Definition and Refinement of Chromosome 11 Regions of Loss of Heterozygosity in Breast Cancer: Identification of a New Region at 11q23.3


Chromosome 11 is frequently altered in several types of human neoplasms. In breast cancer, loss of heterozygosity has been described in two regions of this chromosome, 11p15 and 11q22–23. In this report we have dissected the two regions using high-density polymorphic markers, and have found that there are at least two independent areas of loss of heterozygosity in each region, suggesting that multiple genes on chromosome 11 may be targets of genetic alteration during tumor establishment or progression. The regions defined are: at 11p15, between loci D11S576 and D11S1310 and between D11S988 and D11S1318; at 11q23, between D11S2000 and D11S897 and between D11S528 and D11S990. The narrowing of these regions of loss should facilitate the cloning of the regions in yeast artificial chromosomes to identify the critical tumor suppressor genes.

Introduction

Breast cancer is the most frequent neoplasm in women from Western countries (1). Although several genetic alterations have already been identified (2), clinical applications have thus far been scarce. For most of the genetic abnormalities identified, no genes or mechanisms of action have been discovered. The lack of this essential information is a major limitation to the development of clinical applications.

Loss of genetic material, identified by LOH, is the most frequent genetic alteration found in solid tumors, and breast cancer is no exception to this rule (2, 3). These findings have suggested that restoration of wild-type tumor suppressor genes, defective in tumor cells, might reestablish control over the malignant phenotype, a suggestion that has been supported by several laboratory models: transfection of specific tumor suppressor genes (4, 5) or transfer of chromosomes or subchromosomal fragments (6, 7) could indeed suppress tumorigenicity of human tumorigenic cell lines. These findings have strengthened the suggestion that tumor suppressor genes may eventually be useful in gene therapy of human cancer.

Human chromosome 11 has long been known to be involved in various types of human neoplasms (8). In breast cancer, regions 11p15 (9–12) and 11q22–23 (13, 14) were shown to be frequently affected by LOH, and microcell-mediated chromosome transfer of human chromosome 11 to cells of the MCF-7 breast cancer cell line inhibited tumorigenicity. Also, these microcell-mediated chromosome transfer studies indicated that two regions, one on the short arm and one on the long arm, could affect the tumorigenic behavior of MCF-7 cells (15). Thus, in breast cancer, genetic and biological studies have revealed that at least two regions of chromosome 11 harbor putative tumor suppressor genes. However, no “bona fide” tumor suppressor gene has yet been isolated from either region. Positional cloning efforts to identify these genes have been limited by the complexity of region 11p15 and by the large size, about 20 cm, of the minimal region of LOH at 11q23.

In this study we sought to refine the minimal regions of LOH to establish the basis for positional cloning approaches to the identification of the critical breast tumor suppressor genes of chromosome 11.

Materials and Methods

Tumor Samples. The series of matched tumor:normal specimens used in the course of this study has been described previously (13).

DNA Probes. With the exception of the new 11p15 YAC-derived microsatellites markers listed in Table 1, which were described and mapped in a separate report (16), all other markers were obtained from information available through the Genome Data Base, and exact locations are based on the recently developed radiation hybrid map for human chromosome 11 (17).

LOH Analysis. Microsatellite genotyping was performed as described previously (13). Results were analyzed by densitometric film scanning using a computerized Molecular Dynamics system. Quantification was performed using the program ImageQuanNT. The relative ratio of tumor alleles and normal alleles was determined, normalized, and then compared: when the allele ratio in tumor DNA differed more than 30% (D ≤ 0.30, where D equals difference) from the ratio of alleles in normal DNA, LOH was scored. The possibility that the allelic imbalance was due to gene amplification instead of LOH was ruled out by Southern blot analysis with probes for loci IGF-2 (11p15.5), D11S146 (11q12–13.1), CCND-1 (11q13), FGF-3 (11q13), ALL-1 (11q23), ETS-1 (11q23), and APOC-3 (11q23) as described previously (13).

Statistical Analysis. Possible correlations between clinical parameters and chromosome 11 genotypes were assessed using the χ² test with Yates correction.

Results

Sixty nonselected malignant breast tumors were analyzed for LOH using 11 microsatellites from region 11p15, 1 (FGF-3) from 11q13 and 13 from 11q22–q24. In addition to known microsatellites, LOH analysis of region 11p15 was performed using new microsatellites isolated from YAC clones of the region. Table 1 shows the characteristics of five new microsatellites that were used in the course of this study.

Fig. 1 shows the results of LOH analysis performed on all of the tumor samples with all 25 markers used in the study. Some LOH data for loci TH, D11S860, FGF-3, D11S35, D11S29, and D11S528 are included from a previous report (13). Fig. 2 depicts primary data from some of the critical tumors, showing restricted areas of LOH; results that define the minimal regions of LOH are summarized in Fig. 3.

Two regions of LOH at 11p15 were identified. Case 4 defines the occurrence of an independent telomeric region of LOH in the...
Table 1  PCR primers

<table>
<thead>
<tr>
<th>Primer names</th>
<th>Locus symbol</th>
<th>Primer sequences</th>
<th>Observed heterozygosity</th>
<th>Allele length (bp)/detected frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F9CA-1.1/1.2</td>
<td>D11S2344</td>
<td>TCCCATGGATAGAGCGGAT/</td>
<td>0.60</td>
<td>139 (0.33);141 (0.28);143 (0.17);145 (0.02);151 (0.18);153 (0.02)</td>
</tr>
<tr>
<td>B7CA-1.1/1.2</td>
<td>D11S2346</td>
<td>GCTCTTCATCTTTAAATCC/</td>
<td>0.50</td>
<td>292 (0.22);294 (0.50);296 (0.28)</td>
</tr>
<tr>
<td>D2CA-2.1/2.2</td>
<td>D11S2347</td>
<td>TGCACAGGCTGCGAGATGA/</td>
<td>0.60</td>
<td>155 (0.41);157 (0.26);159 (0.07);161 (0.15);163 (0.11)</td>
</tr>
<tr>
<td>3C3TA-1.1/1.2</td>
<td>D11S2349</td>
<td>TGGCATCAGGTGAAGCTG/</td>
<td>0.60</td>
<td>196 (0.29);200 (0.24);202 (0.47)</td>
</tr>
<tr>
<td>D12CA-1.1/1.2</td>
<td>D11S2351</td>
<td>AGGAGTTCACTGGATCACT/</td>
<td>0.70</td>
<td>155 (0.33);157 (0.18);159 (0.33);161 (0.02);169 (0.12);171 (0.02)</td>
</tr>
</tbody>
</table>

* All STS markers contain a [CA]n repeat, with the exception of 3C3TA, which contains a [TA]n repeat.

telomeric portion distal to locus D11S1318. Case 39, which excludes locus D11S576, suggests that the minimal overlapping region of deletion is between loci D11S576 and D11S1318, with the exclusion of these two loci and centered around the locus D11S922. The size of this telomeric region is estimated to be 3–4 Mb (18). Overall, the frequency of LOH at this region is 20% (8/40) in this series of tumors. Case 54 defines a second region between loci D11S988 and D11S1318. This region overlaps with the region defined by Winquist et al. (12) in breast cancer. By comparing the two sets of data we should conclude that the minimal region is included between D11S988 and TH. The size of this second region is about 3 Mb (16, 18). The frequency of LOH detected at locus D11S860, located between these two loci, in this series of samples is 22% (8/34). A third region of LOH located between loci HBB and PTH was proposed by Ali et al. (9). Our study does not exclude this possibility. Case 19 shows LOH at locus D11S2349, which is centromeric to the retained locus D11S2351 located in the HBB area, suggesting a region of LOH centromeric to HBB. LOH frequency at D11S2349 in these samples is 26% (5/19). However, because we have not defined the centromeric boundary of this potential third LOH region, it could include the remaining entire short arm of the chromosome. Case 19 retains heterozygosity for loci of the long arm.

Two independent regions of LOH were also identified on the long arm of chromosome 11. Cases 10, 17, 24, 45, and 55 define a region of LOH between loci D11S576 and D11S2000. This region is within the previously recognized LOH region between loci D11S35 and D11S29/APOC3 (13, 14). Thus, these results represent a refinement of this LOH region. The size of this region is estimated to be less than 10 Mb (19, 20). The frequency of LOH detected in our cases at D11S1818 is 50% (14/28). A second previously unidentified region of LOH, distal to D11S528 and centromeric to D11S990, is identified by cases 14, 17, 25, and 59. The estimated size is about 20 Mb (17), and the frequency of loss at D11S1284 is 45% (10/22).

Microsatellite instability was detected only at locus D11S1318 in four tumors, and in only one case within a region of LOH. Genetic instability, as shown by microsatellite instability, did not increase the frequency of LOH.

Association between each region of LOH and various clinical parameters, including stage, lymph nodes metastasis, estrogen receptor, progesterone receptor, mitotic index measured by Ki67...
expression, as well as family history was not statistically significant (data not shown).

Discussion

This study demonstrated that multiple regions of human chromosome 11 are subject to LOH during breast cancer development. Overall, about 25% of the cases develop LOH at 11p15 and 40–50% at 11q23–q24. Each of these regions was further subdivided into at least two independent areas of LOH.

At 11p15, the areas of loss are bracketed by loci D11S576–D11S1318 and by TH-D11S988. In addition to these two p-terminal target regions, a previous report (9) and case 19 from our study...
suggest that a third area of LOH centromeric to the HBB locus exists. This finding is not unique, since multiple regions of LOH on the short arm of chromosome 11 have also been identified in lung cancer (21).

In addition to breast cancer, other types of tumors with LOH at the most telomeric region D11S576-D11S1318 are: lung (21, 22), Wilms’ tumor (23), and adrenal adenoma (24). The loss of this region in breast cancer was previously suggested by two studies (10, 11). Our study confirms the previous reports and provides a more accurate definition of the minimal area of LOH.

At region D11S988-TH, other neoplasms showing LOH include lung (21, 22), ovarian carcinoma (25), and rhabdomyosarcoma (7). Two types of chromosome aberrations within this region pinpoint the potential location of important genes. Inversions and translocations at chromosome band 11p15.5, characteristic of the Beckwith-Wiedemann syndrome and rhabdoid tumors, fall within this region (26, 27). A detailed molecular study of chromosomal loci in proximity to those rearrangements may identify a potential tumor suppressor gene, perhaps interrupted by the chromosome rearrangements. This gene could be important for neoplasms either associated or not with the Beckwith-Wiedemann syndrome. The second potentially interesting region may be deduced from the comparison of the results of this study and the analysis of LOH in lung cancer (21). Comparison of the data suggests that a region around the D11S12 locus might contain the critical tumor suppressor gene of the region.

A common region of LOH, which includes all the markers distal to HBB and therefore overlaps with both of the two identified regions of LOH, has also been detected in astrocytoma (28), hepatoblastoma (29), adenocortical carcinoma (30), and Wilms’ tumor (31). These studies do not clarify whether both regions play a role in these cancers. If we include also the region between HBB and PTH (9), chromosome 11p15 harbors three potential tumor suppressor genes.

A previous study has shown that the H19 gene has tumor suppressor activity on rhabdoid tumor cell lines (32). However, results from the present work suggest that the H19 gene does not play an essential role in breast cancer. H19 maps near D11S1318, and the two defined regions of LOH at 11p15.5 are unlikely to include the H19 gene. In addition, analysis of the expression of the H19 gene in the tumorigenic MCF-7 cells in comparison to the tumor suppressed MCF-7/H11 cells did not show any significant difference at the RNA level (data not shown).

At 11q23-q24, the minimal overlapping areas of LOH are defined by loci D11S2000-D11S897 and by D11S528-D11S990. The first LOH area is included within a previously identified LOH area, the second was previously unrecognized and represents a new area of LOH in breast cancer.

The region D11S2000-D11S897 is less than 10 Mb (19, 20). The overlap with the minimal region that includes the A-T locus is intriguing. The report of a 5-fold increased probability of breast cancer development in A-T heterozygotes (33), recently confirmed by two additional independent studies from Norway and Italy (34), along with the present study suggest that the A-T gene might indeed be the target of loss of function in the development of breast cancer.

The second telomeric region of loss on the long arm, between D11S528 and D11S990 is probably about 20 Mb in size (17). The region identified in cervical carcinoma (35) includes both of the two subregions identified in this work, and other types of tumors that have LOH at 11q22-q24, such as colon and ovary carcinoma, neuroblastoma, and melanoma, were not studied using high-density microsatellites, precluding the definition of the region of LOH in these neoplasms.

Recently, the ALL-1 gene, which maps at 11q23 and is involved in the development of acute leukemias, has been shown to be rearranged in a gastric carcinoma cell line (36). The finding of a molecular rearrangement in a solid tumor cell line, and the absence in the same cell line of a functional wild-type allele of the gene, suggested its role as a potential tumor suppressor gene. However, because this gene is not included in either of the two 11q23 LOH regions defined for breast cancer, these results seem to rule out a role for ALL-1 in breast cancer.

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The sizes of the regions of LOH identified at 11p15 may allow an approach to the identification of the critical tumor suppressor gene(s) by positional cloning strategies (37). The recently cloned array of YACs spanning a continuous contig of about 7 Mb of region 11p15, which spans part of the region D11S988-TH, may be used to isolate genes from this region (16).

The regions at 11q23–24 are still considerably large and need further refinement before an attempt to identify the potential tumor suppressor genes. However, the overlap with the A-T locus of the more centromeric LOH region suggests that the A-T region, which has been narrowed to less than 1 Mb (34), could be the location of a tumor suppressor gene inactivated in breast cancer. A YAC contig of 5 Mb at the A-T locus has been developed (38). When the A-T gene is identified, it should be tested to verify its involvement in breast cancer.

With the development of techniques to identify transcriptional units from large genome fragments, the identification of genes from the critical chromosome 11 regions should soon be feasible.

Acknowledgments

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References

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