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BRCA-associated Breast Cancer: Absence of a Characteristic Immunophenotype[1]

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ABSTRACT

To characterize the biological features of breast cancer associated with germ-line mutations in *BRCA1* and *BRCA2*, invasive tumors were studied from 58 Jewish women ascertained through studies of early-onset breast cancer. All women were tested for the *BRCA1* founder mutations 187delAG (commonly known as 185delAG) and 5385insC (commonly known as 5382insC) and the *BRCA2* founder mutation 6174delT. Mutations were detected in 17 of 58 (29.3%) women. Comparing *BRCA*-associated breast cancers (BABCs) to cases arising in women without founder mutations, no differences were noted in tumor size, tumor stage, or frequency of axillary nodal involvement. Infiltrating ductal carcinoma was the predominant histological type in both groups. BABCs were significantly more likely to be of histological grade III (100 *versus* 63%; $P = 0.04$), estrogen receptor negative (75 *versus* 35%; $P = 0.004$), and HER2/*neu* negative (87 *versus* 58%; $P = 0.04$). An associated intraductal component was present in 59% of BABCs and 76% of cancers not associated with mutations (P = not significant). A high Ki-67 labeling index was more commonly observed in BABCs than in cases without mutations (83 *versus* 48%; $P = 0.09$). There were no differences between the two groups in the frequency of expression of epidermal growth factor receptor, cathepsin D, bcl-2, p27, p53, or cyclin D. There were no significant differences in relapse-free or overall survival.

These observations suggest that breast cancers arising in Jewish women with germ-line *BRCA* founder mutations have a greater proliferative potential than cancers in women without such mutations. Additional studies of BABC are required to determine the nature and implications of additional genetic abnormalities occurring in these tumors.

INTRODUCTION

Over 180,000 women will develop breast cancer in 1997 (1). In 5–10% of cases, the disease will occur as part of a hereditary cancer susceptibility syndrome (2). A substantial proportion of hereditary breast cancers can be attributed to mutations in one of two genes, *BRCA1* or *BRCA2*. Studies of *BRCA1* heterozygotes from families with multiple cases of breast and ovarian cancer yielded estimated lifetime breast cancer risks of up to 84% and ovarian cancer risks of up to 44% (3, 4). The penetrance of *BRCA* mutations remains a topic of active investigation, however, because recent studies of less selected families have suggested that the risks may be somewhat lower than these initial estimates (5). *BRCA1* and *BRCA2* mutations are thought to confer a similar susceptibility to breast cancer, but *BRCA2* mutations may pose a lower risk for ovarian cancer.

Histopathological analyses of BABCs[3] have suggested that these tumors may proliferate more rapidly than their sporadic counterparts. Several groups have noted that tumors arising in *BRCA* heterozygotes are of higher overall grade than sporadic breast cancers from controls (6–11). In *BRCA1*-associated tumors, the preponderance of high-grade disease is reflected in an increased mitotic rate (8–11). The S-phase fraction has also been reported to be higher in *BRCA1*-associated breast cancers than in sporadic controls (9, 10). Taken together, these findings suggest that tumors arising in the setting of an inherited predisposition have an increased proliferative capacity when compared to sporadic tumors. This enhancement of proliferative activity may be a direct result of the loss of BRCA1 or BRCA2 function. Alternatively, additional genetic events occurring in conjunction with the loss of BRCA function may be responsible.

In an attempt to further characterize the biological features of breast cancers arising in *BRCA* heterozygotes, this report describes the histopathological and immunohistochemical features of invasive tumors from Jewish women with early-onset breast cancer. Previous studies have demonstrated that approximately 30% of young Ashkenazi Jewish women with breast cancer are heterozygous for one of three *BRCA* founder mutations (*BRCA1* 187delAG, *BRCA1* 5385insC, and *BRCA2* 6174delT; Refs. 12–15). The high prevalence of mutations among affected women reflects the significant frequency (2.5%) of these alleles in individuals of Central/Eastern European Jewish ancestry (5, 14, 16, 17). The subjects of the current series were screened for each of these recurrent *BRCA* mutations. The results of this mutational analysis have been reported previously (12, 13). Tumors from women with and without germ-line mutations were compared.

MATERIALS AND METHODS

Patients. The study cohort was comprised of 58 Jewish women with breast cancer diagnosed before the age of 42 years, for whom pathological material was available for review. These women were ascertained as part of a study of the genetics of early-onset breast cancer conducted at Memorial Sloan-Kettering Cancer Center between February 1992 and February 1996. Most of the subjects of this report were included in previous descriptions of this study (12–14). The sole entry criterion for the study was a diagnosis of early-onset female breast cancer, without regard for family history or ethnicity. The determination of Ashkenazi Jewish ancestry was based on the report of the participant.

Informed Consent. Participants in this study underwent a multistage consent process. Before donating a DNA sample, all subjects provided written informed consent for participation in studies of genetic changes in breast cancer. Once germ-line *BRCA1* testing became available, all participants were again approached and asked to consent to such testing. When their test results became available, participants were offered full genetic counseling, after which they were given the opportunity to decline to learn their result. The consent process was approved by the Memorial Sloan-Kettering Cancer Center Institutional Review Board.

Genetic Testing. Testing for the *BRCA* founder mutations was performed as described previously (12–14). Genomic DNA was isolated from peripheral blood lymphocytes and amplified using standard PCR procedures with the primers and conditions reported. PCR products were subjected to acrylamide gel electrophoresis, and altered migration patterns were detected by either autoradiography or ethidium bromide staining and conventional photography. All samples demonstrating altered migration patterns were directly sequenced to confirm the presence of a mutation.

Histology Review. All cases were reviewed by two pathologists expert in the evaluation of breast disease. The pathologists were blinded to *BRCA* mutation status at the time of review. Tumor size was determined, when

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[3] The abbreviations used are: BABC, *BRCA*-associated breast cancer; EGFR, epidermal growth factor receptor.

possible, by direct microscopic measurement of the largest diameter of invasive tumor. When direct measurement was not possible, size was determined from the original pathology report. Histological grading was performed according to the method of Bloom and Richardson (18). Histological grade I lesions demonstrated a tubular growth pattern with grade 1 nuclei. Histological grade II lesions were characterized by a partial tubular growth pattern with grade 2 or 3 nuclei. Histological grade III lesions manifested little or no tubule formation with grade 2 or 3 nuclei. Nuclear grade was determined by a modification of the method of Black and colleagues based on nuclear size and shape, nucleolar appearance, chromatin pattern, and mitotic rate (19, 20). The presence of an associated *in situ* component was noted, as was the presence of apparent lymphovascular invasion.

Immunohistochemical Studies. Immunohistochemical studies were performed on sections obtained from paraffin blocks of tissues fixed in 10% neutral buffered formalin. The antibodies used in the immunohistochemical profile are listed in Table 1.

In the general protocol used to demonstrate immunoreactivity for the various antigens, 6-μm sections cut from paraffin blocks were placed on polylysine-coated slides and deparaffinized in xylene, 100% ethanol, 95% ethanol, and distilled water. After treatment with 1% hydrogen peroxide for 15 min, the sections were rinsed in PBS. Blocking serum was applied for 30 min and then removed. After suctioning off the blocking serum, the primary antibody was applied at the dilution listed in Table 1, and the slides were refrigerated overnight at 4°C in a closed chamber. After rinsing in PBS, the biotinylated secondary antibody was applied for 30 min. The slides were rinsed again in three changes of PBS. ABC Elite Vectastain (Vector Laboratories, Burlingame, CA) diluted 1:25 in PBS was applied for 30 min, after which the slides were again rinsed three times with PBS. Staining was visualized with 3–3′-diaminobenzidine followed by washing in distilled water, counterstaining with hematoxylin, and dehydration in 95% ethanol, 100% ethanol and xylene.

Positive and negative controls for each study antigen were examined for each group of cases. Interpretation of results was limited to the invasive portion of the tumor. Criteria used to classify a tumor as positive or negative varied from antigen to antigen. Both pathologists were blinded to *BRCA* mutation status at the time of immunohistochemical analysis.

Statistical Analysis. Relationships between mutation status and histological or immunohistochemical variables were evaluated using the χ^2 test or Fisher's exact test.

RESULTS

One of the recurring *BRCA* mutations (*BRCA1* 187delAG, *BRCA1* 5385insC, or *BRCA2* 6174delT) was detected in 17 of 58 subjects (29.3%). The most common mutation was *BRCA1* 187delAG, commonly referred to as 185delAG (10 participants; 17.2%). *BRCA2* 6174delT was detected in 5 of 58 subjects (8.6%), and *BRCA1* 5385insC (also known as 5382insC) was detected in 2 of 58 subjects (3.4%). The clinical features of the women with and without founder mutations are listed in Table 2. There were no significant differences in the median age at diagnosis, primary tumor size, tumor stage, or frequency of axillary nodal involvement.

Table 1 *Antibodies used for immunohistochemical profile*

Antibody	Dilution	Source
Estrogen receptor mouse monoclonal	1:5	Immunotech (Westbrook, ME)
Progesterone receptor mouse monoclonal	1:50	Novocastra/Vector Labs (Burlingame, CA)
p53 mouse monoclonal	1:7000	Oncogene (Cambridge, MA)
p27 mouse monoclonal	1:750	Gift of Dr. Andy Koff (MSKCC,[a] New York)
HER2/*neu* rabbit polyclonal	1:100	Zymed Laboratories (San Francisco, CA)
Ki-67 mouse monoclonal	1:50	Immunotech (Westbrook, ME)
Cyclin D1 mouse monoclonal	1:250	Oncogene (Cambridge, MA)
bcl-2 mouse monoclonal	1:50	DAKO Corporation (Carpenteria, CA)
EGFR mouse monoclonal	1:200	Ciba-Corning (Oraneda, CA)

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Table 2 *Clinical features*

	Mutation positive	Mutation negative[a]
n	17	41
Median age (yr)	36 (25–41)	37 (22–42)
Median tumor size (cm)	1.9 (0.7–4.0)	1.6 (0.3–10.0)
T stage[b]		
T_1	10 (59%)	24 (58%)
T_2	5 (29%)	12 (29%)
$T_{3/4}$	2 (12%)	4 (10%)
Unknown	0 (0%)	0 (0%)
Axillary nodes		
Negative	9 (53%)	21 (51%)
Positive	8 (47%)	20 (49%)

[a] Mutation negative, not heterozygous for *BRCA1* 187delAG or 5385insC or for *BRCA2* 6174delT.
[b] T stage, tumor stage.

Table 3 *Histological features*

	Mutation positive (%) $n = 17$	Mutation negative[a] (%) $n = 41$	P
Histology			
Infiltrating ductal	17 (100)	40 (98)	0.52
Infiltrating lobular	0 (0)	1 (2)	
Associated *in situ* component			
Present	10 (59)	31 (76)	0.20
Absent	7 (41)	10 (24)	
Histological grade			
1	0 (0)	1 (2)	0.04
2	0 (0)	13 (32)	
3	17 (100)	26 (63)	
Unknown	0 (0)	1 (2)	
Nuclear grade			
1	0 (0)	5 (12)	0.13
2	4 (23)	15 (37)	
3	13 (77)	21 (51)	
Unknown	0 (0)	0 (0)	
Necrosis			
Present	7 (41)	11 (28)	0.31
Absent	10 (59)	29 (72)	
Lymphatic invasion			
Present	8 (47)	18 (45)	0.89
Absent	9 (53)	22 (55)	
Lymphocytic infiltration			
None	0 (0)	3 (9)	0.34
1+	4 (27)	8 (23)	
2+	8 (53)	11 (32)	
3+	3 (20)	12 (35)	

[a] Mutation negative, not heterozygous for *BRCA1* 187delAG or 5385insC or for *BRCA2* 6174delT.

Histopathology. Infiltrating ductal carcinoma predominated in women with and without founder mutations. An increased frequency of medullary or atypical medullary carcinoma was not noted among women with mutations. All 17 BABCs were of histological grade III, compared to 26 of 41 (63%) of cases in women without mutations ($P = 0.04$; Table 3). However, there were no significant differences between the two groups in nuclear grade, the presence of necrosis, or the presence of lymphatic invasion. An associated *in situ* component was noted less frequently among cases in women with germ-line *BRCA* mutations (59 *versus* 76% in those without mutations), but the difference was not statistically significant.

Immunohistochemistry. The immunophenotypes of BABCs and those arising in women without founder mutations are contrasted in Table 4. A minority of *BRCA*-associated cases expressed estrogen receptors (6 of 17, 35%) or progesterone receptors (6 of 17, 35%). Estrogen receptor expression was significantly more common among cases without founder mutations (31 of 41, 75%; $P = 0.004$). *BRCA*-associated cases were also significantly less likely to express the HER2/*neu* receptor [2 of 16 (13%) *versus* 17 of 41 (42%); $P = 0.04$]. There were no significant differences in the frequency of expression of EGFR, cathepsin D, or bcl-2.

The majority of evaluable *BRCA*-associated cancers manifested a

Table 4 *Proportion of cases expressing immunohistochemical markers*

	Mutation positive (%)	Mutation negative[a] (%)	*P*
ER[b]	6/17 (35)	31/41 (75)	0.004
PR[c]	6/17 (35)	23/40 (57)	0.25
High Ki-67	10/12 (83)	17/35 (48)	0.09
Cyclin D	6/12 (50)	15/32 (47)	0.85
p27	15/16 (94)	33/40 (83)	0.28
p53	9/17 (53)	14/40 (35)	0.21
HER2/*neu*	2/16 (13)	17/41 (42)	0.04
EGFR	3/17 (18)	2/41 (5)	0.11
Cathepsin D	6/17 (35)	15/40 (38)	0.87
bcl-2	9/16 (56)	31/40 (78)	0.11

[a] Mutation negative, not heterozygous for *BRCA1* 187delAG or 5385insC or for *BRCA2* 6174delT.
[b] ER, estrogen receptor.
[c] PR, progesterone receptor.

high Ki-67 labeling index (10 of 12, 83%). Ki-67 staining could not be assessed in five cases. Among evaluable cases without germ-line *BRCA* founder mutations, only 17 of 35 (48%) demonstrated a high Ki-67 labeling index. The difference between the two groups approached but did not reach statistical significance ($P = 0.09$). There were no differences between the two groups in the frequency of positive staining for p27 (94% in BABCs *versus* 83% in breast cancers without detected mutations), p53 (53 *versus* 35%), or cyclin D (50 *versus* 47%).

DISCUSSION

The present study compares the histopathological and immunohistochemical features of breast cancers arising in young Jewish women with germ-line founder mutations of *BRCA1* or *BRCA2* to that of simultaneously ascertained women without such mutations. Because the cases were selected solely on the basis of age and ethnicity,without regard for family history, the study cohort is likely to be representative of women with early-onset breast cancer. The cohort is a subset of a group of women with early-onset breast cancer evaluated at a single cancer center. The fact that these women were recruited from a tertiary care population raises the possibility that a referral bias may result in an excess of poor prognostic features among the study cohort. However, the distribution of important negative prognostic factors in the group as a whole is comparable to that reported in previous series of breast cancer in younger women (Refs. 21–23; Table 5). There are no data as yet suggesting that the clinical features or outcomes of breast cancer arising in Jewish individuals are distinct from those occurring in other ethnic groups. Thus, there is no obvious bias that would impede the generalization of these results to other cohorts of early-onset breast cancer.

Several series have suggested a relationship between young age at diagnosis of breast cancer and certain histopathological variables. When compared to tumors from older women, breast cancers from younger women more frequently lack hormone receptors (21, 23, 24), are of higher histological grade, and more often show lymphovascular invasion and necrosis (22, 23). Abnormal p53 expression, a high S-phase fraction (21), and overexpression of HER2/*neu* (24) have also been reported to be more common among younger women. Compared to early-onset breast cancers arising in women without detectable *BRCA* mutations, *BRCA*-associated tumors are even more likely to be poorly differentiated, to be hormone receptor negative, and to have an increased proliferative rate. The increased proliferative potential can be demonstrated through the histological assessment of mitotic index (8, 9, 11) or the flow cytometric analysis of the S-phase fraction (9, 10).

As in previous reports (6–11), *BRCA*-associated tumors in the current series were more poorly differentiated than those from women without germ-line mutations. Tumors from *BRCA* heterozygotes were uniformly of histological grade III and were usually estrogen receptor negative. The increased proliferative capacity of *BRCA*-associated tumors was demonstrated by Ki-67 antigen staining. This difference is not explained by deregulation of the products of the cell cycle-regulatory genes *p53*, *p27*, or cyclin D, because the frequency of abnormal expression of these genes did not differ between *BRCA*-associated cases and those without mutations. There were also no differences in the expression of the product of the antiapoptotic gene *bcl-2* or in the expression of the transmembrane receptor for epidermal growth factor. Overexpression of the HER2/*neu* oncogene was less common in *BRCA*-associated cases than among cases without mutations.

Although expression of *BRCA1* and *BRCA2* was not examined in this study, BRCA function is presumed to be absent in tumors arising in women with germ-line mutations. *BRCA1* and *BRCA2* are proposed to function as classic tumor suppressor genes. According to this model, the genetic predisposition to breast and ovarian cancer is expressed only after complete loss of BRCA function, most commonly by mutation of the remaining wild-type allele. Indeed, loss of the wild-type allele has been demonstrated in the overwhelming majority of breast and ovarian cancers from BRCA1-linked kindreds (25, 26). A similar pathway is likely to lead to the loss of BRCA2 function in women with germ-line mutations of *BRCA2*.

Loss of BRCA function may not only predispose to malignancy but may influence the expression of the malignant phenotype. This is consistent with *in vitro* experiments demonstrating acceleration of growth of cultured normal and malignant mammary epithelial cells on antisense inhibition of *BRCA1* (27) and retardation of the growth and tumorigenicity of mammary tumor cell lines on transfection with *BRCA1* (28). However, analysis of other genetic pathways involved in

Table 5 *Clinical features of early-onset breast cancer*

Series	Age (yr)	T[a] < 2 cm	T > 5 cm	Node positive	4+ nodes	HG III[b]	ER negative[c]
Albain *et al.* (Ref. 21)	<30	38%[d]	38%[e]	NR[f]	73%[e]	NR	NR
	30–35	45%[d]	23%[e]		53%[e]		
	35–40	49%[d]	27%[e]		55%[e]		
	40–45	54%[d]	19%[e]		48%[e]		
Nixon *et al.* (Ref. 23)	<35	57%	NR	32%	5%	47%	74%
	35–50	58%		37%	9%	50%	50%
Fisher *et al.* (Ref. 22)	<40	NR	NR	60%	24%	65%	NR
					46%[e]		
Current study	<43	59%	5%	48%	14%	74%	36%
		80%[d]	11%[e]		28%[e]		

[a] T, tumor stage.
[b] HG III, histological grade III.
[c] ER, estrogen receptor.
[d] Node negative only.
[e] Node positive only.
[f] NR, not reported.

the control of cellular proliferation is required before concluding that loss of BRCA1 or BRCA2 function is sufficient to confer a proliferative advantage. In addition, the consequences of loss of function of BRCA1 and BRCA2 may be distinct. In one report, the mitotic rate of *BRCA2*-associated tumors was not increased when compared to sporadic controls (11), suggesting that cancers from *BRCA2* heterozygotes may not share the increased proliferative rate of *BRCA1*-associated disease. There were too few *BRCA2*-associated cases in the current series to make meaningful comparisons. Larger studies will be required to determine whether there are specific clinical correlations with particular mutations in either *BRCA1* or *BRCA2*.

The current series confirms prior observations that breast cancers arising in women with germ-line *BRCA* mutations are more poorly differentiated and proliferate more rapidly than those in women without such mutations. *BRCA*-associated tumors were less likely than sporadic cancers to express estrogen receptors or HER-2/*neu*. No differences were noted when a variety of other biological parameters were examined. Taken together, these data suggest that the loss of BRCA1 or BRCA2 function may, in itself, be sufficient for a tumor to manifest an increased proliferative rate. Studies of larger numbers of *BRCA* heterozygotes are required to further delineate the biology and clinical course of this subset of breast cancers.

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