Loss of Heterozygosity for Chromosome 11 in Primary Human Breast Tumors Is Associated with Poor Survival after Metastasis


Ludwig Institute for Cancer Research [R. W., G. M. H., I. N., W. K. C.]; Departments of Medicine [I. N., W. K. C.] and Family and Preventative Medicine [F. A. W.], Cancer Center [I. N., F. A. W.], and Center for Molecular Genetics [W. K. C.], University of California at San Diego, La Jolla, California 92093-0660; Departments of Medical Genetics [R. W., A. M.], Surgery [H. K.], Pathology [M. A.], and Oncology and Radiotherapy [R. W., G. B., P. J. T.], University of Oulu, FIN-90220 Oulu, Finland; and McDermott Center, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235-8591. [G. A. E.]

ABSTRACT

A common feature of the malignant progression of human tumors is loss of heterozygosity (LOH) for various regions of their genomes. Such events encompassing chromosomes 11p15 and 11q23 are frequent in human breast tumors. Here, we have analyzed genetic and clinical characteristics of a series of primary breast tumors in order to determine: (a) a more finely mapped estimate of the involved regions; (b) whether there is a relationship in the presentation of LOH between the two regions; and (c) whether a correlation exists between such LOH and any of the clinical parameters pertaining to each patient. We found that LOH for 11p15.5 and 11q23 occurred in 35 and 46% of the 86 primary breast carcinomas, respectively, but in none of the 10 benign tumors examined. The minimal region of LOH for 11p15 was in the approximately 2-megabase region between loci TH and D11S988. Twenty-nine percent of the tumors showed LOH simultaneously at both 11p15 and 11q23, 5% had LOH only at 11p15.5, and 15% had LOH only at 11q23. Among these genetic groups, clinical features such as tumor size, involvement of auxiliary nodes, histological subtype, tumor grade, estrogen/progesterone receptor status, and patient age were not markedly different. However, LOH of 11q23 (either alone or in conjunction with LOH of 11p15) in the primary tumor was found to be highly predictive of aggressive postmetastatic disease course with substantially reduced survival (P = 0.0004; log rank test). We also observed a slight trend toward a more rapid development of metastatic lesions, without obvious site specificity, in patients with primary tumors showing LOH for chromosome 11 in the pathogenesis of human breast cancer; we suggest that its effects are late in the progression of this disease.

INTRODUCTION

Breast cancer is the most frequent malignancy in women, with a cumulative lifetime breast cancer risk in an unselected population that has been estimated to be about 10–12% (1, 2). Although the vast majority of cases appears to be sporadic, hereditary factors may account for about 5–10% of all cases (2). Breast cancer is genetically heterogeneous, and a variety of genetic lesions have been identified that tend to accumulate during disease progression (3). By analogy with several other forms of cancer, many of these mutations might be expected to occur in a disease stage- or grade-specific manner. One type of genetic alteration common to many tumor types is LOH, which often seems to unmask recessive mutations in tumor suppressor loci (4). Among the genomic regions commonly undergoing LOH in breast tumors is chromosome 11, in particular, regions 11p15 (5, 6) and 11q23 (7).

LOH for 11p15 has been observed in a variety of different cancers, including breast cancer, suggesting the presence of either a cluster of tumor suppressor-type genes or a single gene with pleiotropic effects. We have shown previously (6) that the critical region of 11p15 in breast tumors lies between the genetic markers TH and HBB at 11p15.5, in a segment appearing to span a maximum of 6–7 Mb of DNA. The involvement of the 11q23 region has been inferred from the location of the cancer-predisposing, DNA repair deficiency syndrome, AT (2). It has been suggested that AT heterozygotes could account for 5–10% of all breast cancer cases (2). This region also shows frequent LOH in malignant melanoma and ovarian, colorectal, and cervical carcinomas (8–11). Recently, we (7) and others (12) have demonstrated a high incidence of LOH in malignant breast tumors. In the present study, we sought to determine whether a more refined position could be gained for the region of LOH, whether the two lesions were conjoint or independent, and whether there was a relationship between the genotype of the primary tumors and their clinical outcome.

MATERIALS AND METHODS

Patients and Tissues. Primary tumor and peripheral blood samples were obtained from 96 randomly selected breast cancer patients from Northern Finland. Treatment of the disease was initiated only after surgical removal of the primary tumor. Once surgically removed the tumor tissue was immediately snap frozen in liquid nitrogen and stored at −70°C until further use. Cell pellets of peripheral blood samples were also stored at −70°C. Each of the fresh breast tumors was histopathologically characterized at the time of diagnosis; when necessary, tumors were further evaluated for the proportion of contaminating normal stromal cells. Clinical and histopathological features of each of the tumors were revealed only after the LOH study had been completed and showed that 10 of the tumors analyzed were benign. Of the 86 malignant tumors studied, 61 were ducal, 11 were lobular, 5 were medullary, 5 were tubular, and 2 were intraductal. One additional case displayed dual tumors (one with ductal and the other with lobular features), and one had an uncertain status. This histological distribution corresponds well to what has been reported previously in the literature (5). The tumor differentiation grade distribution among the ductal carcinomas was: grade I = 16; grade II = 19, and grade III = 25. Grade information was unavailable for one patient. The primary tumor size distribution was: tumor 1 = 28; tumor 2 = 39; tumor 3 = 12, and tumor 4 = 6. There were 32 node-positive and 54 node-negative patients. The estrogen and progesterone receptor status was found to be positive in 56 and 43 cases, respectively, and negative in 24 and 37 of the informative carcinomas. At the time of diagnosis, 9 patients were younger than 40 years, 24 patients were between 40 and 49, 17 patients were between 50 and 59, and 36 patients were age 60 years or older, with an average age of 56.9 years. Thirty-seven of the patients were premenopausal and 49 were postmenopausal. Clinical follow-up data on the patients were available from 3–5 years. Seven of the patients presented with metastatic disease at initial diagnosis, whereas an additional 23 patients displayed metastatic disease during clinical follow-up, (in most cases, typically 1–3 years after the diagnosis of the primary tumor). Fifteen of the patients had died from their breast malignancy before the completion of our analysis. For statistical analysis (see below), time of diagnosis and dates last seen were rounded to the nearest month.
LOH FOR CHROMOSOME 11 IN PRIMARY HUMAN BREAST TUMORS

Table 1 Result of LOH analysis for loci at 11p15 and 11q23 in 96 primary breast tumors. Tumors (numbered 46–158) have been divided into 5 groups based on the molecular findings. Only malignant tumors show LOH for the studied loci on chromosome 11.

<table>
<thead>
<tr>
<th>Molecular Status</th>
<th>Carcinomas (n = 86)</th>
<th>Benign (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOH 11p15 only</td>
<td>64,87,115, and 156</td>
<td>None</td>
</tr>
<tr>
<td>LOH 11q23 only</td>
<td>53,62,69,76,78,86,90,94,109,119,124,126, and 132</td>
<td>None</td>
</tr>
<tr>
<td>LOH 11p15 + 11q23</td>
<td>57,59,65,72,75,82,93,95,96,104,111,112,116,121,123,125,129, 133,134,135,137,139,140, and 143</td>
<td>None</td>
</tr>
<tr>
<td>(a) LOH or (b) No LOH 11p15 and uninformative for 11q23</td>
<td>(a) 55 and 84, (b) 46,49,54, and 70</td>
<td>None</td>
</tr>
<tr>
<td>Normal result</td>
<td>47,51,52,54,58,61,66,67,68,71,77,79,80,81,85,89,92,99,100, 103,105,106,113,118,120,128,130,131,136,141,146,147,149,150, 151,152,153,154,157, and 158</td>
<td>73,74,88,91,97,98,110,127,144, and 155</td>
</tr>
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</table>

DNA Analysis. PCR-based analyses of LOH were performed on DNA extracted from matched primary tumor and peripheral blood leukocyte pairs by conventional methods (13). For each case, 50 ng of DNA were used for PCR amplification of polymorphic short tandem repeat sequences (microsatellite repeats) at the following chromosome 11 loci: HRAS1 (11p15.5), D11S1363 (11p15.5), D11S922 (11p15.5), D11S318 (11p15.5), TH (11p15.5), D11S860 (11p15.5), D11S988 (11p15.5), HBB (11p15.5-15.4), D11S932 (11p15.4), D11S861 (11p15.4), PYGM (11q12), D11S901 (11q14.1), D11S35 (11q22), APOC3 (11q23), and D11S968 (11q24–25). Detailed information about all polymorphic markers used in this study can be obtained from the Genome Database (Johns Hopkins University Medical School, Baltimore, MD), Genethon (Paris, France), and the Cooperative Human Linkage Center (University of Iowa, Iowa City, IO). The conditions for denaturation, annealing, and extension for each primer pair were essentially as reported (Genome Data Base, Genethon, and Cooperative Human Linkage Center) and optimized such that amplification of both alleles in a heterozygote were as equal as possible. The second primer of each pair was end labeled with [y-32P]ATP (Amersham, Amersham, United Kingdom) with the use of T4 polynucleotide kinase (New England BioLabs, Ltd., Mississiauga, Ontario, Canada), and the polymorphic PCR products were resolved by electrophoresis through 6 or 8% denaturing polyacrylamide gels. After electrophoresis, gels were transferred onto paper supports, and autoradiography was performed from 2 h to 3 days.

LOH Analysis. LOH for tumor DNA samples was assessed visually and densitometrically, as described previously (6, 7). Visual inspection was performed independently by at least two individuals who compared their interpretations of the results. This procedure was sufficient in most cases. However, in other cases, imputing tumor LOH was based on diminished intensity of one allele by quantitative scanning laser densitometry. LOH was assigned when the intensity ratio of the two tumor sample alleles differed by at least 30% from that observed for the control sample.

Statistical Analysis. Statistical evaluation of possible correlations between observed genotypes and clinical phenotypes/parameters were performed with the use of either the Fisher exact two-tailed test or the χ² test with Yates correction. Survival curves were generated using the Kaplan-Meier procedure (14) and compared with the use of the log rank test (15).

RESULTS

Assessment of LOH at various polymorphic marker loci from 11p15 and 11q11.2-pter is shown in Table 1. LOH affecting 11p15.5 was found in 35% (30 of 86), whereas LOH for 11q23 was detected in 46% (37 of 80) of the malignant tumors. None of 10 benign tumors showed LOH for either of these 2 chromosomal regions, providing evidence that the observed mutations were restricted to the malignant phase of the disease. It is interesting that 29% (24 of 84) of the breast carcinomas had LOH for both 11p15.5 and 11q23; this occurrence is extremely unlikely by chance alone (P < 0.001; Fisher’s exact test). LOH that was restricted to 11p15.5 was seen in 5% of the informative tumors (4 of 84), and LOH restricted to 11q23 was seen in 16% (13 of 82) of the informative tumors (Table 1). Examples of LOH analysis are shown in Fig. 1. Fig. 2 schematically represents the LOH results obtained for each of the 11p-specific marker loci used in those tumors allowing sublocalization of the involved LOH regions.

In most instances, LOH on 11p15 appeared to be interstitial and, therefore, restricted to a relatively small chromosomal region. There were, however, two cases (55 and 133; Figs. 1 and 2B) with LOH of 11p15.5 that retained normal dosage for markers at distal 11p15.4 but also exhibited LOH for markers at proximal 11p15.4. This, combined with the results obtained from studying other informative tumors, suggests the possibility of two distinct regions of LOH within 11p15, a major region between loci TH and D11S988 at 11p15.5 and a minor region between loci HBB and D11S861 at 11p15.4. The small number of tumors showing aberrations of this region and the limited number of probes used limit our certainty of the size of this region. We cannot be sure whether the two regions act independently or synergistically.

The LOH region on 11p15 did not usually extend further centromeric from loci D11S932 and D11S861 at 11p15.4 (Figs. 1 and 2; data not shown). We have shown previously that the smallest shared LOH region on 11p appears to lie between D11S35 and APOC3 at 11q22–23 (7). Combined results obtained from LOH studies for both 11p and 11q suggest that monosomy 11 was infrequent, occurring in at most 20% of the tumors. In the remaining cases, the alterations appeared to involve only limited portions of chromosome 11.

Examples of data demonstrating 11p15.5-specific LOH (cases 87 and 115) and LOH specific to 11q23 (cases 76 and 78) are presented in Fig. 3A. Comparison of LOH ratios for 11p15 and 11q23 in cases showing simultaneous LOH for both of these chromosomal sites is shown in Fig. 3B. Cases 72, 125, and 140 exhibit very similar LOH ratios for both 11p and 11q, suggesting that the same tumor cells carry both genetic alterations. Although this was the common finding, cases 65 and 82 displayed a higher LOH ratio for 11p than for 11q, and case 112 showed a higher LOH ratio for 11q than for 11p. In the two latter instances it is possible that during tumor evolution one of the two genetic events detectable by LOH occurred before the other one, or that the analyzed tumor specimen contains different subclonal tumor cell populations.

Next, we tested whether these genetic lesions were associated with any of the clinical features of the disease. No strong correlation was found among the genetic groups with respect to patient age, menopausal status, histopathology, tumor size, differentiation grade, lymph node involvement, or estrogen/progesterone receptor status. The results, however, were suggestive of an association between LOH for 11q23 (and 11p15.5) and a more rapid development of metastatic lesions (without obvious site specificity), although the number or cases proved too small for statistical significance. In contrast, LOH of 11q23, alone or in combination with LOH of 11p15.5, was strongly predictive of decreased survival after the development of metastasis (Fig. 4). Although it was not possible to reliably separate the effect of...
Fig. 1. Sublocalization of LOH region(s) on 11p15. The LOH results for cases 64, 125, 133, and 55 (shown schematically in Fig. 2). Left, loci used; T, tumor, N, normal DNA; arrows, alleles showing LOH; NT, not tested; NI, not informative. The decrease in the densitometric ratio observed between the alleles of matching control and tumor DNA is given in percentage below the autoradiographic result for each informative marker locus.

DISCUSSION

The present data allow the target 11p15 LOH region to be narrowed from approximately 6–7 Mb (6) to approximately 2 Mb, based on the estimation of physical distances from Redeker et al. (16). The critical region appears to center around locus D11S860 at 11p15.5. The development of new DNA markers from this chromosomal region will allow further refinement of the position of this alteration, ultimately leading to the identification of the critical gene(s). Available mapping data from recent studies of 11p15 aberrations in rhabdomyosarcoma and Wilms’ tumor, Beckwith-Wiedemann syndrome (17), and lung cancer (18) seem to favor the idea that a shared genetic lesion is involved in all of these cases. It is interesting that in 2 of the breast tumors analyzed (cases 55 and 133; Figs. 1 and 2), there was an additional 11p15 LOH region at 11p15.4, bordered on both sides by loci HBB and D11S861. LOH for this minor region was always present in conjunction with LOH for the major 11p15.5 region, and it remains to be determined whether LOH at this minor region is of any biological significance or whether it only reflects a consequence of the mechanism(s) generating interstitial chromosome deletions at 11p15.5. It also remains to be determined whether the two regions act synergistically and what the exact size of the minor region represents.

LOH on 11q was detected in 46% of the primary breast carcinomas and centers around the APOC3 gene at 11q23. This genomic region coincides with the location of genes involved in the AT syndrome and corresponds well to the smallest overlapping region frequently observed LOH in malignant melanoma and colorectal, ovarian, and cervical carcinomas (8–11).

Although the distance between 11p15.5 and 11q23 is great, most of the breast carcinomas undergoing genetic events detectable by LOH did so simultaneously for both chromosomal regions. We cannot at present exclude the possibility that these genetic alterations could have occurred on the different chromosome homologues. Indirect support for the assumption that both deleted regions reside on the same homologue of chromosome 11 was obtained from densitometric analysis of LOH ratios for 11p15.5 and 11q23 for the same tumors. Most tumors (20 of 24, 83.3%) exhibiting LOH for both 11p15.5 and

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(A) LOCUS/CASE  87  115  76  78
TH, D11S860 or HBB (11p15)
T N  T N  T N  T N
Δ34%  Δ48%  Δ22%  Δ16%

APOCH (11q23)
T N  T N  T N  T N
Δ16%  Δ15%  Δ62%  Δ57%

(B) LOCUS/CASE  72  125  140  65  82  112
TH, D11S860 or HBB (11p15)
T N  T N  T N  T N  T N  T N
Δ79%  Δ71%  Δ91%  Δ73%  Δ79%  Δ52%

APOCH (11q23)
T N  T N  T N  T N  T N  T N
Δ73%  Δ74%  Δ94%  Δ36%  Δ50%  Δ75%

Fig. 3. Examples of LOH analysis of loci at 11p15 and 11q23 in representative breast tumors. Patient numbers, top of each row. Abbreviations are as described in the legend to Fig. 1. A, LOH unique for 11p15 (cases 87 and 115) and 11q23 (cases 76 and 78). B, tumors exhibiting LOH for both 11p15 and 11q23. The allelic ratios for 11p15 and 11q23 show very similar values in the majority of the cases (e.g., cases 72, 125, and 140). However, some tumors show alternatively higher or lower allelic ratios at 11p15 (e.g., cases 65 and 82) or at 11q23 (e.g., case 112).

11q23 displayed very similar allelic ratios for both locations, suggesting that they arose in the same chromosome homologue in the same tumor cells and, perhaps, even simultaneously through the same mechanism. However, there are cases where the allelic ratios on 11p15.5 and 11q23 are clearly discordant (Fig. 3B), suggesting that LOH for 11p15.5 (3 of 24, 12.5%) or 11q23 (1 of 24, 4.2%) occurs independent of one another or, perhaps, in different tumor cell subpopulations.

The relevance of these observations to clinical disease is underscored by our finding that patients whose primary tumors showed LOH for 11q23, either alone or in conjunction with LOH for 11p15.5, displayed a more aggressive postmetastatic disease course than those without these deletions (Fig. 4). Because we could not detect any other significant differences between the two patient groups with respect to other clinical parameters, these results are unlikely to result from an unintended selection of differing subgroups. Most of the patients that exhibited LOH for 11q23 and who developed identifiable metastases died from their disease soon after diagnosis, typically within 2 years. Because none of the 4 patients with LOH for 11p15.5 alone developed metastatic disease during the 3–5-year clinical follow-up period, it seems unlikely that this defect accounts for this dramatically unfavorable disease progression. Therefore, some genetic factor(s) residing at 11q23, alone or in combination with other factors, one of which may be located at 11p15.5, could play a role in generating aggressive breast disease with extremely poor prognosis. If these observations hold true in a larger case study, screening for LOH at 11q23 in primary breast tumors may be of use in identifying those patients who are at potentially higher risk for developing aggressive disease. Although LOH for chromosomal regions that include 11q23 has been identified in several other forms of cancer, these observations appear to be the first to tentatively link this genetic defect to a particular, clinically important feature of the biology of the disease. It is tempting to speculate that the main target gene(s) for the genetic events detectable by LOH at 11q23 could be the same as that responsible for the DNA repair impairment associated with the AT syndrome, where many carriers of the defective gene(s) develop carcinoma of the breast or other organs. It is of interest that Lindblom et al. (Ref. 19) have studied breast cancer families recently in which the disease may be associated with the inheritance of a chromosomal translocation t(11;22), the chromosome 11 breakpoint of which is located in the 11q22–q23 region. Family studies to determine whether the critical genes that are the primary targets for LOH for 11p15.5 and...
11q23 are also involved in predisposition to hereditary/familial breast cancer are now in progress.

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This paper is dedicated to the memory of Dr. Martti Alavaikko.

REFERENCES

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R. Winqvist, G. M. Hampton, A. Mannermaa, et al.


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