Low frequency of microsatellite instability in BRCA1 mutated breast tumours

EDITOR—BRCA1 is one of the major breast and ovarian cancer susceptibility genes. Many studies have suggested that the BRCA1 protein is multifunctional. Notably, it may play a role in DNA repair, especially in double strand break (DSB) DNA repair by homologous recombination because (1) BRCA1 acts in association with Rad51, (2) DNA damage induces its phosphorylation and delocalisation from nuclear foci to the DNA replication forks, and (3) it contains a zinc finger domain that interacts with BARD1, an effector of DNA repair, and two BRCT (BRCA1 C-terminal) domains which are found in various proteins implicated in DNA repair (fig 1A).

Among the various genetic alterations found in breast tumours, one is called microsatellite instability (MI). MI has been shown in a small fraction of sporadic breast tumours, varying from 0 to 30%, and in familial breast tumours conflicting results have been reported with a MI+ tumour frequency of 0% (0/15) according to Lothe et al and 83% (15/18) according to Glebov et al.

MI is characterised by expansion or contraction in one or both alleles of some microsatellites in tumour DNA. First described in hereditary non-polyposis colorectal cancer (HNPCC) and in tumours associated with the HNPCC spectrum, MI is usually the result of defects in DNA mismatch repair. The resulting phenotype is called replication error + (RER+), as in nearly all RER+ HNPCC tumours DNA mismatch repair genes (MMR genes) are mutated. However, in breast tumours, it seems that the nature of instability differs from that described for HNPCC since (1) MI occurs in a lower number of markers, (2) the microsatellite loci affected are mainly trinucleotides and tetranucleotides, and (3) instability is characterised by the occurrence of new allele(s) very different in size from the normal ones. Since no mutation in the MMR genes has been found in breast tumours, it has been proposed that other genes, still unknown and probably implicated in a DNA repair pathway different from mismatch repair, may be involved in this particular type of MI.

In the two studies on familial breast tumours, no information was provided concerning the hereditary predisposition gene implicated in the families, but the analysis of Glebov et al shows frequent loss of heterozygosity (LOH) at the markers surrounding the 17q21 region which contains the BRCA1 locus.

Following these observations, it may be hypothesised that loss of BRCA1 function could lead to abnormal homologous recombination, resulting, when DSB takes place at microsatellite loci, in changes in the repeat number, that is, MI. Therefore, it would be of interest to know whether MI is found in familial BRCA1 tumours, where BRCA1 function is lost consecutively to a germline mutation that inactivates one allele and, in 86% of tumours, to the somatic loss (LOH) of the wild type allele.

We report here the results of the relationship between BRCA1 germline mutation, MI status, and LOH at the BRCA1 locus in 13 BRCA1 mutated breast tumours from 10 unrelated French families.

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### Table 1

<table>
<thead>
<tr>
<th>Family # (cases)</th>
<th>Mutation</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>85 (1,2)</td>
<td>Arg1443ter</td>
<td>16</td>
</tr>
<tr>
<td>279 (3)</td>
<td>Cys812ter</td>
<td>16</td>
</tr>
<tr>
<td>415 (4)</td>
<td>3958del5ins4</td>
<td>15</td>
</tr>
<tr>
<td>588 (5a,5b)</td>
<td>Cys647Tyr</td>
<td>16</td>
</tr>
<tr>
<td>1295-011 (6)</td>
<td>1670delT</td>
<td>*</td>
</tr>
<tr>
<td>153 (7a,7b)</td>
<td>Gln1280ter</td>
<td>†</td>
</tr>
<tr>
<td>192 (8), 201 (10)</td>
<td>5382insC</td>
<td>†</td>
</tr>
<tr>
<td>126 (9), 142 (11)</td>
<td>Glu1753ter</td>
<td>†</td>
</tr>
</tbody>
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Figure 1 (A) Schematic representation of the wild type BRCA1 protein with the regions involved in DNA repair shown in black: (a) zinc finger domain (AA 20-68), (b) interaction with Rad51 (AA 758-1064), (c1 and c2) BRCT motifs (respectively AA 1699-1736 and AA 1818-1855). (B) Schematic representation of the BRCA1 mutated proteins generated by germline mutations in the 10 French breast/ovarian cancer families studied. *A Hardouin, personal communication, †C Maugard, personal communication.
Germline mutations were found by direct sequencing or by the protein truncation test followed by sequencing, (C M Maugard, personal communication, A Hardouin, personal communication). Nine mutations generate truncated proteins and one is a missense mutation which induces a critical change of amino acid in the zinc finger domain (fig 1B). All these alterations disrupt at least one of the four BRCA1 DNA repair domains. Tumour DNA was obtained from paraffin embedded tissues fixed in formalin after microdissection of the tumour foci. The corresponding normal DNA was obtained from peripheral blood lymphocytes. DNA extraction was performed as described previously. Microsatellite analysis was performed with 10 markers, located on eight different chromosomes (fig 2). The majority of the microsatellites studied were tri- and tetrancleotide repeats which have previously shown instability in breast tumours. Three intragenic markers at the BRCA1 locus have been used to identify both MI and LOH of a BRCA1 allele. The Bat-26 repeat was used as a marker for the RER+ phenotype. PCR conditions and microsatellite size analysis (fluorescent technology using a 373A DNA Sequencer, Perkin Elmer) have been described previously. Among the 10 informative cases (10/13), nine tumours (90%) exhibited LOH at one or more intragenic BRCA1 markers (fig 2), confirming the anti-oncogenic nature of the BRCA1 gene. Only one informative tumour has lost no marker. Among the 13 tumours, two cases (tumours 3 and 10, 15.4%) showed MI. This result has been confirmed by duplicate experiments on the same tumour DNA and on an independent extraction. MI corresponds to the appearance of a new allele in tumour DNA different in size from the normal alleles, that is, tumour 3 D17S1323 −2 bp, tumour 10 Mfd 855 −6 bp, DM-1 +15 bp. This does not confirm the frequency of instability observed by Glebov et al, but, since no information concerning the hereditary predisposition was provided, it is possible that the familial tumours studied were not linked to the BRCA1 gene.

It is noteworthy that the mutation identified in tumour 3 led to a protein lacking both BRCT domains and part of the Rad51 interacting region. It is conceivable that this more severe alteration explains the presence of MI, in comparison with proteins lacking only the BRCT domains. Nevertheless, tumour 10, which shows MI at two loci, lacks only half of the BRCT c2 domain and in tumour 6, which lacks all BRCT and Rad51 interaction domains, no MI was found. Finally, as contraction of the Bat-26 marker is found in nearly all RER+ tumours, the absence of instability at this locus in our tumours suggests that none of these exhibit a RER+ phenotype. Thus, it is possible that the observed instabilities may be fortuitous events.

In conclusion, MI seems to correspond to a rare event in BRCA1 breast tumours and the frequency found (15.4%) is similar to that previously reported in sporadic breast cancers. Our results suggest that MI in breast tumours may not be attributable to the inactivation of the potential BRCA1 DNA repair function. Nevertheless, loss of the BRCA1 DNA repair function might favour another type of genomic instability, leading either to activation of oncogenes or inactivation of anti-oncogenes, in agreement with the putative caretaker function of the BRCA1 gene.

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