Advances in Brief

Detection of Germline BRCA1 Mutations in Breast Cancer Patients by Quantitative Messenger RNA in Situ Hybridization¹

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Abstract

Mutations in the breast cancer susceptibility gene 1 (BRCA1) may account for one half of all familial breast cancers. Because of the wide spectrum of different germline mutations, identification of BRCA1 mutation carriers using current techniques is laborious and difficult. The majority of the identified mutations, however, lead to aberrant expression of the gene product in tumor tissue, potentially allowing the detection of BRCA1-linked breast cancers using simple histochemical techniques. We performed quantitative mRNA in situ hybridization on archival paraffin-embedded tumor specimens from 25 patients with characterized germline BRCA1 mutations or linkage and from 29 patients with sporadic breast cancers. BRCA1 mRNA levels were invariably low in tumors from BRCA1 mutation carriers. Normal breast epithelium surrounding the BRCA1 tumors showed higher mRNA levels than the tumor tissue, indicating that the low mRNA levels were due to somatic inactivation of the wild-type BRCA1 allele in the tumor tissue. The expression levels in the sporadic tumors were, on average, six times higher than in the BRCA1 mutated and sporadic tumors with more than 95% specificity and sensitivity. We conclude that the analysis of BRCA1 gene expression by mRNA in situ hybridization may be useful in screening for patients with BRCA1-linked breast cancer.

Introduction

Approximately 5–10% of women diagnosed with breast cancer have a familial history of the disease, compatible with the segregation of a dominant trait. Germline mutations of the BRCA1 gene (1) are estimated to account for approximately one half of these cases and for the majority of cases with inherited breast and ovarian cancer. Identifying these patients would be extremely important because the patients themselves are highly prone to subsequent acquisition of either bilateral breast cancer or ovarian cancer, and their family members have a substantially increased risk for developing breast cancer. It has been estimated that 1 of 300 to 1 of 800 people in the United States may be carriers of BRCA1 mutations (2). With the exception of genetic isolates (3), germline BRCA1 mutations, analyzed in more than 100 families, have turned out to be ubiquitously distributed throughout the gene (2). Because of the large size of the gene, the search for BRCA1 mutations is a daunting task even in affected families, and large scale population studies of unselected patients or individuals are virtually impossible. Individual methods, such as SSCP⁴, denaturing gradient gel electrophoresis, or protein truncation testing, typically detect only about 60–70% of the mutations, and the combined use of several methods is required to achieve sufficient sensitivity (2, 4, 5).

Since BRCA1 is thought to act as a classical tumor suppressor gene, a germline mutation in the BRCA1 gene is likely to be followed by a second somatic genetic event, either loss or mutation of the remaining allele, leading to complete inactivation of BRCA1 in the tumor tissue. Many of the germline mutations of the BRCA1 gene are either frame-shift or nonsense mutations causing premature translation termination (5). The resulting truncated proteins may lack some or all of the biochemical functions of the wild-type protein, may be nonfunctional due to abnormal subcellular localization, or physically absent due to rapid degradation (1, 6, 7). Moreover, mutations leading to loss of transcript, either by affecting gene regulatory elements or stability of the transcript, have also been implicated (1, 2). This opens up the possibility of using a simple histochemical method, mRNA in situ hybridization, to detect BRCA1 mutations.

Materials and Methods

The patient material consisted of 25 women (mean age, 45 years; range, 29–73) who were classified as having familial breast or breast-ovarian cancer on the basis of having at least three first-degree family members with breast or ovarian cancer and at least one case of cancer diagnosed below the age of 50 years. The patients were from 10 families from South Sweden diagnosed as having a germline BRCA1 mutation by SSCP, heteroduplex analysis, and protein truncation testing followed by DNA sequencing (8). Four patients from one family manifested linkage to BRCA1 (multipoint LOD score 1.6 to markers THRA1, D17S855, and D17S579) and allelic loss of wild-type chromosomes in the associated tumors, but no mutation was found in the BRCA1 coding region (Ref. 8; Table 1).

All subjects gave written informed consent for participation in the study. Lymphocyte genomic DNA from one affected individual per family was used as a template in BRCA1 mutation analysis. Paraffin-embedded tissues from breast tumors were obtained from different pathological departments of the south Swedish health care region. Reference material from an unselected cohort of breast cancer patients (mean age 63 years; range, 29–85), representing 29 sporadic breast cancer cases, was used as a control group. All tumors in both groups were histologically classified as invasive ductal carcinomas.

Five-µm paraffin-embedded tissue sections were cut onto SuperFrost slides (Menzel-Gläser, Germany), deparaffinized, rehydrated, and air dried prior to hybridization. Oligonucleotide probes (nucleotides 556–598 and 3457–3501, corresponding to exons 5 and 11, respectively; Ref. 1) from regions of the BRCA1 gene unaffected by mutations in the present material (Table 1) were labeled with [³²P]dATP (DuPont New England Nuclear) to a specific activity of >1x10⁶ cpm/µg using terminal deoxynucleotidyl transferase (Pharmacia Biotech, Uppsala, Sweden).

Seventy-five µl of the hybridization solution [consisting of 1 x 10⁶ cpm/ml of the labeled BRCA1 probe, 50% deionized formamide, 10% dextran sulfate, 1% Sarkosyl, 0.02 M sodium phosphate (pH 7.0), 4X SSC, 1X Denhardt’s solution, and 10 mg/ml single-stranded DNA] were applied to each slide. The hybridization was performed overnight in a humidified chamber at 42°C. The sections were washed in 1X SSC to remove the unbound probe, briefly dehydrated, and covered either with Molecular Dynamics Phosphorimager

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⁴ The abbreviations used are: SSCP, single-stranded conformational polymorphism; LOD, logarithm of the odds of linkage.

2912
Table 1  Germline BRCA1 mutations found in south Swedish breast and ovarian cancer kindreds

<table>
<thead>
<tr>
<th>Family</th>
<th>Disease</th>
<th>Type</th>
<th>Location</th>
<th>Amino acid change</th>
<th>Predicted effect</th>
<th>No. of tumors studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lund 1</td>
<td>br-ov&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>Regulatory ?</td>
<td>Not found&lt;sup&gt;c&lt;/sup&gt;</td>
<td>845 → Stop</td>
<td>Protein truncation</td>
<td>4</td>
</tr>
<tr>
<td>Lund 3</td>
<td>br-ov</td>
<td>Frameshift</td>
<td>2395 delA</td>
<td>Ser 361 → Stop</td>
<td>Protein truncation</td>
<td>4</td>
</tr>
<tr>
<td>Lund 8</td>
<td>br-ov</td>
<td>Frameshift</td>
<td>1201 del11</td>
<td>Gln563 → Stop</td>
<td>Protein truncation</td>
<td>5</td>
</tr>
<tr>
<td>Lund 9</td>
<td>br-ov</td>
<td>Nonsense</td>
<td>C 1806 T</td>
<td>1025 → Stop</td>
<td>Protein truncation</td>
<td>1</td>
</tr>
<tr>
<td>Lund 24</td>
<td>br-ov</td>
<td>Frameshift</td>
<td>3166 ins 5 bp</td>
<td>1025 → Stop</td>
<td>Protein truncation</td>
<td>1</td>
</tr>
<tr>
<td>Lund 30</td>
<td>br</td>
<td>Missense</td>
<td>T 300 G</td>
<td>Cys 61 → Gly</td>
<td>Lost Zn-bind motif</td>
<td>2</td>
</tr>
<tr>
<td>Lund 36</td>
<td>br</td>
<td>Nonsense</td>
<td>C 1806 T</td>
<td>Gln 563 → Stop</td>
<td>Protein truncation</td>
<td>2</td>
</tr>
<tr>
<td>Lund 44</td>
<td>br-ov</td>
<td>Frameshift</td>
<td>3166 ins 5 bp</td>
<td>1025 → Stop</td>
<td>Protein truncation</td>
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</tr>
<tr>
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<td>Frameshift</td>
<td>1675 delA</td>
<td>531 → Stop</td>
<td>Protein truncation</td>
<td>1</td>
</tr>
<tr>
<td>Lund 79</td>
<td>br-ov</td>
<td>Frameshift</td>
<td>1201 del11</td>
<td>Ser 361 → Stop</td>
<td>Protein truncation</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> br-ov, breast and ovarian cancer; br, breast cancer; ?, not known.

<sup>b</sup> No mutations in coding regions of the gene but positive by linkage analysis (LOD score, 1.6).

 screens (exposure time, 4 days) for quantitative analysis of the hybridization signal or with Amersham β-max hyperfilm (Amersham International, Buckinghamshire, United Kingdom) for morphological visualization of mRNA expression at the tissue level (exposure time, 3 weeks). All tissue sections were hybridized in duplicate and analyzed concurrently. Hybridization with both of the probes used gave similar results. To confirm that reduced BRCA1 mRNA levels were not caused by general RNA degradation, each paraffin-embedded tissue block was tested with mRNA in situ hybridization using a control probe to the metabolic enzyme glyceraldehyde-3-phosphate dehydrogenase (nucleotides 1022–1064; GenBank accession no. M33197). Nearly equal glyceraldehyde-3-phosphate dehydrogenase hybridization signals were present in all tissue blocks studied.

<sup>c</sup> BRCA1 mRNA

Fig. 1. Quantitative in situ hybridization analysis showing a low BRCA1 mRNA level (480 counts/mm<sup>2</sup>) in the breast cancer tissue of a patient carrying a germline BRCA1 mutation (A), in contrast to the moderate level (4520 counts/mm<sup>2</sup>) observed in the tumor tissue of a sporadic breast cancer patient (B) and in normal mammary epithelium (C). The normal mammary epithelium from a BRCA1 mutation carrier shows a similar BRCA1 mRNA level (D) as the normal mammary epithelium of an unaffected individual (C). Boxes, areas shown in the right panels after H&E staining. Bar, 0.15 cm.
The statistically significant difference in *BRCA1* mRNA levels between the sporadic and *BRCA1*-linked breast carcinomas indicates that, although decreased *BRCA1* expression has also been implicated in sporadic breast cancer progression (10), the very low transcript levels in the mutated cases are in part caused by the loss of the remaining allele per se. Previous studies have clearly demonstrated the high frequency and almost invariable loss of wild-type *BRCA1* alleles in breast tumors from families with *BRCA1* linkage and/or mutation (11). The low *BRCA1* mRNA levels in tumor cells, but not in normal breast epithelium, of mutation carriers suggest that the residual *BRCA1* mRNA present in the tumor tissue reflects transcripts of the mutant alleles. The majority of *BRCA1* mutations are of the frameshifting or nonsense type, transcripts of which have decreased stability as compared to wild-type transcripts (6). A regulatory system for recognition and accelerated degradation of defect transcripts may also be active in mammalian cells, similar to the “surveillance” pathway described in yeast cells (12). One may also speculate about a positive feedback mechanism by which the wild-type *BRCA1* protein might increase its own mRNA stability or transcriptional activity, as described for several other genes (6).

The present results indicate that both structural and inferred regulatory *BRCA1* mutations are associated with very low *BRCA1* transcript levels in tumor cells. Although the present material only includes seven different *BRCA1* mutations, they are representative of the spectrum of *BRCA1* mutations identified thus far (2). Thus, with regard to the 6-fold difference between the mean expression levels of the sporadic and *BRCA1*-linked breast cancers, significantly reduced *BRCA1* mRNA levels in breast tumor tissue are highly indicative of a germline *BRCA1* mutation. These results are in line with those of Serova et al. (13), who showed that lymphocytes of *BRCA1* mutation carriers exhibited decreased mutant *BRCA1* transcript levels in 8 of 16 cases. In tumor cells, decreased transcript levels have been demonstrated with mutations of other tumor suppressor genes, e.g., the *RB1* and *APC* genes (14, 15). Moreover, Chen et al. (7) observed loss of *BRCA1* protein using immunohistochemistry in 2 of 50 breast cancer samples; a percentage that is in line with the suggested prevalence of *BRCA1* mutation carriers in the American population (2).

According to the present results, mRNA in situ hybridization may be of significant value in screening for patients with *BRCA1* mutations. As a simple histochemical technique, mRNA in situ hybridization is more easier and cost efficient to perform than other currently available methods used for mutation screening (such as the protein truncation test or SSCP combined with direct sequencing). In situ hybridization can be automatized with commercially available staining robots, but even the present technology makes it possible to analyze up to 100 patient samples in a single hybridization batch. These practical aspects emphasize the usefulness of in situ hybridization for screening of *BRCA1*-linked breast cancers.

Even though the clinical benefits of diagnosing *BRCA1* mutations are apparent, population level screening for hereditary diseases has obvious ethical concerns that must be considered. The present approach is applicable only to persons with a clinically presented breast cancer. However, its simplicity would make possible the screening of large numbers of patients, not just those at high risk for hereditary breast cancer. It would alleviate the need to set strict criteria as to which patients can be analyzed for mutations (16), allowing detection of mutations also in patients whose disease does not appear to be familial in origin (e.g., due to small kindred size).
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References


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