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Expression of BRCA1, HER-1 (EGFR) and HER-2 in Sporadic Breast Cancer and Relationships to Other Clinicopathological Prognostic Features

YAN ANSQUER[1,5], LAURENT MANDELBROT[1], THÉRESE LEHY[6], LAURENCE SALOMON[2], CAROLINE DHAINAUT[4], PATRICK MADELENAT[4], GÉRARD FELDMANN[5] and FRANCINE WALKER[3,6]

Hôpital Louis Mourier, [1]Service de Gynécologie Obstétrique and [2]Département de Santé Publique, 178 rue des Renouillers 92 701, Colombes Cedex; Hôpital Bichat, [3]Service d'anatomopathologie and [4]Service de Gynécologie Obstétrique, 46 rue Henri-Huchard, 75 018 Paris; [5]INSERM U481 and [6]INSERM U 410, faculté de Médecine Xavier Bichat, 16 rue Henri Huchard, 75018 Paris, France

Abstract. *Background: The BRCA1 caretaker gene is associated with poor prognostic features in hereditary breast cancer and may also play a role in sporadic breast cancer (SBC). HER-1 and HER-2 overexpression is associated with adverse prognosis in SBC. We studied whether BRCA1 expression was associated with HER1, HER2 and other prognostic features in SBC. Patients and Methods: Fifty newly-diagnosed SBC patients were studied for prognostic features and immunohistochemical expressions of BRCA1, HER-1 and HER-2. Results: Tumors were positive for BRCA1 in 26%, HER-1 in 32% and HER-2 in 20% of cases. Lack of BRCA1 expression was associated with node metastases and decreased estrogen receptor. HER-2 expression was associated with young age, HER-1, Ki67 and decreased hormone receptors. No correlation was observed between BRCA1 and HER-1 or HER-2. Conclusion: In SBC, the lack of BRCA1 expression was associated with poor prognostic features, but unrelated to HER-1 and HER-2. HER2 and HER-1 were, however, highly correlated.*

BRCA1 was isolated by positional cloning methods as a gene linked to breast cancer in families with a pattern of autosomal dominant inheritance of the disease (1). Inherited BRCA1 breast cancer is associated with poor prognostic features and decreased survival (2, 3).

Correspondence to: Yan Ansquer, Hôpital Louis Mourier, Service de Gynécologie Obstétrique, 178 rue des Renouillers, 92 701 Colombes Cedex, France. Tel: 00 33 1 47 60 63 40, Fax: 00 33 1 47 60 63 38, e-mail: yan.ansquer@lmr.ap-hop-paris.fr

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The product of the BRCA1 gene is a 220-kD a nuclear phosphoprotein that has been implicated in the regulation of cell proliferation, cell cycle progression, apoptosis, DNA repair and recombination (4). These functions of BRCA1 support the role of BRCA1 as a tumor suppressor gene, or more precisely as a "caretaker gene", since it is involved in genome integrity maintenance (5), therefore raising the question of the role of BRCA1 in sporadic breast cancer.

HER-1 was the first identified of a family of receptors known as the HER family or ErbB tyrosine kinase receptors. This receptor family comprises four homolog receptors: HER-1 (also called EGFR, ErbB-1), HER-2 (HER2/neu, ErbB-2), HER-3 (ErbB-3) and HER-4 (ErbB-4). Expression of HER-1 and overexpression of HER-2 are associated with poor prognosis in breast cancer patients (6).

Particular interest in the HER family comes from the demonstration of improvement in overall survival in advanced HER-2-overexpressing breast cancer using anti-HER-2 monoclonal antibody (7), and the development of promising inhibitors of HER-1 (8).

Few studies have explored the potential association of HER family expression with BRCA1. Information currently available only concerns BRCA1 inherited breast cancer and HER-2 and remains inconclusive, with findings of no association or an inverse correlation between BRCA1 gene mutation status and the amplification of the HER-2 gene (9-12).

The aim of the present study was to explore, in the setting of sporadic breast cancer, the potential link between BRCA1, HER-1 and HER-2 expressions and their relationships with other clinicopathological prognostic features.

Patients and Methods

Patients and samples. Paraffin-embedded tissue was obtained from 50 newly-diagnosed patients, who underwent surgery at the Department of Obstetrics and Gynecology of Bichat Hospital (Paris, France) between January 2001 and December 2002. Information about the patients' clinical history was obtained from their charts. All patients had histological evidence of invasive breast carcinoma. Three patients (6%), aged 61, 41 and 29 years, had a family history of breast cancer in a first degree relative, as elucidated by questioning at the time of admission for surgery. Only the 29-year-old patient was referred for genetic counseling. The estimated risk for a BRCA1 mutation carrier is 25%, however no mutation could be evidenced. The patient and tumor characteristics are shown in Table I. The patients were not treated with neoadjuvant chemotherapy, hormonal therapy, or irradiation prior to tumor excision. Surgical treatment was lumpectomy with axillary lymph node dissection, or mastectomy with axillary lymph node dissection, except in three cases that were treated by mastectomy only because of advanced age (≥85 years) and the associated morbidity. The size of the primary tumor was the largest tumor diameter observed after surgical excision. Lymph node status was determined with histological evidence of metastatic breast carcinoma. Histological typing and grading were done according to the WHO classification (13) and the Nottingham scheme, respectively (14).

Immunohistochemical studies. Four-μm-thick sections were cut from paraffin blocks which contained representative histology of the breast carcinoma. Paraffin sections on silane-coated slides were dewaxed and rehydrated. Then, endogenous peroxidase activity was blocked in absolute methanol solution containing 1% hydrogen peroxide for 35 minutes and the slides were washed in 10 mM phosphate-buffered saline (PBS), pH 7.4. For antigen retrieval, they were immersed in 1 mM citrate-phosphate buffer and microwaved at 100˚C for 15 minutes. The tissue sections were incubated with various mouse monoclonal primary antibodies overnight at 4˚C in a humidified chamber. The details of these antibodies were as follows: BRCA1 Ab1 antibody (clone MS110) (Oncogene Research, San Diego, CA, USA) used at 1:100 dilution, HER-2 antibody (Dakopatt, Glostrup, Denmark) used at 1:1600 dilution, HER-1 antibody (Kit EGFR pharmDx TM, Dakopatt), used not diluted, Ki67 antibody (clone MIB 1, Dakopatt) used at 1:100 dilution, estrogen receptor antibody (Dakopatt) used at 1:10 dilution and progesterone receptor antibody (Novocastra, Newcastle-Upon-Tyne, UK) used at 1:100 dilution. After reaction with a mouse biotinylated secondary antibody, antigen-antibody reactions were revealed by the avidin-biotin-peroxidase complex (ABC) procedure with a Vectastain ABC kit (Vector, Burlingame, CA, USA) with diaminobenzidine as the chromogen. Immunostaining specificity was checked by omission of primary antibodies for BRCA1, estrogen receptors, progesterone receptors and Ki67 detection in the normal adjacent breast tissue. In these cases, the signal was abolished in that tissue. Only nuclear staining of neoplastic cells was scored for BRCA1. Membrane staining was scored for HER-1 and HER-2. Tumors were scored depending on the percentage of malignant cells labelled. Morphoquantitative analysis was performed at 400 magnification on ten consecutive fields. The cut-off for antibody positivity was chosen in accordance with previously published articles. Immunostaining for BRCA1, estrogen and progesterone receptors was considered as positive when tumor immunostained cells were >10% (15, 16). Immunostaining was considered as positive when scored 3+ for HER-2 (15, 17), and when tumor immunostained cells were ≥1% for HER-1 (17).

Table I. *Patients' characteristics.*

Clinicopathological features	
Mean age (years)	57.3±16 (range: 29-97)
Postmenopausal	31 (62%)
Histological type	
Infiltrating ductal carcinoma	44 (88%)
Infiltrating lobular carcinoma	3 (6%)
Other	3 (6%)
Mean tumor size (mm)	25±30 (range: 6-200)
Number of tumor-positive lymph nodes	
unknown	3 (6%)
0	28 (56%)
≤3	12 (24%)
>3	7 (14%)
Tumor grade (Scarff Bloom Richardson)	
I	9 (18%)
II	35 (70%)
III	6 (12%)
Estrogen receptor-positive (>10% of cells stained)	38 (76%)
Progesterone receptor-positive (>10% of cells stained)	36 (72%)
BRCA1-positive (>10% of cells stained)	13 (26%)
HER-2-positive (Hercept test ≥3+)	10 (20%)
HER-1-positive (>1% of cells stained)	16 (32%)
Mean percentage of cells Ki67-positive	26%±23 (range: 1%-80%)

Statistical analysis. A univariate analysis was performed to study the relationship between the immunohistochemical expressions of BRCA1, HER-1 and HER-2 proteins, and the other clinicopathological prognostic features (*i.e.* age, primary tumor size, nodal involvement, histological grading, Ki67 and hormonal receptor status). Fisher's exact test or Mann-Whitney statistics were used when appropriate. The non-parametric Spearman Rank correlation was also used to evaluate the correlations between BRCA1, HER1 and HER2. All *p*-values were two-tailed and the 0.05 level was considered statistically significant. Considering the size of our sample, multivariate analysis was not

Table II. *Association of BCRA1 expression with the clinicopathological profile in sporadic breast cancer.*

Features	BRCA1-negative (N=37)	BRCA1-positive (N=13)	*p*
Mean age (years)	57±17	58±14	0.76
Mean tumor size (mm)	28±34	16±5	0.40
Positive nodes	17/34 (50%)	2/13 (15.4%)	**0.0463***
Tumor grade 3	5/36 (14%)	1/11 (9%)	0.999
Estrogen receptor-positive	26/37 (70%)	12/13 (92%)	0.15
Mean percentage of cells estrogen receptor-positive	50.4±37%	75.6±27%	**0.0196***
Progesterone receptor-positive	27/37 (73%)	9/13 (69%)	0.999
HER-1-positive	12/37 (32%)	4/13 (31%)	0.999
HER-2-positive	9/37 (24%)	1/13 (7.7%)	0.26
Mean percentage of cells Ki67-positive	28.7±25%	19.8±20%	0.36

BRCA1-positive staining was defined as a signal present in >10% of the cells.
*Difference statistically significant.

Table III. *Association of HER-2 expression with the clinicopathological profile in sporadic breast cancer.*

Features	HER-2-negative (< 3+) (N=40)	HER-2-positive (= 3+) (N=10)	*p*
Mean age (years)	60±16	47±11	**0.0392***
Mean tumor size (mm)	26±33	21±9	0.30
Positive nodes	13/38 (34%)	6/9 (67%)	0.13
Tumor grade 3	3/38 (8%)	3/10 (30%)	0.09
Estrogen receptor-positive	34/40 (85%)	4/10 (40%)	**0.0073***
Progesterone receptor-positive	33/40 (82.5%)	3/10 (30%)	**0.0026***
HER-1-positive	9/31 (29%)	7/10 (70%)	**0.0074***
Mean percentage of cells Ki67-positive	19.8±19	52.5±26	**0.001***

HER-2-positive staining was defined as a signal = 3+.
*Difference statistically significant.

performed. A computer program package (Stat View 4.0, Abacus Concepts, Berkeley, CA, USA) was used for statistical testing and management of the database.

Results

BRCA1 expression. In normal tissue surrounding tumors, BRCA1 was always present in epithelial lobular and ductal components with a nuclear localization (Figure 1A). This immunoreactivity served as control. When a tumor expressed BRCA1, the immunostaining was heterogeneous and frequently less intense than in normal cells (Figure 1B, C, D). Positive staining ranged from 0 to 50% of the tumor cells and was <5% of tumor cells in 27 cases (54%), from 5 to ≤10% tumor cells in 10 cases (20%), from 10 to <40% tumor cells in 8 cases (16%) and ≥40% tumor cells in 5 cases (10%). So, according to the definition described above (Patients and Methods), 74% of cases were considered as negative (0 to 10% positive cells). The relationships between BRCA1 expression and clinicopathological parameters are summarized in Table II. Reduced BRCA1 expression was significantly associated with axillary lymph nodes metastases and decreased percentage of tumor cells expressing estrogen receptors.

HER-2 expression. No overexpression of HER-2 was observed in the normal tissue surrounding tumors. Positive membrane staining in tumor cells ranged from 0 to 3+, being 0 in 30 cases (60%), 1+ in 8 cases (16%), 2+ in 2 cases (4%) and 3+ in 10 cases (20%) (Figure 1F). So 10 (20%) of the cases were considered positive for HER-2 overexpression. The relationships between HER-2 expression and clinicopathological parameters are summarized in Table III. Overexpression of HER-2 was associated with younger age, lack of estrogen and progesterone receptors and Ki67 labelling was an indicator of cell proliferation.

HER-1 expression. Normal tissues surrounding the tumors were slightly positive for HER-1. Positive membrane staining in tumor cells ranged from 0 to 100% of the cells, being <1% in 34 cases (68%), from 1 to <5% in 4 cases (8%), from 5 to ≤10% in 6 cases (12%), from 10 to ≤40% in 2 cases (4%), from 40 to ≤70% in 1 case (2%) and ≥70% in 3 cases (6%) (Figure 1E). So 16 (32%) of the cases were considered positive for HER-1 expression (≥1% of positive cells). The relationships between HER-1 expression and clinicopathological parameters are summarized in Table IV. None of the studied variables was statistically significantly associated with HER-1 expression.

Table IV. *Association of HER-1 expression with the clinicopathological profile in sporadic breast cancer.*

Features	HER-1 negative (N=34)	HER-1 positive (N=16)	*p*
Mean age (years)	59±17	54±10	0.31
Mean tumor size (mm)	29±36	17±9	0.21
Positive nodes	13/32 (41%)	6/15 (40%)	0.99
Tumor grade 3	4/34 (12%)	2/16 (13%)	0.99
Estrogen receptor-positive	28/34 (82%)	10/16 (63%)	0.16
Progesterone receptor-positive	26/34 (76%)	10/16 (63%)	0.33
Mean percentage of cells Ki67-positive	20±20	29±18	0.07

HER-1-positive staining was defined as a signal present in >1% of the cells.

Correlation between variables. No correlation could be demonstrated between BRCA1 expression and HER-1 or HER-2 expressions (Table II). The Spearman rank correlation coefficients were r=0.060 and r=0.08, respectively (NS). By contrast, HER-2 expression was highly correlated to HER-1 expression (Table III). The Spearman rank correlation coefficient was r=0.39, $p<0.005$.

Discussion

This study provides original findings comparing the expressions, distribution and association of BRCA1, HER-1 and HER-2 proteins in sporadic breast cancers. In our series, 74% of the cases were considered as negative for BRCA1. The lack of BRCA1 immunostaining was associated with axillary node metastases and decreased estrogen receptor expression. HER-2 overexpression was associated with poor prognostic features (young age, decreased hormone receptors and increased Ki67 labelling), but not with axillary node metastases. In addition, a close correlation was found between HER-1 and HER-2 expressions. However, we failed to demonstrate any association between BRCA1 and HER-1 or HER-2 expressions.

The shortcomings of our study may derive from the sample size and the lack of a test for BRCA1 mutation carriers (except in one case). However, the estimated prevalence of BRCA1 germline mutations is 0.1% in the general population, so less than one patient in our study would be expected to be a BRCA1 mutation carrier (18). In previous studies, the lack of high specificity of anti-BRCA1 antibodies was a limitation when studying the correlation of BRCA1 protein expression and other prognostic features. This lack of specificity has been exemplified with COOH terminal antibodies with false positivity or cross reaction with HER-1 (19, 20). Therefore, after testing different antibodies, we chose to use MS110, which is a monoclonal antibody that was recommended in two comparison studies focusing on the choice of BRCA1 antibody (19, 20).

BRCA1 expression was reported to be dramatically reduced in sporadic breast cancer compared to normal breast, both at the mRNA and protein levels (20, 21). In our series, 74% of the tumors harbored a weak BRCA1 staining with less than 10% of the cells stained, which was consistent with the 63% negative BRCA1 staining reported by Yang *et al.* using the same MS110 antibody (15). Although BRCA1 somatic mutations are rare in sporadic breast cancer (22), allelic loss of BRCA1 and epigenetic silencing by promoter hypermethylation of BRCA1 were demonstrated in this setting and may account for the decreased BRCA1 expression (23, 24). Therefore, this raises the question of whether the pathobiological characteristics of sporadic breast cancers negative for BRCA1 are comparable to those of BRCA1-inherited breast tumors. Controversial results have been previously reported in the literature. Thus, the lack of BRCA1 expression in sporadic breast cancer was found not to correlate with other clinicopathological parameters (25). On the contrary, BRCA1 expression was associated with adverse prognostic features such as high histological grade (15, 19), decreased expression of estrogen and/or progesterone receptors (15, 26), increased proliferation (26) and axillary lymph node metastases (15). The sample size may account for the discrepancies between the studies, however, reduced BRCA1 expression was commonly associated with poor prognostic features in sporadic breast cancer. Herein, we also reported a decreased estrogen receptor expression and an increased risk of axillary nodes metastases in BRCA1-negative sporadic breast cancers. This last finding may be of interest since lymph node status was reported to be the most important predictor of survival (27). Moreover, the poorer characteristics of negative BRCA1 sporadic breast tumors compared well with the main characteristics of BRCA1-inherited breast tumors, as previously reported: high grade (28, 29), low level of estrogen and progesterone receptors (28, 30), high mitotic count (3). This similarity between the characteristics of negative BRCA1 sporadic tumors and BRCA1-inherited tumors argues for a role for BRCA1 in sporadic breast cancer.

No relationship was observed in this series between BRCA1 and HER oncogene family expression. Although, to date, the relationship between BRCA1 and the HER family has yet not been addressed in sporadic breast cancer, investigations of HER-2 status in BRCA1-inherited breast

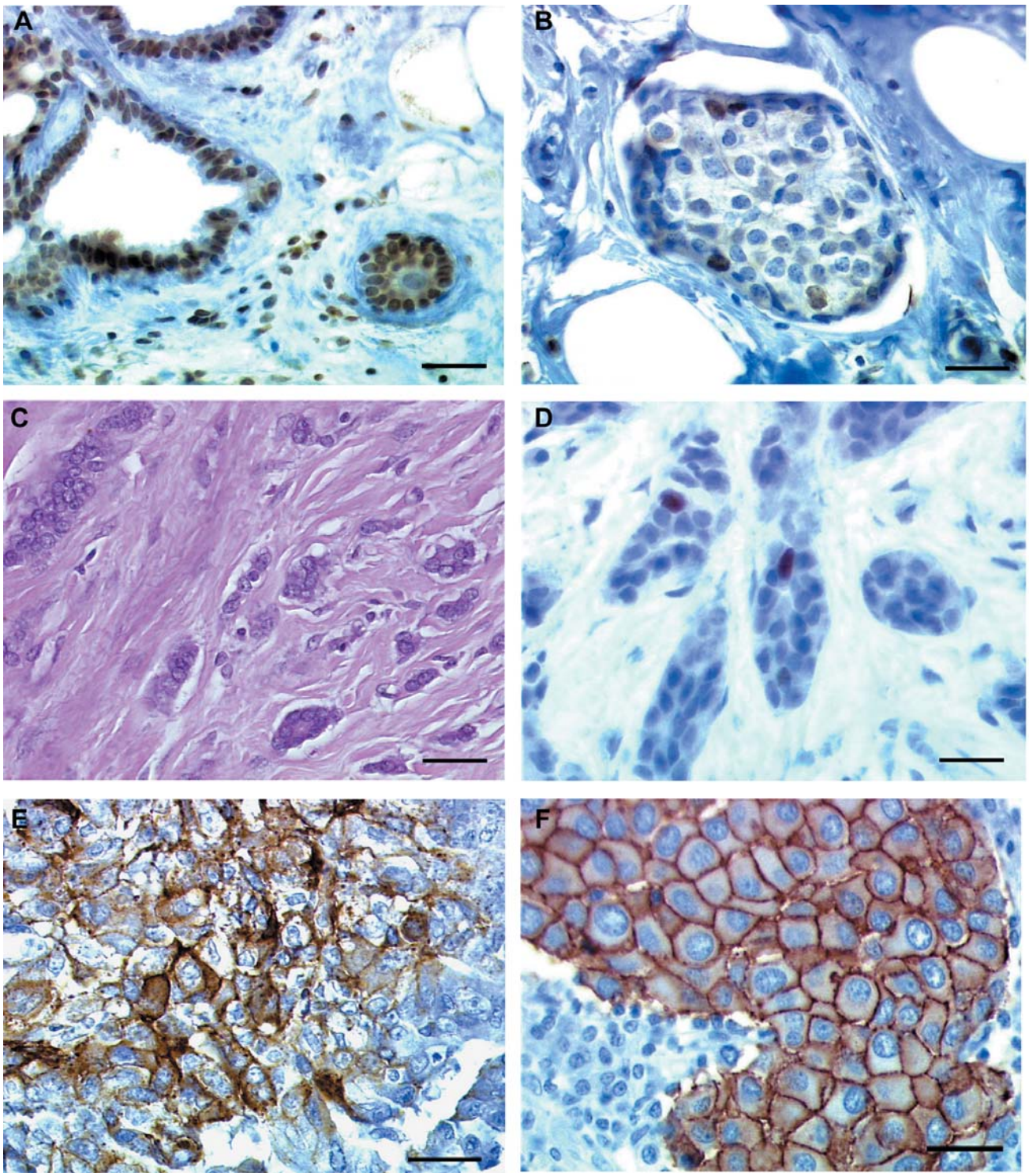

Figure 1. *A, nuclear BRCA1 immunohistochemical staining in lobular and ductal structure of normal breast; B, weak positive BRCA1 immunostaining in few cells (<10%) in a sporadic breast cancer; C, trabecular pattern of one invasive ductal carcinoma (HES staining); D, adjacent section of the same cancer showing few cells (<10%) strongly BRCA1-immunostained; E, HER1 cell membrane immunostaining of breast tumor cells (>50% of positive cells); F, HER2 cell membrane immunostaining of breast tumor cells (scored 3+) Bar=30 µm.*

cancer have either found no difference in HER-2 overexpression (9) or a lower frequency of overexpression in BRCA1-inherited tumors compared to sporadic breast cancer (10-12).

The introduction of anti-HER-2 monoclonal antibodies in the treatment of advanced breast cancer was a demonstration of how molecular biology may translate into clinical practice (7). Anti-HER-2 monoclonal antibodies offer a clinical benefit only in those patients with high levels of HER-2 receptor overexpression, whereas the clinical activity of anti-HER-1 seems also to be observed in low HER-1 receptor-expressing tumors (31). This has led to different definitions of positive staining for HER-2 (positive when signal equal to 3+) and HER-1 (positive when >10% of the cells stained) (17). In our study, 20% of the tumors overexpressed HER-2 and this was associated with young age, lack of estrogen and progesterone receptors and high proliferation index. Our results were in accordance with previous reports of 20-30% of breast cancers overexpressing HER-2 (32). Poorer prognostic features were also previously reported to be associated with HER-2 overexpression, namely lack of estrogen and progesterone receptors (33, 34), high S-phase percentage (34) and grade III tumors (33, 34). As in our study, most series did not find a correlation between HER-2 overexpression and nodal status, suggesting that HER-2 may confer a high proliferative capability that would not necessarily be associated with high metastatic potential (33, 34).

In our series, 32% of tumors were positive for HER-1. HER-1 was reported to be expressed in 20-58% of the breast cancer specimens (35) and correlated with adverse prognostic features such as a decrease in estrogen receptor expression (36). HER-1 expression was also associated with early recurrence and death from breast cancer (37). In our study, no association could be evidenced between HER-1 and the studied variables except with HER-2.

The lack of association between BRCA1 and HER-1 or HER-2 expressions contrasted in our study with the clear-cut correlation between HER-1 and HER-2. This correlation, consistent with previous reports (6, 17), highlights the interactions between the members of the HER family. HER-2 lacks a known direct ligand and was demonstrated to heterodimerize with other HER receptors that possess stimulatory ligands (38). Moreover, some experimental studies suggested that the HER-1 receptor is a key regulator of the HER family and that inhibition of the HER-1 tyrosine kinase prevents HER-2 activation in breast cancer (31). A dual HER-1/ HER-2 tyrosine kinase inhibitor is also currently under development (39).

Further studies should confirm the lack of association between BRCA1 and HER family expression, and identify the respective roles of HER-1 and HER-2 as independent prognostic factors and therapeutic targets in sporadic breast cancer patients.

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