Genomic Loss of 18p Predicts an Adverse Clinical Outcome in Patients with High-Risk Breast Cancer

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ABSTRACT

The impact of the genomic imbalances on the clinical outcome of 34 patients with lymph-node positive high-risk breast cancer (HRBC) was investigated using comparative genomic hybridization. All of the patients were uniformly treated with high-dose chemotherapy and autologous stem cell transplantation. The average number of chromosomal imbalances per tumor was 11 (range, 2–24), including DNA overrepresentation on chromosomes 1q (59%), 17q (38%), 8q and 16p (35% each), 20q (32%), and 13p (26%), and genomic losses involving 9p and 18q (41%), 8p, 11q, and 18p (38%), 17p (32%), 4p and Xq (29%), and 16q (26%). The most significant association among genomic changes and clinical-pathological features was the correlation of the loss of 8p with progesterone receptor positivity (P < 0.005). With a median follow-up time of 74 months, 15 patients (44%) have relapsed. In the univariate analysis, patients with gain/amplification of 17q including the HER-2/neu gene locus had a longer disease-free survival (P = 0.02), whereas those with genomic loss of 18p had a higher probability of relapse (P = 0.003). In multivariate analysis, the loss of 18p was the only parameter correlated with shorter disease-free survival (relative risk, 4.8; 95% confidence interval, 1.57–14.8; P = 0.006). In summary, our data indicate that the tumoral genomic profile may represent a valuable marker for predicting the clinical outcome in HRBC. Furthermore, the genomic loss of 18p may identify a poor prognostic subgroup of patients with HRBC.

INTRODUCTION

The presence of axillary lymph nodes affected at diagnosis is the most significant factor in overall survival of breast cancer patients (1). Adjuvant chemotherapy has been demonstrated to improve outcome of these patients, but it is still controversial whether the use of HDC followed by ASCT may be beneficial (2–5). Little is known about the impact of the tumor genetic changes on the outcome of patients with lymph node-positive HRBC. Most studies have evaluated single genetic markers that in some cases have been associated with increased risk of relapse (6–9). Despite these efforts, based on current knowledge it is not possible to predict the outcome of patients with HRBC after intensive therapy.

Using CGH, tumors can be screened for DNA copy number variation genome-wide (10). This technique has revealed typical aberrant genomic profiles that include amplification and deletion sites unknown previously in most cancer categories (11, 12). In breast carcinomas, distinct patterns of genomic imbalances have been described in the different clinical-pathological subgroups (13–27). However, the correlation of the CGH data with patient survival has been scarce. Isola et al. (14) reported that genetic aberrations detected by CGH predict outcome in lymph node-negative breast cancer. In a recent report, genomic profiles were valuable prognostic parameters in patients with HRBC (21). The identification of recurrent genomic changes associated with the outcome of patients with HRBC would reveal novel clinically useful markers.

We report on the CGH analysis of a group of patients with lymph node-positive HRBC treated with a uniform protocol. Our aims were to define the pattern of genomic imbalances in this cohort, to correlate CGH findings with clinical-pathological features, and to identify possible genetic aberrations that might influence in patient outcome.

MATERIALS AND METHODS

Tumor Samples. Breast tumor samples obtained at diagnosis from 34 patients were studied. They were consecutively diagnosed at HRBC stages II or III. Those with >10 lymph nodes affected at diagnosis (n = 24) received six courses of FAC/FEC (5-fluorouracil 600 mg/m²/day and doxorubicin/epirubicin 50/75 mg/m²/day, respectively, and cyclophosphamide 600 mg/m²/day), whereas patients with 4–10 lymph nodes affected (n = 10) received induction chemotherapy (four courses of FEC 75), followed by breast resection and two additional courses of FEC 75. Subsequently, all of them re-

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3 The abbreviations used are: HDC, high-dose chemotherapy; ASCT, autologous stem cell transplantation; HRBC, high-risk breast cancer; CGH, comparative genomic hybridization; FISH, fluorescence in situ hybridization; IHC, immunohistochemical; DFS, disease-free survival; RR, relative risk; CI, confidence interval.

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tumor:test ratio exceeded 1.2, genomic losses when the ratio was interpreted as genomic gains when DNA analysis was performed as described previously (28). Chromosomal imbalances were evaluated in Kaplan-Meier survival curves to identify factors associated with DFS after diagnosis. There was no correlation between the total number of genomic imbalances and the outcome of patients. However, the number of genomic imbalances with themselves and with clinical-pathological parameters were analyzed through 2 × 2 contingency tables using Pearson’s χ² test unless there was an inadequate number of observations, in which case a Fisher’s exact test was used. All of the resulting Ps were two-tailed. DFS time was calculated according to the Kaplan-Meier survival curves and Ps with the log-rank test. Multivariate analysis using the Cox regression model was performed only on the variables with a P <0.05 in the univariate analyses. Both backward and forward analyses removed the same nonsignificant variables. Finally, the factors were removed one at time, based on the Wald test that was used to determine the level of significance; only statistically significant factors remained (P < 0.05). All of the clinical variables included in statistical analysis were dichotomized, i.e., estrogen and progesterone receptors were considered positive or negative when protein level were higher or lower than 10 fmol/mg, or age that was considered as ≤50 or >50 years. The statistical analyses were carried out using SPSS 10.0 software for Windows 98.

RESULTS

All 34 of the tumors showed genomic changes (Fig. 1). The average number of chromosomal imbalances per tumor was 11 (range, 2–24), including 5 gains (0–11) and 6 losses (0–15). A variable spectrum of genomic aberrations occurring across the entire genome was observed, including DNA overrepresentation on chromosomes 1q (59%), 17q (38%), 8q (35%), 16p (35%), 20q (32%), 19p (26%), and 11q (24%), and chromosomal losses involving 9p (41%), 18q (41%), 18p (38%), 8p (38%), 11q (38%), 17p (32%), Xq (29%), 4p (29%), 16q (26%), 4q (24%), and 22q (24%). In addition, three amplification events were observed in 6q21-q22, 8q24, and 20q13.

A number of associations among the most common genomic changes were detected: the loss of chromosome 8p and gain of 8q (P = 0.002), possibly as a consequence of an isochromosome 8q; losses of 18p and 9p (P = 0.01) or 4q (P = 0.002); losses of 18q and 19p (P = 0.005); and loss of 18q and gain of 20q (P = 0.002; Table 2). Significant correlations between recurrent genetic changes and clinical-pathological characteristics were investigated. Loss of 8p was significantly correlated with progesterone receptor positivity (P < 0.005). In addition, gain on chromosome 8q was detected in 73% of tumors and was correlated with stage III, whereas all of the samples with 19p gain corresponded with tumors in stage II; however, the few tumors in the study prevented us to adequately perform the χ² test. There was no relationship between any other single chromosomal abnormality and the clinical-pathological variables, including changes occurred at high frequency such as gains of 1q, 17q, 16p, and 20q, and losses of 11q and 17p.

With a median follow-up from diagnosis of 74 months (range, 18–96), 19 patients (56%) are alive, and disease-free and 15 patients (44%) have relapsed. DFS for the whole series is shown in Fig. 2. Clinical-pathological features and genomic imbalances were evaluated in Kaplan-Meier survival curves to identify factors associated with DFS after diagnosis. There was no correlation between the total number of genomic imbalances and the outcome of patients. However, the number of genomic

### Table 1

<table>
<thead>
<tr>
<th>Clinical Pathological Features</th>
<th>Patients n = 34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years) (range)</td>
<td>50 (36–62)</td>
</tr>
<tr>
<td>≤50 yr</td>
<td>16 (47%)</td>
</tr>
<tr>
<td>&gt;50 yr</td>
<td>18 (53%)</td>
</tr>
<tr>
<td>Hormonal status</td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>16 (47%)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>16 (47%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Lymph nodes affected</td>
<td></td>
</tr>
<tr>
<td>4–9</td>
<td>10 (30%)</td>
</tr>
<tr>
<td>10–15</td>
<td>17 (50%)</td>
</tr>
<tr>
<td>16–20</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>&gt;20</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>20 (59%)</td>
</tr>
<tr>
<td>IIIA</td>
<td>11 (32%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>17 (50%)</td>
</tr>
<tr>
<td>Negative</td>
<td>10 (29%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>7 (21%)</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14 (41%)</td>
</tr>
<tr>
<td>Negative</td>
<td>13 (38%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>7 (21%)</td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>II</td>
<td>16 (47%)</td>
</tr>
<tr>
<td>III</td>
<td>8 (23%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (18%)</td>
</tr>
<tr>
<td>Histologic type</td>
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<tr>
<td>Ductal</td>
<td>29 (85%)</td>
</tr>
<tr>
<td>Lobular</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>Tubular</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Patient survival status</td>
<td></td>
</tr>
<tr>
<td>Relapsed</td>
<td>15 (44%)</td>
</tr>
<tr>
<td>Disease free</td>
<td>19 (56%)</td>
</tr>
</tbody>
</table>

**Statistical Analyses.** Significant correlation between the genomic imbalances with themselves and with clinical-pathological parameters were analyzed through 2 × 2 contingency tables using Pearson’s χ² test unless there was an inadequate number of observations, in which case a Fisher’s exact test was used. All of the resulting Ps were two-tailed. DFS time was calculated according to the Kaplan-Meier survival curves and Ps with the log-rank test. Multivariate analysis using the Cox regression model was performed only on the variables with a P <0.05 in the univariate analyses. Both backward and forward analyses removed the same nonsignificant variables. Finally, the factors were removed one at time, based on the Wald test that was used to determine the level of significance; only statistically significant factors remained (P < 0.05). All of the clinical variables included in statistical analysis were dichotomized, i.e., estrogen and progesterone receptors were considered positive or negative when protein level were higher or lower than 10 fmol/mg, or age that was considered as ≤50 or >50 years. The statistical analyses were carried out using SPSS 10.0 software for Windows 98.
losses was associated with inferior outcome: patients with tumors containing \(18p^\#\) losses had a better DFS than those with \(18p^\#\) gains (\(P = 0.03\); Fig. 3A). When individual genomic imbalances were analyzed separately, two different associations were found. Gain of chromosomal material on 17q was correlated with a lower risk of relapse (\(P = 0.02\)), whereas patients with tumors displaying losses of chromosome 18p showed higher risk of relapse (\(P = 0.003\); Fig. 3, B and C). No other association between chromosomal aberrations and DFS was found. A multivariate analysis (Cox model) was applied to analyze those parameters previously found statistically significant in the univariate analysis. Both backward and forward analyses retained only the loss of 18p associated with increased risk of relapse (RR = 4.8; 95% CI, 1.57–14.8; \(P = 0.006\)). The correlation of gain of 17q with superior DFS retained a borderline significance (RR = 3.24; 95% CI, 0.87–12.10; \(P = 0.06\)) in the multivariate analysis, whereas the number of genomic losses was not statistically significant (\(P = 0.146\)).

Because the HER-2/neu gene is located in band 17q12, we evaluated the status of this gene in the patients. All but one of the samples with genomic gain of 17q showed amplification of HER-2/neu gene documented by FISH and/or augmented protein expression according to immunoblotting or IHC. Among the 21 remaining tumors without genomic abnormalities of 17q, 5 had HER-2/neu alteration (data not shown). No differences in DFS were observed when patients with and without HER-2/neu abnormality detected by FISH, immunoblotting, or IHC were compared.

DISCUSSION

Patients with lymph node-positive breast cancer have markedly different clinical courses and treatment responses...
Genomic Loss of 18p in High-Risk Breast Cancer

Despite the few patients in the series, we found some association among genetic changes and clinical-pathological characteristics. The loss of 8p was correlated with positive progesterone receptors. This association showed a small $P (<0.005)$ in the Fisher’s exact test, therefore supporting that it is not coincidental. Other correlation included the gain of 8q that was frequent in tumors in stage III. This alteration has been associated with tumor progression (14, 20) and is present not only in the majority of breast tumors but also in other malignancies (11). On the contrary, gain of 19p was characteristic of tumors in stage II. However, in the associations among the gains of 8q and 19p with tumors in stage III or II, respectively, we cannot exclude a coincidence because of random sampling because of the few patients. The association between 8p loss and 8q gain as a consequence of an isochromosome 8q formation was already known (14, 21).

The number of genomic aberrations did not influence the clinical outcome of patients. In contrast, Isola et al. (14) reported that relapsed patients with lymph node-negative breast cancer had a greater number of genomic changes than those that were disease free. However, in our series, the number of genomic losses was associated with higher risk of relapse, confirming previous studies of breast tumors (14) and of other malignancies (12). This finding supports the hypothesis that the loss of genomic material affecting tumor suppressor gene loci may play a critical role in tumor progression.

In a univariate analysis, gain in 17q was associated with favorable patient outcome. The long arm of chromosome 17 at 17q12 is the locus of HER-2/neu gene. This oncogene is activated in 20–30% of breast tumors through amplification and overexpression. HER-2/neu positivity is associated with adverse prognostic factors and shorter survival of breast cancer patients (26, 31). Nieto et al. (6) reported that HER-2/neu overexpression was an independent negative predictor of relapse in HRBC treated with HDC and ASCT. In contrast, in other reports HER-2/neu overexpression seemed to increase tumor sensitivity to intensive chemotherapy regimens containing doxorubicin or paclitaxel (7–9, 32). In agreement with these studies, we report here that the gain of 17q containing the HER-2/neu gene locus is associated with a superior DFS in HRBC treated with intensive chemotherapy including doxorubicin. However, in our series, when HER-2/neu status was evaluated by FISH, Western blotting, or IHC, it did not influence patient outcome. Therefore, according to our data it is the genomic gain of 17q and not the HER-2/neu alteration that the predictive parameter correlated with prolonged survival in HRBC. In 17q, two different regions of amplification have been reported. These loci harbor a number of additional genes that are also amplified in breast tumors and may be critical in the pathogenesis of the disease: RAD51C, S6K, PAT1, and TBX2 in 17q23, and GRB7, MLN64 and TopoIIa in the HER-2/neu amplicon in 17q12 (33–37). TopoIIa gene amplification is associated with a frequent occurrence of amplification. It has been postulated that the increased gene dosage of TopoIIa may relate to an increased sensitivity to TopoII inhibitors such as doxorubicin in patients with breast cancer (35–37). Whether the amplification of other genes in the 17q12 amplicon, in addition to frequently and TopoIIa, may be of clinical significance in HRBC is at present unknown.

Despite the results of the univariate analysis, the only parameter correlated with shorter DFS in the multivariate analysis was the loss of 18p, whereas the association of the gain of 17q with superior DFS had a borderline significance. For the multivariate analysis, ~10 events per independent variable (in our case, tumor relapses) are required to produce a statistical model of reasonable accuracy (38). As it was not possible in our series, results of the multivariate analysis should be interpreted carefully. Nevertheless, in our series
the status of 18p was the only significant predictive factor for clinical outcome, and it was independent from other genomic changes. Thus, loss of 18p may be an important indicator of adverse outcome in HRBC. The status of 17q was a marginally significant indicator of response to the treatment and seems to show trends to favorable clinical outcome; this is probably related to the few patients included in the series and to the $P$ of 0.02 in the univariate analysis. On the contrary, in the multivariate analysis the 18p loss retained its statistically significance because of the lower $P$ (0.003) observed for this specific correlation in the univariate analysis. Nevertheless, because of the limited number of patients, these results should be confirmed in larger trials.

The deletion of 18p has not been reported as a frequent change in previous CGH studies of breast cancer patients (13–27), but it was a common event in breast tumor cell lines (39). Loss of heterozygosity on 18p was detected in patients with breast tumors (40–42). In one report, the allelic loss on 18p11.3 occurred early in ductal carcinoma *in situ* (40). This genomic loss is also common in other malignancies (12, 41). The association of the genomic loss of 18p and shorter DFS has not been reported previously in breast cancer. Our results suggest the presence of one or more tumor suppressor gene(s) on 18p with a role in breast cancer progression.

In summary, using CGH we have identified a subgroup of patients with HRBC with 17q gain or with 18p loss who have a significantly different clinical outcome after HDC and ASCT. The biological importance of these two regions and their definite clinical relevance warrants additional evaluation in a larger series of patients. Our results indicate that HRBC patients with genomic loss of 18p have an adverse clinical outcome and may benefit from more intensive or

![Fig. 3  DFS of HRBC patients according to the pattern of genomic imbalances in the tumors. A, comparison of tumors with <4 genomic losses versus tumors with <4 losses. B, tumors with gain in 17q chromosomal region versus tumors without this genomic gain. C, tumors with 18p genomic loss versus tumors without this genomic loss. D, tumors with genomic gain in 17q and without 18p loss versus the remaining tumors.](image-url)
novel experimental therapies. Future studies using large-scale CGH to microarrays and gene expression profiling will help in the identification of genes at these regions targeted by gene amplification and deletion.

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