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Histological features of extratumoral breast lesions as a predictive factor of familial breast cancer

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Abstract. The aim of this study was to verify whether histopathological features of extratumoral and of primary tumor breast tissue could play a role in identifying patients with familial characteristics. We examined the clinicopathological features of 504 patients with sporadic or familial breast cancer stratified for risk of BRCA mutation. Patients with a higher risk of being carrier of BRCA gene mutations were significantly associated with tumor poor differentiation (p=0.003), positive lymph node invasion (p=0.02) and presence of vascular peritumoral invasion (p=0.008). Among the extratumoral lesions, only the epithelial proliferative lesions were related to higher mutation risk both in the overall series and familial patients (p<0.0001 and p=0.003, respectively). Interestingly, a significant difference in terms of high mutation risk was observed in usual ductal hyperplasia lesions (UDH), (p=0.002). We suggest that vascular peritumoral invasion and UDH lesions could predict a higher mutation risk of BRCA1 and BRCA2 genes and help in individuating patient candidates to further molecular analysis.

Introduction

Family history of breast cancer is a well established risk factor associated to mutations in high and low penetrance breast cancer susceptibility genes such as BRCA1, BRCA2, p53, ATM or PTEN but also to alterations not yet identified (1,2). Furthermore, the study of Easton *et al* (3) demonstrated that common susceptibility loci can be reliably identified, and that they may together explain an appreciable fraction of the genetic variance in breast cancer risk. Consequently, there is a clear need for identification of phenotypic and molecular traits explaining the remaining familial cases of breast cancer.

Biological characteristics and clinical course of family history of breast cancer have been extensively reported (4-6), while the pathological characteristics of the surrounding non-malignant tissue have been few investigated. Proliferative lesions in breast parenchyma include many histological changes and for some of them [ductal carcinoma *in situ* (DCIS), atypical hyperplasia and sclerosing adenosis] a potential interest for the progression of cancer has been demonstrated (7). Moreover, women with an autosomal dominant family history of breast cancer, independently from BRCA mutation, are prone to develop high-risk epithelial lesions (8).

Studies examining a possible association between presence of extratumoral lesions and presence of family history for breast cancer have been up to now performed in incomplete information on family history of disease (9), small series of hereditary breast cancer patients (10) and patients with basic information from only breast tissues obtained from prophylactic surgery (11).

The knowledge of histopathological characteristics of normal tissue where primary tumor of familial breast cancer patients is developed could be of particular interest. In fact, altered characteristics of the non-malignant surrounding tissue could represent the basis for genesis and progression of cancer disease in subjects with germline alterations and with hereditary susceptibility.

In the present study, we investigated the histopathological features of tumor and of surrounding tissue in patients with sporadic or familial breast cancer stratified for risk of BRCA mutation. Furthermore, we looked for a potential association between the normally considered 'breast cancer related risk factors' and the results of histological examination.

Materials and methods

Patients. We studied a retrospective series of 504 patients with a first diagnosis of breast cancer treated surgically in our Institute between February 2002 and March 2003 and all

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Abbreviations: UDH, usual ductal hyperplasia; DCIS, ductal carcinoma *in situ*; H&E, haematoxylin and eosin; WHO, World Health Organization; IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; ADH, atypical ductal hyperplasia; ER, estrogen receptor status; PgR, progesterone receptor status

Key words: extratumoral breast lesions, BRCA genes risk mutation, vascular peritumoral invasion, UDH

followed in genetic counseling program for familial breast/ovarian cancer (12). Briefly, patients eligible for genetic counseling program were transferred to genetic counseling out-patients clinics where *ad hoc* teams updated their medical history and obtained informed consent to perform the molecular analyses. Patients were classified as having a family history of breast cancer according to criteria previously described (13). The risk of being a BRCA mutation carrier was calculated for each patient using the New Myriad II program (14). Patients were classified as having an 'increased risk' when the probability of finding a BRCA mutation was ≥10%.

Histological examination. After surgical removal of biological tissues, the pathologist selected from the primary tumor and from surrounding macroscopically uninvolved breast tissues, samples destined for routine diagnostic practice at Pathology Department of our Institute. The extratumoral lesions were examined on the selected samples from the breast parenchyma at different distances from the primary nodule. Stored haematoxylin and eosin (H&E) stained slides from each patient were evaluated by two pathologists (F.A.Z. and G.S.) who were unaware of the initial histological diagnoses and patient outcomes.

All tumors included in the study were classified according to the guidelines of the World Health Organization (WHO), (15) as infiltrating ductal carcinoma (IDC) not otherwise specified, infiltrating lobular carcinoma (ILC), DCIS and other carcinoma types (mixed tubular, tubular, medullary, atypical medullary, cribriform, mucinous, etc.).

Different histotypes, tumor size (≤1.5 cm, >1.5 to ≤3 cm, >3 cm), histological grading classified in 1, 2, 3 grade (performed according to the criteria described by Elston and Ellis) (16), axillary lymph node invasion (number of involved nodes in relation to total examined number), multifocality (absent, present), perineural invasion (absent, present), tumor necrosis (absent, present), calcification (absent, present), and vascular peritumoral invasion (absent, present) were recognised and reported. In H&E-stained sections, vascular peritumoral invasion was considered evident if at least one tumor cell cluster was clearly visible in the vascular space with erytrocytes identified in the endothelial-lined space.

The histological classification of the benign extratumoral breast deseases was summarized into three categories: i) fibrocystic lesions (atrophy, fibrosis, fibrocystic changes); ii) epithelial proliferative lesions [usual ductal hyperplasia (UDH), ductal hyperplasia with columnar changes, flat epithelial atypia, atypical ductal hyperplasia (ADH), sclerosing adenosis and DCIS]; iii) benign tumors (fibroadenoma, papilloma).

Tumor proliferative activity (MIB-1 labeling index), estrogen (ER) and progesterone (PgR) receptor status were previously determined by immunohistochemistry (13,17), and a cut-off of 10% of immunostained tumor cells was established for a tumor to be classified as negative or positive receptor and to differentiate between low or high proliferating tumors (13). All the analyses were performed in the Clinical Experimental Oncology Laboratory of the National Cancer Institute of Bari which participates in the INQUAT Quality Control programs (18) and is ISO 9001-2000 certified (DNV Certificate No. CERT-17885-2006-AQ-BRI-SINCERT).

Table I. Patients with sporadic breast cancer or with family history of breast and/or ovarian cancer.

	No. of patients (%)
Overall series	504
Sporadic breast cancers	336 (66.7)
Family history	168 (33.3)
One first-degree relative, two or more second-degree relatives	82 (49)[a]
One second-degree relative	77 (45.8)
Two or more first-degree relatives	9 (5.2)

[a]Eight cases with both breast and ovarian cancer.

Statistical analysis. The association between clinicopathological features and risk of being carrier of BRCA gene mutations or presence of family history for breast cancer was assessed using the χ^2 test. Differences were considered to be significant when p-value was <0.05. Statistical analyses were carried out with the SPSS statistical software (SPSS, Inc., Chicago, IL, USA).

Results

The characteristics of 504 patients are described in the Table I. Patients with familial breast cancer presented more frequently an ER-negative phenotype (34%) with respect to sporadic patients (23%), (p=0.01). Moreover, MIB-1 positivity was significantly higher in sporadic cases (73%) compared to familial cases (62%), (p=0.04).

Table II summarizes the risk of BRCA gene mutations in relation to clinicopathological features of all patients. The risk of being a BRCA gene mutation carrier is higher in patients aged between 35-50 years both considering the overall series (p<0.0001) and the familial cases (p=0.000). The patients with a higher risk showed to be significantly associated with higher histological grade (p=0.003), and positive lymph node invasion (p=0.02). Similar result was also found when the analysis was restricted to the group of familial women: in fact, the 168 familial patients with a higher risk of BRCA genes mutation were significantly associated with histological grade and positive lymph node invasion (p=0.047 and p=0.008, respectively). Moreover, the presence of vascular peritumoral invasion was significantly associated with a higher risk of BRCA gene mutations both in overall series and the familial subgroup (p=0.008 and p=0.048, respectively).

When benign extratumoral lesions were examined (Table III), fibrocystic lesions were significantly associated with BRCA low mutation risk (p<0.0001) in all patients. In particular, atrophy lesion was specifically related to a low mutation risk (14% low risk vs. 0% high risk patients, p=0.0468); and, atrophy and fibrosis were significantly present in sporadic patients with low risk gene mutation (p=0.04, data not shown). Instead, when considering epithelial proliferative lesions in overall and familial series of patients, these were related to BRCA high mutation risk (58% high

Risk of BRCA gene mutations in relation to clinicopathological features.

Variables	All cases	Risk of BRCA gene mutations		p-value
		Low	High	
	504	459	45	
Age (years)				
≤35	21	13	8	
>35 and <50	142	115	27	0.0001
≥50	341	331	10	
Histotype				
IDC	408	372	36	NS
ILC	26	23	3	
DCIS	23	22	1	
Others	47	42	5	
Tumor size (cm)				
≤1.5	108	99	9	NS
1.5 <T≤3	268	243	25	
>3	104	94	10	
Histological grade				
I	78	75	3	
II	227	212	15	
III	141	119	22	0.003
Lymph node invasion				
Negative	221	209	12	
Positive	243	214	29	0.02
Multifocality				
Absent	350	320	30	NS
Present	124	111	13	
Perineural invasion				
Absent	420	381	39	NS
Present	17	15	2	
Tumor necrosis				
Absent	193	176	17	NS
Present	266	243	23	
Calcification				
Absent	228	205	23	NS
Present	233	216	17	
Vascular peri-tumoral invasion				
Absent	318	296	22	
Present	121	102	19	0.008
Steroid receptors				
ER^- (≤10%)	130	115	15	NS
ER^+ (>10%)	359	29	30	
PgR^- (≤10%)	209	192	17	NS
PgR^+ (>10%)	280	252	28	
MIB-1				
Negative (≤10%)	166	156	10	NS
Positive (>10%)	323	289	34	

NS, not significant (p>0.05).

risk vs. 26% low risk patients, p<0.0001 and 45% high risk vs. 21% low risk patients, p=0.003, respectively).

Among epithelial proliferative lesions, the higher mutation risk resulted significantly associated to UDH (42% high risk vs. 17% low risk patients, p=0.002), while a significant low risk is associated to ADH and sclerosing adenosis (44 vs. 24%; p=0.0397; 19 vs. 3%, p=0.0273, respectively). Any significant association was found analyzing singularly the presence of UDH, ADH and sclerosing adenosis both in sporadic and in familial patients group. Benign tumors were all low risk patients and all of them had familial characteristics.

Discussion

In this study, we verified whether histopathological features of primary tumors and non-malignant surrounding breast tissue could be of some help in identifying risk familial patients. This information could be of great interest for several reasons: it could contribute to better understanding of the process of breast carcinogenesis and it could also represent a useful method to select patient candidates to further molecular studies.

We evaluated clinicopathological issues of 504 breast cancer patients with or without familiarity and with different risk of being a BRCA mutation carrier, focusing our attention on morphological features currently observed in clinical practice.

According to other studies (19), individual data on breast cancer in first and second-degree relatives of all the patients were collected, checked and analysed. We considered clinical features of familiarity to avoid exclusion of patients with hereditary breast cancer independent of BRCA, but dependent on other unknown genes.

The pathological features of BRCA associated cases are reported as being less favorable as compared to sporadic cases, with higher frequency of high grade (20), negative oestrogen and progesterone receptor (21) and rapidly proliferating tumors (22) as previously found (13). Moreover, Aaman *et al* (9) evidenced that the prevalence of proliferative breast diseases was slightly but not significantly lower in familial patients compared to sporadic. Differently from our study, Mohammed *et al* (20) and Molino *et al* (23) reported a significantly higher percentage of small tumors in women with family history.

In this study, familial cases resulted predominantly ER negative, while sporadic breast carcinomas showed higher proliferative activity. Furthermore, in agreement with Russo *et al* (24), we found no significant difference with other clinicopathological features.

When we analysed the relationship between the clinicopathological characteristics and risk of being a BRCA mutation carrier, the risk was lower in elderly patients as expected from other population-based studies. This is consistent with findings from other populations, indicating that the frequency of BRCA1 mutations is higher in early-onset breast cancer (25-27). According to Veronesi *et al* (28), higher histological grade and lymph node invasion were significantly more frequent in patients with high genetic risk. Moreover, we also found that patients with higher risk mutation proved to be significantly associated with the presence of vascular peri-

Table III. Extratumoral breast lesions in relation to risk of BRCA genes mutation in 504 patients.

Type of parenchyma breast lesions	All patients	Mutation risk		P-value
		Low (%)	High (%)	
Fibrocystic lesions	339	315 (70)	24 (42)	<0.0001
Atrophy	45	45 (14)	0 (0)	0.0468
Fibrosis	206	191	15	NS
Fibrocystic changes	88	79	9	NS
Epithelial proliferative lesions	151	118 (26)	33 (58)	<0.0001
Usual ductal hyperplasia (UDH)	34	20 (17)	14 (42)	0.0020
Ductal hyperplasia with columnar changes	21	13	8	NS
Flat epythelial atypia	7	6	1	NS
Atypical ductal hyperplasia (ADH)	60	52 (44)	8 (24)	0.0397
Sclerosing adenosis	23	22 (19)	1 (3)	0.0273
DCIS	6	5	1	NS
Benign tumors	14	14	0	NS
Fibroadenoma	9	9	0	NS
Papillomas	5	5	0	NS

NS, not significant (p>0.05).

tumoral invasion that is an essential step in the metastatic cascade (29), and it has long been demonstrated that the detection of presence of tumor cells within vessels is a marker of a tumor with metastatic potential (30).

We further examined the role and the prevalence of benign proliferative lesions. No statistical difference was found when features of each extratumoral lesion in familial or sporadic breast cancer were compared. Moreover, examining the three histological categories of parenchyma breast lesions, the percentage of fibrocystic lesions, epithelial proliferative lesions and benign tumors were similar in patients with or without family history of breast cancer. Interestingly, also when the different criteria adopted to classify familiarity were considered, no difference was present regarding the type of parenchyma breast lesions (data not shown).

We found that the association of the fibrocystic lesions with low risk of mutation in all patients, and, in particular, of the atrophy with a lower mutation risk was due to the presence of atrophy and fibrosis in a higher number of low risk sporadic patients. Moreover, Worsham *et al* (31) demonstrated that the presence of fibrosis was protective against breast cancer, in fact women with fibrosis had a reduced risk for progression to breast cancer as compared with women without fibrosis.

In our study, the epithelial proliferative lesions of the overall series compared to fibrocystic lesions and benign tumors, were significantly associated with a high risk of mutation. This data was confirmed, also, analyzing mutation risk in familial patients.

ADH and sclerosing adenosis were significantly associated with a lower mutation risk. Our findings are in accord with those of Aaman *et al* (9) showing that patients with a family history of breast cancer had a slightly, but not significantly, decreased prevalence of ductal atypia (DCIS or ADH) and sclerosing adenosis when compared with patients with no family history of cancer. Instead, the study of Webb *et al* (32) showed that women with proliferative benign breast diseases, in particular with atypia, were significantly associated with family history. Other studies also showed that women with a hereditary predisposition for breast cancer (11) and women with BRCA mutations (32) are prone to develop high risk lesions in their breasts, in particular lesions associated with an increased risk of invasive carcinoma (ADH, atypical lobular hyperplasia and lobular carcinoma *in situ*). The study of Hoogerbrugge *et al* (8) reported that women with familial breast cancer with a BRCA mutation had the highest predicted probability for the presence of high risk lesions in their breasts (ADH, DCIS). Moreover, Kroiss (6), in agreement with Hoogerbrugge *et al* (8) and Kauff *et al* (33), but differently from Adem *et al* (10), reported that BRCA gene mutation carriers had greater prevalence of pre-malignant lesions.

Several studies showed that the transformation of a benign breast lesion into an invasive carcinoma may occur more rapidly in BRCA carriers than in women suffering from breast cancer, perhaps because the environmental carcinogenesis is bypassed or accelerated in women with germline mutations in DNA repair genes. However, in our study, this hypothesis is contradicted by the high prevalence of epithelial proliferative lesions in high risk patients, in accord with Kroiss *et al* (6), and in particular by the high prevalence of UDH. In fact, we demonstrated that the UDH was significantly predominant in all patients with a higher risk of BRCA gene mutations, and indirectly confirmed by absence of a relationship between risk and fibrocystic lesions. These are more frequent in benign breast pathology, they do not normally include areas of ductal hyperplasia, or only in limited areas of the parenchyma.

nclusion, our data show that vascular peritumoral could be a predictive factor of high risk, particularly, in familial patients with BRCA1 and BRCA2 genes mutation risk. Moreover, only the epithelial proliferative lesions, in particular UDH, are associated to a higher mutation risk patients. Intriguingly, UDH is a lesion widely observed both in sporadic and in familial breast cancer. Therefore, UDH should be considered as a part of the BRCA associated tumor spectrum and for this reason, we hypothesize that UDH could be the real target of malignant progression of this lesion. Our findings suggest that these women could be patient candidates to further molecular studies.

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