Identification of a Possible Somatic BRCA1 Mutation Affecting Translation Efficiency in an Early-Onset Sporadic Breast Cancer Patient

Mutations in the BRCA1 gene account for up to 80% of cases of familial breast cancers (1). However, the majority of breast cancers are sporadic and only 10% of cases show a familial basis. No alterations of the BRCA1 gene have been associated with sporadic breast cancer cases, although other cancers, like sporadic ovarian cancers, display somatic inactivation of BRCA1 (2). However, decreased BRCA1 gene expression is frequently found in sporadic breast cancer in the absence of direct mutations in the coding region (3). It is plausible that BRCA1 down-modulation occurs by epigenetic mechanisms involving altered upstream regulatory regions, such as hypermethylation of the promoter region (4), or other factors implicated in the complex regulatory mechanism. We report here the description of a novel BRCA1 gene mutation potentially affecting BRCA1 gene expression in a woman with sporadic breast cancer who was a case subject in a population-based retrospective screening for BRCA1 gene involvement in 96 sporadic breast cancer cases.

The women, 32 years of age, was diagnosed with a grade III infiltrating ductal carcinoma of the breast and treated with mastectomy, ancillary lymph node resection, radiotherapy, and six cycles of standard adjuvant chemotherapy. The patient was then cancer free for 10 months. However, the tumor recurred and the patient, who was treated with palliative chemotherapy, died 2 years later.

This woman’s genomic DNA was available from the primary tumor and one metastatic lymph node. Full-length RNA single-strand conformation polymorphism analysis of each of the 24 BRCA1 gene exons revealed several common sequence polymorphisms and a unique aberrant banding pattern within exon 2 in both tumor samples. The exon 2 sequences were amplified by use of the polymerase chain reaction and the product subjected to direct sequencing. DNA sequence information was obtained from both tumor samples and the sequence was confirmed on both strands. A single base transversion (G to C) was identified at nucleotide position 117, three nucleotides 5' of the ATG initiation codon, within a “Kozak” consensus motif (5) for the start of translation (Fig. 1, A). Direct sequencing of the BRCA1 gene confirmed by multiple sequence reactions from independent polymerase chain reaction reactions (both manual and automated analysis using a Vistra DNA Sequencer 725, Amersham, Little Chalfont, Buckinghamshire, U.K.) showed two bands, a more intense band corresponded to the mutated C and a fainter band corresponding to the normal G. The presence of both bands are consistent with the fact that each tumor sample was composed of a mixture of malignant cells and of nonmalignant stromal cells.

No messenger RNA translation studies were performed based on this information. However, it is known that a purine to pyrimidine base change located three nucleotides 5' of Kozak consensus sequence motif severely affects translation efficiency (Fig. 1, B) (6). These data are consistent with the observation that 97% of all vertebrate messenger RNAs carry a purine at this ‘‘-3’’ position of the translation start motif (7). It is also noteworthy that the sequence context is well conserved for the major translation initiation site of the BRCA1 gene among human, mouse, and rat gene homologues (GenBank accession numbers: U14680, U32446, and AF036760, respectively).

Because of the early onset of the tumor, we investigated the possibility that

![Fig. 1. A)](image)

DNA sequence analysis of polymerase chain reaction products from BRCA1 gene exon 2 from a breast cancer patient. The vertically oriented sequence to the left of the panel shows the sequence of the region of interest from the human BRCA1 gene and the arrow indicates the position of the mutation, a G to C transversion at nucleotide 117 within the consensus sequence for translation initiation (-3 nt 5' to the first ATG). To the right of the sequence are shown banding patterns obtained from DNA samples prepared from the patient’s primary breast cancer (T) and metastatic lymph node (LFN) compared with DNAs from peripheral blood lymphocytes obtained from the mother, father, and brother. Sequencing was performed using double-stranded DNA templates and deoxy-termination reactions (Sequenase PCR product sequencing kit, Amersham-USB). B) Schematic representation of the translation initiation (Kozak) consensus sequences from normal and mutant BRCA1 genes compared with Kozak sequences of the α-globin gene and α-thalassemia form of the α-globin gene (6). Pur = purine; Pyr = pyrimidine; bold letters = the most conserved nucleotides within the translation initiation consensus motif; underlined = mutation site.
this mutation was inherited. Genomic DNA was isolated from blood samples obtained from both living, unaffected parents and from the healthy brother and sequenced (Fig. 1, A). None of the immediate family members carried the mutation found in the DNA from the patient’s tumor. This finding argues against a new familial form of breast cancer. However, we cannot definitively exclude the occurrence of a mutation in the germline of one of the parents, since the lack of a normal DNA sample from the patient hampers verification of a possible somatic origin of the mutation.

Our finding supports the hypothesis that somatic or constitutive mutations affecting BRCA1 gene expression could identify a subset of ‘‘sporadic’’ breast cancers and that such mutations could potentially represent a useful prognostic marker. We are currently investigating the functional effect of this candidate BRCA1 gene mutation.

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Notes

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Human Herpesvirus 8 Seroprevalence in Sardinia

Italy has one the highest rates of classic Kaposi’s sarcoma (KS) in the world, with a particularly high incidence occurring in Sicily, in the Po valley, and in Sardinia (1–3). In a recent issue of the Journal, Whitby et al. (4) reported a higher prevalence of antibodies against human herpesvirus 8 (HHV-8) among blood donors from southern Italy (i.e., Apulia, Calabria, and Sicily; 24.6%) compared with blood donors from northern and central Italy (i.e., Lombar- dia, Friuli-Venezia Giulia, and Emilia-Romagna; 7.3%). Although it is not clear from the data presented by the authors that these blood donors from different regions in Italy were comparable in age and sex, the geographic distribution of HHV-8 seropositive individuals seems to parallel the national rates of incidence for classic KS (1–3). Their findings suggest that an association exists between the prevalence of HHV-8 antibodies and the increased incidence of classic KS. These data also support a role for HHV-8 in determining the risk for KS development (4,5).

We report similar data derived from pregnant women in Sardinia, where a bank of sera has been collected since 1986, within the framework of the Sardinia Insulin Dependent Diabetes Mellitus Study (6). To determine the prevalence of HHV-8 infection in pregnant women living in Sardinia, 100 individual sera were prepared from umbilical cord blood taken at delivery in 1993 and 1994. These sera were assayed for antibodies directed against both latent nuclear antigens (antilatent antibodies) and cytoplasmic antigens (antilytic antibodies) of HHV-8, using the method described by Lennette et al. (7).

We determined that 25 of 100 sera were seropositive for HHV-8 antilytic antibodies (95% confidence interval [Cl] = 16.5%–33.5%) (Table 1). HHV-8 seropositivity for the cytoplasmic antigens ranged from 29.1% in women under 30 years of age to 21.6% in women greater than or equal to 30 years of age (data not shown). A greater percentage of women living in northern Sardinia (i.e., the provinces of Sassari and Nuoro), an area with especially higher incidence rates of classic KS (2), were seropositive (31.0%) than those women living in the southern part of the region (i.e., Cagliari province (12.0%)) (Table 1). This difference between the two groups persisted after adjustment by quinquennia of age (X² = 4.13; P = .04). The geometric mean titer was 183

Table 1. Prevalence of HHV-8 infection in pregnant women, at time of delivery, according to type of antibodies and area of residence in Sardinia 1993 and 1994

<table>
<thead>
<tr>
<th>Residence*</th>
<th>No. of sera tested</th>
<th>Sera positive for antilytic antigens (No. %)</th>
<th>Sera positive for antilatent antigens (No. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Sardinia</td>
<td>71</td>
<td>22 (30.1)</td>
<td>3 (4.2)</td>
</tr>
<tr>
<td>Southern Sardinia</td>
<td>25</td>
<td>3 (12.0)†</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>25 (25.0)</td>
<td>3 (3.0)</td>
</tr>
</tbody>
</table>

*The sum does not add up to the total because of missing values.
†X², adjusted for age = 4.13, P = .04, two-sided.