

PREFERENTIAL ALLELIC EXPRESSION CAN LEAD TO REDUCED EXPRESSION OF *BRCA1* IN SPORADIC BREAST CANCERS

Hilmi ÖZÇELİK^{1,2,3}, Minh D.To^{1,4}, Jean COUTURE^{1,5}, Shelley B. BULL^{1,6} and Irene L. ANDRULIS^{1,2,3,4,7*}

¹Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada

²Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Canada

³Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada

⁴Department of Molecular and Medical Genetics, University of Toronto, Toronto, Canada

⁵Department of Surgery, Mount Sinai Hospital, Toronto, Canada

⁶Department of Public Health Sciences, University of Toronto, Toronto, Canada

⁷Cancer Care Ontario, Toronto, Canada

BRCA1 is considered to be a tumor-suppressor gene, yet mutations in this gene are uncommon in sporadic breast tumors. We investigated whether mechanisms other than DNA mutations that affect the coding region might be involved in breast carcinogenesis. Since loss of expression of the *BRCA1* gene would lead to lack of protein, we evaluated the level of *BRCA1* mRNA in 21 normal epithelial specimens and in 74 breast carcinomas using quantitative reverse-transcription-polymerase-chain-reaction (RT-PCR). All normal breast epithelial samples expressed *BRCA1* mRNA. On the other hand, the tumor specimens exhibited approximately 10-fold range of levels of *BRCA1*, with some specimens expressing barely detectable amounts of *BRCA1* mRNA. The distribution in levels was significantly higher in normal breast epithelial cells than in tumor specimens ($p = 0.004$). Examination of the *BRCA1* locus indicated that deletion of the *BRCA1* gene may account for low levels of *BRCA1* in a number of specimens. In addition, analysis of samples with relatively reduced levels of *BRCA1* expression revealed preferential allele-specific expression in a number of cases, suggesting the presence of regulatory mutations. Our data suggest that the *BRCA1* gene may be involved in sporadic breast carcinogenesis through a reduction in gene expression. *Int. J. Cancer* 77:1–6, 1998.

© 1998 Wiley-Liss, Inc.

The identification of mutations in the breast- and ovarian-cancer-susceptibility gene *BRCA1* that co-segregated with tumors in high-risk families was an early criterion for associating the *BRCA1* gene with cancer predisposition (Castilla *et al.*, 1994; Freidman *et al.*, 1994; Miki *et al.*, 1994; Simard *et al.*, 1994; Shattuck-Eidens *et al.*, 1995). Since breast and ovarian tumors from carriers with germline *BRCA1* mutations were observed to have lost the second wild-type allele (Smith *et al.*, 1992) it has been suggested that *BRCA1* acts as a tumor-suppressor gene. Several studies have shown that loss of heterozygosity (LOH) is a common event at the 17q21 region in the sporadic form of these diseases, as well as in hereditary breast and ovarian cancers (Saito *et al.*, 1993; Cropp *et al.*, 1994), and it has therefore been postulated that *BRCA1* may have an important role not only in hereditary but also in sporadic breast and ovarian cancers.

BRCA1 somatic mutations have been observed in a small number of ovarian tumors (Hosking *et al.*, 1995; Merajver *et al.*, 1995), but not in sporadic breast tumors (Futreal *et al.*, 1994). Nevertheless, it is possible that *BRCA1* plays a role in sporadic breast carcinomas through alterations other than coding-region mutations. One mechanism of aberrant regulation may be through lower levels of *BRCA1*-mRNA expression, as has been observed for breast-adenocarcinoma cells in comparison with normal mammary epithelial cells (Thompson *et al.*, 1995). Inhibition of *BRCA1* expression with anti-sense oligonucleotides indicated that reduced expression of *BRCA1* increased the proliferative rate of benign and of malignant breast epithelial cells (Thompson *et al.*, 1995). These findings suggest that *BRCA1* may play a role in the development of sporadic breast cancer through growth stimulation induced by low levels of *BRCA1* mRNA.

The *BRCA1* gene encodes a 1863-amino-acid polypeptide that has a potential granin motif (Jensen *et al.*, 1996) as well as a possible RING finger motif (Lovering *et al.*, 1993), suggestive of a role in secretory pathway and transcriptional regulation respectively. Chen *et al.* (1995) have reported an aberrant sub-cellular localization of the *BRCA1* protein in breast-cancer cell lines and biopsies. Moreover, the *BRCA1*-protein staining was significantly low in 4% of the breast-cancer biopsies, supporting the hypothesis of reduced *BRCA1* expression in the etiology of sporadic breast cancer.

To investigate the importance of *BRCA1* expression in sporadic breast cancers, we have evaluated the level of expression in 21 normal breast epithelial specimens and in 74 primary tumors from axillary-node-negative (ANN) breast-cancer cases. We found that the levels of *BRCA1* mRNA expression were reduced in some tumor specimens, and we further investigated the possible roles of deletions, preferential allelic expression and regulatory mutations in the low levels of *BRCA1* expression.

MATERIAL AND METHODS

Tissue samples

Axillary-node-negative breast-carcinoma samples were obtained in collaboration with Dr. D. Sutherland (Sunnybrook Steroid-Receptor Laboratory, Toronto) and hospitals in Toronto. Normal tissue used in this study was extracted from mammary-reduction samples and normal tissue adjacent to the tumor. DNA and RNA were extracted from quick-frozen tumor and adjacent histopathologically normal tissue and mammary-reduction samples by conventional methods.

Quantitation of *BRCA1* mRNA levels

cDNA was transcribed from cellular RNA by using random hexamers (Boehringer, Mannheim, Germany) and Moloney-murine-leukemia-virus reverse transcriptase (GIBCO-BRL, Gaithersburg, MD). Quantitative RT-PCR (Noonan *et al.*, 1990) was performed by multiplexing a region of *BRCA1* cDNA in exons 12 and 13 with a region of an internal control gene, porphobilinogen deaminase (*PBGD*) (Finke *et al.*, 1993). Expression of the housekeeping gene, *PBGD*, was evaluated in each reaction and compared with that of *BRCA1* to control for variations in the quality and quantity of RNA obtained from the tumor specimens. Although *PBGD* is located on the long arm of chromosome 11 at q23.3, it does not map to regions

Grant sponsors: Canadian Breast-Cancer Research Initiative/National Cancer Institute of Canada.

*Correspondence to: Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Room 870, Toronto, Ontario, M5G 1X5, Canada. Fax: (416) 586 8663. E-mail: andrulis@mshri.on.ca

Received 10 October 1997; Revised 2 March 1998

found to be amplified (11q13; Kallioniemi *et al.*, 1994) or deleted (11q22-q23.1 and 11q25-qterm; Koreth *et al.*, 1997) in breast tumors. The primers for *BRCA1* (BRCA1-1F, 5'-GGCTATCCTCT-CAGAGTGACATT-3' and BRCA1-4R, 5'-CTGATGTGCTTTGTTC-TGGA-3') and *PBGD* (PBGD-1, 5'-TGTCTGGTAACGGCAAT-GCG-3' and PBGD-2A, 5'-TTGCCACCACACTGTCCGTCT-3') amplify fragments of 201 and 120 bp respectively.

PCR reactions were carried out in a 12- μ l reaction volume containing 1 \times PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.01% gelatin), 1.0 mM MgCl₂, 112.5 mM of each deoxyribonucleoside triphosphate (dNTP), 10 pmol of each *BRCA1* and *PBGD* primers, 1 μ of AmpliTaq DNA polymerase (Perkin Elmer/Cetus, Foster City, CA) and 4 μ l of cDNA reaction containing 50 ng of total RNA. The thermal cycling conditions were 94°C for 30 sec, 55°C for 30 sec and 72°C for 1 min. The PCR products were separated on 10% polyacrylamide gels.

For each sample, PCR was carried out at 3 different cycles in the exponential phase of amplification to ensure quantitative evaluation of the PCR reaction. The band intensities of *BRCA1* and *PBGD* were determined by densitometry and the ratio of *BRCA1*/*PBGD* mRNA expression was averaged over at least 2 cycles in the logarithmic phase of amplification.

Deletion analysis at the *BRCA1* locus

The presence of deletions at the *BRCA1* locus was evaluated by multiplex analysis of the PCR products of the *BRCA1* gene and an internal control gene, asparagine synthetase (*AS*). The *AS* gene serves as a control for DNA copy number, since it maps to chromosome 7q21.3 (Heng *et al.*, 1994), a region that is not commonly amplified or deleted in breast cancers. Although there is a region on the long arm of chromosome 7 (7q31) that is often subject to loss of heterozygosity (LOH) in breast cancers, *AS* is located beyond the region of LOH. Reactions were carried out in a 15- μ l volume containing 1 \times PCR buffer, 1.6 mM MgCl₂, 100 μ M of each dNTPs, 10 pmoles of each *BRCA1* and *AS* primers, 1 μ of AmpliTaq DNA polymerase (Perkin Elmer/Cetus) and 50 ng of genomic DNA. The *BRCA1* (BRCA1-3F, 5'-AGCAGAGGGA-TACCATGC-3' and BRCA1-4R) and *AS* (AS-1, 5'-ACATTGAAG-CACTCCGCGAC-3' and AS-5, 5'-TGCAACTTTGCCATTTG-GCT-3') primers yield fragments of 168 and 96 bp respectively. The cycling conditions and evaluation of the PCR results were carried out as described above. The quality of the genomic DNA samples was assessed by running 50 ng of each sample on 1.2% agarose gels; samples that appeared to have been degraded were excluded from the study.

Allelic expression analysis

The *BRCA1* primers for DNA (BRCA1-3F and BRCA1-4R, 168 bp) and RNA (BRCA1-1F and BRCA1-4R, 201 bp) amplifying the region containing the C-T polymorphism at nucleotide 4427 in the *BRCA1* gene (Miki *et al.*, 1994) were used to carry out a PCR reaction as described above. PCR products (8 μ l) were digested with 5 μ EarI restriction enzyme at 37°C overnight. The DNA (128 and 40 bp) and the RNA (161 bp and 40 bp) products were run on 10% polyacrylamide gels. The intensity of the digested PCR fragments representing each allele were measured by densitometry for DNA and for RNA. PCR reaction and digestion analysis for each RNA and DNA sample were repeated twice, and results were in complete concordance.

Analysis of the promoter mutations

The potential promoter region of the *BRCA1* gene was analyzed by single-strand-conformation polymorphism (SSCP) using 4 sets of primers, BRCA1-P1 (5'-GGCTACCGCTAAGCAGCAGC-3') and BRCA1-P2 (5'-GCGAACTCAGGTAGAAATTCT-3'), BRCA1-3P (5'-CGAAAGGCCTTGCCACACT-3') and BRCA1-4P (5'-GTCTTCCTAGGAGTTGTAGG-3'), BRCA1-5P (5'-GGCCCACTGTCCCTTTCC-3') and BRCA1-6P (5'-TCCCATCC-TCTGATTGTACC-3'), BRCA1-7P (5'-GGATGACGTAAGAG-GAAAGA-3') and BRCA1-8P (5'-CATTAGGGCGAAAGAG-TGG-3') producing 170-, 220-, 262- and 222-bp fragments respectively. The PCR reaction for each primer set was carried out in a 30- μ l volume containing 1 \times PCR buffer, 50 μ M of each dNTPs, 6 pmol of each primers, 2 μ Ci of [³²P]deoxycytidine triphosphate (dCTP; 3000 Ci/mmol, Du Pont, Markham, Canada), 1 μ of AmpliTaq DNA polymerase (Perkin Elmer/Cetus) and 50 ng of genomic DNA. The PCR reaction was performed at 94°C for 40 sec, 57°C for 40 sec and 72°C for 70 sec for 33 cycles. The PCR products were diluted 1:3 with formamide-loading buffer (95% formamide, 2 mM EDTA, pH 8.3, 0.05% bromophenol blue, 0.05% xylene cyanol), loaded on 6% non-denaturing acrylamide (40:1 acrylamide:bisacrylamide) gels in 10% glycerol and 0.5 \times Tris-borate EDTA (TBE) buffer and electrophoresed for 17 hr at 15°C.

RESULTS

Evaluation of *BRCA1* mRNA expression in ANN breast carcinomas

Since the limitation in material is a major concern in studies of human primary-tumor specimens, we developed a quantitative RT-PCR assay for the analysis of *BRCA1* mRNA levels, as described in "Material and Methods". The level of *BRCA1* mRNA was determined in 74 ANN breast carcinomas (Fig. 1). *BRCA1*

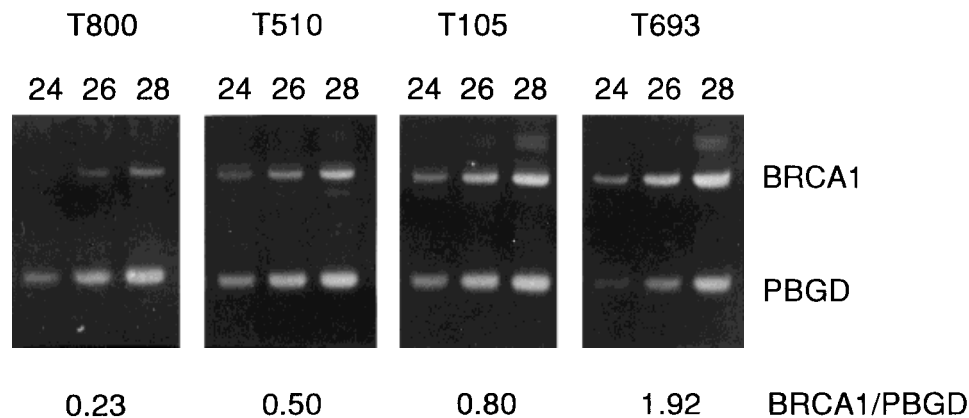


FIGURE 1 – Evaluation of *BRCA1* mRNA expression in ANN breast carcinomas (T800, T510, T105, T693) by quantitative RT-PCR. Each sample was analyzed at 3 different PCR cycles (24–26–28) and the intensities of bands were measured by densitometry. The *BRCA1*/*PBGD* ratio was calculated at each cycle, and only values within the logarithmic phase of PCR amplification were used in determining the average relative expression level as indicated for each sample (see "Results").

mRNA expression varied greatly among tumor specimens (relative units of 0.02 to 2.3), with some tumors exhibiting barely detectable amounts of *BRCA1* mRNA. In contrast, analysis of 21 histopathologically normal breast epithelial samples indicated that normal specimens expressed *BRCA1* mRNA at levels ranging from 0.8 to 2.5 relative units. Comparison of the levels of *BRCA1* expression in the tumor and the normal samples (Fig. 2) revealed that the distribution of *BRCA1*-expression levels exhibited by tumors was significantly lower than the distribution of mRNA levels for the normal tissues (2-sided Wilcoxon-Mann-Whitney test; p value = 0.004).

RNA degradation, which can lead to preferential degradation of larger RNA fragments, can be a concern when analyzing mRNA expression from primary tumors. To control for this variable,

quantitative multiplex PCR assays were also performed in which a *BRCA1* fragment smaller than that of the PBGD internal control was amplified (data not shown). The results of the latter were in complete concordance with those of the first set of experiments, and exclude the presence of RNA degradation as a cause for low levels of *BRCA1* expression observed in some tumors.

Deletion analysis of the BRCA1 gene

To address the possibility that the reduction in *BRCA1* mRNA expression resulted from allelic loss, a sub-group of 47 tumor samples was analyzed for DNA deletion at the *BRCA1* locus, by means of a quantitative PCR assay (Fig. 3). Of the 47 tumor DNAs, 40 (85%) exhibited a ratio of copies of *BRCA1* to asparagine synthetase (AS) above 0.69 (range 0.7–1.2), and were interpreted

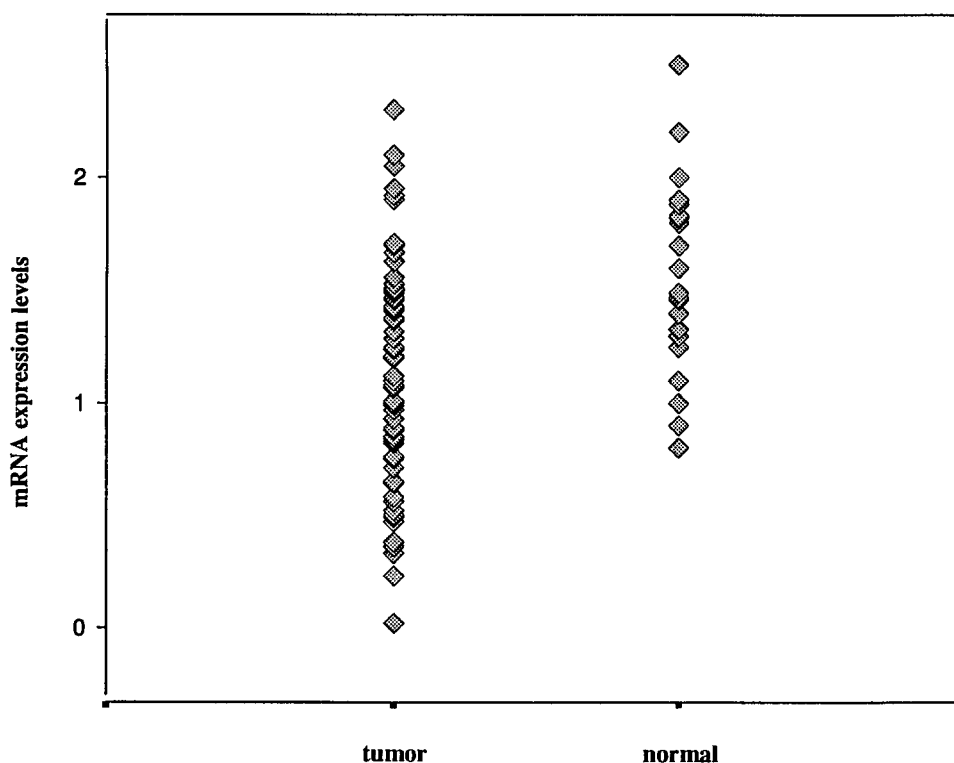


FIGURE 2 – Distribution of relative expression levels of *BRCA1* mRNA expression in tumor ($n = 74$) and normal breast epithelial ($n = 21$) samples. The reduced expression in the tumors was found to be statistically significant ($p = 0.004$ by the Wilcoxon-Mann-Whitney test).

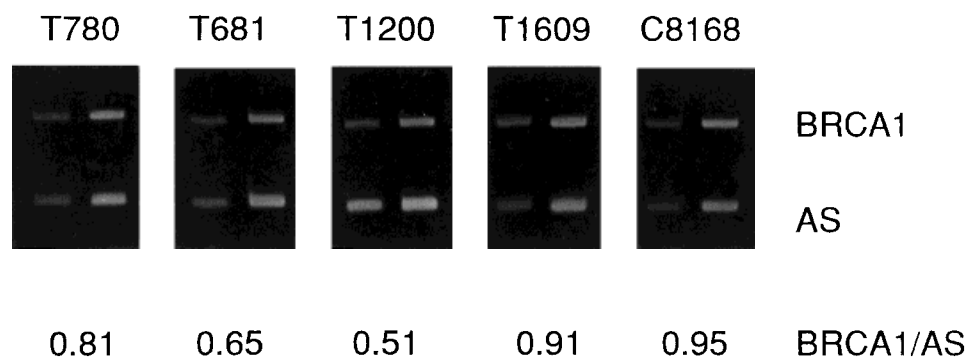


FIGURE 3 – Deletion analysis of *BRCA1* gene by quantitative PCR. For each DNA sample, the *BRCA1*/*AS* ratio was analyzed at 2 different PCR cycles within the logarithmic phase of the PCR reaction. The average ratios of *BRCA1*/*AS* are shown in representative tumor (T780, T681, T1200, T1609) and normal (C8168) samples in the figure. Samples T681 and T1200 had markedly lower *BRCA1*/*AS* ratio than normal samples, indicating the presence of deletions.

as not having deletion at the BRCA1 locus. The mean BRCA1/AS ratio of 0.93 ± 0.12 for these 40 samples was comparable with the value of 0.99 ± 0.3 found for non-cancerous DNA samples. On the other hand, 7 tumors (15%) had a BRCA1/AS ratio below 0.69 (range 0.35–0.65), and were interpreted as having potential small intragenic or large chromosomal deletions at the BRCA1 locus. Examination of the levels of BRCA1 mRNA in the 7 latter specimens (tumors 850, 932, 1200, 800, 617, 681 and 1035 in Table I) indicated that the distribution of levels of BRCA1 mRNA in these specimens with deletions was significantly different from that in the specimens without deletions (2-sided Wilcoxon-Mann-Whitney test; p value = 0.001), in agreement with the hypothesis that allelic deletion may lead to low BRCA1 mRNA levels in some cases. On the other hand, DNA deletion did not account for all cases of low BRCA1 mRNA, suggesting that additional mechanisms contribute to a reduction in BRCA1 expression.

Allelic expression analysis of the BRCA1 gene

We explored the role of regulatory mutations, including those affecting mRNA stability, in decreasing BRCA1 expression. Allelic analysis was performed by taking advantage of a C-T polymorphism at nucleotide 4427 that changes the recognition site of the *EcoRI* restriction enzyme. Tumor-DNA samples (65 in all) representing cases of low and high BRCA1 mRNA levels were analyzed by enzyme digestion of PCR products, and 21 were found to be heterozygous at the *EcoRI* recognition site. Digestion analyses of PCR products from these 21 informative cases were carried out for DNA and for cDNA in order to detect differences in the allelic expression patterns (Fig. 4). The intensities of the bands representing each allele were evaluated by densitometry both for RNA and for DNA samples. In the 21 informative cases, 12 showed equivalent expression of both alleles (Table I). In contrast, preferential allelic expression was observed in 9 samples; 7 of these showed an approximately 2–5-fold, and 2 a more than 5-fold, difference in expression of one allele. Comparison of BRCA1 mRNA expression in these 21 cases (Table I) revealed that the distribution of expression levels in tumors with preferential allelic expression was

significantly lower than in tumors with equivalent allelic expression (2-sided Wilcoxon-Mann-Whitney test; exact p value = 0.001).

Although preferential allelic expression can be due to allelic deletion, this does not appear to be the case for 4 of the samples. These 4 specimens also exhibited significantly lower mRNA expression (2-sided Wilcoxon-Mann-Whitney test; exact p value = 0.03) without reduced amounts of BRCA1 DNA, suggesting that other allelic silencing mechanisms are involved.

Analysis of mutations in the potential promoter region of the BRCA1 gene

Mutations in the promoter region can interfere with the transcriptional process and considerably reduce the amount of mRNA produced. Although not well characterized, the DNA sequence upstream of the 5' region of exon 1 of the *BRCA1* gene has been shown to lie in head-to-head orientation with the adjacent 1A1-3B gene, the 5' region of which contains several potential regulatory sequences (Brown *et al.*, 1994). To assess the possibility of regulatory mutations as a cause of reduced expression, this potential 5' promoter region of the *BRCA1* gene was analyzed in 25 samples exhibiting relatively reduced levels of BRCA1 mRNA. Single-strand-conformation-polymorphism analyses were performed for the entire exon 1 of the *BRCA1* gene, the region lying between exon 1 of BRCA1 and exon 1A of the 1A1-3B gene and most of exon 1A of the 1A1-3B gene. In addition, the region between exons 1A and 1B of 1A1-3B was analyzed, since it contains CAT-regulatory elements. We did not observe any SSCP shifts in the regions analyzed (data not shown).

DISCUSSION

In this study, we found that a proportion of human primary breast tumors express low levels of BRCA1 mRNA. Reduction in BRCA1 expression has been associated with a proliferative phenotype in mammary epithelial cells, and may have an important role in breast-cancer progression (Thompson *et al.*, 1995). It has been shown that down-regulation of BRCA1 expression through anti-sense inhibition leads to proliferative stimulation (Thompson *et al.*, 1995). Thus, reduction in mRNA expression may be a way in which BRCA1 is involved in the development of sporadic breast cancer.

In investigating the possible mechanisms underlying the observed reduction in BRCA1 expression, we evaluated the BRCA1 locus for deletion, since LOH has been shown to be a common event at the 17q21 region. A reduction in BRCA1 DNA level was detected in 15% of specimens, and was associated with relatively low levels of BRCA1 mRNA expression. Thus, LOH or deletion events at the BRCA1 locus may contribute to sporadic-breast-cancer tumorigenesis through down-modulation of expression of the *BRCA1* gene.

Since low BRCA1 mRNA expression was not always accompanied by a deletion event, it is likely that other factors are involved in down-regulating the expression of BRCA1. Other studies have described preferential expression of mRNA from one allele, despite the presence of both copies of the gene at the DNA level (Miki *et al.*, 1994; Thompson *et al.*, 1995). Our analysis showed that a number of tumors with low BRCA1 mRNA levels exhibited preferential allelic expression. However, not all tumors displaying allelic-specific expression can be accounted for by DNA deletion, suggesting the presence of mutations that affect either the transcriptional regulation of the gene or the stability of the mRNA.

To further explore the possibility of transcriptional regulatory defects, we analyzed the 5' end of the *BRCA1* gene for the presence of potential promoter mutations by SSCP. We did not observe any SSCP shifts, suggesting that mutation is not a frequent event in this region of the *BRCA1* gene. However, the transcriptional regulatory elements of BRCA1 have not been fully characterized, and we

TABLE I – ALLELIC EXPRESSION STATUS AND mRNA LEVELS OF BRCA1*

| Tumor specimen | Allelic expression | Expression levels |
|----------------|--------------------|-------------------|
| 850 | NI | 0.02 |
| 932 | NI | 0.56 |
| 1200 | NI | 0.82 |
| 800 | DE | 0.23 |
| 617 | DE | 0.38 |
| 1192 | DE | 0.5 |
| 1817 | DE | 0.71 |
| 681 | DE | 0.76 |
| 1035 | DE | 0.83 |
| 1647 | DE | 0.97 |
| 1835 | DE | 1.08 |
| 1724 | DE | 1.12 |
| 1800 | EE | 0.65 |
| 1410 | EE | 0.88 |
| 1173 | EE | 1.07 |
| 1741 | EE | 1.37 |
| 1935 | EE | 1.41 |
| 1070 | EE | 1.46 |
| 1399 | EE | 1.5 |
| 1916 | EE | 1.51 |
| 1863 | EE | 1.92 |
| 1226 | EE | 1.95 |
| 1711 | EE | 2.3 |
| 912 | EE | 2.4 |

*NI, not informative; EE, equivalent allele expression; DE, differential allele expression.

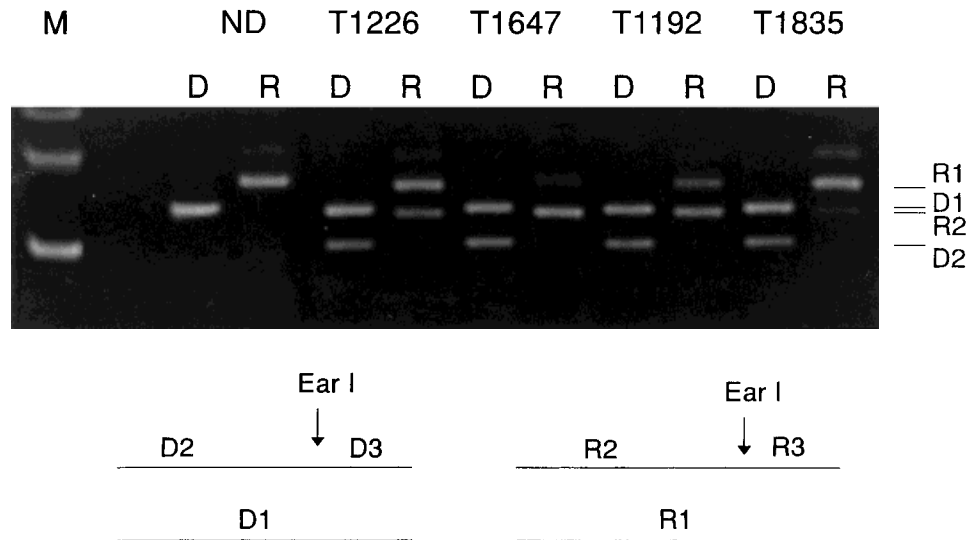


FIGURE 4 – Allelic expression analysis of the *BRCA1* gene. PCR products from DNA (D) and RNA (R) from each sample were digested with the EarI enzyme (diagram below the picture) and the band intensities of the corresponding alleles in DNA and RNA of each sample were compared. Sample T1226 represents equivalent expression, whereas samples T1647, T1192 and T1835 show preferential expression of one allele. M, marker is a 123-bp ladder.

cannot rule out the possibility of mutations in other regulatory regions affecting *BRCA1* transcription.

The data presented here support the hypothesis that *BRCA1* may be involved in sporadic breast cancer through a reduction in *BRCA1* mRNA levels. Several mechanisms can lead to reduced expression of a gene *in vivo*. In this study, we found deletion at the *BRCA1* locus and preferential allelic expression in tumors with low *BRCA1* mRNA levels. Future studies should involve more stringent analysis of the promoter and mRNA stability elements, as well as investigation of the clinical significance of low levels of *BRCA1* mRNA expression.

ACKNOWLEDGEMENTS

We thank the pathologists and the surgeons at the co-operating hospitals (Mount Sinai Hospital, Toronto Hospital, Women's College Hospital, Toronto East General Hospital, Saint Joseph's Hospital, North York General Hospital, and Credit Valley Hospitals), also Dr. P. Watson (Manitoba Breast Tumor Bank) for the normal epithelial cDNA samples. This work was supported by the Canadian Breast-Cancer Research Initiative/National Cancer Institute of Canada (I.L.A. and S.B.B.). Dr. S.B. Bull is a National Health Research Scholar (Health Canada).

REFERENCES

- BROWN, M.A., NICOLAI, H., XU, C.-F., GRIFFITHS, B.L., JONES, K.A., HOSKIN, L., TROWSDALE, J., SOLOMON, E., BLACK, D.M. and MCFARLANE, R., Regulation of *BRCA1*. *Nature (Lond.)*, **372**, 733 (1994).
- CASTILLA, L.H., COUCH, F.J., ERDOS, M.R., HOSKINS, K.F., CALZONE, K., GARBER, J.E., BOYD, J., LUBIN, M.B., DESHANO, M.L., BRODY, L.C., COLLINS, F.S. and WEBER, B.L., Mutations in the *BRCA1* gene in families with early-onset breast and ovarian cancer. *Nature Genet.*, **8**, 387–391 (1994).
- CHEN, Y., CHEN, C.-F., RILEY, D.J., ALLRED, D.C., CHEN, P.-L., VON HOFF, D., OSBORNE, C.K. and LEE, W.-H., Aberrant subcellular localization of *BRCA1* in breast cancer. *Science*, **270**, 789–791 (1995).
- CROPP, C.S., NEVANLINNA, H.A., PYRHONEN, S., STENMAN, U.-H., SALMIKANGAS, P., ALBERTSEN, H., WHITE, R. and CALLAHAN, R., Evidence for involvement of *BRCA1* in sporadic breast carcinomas. *Cancer Res.*, **55**, 2548–2551 (1994).
- FINKE, J., FRITZEN, R., TERNES, P., LANGE, W. and DOLKEN, G., An improved strategy and a useful housekeeping gene for RNA analysis from formalin-fixed, paraffin-embedded tissues by PCR. *Biotechniques*, **14**, 448–453 (1993).
- FREIDMAN, L.S., OSTERMEYER, E.A., SZABO, C.I., DOWD, P., LYNCH, E.D., ROWELL, S.E. and KING, M.-C., Confirmation of *BRCA1* by analysis of germline mutations linked to breast and ovarian cancer in 10 families. *Nature Genet.*, **8**, 399–404 (1994).
- FUTREAL, P.A. and 26 OTHERS, *BRCA1* mutations in primary breast and ovarian carcinomas. *Science*, **266**, 120–122 (1994).
- HENG, H.H.Q., SHI, X.-M., SCHERER, S.C., ANDRULIS, I.L. and TSUI, L.-C., Refined localization of the asparagine synthetase gene (*ASNS*) to chromosome 7, region q21.3, and characterization of the somatic-cell hybrid line 4AF/106/K015. *Cytogenet. Cell Genet.*, **66**, 135–138 (1994).
- HOSKING, L., TROWSDALE, J., NICOLAI, H., SOLOMON, E., FOULKES, W., STAMP, G., SIGNER, E. and JEFFREYS, A., A somatic *BRCA1* mutation in an ovarian tumour. *Nature Genet.*, **9**, 343–344 (1995).
- JENSEN, R.A., THOMPSON, M.E., JETTON, T.L., SZABO, C.I., VAN DER MEER, R., HELOU, B., TRONICK, S.R., PAGE, D.L., KING, M.-C. and HOLT, J.T., *BRCA1* is secreted and exhibits properties of a granin. *Nature Genet.*, **12**, 303–308 (1996).
- KALLIONIEMI, A., KALLIONIEMI, O.P., PIPER, J., TANNER, M., STOKKE, T., CHEN, L., SMITH, H.S., PINKEL, D., GRAY, J.W. and WALDMAN, F.M., Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. *Proc. nat. Acad. Sci. (Wash.)*, **91**, 2156–2160 (1994).
- KORETH, J., BAKKENIST, C.J. and MCGEE, J. O'D., Allelic deletions at chromosome 11q22–q23.1 and 11q25–qterm are frequent in sporadic breast but not colorectal cancers. *Oncogene*, **14**, 431–437 (1997).
- LOVERING, R., HANSON, I.M., BORDEN, K.L.B., MARTIN, S., O'REILLY, N.J., EVAN, G.I., RAHMAN, D., PAPPIN, D.J.C., TROWSDALE, J. and FREEMONT, P.S., Identification and preliminary characterization of a protein motif related to the zinc finger. *Proc. nat. Acad. Sci. (Wash.)*, **90**, 2112–2116 (1993).
- MERAJVER, S.D., PHAM, T.M., CADUFF, R.F., CHEN, M., POY, E.L., COONEY, K.A., WEBER, B.L., COLLINS, F.S., JOHNSTON, C. and FRANK, T.S., Somatic mutations in the *BRCA1* gene in sporadic ovarian tumors. *Nature Genet.*, **9**, 439–443 (1995).
- MIKI, Y. and 44 OTHERS, A strong candidate for the breast- and ovarian-cancer-susceptibility gene *BRCA1*. *Science*, **266**, 66–71 (1994).

- NOONAN, K.E., BECK, C., HOLZMAYER, T.A., CHIN, J.E., WUNDER, J.S., ANDRULIS, I.L., GAZDAR, A.F., WILLMAN, C.L., GRIFFITH, B., VON HOFF, D.D. and RONINSON, I.B., Quantitative analysis of MDR1 (multidrug resistance)-gene expression in human tumors by polymerase chain reaction. *Proc. nat. Acad. Sci. (Wash.)*, **87**, 7160–7164 (1990).
- SAITO, H., INAZAWA, J., SAITO, S., KASUMI, F., KOI, S., SAGAE, S., KUDO, R., SAITO, J., NODA, K. and NAKAMURA, Y., Detailed deletion mapping of chromosome 17q in ovarian and breast cancers: 2 cM region on 17q21.3 often and commonly deleted in tumors. *Cancer Res.*, **53**, 3382–3385 (1993).
- SHATTUCK-EIDENS, D. and 42 OTHERS, A collaborative survey of 80 mutations in the *BRCA1* breast- and ovarian cancer susceptibility gene. *J. Amer. med. Ass.*, **273**, 535–541 (1995).
- SIMARD, J. and 15 OTHERS, Common origins of *BRCA1* mutations in Canadian breast- and ovarian-cancer families. *Nature Genet.*, **8**, 392–398 (1994).
- SMITH, S.A., EASTON, D.F., EVANS, D.G.R. and PONDER, B.A.J., Allele losses in the region 17q12-21 in familial breast and ovarian cancer involve the wild-type chromosome. *Nature Genet.*, **2**, 128–131 (1992).
- THOMPSON, M.E., JENSEN, R.A., OBERMILLER, P.S., PAGE, D.L. and HOLT, J.T., Decreased expression of *BRCA1* accelerates growth and is often present during sporadic breast cancer progression. *Nature Genet.*, **9**, 444–450 (1995).