Two Distinct Regions Involved in 1p Deletion in Human Primary Breast Cancer

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Abstract

Alteration of chromosome 1 is the most consistent cytogenetic abnormality found in human breast carcinoma. Cytogenetic studies have shown independent alterations on the two arms of chromosome 1, increased copy number of the long arm and loss of the short arm of chromosome 1. These deletions are thought to coincide with the location of tumor suppressor gene(s). We carried out deletion analysis of the 1p region by using restriction fragment length polymorphism markers mapping to the long (six markers) and short arm (22 markers). Thirty-five of the 74 (47.3%) human breast tumors tested showed somatic loss of heterozygosity at one or more loci on the short arm. Two commonly deleted regions, 1p13-p21 and 1p32-pter, were identified. The latter region is frequently involved in other types of tumors, suggesting that it harbors a common tumor suppressor gene. Our findings suggest that two tumor suppressor genes involved in the development of human breast carcinoma may occur on the short arm of the chromosome 1.

Introduction

Somatic deletions of chromosomal sequences specifically affecting certain regions of the genome are the most frequent genetic alteration observed in solid tumors. The most common cytogenetic abnormalities in breast cancer affect chromosome 1, leading principally to gain of 1q and loss of 1p. Several karyotypes representing common structural and numerical alterations occur: polysomy 1q; isochromosome 1q; monosomy 1p or 1p; and deletions or translocations both on the long and short arms (1, 2). There does not appear to be a uniform chromosome band for these rearrangements, although a study has shown that the 1p13 band is the most frequent breakpoint observed in breast cancer (3). Deletions of the 1p region have been confirmed as LOH by RFLP analysis (4–9). We have previously shown that a high frequency (37%) of LOH occurs at the L-myc locus on chromosome 1p32 (5). Reported estimates of LOH on 1p, however, vary from 3 (9) to 14 (7), 27 (8), and 41% (4). This variation may be due to the use of different probes as well as to differences in the stages of breast tumors studied. It is thought that these deletions can remove or reveal recessive mutations affecting tumor suppressor genes which are required for the normal regulation of cell proliferation during development.

Cell fusion experiments have indicated the presence of one or more genes on chromosome 1 with the ability to suppress tumorigenicity of transformed cells (10). Furthermore, the introduction of a single copy of human chromosome 1 into hamster cells by microcell fusion caused typical signs of cellular senescence (11). If aging is the counterpart of malignant transformation, “senescence genes” would be tumor suppressor genes overexpressed in senescent cells. Studies of colorectal adenoma cell lines (12), and human breast tumor biopsies (5, 8, 13), have implicated abnormalities of chromosome 1 in the progression of human tumors.

These data suggest that chromosome 1p harbors nonidentified tumor suppressor genes, the inactivation of which may be involved in human breast tumorigenesis. There are, however, few data on the extent of deleted region(s) of chromosome 1p in human breast cancer; most workers have used only one genetic marker in studies of chromosome 1p. We examined 74 human breast tumors for evidence of allelic loss in chromosome 1p, using RFLP analysis (22 polymorphic markers mapping to the 1p), in order to produce a deletion map of this arm and to locate the gene(s) of interest. In addition, we used six polymorphic DNA markers mapping to chromosome 1q to get an overall picture of chromosome 1 and to compare our results with cytogenetic data. We identified two distinct 1p regions (1p13–p21 and 1p32–pter) frequently deleted in human breast cancer.

Materials and Methods

Tumor and Blood Samples. Samples were obtained from 74 primary breast tumors surgically removed from patients at the Centre René Huguenin, none of the patients had undergone previous radiotherapy or chemotherapy. Immediately following surgery, the tumor samples were placed in liquid nitrogen and stored at −80°C until extraction of high molecular weight DNA. A blood sample was also taken from each patient.

DNA Analysis. DNA was extracted from tumor tissue and blood leukocytes of each patient, according to standard methods (14). Ten μg of DNA from each sample were digested with the appropriate restriction endonuclease. The resulting fragments were separated by electrophoresis in agarose gel (leukocyte and tumor DNA samples from each patient were run in adjacent lanes) and blotted onto nylon membrane filters, according to standard techniques. The membrane filters were hybridized with nick-translated 32P-labeled probes, washed, and autoradiographed at −80°C for an appropriate period.

DNA Probes. We used 28 polymorphic DNA probes (see Ref. 15), specific for known genes, chromosome 1 specific repetitive sequences, or anonymous DNA sequences. These and the appropriate restriction enzymes are listed in Table 1. Probes were positioned and ordered by genetic linkage analysis (16, 17).

Determination of Allele Loss. Paired normal and tumor DNA from each patient was analyzed using probe–enzyme combinations which identify RFLP in a large proportion of individuals. Normal DNA samples which were polymorphic at a given locus were considered to be “informative,” whereas the homozygotes were declared “uninformative.” The signal intensity of fragments was determined by densitometry. LOH was considered to occur when the intensity of the allele in tumor DNA was less than 50% of that in corresponding normal tissue DNA. A complete loss of heterozygosity was observed in some cases, presumably because the total DNA extracted was derived from cells harboring DNA deletions. In most patients, however, only a partial loss of heterozygosity was observed, with the band being fainter than the conserved allele but still visible. This partial loss is likely to be due either to contaminating normal tissue or to tumor heterogeneity. When extra bands were observed using certain probes, their validity was checked by using different restriction enzymes.
Table 1 Chromosomal locations of polymorphic DNA probes used to study LOH and appropriate restriction enzymes

<table>
<thead>
<tr>
<th>Chromosome location</th>
<th>Locus</th>
<th>Probe</th>
<th>Enzyme</th>
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<td>p36.3-p35</td>
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Results

We analyzed normal DNA (peripheral blood lymphocytes) and autologous tumor DNA from 74 breast cancer patients, using 22 polymorphic DNA markers spanning the length of the short arm of chromosome 1 and 6 polymorphic markers mapping to the long arm.

All patients were informative at 8 or more loci. LOH occurred in at least one locus on the short arm of chromosome 1 in 35 of the 74 tumors (47.3%). Of these 35 deleted tumors, 16 (46%) showed LOH at all informative loci tested on 1p, indicating deletion of the entire short arm of chromosome 1, while the other 19 (54%) showed partial (interstitial or/telomeric) deletions on 1p.

The 16 tumors in which the whole short arm was lost comprised seven monosomies 1 (all informative loci on chromosome 1 had lost heterozygosity); six monosomies 1p (the six informative loci on the long arm retained constitutional heterozygosity); and three isochromosomies 1q (increased copy number of informative loci on the long arm). In one of the six tumors with a monosomy 1p, LOH was observed in the most distal loci on the long arm (REN, ATP1B1, D1S61), but not in the proximal loci (SPTA1, MUC1, D1S57). In addition to the loss of an entire short arm in this tumor, we observed a distal deletion on 1q with a breakpoint between DIS61 and SPTA1 markers.

One of the seven tumors with a monosomy 1 appeared to be due to a double genetic event, since the signal intensities of 1p and 1q markers were decreased by 50 and 90%, respectively, relative to normal tissue. The 19 tumors showing partial deletions on chromosome 1p proved to be of greater interest than those showing total deletions (Fig. 1 shows examples of the most common patterns of genetic changes; Fig. 2 summarizes LOH data in the form of a deletion map). The tumors were subdivided into three groups on the basis of the type of deletion: A; telomeric deletions (n = 7); B; interstitial deletions (n = 7); and C, tumors with both these deletions (n = 5).

We compiled a composite group comprising all 12 tumors with telomeric deletions on 1p (groups A and C) and determined the relative position of breakpoints between two markers for each tumor. In tumor T1210, for example, the DIS2 locus remained heterozygous, whereas the next distal locus, DIS18, had lost one allele, suggesting a breakpoint located between these two loci. Using the same method, we located breakpoints between NRAS and NGFB (tumor T455), D1S9 and DIS14 (tumor T231), D1S62 and DIS100/101 (tumor T1246), and D1S77 and DIS59596 (tumor T465) (Fig. 2). One tumor (T206) showed an intragenomic DNA fragment breakpoint within D1S9; this was confirmed by other restriction digests. Unfortunately, we did not observe a common involved breakpoint in any of the tumors tested. However, all these tumors showed LOH at the loci DIS95, DIS96, and DIS80 adjacent to pter, suggesting a common deleted region. The limits of this smallest common deleted region were defined by locus DIS77 and the 1p telomere distally.

On the other hand, analysis of groups B + C identified tumors with interstitial deletions of various sizes, without common breakpoints. The 12 tumors in these groups (except T589) displayed a consensus for a second smaller common deleted region proximal to locus DIS11 and distal to DIS14. This region, which involved the loci DIS9 and DIS13, spanned the chromosome bands 1p13-p21. Several genes have been mapped to these two common deleted regions, including RAP1A/Krev-1 (1p13-p21) and JUN, LCK, and FGR (1p32-pter) (15). Using probes for these genes (which are poorly or not polymorphic), we detected no major abnormalities in any of the human breast tumors tested (data not shown).

One tumor (T465) showed extra bands with both DIS10 and DIS13 probes, the validity of which were confirmed using other restriction enzymes. This abnormal case had an interstitial deletion 1p13-p21, and the DIS10 and DIS13 loci were fused.

Some of the tumors with partial 1p LOH showed LOH on the long arm (T206, T213, T589, and T1303) and amplifications (T605, T330, T1246, and T465). These alterations of chromosome 1 seemed to involve the entire long arm, except for tumors T206 (LOH) and T465 (amplification). In conclusion, our findings suggest that tumor suppressor genes involved in the genesis of breast and possibly other carcinomas are located in two distinct regions (1p13-p21 and 1p32-pter) of chromosome 1p.

Discussion

There are various approaches to the search for genes the deletion of which may be responsible for tumorigenesis. Cytogenetic analysis has allowed the localization of distinct chromosomal regions which might harbor genes contributing to tumorigenesis. Fine scale molecular mapping of deleted regions is needed to locate such genes precisely and can be done by studying loss of heterozygosity. We thus carried out a molecular analysis of 1p deletions previously associated with human breast cancer in cytogenetic studies. We found allelic loss in the chromosome 1p in 35 of the 74 human breast tumors studied (47.3%), using 1p specific DNA probes. We found no amplification on this arm of chromosome 1. Tumor alterations could be placed in two groups: loss of a whole chromosome 1 or the 1p region (due to mitotic nondisjunction); and structural rearrangements of 1p.

Our analysis of tumors from which all of chromosome 1p was lost is consistent with previous cytogenetic studies: the frequency was similar and most rearrangements involved either entire 1p translocations or deletions or isochromosomes. In most cases the rearrangements were associated with a breakpoint in the constitutive heterochromatin. Indeed, we used two probes located near to the heterochromatin: DIS67 on 1qcen and DIS13 on 1pcent. DIS13 was lost when an entire 1p deletion occurred. DIS67 was duplicated when an isochromosome 1q occurred and lost in the case of an entire 1q deletion. Nevertheless, in two of the 16 tumors showing centromeric breakpoints, the latter were located within the euchromatin. This first
The 19 tumor DNAs showing partial deletions of 1p provided more data in this respect. Deletion mapping revealed that breakpoints on chromosome 1p differed between tumors, thus ruling out the hypothesis that a chromosome 1p breakpoint itself might play an important role in the development of human breast tumors. LOH data for 12 breast tumors with telomeric deletions indicated that the smallest common deleted region was located between the DIS57 locus and the 1p telomere distally. Karyotypic abnormalities in this distal location (1p32-pter) have been reported in a wide variety of tumors (18), including breast tumors (2). Loss of heterozygosity on this region is found in neuroblastoma, melanoma, sporadic pheochromocytoma, multiple endocrine neoplasia type 2a, and carcinomas of the liver, stomach, pancreas, and colon (19, 20). Our findings are consistent with the high frequency of telomeric LOH previously observed in breast cancer in studies using only one genomic marker (4, 8). The striking correlation between the distal deletions in these different cancers suggests that this region harbors putative tumor suppressor gene(s) with relevance to the induction of more than one tumor type, although it cannot be predicted whether the same gene is involved in these different tumor types. While several oncogenes (including JUN, LCK, and FGR) are located in this region (15), there are no obvious candidate tumor suppressor genes. A putative melanoma susceptibility gene was identified in this region (21), but this finding has been challenged by other workers (22). By using large number of paired breast cancer samples, and numerous polymorphic markers for the entire chromosome 1p, we show that a second distinct region (1p13-p21), still unidentified, is involved in breast tumorigenesis. We observed a second smallest common deleted region in tumors with partial interstitial deletions. This region is located between the DIS16 and DIS73 loci, which are estimated by means of linkage analysis to be 16 cM apart (17). As no common deletions have been reported in this region in any other cancer, it may be specific to breast cancer. None of known genes suspected to be localized in this region are obvious candidates for tumorigenicity in breast cancer. The only tumor suppressor gene located here (RAPlAIKrev-1) (15) shows no major rearrangement. However, we cannot rule out the possibility that the second allele of RAP1A/Krev-1 may be inactivated by point mutations but go undetected by Southern blotting. The 1p13 band has been identified as the most frequent site for structural chromosome changes in human breast tumors (3), and our findings suggest that a tumor suppressor gene is located in this region.

It is noteworthy that two (and even three or four) genetic alterations were observed in the same tumor, including one or two LOH on the short arm of chromosome 1 and/or one LOH (or one amplification) on 1q. Several mechanisms are possible: it could arise in one clone by two simultaneous events (this would result in a single clone with two alterations, with probably similar signal intensities) or as two independent alterations in two subclones. Two successive events could also give rise to a subclone with two alterations (these would likely have different signal intensities).
We observed differences in signal intensity in several tumors, thus confirming intratumoral polyclonality. Moreover, the two alterations in the same clone (or subclone) could be syngenic or on each chromosome of the pair. This last hypothesis is the only one which could account for tumor T465 (Fig. 2).

In conclusion, heterozygosity is frequently lost in two different regions of the short arm of chromosome 1 in human breast cancer. This suggests that genes in these regions are involved in the initiation and/or progression of human breast cancer. One region (1p32-pter) is frequently involved in other types of tumors, suggesting it may harbor a common tumor suppressor gene. The other regions (1p13-p21) may contain gene(s) specific for breast cancer. Although we have narrowed down the search for likely tumor suppressor genes to two regions, the length of DNA to be searched is of the megabase order. Identification of these putative suppressor genes will require further LOH studies using fine scale genetic mapping with RFLP or microsatellite markers and the construction of a physical mapping of the regions. Unequivocal evidence that these regions harbor tumor suppressor genes will require isolation of the gene and analysis of its products.

Acknowledgments

We are indebted to Dr. N. C. Dracopoli for kindly providing probes DIS9, DIS11, DIS13, DIS14, DIS15, DIS16, DIS17, DIS18, and DIS21; to Dr. B. G. Forget for probe SPTA1; to Dr. S. J. Gendler for probe MUC1; to Dr. J. D. Minna for probe MYCL1; to Dr. A. Weith for probes DIS95 and DIS96; to Dr. D. Mathieu-Mahul for probes DIS100 and DIS101; and to Dr. J. B. Lingrel for probe ATP1B1. Other probes were obtained from the American Tissue Type Culture Collection and the Japanese Cancer Research Resources Bank.

References


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