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The hereditary breast cancer susceptibility genes, BRCA1 and BRCA2, are likely to be tumor suppressor genes. It is through their loss of function that these genes confer a high-risk for developing the disease. Among a relatively large number of non-hereditary breast cancers that have been examined, BRCA1 and 2 mutations are very rare. Therefore, the conclusion has been made that these genes do not play a significant role in the more common sporadic forms of the disease. There are, however, other mechanisms besides mutations that can serve to inactivate the function of a gene. A common one is loss of expression due to hypermethylation of the associated CpG island. In this study, Dr. Magee measured the levels of BRCA1 and BRCA2 mRNA in an unselected set of 101 primary breast cancers and normal breast epithelial specimens. Dr. Magee found that these levels varied over a 20 fold range after normalization. In specimens that had very low levels of one or the other gene, the methylation status was examined to determine if a correlation existed between expression and hypermethylation. No correlation was found suggesting implying that the low levels of BRCA1 and 2 found in a subset of cancers is not due to aberrant methylation.

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Kendra Magee Meirose 7/28/99

PI - Signature Date

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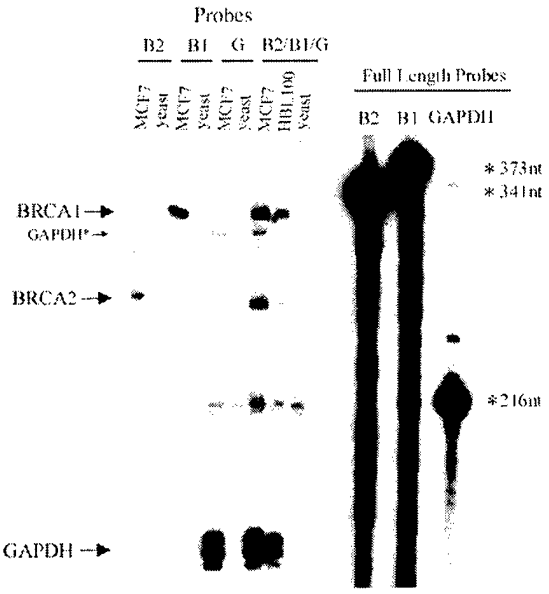
Introduction:

It has already been clearly established that BRCA1 and BRCA2 mutations are exceedingly rare in sporadic breast cancers, and when present, they are almost invariably germ-line mutations occurring outside of a recognized cancer family syndrome. However, there are other mechanisms by which tumor suppressor genes can be inactivated. In particular, an increasingly recognized type of inactivation occurs via transcriptional silencing (frequently through hypermethylation of the promoter region). This proposal was designed to test whether expression of BRCA1 and/or 2 was diminished in primary breast cancers and whether this was related to hypermethylation.

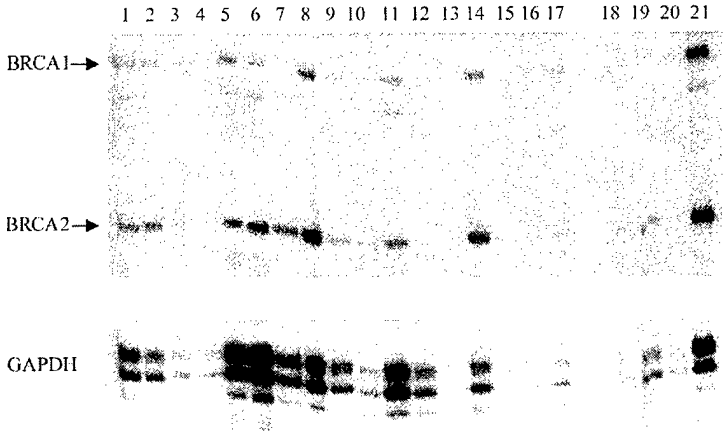
Body:

We have measured the expression (by Rnase protection assays) of both BRCA1 and BRCA2 in a series of 101 primary breast sporadic breast cancers and a series of normal breast epithelial specimens. Among these specimens were 86 invasive ductal cancers, 4 lobular invasive, 1 medullary, and 10 ductal carcinoma in-situ (DCIS). Specimens were examined for percent tumor content and, if greater than 50% of the tissue was composed of malignant cells, RNA was extracted from the tissues by the Trizol method. RNA was quantitated by spectrophotometry and hybridized to a mixture of ^{32}P end-labeled single stranded RNA probes specific for RBCA1, BRCA2, and GAPDH (for normalization). After hybridization, single-stranded regions were digested with RNase and the products were electrophoresed on 7% sequencing gels. The gels were dried and bands intensities were quantitated by phosphorimage analysis. This RNase protection assay approach allowed us to quantitate and normalize the levels of BRCA1 and 2 using a single aliquot of RNA and one lane of a sequencing gel. A test set of samples is shown below that validates band assignment as BRCA1, BRCA2, and GAPDH.



Full-length probes are 373, 341, and 216 nucleotides (BRCA1, BRCA2, and GAPDH respectively). Protected probes are indicated by arrows on the left. GAPDH reproducibly yielded an additional band (GAPDH*) both in the full length transcription product and protected products.

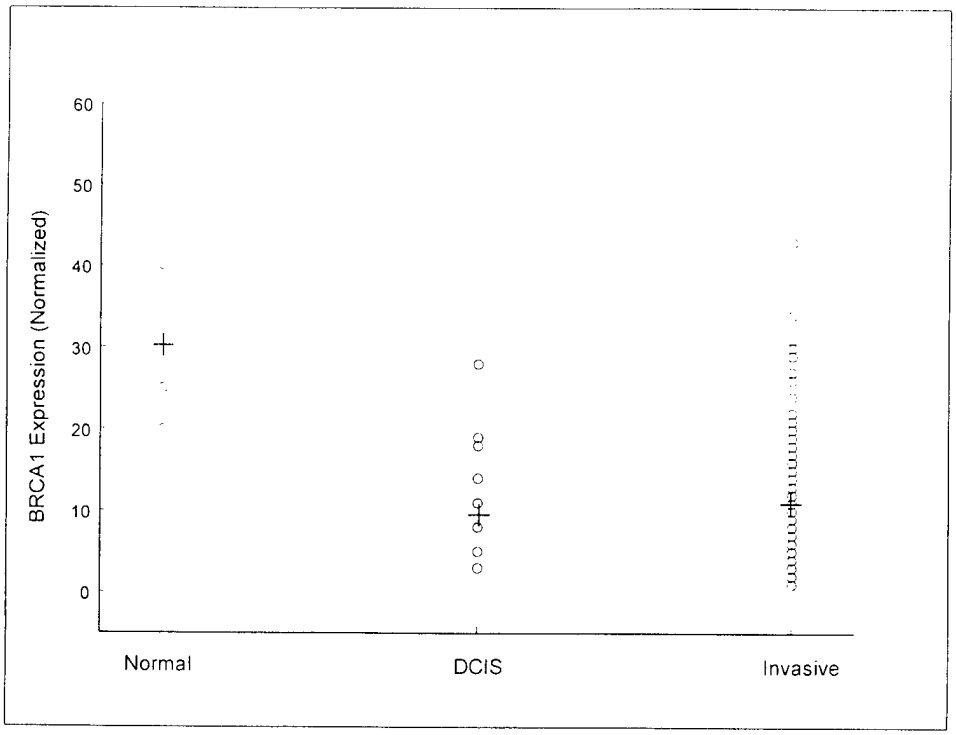
All samples (101 cancers and 4 normal breast epithelial preparations from reduction mammoplasties) that contained sufficient malignant cell content were hybridized as above using the three probes together. A representative gel is shown below.

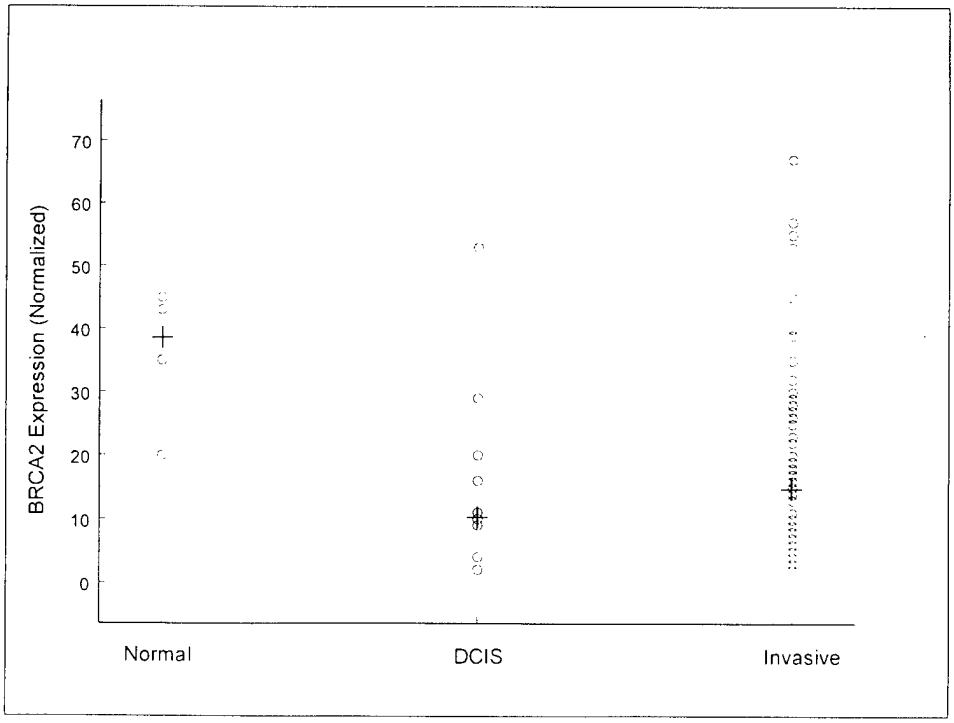


Since our lab has previously found that both genes are cell cycle regulated genes that are induced late in G1, we expected that if one of these genes was transcriptionally silenced, it would show up in the ratio of expression between BRCA1 and 2. This approach should eliminate the variability in expression due to the proliferation state of the cancer. Of the 101 cancers analyzed in this manner, we found 5 specimens that had 5 fold (or greater) more BRCA2 transcript that BRCA1 (lane 7 above is one such example where BRCA1 expression was much lower than BRCA2 mRNA). None of the cancers reached this level in reverse, *i.e.*, had 5 fold more BRCA1 than 2.

I specifically examined the CpG island upstream of the BRCA1 gene for methylation differences between cancers that had relatively high levels and those that had diminished relative levels of BRCA1. This was accomplished by methylation sensitive restriction digests (MspI vs. HpaII) followed by Southern blotting of the cut genomic DNA. There were no differences in the restriction pattern between high and low expressors indicating that methylation was likely not a factor in the differential expression observed.

An early report on BRCA1 expression suggested that expression was elevated in DCIS but reduced in invasive cancers. In our series, I observed no significant difference in the levels of BRCA1 or 2 between intraductal and invasive cancers. This is graphically represented on the scatter plots on the next page. The mean values for both BRCA1 and 2 were slightly lower in the DCIS compared to invasive cancers. The normal breast epithelial samples did have a slightly higher mean than either the DCIS or invasive samples. In collaboration with Gloria Broadwater (Cancer Center Biostatistics), I will have a complete report on the relationship between expression and clinico-pathologic variables. This will be completed in the next several weeks at which point I will begin preparing the manuscript for peer-reviewed publication. The manuscript will detail the above outlined data and include all relevant statistical analyses.


BRCA1 Expression (Normalized)
60
50
40
30
20
10
0
Normal
DCIS
Invasive


BRCA2 Expression (Normalized)
70
60
50
40
30
20
10
0
Normal
DCIS
Invasive

Key Research Accomplishments

- Developed an Rnase protection assay to simultaneously quantitate BRCA1 and BRCA2 expression from primary tissue specimens
- Measured the expression of BRCA1 and BRCA2 in 101 primary breast cancers and normalized expression to GAPDH
- Collected clinico-pathologic information associated with the breast cancer specimens
- In collaboration with a bio-statistician, examined correlations between BRCA1 and 2 expression and other parameters
- Examined the methylation status of cancers that underexpress BRCA1 in relation to those tumors that have "normal" levels of the mRNA
- Manuscript preparation of the above data is in progress