The Accumulation of Specific Amplifications Characterizes Two Different Genomic Pathways of Evolution of Familial Breast Tumors

Lorenzo Melchor, Sara Álvarez, Emiliano Honrado, José Palacios, Alicia Barroso, Orland Díez, Ana Osorio, and Javier Benítez

Abstract

Purpose and Methods: High-level DNA amplifications are recurrently found in breast cancer, and some of them are associated with poor patient prognosis. To determine their frequency and co-occurrence in familial breast cancer, we have analyzed 80 tumors previously characterized for BRCA1 and BRCA2 germ-line mutations (26 BRCA1, 18 BRCA2, and 36 non-BRCA1/2) using high-resolution comparative genomic hybridization.

Results: Twenty-one regions were identified as recurrently amplified, such as 8q21-23 (26.25%), 17q22-25 (13.75%), 13q21-31 (12.50%), and 8q24 (11.25%), many of which were altered in each familial breast cancer group. These amplifications defined an amplifier phenotype that is correlated with a higher genomic instability. Based on these amplifications, two different genomic pathways have been established in association with 8q21-23 and/or 17q22-25 and with 13q21-31 amplification. These pathways are associated with specific genomic regions of amplification, carry specific immunohistochemical characteristics coincident with high and low aggressiveness, and have a trend to be associated with BRCA1 and BRCA2/X, respectively.

Conclusion: In summary, our data suggest the existence of two different patterns of evolution, probably common to familial and sporadic breast tumors.

Germ-line mutations of BRCA1 (OMIM 113705) or BRCA2 (OMIM 600185) confer an estimated cumulative lifetime risk of 56% to 84% of breast cancer and a 15% to 45% risk of ovarian cancer (1, 2). High-risk families are usually selected based on specific clinical criteria to perform a genomic screening of these genes, but their mutations only explain 30% of familial breast cancers (3). Several candidate loci such as 8p12-p22 (4), 13q21 (5), and 2q32 (6) have been postulated to represent the genetic background of the remaining 70% of families (group named BRCAX) without definitive results.

Immunohistochemical and expression profiles have been defined for familial breast cancers associated with distinct classes of BRCA tumors. Briefly, BRCA1 tumors are high grade, negative for hormone receptors and HER-2, positive for p53, E2F6, cyclins A, B, and E, SKP2, and topoisomerase I; and display a high expression of P-cadherin (7–10). In contrast, BRCAX tumors have a lower grade and an opposite phenotype which is hormone receptor and HER-2 positive, a low proliferation rate, and undetectable P-cadherin expression (9, 10). BRCA2 tumors show an intermediate phenotype with a higher proliferation rate than BRCAX, and positive markers different from BRCA1 such as cyclins D1 and D3, p27, p16, p21, and cyclin-dependent kinases 4, 2, and 1 (7–10).

Hereditary breast tumors have also been studied characterizing their genomic changes using comparative genomic hybridization (CGH; ref. 11). Some of the genomic changes present in these tumors have been used to build classifiers that distinguish between sporadic and BRCA1/2 tumors (12, 13) or between BRCAX and BRCA1/BRCA2 tumors (14). This last predictor, which uses high-resolution CGH (HR-CGH), allowed us to identify BRCAX cases with a genomic changes profile similar to BRCA1 tumors, probably due to aberrant methylation of the BRCA1 promoter (14).

These studies have also shown the existence of common alterations such as high-level DNA amplifications that, in fact, are recurrently found in familial and sporadic breast cancer (15). Some of these amplified regions include known oncogenes, such as MYC (8q24), ERBB2 (17q12), FLG (8p12), CCND1 (11q13), and IGF1R/FES (15q24-q25; refs. 16, 17); some of these oncogenes, such as MYC and ERBB2, have largely been correlated with poor prognosis (18, 19). However, in other cases, specific high-level amplifications, such as 8q23, 17q23-q25, and 20q11-q13, have been observed at chromosomal sites that do not coincide with the locations of the classic...
breast cancer oncogenes (20). The comprehensive characterization of some of these amplicons has already started (21, 22) but their meaning remains unclear, especially because some studies have shown that there are tumors with a trend to accumulate high-level DNA amplifications (23–25).

In the present study, we have used HR-CGH to estimate the frequency and distribution of regions with high-level amplifications in familial breast tumors because this genomic event may have important prognostic value. We have classified the cases according to the number of amplifications and defined their immunohistochemical characteristics and the immunohistochemical profiles associated with specific amplification pathways.

Materials and Methods

Patients and tumor samples. Eighty breast tumors were collected from patients selected from three centers in Spain: the Spanish National Cancer Centre (CNIO), the Fundación Jimenez Diaz in Madrid, and the Hospital Sant Pau in Barcelona. Patients belonged to families with at least three women affected with breast/ovarian cancer and at least one of them diagnosed before 50 years, or to families with female breast/ovarian cancer and at least one case of male breast cancer. All cases had been studied for mutations in the BRCA1 and BRCA2 genes using standard procedures (26). Twenty-six cases presented mutations in the BRCA1 gene, 18 cases had mutations in the BRCA2 gene, and 36 cases were negative for germ-line mutations in these genes and were considered as BRCA.

High-resolution comparative genomic hybridization analysis. Genomic DNA was isolated from 4 × 10^-6 sections of 80 paraffin-embedded tumors using a commercially available DNaise Tissue Kit (Qiagen, Chatsworth, CA) according to the recommendations of the manufacturer. HR-CGH was carried out as described in our recent study that included 72 of these cases, and for genomic studies, we used the 63 regions previously defined (14). Briefly, we chose as the most common minimal regions of involvement 50 regions including imbalances in at least 30% of the BRCA cases used to build our previous predictor (14) and with at least three cases defining the cytogenetic thresholds. To include the rest of the genome not fitting the previously defined criteria, we grouped the unselected areas on 13 chromosomal regions. Those chromosomal regions with CGH ratios >1.5 were defined as high-level amplifications and considered as recurrent when they were found in two or more cases.

Tissue microarray and immunohistochemistry. For immunohistochemistry studies, we used a previously published tissue microarray (8, 9) that included 74 of the breast tumors here analyzed by HR-CGH; 23 were BRCA1, 18 were BRCA2, and 33 were BRCA.

Before tissue microarray construction, total sections of each H&E-stained tumor were evaluated and classified according to the WHO classification. Grade was assessed using the Nottingham grading system.

Immunohistochemical staining was done by the Labeled Streptavidin Biotin method (DAKO, Glostrup, Denmark) with a heat-induced antigen retrieval step. Sections from the tissue array were immersed in boiling 10 mmol/L sodium citrate at pH 6.5 for 2 minutes in a pressure cooker. Antibodies, dilutions, and suppliers are listed in Supplementary Table S1. Two pathologists (E.H. and J.P.) independently evaluated in a blind study the immunohistochemical staining of nine antibodies. The percentage of stained nuclei, independent of the intensity, was scored for estrogen receptor, progesterone receptor, p53, and Ki-67. In the same way, the percentage of cells with cytoplasmic stain was scored for Bcl-2.

We took the mean of the percentage of stained cells as the cutoff point. Thus, when the percentage of stained cells was ≥10%, we considered the tumor as positive for estrogen receptor and progesterone receptor; ≥25%, positive for p53; and ≥70%, positive for Bcl-2. Three categories were defined for Ki-67: 0% to 5%, 6% to 25%, and >25% of stained nuclei.

A tumor was considered to have preserved expression of E-cadherin and catenins (γ-catenin and p120CREM) when >75% of the cells showed complete membranous staining of similar intensity as normal breast epithelium (27).

Statistical analysis. We used a nonparametric Mann-Whitney U test to identify differences in the number of genomic changes (chromosomal gains or losses) among the patient groups (based on the BRCA class or the number of high-level amplifications). Differences in the frequency of involvement of individual chromosomal regions among the three familial breast cancer classes were tested with Fisher’s exact test. The indicated P values were calculated using the Stat POMELO (28). This tool is available at http://pomelo.bioinfo.cnio.es. To determine immunohistochemical differences between groups based on the main amplified region, the χ2 test was used with Fisher’s exact test correction when necessary. The SPSS for Windows statistical program (SPSS, Inc., Chicago, IL) was used for this analysis. Hierarchical unsupervised clustering was done using the UPGMA method (28). The statistical test and the clustering are implemented in the GEPAS package (http://bioinfo.cnio.es).

Results

In the present study analyzing 80 tumors by HR-CGH, we have found similar results with our previous work that included 72 of them (14). Briefly, the mean number of changes was higher in BRCA1 and BRCA2 than in BRCAx tumors. Four chromosomal regions were commonly altered in ~50% of the three BRCA groups: gains at 1q and 8q21-23 and losses at 8p21-23 and 11q22-25. There were also specific changes that significantly distinguished each BRCA class in the intergroup comparisons (P < 0.05, nonadjusted Fisher’s exact test). The overall frequencies of changes in the 63 regions are shown in Supplementary Table S2.

Identification of recurrent regions with high-level DNA amplification

We found 21 chromosomal regions recurrently amplified (HR-CGH ratio > 1.5, present in two or more cases). The most frequently involved cytogenetic regions were 8q12-23 (21 of the 80 cases studied), 17q22-25 (11 cases), 13q21-31 (10 cases), 8q24 (9 cases), 12q15-21 (7 cases), and 20q12-13, 15q22-26, and 3q25-26.3 (6 cases each; Fig. 1). The most frequently altered regions were common to all the BRCA groups except 17q22-25 and 20q12-13, which were not present in the BRCA2 mutation carriers.

No amplified regions were found in ~50% of cases of the three groups (39 cases); these cases were named “non-amplifier” (NA) tumors, in contrast to the rest of cases that had at least one amplified region. Based on the median number of amplifications, we established a cutoff that distinguished two further categories: “low-amplifier” (LA) tumors that showed one or two regions with high-level DNA amplifications (lower than the median number; 18 cases) and “high-amplifier” (HA) tumors which had three or more amplified regions (equal to or more than the median number; 23 cases). The distribution of these categories within the distinct BRCA classes is shown in Fig. 2. BRCA1 tumors seem to have a higher trend to accumulate amplifications than BRCA2 or BRCAx tumors although the three groups do not present significant differences. As expected, those tumors with more amplified regions had significantly more genomic alterations. HA tumors have a higher genomic instability than the other phenotypes, and the same occurs with LA tumors.
that present higher instability than NA tumors (Supplementary Fig. S1).

**Two different pathways of genomic evolution**

*Association with specific amplifications.* We have analyzed the association of the amplified regions with each other using an unsupervised clustering (Fig. 3A). Two main branches were separated, one of them associated with 13q21-31 amplification which presented a trend to accumulate specific genomic amplifications such as 6q12-15, 5q15-22, 2q23-32, or 4q26-28, and a second one associated with amp8q21-23 and/or 17q22-25, with 15q22-26 or 8q24 regions in the amp8q21-23 group and the 20q12-13 region in the amp17q22-25 tumors. When comparing the frequencies of amplification of the regions between both branches, we observed significant differences (adjusted \( P < 0.05 \)) for 13q21-31 \( (P = 0.000) \), 8q21-23 \( (P = 0.0017) \), and 6q12-15 \( (P = 0.017) \); data not shown). Other genomic changes (gains and losses) were also distributed in a significantly different fashion between both branches (data not shown). When analyzing the distribution of the three BRCA groups, we observed that they were randomly distributed among the two main branches.

*Association with immunohistochemical markers.* To determine if there were specific characteristics associated with the acquisition of the amplified regions, we defined the immunohistochemical profile by analyzing histologic variables and some markers of proliferation (Ki-67), hormone receptors (estrogen receptor and progesterone receptor), the cell cycle (p53), cell adhesion (p120ctn, CAD-E, CAD-P, and G-CAT), and apoptosis (Bcl-2). Statistical comparisons were made to identify the markers that were significantly different in these groups (Table 1). Tumors with amp8q21-23 and/or 17q22-25 were characterized by a higher grade and mitosis number, a high expression of Ki-67, and negative staining for p120ctn and E-cadherin. By contrast, cases with amp13q21-31 mainly presented a low grade and mitosis, a low level of Ki-67, positive staining for hormone receptors (estrogen receptor and progesterone receptor) in nearly 100% of the cases, negative

---

**Fig. 1.** Distribution of the 21 chromosomal regions recurrently found amplified among the 80 familial breast cancer samples studied.

**Fig. 2.** Distribution of the three genomic phenotypes (HA, LA, and NA tumors) described in this study within the different BRCA classes.
staining for p53 and p120 ctn, and positive staining for E-cadherin; this latter marker was significantly different from the NA tumors. In addition, NA tumors presented an immunohistochemical phenotype similar to amp13q21-31 tumors although with a lower percentage of cases positive for hormone receptors and negative for p120 ctn (Table 1).

Using the studied immunohistochemical variables, we made with all cases an unsupervised cluster to determine if these tumors are well separated (Fig. 3B). The cluster had two main branches: one was associated with high grade and mitosis, a high level of Ki-67, negativity for hormone receptors and Bcl-2, and positive for p53 (right); the other branch showed an opposite phenotype with lower grade and mitosis, a lower level of Ki-67, positivity for hormone receptors and Bcl-2, and negative for p53 (left). The first branch contained the NA BRCA1 tumors and the majority of cases with amp8q21-23 and/or 17q22-25 (18 cases) independent of the BRCA type. In the left branch, there was a mix of NA BRCA2/X tumors (23 cases), amp13q21-31 (5 cases), and amp8q21-23 (5 cases).

**Discussion**

In the present study, we have established the most frequently found somatic alterations in familial breast tumors by analyzing 63 chromosomal regions by HR-CGH. We have defined the existence of a genomic phenotype that is characterized by the accumulation of DNA amplifications that are commonly found in the three BRCA groups. Finally, we have shown three major amplified regions that define two different genomic pathways that seem to be associated with specific immunohistochemical characteristics and prognosis.

We have found four common genomic alterations present in the three classes of BRCA tumors: gains at 1q and 8q21-23 and losses at 8p21-23 and 11q22-25. These alterations coincide with those described in other studies on BRCA mutation carriers (11, 13) and with our previous study, although the gain at 8q21-23 had lower frequency in BRCA2 cases (14). It is interesting to note that van Beers et al. (13) included 80 regions with alterations defined by CGH in a similar way as described in the present study, and the majority of these regions coincide with ours. Therefore, these altered regions could represent a core of abnormalities common to familial and sporadic breast cancer.

We also found specific regions associated with the three BRCA groups (Supplementary Table S2). However, only loss at 5q11-23 in BRCA1 and gain at 3q11-23 in BRCA2 coincide with the specific changes associated with some of the BRCA classes defined by van Beers et al. (13). These specific chromosomal regions warrant further analysis.

Finally, we have defined three different genomic phenotypes: NA, with no amplification (48% of the total number of
samples); LA, with one or two amplified regions (23%); and HA, with three or more amplifications (29%). As expected, tumors with high-level DNA amplifications presented higher instability than NA tumors. These results support the idea that tumors with a high genomic instability have an increased probability to develop multiple amplifications (18). In this scheme, LA tumors would represent the first level of genomic instability to develop more amplifications.

**Different pathways of genomic evolution.** Among the 21 regions with high-level DNA amplification that we have identified on familial breast cancer, three amplified regions characterize two different genomic pathways. 8q21-23 and 13q21-31 are the two regions with the highest frequency of amplifications in both LA and HA tumors and may represent the earliest amplified regions that drive the tumors through distinct pathways of evolution (Fig. 4). In contrast, amp 17q22-25, the second most frequent amplification, may be associated with tumor progression because it seemed to be significantly more frequent in HA tumors and was often amplified together with 8q21-23 (Fig. 4). By using unsupervised clustering, we

---

**Table 1.** Comparison of immunohistochemical variables among familial breast tumors with amplification at 8q21-23 and/or 17q22-25, amplification at 13q21-31, and without amplifications

<table>
<thead>
<tr>
<th></th>
<th>Cases with amp 8q21-23 and/or amp 17q22-25</th>
<th>Cases with amp 13q21-31</th>
<th>Nonamplifier cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n (%) )</td>
<td>( P^{*} )</td>
<td>( n (%) )</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 (9.1)</td>
<td>NS</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>2</td>
<td>6 (27.3)</td>
<td></td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>3</td>
<td>14 (63.6)</td>
<td></td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>Mitosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 (15.0)</td>
<td>NS</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>2</td>
<td>4 (20.0)</td>
<td></td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>3</td>
<td>13 (65.0)</td>
<td></td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>Ki-67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5%</td>
<td>7 (30.4)</td>
<td>NS</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>6-25%</td>
<td>9 (39.1)</td>
<td></td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>&gt;25%</td>
<td>7 (30.4)</td>
<td></td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>11 (47.8)</td>
<td>0.028</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>12 (62.2)</td>
<td></td>
<td>8 (100)</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>14 (60.9)</td>
<td>0.037</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>9 (39.1)</td>
<td></td>
<td>7 (87.5)</td>
</tr>
<tr>
<td>p53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>16 (69.6)</td>
<td>NS</td>
<td>7 (87.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>7 (30.4)</td>
<td></td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>p120(^{cm})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>15 (78.9)</td>
<td>NS</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Positive</td>
<td>4 (21.1)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>E-Cadherin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>12 (60.0)</td>
<td>NS</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>8 (40.0)</td>
<td></td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>P-Cadherin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>16 (80.0)</td>
<td>NS</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Positive</td>
<td>4 (20.0)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>g- Catenin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13 (72.2)</td>
<td>NS</td>
<td>6 (75.0)</td>
</tr>
<tr>
<td>Positive</td>
<td>5 (27.8)</td>
<td></td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>Bcl-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>14 (63.6)</td>
<td>NS</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>8 (36.4)</td>
<td></td>
<td>3 (37.5)</td>
</tr>
</tbody>
</table>

**NOTE:** All \( P \) values were obtained by the \( \chi^2 \) contingency test using Fisher’s exact test correction when necessary. \( P < 0.05 \) was considered significant. NS, non-significant differences.

* \( P \) obtained by comparison of cases with amp 8q/17q versus amp 13q.

\( P \) obtained by comparison of cases with amp 13q versus nonamplifier tumors.

\( P \) obtained by comparison of cases with amp 8q/17q versus nonamplifier tumors.
could confirm these points: we observed that the branch represented by amp8q21-23 is associated with specific regions such as 15q22-26 and 8q24, and that the second branch defined by amp13q21-31 is associated with 6q12-15, 5q15-22, 2q23-32, and 4q26-28 (Fig. 3A). Therefore, the pathways defined by 13q21-31 and 8q21-23, with or without 17q22-25, might influence the future accumulation of other specific amplified regions and would determine the genomic evolution of these tumors. Genomic evolution is a frequent event in breast cancer (25, 29–32). In this way, Courjal and Theillet (29) and Courjal et al. (32) described the genetic evolution of a set of sporadic breast tumors based on amplified regions. Because the amplified regions they found affected the same chromosomes and in similar regions as in our genomic pathways, we think that 13q21-31, 8q21-23, and 17q22-25 may be common genomic amplification pathways in breast cancer.

Because of the genomic differences between the two pathways, we have tried to define the immunohistochemical characteristics associated with each one of them. We analyzed 11 immunohistochemical variables and found that tumors with amp13q21-31 presented a profile defined by positive estrogen receptor and progesterone receptor staining in 100% and 90% of cases, respectively, a low Ki-67 expression, and negative p53 and p120ctn staining (Table 1). The majority of these markers have been previously correlated with good prognosis and low malignant potential in BRCA2 and BRCA1 familial tumors (7–10), and although the number of tumors here analyzed is small, the majority correspond to these subtypes. In contrast, the group associated with amp8q21-23 with or without amp17q22-25 showed immunohistochemical variables of aggressiveness, such as a high grade and mitosis number, high expression of Ki-67, and negative expression of hormone receptors and E-cadherin. Moreover, recent studies by our group and others have shown that the immunohistochemical characteristics of BRCA1 tumors are in general represented by this profile (7–10), and although in the amp8q21-31/17q22-25 group all three subtypes are represented, the majority are BRCA1 tumors. Regarding NA tumors, the immunohistochemical profile was similar to that of the amp13q21-31 group.

The unsupervised cluster showed the same trend (Fig. 3B): the right branch included phenotypic markers of aggressiveness and mainly contained NA BRCA1 tumors (8 cases) and BRCA1 and BRCA2/X tumors with amp8q21-31/17q22-25 (10 and 8 cases, respectively); in the left branch, we found NA BRCA2/X tumors (23 cases) and BRCA1 and BRCA2/X tumors with amp13q21-31 (1 and 4 cases, respectively). Only a minority of cases was incorrectly located according to the amplified region or BRCA type. All these data suggest that the genetic mutation in familial breast tumors is mainly responsible for the immunohistochemical phenotype, although a small group can present different phenotypes (more or less aggressive) probably due to their own genetic background. During tumor evolution, the genetic phenotype may induce a set of genomic changes through two main pathways: amp13q21-31, which is associated with less aggressive tumors and good prognosis, and amplification of the 8q21-23/17q22-25 region, which is associated with highly aggressive tumors and bad prognosis. Thus, BRCA1 cases that amplify 8q21-23 and/or 17q22-25 may represent a more aggressive subgroup within the heterogeneous population of BRCA1 tumors, and this should be taken into account in future searches for genes responsible for BRCA1.

Why this occurs is currently unknown but different studies have pointed out that the gain of 8q is a recurrent event in sporadic breast cancer with poor prognosis (23, 24, 33, 34). Our results support this correlation and suggest that 8q amplification may be a universal bad prognostic marker for breast cancer.

The same occurs with the amplification 17q22-25, which is distal to ERBB2 and BRCA1 genes and seems to be a major amplification site in sporadic breast cancer (20, 29, 35–37). This amplified region has also been correlated with poor patient prognosis (24, 33) and detailed characterizations of

![Fig. 4. Frequency of the 21 recurrent amplified regions found in the HA (black columns) and LA tumors (white columns). Amplifications at 8q21-23 and 13q21-31 are present with a high frequency in both groups. However, amplifications at 8q24 and 17q22-25 occur more frequently on HA tumors and may be considered as late alterations in the tumor development. * significant differences in the statistical comparison of the frequencies between HA and LA tumors.](https://www.aacrjournals.org/clinicancerres/article-pdf/11/24/8574/10880715/clinican10880715.pdf)
the amplification and overexpression of genes located at this region have already been reported (21, 38–41). Finally, the amp13q21-31 group and its correlation with good prognosis still have to be studied in detail. Different candidate genes are located in this region, such as protocadherins (PCDH9 and PCDH17), mitotic control protein genes (KIAA1008), Krüppel-like transcription factors (KLF5 and KLF12), transcription regulatory function genes (LMOT), or inhibitors of natural killer activity and prostaglandin synthesis during pregnancy (PIBF1; refs. 5, 42, 43). 13q21-31 amplification has also been reported in breast cancer cell lines (20, 44, 45) but little is yet known on its role. Our findings about the recurrence of this amplification in all BRCA1 groups, but mainly in BRCA1 and BRCA2, the strong correlation with estrogen receptor–positive tumors (100%), and the good prognosis immunohistochemical profile indicate that this amplified region could serve as a marker for tumor evolution and follow-up of the patient.

In summary, we have defined 21 recurrent amplification sites and described an amplifier phenotype that is probably common not only to familial but also to sporadic breast tumors and that is correlated with a higher genomic instability. In addition, two different genomic pathways associated with 8q17q17 and 13q amplification have been established. They are associated with specific genomic regions of amplification, carry specific immunohistochemical characteristics, and have a trend to be associated with BRCA1 and BRCA2/X, respectively. According to these data, tumors with 13q amplifications are associated with low aggressiveness and good prognosis whereas 8q17q17 amplification defines tumors with high aggressiveness and poor prognosis. Because these amplifications have been previously identified in sporadic breast tumors and correlated with prognosis, the two pathways may represent a general mechanism of evolution in breast tumors and may contain key genes for tumor evolution.

Acknowledgments

We thank the Spanish National Tumor Bank Network, the Immunohistological Unit of the Spanish National Cancer Centre, and Carmen Martin from the Cytogenetics Unit for their technical support; Roger Milne and Ramón Díaz-Uriarte for their assistance in the statistical analysis; and Miguel Urioste, Laura Valle, and Raquel Rodriguez for the selection of families.

References


The Accumulation of Specific Amplifications Characterizes Two Different Genomic Pathways of Evolution of Familial Breast Tumors

Lorenzo Melchor, Sara Álvarez, Emiliano Honrado, et al.