Loss of Heterozygosity in Sporadic Human Breast Carcinoma: A Common Region between 11q22 and 11q23.3

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Abstract

The development of sporadic human breast cancer is associated with the accumulation of genetic alterations on several chromosomes. In the case of chromosome 11, loss of heterozygosity (LOH) at loci on the short arm has been well documented and suggests the presence of a suppressor gene(s) at 11p15.5. However, the evidence for similar events on the long arm is less compelling. Here, we determined the prevalence of LOH for gene(s) at HplS.5. However, the evidence for similar events on the long arm has been well documented and suggests the presence of a suppressor case of chromosome 11, loss of heterozygosity (LOH) at loci on the short arm. In the accumulation of genetic alterations on several chromosomes. In the case of chromosome 11, loss of heterozygosity (LOH) is common in the pathogenesis of breast cancer as 19 of 44 (43%) malignant tumor specimens exhibited LOH. Eleven (58%) of these genetic alterations were specific to the long arm of the chromosome. The smallest region of shared LOH places the target between 11q22 and 11q23.3, the same general region frequently altered in cancers of the ovary, colon, skin, and uterine cervix, perhaps indicating the location of a tumor suppressor gene or genes of importance in each of these different tumor types.

Introduction

Breast cancer is the second most common cause of cancer-related death among women in the United States (1). Although the majority of cases appear to be sporadic, a sizable proportion (5–10%) may involve inherited predisposition (2). Cytogenetic and molecular genetic analyses of breast tumor cells suggest that the development of human breast cancer involves the accumulation of alterations in genes that normally serve to control growth and differentiation (reviewed in Ref. 3). LOH, which has proven indicative of the presence of tumor suppressor genes, has been observed for several particular loci in breast cancer with frequencies ranging between approximately 20 and 60% (4, 5). This is in contrast to a generally low “background” rate of “non-causal” LOH of approximately 5% or less (4, 5).

The involvement of a gene or genes on chromosome 11p in the pathogenesis of breast cancer is well established (6, 7), by both molecular genetic analysis, which has led to the fine mapping of a putative suppressor gene in the 11p15.5 region (8, 9), and by microcell-mediated transfer of chromosome 11 to breast cancer-derived cell lines such as MCF-7 (10). However, the cumulative evidence for LOH on the long arm remains unconvincing. Depending on the position of the polymorphic markers used, the frequency of LOH reported varies between 4% at 11q13 in one study (11) and 59% at the 11q23 locus, D11S29 (12). Recent cytogenetic analyses of 34 cases of metastatic breast cancer samples have shown that the most frequent chromosome losses are 1p, 6q, 7, and 11q (13). This contrasts with cytogenetic analyses of 28 cases of localized disease in which alteration of 11q does not appear to be common (14). Recently, LOH has also been reported at 11q22-qter in a sizable fraction of colon, ovarian, and skin cancers (15–17). In addition, some of us have recently reported a similar finding in cervical carcinoma (18). Here, our results show that LOH for 11q occurs in more than 40% of malignant tumors and the minimal region of shared LOH is distal to 11q22 and proximal to 11q23.3, the same general region observed to be a target in the other tumor types.

Materials and Methods

Patient Materials. Tumor and peripheral blood samples were obtained from 47 unselected primary breast cancer patients from Northern Finland and processed as described previously (9). Invasive tumors included 29 cases of ductal carcinoma, 4 cases of medullary carcinoma, 3 cases of ciberriform-papillary carcinoma, and 2 cases each of intraductal, lobular, and tubular carcinomas. Histological information was not available for 2 cases and 3 cases were benign fibroadenoma.

PCR Analysis. Each of the matched pairs of normal and tumor DNAs were subjected to PCR analysis using chromosome 11q microsatellite loci: D11S901 [11q14.1] (19), D11S35 [11q22] (20), APOC3 [11q23] (21), and D11S968 [11q24-q25] (19). In some cases, matched normal tumor DNAs were also analyzed using oligonucleotide primers flanking a microsatellite polymorphism within the PYGM gene [11q12] (22). PCR reactions were carried out as described previously with the inclusion of one [γ-32P]ATP-end-labeled primer (18). PCR products were resolved by electrophoresis through 6 to 8% polyacrylamide gels as described by Litt et al. (23).

LOH Analysis. In cases where a particular marker was informative (i.e., heterozygous) in normal peripheral blood DNA, LOH was assessed in the corresponding tumor sample by two independent observers, and for most cases described here, LOH was visually obvious. However, for cases in which there was a reasonable doubt, or in which the mapping information obtained was crucial, scanning laser densitometry was carried out. Ratios of integrated allelic intensity were calculated by published methods (24) and LOH was imputed if the effective decrease in one allele was equal to or greater than 30%. In the least obvious cases, we could not distinguish between the loss of an allele or the amplification of an allele (or a combination of both); these results are therefore equivocal.

Results

The 47 paired normal-tumor DNAs were initially assessed for LOH at four chromosome 11q-specific microsatellite loci: D11S901, D11S35, APOC3, and D11S968. These markers encompass chromosomal region 11q14-qter; this region most frequently exhibits LOH in cervical carcinoma (18) and has been implicated in breast cancers by...
limited analyses (12). The frequency of LOH at each of these four loci is shown in Table 1, and representative examples of the data are displayed in Fig. 1 with the composite data shown in Fig. 2.

LOH was detected in 19 of 44 (43%) malignant tumors and in none of the 3 benign tumors. Fifteen of the 19 tumors exhibiting LOH at any locus showed LOH at the D11S901 locus or were uninformative, whereas the remaining 4 of 19 cases retained heterozygosity at D11S901 or at the more telomeric D11S35 locus. Thus, in 15 cases, we were unable to assess whether the genetic alterations leading to LOH at chromosome 11q markers were restricted to this chromosomal arm. To test this, they were analyzed for LOH using a highly polymorphic microsatellite within the PYGM gene close to the centromere at 11q12 (Figs. 1 and 2). Twelve of the 15 cases were informative at this locus and 5 exhibited LOH. Overall, these results imply that the chromosomal alterations in at least 11 of the 19 cases (58%) were restricted to the long arm of the chromosome.

Five of the 19 tumors exhibiting LOH on 11q, i.e., 104, 116, 124, 134, and 139, exhibited chromosomal aberrations leading to LOH for only a subset of the four loci examined (see Figs. 1 and 2). Assuming that each of these 5 genetic events leading to LOH represent the loss of the same suppressor locus (loci) encompassed by the other more gross genetic alterations, the likely position of such a gene(s) is distal to 11q22 and proximal to 11q23.3.

Discussion

The results reported here show that chromosomal alterations leading to LOH on 11q are relatively common in sporadic breast cancer (i.e., 43% of malignant cases). This is a conservative estimate because we confined the assignment of LOH to tumors where the reduction in intensity of one allele was clear. However, there were a small number of tumors that did not meet our criteria, but did nonetheless show a weak reduction in the intensity of one or more alleles for informative markers on 11q. In these latter cases, the weak reduction of the allelic signal may be simply due to the presence of a large proportion of contaminating nonneoplastic cells. However, it may also be due to the fact that not all of the tumor cells contain the genetic alteration, an observation recently described for 11q alterations in cervical carcinoma (18) and for other genetic alterations in breast cancer (25). Thus, the actual frequency of LOH may be in the range of 50%, in line with the 59% LOH observed at the D11S29 locus (11q23) by Stickland et al. (12). The tumor specimens described here were not enriched for neoplastic cells prior to the purification of DNA, and it is also likely that there are cases in this series in which LOH was not detected because of too high a proportion of contaminating nonneoplastic cells.

A large proportion (58%) of the chromosomal alterations on 11q described here were found to be specific to the long arm. LOH has been analyzed also on chromosome 11p in this collection of 47 tumors. These analyses suggest that whole chromosome loss is actually quite infrequent and that the genetic alterations in as many as 15 of the 19 (79%) tumors described here may in fact represent the selective loss of chromosome 11q material.

\[3 \text{ R. Winquist, personal communication.}\]
Overall, our data on 11q are supported by a number of recent observations. Cytogenetic studies in metastatic breast carcinomas indicate that loss of chromosome 11q is quite common, perhaps suggesting that such alterations occur late in tumor progression (14). In addition, Negrini et al. have shown by chromosome transfer experiments that loss or alteration of a gene(s) on 11q, in addition to 11p, in the breast carcinoma-derived cell line MCF-7, appears to have been an essential part of the tumorigenic pathway (26). Restoration of the defect(s) on 11q which apparently maps distal to 11cen and proximal to 11q23.1 by hybrid analysis, leads to generally smaller tumors than the parental cell line in immune-deficient mice, some of which tend to regress. Whether or not mutation of the putative gene we have mapped in this series of breast tumors represents the target gene resulting in the apparent phenotypic effect reported by Negrini et al. (26) remains to be determined.

Although the majority of tumors in this series exhibited LOH for all of the four key 11q loci, 5 tumors have allowed us to suggest a tentative map location for the putative tumor suppressor gene(s) distal to 11q22 and proximal to 11q23.3. Even though this chromosomal segment was defined by the use of only four polymorphic loci in this analysis, the region nonetheless coincides with that recently described in cervical carcinoma (18) and the analysis might suggest that loss or alteration of the same gene(s) is important in the pathogenesis of both tumor types. This conjecture becomes more interesting in light of studies that demonstrate a high frequency of LOH around 11q22-qter in ovarian cancer (15), colorectal carcinoma (17), and malignant melanoma (16). More recently, Lindbolm et al. (27) have reported families in which there appears to be a significant increase in breast cancer risk associated with the presence of the constitutional t(11; 22)(q23;q11) translocation. The authors posit that involvement of a gene or genes on 11q23 and/or 22q11 may therefore be involved in the pathogenesis of breast cancer. More extensive genetic analysis of 11q alterations in breast cancer, in addition to each of these other tumor types, will be important to establish whether loss or alteration of the same gene or of different genes is involved in each of these cases.

References


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