Somatic and Germline Mutations of the BRCA2 Gene in Sporadic Ovarian Cancer


Cancer Research Campaign Human Cancer Genetics Research Group, Box 238, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ [K. A. F., P. H., P. R., B. A. J. P., S. A. G.]; United Kingdom; The Queensland Institute of Medical Research, 300 Herston Road, Brisbane, QLD 4006, Australia [J. K., G. C. T.]; Department of Gynecological Oncology Research, The Roswell Park Cancer Institute, 5709 Simpson Building, Elm and Carlton Streets, Buffalo, New York 14263 [R. A. D.]; Department of Obstetrics and Gynaecology, Derby City General Hospital, Uttoxeter Road, Derby DE22 3NE [I. V. S.]; United Kingdom; and Ovarian Cancer Screening Unit, The Royal Hospitals National Health Service Trust, St. Bartholomew’s Hospital, West Smithfield, London EC1A 7BE [I. J.], United Kingdom

Abstract

The breast and ovarian cancer susceptibility gene BRCA2 has recently been isolated. A role for BRCA2 in sporadic breast and ovarian cancer has been suggested by loss of heterozygosity (LOH) studies which show frequent LOH in the BRCA2 region at chromosome 13q12. In addition, the observation of nonrandom loss of the wild-type chromosome in a breast/ovarian cancer family which shows linkage to BRCA2 suggests it may act as a tumor suppressor gene. To determine the extent of somatic alteration involving BRCA2 in sporadic ovarian cancer, 50 tumors were analyzed for mutations throughout the entire BRCA2 coding region. Mutations predicted to result in truncation of the BRCA2 protein were detected in four tumors. Analysis of germline DNA revealed two of these alterations to be of somatic origin. In addition, all four tumors exhibited loss of the second BRCA2 allele as predicted by Knudson’s hypothesis for a tumor suppressor gene. These results suggest that, as is the case with BRCA1, somatic mutations of BRCA2 are infrequent in sporadic ovarian cancer, despite the relatively high frequency of LOH detected around the BRCA2 locus.

Introduction

The BRCA2 gene was localized in 1994 to chromosome 13q12−13 through linkage analysis in breast and ovarian cancer families (1) and was cloned at the end of 1995 (2). It is a large gene comprising 10,254 nucleotides encoded by 26 exons, with almost half of the coding sequence contained within exon 11 (3). The predicted protein consists of 3,418 amino acids and has little homology to previously identified proteins (3). Germline mutations in BRCA2 confer an 85% lifetime risk of breast cancer as well as an elevated risk of ovarian cancer (1). Epidemiological studies suggest that BRCA2 and BRCA1, the first breast and ovarian cancer susceptibility genes to be isolated (4), account for the majority of familial breast and ovarian cancer (1, 5). Tumors from families linked to BRCA1 or BRCA2 show consistent loss of the wild-type allele, suggesting that both genes behave as tumor suppressors (6, 7). LOH studies indicate that both genes are also frequently deleted in sporadic breast and ovarian cancer (8−11). However, few somatic BRCA1 mutations have been found in sporadic ovarian tumors and none in sporadic breast tumors (12−14).

To determine the frequency of somatic alterations of BRCA2 in sporadic ovarian cancer, we have analyzed a series of ovarian tumors for LOH in the BRCA2 region and for mutations throughout the entire coding sequence of the gene. Eighty-five tumors collected from Australia, the United Kingdom, and the United States were analyzed for LOH at the BRCA2 locus. Loss was detected in 46% of the tumors. Fifty of these tumors, 31 with loss and 19 without, were subsequently analyzed for BRCA2 mutations using a combination of HA and the PTT. Two somatic and two germline mutations predicted to result in truncation of the BRCA2 protein were detected. Loss of the wild-type allele was evident in all four tumors, supporting the hypothesis that BRCA2 acts as a tumor suppressor gene.

Materials and Methods

Patient Material. Forty-three ovarian tumors collected in Australia, 30 tumors collected in the United Kingdom, and 12 tumors collected in the United States, all with matched peripheral blood or normal tissue, were used in this analysis. All tumors were confirmed as epithelial ovarian carcinomas from histology reports. Ovarian cancer cases collected in the United Kingdom were selected on the basis of no family history of ovarian cancer. Tumors collected in Australia and the United States were selected on no basis other than availability. Australian tumors were enriched for tumor cells by treatment with collagenase prior to storage in liquid nitrogen, and DNA was extracted as previously described (15). Tumors collected in the United Kingdom and the United States were frozen in liquid nitrogen immediately following surgery. Tumors from the United Kingdom were sectioned and microdissected to minimize contaminating normal tissue. Tumor tissue was placed in extraction buffer (1X PCR buffer, 1.5 mM MgCl2, 0.45% Tween 20, and 0.45% NP40) and treated with proteinase K (200 μg/ml). Tumors from the United States were homogenized without preselection for tumor cells in STE buffer [0.05 M Tris-HCl, pH 7.5, 0.1 M NaCl, 1 mM EDTA, and 1% SDS] and treated with proteinase K (100 μg/ml). DNA was phenol-chloroform extracted and ethanol precipitated.

LOH Analysis. LOH analysis of 30 tumors from the United Kingdom and 12 tumors from the United States was performed using the polymorphic microsatellite markers D13S171, D13S267, and D13S260 (16), which are ordered centromere-D13S260-1cM-D13S171-2cM-D13S267-telomere. BRCA2 is located between D13S260 and D13S171 (2). Forty-three ovarian tumors from Australia were initially assessed for LOH at 13q12 using D13S267. Fourteen tumors from this study that showed LOH were included in further LOH analyses with markers D13S171 and D13S260. Following PCR amplification of matched blood and tumor DNAs, products were electrophoresed through 8−12% nondenaturing polyacrylamide gels and stained with silver as previously described (17). LOH was scored visually as either complete loss of an allele or as significant reduction in intensity of a single allele relative to the second allele. A recent comparison of two methods of scoring found visual scoring to be compatible with densitometric analysis (18). All LOH scoring was done by a single investigator and independently confirmed by a second investigator. Tumors with loss of at least one of the three markers examined were considered to have LOH in the BRCA2 region. No cases of interstitial loss excluding BRCA2 were seen.

Mutation Analysis of BRCA2. Oligonucleotide primers were designed to perform the PCR and HA from genomic DNA on all coding exons and

Received 4/22/96; accepted 7/1/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The laboratory work in this study was funded by a program grant from the CRC, the Queensland Cancer Fund, the National Health and Medical Research Council, Core Grant CA16056 of the National Cancer Institute, and the Gustavus and Louise Pfeiffer Foundation (R. A. D.). K. F. was supported by the Overseas Research Student Award Scheme and Medical Research Council Research Fellow. To whom requests for reprints should be addressed. Fax: 44 1223 336902.

1 The laboratory work in this study was funded by a program grant from the CRC, the Queensland Cancer Fund, the National Health and Medical Research Council, Core Grant CA16056 of the National Cancer Institute, and the Gustavus and Louise Pfeiffer Foundation (R. A. D.). K. F. was supported by the Overseas Research Student Award Scheme and the National Science Foundation Graduate Fellowship Program. B. A. J. P. is a Gibb Fellow of the CRC. I. J. is an McLlwain Fellow of the CRC. G. C. T. is a National Health and Medical Research Council Research Fellow.

2 To whom requests for reprints should be addressed. Fax: 44 1223 336902.

PTT, protein truncation test; CRC, Cancer Research Campaign.
splicing boundaries except for exon 11. Exon 11 was screened using a combination of PTT to search for frameshift and nonsense mutations and HA to look for putative splice site mutations. Primer sequences are available on request.4 HA was performed as described previously (17), except that gels were visualized under UV light following treatment with SYBR Green nucleic acid stain (Flowgen). The PTT was carried out on exon 11 in five overlapping fragments using genomic DNA as a template for PCR amplification. PCR products were translated in vitro using a T7-coupled reticulolysate system (Promega) with [35S]methionine. Protein products were electrophoresed through 10% SDS polyacrylamide gels, fixed, and treated with Amplify (Amersham). Gels were visualized following autoradiography. Sequence analysis of variant samples was performed using the ABI 373A DNA sequencer by dye terminator cycle sequencing with AmpliTaq DNA polymerase (Perkin Elmer).

Results and Discussion

Eighty-five matched germline and tumor DNA samples from sporadic ovarian cancers collected in Australia, the United Kingdom, and the United States were analyzed for LOH around the BRCA2 locus. At least one polymorphic microsatellite marker from a 3-cM region containing the BRCA2 gene (1) detected LOH in 41 tumors (46%). These data are summarized in Table 1. DNA from 50 tumors, 31 with LOH and 19 without, was screened for mutations throughout the BRCA2 coding sequence.

PTT of exon 11 revealed truncated proteins in three tumors (Fig. 1, a and b). Sequence analysis characterized all three variants as frameshift mutations resulting from deletion or insertion of between 1 and 4 bp (Table 2 and Fig. 2). HA of the remainder of the gene identified several variants which occurred frequently throughout the sample set, indicating that they are common polymorphisms (data not shown; Ref. 3). One unique heteroduplex variant in tumor A7 was characterized as a 16-bp deletion that removes the last 9 bp of exon 24 and the splice donor consensus sequence (Fig. 1c and Table 2). cDNA was not available from this tumor to assess the effect of this mutation, although the deletion would be predicted to result in either exon skipping or the activation of a cryptic splice site leading to premature chain termination.

Analysis of lymphocyte DNA for each of the four mutations revealed two to be present in the germline whereas the remaining two were somatic. Sequence analysis confirmed loss of the wild-type allele in all four tumors. Both somatic mutations and one germline mutation are from the set of tumors collected in Australia. The remaining germline mutation is in 1 of 24 tumors from the United Kingdom that were analyzed. No mutations were detected in the series of tumors from the United States. Both individuals with germline mutations have no previously reported history of cancer in their families.

These results suggest that somatic mutations of the BRCA2 gene are an uncommon event in the progression of sporadic ovarian cancer, despite frequent allele loss around the BRCA2 locus. Three recently published studies indicate that somatic BRCA2 mutations also occur infrequently in sporadic breast cancers (19-21). One of these studies also analyzed 55 sporadic ovarian tumors but failed to detect either germline or somatic mutations (19). Together, these results are similar to those of BRCA1 in which only a small number of somatic mutations have been detected (12, 13). There are several explanations which may account for the paucity of somatic BRCA2 mutations in sporadic ovarian cancer. Mutations may have been missed either as a result of the techniques used in this study or because they are in regions eliminated from the analysis. Large deletions, insertions, or duplications are unlikely to be detected with the PCR-based mutation detection techniques used, whereas intronic or regulatory sequences which may harbor mutations affecting splicing efficiency or mRNA processing were not analyzed. In addition, any missense mutations in exon 11 would not have been detected by PTT which was used to screen this region of the coding sequence. Alternatively, BRCA2 may not be the target for the LOH observed in ovarian cancer. The region of LOH on 13q14 characterized by many investigators extends for the length of the chromosome arm with few cases of interstitial deletion and is therefore likely to result in the loss of several genes (10, 11, 22). One candidate, the retinoblastoma gene at 13q14, also shows frequent LOH in ovarian cancers accompanied by infrequent somatic mutations and normal levels of expression (22, 23). It remains to be seen whether expression levels of BRCA2 are altered in both breast and ovarian tumors.

---

4 Fax: 44 (0)1223 336902; E-mail: sg200@cus.cam.ac.uk.

---

Table 1 Summary of LOH analysis at the BRCA2 locus on chromosome 13q12

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>13q LOH analysis</th>
<th>BRCA2 mutation analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of tumors</td>
<td>% tumors with LOH</td>
</tr>
<tr>
<td>Australia</td>
<td>43</td>
<td>20</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>United States</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>

---

Table 2  Somatic and germline mutations of the BRCA2 gene in sporadic ovarian cancers

<table>
<thead>
<tr>
<th>Tumor DNA</th>
<th>13q LOH status</th>
<th>Lymphocyte DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mutation</td>
<td>Predicted effect</td>
</tr>
<tr>
<td>A6</td>
<td>3866 del A</td>
<td>Loss</td>
</tr>
<tr>
<td>A2</td>
<td>5803 del ATTA</td>
<td>Truncation codon</td>
</tr>
<tr>
<td>UK30</td>
<td>6064 ins A</td>
<td>Truncation codon</td>
</tr>
<tr>
<td>A7</td>
<td>9476 del 16</td>
<td>Loss of exon 24</td>
</tr>
</tbody>
</table>

---

Fig. 1. Examples of HA and the PTT performed for BRCA2 in sporadic ovarian cancers. a, tumor sample A2 (T) exhibits a truncated protein with PTT fragment 4 of exon 11 compared with the wild-type full-length protein (WT) in a normal sample (N); this results from the mutation 5803delATTA. b, PTT fragment 2 of exon 11 reveals a truncated mutant protein product (Mut) in tumor sample A6 (T) compared with wild-type full-length protein (WT) in a normal sample (N); a 1-bp deletion at nucleotide 3866 of BRCA2 is the cause of this variant. c, HA of exon 24 exhibits significant heteroduplex variation (Het) and mutant homoduplex variation (Mut-Hom) in tumor sample A7 (T) compared with the wild-type homoduplex pattern (WT-Hom) exhibited by normal fragments (N) from the same sample set. This variant was characterized as a 16-bp deletion beginning at nucleotide 9476 of BRCA2.
BRCA2 MUTATIONS IN SPORADIC OVARIAN CANCER

Fig. 2. Sequence analysis of tumor and lymphocyte DNA in two samples with PTT variants: a, sequence of the tumor A6 shows the mutation 3866delA to be present in tumor DNA but not in lymphocyte DNA, indicating this is a somatic mutation; the mutant sequence in tumor DNA is prominent due to loss of the second allele, b. sequence of tumor and lymphocyte DNA for the sample UK30 shows the mutation 6064insA to be present in both, indicating that this is a germline mutation; loss of the wild-type sequence in the tumor DNA results in a prominent mutant sequence.

Acknowledgments

We thank Jo Dearden, Carole Pye, Samantha Crockett, Anne Wilson, Linda Hubbold, Ian Symonds, E. Symonds, and M. Steven Piver for help with collecting patient material: Mike Straton and Richard Wooster for sharing primer information; Lori Friedman for technical assistance and critical review of the manuscript; and Tania Coombs and Terry Hurst for technical assistance.

References


BRCA2 MUTATIONS IN SPORADIC OVARIAN CANCER


Somatic and Germline Mutations of the BRCA2 Gene in Sporadic Ovarian Cancer

Karen A. Foster, Patricia Harrington, Judith Kerr, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/16/3622