A Large 6q Deletion Is a Common Cytogenetic Alteration in Fibroadenomas, Pre-malignant Lesions, and Carcinomas of the Breast

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ABSTRACT

To assess whether early breast lesions are the precursors of invasive carcinomas, three classes of breast lesions, namely benign tumors (including fibroadenomas), putative premalignant lesions (including cases of atypical hyperplasia), and invasive carcinomas, were compared at the cytogenetic and molecular cytogenetic levels. Genetic relatedness was clearly demonstrated by the sharing of several anomalies, among which 6q deletions outnumbered all of the other alterations detected. Indeed, deletions of the long arm of chromosome 6, most likely occurring in epithelial cells, were present in 83.9% of benign breast tumors, 64% of putative premalignant lesions, and 77.4% of analyzable carcinomas. Furthermore, the interval between 6q24 and qter appeared to be the common region of deletion in all three classes of breast lesions, whereas the minimal common region of deletion was 6q27-qter. Interestingly, the latter region was reported previously to be deleted in benign ovarian tumors and recently found to harbor a gene (SEN6) that is important for SV40-mediated immortalization of human cells.

INTRODUCTION

Breast CA is among the most common and lethal malignancies in women in western countries. Because this malignancy is very common and difficult to treat, there is growing interest in studying early premalignant changes of invasive breast cancer to prevent and control this disease.

PBLs constitute a large group of abnormalities, the nature and contribution to overt CA of which are not well established yet. The most convincing evidence favoring a causal relationship between these two pathological entities (putative precursors and invasive CA) is mainly derived from epidemiological studies: women with PBLs such as usual hyperplasia, atypical hyperplasia, and in situ CA have approximately 2-, 4-, and 10-fold increased relative risks, respectively, of eventually developing invasive breast cancer (1–4).

Clearly, the presence of genetic abnormalities in PBLs would implicate these lesions as potential precursors of breast cancers and could shed light on genetic abnormalities that may play a role in early breast tumorigenesis. Recent insights concerning a probable common genetic pathway between benign lesions and CAs of the breast were gained from molecular studies employing LOH at 15 polymorphic loci in 399 putative precursor lesions (including UDH, ADH, and ductal CA in situ; Ref. 5). Loss of material from several loci was reported, and more importantly, a sharing of LOH phenotypes between hyperplastic breast disease and synchronous cancer has strengthened the concept that hyperplasias may be precursors of breast cancer (5–7).

Among benign lesions of the breast, one of the most common is fibroadenoma, which is considered a neoplasm composed of epithelial and stromal components (8). Women can present with this lesion at any age, but the tumor is most commonly diagnosed when the patients are in their twenties, an age that is very unusual for breast cancer. Perhaps for this reason, fibroadenomas have traditionally been thought to be unrelated to breast cancer. Nevertheless, several investigators have found that women with fibroadenomas, compared with women of similar age in the general population, have two to three times the general risk of breast cancer (9–13).

To date, only 33 fibroadenomas with clonal chromosome aberrations have been reported (14–21). In all cases, the cytogenetic analysis was performed using short-term cultures, and

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3 The abbreviations used are: CA, carcinoma; ADH, atypical ductal hyperplasia; BBT, benign breast tumor; BL, breast lesion; cDCIS, comedo ductal carcinoma in situ; ncDCIS, noncomedo ductal carcinoma in situ; FISH, fluorescence in situ hybridization; LOH, loss of heterozygosity; PBL, proliferative breast lesion; PPL, putative premalignant lesion; UDH, usual ductal hyperplasia; YAC, yeast artificial chromosome.
the origin (epithelial or stromal) of the clonal abnormalities was not evaluated. No fibroadenoma-specific aberrations have emerged from the reported studies, although rearrangements of chromosomes 1, 6, and 3 have been repeatedly involved in clonal changes (14, 19–21). Numerical alterations and, in particular, monosomy for chromosome 6 and trisomy for chromosomes 4, 7, and 20 were also reported (14–21). However, studies using molecular approaches failed to detect alterations in this class of benign neoplasms (22).

To provide additional evidence for a genetic relatedness between putative precursor lesions and breast CAs, we performed cytogenetic and FISH analyses on fibroadenomas, hyperproliferative lesions, and breast cancers. Additionally, we also studied a series of cases in which a double sampling in morphological different areas (cancerous and noncancerous) was performed. All cytogenetic results described in this report were obtained following direct harvesting and short-term incubation of the tumor cells. We found that this methodological approach is the most appropriate to study the chromosome pattern of solid tumors because it avoids selection biases due to culturing (23, 24).

Among several cytogenetic changes detected in all three classes of BLs, the most frequent lesions involved chromosome 6q. This finding, besides adding further evidence for a progression mechanism reminiscent of that identified in colon cancer, suggests that alterations of genes located in 6q are among of the earliest events in the pathogenesis of breast cancer.

MATERIALS AND METHODS

Samples

During the period from June 1996 to May 1998, a series of 80 BLs was investigated. Tumor and nontumoral samples were obtained from patients after surgical removal and sent under sterile conditions for cytogenetic and histological investigations, which were performed on contiguous areas. All BLs were newly diagnosed, previously untreated, and histologically well characterized.

Three basic microscopic patterns were observed: (a) BBTs, including 28 fibroadenomas (19 of which were classified as complex according to the criteria reported in Ref. 8), 1 tubular adenoma, 1 intraductal papilloma, and 1 benign phyllodes tumor; (b) PPLs, including proliferative lesions (two adenosis and one fibrocystic disease) in noncancerous breast and proliferative lesions in cancerous breast, such as adenosis (6 cases) and atypical hyperplasia (5 cases); and (c) CAs, including 4 in situ CAs (2 low grade and 2 high grade) and 31 invasive CAs (10 moderately differentiated and 10 poorly differentiate ductal CAs, 6 tubulolobular CAs, 2 mucinous CAs, 2 comedocarcinomas, and 1 medullary CAs).

In eight cases, a double sampling and karyotyping in morphologically distinct areas of the same cancerous breast were performed. These samples included hyperplastic and cancerous areas, fibroadenomas and cancerous areas, in situ CA and invasive CA areas, epithelial hyperplastic lesions, and benign phyllodes tumor areas.

In summary, we cytogenetically evaluated 80 samples of BLs, including 31 BBTs, 14 PPLs, and 35 CAs from 72 Caucasian patients. The mean age of patients with BBTs was 32.07 years (range, 16–57), whereas the mean age of patients with PPLs and CAs was 54.0 (range, 29–71) and 57.7 years (range, 33–85), respectively.

Histological and Immunohistochemical Analysis

After formalin fixation and paraffin embedding, the mammary lesions were classified on H&E-stained sections according to the criteria reported in Ref. 8. Proliferative index of epithelial and stromal components was investigated immunohistochemically using MIB-1 (and monoclonal detecting Ki67 antigen antibody; Immunotech, Marseilles, France; diluted 1:110) after microwave treatment and diaminobenzidine immunostaining. The MIB-1 proliferation index was calculated based on a count of a minimum of 500 nuclei of neoplastic, epithelial hyperplastic, or stromal cells in the highest proliferative areas and expressed as a percentage of positive cells. Mitotic index was evaluated as number of mitoses found in 10 high-power (×400) fields of highly cellular areas using an Olympus microscope.

Statistical Analysis

Student’s t test was performed in all three classes of BLs to evaluate differences as follows: (a) the comparison of MIB-1 and mitotic indexes found between epithelial and stromal components; and (b) the comparison of 6q deletion in relation to the MIB-1 value.

Cytogenetic Analysis

Chromosome analysis was performed in each case on direct preparations as described in Ref. 23. Briefly, the suspension of tumor cluster cells obtained by mincing small pieces of the tumor was incubated for 24 or 48 h at 37°C with 5% CO2 and harvested after overnight colcemid treatment (0.02 μg/ml). Five cases of normal breast tissues surgically removed for mammoplasty were harvested by the same method, but no spontaneous metaphases were found in any of the cases.

Karyotype evaluation was performed using Q-bands by fluorescence using quinacrine and G-Wright banding techniques. Chromosome abnormalities were described according to the Cancer Cytogenetics Supplement recommendations (25). In particular, only clonal abnormalities were considered in the description of the tumor karyotype: more specifically, the same structural rearrangement or chromosomal gain had to be present in at least two metaphases, whereas loss of a chromosome had to be detected in at least three metaphases. When different tumor cell populations were identified, the chromosome complement of each population was described. A cell line was classified as haploid when the chromosome number ranged from 10 to 34 or as diploid when the chromosome number ranged from 35 to 57.

FISH Analysis

FISH. FISH using simultaneously chromosome 6 α-satellite (purchased from Oncor) and YACs as probes was performed using the method of Pinkel et al. (26) with modifications. The chromosomal DNA was denatured in 70% formamide, 2× SSC, pH 7.0, at 72°C for 2 min. Cot-1 and YAC DNA were dissolved and denatured at 80°C for 10 min and preannealed for 1 h at 37°C. YAC DNA was mixed with denatured α-satellite probe. Hybridization was done at 42°C
In the present study, we have investigated 31 BBTs, 14 PPLs, and 35 CAs. The mitotic and the MIB-1 indexes were evaluated separately on the epithelial and stromal components in the three classes of lesions. The MIB-1 index was significantly higher ($P < 0.01$) in the epithelial than in the stromal component in all BL classes. Likewise, the mitotic index showed significantly higher values in the epithelial than in the stromal component in BBT, PPL, and CA classes ($P < 0.05$, $P < 0.05$, and $P < 0.001$, respectively). These findings indicate that in these BLs, the epithelial cells grow more rapidly than the fibroblastic ones.

In all three groups of BLs, a diploid and haploid chromosome complement were observed. In particular in the majority of BBTs (18 of 31), PPLs (12 of 14), and CAs (22 of 35), a haploid cell line in addition to the diploid one was observed. The mean percentage of cells with diploid complement (35–57 chromosomes) was 75.2% for BBTs, 59.3% for PPLs, and 70.1% for CAs. The asymmetric distribution of chromosome number indicates that the haploid cell line derives from the diploid one following progressive loss of chromosomes. The three BL classes showed the same distribution and a high degree of karyotypic heterogeneity. The tumoral karyotypes of the three classes are listed in Tables 1–3. A normal chromosome complement was identified only in one BBT and in two PPLs. Clonal chromosome anomalies were found in 30 of 31 (96.8%) BBTs, in 12 of 14 (85.7%) PPLs, and in all cases of CA.

Four cases of breast CA showed complex karyotypes: metaphases of these tumors contained fragmented chromo-
monosomies (for chromosomes 8, 10, 15, 16, 17, 20, 21, 22, and 23) were more frequent than the trisomies; many of the abnormalities of the diploid cell lines only. In all cases, the karyotype description (Table 3, cases 24–26, and Table 4, cases 27–31) was extremely heterogeneous, we have considered the chromosomal abnormalities of the diploid cell lines only. In all cases, monosomies and/or trisomies of chromosomes 1 and trisomy of chromosomes 1, 14, and 15 was detected only in CAs (Fig. 1).}

### Table 2 Cytogenetic analysis of PPL

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</tr>
<tr>
<td>2</td>
<td>46,XX</td>
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<tr>
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<td>35–46,XX,−9,−10,−13,−14,−16,−17,−20,−22/18–30,X,+2,+3,+4,+5,+8,−9,−10,+11,+12,+13,+17,−18,−19,−20,+21,+22[cp9]</td>
</tr>
<tr>
<td>6</td>
<td>36–46,XX,−X,X,del(6)(q24-pter)[cp8]/19–32,X,−X,+1,+2,+3,+4,+5,+6,−6,−7,−12,−15,+16,+17,+18,+21[cp6]</td>
</tr>
<tr>
<td>7</td>
<td>39–45,XX,del(6)(q24-pter),−16[cp6]/19–31,X,+1,+2,+3,+4,+5,+8,−8,+9,−10,+11,+15,+17,+19,−20,+21,+22[cp6]</td>
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<tr>
<td>8</td>
<td>39–46,XX,del(6)(q23-pter),−8,−10,−19,−20[cp12]/21–34,XX,−X,+3,+4,+5,+6,−7,−8,−10,+11,+13,+14,+16,−19,+20,+21,+22[cp6]</td>
</tr>
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<td>9</td>
<td>36–46,XX,del(6)(q25-pter),−16,−17,−18,−19,−20,+21,+22[cp6]</td>
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### Table 3 Cytogenetic analysis of CA*

<table>
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<td>3</td>
<td>47,XX,+12[12]</td>
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<td>38–46,XX,−17[cp6]</td>
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<td>43–47,XX,del(6)(q25-pter),+12[cp6]</td>
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<td>39–45,XX,del(6)(q24-pter),−21[cp5]</td>
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<td>7</td>
<td>35–46,XX,−4,−6,del(6)(q25-pter),−8[cp8]</td>
</tr>
<tr>
<td>8</td>
<td>46,XX,del(6)(q24-pter)[3]/10–13,XX,+7,−11,−16,+18[cp4]</td>
</tr>
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<td>9</td>
<td>37–47,XX,del(6)(q25-pter),+mar[cp9]/27–34,XX,+2,+4,+9,+10,+11,+13,+14,+15,+18[cp3]</td>
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<td>35–46,XX,del(6)(q24-pter),−15[cp6]/26–32,X,−X,+1,+3,+4,+5,+8,+9,+11,+12,+15,+18,−19,+21,+22[cp5]</td>
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<td>12</td>
<td>37–46,XX,−6,del(6)(q25-pter),−15,−16,−17,−19[cp8]/12–32,XX,−X,+1,+2,+3,+4,+5,−5,+7,−8,+9,+10,+10,+11,+11,−12,+13,+14,−16,+18,−19,−20,+21,+22[cp8]</td>
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<td>13</td>
<td>46,XX,del(6)(q24-pter),del(8)(q24-pter)[9]/15–34,XX,−X,+3,+4,+5,+6,−7,+9,+10,+11,+12,+13,+14,+16,+19,−22[cp6]</td>
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<td>14</td>
<td>36–46,XX,−8,−19,−21[cp9]/24–34,XX,+1,+2,+3,+5,+7,+10,+11,+16,+18,+19,[21[cp5]</td>
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<td>36–43,XX,−1(17),−3,−4,del(6)(q25-pter),−17[cp5]/25–31,XX,+2,+4,−5,+7,−12,−16[cp3]</td>
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<td>16</td>
<td>45,XX,−X,−2.dup(5)[q12.2][13]/34命题,del(6)(q25-pter),−19–32,XX,−X,+1,+3,+4,+11,−13,−14[cp4]</td>
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<td>17</td>
<td>45,XX,−X,−del(6)(q25-pter)[3]/46,XX,del(6)(q25-pter)[6]/19–34,XX,−X,+1,+2,+3,+6,−7,+9,+10,+11,+12,+14,+15,+21[cp4]</td>
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<tr>
<td>18</td>
<td>39–46,XX,−6,del(6)(q25-pter),−15,−16,−17,−19[cp13]/24–31,XX,+5,+14,+16,+21,+22[cp3]</td>
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<td>19</td>
<td>38–46,XX,del(6)(q24-pter),del(6)(q25-pter)−22/27–34,XX,−X,+1,+2,−3,+3,+4,+5,+8,+11,+12,+13,+14,+16,+17,−18,+20,+21,+22[cp4]</td>
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<td>21</td>
<td>46,XX,del(6)(q26-pter)[2]/18–32,X,−X,+1,+2,+3,+5,+7,+9,+10,+11,+13,−16,−18,+19,+20,+22[cp6]</td>
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<td>22</td>
<td>A: 46,XX,del(6)(q25-pter),−15,−18,−19[cp8]/25–31,XX,+1,+3,+4,+5,+7,+10,+11,+12,+15,+17,+18,+20[cp4]</td>
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<tr>
<td></td>
<td>B: 46,XX,del(6)(q24-pter)[7]</td>
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<tr>
<td>23</td>
<td>A: 37–46,XX,del(6)(q24-pter),−21,−22,−23[11]/15–29,X,+1,+2,−3,+4,+5,+6,−16[cp5]</td>
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</tr>
</tbody>
</table>

- In brackets is the number of mitoses analyzed.
- A and B are different samples from the same case.

By haploid cell lines in the three groups of BLs were extremely heterogeneous, we have considered the chromosome abnormalities of the diploid cell lines only. In all cases, monosomies were more frequent than the trisomies; many of the monosomies (for chromosomes 8, 10, 15, 16, 17, 20, 21, 22, and 23) were common in BBT, PPL, and CA groups. Some aneuploidies were peculiar to a single class of BLs; in particular, monosity of chromosome 1 and trisomy of chromosomes 1, 18, and 21 were detected only in BBTs, trisomy of chromosome 4 was detected only in PPLs, and trisomy of chromosomes 11 and 12 was detected only in CAs (Fig. 1). Concerning chromosome rearrangements, anomalies of chromosomes 1, 3, 5, 8, and 10 were found in CAs samples only, whereas structural aberrations of chromosome X were
present restricted to the PPL group. Rearrangements of chromosome 2 were present both in CBAs and BBTs.

Worth noting is that 6q deletions with different breakpoints along the long arm were the most frequent and the sole common anomaly in all three classes of BLs, and more precisely, a 6q deletion was present in 26 of 31 (83.9%) BBTs, in 9 of 14 (64.3%) PPLs, and in 24 of 31 (77.4%) analyzable CBAs. Furthermore, in three cases of BBT and two cases of CA, 6q deletion was found as the sole chromosome anomaly (Table 1, cases 2–4; Table 3, cases 1 and 2). In four cases (two CBAs, one BBT, and one PPL; Table 3, cases 19 and 22; Table 2, case 7; Table 1, case 28), allelic loss heterogeneity with two different clonal 6q deletions was observed, which most likely suggests intralesional progression. Furthermore, 6q deletions with different breakpoints were also detected in the haploid cell lines, suggesting that this chromosome abnormality arises before the aneuploid state. Other single anomalies found in two cases of CBAs (Table 3, cases 3 and 4) involved trisomy of chromosome 12 and monosomy of chromosome 17.

The extent of the 6q deletions was determined by conventional cytogentic and FISH analysis and is summarized in Table 3, cases 5 and 6. In all three BL classes the deletion spanned from 6q23 to 6qter, and the breakpoints most frequently involved in these terminal deletions were 6q24, 6q25, and 6q26. The interval between 6q24 and 6qter appears to be the common region of deletion in all three classes of BLs. Moreover, the minimal common region of deletion was 6q27–qter, whereas the region defined by band 6q23 was deleted only in CA and PPL samples.

Among the 8 cases in which a double sampling of the breast tissue was performed (see “Materials and Methods”), we found similar chromosome abnormalities in in situ CA and invasive CA areas (one case), in ADH and invasive CA areas (one case), and in fibroadenoma and tubulobular CA areas (two cases). The karyotypic profiles of these cases is shown in Table 4. Again, in all of the cases mentioned above, 6q deletion was the most prominent finding. We then compared the detection of 6q deletion with the MIB-1 value in each BL classes, but no statistically significant relationship was found.

In conclusion, chromosome 6 deletions, comprising the 6q24–qter interval, were the most consistent and frequent alterations detected, although they did not correlate with all clinicopathological parameters, such as grade, size, and axillary lymph node involvement. These deletions, besides being supporting evidence for the hypothesis that putative precursors and cancers are genetically related proved that abnormalities of 6q, are among the earliest lesions in the pathogenetic process leading to frank CAs.

To better define and confirm the 6q deletion, we have investigated a selected group of cases from the three BL classes by FISH analysis using YAC clones as probes. The YAC recombinants were mapped to several 6q regions known to be deleted in malignant BLs, and they contained the same markers found by LOH analysis to be lost. The YAC clones were localized to the 6q26–27 (clones 962b4, 17a12, 4e8, and 74e9), 6q25 (clone 823b11), 6q24 (clone 962 g7), 6q21 (clone 962 g10), and 6q13 (clone 765a3) regions. Table 2 shows cases from the three different groups of BLs in which YAC clones

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**Table 4: Cytogenetic analysis of double sampling tumors**

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<th>Cases</th>
<th>Histotype</th>
<th>Karyotype</th>
</tr>
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<tr>
<td>3 A: atypical hyperplasia</td>
<td>A: 35–46,XX,–4,–5,–6,del(6)(q26-qter),–17,–18,–21[cp10]12–31,XX,–1,–1,–2,–3,–4,–4,–5,–6,–8,–9,–10,–10,–11,–12,–13,–13,–14,–15,–16,–17,–17,–17,–18,–22,–22[cp8]</td>
<td>B: ductal carcinoma</td>
</tr>
</tbody>
</table>

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*In brackets is the number of mitoses analyzed.

* A and B are different samples from the same case.
were lost from one chromosome 6 homologue. Moreover, Fig. 2 shows an example of FISH analysis depicting a loss of the terminal region on one homologue evidentiated by YAC clone 4he8.

As expected, the molecular cytogenetic approach confirmed the conventional cytogenetic results and allowed us to localize, in all three classes of BLs, the proximal most commonly involved breakpoint, which is tentatively located between YAC clones 962 g7 (6q24) and 823b11 (6q25). The definition of the distal breakpoint is inconclusive, as YAC clone 74e9 (the...
most telomeric clone used in this study) is deleted in the majority of the analyzed samples. The involvement of other 6q areas known to be deleted in CAs (e.g., 6q13 and 6q21) was found to be normal in those cases (three PPLs and three benign BLs) not showing any gross abnormality of 6q (data not shown).

Furthermore, we investigated with YAC clones from the previously mentioned regions four cases showing normal chromosome 6 with conventional cytogenetics (case 2 in Table 2, case 14 in Table 3, case 26 in Table 1, and sample 6B in Table 4): two of these (case 2 in Table 2 and case 14 in Table 3) failed to show deletion at the molecular level, whereas the other two cases (case 26 in Table 1 and case 6B in Table 4), showed deletion of YAC clones 4He8 and 74e9, respectively. Unfortunately, identification of the molecularly deleted region was not possible because of the very small number of metaphases available for FISH analysis.

DISCUSSION

The possibility of detecting precursor lesions of invasive neoplasms is of paramount importance from the diagnostic, prognostic, and therapeutical points of view. This seems to be particularly true for breast cancer, which, among other malignancies, raises serious therapeutical problems once it is diagnosed. It is still uncertain whether the pathogenesis of breast cancer can be assimilated to the multistep model of carcinogenesis, according to which tumors develop and progress as a result of changes in oncogene and tumor suppressor gene loci (27). This model has successfully explained the benign to malignant transition of colon cancers with recognizable molecular changes involving the above classes of genes (27). Unfortunately, in breast cancer (and other solid malignancies like ovarian neoplasms), the colon paradigm cannot be applied because little is known about the natural history of the disease.

Up to now, evidence favoring a direct relationship between breast benign and malignant lesions relied mainly on histological grounds, where a coexistence and a continuum between these lesions is seen (28, 29). Further evidence, although indirect, was provided by epidemiological studies showing that women with usual hyperplasias, atypical hyperplasias, and in situ CAs have 2-, 4-, and 10-fold increased relative risks of eventually developing invasive CAs (1-4).

However, recent cytogenetic and molecular studies of benign BLs seem to indicate the presence of recognizable specific alterations that are also present in invasive CAs (5-7, 30). In particular, in one of these studies (5), LOH at several loci was observed in 37% of UDH cases and in 42% of ADH cases from noncancerous breasts, whereas higher rates of LOH were observed in noncancerous ncDCIS (70%) and cDCIS (79%). Interestingly, sharing of LOH between the hyperplastic lesions (UDH and ADH) as well as cDCIS/ncDCIS lesions and synchronous cancer was clearly established, with an increased frequency ranging from UDH to cDCIS.

Clearly, the message gathered from this study gives a solid basis to the concept that hyperplasias may be direct precursors of breast CAs.

In the present study, we have extended and added further evidence for such relationship by analyzing and comparing, at the cytogenetic level, 31 BBTs, 14 putative precancerous lesions, and 35 CAs. Among several chromosome alterations observed and shared by the three classes of breast abnormalities, chromosome 6q deletions were by far the most frequent, being present in 83.9, 64.3, and 77.4% of BBTs, PPLs, and CAs, respectively. As 6q alterations are a frequent finding among breast CAs (and several other solid and hematological malignancies; Ref. 31), it is very likely that the development of BBTs, PPLs, and CAs involves the same or similar genetic pathways (at least in a temporal fashion; see below). This concept is further strengthened by the finding, in the same breast, of coexisting histologically different lesions (such as in situ invasive CA and ADH or fibroadenoma and tubulolobular CA), the majority of which show 6q deletion as the common shared lesion.

We have also tried to address the genetic bases of another frequent BL, namely fibroadenoma. This type of BL represents a separate entity among the breast neoplasms in that, unlike other breast neoplasms, the target of which is the epithelium,
fibroadenomas are considered benign lesions of biphasic origin. Information concerning these neoplasms is still scanty; for instance, studies using molecular techniques have not found any evidence of genetic alteration (22). More insights were gained by conventional cytogentic analysis using short-term cultures: to date, only 33 fibroadenomas revealed clonal chromosome aberrations (14–21). Among the cytogenetically detected lesions, several (such as rearrangements of 1p or 3p or trisomy 20, and so forth) are also present in CAs (14–21).

The majority of these studies, however, did not address the nature of the cells, i.e., epithelial or stromal harboring the clonal anomalies. In the present study, we have studied 28 fibroadenomas and tried to solve two of the limitations of the previous studies, namely short-term culturing and undefined nature of the involved cells. Although they are indirect, immunohistochemical data tell us that the epithelial component of fibroadenomas has a higher proliferative activity than the stromal one, suggesting that, very likely, the direct method metaphases are epithelial in origin. As mentioned above, the vast majority (83.9%) of fibroadenomas showed deletions or rearrangements of 6q. To our knowledge, this is the first report describing the substantial contribution of chromosome 6 alterations among the genetic lesions present in fibroadenomas.

Taken together, the data that we have collected in this study raise another interesting point concerning the temporal aspects of breast tumorigenesis that implies the occurrence of 6q alterations as one of the earliest events in the pathogenetic process leading to CA. This is not in contradiction with an increasing body of evidence indicating that the presence of one or more genes on 6q controls the immortal growth/senescence phenotype typical of human and murine cells (32–37). Evidence obtained primarily from chromosome transfer studies indicates that chromosome 6 is capable of restoring senescence in human SV-40 immortal ovarian tumor cells (36). The emergence of spontaneous revertants from murine fibroadenomas (33) and ovarian cell lines (36) was associated with the loss of sequences from 6q that were localized to 6q26–27, 6q21–23 (33), and 6q13–21 (36). On the other hand, these data agree remarkably well with LOH analyses on breast CAs in which the same regions, with the addition of 6q23–q25, are found deleted in a high proportion of tumors.

The 6q27 region has recently been the subject of intensive investigation, which resulted in the mapping of a senescence gene (SEN6) in the interval defined by markers D6S133 and D6S281, positioned 3.2 cM apart (33). Finer narrowing, within 6q27, for this gene or another with similar features was provided by our group with the construction of a YAC contig and a genomic long-range restriction map around the D6S149–D6S193 interval, which is included in the larger D6S133–D6S281 region (24). This study has indeed provided for the first time evidence for 6q27 involvement in benign ovarian tumors, a situation that parallels what is described in this study for BLs (i.e., BBTs and PPLs), and has highlighted a genomic region of no more than 450 kb as the shortest region of deletion for these type of tumors. Worth noting is another benign condition, namely parathyroid adenomas, which has yielded similar findings, with losses overlapping the same area (Ref. 45; LOH analysis in parathyroid adenomas revealed deletions for markers D6S264 and D6S37, which are positioned either centromeric or telomeric to the D6S149–D6S193 interval including it). Because all of the conditions mentioned are benign in nature and all have the epithelium as the target of the carcinogenic process, it is tempting to speculate that a gene(s) located in 6q27 and exerting a restricted action on the epithelium could play an important role in the early steps of the tumorigenic process, as does immortalization, which in some tumors is the primary detectable event.

To date, very few candidate genes have been mapped to the 6q27 genomic region encompassing the deletion areas found in the benign ovarian tumors and common to the above mentioned benign conditions. IGF2R and AF-6 are two interesting candidate genes positioned in 6q26–27 (46–48) and found to harbor deleterious mutations in several malignant tumors [AF-6 is disrupted in a t(6;11)(q27;q23) chromosomal translocation frequently observed in acute myeloid leukemia (48), whereas IGF2R is found to be altered in tumors exhibiting the microsatellite instability phenotype (49)]. Both genes were positioned either distally (AF-6) or proximally (IGF2R) to the deleted area, thereby excluding them as candidates for being altered in benign tumors.

Within the ovarian neoplasm deleted area, only the genes coding for a member of the Rh/T2S/glycoprotein RNase family (50), for a G-protein coupled receptor (51), and for the human homologue of the testes-expressed locus tcp10 (TCP-10) were recently mapped (52). Extensive screening of the coding regions of these genes in tumors in which at least one chromosome 6 homologue was present did not reveal any mutation (excluding regulatory mutations or gene dosage effects).4 Additional transcripts in the restricted 450-kb region that could be assessed either by mutation screening in tumors or by functional assays after introduction into suitable immortal or tumoral cell lines are being isolated in our laboratory by gene search methodologies such as cDNA selection and exon trapping techniques.

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A Large 6q Deletion Is a Common Cytogenetic Alteration in Fibroadenomas, Pre-malignant Lesions, and Carcinomas of the Breast

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