussion. We are grateful to Nancy Glover for technical assistance.

References

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