Loss of heterozygosity in sporadic breast tumours at the **BRCA2** locus on chromosome 13q12–q13

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**Summary** Loss of heterozygosity (LOH) on chromosome 13 occurs on 25–30% of breast tumours. This may reflect the inactivation of the retinoblastoma susceptibility gene **RB1**. However, recently another candidate tumour-suppressor gene has been identified on chromosome 13 by linkage analysis, the breast cancer susceptibility gene **BRCA2**. To investigate the involvement of **BRCA2** in sporadic breast cancer 200 breast tumours were tested for LOH on chromosome band 13q12–q14, using 11 highly polymorphic microsatellite markers. LOH was found in 65 tumours, which all showed simultaneously loss of **BRCA2** and **RB1**. Of 12 breast tumour cell lines tested with polymorphic microsatellite markers, seven showed a contiguous region of homozygosity on 13q12–q14, suggesting LOH in the tumour from which the cell line had been derived. One cell line showed homozygosity in the **BRCA2** region and heterozygosity at **RB1**. This is the only indication that **BRCA2** is a distinct target for LOH on chromosome 13 in addition to **RB1**.

**Keywords**: **BRCA2**, **RB1**; loss of heterozygosity; breast cancer; tumour-suppressor gene

Loss of heterozygosity (LOH) on chromosome 13 occurs in approximately 25% of primary breast tumours (Devilee and Cornelisse, 1994). As LOH is thought to reflect the inactivation of one allele of a tumour-suppressor gene, the retinoblastoma susceptibility gene (**RB1**), present on chromosome band 13q14 could be the target for these LOH events. Structural abnormalities in **RB1** have been reported in primary breast cancers and in breast cancer cell lines (Lee et al., 1988; T’Ang et al., 1988; Varley et al., 1989). However, allele loss at the **RB1** locus in breast and ovarian cancers is not correlated with loss of **RB1** protein expression (Borg et al., 1992; Dodson et al., 1994; Kim et al., 1994). This suggests the presence of another tumour-suppressor gene adjacent to **RB1**.

The breast cancer susceptibility gene, **BRCA2**, has recently been localised to 13q12–q13 by genetic linkage analysis (Wooster et al., 1994a). In common with the **BRCA1** gene on chromosome 17q21 (Miki et al., 1994), mutations in **BRCA2** confer susceptibility to early-onset breast cancer in women and to ovarian cancer, but the latter risk is probably lower than for **BRCA1**. In contrast to **BRCA1**, **BRCA2** is associated with a substantially increased risk of breast cancer in men.

To investigate further the roles of **BRCA2** and **RB1** in breast cancer development we have determined LOH in the 13q12–q14 chromosomal region in 200 sporadic breast carcinomas using 11 polymorphic microsatellite markers.

**Materials and methods**

The 13q12–q14 polymorphic microsatellite markers used were D13S289, D13S250, AFM238z9, AFM109z9, D13S260, D13S171, D13S267, D13S219, D13S218, D13S155 and D13S153 (within **RB1**). (Gyapay et al., 1994; J Weissenbach, personal communication). Polymerase chain reaction (PCR) was performed as described previously (Abeln et al., 1994; Wooster et al., 1994b). The PCR products were electrophoresed on denaturing polyacrylamide gels and visualised by autoradiography. Signal intensities were measured on a Molecular Dynamics PhosphorImager 445 Si. Molecular Dynamics ImageQuaNT Software was used for quantification of PCR products. The allelic imbalance (AI) factor is the quotient of the peak ratio from tumour and constitutional DNA. An AI factor of 1.5 or lower is interpreted as retention of heterozygosity, whereas an AI of 2.0 or higher signifies LOH (Devilee et al., 1994).

DNA was isolated from 200 freshly frozen breast tumours and 12 breast tumour cell lines. The corresponding constitutional DNA was obtained from peripheral blood samples or from skin samples. The breast tumour cell lines used in this study are listed in Table I. The tumour cell lines had no corresponding normal DNA with which to compare the results. Therefore the published allele frequencies of each allele at all markers were used to calculate the probability of the wild-type DNA being homozygous at each locus. It was then possible to calculate the probability of the DNA being homozygous throughout the **BRCA2** and **RB1** regions in the germline DNA (Table I), e.g. the probability that cell line MDA157 has a contiguous zone of homozygosity for the markers D13S260, S171, S267, S219 and S218 is 1:895 656.

**Results and discussion**

Table I represents the results obtained from the LOH analysis of 200 sporadic breast carcinomas using 11 polymorphic microsatellite markers in the 13q12–q14 chromosomal region. In total 65 tumours (32.5%) show LOH for at least one informative marker on chromosome 13q. The results confirm cumulative results of LOH on 13q from several literature reports, i.e. 25% LOH in 450 informative breast tumours (Devilee and Cornelisse, 1994).

Evaluation of the role of somatic mutations of **BRCA2** in sporadic cancers is complicated by the presence of the **RB1** gene in the vicinity. Since **RB1** shows structural abnormalities in 15% of the primary breast tumours tested (T’Ang et al., 1988; Varley et al., 1989), much of the LOH on 13q could be directed at **RB1**. However, expression of the **RB1** gene on chromosomal band 13q14 in tumours of the breast or the ovary does not correlate with LOH on chromosome
Absence of RBJ staining is found in 15% of the breast tumours tested, and detected predominantly in tumours without LOH on 13q (Kim et al., 1994). The incongruity between LOH in the RBJ chromosomal region and RBJ expression may reflect the involvement of BRCA2 as a target for LOH.

The results shown in Table II indicate that all tumours which showed LOH at RBJ also showed LOH within the BRCA2 region. Similarly, all tumours which showed LOH at

![LOH results for the three tumours with a breakpoint in the BRCA2 region. Phospholmager traces are shown for those markers that determine the breakpoints and for several adjacent markers. The upper graph represents the signal intensities for the two alleles in constitutional DNA, the lower graph is tumour DNA. The peaks representing the two alleles are indicated '1' and '2'. L, loss of heterozygosity; R, retention of heterozygosity; N, not informative; NA, no available data. Numbers in the graphs signify the allelic imbalance factors, e.g. marker D13S267 in tumour BT19 has lost allele '1' and the allele imbalance factor is 4.6.](image-url)
Table I Results of 12 breast tumour cell lines tested with microsatellite markers in the 13q12–q14 region: allele frequencies are shown between brackets for cell lines with a contiguous stretch of homozygous markers

| Cell line     | AFM       | D13S260 | D13S171 | D13S267 | D13S219 | D13S218 | D13S155 | D13S153 |
|---------------|-----------|---------|---------|---------|---------|---------|---------|---------|
| MDA-MB-157    | (0.24)    | (0.11)  | (0.01)  | (0.29)  | (0.01)  | (0.35)  |         |         |
| MDA-MB-231    | (NA)      | (0.01)  | (0.01)  | (0.44)  | (0.36)  | (0.42)  |         |         |
| MDA-MB-361    | (0.02)    | (0.09)  | (0.32)  | (0.29)  | (0.36)  | (0.42)  |         |         |
| MDA-MB-468    | (0.16)    | (0.04)  | (0.32)  | (0.29)  | (0.36)  | (0.14)  |         |         |
| T47D          | (0.28)    | (0.13)  | NA      | (0.44)  | (0.36)  | (0.14)  |         |         |
| BT20          | (0.28)    | (0.41)  | (0.32)  | (0.17)  | (0.36)  | (0.14)  |         |         |
| BT474         | (0.17)    | (0.11)  | (0.32)  | (0.17)  | (0.36)  | (0.14)  |         |         |
| MDA-MB-415    |          |         |         |         |         |         |         |         |
| SKBR3         |          |         |         |         |         |         |         |         |
| DU44-77       |          |         |         |         |         |         |         |         |
| MCF7          | NA        |         |         |         |         |         |         |         |
| ZR-75-1       | (0.16)    | (0.41)  | (0.32)  | (0.17)  | (0.36)  | NA      |         |         |

-□, Heterozygous; ■, homozygous; NA, no available data.

Table II LOH results in the 13q12–q14 region obtained with 11 microsatellite markers in 200 breast tumours

| Category                     | No. of tumours | Percentage |
|------------------------------|---------------|------------|
| Retention of all 13q markers | 135           | 67.5       |
| LOH of all informative markers | 62            | 31         |
| LOH only in the BRCA2 region | 0             | 0          |
| LOH only in the RBI region | 0             | 0          |
| Breakpoint within the BRCA2 region | 3 | 1.5 |

BRCA2 showed LOH at RBI. Analyses using markers between the two loci (D13S218 and D13S219) indicate that the allele losses involve a contiguous region of the chromosome including both BRCA2 and RBI. Since LOH usually involves large chromosomal regions and because BRCA2 and RBI are only 20 cm apart, the loss of both loci has most probably occurred through a single genetic event. It is, however, possible that a growth advantage is conferred upon neoplastic cells by simultaneous inactivation of both genes. Either way, our results do not clarify whether BRCA2 or RBI is the predominant target of the LOH on chromosome 13q.

Three tumours were identified which showed a transition from retention of heterozygosity to LOH at an adjacent marker, defined as a 'breakpoint', within the BRCA2 region as defined by linkage analysis (Wooster et al., 1994a). BT19 had a breakpoint between D13S260 and D13S171. BT42 and BT589 were not informative for D13S171. They showed a breakpoint between D13S260 and D13S267. Figure 1 shows the allelic imbalance factors and the Phosphorimager traces of the PCR products obtained from these tumours using the markers that border the breakpoints. On the assumption that BRCA2 is the target for LOH in these tumours the results suggest that the candidate region for this gene is now decreased to 3 cm and defined by D13S260 (proximal) and D13S267 (distal). However, since all three breakpoint tumours also show LOH of RBI, and hence RBI may be the target of LOH, this mapping information may be misleading.

In addition to the 200 primary breast tumours, 12 breast cancer cell lines were examined. Although corresponding DNA from non-neoplastic tissue was not available, each polymorphic marker used is heterozygous in a minimum of 64% of the cases (and usually more). It is therefore extremely unlikely that an individual will be constitutionally homozygous for all polymorphisms in the chromosome 13q12–q14 region. Since 7 of the 12 cell lines were homozygous at all markers examined it is likely that allele loss has occurred during development of the tumour or subsequently during culture in vitro. Table I shows the cell lines and the allele frequencies for cases with a contiguous region of homozygosity. The high frequency of plausible allele loss in the RBI region in cell lines (7 out of 12, 58%) compared with that in primary tumours (32.5%) corresponds well with the observation of T'Ang et al. (1988) that rearrangements in RBI occur more frequently in breast cancer cell lines than in primary breast tumours, suggesting an enhancement of cell line establishment as a result of RBI inactivation.

Of particular interest is ZR-75-1. This cell line is homozygous at all markers within or adjacent to the BRCA2 region but is heterozygous at both polymorphisms close to RBI, D13S155 and D13S153. The probability of such a contiguous zone of homozygosity in the BRCA2 region is less than 1:4000. This number is the multiplication sum of the allele frequencies for all markers in the BRCA2 region (Table I) and additionally from marker D13S220 (located between D13S267 and D13S219), of which the frequency for the allele in ZR-75-1 is 0.18. The results therefore suggest that this individual was constitutionally heterozygous at at least one marker but that LOH has occurred in the BRCA2 region and not at RBI. Consequently ZR-75-1 is the only breast cancer in this series indicating that BRCA2 is a distinct target for LOH on chromosome 13q in addition to RBI. However, we cannot exclude that LOH in the BRCA2 region occurred during cell line establishment and was not present in the primary tumour. The results from ZR-75-1 do not decrease the candidate region for BRCA2.

We have recently analysed allele loss on chromosome 13q in breast cancers from a family showing strong evidence of linkage to BRCA2. Seven out of eight informative tumours showed LOH and in all cases it was the wild-type allele that was lost (Collins et al., 1995). This observation in familial breast cancer and the presence of LOH on 13q above background rates in sporadic breast cancer support the hypothesis that BRCA2 is inactivated during oncogenesis. However, the role of mutations of BRCA2 in sporadic breast cancer remains unclear. The results presented here do not support or exclude the possibility that BRCA2 is the target of LOH on chromosome 13q in sporadic breast cancer.

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