ORIGINAL PAPER

BRCA1 expression in invasive breast carcinomas and clinicopathological correlations

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Abstract

BRCA1-positive tumors characterize a heterogeneous group of breast cancers with a specific range of histopathological and immunohistochemical features. We evaluated the relationship between morphological characteristics and immunohistochemical profile of 14 BRCA1-positive breast tumors from an initial study group of 40 breast tumors. Morphological parameters of all cases were studied on Hematoxylin–Eosin-stained sections and their immunophenotypic profile was assessed using antibodies against estrogen receptors (ER), progesteron receptors (PR), c-erbB2 oncoprotein (Her2/neu), BRCA1-protein, and the proliferative rate of the tumoral cells was assessed by Ki-67-proliferative index. All patients were females with an average age of 43.71-year-old. Fourteen out of the 40 tumors were BRCA1-positive. All breast carcinomas in this study were invasive. The most common histological type in our study was invasive ductal carcinoma. The majority of the tumors were high-grade G3. The examination of the lymph node found metastasis in eight cases. We found 11 cases of triple negative (ER, PR and Her2/neu), while the rest showed positivity for all these three markers. We observed a high-celullar proliferation index in all cases. The immunohistochemical study of BRCA1-protein is important to the study of the development and progression of the disease.

***Keywords*:** breast, BRCA1, ductal carcinoma, estrogen receptors, progesteron receptors, c-erbB2 oncoprotein.

Introduction

The mammary gland is a complex cellular ecosystem in which every cellular type is constantly transformed. By extension, breast tumors represent a complex cellular ecosystem in which cellular populations are renewed constantly and the aggressivity of the tumor increases.

Breast cancer is one of the most challenging problems of pathology, as the treatment still represents a problem with many unknown issues that affects patients both physical and psychical.

The aim of the paper was to evaluate the relationship between morphological characteristics and immunohistochemical profile of BRCA1-positive breast tumors in a series of breast cancers.

Material and Methods

The studied material was retrieved from a consecutive series of 40 specimens of invasive breast cancers received in the Research Center of Microscopic Morphology and Immunology of the University of Medicine and Pharmacy of Craiova.

We performed morphological and immunohistochemical studies on the initial group of 40 breast tumor specimens. For morphological studies, tumors were evaluated for tumor size; tumoral histological type and grade (1–3) using the modified Scarff–Bloom–Richardson grading system comprising an architectural grade, nuclear grade and mitotic grade; the presence of geographic necrosis, apoptotic tumoral cells, vascular or nervous invasion, lymphocytic stromal response, chromatinian pattern of the nuclei and the presence of large central acellular zone.

For immunohistochemical studies, sections of 5-μm thickness from formalin-fixed and paraffin-embedded tumors were cut and mounted. Following deparaffinization in xylene, slides were rehydrated through graded ethanol series, and then placed in running water. Endogenous peroxidase activity was blocked with 6% hydrogen peroxide and methanol. Samples were steamed for antigen retrieval with 10 mM citrate buffer (pH 6.0) for 30 minutes. Following protein block, slides were stained with antibodies against ER (clone 1D5, 1:50, DAKO), PR (clone PgR 636, 1:50, DAKO), c-erbB2 (polyclonal, 1:250, DAKO), BRCA1-protein (clone GLK-2, 1:50, DAKO), Ki-67-protein (clone MIB-1, 1:50, DAKO) (Table 1), and incubated with streptavidin–conjugated horseradish peroxidase using DAKO EnVision kit protocol.

Table 1 – ***Antibodies used in the study***

Antibody	Manufacturer	Clone	Dilution
ER	DAKO	1D5	1:50
PR	DAKO	PgR 636	1:50
c-erbB2	DAKO	Polyclonal	1:250
BRCA1	DAKO	GLK-2	1:50
Ki-67	DAKO	MIB-1	1:50

For the visualization of the antibody–enzyme complex, we used 3,3'-diaminobenzidine tetrahydrochloride (DAB) counterstained with Hematoxylin and examined by light microscopy. Immunohistochemical results were scored as follows: tumors were considered positive for hormone receptors if at least 10% of the tumor cells showed nuclear staining. Tumor immunoreactivity for c-erbB2 was scored as: 0 – negative; 1+ – when more than 10% of cells showed weak and incomplete membrane staining; 2+ – when moderately strong membrane staining was present in >10% of the tumor cells; 3+ – when strong membrane staining was present in >10% of the tumor cells.

For BRCA1-protein, we consider that diffuse cytoplasmic immunostaining within the tumoral cells was positive for gene BRCA1-mutations and nuclear or nuclear and cytoplasmic immunostaining within tumoral cells as negative for gene BRCA1-mutations. We used the scoring BRCA1-staining system of Yoshikawa K *et al.* [1], that scores on a discontinuous four grade scale based on the proportion of cells with moderate or strong staining: score 0 – no cells immunostained; score 1 – 0–20%; score 2 – 20–80%; score 3 – >80%.

Proliferative activity was assessed by calculating Ki-67-labeling index. We counted at high-power (400×) 1000 nuclei for each case, from the tumoral zone with the highest proliferative activity, and interpreted as positive the nuclei stained brown to black. The proliferative index was the result of the ratio between the number of positive nuclei and the total number of counted cells (positive and negative) multiplied by 100 and percentage-reported.

Results

All patients included in this study had been diagnosed with unilateral invasive breast cancer and all specimens were obtained from total mastectomies. Our study was focused on 14 tumors, which showed positivity for BRCA1 within tumoral cells.

The patients were females with age between 31 and 62-year-old (mean 43.71). For each case, there were an average number of 12 slides, excluding the lymph nodes.

The most common histological type in our study was ductal carcinoma in 11 cases (78.58%), characterized by the presence of tumoral round shaped cells with abundant eosinophilic cytoplasm with pleomorphic nuclei with visible nucleoli and variable mitotic activity. The architectural pattern showed nests, tubules, sheets and isolated tumoral cells in an abundant stroma. The surrounding stroma showed desmoplastic response and in eight cases (57.14%), we noticed the presence of foci of elastosis disposed periductal and perivascular.

The second most common histological type was lobular carcinoma in two cases (14.28%), showing the presence of small ovoid cells with scarce cytoplasm and eccentric placed nuclei, with little cytological pleormophism, aligned in strands or single files ("Indian files").

In one case (7.14%), the tumor showed highly pleomorphic tumor cells (nuclear score 3) with a syncytial pattern of growth and the presence of a moderate lymphoplasmocytic infiltrate throughout the whole tumor, which was diagnosed as medullar carcinoma (Table 2).

Table 2 – ***Morphological and immunohistochemical features of the tumors in the study***

Histological type	No. of cases (%)
Ductal	11 (78.58)
Lobular	2 (14.28)
Medullar	1 (7.14)
Histological grade	
G1	1 (7.15)
G2	5 (35.71)
G3	8 (57.14)
Dimensions (maximum diameter)	
<2 cm	5 (35.71)
>2 cm	9 (64.29)
Axillary lymph node	
Negative	6 (42.86)
Positive	8 (57.14)
Receptor status	
ER+ / ER-	3 (21.43) / 11 (78.57)
PR+ / PR-	3 (21.43) / 11 (78.57)
c-erbB2 (Her2/neu) score	
0	11 (78.57)
1+	0 (0)
2+	1 (7.14)
3+	2 (14.29)

The vast majority of these tumors were high-grade G3, eight cases (57.14%) (Figure 1), while just one case was grade 1 (7.15%). We also encountered five cases (35.71%) of breast cancers grade G2. Other additional morphological features encountered were geographic tumor necrosis, infiltrative margins (Figure 2), and lymphoid stroma (Figure 3). The inflammatory infiltrate consisted mainly of mature lymphocytes, few plasma cells and very rare histiocytes.

The diameter of the tumor varied between 0.5 cm and 11 cm, with an average of 3.25 cm. The majority of the tumors were larger than 2 cm in diameter – nine cases (64.29%).

The number of lymph nodes varied in each case and ranged from two to 16. The examination of the lymph node found metastasis present in eight cases (57.14%), and six cases (42.88%) where lymph node negative. We did not encounter any lymph node micrometastasis (<0.2 cm) or lymph node metastasis larger than 2 cm.

The presence of angiolymphatic invasion was not detected in the breast tissue immediately adjacent to the tumor.

The majority of the tumors in our study were triple negative for ER, PR and c-erbB2, 11 cases (78.57%), while the rest of the cases (21.43%) showed positivity for all these three markers (Figures 4 and 5).

The immunostaining score for c-erbB2 was 0 in 11 cases (78.57%) and 2+ (moderate membranous staining in one case (7.14%). Two cases (14.29%) were characterized by a strong membranous reaction visible on 10× magnification in at least 30% of tumor cells (3+) (Figure 6).

There was no case showing 1+ score.

The immunostaining for BRCA1 was intense, into the cytoplasm of the tumoral cells and diffuse within the tumor, in all 14 cases assessed in this study (Figure 7). The Ki-67-proliferative index was high in all cases (>30%) (Figure 8).

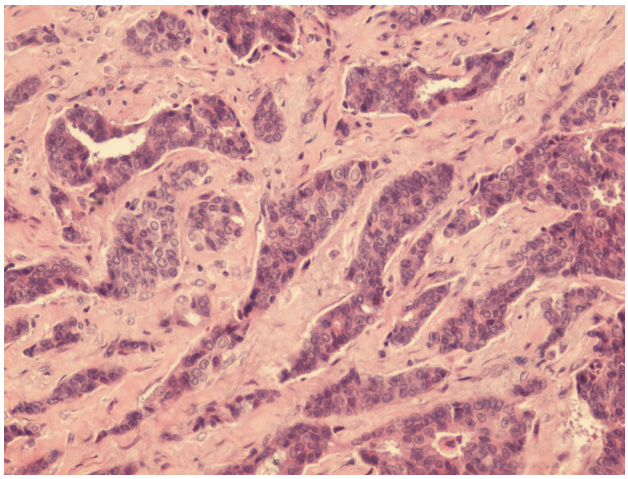

Figure 1 – ***Invasive ductal breast carcinoma, poorly differentiated (G3). H&E stain, 100×.***

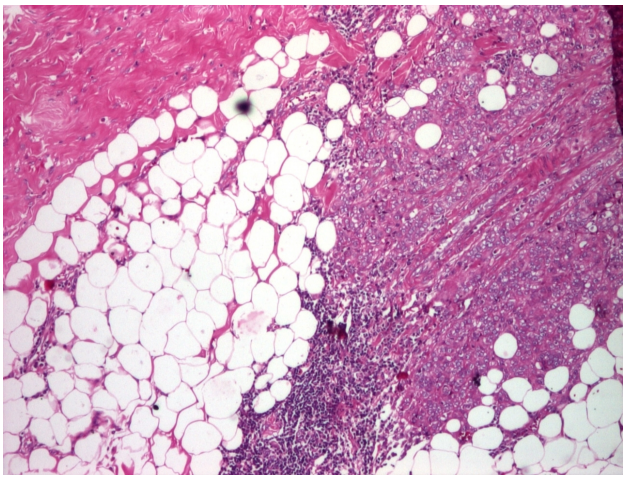

Figure 2 – ***Invasive ductal breast carcinoma with infiltrative margins. H&E stain, 40×.***

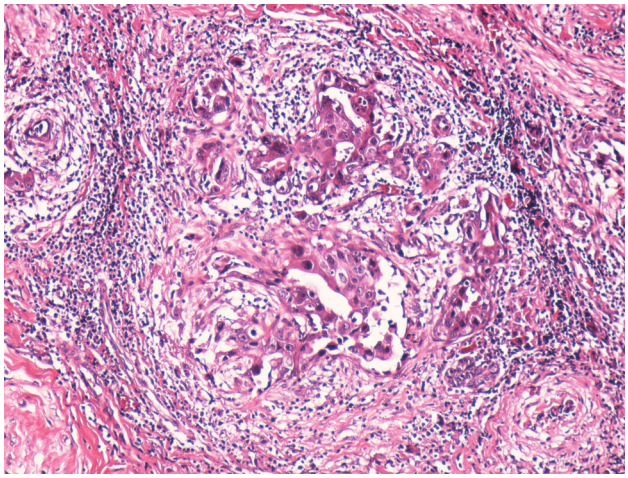

Figure 3 – ***Invasive ductal breast carcinoma with lymphoid stroma. H&E stain, 100×.***

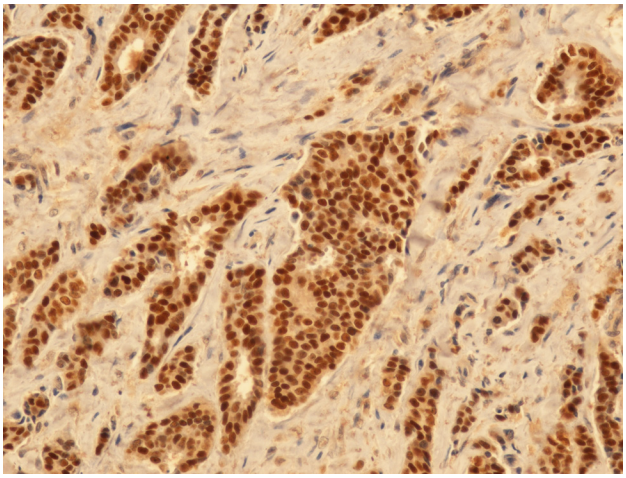

Figure 4 – ***Invasive ductal breast carcinoma: positive nuclear immunostaining for estrogen receptors. LSAB technique, 100×.***

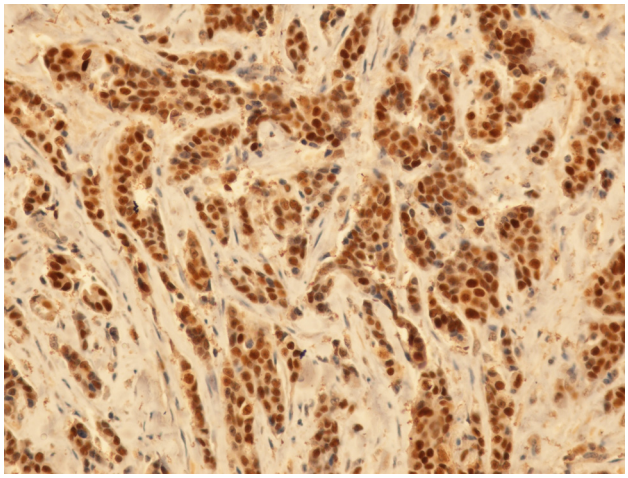

Figure 5 – ***Invasive ductal breast carcinoma: positive nuclear immunostaining for progesteron receptors. LSAB technique, 100×.***

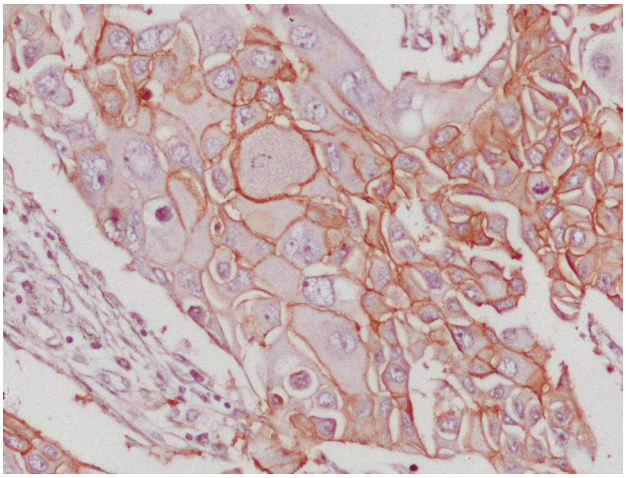

Figure 6 – ***Invasive ductal breast carcinoma: positive membranary immunostaining for c-erbB2 protein (score 3+). LSAB technique, 200×.***

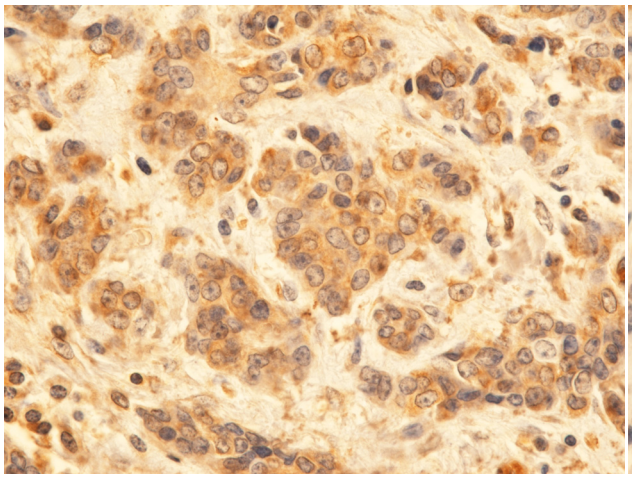

Figure 7 – ***Invasive ductal breast carcinoma: positive cytoplasmatic immunostaining within tumoral cells for BRCA1. LSAB technique, 200×.***

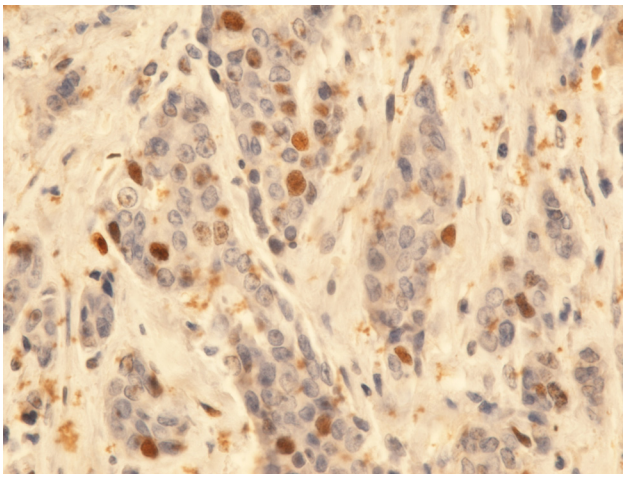

Figure 8 – ***Invasive ductal breast carcinoma: Ki-67-proliferative index higher than 30% within the tumor. LSAB technique, 200×.***

Discussion

Breast cancer is one of the most frequent neoplasia and the second cause of mortality in women. It represents a multi-stage process, which involves alterations of the normal cellular functions.

The *World Health Organization* [1] uses histological typing as the gold standard for classification of breast carcinoma, although recent studies [2], using molecular portraits revealed in the patterns of gene expression, have separated breast carcinomas in five major molecular subgroups of breast cancer – normal-like, luminal A and B, basal-like or c-erbB2+. The morphology-based approaches are clinically validated and are used in prognosticating patient outcome.

Most cases of breast cancer are sporadic and do not result from a hereditary genetic predisposition, but about 5–10% of all cases are caused by a single gene mutation that increases the susceptibility to develop breast cancer [3]. The identification of these breast cancer susceptibility genes has contributed to major modifications in the treatment of women with inherited predisposition to breast cancer.

BRCA1 is a cancer susceptibility gene located on the long (q) arm of the chromosome 17. The normal gene plays a role in repairing breaks in DNA but when is mutated this repair function may become disabled thus leading to more DNA-replication errors and cancerous growth. The temporal patterns of BRCA1 expression in human fetuses imply a role for BRCA1 in the morphogenesis and differentiation of the human mammary gland [4]. Germ line mutations of BRCA1 gene confer an estimated cumulative risk of breast cancer of around 50% until the age of 50 [5] and 85% lifetime risk in families with relatives with breast or ovarian cancer [6, 7].

BRCA1-positive tumors encompass a heterogeneous group of tumors that show distinctive pathological and clinical features. BRCA1-positive breast carcinomas are usually high-grade invasive ductal carcinomas, and can sometimes have features of medullary typical or atypical carcinoma [8]. In our study, the vast majority of the tumors were high-grade invasive ductal carcinomas – 11 cases (78.58%). According to Honrado E *et al.* typical medullary carcinomas have been observed in 8% to 13% of BRCA1-positive breast tumors compared with 3% for sporadic breast cancer [9]. Only one of our cases was of medullary carcinoma (7.14%), but the low number of patients in the study group could explain the smaller percent. We also found two cases of lobular carcinoma but in these cases hormone receptors and c-erbB2 were positive. In a study performed on two groups of BRCA1-positive breasts carcinomas, Butler WM *et al.* found only 1% of the tumors to be invasive lobular carcinomas in the group of African American women and 11% in the group of the European American women [10].

BRCA1-positive breast tumors are generally observed at younger age and have distinct pathological features when compared with sporadic breast cancer [11]. The mean age of our patients was around 43-year-old. We believe this age not to be statistically significant, as all the tumors in our study were high grade and in some, the clinical history revealed the presence of a breast lump for a period before the presentation to the physician.

Immunohistochemical assessment of BRCA1-mutation is less expensive and time consuming than genetic testing. There are also several histopathological aspects that may suggest the presence of this mutation. BRCA1-associated breast carcinomas are described as having a high mitotic index, a high histological grade and a low hormone receptor-positive rate [12]. The histopathology study of our cases showed only aggressive, invasive tumors, with no case with only in situ tumor.

Breast cancers in patients with BRCA1-germline mutations are more often negative for estrogen receptor, progesterone receptor and Her2 [6, 13]. The gold standard of tumor markers is represented by the use of the hormone receptors (ER, PR) to predict benefit from endocrine therapy. In our study, the majority of the specimens (78.57%) showed negativity for the hormone receptors and for c-erbB2 oncoprotein. Lynch BJ *et al.* [14] reported that BRCA1-associated breast tumors are

more likely to lack hormone receptors and to have an expression of p53-positive more frequently than in sporadic tumors. Hormone receptors play an important role as they represent a successful target for therapy, thus causing a decline in the mortality of breast cancer.

The c-erbB2 oncogene is involved in the regulation of cell proliferation and invasion, its amplification or protein overexpression being a genetic aberration occurring in 30% of all breast carcinomas and so correlating with a poor clinical outcome [15]. According with data, in our study the vast majority of the tumors have had score 0 for c-erbB2 oncoprotein – 11 cases (78.57%), but we also found two cases (14.29%), which overexpressed this protein (score 3+). This over-expression can predict the response to trastuzumab (Herceptin), a humanized monoclonal antibody that acts against cells that express Her2/neu.

These triple negative tumors are not expected to be endocrine responsive and also do not benefit of the treatment with Herceptin. In the attempt to further reducing the mortality of breast cancer, it is necessary to characterize this subset of triple negative tumors. There are data in the literature, which have showed high resemblances between these triple negative breast cancers and the newly described basal subtype [16], but an equality mark between these two entities was not proven.

Rakha EA *et al.* (2007) examined invasive breast cancers by microarray techniques as well as immuno-histochemistry (ER, PR, Her2, androgen receptor, EGFR, P-cadherin, E-cadherin, basal cytokeratin CK5/6 and CK14); in that study, 16.3% of the cases proved to be triple negative and associated with high tumoral diameter, invasive margins, low Nottingham prognostic index, recurrences and metastasis and poor prognosis [17]. Only the immunohistochemical assessment of the basal like phenotype using hormone receptors and basal cytokeratins does not represent an ideal surrogate for the microarray technique. This group study is part of a research project and further molecular analysis will be performed in order to define similarities between the molecular subtypes and histopathological and immunohistochemical features, as molecular techniques are rather difficult in routine practice.

The study was limited by the small size of the group in our study, which implied limited information for statistical analysis. Our study group was not a population-based cohort as the generated cancer population is often bias for younger age, but a certain preponderance for younger age at diagnosis could be subjectively appreciated.

Conclusions

Although it does not represent a routine assessment, the immunohistochemical study of BRCA1-protein represents important information in what concerns the development and progression of the disease. Validation of the imunohistochemical results by using a larger sample and molecular confirmation, will allow using immunohistochemistry to decide which are high-risk patients providing a better patient selection and so increasing the possibilities of obtaining more rapidly informative genetic results.

Identification of morphological and immunohisto-chemical features of breast tumors that are associated with BRCA1 mutation would be of assistance for the clinical and therapeutically follow-up.

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