Loss and Gain of Distinct Regions of Chromosome 1q in Primary Breast Cancer

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ABSTRACT

Alterations of the long arm of chromosome 1 are the most frequent cytogenetic abnormalities found in human breast carcinoma. We examined genetic alterations on chromosome 1q in 124 human breast tumors, using restriction fragment length polymorphism markers mapping to the long arm (13 markers) and short arm (4 markers). Imbalance of heterozygosity at one or more loci on the long arm was observed in 80 (65%) of the 124 tumors. Among these 80 tumor DNAs, 38 showed an allele gain, 16 a loss of heterozygosity, and 1 both allele gain and loss of heterozygosity at each locus on the long arm, indicating that 55 tumor DNAs had a gain and/or loss of the entire long arm of chromosome 1. Detailed alteration mapping of the other 25 tumors showing partial alterations of chromosome 1q identified two distinct altered regions: a smallest common deleted region at 1q21-31 and a smallest common overrepresented region at 1q41-q44. The results suggest that both oncogenes and tumor suppressor genes are present on chromosome 1q and are associated with breast tumorigenesis.

INTRODUCTION

Carcinogenesis is now considered to be a highly complicated process in which accumulation of genetic mutations is required to transform a normal cell into a malignant cell. Any one of several mechanisms, such as point mutations, translocations, gene amplifications, and loss or gain of a whole chromosome or part of it, can activate oncogenes or inactivate tumor suppressor genes, thereby allowing a cell to escape from normal growth control.

One of the first indications that a "cancer gene" was present on a specific chromosome consisted of structural abnormalities and gain or loss of a specific chromosome detected by karyotypic analysis of tumor cells. Using molecular analysis of the allelic pattern of tumor DNAs with polymorphic probes mapping to the suspected chromosome, it is possible to locate a common region for allelic alterations, which could lead to the identification of cancer genes (1).

Breast cancer comprises several separate entities. The molecular pathogenesis of breast tumors is complex and involves at least 10 genes (2). Cytogenetic analyses have indicated that alterations to the long arm of chromosome 1 are the most frequent type of abnormality associated with breast cancer. There are several karyotypes with common structural and numerical alterations: polysomy 1q, isochromosomy 1q, or monosomy 1, and deletions, amplifications, or translocations on the long arm of chromosome 1 (3–7). Dutrillaux et al. (6) and Pandis et al. (7) have suggested that gain of 1q is an early chromosomal abnormality in breast carcinomas, preceding the acquisition of more complex changes.

Chromosome 1q alterations (gain and loss of DNA sequences) have been confirmed by RFLP3 analysis in breast tumors (8–11). We have previously shown that a high frequency (40%) of allelic imbalance (29% of LOH and 11% of allele gain) occurs at the MUC1 locus on chromosome 1q21-23 (12).

Cell fusion experiments have indicated the presence of one or more genes on chromosome 1 with the ability to suppress the tumorigenicity of transformed cells (13). Furthermore, the introduction of a single copy of human chromosome 1 into hamster cells by microcell fusion caused typical signs of cellular senescence. Detailed analysis of somatic cell hybrids showing chromosome 1 translocations indicated that the senescence gene(s) was on the long arm of chromosome 1 (14). These data suggest that chromosome 1q harbors unidentified cancer genes whose activation (oncogenes) or inactivation (tumor suppressor genes) may be involved in human breast tumorigenesis. There are no data on the extent of the altered region(s) of chromosome 1q in human breast cancer, because most studies have only analyzed one or a few genetic markers of chromosome 1q.

In this study, we examined 124 human breast tumors for evidence of gain or loss of DNA sequences on chromosome 1q, using RFLP analysis (13 polymorphic markers mapping to 1q), in order to produce an alteration map of this arm and to locate the gene(s) of interest. In addition, we used 4 polymorphic DNA markers mapping to chromosome 1p to get an overall picture of chromosome 1 and to compare our results with cytogenetic data.

PATIENTS AND METHODS

Tumor and Blood Samples. Samples were obtained from 124 primary breast tumors surgically removed from patients at the Centre Rene Huguenin (France); none of the patients had undergone previous radiation therapy or chemotherapy. Immediately following surgery, the tumor samples were

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: RFLP, restriction fragment length polymorphism; LOH, loss of heterozygosity.
placed and stored in liquid nitrogen 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 by using standard methods (15). Ten μg DNA from each sample was 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 17 polymorphic DNA probes (see Ref. 16), specific for known genes, chromosome 1-specific repetitive sequences, or anonymous DNA sequences. These probes and the appropriate restriction enzymes are listed in Table 1. Probes were positioned and ordered by genetic linkage analysis (17, 18).

**Determination of Allelic Dosage.** Paired normal and tumor DNA from each patient were analyzed using probe-enzyme combinations which identify RFLPs 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 considered to be “uninformative.” Only cases of constitutional heterozygosity were used in the evaluation of allelic dosage. The signal intensity of the polymorphic alleles was determined by visual examination and confirmed by means of densitometry. The loading of the paired normal and tumor DNA from each patient was taken into account in judging allele gain or loss in tumor DNA. The loading of DNA in each lane was evaluated by using control probes on other chromosomes.

Allele gain or LOH was considered to occur when the intensity (peak area corresponding to the hybridizing signal) of one allele in tumor DNA was more or less than 50% of that in corresponding normal tissue DNA, respectively.

Loss of heterozygosity was partial in most cases of 1q LOH, the band being fainter than the conserved allele but still visible. This partial loss is due either to contaminating normal tissue or to tumor heterogeneity.

Allele gain was quantified by serial dilutions of the tumor DNA samples to obtain a Southern blot hybridization signal of about one copy.

**RESULTS**

We analyzed normal DNA (peripheral blood leukocytes) and autologous tumor DNA from 124 patients with breast cancer using 13 polymorphic DNA markers spanning the entire long arm of chromosome 1, and 4 polymorphic markers mapping to the short arm.

All patients were informative for 5 or more loci. Allele gain or LOH occurred in at least one locus on the long arm of chromosome 1 in 80 (65%) of the 124 tumors. Of these 80 altered tumors, 38 (48%) showed allele gain, 16 (20%) LOH, and 1 both allele gain and LOH at all informative loci tested on 1q, indicating polysomy and/or deletion of the entire long arm of chromosome 1, while the other 25 (31%) showed partial (interstitial and/or telomeric) alterations on 1q. Fig. 1 shows examples of the most common patterns of genetic changes. The 16 tumors in which the whole long arm was lost comprised 7 cases of monosomy 1 (all informative loci on chromosome 1 had lost heterozygosity) and 9 of monosomy 1q (informative loci on the short arm retained constitutional heterozygosity).

The 38 tumors in which the whole long arm was gained comprised 11 cases of isochromosomy 1q (all informative loci on chromosome 1p had lost heterozygosity) and 27 of polysomy 1q (informative loci on the short arm retained constitutional heterozygosity).

Finally, one tumor had LOH on one arm and allele gain on the other, for all informative loci on chromosome 1q.

Densitometric quantification of the polisomies showed that one allele was multiplied 2–7-fold in the tumor DNA.

The 25 tumors showing partial alterations (deletions or polysomy) on chromosome 1q proved to be of greater interest than those showing entire arm rearrangements. Fig. 2 summarizes imbalance data in the form of an alteration map. The tumors were subdivided into three groups on the basis of the type of alteration: group A, partial polysomies (n = 11); group C, partial deletions (n = 9); and group B, tumors with both these alterations (n = 5).

We compiled a composite group comprising all 14 tumors with partial deletions on 1q (groups B and C). Twelve tumors in this panel had a common deleted region located in the center of the long arm of chromosome 1 (1q21-31). This region is proximal to locus D1S65 and distal to locus D1S75, and involves the locus D1S61.

In group B, four tumors (T407, T588, T1156, and T1241) showed one or several loci with both LOH of one allele and allele gain of the other allele in the telomeric part of chromosome 1q. Three of these tumors (T407, T588, and T1156) showed partial telomeric deletions on one long arm of chromosome 1 and polysomy of the entire (T407 and T588) or partial (T1156) long arm of the other chromosome 1. Conversely, tumor T1241 showed partial telomeric duplication on one long arm of chromosome 1 and deletion of the entire long arm of the

**Table 1 Chromosomal locations of polymorphic DNA probes used in this study and appropriate restriction enzymes**

<table>
<thead>
<tr>
<th>Chromosomal location</th>
<th>Locus</th>
<th>Probe</th>
<th>Enzyme</th>
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</thead>
<tbody>
<tr>
<td>p36.3-p35</td>
<td>D1S80</td>
<td>pMCT118</td>
<td>PvuII</td>
</tr>
<tr>
<td>p35-p33</td>
<td>D1S73</td>
<td>pEFD53.2</td>
<td>TaqI</td>
</tr>
<tr>
<td>p32</td>
<td>MYC1L</td>
<td>MYC1L</td>
<td>EcoRI</td>
</tr>
<tr>
<td>p21-p13</td>
<td>D1S73</td>
<td>pEFD53.2</td>
<td>TaqI</td>
</tr>
<tr>
<td>q21</td>
<td>D1S67</td>
<td>pHHH106</td>
<td>MspI</td>
</tr>
<tr>
<td>q21-q23</td>
<td>MUC1</td>
<td>pMUC10</td>
<td>BamHI/EcoRI</td>
</tr>
<tr>
<td>q21-q23</td>
<td>SPTA1</td>
<td>302E1I</td>
<td>PvuII/MspI</td>
</tr>
<tr>
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<td>D1S26</td>
<td>LR67</td>
<td>Bell</td>
</tr>
<tr>
<td>q21-q23</td>
<td>D1S75</td>
<td>OS-6</td>
<td>TaqI</td>
</tr>
<tr>
<td>q21-q31</td>
<td>D1S61</td>
<td>pMLAJ1</td>
<td>TaqI/PstI</td>
</tr>
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<td>pEKH7.4</td>
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<td>ATP1B1</td>
<td>HH1.2</td>
<td>PvuII/MspI</td>
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<td>REN</td>
<td>pHRnES1.9</td>
<td>HindIII</td>
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<td>D1S58</td>
<td>pYNZ23</td>
<td>MspI</td>
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<td>CRI-L461</td>
<td>TaqI</td>
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<td>q32-q43</td>
<td>D1S51</td>
<td>CRI-L1191</td>
<td>MspI</td>
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<tr>
<td>q42-q43</td>
<td>D1S8</td>
<td>pMS32</td>
<td>TaqI</td>
</tr>
</tbody>
</table>

*Figure 1* shows examples of the most common patterns of genetic changes. The 16 tumors in which the whole long arm was lost comprised 7 cases of monosomy 1 (all informative loci on chromosome 1 had lost heterozygosity) and 9 of monosomy 1q (informative loci on the short arm retained constitutional heterozygosity).
Case 588 shows both a partial telomeric deletion on one long arm of chromosome 1 and polysomy of the entire long arm of the other copy differently modified relative to normal tissue.

These double alterations appeared to be due to a double genetic event, since the signal intensities of ip and iq markers were not differentially modified relative to normal tissue. This is consistent with the high frequency of allelic imbalance previously observed in breast cancer in studies using only one or a few genomic markers, especially concerning the MUC gene located in 1q21-23 (8, 10, 11). Tumor DNA alterations could be placed in two groups: loss of at least the whole chromosome 1q and/or gain of multiple copies of chromosome 1q (due to mitotic nondisjunction) and structural rearrangements of 1q.

Our fine analysis of chromosome 1q alterations identified a first group of 55 human breast tumors which showed loss (16 tumors), gain (38 tumors), or both loss and gain (1 tumor) of the whole copy of chromosome 1q. This is consistent with the results of previous cytogenetic studies: the frequency of chromosomal rearrangements was similar and most involved entire 1q deletions, polysomy or translocations (especially involving chromosome 16), or isochromosomy 1q (3–7). The rearrangements were mostly associated with a break point in the constitutive heterochromatin (21). We used probe DIS70, located near the heterochromatin on 1qcen, and this locus was consequently informative in 80% of the tumors studied. We found allelic imbalance in chromosome 1q in 80 (65%) of the 124 human breast tumors studied using 1q-specific DNA probes. This incidence is higher than that of other frequently altered regions in breast tumors, which are essentially deletions: 1p32-pter, 3p13-14.3, 7q31, 16p22-23, 17p13, and 17q21 (2, 20).

DISCUSSION

Various approaches can be used to detect genes whose alteration may lead to tumorigenesis. Cytogenetic analysis has been used to locate distinct chromosomal regions which might harbor genes contributing to tumorigenesis. Fine-scale molecular mapping of altered regions is needed to locate such genes precisely, and this can be done by studying the imbalance of heterozygosity. We thus carried out a molecular analysis of chromosome 1q alterations previously associated with human breast cancer in cytogenetic studies. We found allelic imbalance in chromosome 1q in 80 (65%) of the 124 human breast tumors studied using 1q-specific DNA probes. This incidence is higher than that of other frequently altered regions in breast tumors, which are essentially deletions: 1p32-pter, 3p13-14.3, 7q31, 16p22-23, 17p13, and 17q21 (2, 20). However, our findings are consistent with the high frequency of allelic imbalance previously observed in breast cancer in studies using only one or a few genomic markers, especially concerning the MUC gene located in 1q21-23 (8, 10, 11). Tumor DNA alterations could be placed in two groups: loss of at least the whole chromosome 1q and/or gain of multiple copies of chromosome 1q (due to mitotic nondisjunction) and structural rearrangements of 1q.

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Imbalance analysis at various chromosome 1q loci in human breast cancer. Left, probes used and their relative locations (16–18). Circles, informative loci; dots, homozygous loci. Some combinations were not determined (especially for D1S51, D1S70, and D1S26) for technical reasons or because limited amounts of DNA were available. □ (LOH), ○ (allele gain), and ■ (both LOH and allele gain), maximum extents of the alterations, as defined by one or more probes showing LOH, allele gain, or both. Right, smallest common overrepresented (SCOR) and deleted (SCDR) regions.

Fig. 2

Somatic changes in these tumors involved trisomy, tetrasomy, or polysomy of the entire chromosome 1q, in keeping with cytogenetic studies in which up to 9 copies of 1q were observed per cell (4). This high frequency of gain of a whole chromosome 1q in our panel of tumors suggests a putative dosage effect of the 1q gene in breast tumor development. This first group of 55 human breast tumors thus showed a perfect correlation between molecular and cytogenetic data but yielded no information on the delineation of the 1q alteration and, therefore, on the location of putative tumor genes.

The other 25 tumor DNAs showing partial alterations of 1q provided more information on the location of such genes. Alteration mapping revealed that break points on chromosome 1q differed between tumors, except for tumors T392 and T1156 (between the D1S65 and D1S61 loci), thus ruling out the hypothesis that a chromosome 1q break point itself plays an important role in the development of human breast tumors. LOH data for 12 breast tumors with partial deletions indicated that most partial deletions are proximal, whereas allele gain is mostly distal. The smallest common deleted region was located between loci D1S75 and D1S65, which are estimated, by means of linkage analysis, to be 16 centiMorgan apart (18). Deletions in this central location (1q21-31) have been detected by the use of both molecular and cytogenetic approaches in a wide variety of tumors (reviewed in Refs. 22 and 23), including breast tumors (8). The striking concordance between the central deletions in these different cancers suggests that functional inactivation of the same suppressor gene might be involved in the etiology of these different tumors. While only two oncogenes (SKI and NTRK1) are located in this region (19), there are no obvious candidate tumor suppressor genes.

By using large numbers of polymorphic markers for the entire chromosome 1q, we found that another distinct region (1q41-44) is affected by allele gain in breast tumors. We observed a smallest common overrepresented region in tumors with partial polysomy. The D1S8 and D1S51 loci appeared to be affected in all but one (T1343) of the cases showing 1q allele gain, but the length of the allele gain differed from one tumor to another. None of the genes suspected of being located in this region (ADP-ribosyltransferase NAD+, RAB4, and transforming growth factor β2) are obvious candidates for tumorigenicity in breast cancer, and showed no major rearrangements. However, we cannot rule out the possibility that these genes may also be altered by point mutations and go undetected by Southern
blotting. Major oncogenes in this region must regulate growth in a number of tissues, because cytogenetic analysis has shown that this region is frequently partially duplicated in various cancers (reviewed in Ref. 22), and their activation must therefore be required for the development of multiple tumor types.

It is noteworthy that two (and sometimes three or four) genetic alterations were observed in a single tumor, including an allele gain and/or a LOH on the long arm of chromosome 1 and/or a LOH on 1p. 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 syngeneic or on each chromosome of the pair as in the case of four tumors (T407, T588, T1156, and T1241) (Fig. 2) in which we observed one or several loci with both LOH of one allele and allele gain of the other allele in the telomeric part of 1q.

This study shows a high frequency (65%) of complex and multiple genetic alterations on the long arm of chromosome 1 in human breast cancer, suggesting that genes in this region are involved in human breast tumorigenesis. One region (1q41-44) was frequently overrepresented, indicating that it may harbor oncogenes. The other region (1q21-31), which was frequently deleted, may contain tumor-suppressor genes. These two alterations are common to tumors of different cellular origins. Unfortunately, the limitations of cytogenetic resolution and the small number of RFLP markers for use in molecular studies make it impossible to determine if these alterations involve a single tumor gene in different tumor types or distinct molecular lesions involving distinct genes. Identification of these putative genes will require further molecular studies using fine-scale genetic mapping with RFLP or microsatellite markers, and the construction of a physical map of these altered regions.

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