Germline BRCA1 Mutations and a Basal Epithelial Phenotype in Breast Cancer

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A basal epithelial phenotype is found in not more than 15% of all invasive breast cancers. Microarray studies have shown that this phenotype is associated with breast cancers that express neither estrogen receptor (ER) nor erbB-2 (HER2/neu) (i.e., ER/erbB-2–negative tumors). The ER/erbB-2–negative phenotype is also found in breast cancers occurring in BRCA1 mutation carriers (i.e., BRCA1-related breast cancers). We tested the hypothesis that BRCA1-related breast cancers are more likely than non–BRCA1/2-related breast cancer to express a basal epithelial phenotype. Among 292 breast cancer specimens previously analyzed for ER, erbB-2, p53, and germline mutations in BRCA1 and BRCA2, we identified 76 that did not overexpress ER or erbB-2. Of the 72 specimens with sufficient material for testing, 40 expressed stratified epithelial cytokeratin 5 and/or 6 (5/6). In univariate analysis, the expression of cytokeratin 5/6 was statistically significantly associated with BRCA1-related breast cancers (odds ratio = 9.0, 95% confidence interval = 1.9 to 43; $P = .002$, two-sided Fisher's exact test). Thus, germline BRCA1 mutations appear to be associated with a distinctive breast cancer phenotype. [J Natl Cancer Inst 2003;95:1482–5]

Recent microarray studies of breast cancer have identified new sub-phenotypes of breast cancer that were not identified by traditional histopathologic methods. From microarray expression studies *(1–3),* the first-level classification of breast cancers separates estrogen receptor (ER)–negative tumors from ER-positive tumors. Further subdivision of the ER-negative group has been attempted. Perou et al. *(1)* suggested that ER-negative tumors can be divided into groups that do and do not overexpress erbB-2 (HER2/neu). van't Veer et al. *(2)* used ER-negative status as a starting point to define an expression profile for breast cancers in women carrying germline BRCA1 mutations because such tumors are usually ER-negative *(4).* A notable feature of tumors in the ER-negative and erbB-2–negative (i.e., ER/erbB-2–negative) subgroup, as defined by Perou et al. *(1),* was the expression of certain cytokeratins that indicated a basal differentiation for these breast tumors. A basal epithelial phenotype (referred to as basal-like or basaloid) was found in 15% of the breast cancers studied.

Breast cancers can be broadly divided into those that express luminal keratins or the so-called simple epithelial-type keratins (such as cytokeratins 7, 8, 18, and 19) and those that express high levels of the stratified epithelial cytokeratins (such as cytokeratins 5, 6, 14, 15, and 17), which are characteristic of the basal epithelial cells of the normal mammary gland. Other markers, such as smooth muscle actin, glial fibrillary acidic protein, and calponin may also be present in basal-like breast cancers *(5–7),* which account for between 3% and 15% of all invasive ductal breast cancers of no special type. Conventional histopathologic and molecular studies of breast cancers with basaloid/myoepithelial cell differentiation patterns have shown that these tumors are often high-grade *(6),* have areas of necrosis *(8),* may have a typical *(7,9)* or an atypical *(7)* medullary phenotype, and have a distinct pattern of genetic alterations *(6),* including frequent TP53 mutations *(10).* Most *(10–13)* but not all *(9)* studies of outcome have also indicated that basal-like breast cancers often have a poor prognosis.

These features are similar to those observed in breast cancers arising in BRCA1 mutation carriers (hereafter BRCA1 carriers) *(4,14).* The purpose of this study was to determine whether the ER/erbB-2–negative basaloid phenotype of breast cancers was, in part, associated with the presence of germline BRCA1 mutations (BRCA1-related breast cancers). Moreover, identification of a basal phenotype for BRCA1-related breast cancer could help identify the cell of origin of these breast cancers.

We studied 292 specimens of first primary invasive breast cancers diagnosed in Ashkenazi Jewish women younger than 65 years at the Sir Mortimer B. Davis-Jewish General Hospital, McGill University, in Montreal between January 1, 1980, and November 1, 1995. The study used an anonymized design whereby the mutation results were separated from any personal identifiers in a manner approved by the Hospital Research Ethics Review Board. All cases were initially classified histopathologically by one pathologist (L. R. Bégin), and samples were immunostained for ER, erbB-2, and p53 proteins. Molecular testing for the three founder mutations, which account for 95% of all germline BRCA1 and BRCA2 mutations in this population, was also performed. In this series, we identified 31 BRCA1 carriers and 10 BRCA2 carriers, as described previously *(15–17).* Of the 292 patients, we excluded all 10 BRCA2 carriers because specimens of all but one were positive for ER and/or erbB-2. An additional 14 patients, including two BRCA1 carriers, were excluded because of missing ER and/or erbB-2 data. Of the remaining 268 patients, 29 had BRCA1 mutations (11%), 96 were ER negative (36%), and 238 (89%) did not overexpress erbB-2 as determined by immunohistochemical staining. ER status and erbB-2 status were negatively associated in this series (odds ratio [OR] = 0.23, 95% confidence interval [CI] = 0.10 to 0.51; $P<.001$), so only 76 cases (28% of those with complete data) were found to lack staining for both ER and erbB-2, indicating that they might

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be part of the group of basal-like breast cancers. There were 17 BRCA1 carriers and 55 patients without a BRCA1 or BRCA2 mutation among the 72 patients with sufficient tissue available for testing. We immunostained these tumors with a mouse anti-human cytokeratin 5/6 monoclonal antibody (clone D5/16 B4; product M 7237; DakoCytomation, Oslo, Norway) (Fig. 1). We defined a staining index (values = 0–9) as the product of staining intensity (values = 0–3) and the proportion of immunopositive tumor cells (<10% = 1; 10%–50% = 2; >50% = 3). Specimens with staining indices of 1–9 were defined as positive; those with a staining index of 0 were defined as negative.

Cytokeratin 5/6 was detected immunohistochemically in 40 (56%) of the 72 ER/erbB-2–negative breast cancers. The presence of cytokeratin 5/6 (as indicated by a positive index) was statistically significantly associated with high-grade, lymph node–negative tumors and the expression of p53 (Table 1). Among the 55 non–BRCA1/2-related breast cancers with an ER/erbB-2–negative phenotype, 25 (45%) expressed cytokeratin 5/6. Of the 17 specimens from BRCA1 carriers with an ER/erbB-2–negative phenotype, 15 (88%) expressed cytokeratin 5/6 (Fig. 1, A), indicating that the ER/erbB-2–negative phenotype in BRCA1-related breast cancers was statistically significantly associated with the expression of cytokeratin 5/6 (OR = 9.0, 95% CI = 1.9 to 43; P = .002) (Table 1). These results support the hypothesis that the ER/erbB-2–negative basaloid phenotype is associated with BRCA1-related breast cancers. The results obtained with respect to histologic grade, mitotic count (data not shown), and p53 status are consistent with previous reports *(10,12)*. Recently, we showed *(19)* that 68 (25%) of 268 specimens in this breast cancer series expressed p53 by immunohistochemistry. Of the 15 ER/erbB-2–negative, cytokeratin 5/6-positive specimens from BRCA1 carriers, 10 (67%) expressed p53. This finding is consistent with previous studies of tumors with the ER/erbB-2–negative, cytokeratin-5/6–positive phenotype *(10)*.

A previous study *(20)* suggested that, among those diagnosed with breast cancer at age 35 years or younger, an ER-negative status and a histologic tumor grade of 3 could efficiently select candidates for BRCA1 mutation analysis. In our series of 292 tumors from Ashkenazi Jewish women with breast cancer, only five of 31 (16%) BRCA1 carriers were diagnosed in this age group. However, germline BRCA1 mutations accounted for 15 (38%) of 40 ER/erbB-2–negative tumors that expressed basal keratins. Sixteen BRCA1-related tumors were found among the remaining 252 specimens (two-sided χ^2 test, P<.001). The presence of a BRCA1 mutation has been associated with high-grade breast cancers that express p53 *(4)*. To determine whether cytokeratin 5/6 and/or p53 expression were independent strong predictors of BRCA1 mutation status, we performed a multivariable analysis among the 72 patients with ER/erbB-2–negative breast cancer, with the following variables: tumor type (ductal/others), tumor size (<2 cm, ⩾2 cm), histologic grade (defined continuously), lymph node positivity (yes/no), p53 expression (yes/no), and cytokeratin expression (yes/no). In the final model, we included only those variables that were statistically significant predictors of BRCA1 mutation status in univariate analysis (i.e., histologic grade and p53 and cytokeratin 5/6 status). In this model, expression of p53 was not statistically significantly associated with BRCA1-related breast cancers (OR = 1.61, 95% CI = 0.60 to 4.30; P = .34), whereas the presence of cytokeratin 5/6 was statistically significantly associated with BRCA1-related

Table 1. Basal keratin expression in 72 estrogen receptor (ER)/erbB-2–negative breast cancers*

Variable	Cytokeratin 5/6 index: Negative (index = 0)	Cytokeratin 5/6 index: Positive (index = 1–9)	OR (95% CI)	*P*
Tumor type, No. (%)				
Ductal	25 (42)	35 (58)	—	—
Others	6 (54)	5 (46)	0.59 (0.16 to 2.2)	.51
Tumor size, No. (%)				
<2 cm	16 (52)	15 (48)	—	—
⩾2 cm	13 (34)	25 (66)	2.1 (0.78 to 5.4)	.22
Histologic grade, No. (%)				
1	13 (76)	3 (24)	—	—
2	12 (48)	13 (52)	4.7 (1.1 to 2.1)	.050
3	7 (23)	24 (67)	14.9 (3.2 to 67)	<.001
Lymph node status, No. (%)				
Negative	11 (29)	27 (71)	—	—
Positive	17 (61)	11 (39)	0.27 (0.09 to 0.74)	.013
p53 status, No. (%)				
Negative	25 (46)	20 (64)	—	—
Positive	7 (28)	20 (72)	3.6 (1.3 to 10)	.016
BRCA1 mutation, No. (%)				
No	30 (55)	25 (45)	—	—
Yes	2 (12)	15 (88)	9.0 (1.9 to 43)	.002

*Mantel–Haenszel odds ratios (ORs) and confidence intervals (CIs) were calculated. — = referent. *P* values were derived from two-sided Fisher's exact tests. Histologic grade was evaluated by the Nottingham criteria *(18)*.

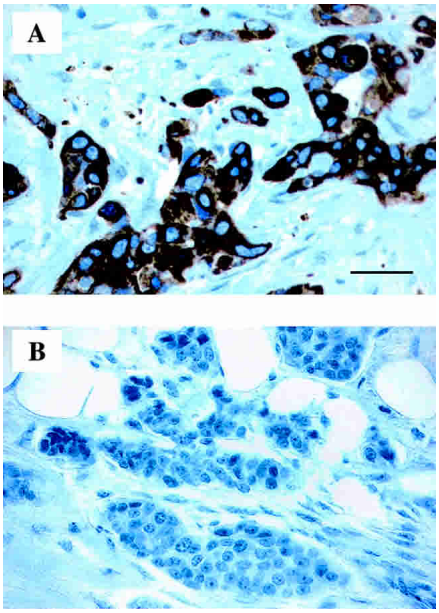

Fig. 1. Breast cancer specimens and cytokeratin 5/6. Sections (5 μm) of formalin-fixed, paraffin-embedded tumor tissue were stained with a mouse cytokeratin 5/6 monoclonal antibody after antigen retrieval by microwave treatment (750 W for 15 minutes and then 500 W for three 5-minute periods). Slides were incubated with cytokeratin 5/6 antibody at room temperature for 25 minutes (dilution 1 : 25); bound antibodies were detected by the avidin–biotin method and processed on DAKO TechMate 500 equipment. **A**) Strong uniform staining for cytokeratin 5/6 in a BRCA1-related invasive ductal breast cancer. **Scale bar** = 35 μm. **B**) Completely negative staining in a non–BRCA1/2-related breast carcinoma. Tumors in **panels A** and **B** expressed p53 (data not shown).

breast cancers (OR = 5.7, 95% CI = 1.07 to 30.5; $P = .04$). Because the BRCA1 gene may also be inactivated somatically *(21),* it will be interesting to determine whether the presence of cytokeratin 5/6 is associated with the loss of BRCA1 protein of either germline or somatic origin.

Areas of necrosis are more likely to be observed in BRCA1-related breast cancers than in sporadic breast cancers *(22,23).* These acellular regions have been observed in high-grade, basal keratin–expressing breast cancers that were prone to metastasize to the lung and brain *(8).* Consequently, it should be determined whether the basal epithelial phenotype of BRCA1-related breast cancers is associated with the poor prognosis that we *(24)* and others *(25–27)* observed for BRCA1 carriers with breast cancer. Interestingly, seven of the 72 tumors in this cohort had an atypical medullary phenotype, and four of these seven occurred in BRCA1 carriers. Thus, the relationship of cytokeratins, atypical medullary breast cancer, and outcome should be further investigated in prospective studies.

Because the cytokeratin profile of breast cancer tumors may not change over time *(28),* the positive cytokeratin 5/6 profile that we observed in BRCA1-related breast cancers is likely to be present *ab initio.* Furthermore, it has been proposed that breast stem cells in rodents *(29)* and humans *(28)* have a cytokeratin 5/6–positive profile. The patterns of genetic alterations identified in cytokeratin 5/6–positive breast cancers (low-level expression of BCL2, $p21^{Cip1}$, $p27^{Kip1}$, ER, progesterone receptor, and erbB-2, combined with high-level expression of Ki-67, epidermal growth factor receptor, and p53) *(30)* are similar to those observed in the BRCA1-related breast cancers, as described above and elsewhere *(4,17,22,31).* The interrelationships between the function of BRCA1 in breast stem cells, in normal breast development, and in breast cancer deserve further consideration. For example, the cleared mammary fat pad model *(29)* could be used to analyze the behavior of single murine breast cells carrying conditionally regulated alleles of Brca1. This system would enable detailed investigation of the role of both wild-type and mutated BRCA1 in breast development and breast cancer.

Note added in proof. Our conclusion that most BRCA1-related breast cancers show a basal-like phenotype is supported by the recent publication by Sørlie et al. *(32),* where all 18 BRCA1-related breast cancers had a gene expression profile consistent with a basal-like phenotype. Interestingly, two of the tumors were estrogen receptor–positive. Two BRCA2 tumors, which were also studied, had a luminal phenotype.

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