Two Prognostic Groups of Inflammatory Breast Cancer Have Distinct Genotypes

Florence Lerebours, Philippe Bertheau, Ivan Bieche, Louis-François Plassa, Marie-Hélène Champeme, Kamel Hacene, Christine Toulas, Marc Espie, Michel Marty, and Rosette Lidereau


ABSTRACT

Purpose: The prognosis of inflammatory breast cancer (IBC) remains poor despite the use of multimodality treatments, with a 10-year survival rate of not >30%. Clinicopathological and biological predictors of outcome are inadequate in this setting. Analysis of loss of heterozygosity (LOH) can provide a molecular portrait of the genetic alterations underlying stepwise cancer progression. We tested the value of LOH patterns as diagnostic and prognostic markers in IBC.

Experimental design: In a previous study of 64 patients with IBC who were treated homogeneously between 1988 and 1999, we determined LOH frequencies at 71 loci located in 20 chromosomal regions associated with primary breast cancer. Six of these regions bore alterations that were less frequent in non-IBC. In the present study, we sought correlations between these molecular data and the clinicopathological features and clinical outcome of the same 64 patients.

Results: With the exception of stage IV disease, extensive breast inflammation at first clinical examination was the main factor associated with poor outcome (P = 0.00065 versus localized inflammation). The overall frequency of LOH was also higher in this group (P = 0.000073), LOH patterns differed between patients with localized and extensive breast inflammation.

Conclusion: Patients with IBC can be separated into two major prognostic groups on the basis of initial clinical signs, which appear to be subverted by different molecular alterations.

INTRODUCTION

The most lethal form of primary breast cancer is IBC3 (1). IBC is diagnosed on the basis of rapidly progressive signs, such as localized or generalized induration, redness, and edema of the breast (2). The 3-year survival rate of IBC patients is ~40%, compared with 85% among patients with non-IBC (1). Most of the main prognostic parameters used in non-IBC are not relevant to IBC: (a) precise IBC tumor measurement is often impossible at diagnosis; (b) most patients with IBC have lymph node involvement at diagnosis, and ≥30% have distant metastases (1); and (c) because IBC and other forms of locally advanced breast cancer necessitate first-line chemotherapy instead of surgery, the prognosis of IBC is rarely studied separately, despite its specific incidence rate, clinical presentation, and histological features, such as dermal lymphatic emboli (2). It is highly likely that specific patterns of genetic changes account for the clinicopathological characteristics and aggressiveness of most IBCs. However, the biology of IBC is poorly documented (3). We studied previously LOH patterns in 64 patients with IBC and found that 6 of 20 chromosomal arms or regions associated with breast cancer (3p21.2-p14.2, 6p, 8p22, 11q22–25, 13q14, and 17q21) showed particularly frequent alterations (4). We concluded that some of these regions might contain genes specifically involved in IBC. Here, by further studying the same series of 64 IBC patients, we sought to correlate these frequent molecular alterations with clinicopathological characteristics and outcome.

MATERIALS AND METHODS

Patients and Samples. Pretreatment tumor specimens were obtained from 64 women with IBC by surgical biopsy. The patients were all diagnosed and treated at Saint-Louis Hospital (Paris, France) and Institut Claudius Regaud (Toulouse, France) between 1988 and 1999. All of the tumors were classified T4d (UICC classification, 1997). Seven patients had distant metastasis at diagnosis. All of the tumors were infiltrating ductal carcinomas, apart from one lobular carcinoma. Clinical and pathological data are summarized in Table 1. Twenty-three patients had inflammatory signs throughout the affected breast (Gustave Roussy stage PEV3; Ref. 5), whereas 41 had only localized inflammatory signs (stage PEV2). Median age at diagnosis was 50 years (range 11–76 years). All of the patients were treated with a first-line anthracycline-based chemotherapy, followed by mastectomy and radiotherapy. At the time of this
analysis, 41 patients had relapsed, and 23 remained disease free. The median RFI among the 41 patients who relapsed was 20 months (range 3–116 months). The median follow-up of the 23 patients who remained relapse free was 81 months (range 35–163 months).

Tumoral and normal DNA was prepared as described previously (4).

**Oncogene Amplification.** DNA from 34 patients was available for this analysis. The DNA copy number of the ERBB2, MYC, and CCND1 oncogenes was determined by means of real-time PCR with TaqMan fluorescence methodology (6).

**LOH.** DNA from all 64 patients was available for this analysis. LOH analysis was performed as described previously with 71 microsatellite markers covering 13 chromosome arms associated with primary breast cancer (4). The 13 chromosome arms were divided into a total of 20 regions (listed in Table 2) according to the published locations of the 71 markers (7).

A genomic region was considered lost when at least one marker located within it showed LOH.

**Statistical Analysis.** Differences in the distribution of LOH between the different groups of tumors were tested for significance by using the $\chi^2$ test with Yate’s correction to adjust the continuity of the $\chi^2$ distribution (Fisher’s exact test).

Possible links between the HI and LOH at specific loci were tested using the Mann-Whitney U test. RFI was measured by using the Dextran-coated charcoal method with a cutoff of 10 fmol/mg.

### RESULTS

**Correlations between Molecular Alterations and Clinicopathological Features of IBC.** The frequencies of ERBB2 (18%), MYC (6%), and CCND1 (15%) amplification have been shown previously to be similar in IBC and non-IBC (4). No association was observed between amplification of one or more of these markers and any clinicopathological parameter.

An HI was calculated for each tumor, as the ratio between the number of loci showing LOH and the number of informative loci tested. This index ranged from 0 (one tumor showed no LOH at any of the loci tested but bore other distinct genetic alterations) to 100% (three tumors showed LOH at all of the loci tested), with a mean value of 49%. We found no correlation between the HI and age or distant metastasis at diagnosis, hormone receptor status, or histological grade. In contrast, the mean HI value was significantly higher in stage PEV3 tumors (49.0 ± 12.1) than in stage PEV2 tumors (44.5 ± 10.1) ($P = 0.000073$).

Further analysis showed that the distribution of HI values differed between PEV3 and PEV2 tumors (Fig. 1). PEV2 tumors showed a homogeneous distribution of HI values, with an HI value $>60%$ in 30% of cases. In contrast, HI values were $\geq 60%$ in 61% of PEV3 tumors.

We had found previously that 6 of the 20 regions listed in Table 2 were more frequently deleted in IBC than in non-IBC (4). Among these six regions, 17q21 was the only one showing a significantly higher frequency of LOH in PEV3 tumors (Fish-
er’s exact test, \( P = 0.022 \); Table 2). The only other association observed between a clinicopathological variable and a molecular alteration was higher grade in 6p22-deleted tumors (\( P = 0.042 \), Fisher’s exact test).

Here we sought associations between HI values and LOH at these six regions (Table 3). LOH was significantly associated with high HI values at 8p22, 11q22-q25, 13q14, and 17q21 but not at 3p21.2-p14.2 or 6p. The same significant associations with LOH at 8p22, 11q22-q25, and 13q14 were observed in the subgroup of PEV2 tumors, whereas LOH at 17q21 only correlated with high HI values in PEV3 tumors. 8p22 was the only other region at which LOH was significantly associated with high HI values in PEV3 tumors (Table 3).

Correlations were also found between nonsyntenic allelic losses. In the overall population, a significant association was found between allelic loss occurring at all sites except 17q21 (data not shown). As LOH at 17q21 was more frequent in PEV3 tumors, concerted nonsyntenic allelic losses in PEV2 and PEV3 tumors were then analyzed. The same correlations found in the total population were also found in the subgroup of PEV2 tumors (Table 4a). The only two significant groups of chromosomes subject to concerted nonsyntenic losses in PEV3 tumors were 13q14 with 3p21.2-p14.2 and 13q14 with 6p (Table 4b).

**Correlations of clinicopathological and molecular parameters with clinical outcome.** Correlations between outcome and clinicopathological parameters are shown in Table 1. The median RFI was shorter in patients who were metastatic at diagnosis (RFI 18 versus 43 months among nonmetastatic patients, Log-rank exact test, \( P = 0.00014 \)). Inflammation of the entire breast (stage PEV3) was also associated with poorer outcome (RFI 20 versus 95 months among patients with PEV2 tumors, \( P = 0.00065 \), Log-rank exact test), and so was younger age at diagnosis (\( \leq 35 \) years: Log-rank exact test, \( P = 0.025 \)). Neither histological grade nor hormone receptor status was associated with outcome (Table 1).

Pathological and molecular findings did not differ significantly between the seven patients with metastases at diagnosis and the other 57 patients. The analysis was therefore performed on the overall population of 64 patients.

Oncogene amplification levels did not influence outcome. No relationship was observed between HI values and RFI in the overall population. However, women with PEV2 tumors and HI values below the mean (46%) for this tumor type had shorter RFI, whereas patients with PEV3 tumors and HI values below the mean (55%) for this tumor type had longer RFI (\( P = 0.0017 \), Log-rank exact test; Fig. 2). All 14 patients with PEV3 tumors and HI values above the mean for this tumor type had relapsed at the time of this analysis.

No correlation was found between outcome and LOH at any of the six chromosomal regions of interest.

**DISCUSSION**

Genetic instability is thought to underlie the cancerous phenotype. This instability is of two different types. About 15% of human tumors show microsatellite instability, which can be demonstrated by nucleotide sequence analysis. However, most solid cancers show chromosomal instability, their tumor cells bearing aberrant numbers of chromosomes that also frequently have gross structural alterations (translocations, deletions, and amplifications). This is the case of breast tumors, which show an average of 30% genome-wide DNA amplification as well as LOH (8, 9).

We found that amplification levels of ERBB2, CCND1, and MYC were not higher in IBC than reported previously in non-IBC, and none of these oncogenes had any prognostic influence. In contrast, we found a high frequency of LOH in these 64 patients with IBC. We initially thought that this high frequency of allelic loss was the hallmark of increased genomic instability correlating with tumor aggressiveness. Moreover, the highest LOH frequencies were found in tumors associated with extensive breast inflammation, a clinical parameter associated with greater aggressiveness (as defined by a shorter RFI). These findings are in keeping with correlations reported previously between a high frequency of LOH and several markers of breast tumor aggressiveness (10–12).

The two main prognostic factors in early breast cancer (stages I and II) are the number of involved axillary nodes and tumor size. By definition, patients diagnosed with IBC have at least stage IIB disease. In an attempt to refine the prognosis of IBC, Gustave Roussy investigators developed a staging system named PEV (5), based on signs of inflammation and tumor aggressiveness. At diagnosis, about one-third of IBC patients have PEV3 tumors, with inflammation involving the entire breast, whereas the remaining patients (PEV2) have only localized breast inflammation. Respectively, one-third and two-thirds of the 64 patients in this series had PEV3 and PEV2 tumors. We found that PEV3 tumors were associated with poorer outcome than PEV2 tumors (Table 1), in keeping with previous studies (13–15).

Unexpectedly, a high HI had no clear prognostic influence in the overall patient population. High HI values (above the PEV3 subgroup mean of 55%) were associated with shorter RFI in patients with PEV3 tumors, whereas low HI values (below the PEV2 subgroup mean of 46%) were associated with shorter RFI in patients with PEV2 tumors. This suggests that distinct genes and/or types of genetic alteration are targeted by LOH in PEV2 and PEV3 tumors and account for these prognostic differences. However, as the prognosis of IBC may be improved by a
favorable response to induction chemotherapy (16–18), and as these responses were not specifically evaluated here, we cannot rule out their influence on the prognosis of PEV2 and PEV3 tumors.

Several findings support the hypothesis that PEV2 and PEV3 tumors may occur through distinct molecular pathways: (a) most PEV3 tumors had high HI values, whereas PEV2 tumors seemed to be genetically more heterogeneous; and (b) although LOH frequencies at five of the six regions of interest did not differ significantly between PEV2 and PEV3 tumors, chromosome arm 17q21 was more frequently altered in PEV3 than in PEV2 tumors. Among the genes located in this region, BRCA1 is currently considered as the main candidate for a causative role in sporadic breast tumors (19). LOH at 17q21 correlates with a more aggressive clinical phenotype of breast cancer (20–22), and its frequent detection in PEV3 tumors was not therefore surprising. However, LOH at 17q21 did not correlate with shorter RFI, suggesting that the prognosis of 17q21-deleted tumors may be influenced by other molecular alterations which remain to be identified. Alternatively, the number of tumors studied here may have been too small to show a prognostic role of 17q21 LOH, especially in PEV3 tumors, only two of which lacked 17q21 LOH. A classification according to the status of each specific chromosome region revealed that the average HI value was usually higher in the group of tumors with the deleted region. However, a difference between PEV2 and PEV3 tumors was observed. 17q21 LOH was associated with higher HI values in PEV3 tumors, whereas a similar association was observed for regions 11q22–25 and 13q14 in PEV2 tumors. Alteration of target genes at 17q21 and 11q22–25/13q14 may specifically increase genomic instability in PEV3 and PEV2 tumors, respectively, whereas the target gene(s) at 8p22 seemed to be involved in both tumor types. The lack of any significant association between 3p21.2-p14.2 and 6p LOH and high HI values may reflect early involvement of the relevant genes in the genesis of IBC. Finally, most nonsyntenic allelic losses were concerted in PEV2 tumors, indicating that the corresponding genes may cooperate in tumorigenesis. Only two concerted nonsyntenic allelic losses were observed in PEV3 tumors, between 3p14-p21 and 13q14 and between 6p and 13q14.

Taken together, these results show a high rate of chromosomal instability in IBC, based on mean frequencies of allelic loss. However, this instability, and the corresponding target genes, may differ between PEV3 and PEV2 tumors. These two major prognostic groups of IBC may have distinct genetic bases and be regarded as separate diseases. However, as five of the six chromosomal regions studied here were deleted with similar frequency in both tumor types, we deduce that alterations of several genes are necessary for the occurrence of IBC, whereas alterations of several other genes are specifically required for the basic tumor to progress to either PEV2 or PEV3. There is no firm evidence that PEV2 tumors always progress to stage PEV3. PEV2 may thus represent a heterogeneous subset of IBC tumors, only some of which progress to PEV3.

LOH is now recognized as an invaluable tool for cancer diagnosis and prognostication, regardless of whether the corre-
sponding target genes have been identified (23–25). Additional investigations are required to determine the prognostic value of LOH analysis in IBC.

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