Re: Molecular Basis for Estrogen Receptor α Deficiency in BRCA1-Linked Breast Cancer

We read with great interest the article by Hosey et al. (1), which proposed BRCA1-mediated transcriptional activation of ESR1 in breast cancer cell lines, thus suggesting a direct link between the BRCA1 gene and estrogen response that involves OCT-1. We assessed whether these in vitro results could be clinically confirmed in an independent cohort of primary breast cancer specimens that included 23 sporadic and 27 hereditary breast tumors in which full DNA sequencing and multiplex ligation-dependent probe amplification of the BRCA1 gene detected no mutations. We assessed estrogen receptor (ER) status as previously described (2) and determined the mRNA expression levels of the ESR1, BRCA1, and OCT-1 genes by real-time quantitative polymerase chain reaction (TaqMan gene expression assay identification numbers Hs00174860_m1, Hs00173233_m1, and Hs00231250_m1, respectively; Applied Biosystems, Foster City, CA), using the housekeeping gene 18S ribosomal RNA (4319413E) for normalization.

BRCA1 mRNA levels in the 34 ER- positive (ER+) samples overlapped with those of the 16 ER-negative (ER-) samples (range of log2-transformed BRCA1 expression: −19.8 to −15.95 in ER− and −19.6 to −14.7 in ER+), resulting in a weak association between BRCA1 expression and ER status (ratio of mean BRCA1 expression of ER+ to ER− samples = 1.61, 95% confidence interval [CI] = 0.99 to 2.56, P = .054 [two-sided t test with unequal variances using the log-transformed data]). There was no association between OCT-1 expression and ER status (mean expression ratio = 0.83, 95% CI = 0.58 to 1.19, P = .30). These results were further confirmed in publicly available datasets (3): neither BRCA1 nor OCT-1 expression was associated with ER status in sporadic breast cancers (P < .05 for two of 13 datasets for BRCA1 and for three of 11 datasets for OCT-1).

On average, ER+ samples that had higher BRCA1 expression also had higher ESR1 expression (P = .004, Wald test), consistent with the proposal of Hosey et al. (1). In a series of 19 BRCA1-mutant samples from our institution for which ER status and ESR1 and BRCA1 mRNA expression levels were available, we found four ER+ samples; interestingly, in those four samples, BRCA1 expression was positively associated with ESR1 expression.

To see whether the existence of ER+, BRCA1-mutant cases could be explained by the presence of specific deleterious BRCA1 mutations that disrupt protein function but do not abrogate BRCA1-mediated ESR1 transcription, we analyzed the type and location of the BRCA1 mutation in a series of BRCA1-mutant patients with primary breast cancer who had no personal history of ovarian cancer (including 24 ER+ and 65 ER− BRCA1-mutant patients). We identified 41 different mutations of the BRCA1 gene—20 in the ER+ patients and 31 in the ER− patients—that were distributed along the entire coding sequence, with no evidence of preferential grouping of mutations according to the ER status. Twelve of these mutations were present in both the ER+ and ER− patients, including the 5382insC mutation [the mutation that was present in the HCC1937 cell line that Hosey et al. used for the in vitro experiments (1)], which we detected in three ER+ patients and 12 ER− patients. Finally, we observed that patients who were members of the same family (and thus carried the same BRCA1 mutation) had tumors that differed with respect to ER status, which indicates that the type of BRCA1 mutation alone does not determine the ER status of breast tumors.

Our results suggest that ESR1 expression levels might be influenced by genes other than BRCA1 and OCT-1 and/or by mechanisms (4–6) other than those proposed by Hosey et al. (1).
We thank Lusa et al. for their interest in our study and would like to address some of their findings. They provide data on the correlation between BRCA1 mRNA levels and estrogen receptor (ER) status as assessed by immunohistochemistry (IHC). Tumor sections were scored as ER positive if at least 10% of the tumor cells were labeled (1). They found that tumor expression of BRCA1 mRNA was weakly associated with positive ER status. IHC is not an intrinsically quantitative measure for the actual ER tumor content; therefore, we think that to obtain a more robust dataset it is necessary to quantitatively measure ER mRNA and/or protein expression levels for comparison to the measured BRCA1 mRNA levels.

Using real-time quantitative polymerase chain reaction (qPCR), Roldan et al. (2) found a positive correlation between the levels of BRCA1 and ESR1 mRNA expression in 40 sporadic breast cancers (Spearmann $\rho = 0.75$, 95% confidence interval = 0.57 to 0.86; $P < .0001$). In addition, we have carried out a retrospective analysis in an independent cohort of 61 sporadic breast cancers to examine the relationship between levels of BRCA1 and ESR1 mRNA expression. In agreement with the findings of Roldan et al., our qPCR analysis revealed a statistically significant positive association between the levels of BRCA1 mRNA and ESR1 mRNA in these tumors ($P < .002$) (Figure 1). Other studies (3–5) have shown that reduced expression of BRCA1 (by mechanisms that include epigenetic silencing) in sporadic cancers is associated with ER negativity. It is interesting that when Lusa et al. analyzed ESR1 mRNA levels in the ER-positive samples (34 of the 50 breast tumors), they found that tumors with higher BRCA1 mRNA expression levels also had higher levels of ESR1 mRNA expression, which is consistent with our proposal (6) and the findings reported here (Figure 1).

Lusa et al. also examined whether specific BRCA1 gene mutations are associated with ER-positive status in a series of BRCA1-mutant tumors, of which 24 were classified as ER positive and 65 as ER negative by IHC. They found no preferential grouping of mutations according to ER status. However, they noted that of the 15 patients who carried the 5382insC mutation, 12 were ER negative and three were ER positive. This interesting finding suggests that ER-positive status in BRCA1-mutant tumors may be influenced by factors in addition to the BRCA1 gene mutation status and is in agreement with the published literature, which consistently identifies a subpopulation of BRCA1-mutant tumors that are ER positive. A concern remains, however, regarding the manner in which the studies by Lusa et al. were performed, particularly the manner in which ER positivity was determined by IHC.

We agree with Lusa et al. that mechanisms that do not involve BRCA1 expression are also likely to influence ER expression in breast tumors. Other factors that regulate ER expression may act independently of BRCA1, synergize with BRCA1, or antagonize the effect of BRCA1. Our study has illustrated one of the mechanisms of ER regulation and has brought us closer to understanding the frequently observed phenomenon of reduced ER expression in breast tumors that have lost BRCA1 function.

References

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