

## CORRESPONDENCE

### Re: Germline BRCA1 Mutations and a Basal Epithelial Phenotype in Breast Cancer

Foulkes et al. (1) reported that the expression of cytokeratin 5/6, indicating a basal epithelial phenotype, was statistically significantly associated with germline BRCA1 mutations in estrogen receptor (ER)- and erbB2-negative invasive breast cancers. It has recently been shown that the epidermal growth factor receptor (EGFR) is often expressed in basal-type ("stem") cells of the breast (2), extending the phenotype of basal cells to ER<sup>-</sup>/erbB2<sup>-</sup>/EGFR<sup>+</sup> breast cells. We examined the expression of EGFR by immunohistochemistry in the invasive breast cancers from 21 proven carriers of BRCA1 germline mutations and five proven carriers of BRCA2 germline mutations, as well as from a control group of 430 invasive breast cancers from patients unselected for a family history of breast cancer. Only clear membrane staining for EGFR was considered as overexpression. Of the 21 BRCA1-related breast cancers, 14 (67%) showed EGFR overexpression, 19 (90%) were ER negative, and 17 (81%) were erbB2 negative. Eleven (52%) of 21 tumors were ER<sup>-</sup>/erbB2<sup>-</sup>/EGFR<sup>+</sup>.

All five (100%) breast cancers in BRCA2 mutation carriers showed EGFR overexpression, four (80%) were ER negative, and three (75%) of four were erbB2 negative (one could not be characterized). Two (50%) of four tumors were ER<sup>-</sup>/erbB2<sup>-</sup>/EGFR<sup>+</sup>. In the control group of 430 tumors, EGFR overexpression was found in only 70 (16%), and only 28 (7%) of 422 tumors were ER<sup>-</sup>/erbB2<sup>-</sup>/EGFR<sup>+</sup>. (Eight tumors could not be characterized for both proteins.) EGFR overexpression was statistically significantly higher in breast cancers in BRCA1 ( $P < .001$ , two-sided Fisher's exact test) and BRCA2 ( $P < .001$ ) mutation carriers than in the control tumors. Also, the full ER<sup>-</sup>/erbB2<sup>-</sup>/EGFR<sup>+</sup> phenotype was statistically signifi-

cantly more frequent in BRCA1/2 mutation carriers ( $P < .001$ ).

The high frequency of EGFR overexpression fits with the poor prognosis for patients with hereditary breast cancer, but the underlying mechanism is yet unclear. Amplification of EGFR seems to be very rare in invasive breast cancer (Buerger H, unpublished data). Further, by comparative genomic hybridization, amplification at the EGFR locus was not observed (3). Genetic alterations in an expression-regulating CA repeat in the first intron of the EGFR gene (4) have not yet been studied in hereditary breast cancer. In two gene expression studies in hereditary breast cancer (5,6), increased EGFR mRNA expression was not observed, indicating that EGFR expression is probably largely posttranscriptionally regulated.

We conclude that invasive breast carcinomas in patients with a BRCA1 or BRCA2 germline mutation show a high frequency of EGFR overexpression, compatible with the previously (7) established predominantly basal phenotype (ER<sup>-</sup>/erbB2<sup>-</sup>) of these cancers and their aggressive clinical behavior. Thus, we urge further investigation into the mechanisms of EGFR overexpression and into new preventive strategies through EGFR targeting.

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### NOTES

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Several reports have established that sporadic breast carcinomas with expression of basal cell (myoepithelial cell) markers, such as P-cadherin and high molecular weight cytokeratins CK5/6, CK14, and CK17, show specific morphologic and prognostic characteristics (1,2). In cDNA studies, Perou et al. (3) also defined a subgroup of estrogen receptor (ER)-negative/HER2-negative breast cancers characterized by the expression of the basal cell markers. Recently, Sorlie et al. (4) reanalyzed cDNA microarray data from van't Veer et al. (5) and observed that most carcinomas with a BRCA1 mutation had the gene expression profile of basal type cells. In a recent issue of this Journal, Foulkes et al. (6) reported that the expression of CK5/6 was statistically significantly associated with ER-negative/HER2-negative BRCA1-related cancers (15 [88%] of 17 tumors), when compared with ER-negative/HER2-negative sporadic cancers (25 [45%] of 55 tumors) in Askenazi Jewish women younger

than 65 years. In addition, we have recently reported that the expression of P-cadherin is also most common in BRCA1-related cancers than in familial BRCA2-related and non-BRCA1/2-related cancers (7).

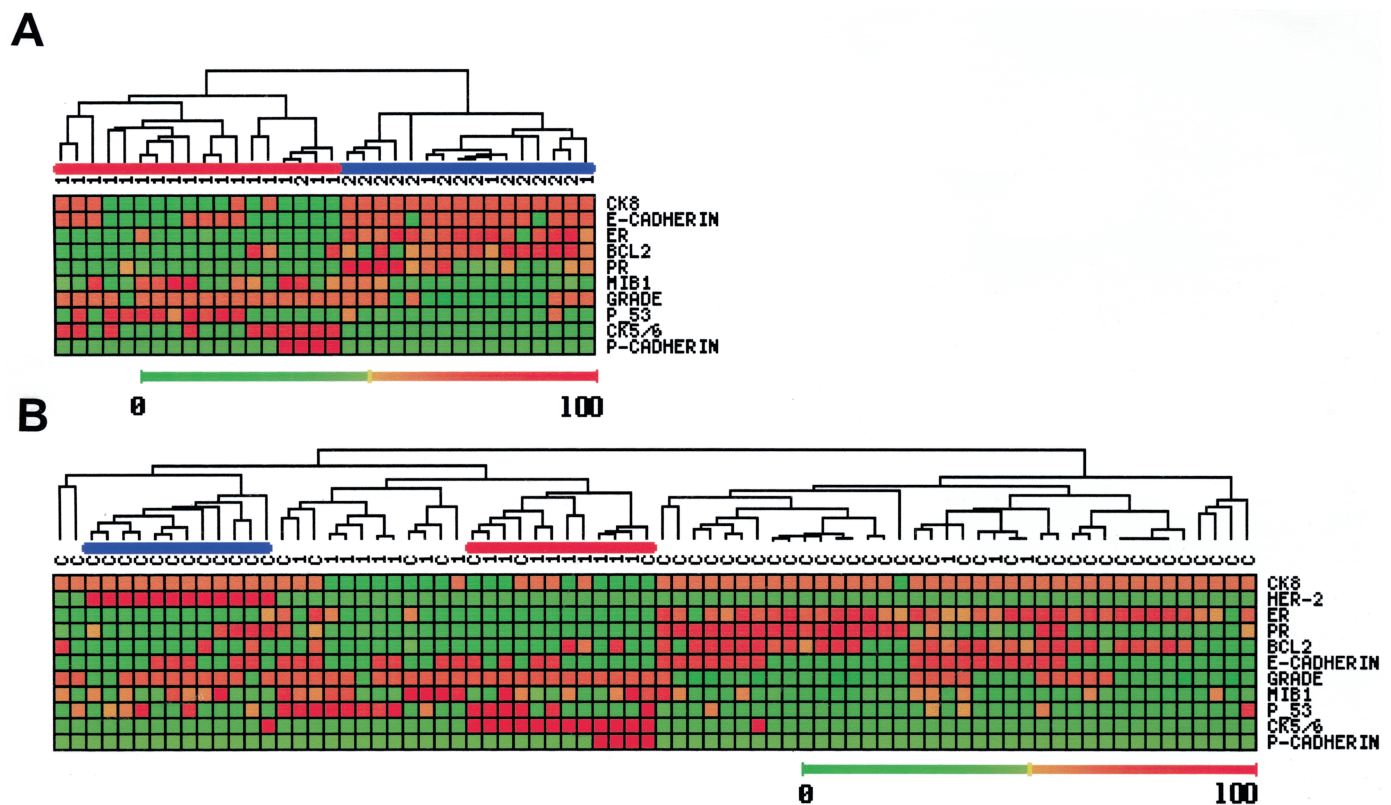
To further test the hypothesis that the basal cell phenotype is BRCA1-related, we analyzed the basal cytokeratin CK5/6 and the ductal cytokeratin CK8 in infiltrating ductal carcinomas (IDCs) from 20 patients with a BRCA1 mutation (mean age = 42 years) and 14 patients with a BRCA2 mutation (mean age = 42.6 years) genes. The morphological and some immunohistochemical features of this series have been recently published (7). In addition, 59 patients with non-familial IDCs (mean age = 42.6 years) were studied, as an age-matched control group. All tumors were included in a tissue microarray and subjected to immunohistochemistry as previously described (7). Positive CK5/6 expression was as reported by Foulkes

et al. (6). Reduced expression of CK8 was recorded when less than 50% of tumor cells expressed this marker.

In our series, CK5/6 was expressed in nine (45%) of 20 BRCA1-related carcinomas, one (7%) of 14 BRCA2-related carcinomas, and five (8%) of 59 sporadic breast carcinomas, respectively ( $P < .001$ ) (Fig. 1). The ER-negative/HER2-negative phenotype was present in 15 (75%), one (7%), and eight (14%) of the BRCA1-related, BRCA2-related, and sporadic breast cancers, respectively ( $P < .001$ ). The complete ER-negative/HER2-negative and CK5/6-positive phenotype was observed in nine (45%), one (7%), and three (5%) BRCA1-related, BRCA2-related, and sporadic breast carcinomas, respectively ( $P < .001$ ). When we considered only cases with an ER-negative/HER2-negative phenotype, CK5/6 was expressed in nine (60%) of 15 BRCA1-related carcinomas and three (37%) of eight sporadic breast carcinomas, although

this difference was not statistically significant, in contrast to the results of Foulkes et al. (6). The smaller number of case patients and the different ethnic population analyzed in our study may explain the discrepancies between series. In this sense, most case patients in the study of Foulkes et al. (6) were probably related to the BRCA1 Ashkenazi Jewish founder mutations, which could be associated with a more homogeneous breast cancer phenotype. In any case, the present study supports the existence of a subtype of high-grade, ER-negative/HER2-negative breast carcinomas with a basal phenotype that occurs more frequently in BRCA1-related than in sporadic carcinomas. This phenotype can also occur, although very infrequently, in BRCA2-related breast cancer.

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**Fig. 1.** A) Differential expression profiles between BRCA1 (column 1) and BRCA2 (column 2) tumors. Using the immunohistochemical expression of the markers shown on the right, the majority of the BRCA1 tumors (red bar) and the majority of the BRCA2 tumors (blue bar) were grouped in distinct clusters. Only the BRCA2-related carcinoma expressing cytokeratin (CK) 5/6 is grouped with BRCA1-related carcinomas. B) Cluster analysis of BRCA1 tumors (column 1) and sporadic breast carcinomas (column c) defines two main branches, one composed of estrogen receptor

(ER)-positive carcinomas and the other one of ER-negative carcinomas. In this later branch, sub-branches containing HER2-positive (blue bar) and CK5/6-positive tumors (red bar) are clearly distinguished. Statistical test and the clustering were implemented in the GEPAS package (<http://gepas.bioinfo.cnio.es>). Red = positive expression; green = negative expression. Intensity of color is a function of immunohistochemical expression level. Grade = histological grade assessed by Nottingham Grading System.

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## RESPONSE

We note with interest that data from Palacios et al. broadly support our orig-

inal observation, and that the data from van der Groep et al. extend our findings to include other basal-associated markers, in this case, epidermal growth factor receptor (EGFR). It is notable that van der Groep et al. found that BRCA2-related breast cancers also tended to share the basal epithelioid phenotype previously identified only in BRCA1-related breast cancers. This result should be interpreted cautiously, particularly because only four tumors were studied and because all four BRCA2 tumors were estrogen receptor (ER)-negative, which is unusual. Most BRCA2-related breast cancers are ER-positive (1). Moreover, the reports of Sørlie et al. (2) and Palacios et al. do not suggest that the basal phenotype of BRCA1-related breast cancer is likely to be extendible to BRCA2-related breast cancer.

The data provided by Palacios et al. suggest that the complete ER/erbB2-negative, cytokeratin (CK) 5/6-positive phenotype may not be quite so discriminatory of a BRCA1 mutation if age is taken into account. However, in light of our findings and those reported by Palacios et al. and van der Groep et al. we suggest that basal markers, such as CK5/6 and its partner CK14, as well as P-cadherin and EGFR, be evaluated further as first-line immunohistochemical tests for the presence of a germ-line BRCA1 mutation. The finding of van der Groep et al. that the BRCA1-related basal phenotype can be extended to include the basal-associated marker EGFR is consistent with the work of Santini et al. (3), who noted that EGFR expression was frequently observed only in breast cancers with a high nuclear grade that also expressed basal epithelial markers (3). In a more recent study, long-term treatment of basal mammary epithelial cells with EGF resulted in a mobile rather than a stationary phenotype (4), possibly indicating a mechanism by which expression of EGFR influences the behavior of breast cancer cells that possess a basal epithelial phenotype.

One question raised by these observations is whether the basal phenotype of BRCA1-related breast cancer reflects the possible cell of origin of

these tumors (5), or instead, the presence of CK5/6 merely represents a particular pattern of differentiation, uncoupled from histogenesis (6). Moreover, whether the CK5/6 intermediate filaments are simply biological bystanders (7) or contributors in their own right to the phenotype of BRCA1-related breast cancer is a key question in determining the true significance of basal cytokeratin staining in hereditary breast cancer.

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