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Prognostic value of loss of heterozygosity at *BRCA2* in human breast carcinoma

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Summary To confirm several recent studies pointing to loss of heterozygosity (LOH) at *BRCA2* as a prognostic factor in sporadic breast cancer, we examined this genetic alteration in a large series of human primary breast tumours for which long-term patient outcomes were known. LOH at *BRCA2* correlated only with low oestrogen and progesterone receptor content. Univariate analysis of metastasis-free survival and overall survival (log-rank test) showed no link with *BRCA2* status ($P = 0.34$, $P = 0.29$ respectively). LOH at *BRCA2* does not therefore appear to be a major prognostic marker in sporadic breast cancer.

Keywords: *BRCA2*; loss of heterozygosity; prognostic value; breast cancer

Breast cancer, one of the most common life-threatening diseases in women, occurs in hereditary and sporadic forms. The two major breast cancer susceptibility genes, *BRCA1* and *BRCA2*, were recently isolated (Miki et al, 1994; Wooster et al, 1995; Couch et al, 1996). Both are considered to be tumour-suppressor genes and are thought to be inactivated by a 'two-hit' mechanism originally proposed by Knudson to explain the tumorigenesis of retinoblastoma. In hereditary cancer, the first hit would be a germline mutation in a specific cancer gene, whereas in sporadic cancer the first hit would be a somatic mutation or another inactivating molecular event. The second hit would be a loss of the second gene copy in the somatic cell, in both hereditary and sporadic forms. A number of germline mutations in the *BRCA1* and *BRCA2* genes have been identified in families prone to breast cancer (Shattuck-Eidens et al, 1995; Couch et al, 1996, Phelan et al, 1996). In sporadic forms, somatic *BRCA2* mutations, like somatic mutations in the *BRCA1* gene, are rare (Lancaster et al, 1996; Miki et al, 1996; Teng et al, 1996). However, aberrant subcellular location (Chen et al, 1995) and reduced expression of *BRCA1* (Thompson et al, 1995), together with high frequencies of loss of heterozygosity (LOH) on 17q12–q21 and 13q12–q13 (sites of *BRCA1* and *BRCA2*) (Bièche and Lidereau, 1995), point to a significant role of these two genes in the tumorigenesis of sporadic breast cancer, but through a mechanism other than structural mutation. LOH on 13q12–q13 occurs in 30–60% of somatic breast tumours (Cleton-Jansen et al, 1995; Kerangueven et al, 1995; Beckmann et al, 1996; Hamann et al, 1996; Kelsell et al, 1996). These LOH studies identified a consensus region of deletion involving *BRCA2* and excluding the RB1 locus. Several studies have pointed to a link between *BRCA2* inactivation (mutation and/or LOH) and Scarff, Bloom and Richardson (SBR) histopathological grade 3, in both hereditary and sporadic forms of breast cancer, suggesting the involvement of this gene in the aggressiveness of breast tumours (Bignon et al, 1995; Beckmann et al, 1996; Kelsell et al, 1996). Recently, in a pilot study, LOH at *BRCA2* was found to be an independent prognostic factor (van den Berg et al, 1996). However, this study involved a heterogeneous population of 84 primary tumours from both familial ($n = 45$) and sporadic ($n = 39$) cases of breast cancer.

To confirm this pilot study, we examined a larger series of human primary sporadic breast tumours ($n = 102$) with longer follow-up.

We reviewed excised primary breast tumours from 102 women treated at the Centre René Huguenin from 1977 to 1989. These patients (mean age 57 years; range 34–86) met the following criteria: primary unilateral invasive breast carcinoma; no other primary cancer or metastasis (supraclavicular nodes included); no radiotherapy or chemotherapy before surgery; and complete clinical, histological and biological data. According to the 1979 UICC criteria, 11 women were in stage I, 70 in stage II, 19 in stage IIIa and two in stage IIIb. The main tumour characteristics are presented in Table 1. Oestrogen and progesterone receptor assays were performed using the method described by the European Organization for Research and Treatment for Cancer (EORTC, 1980), with a detection limit of 10 fmol mg^{-1} cytosolic protein. Eighty (78.4%) tumours were infiltrating ductal carcinomas. All the patients underwent a physical examination and routine chest radiography every 3 months for the first 2 years and annually thereafter. Liver scintigraphy, bone scans and mammograms were performed annually. The median follow-up was 9 years (range 1.4–16.2). The cut-off date for the analysis was January 1996. All but two of the 27 deaths were related to breast cancer; 37 patients relapsed (eight local and/or regional recurrences, 22 metastases, three both and four contralateral breast tumours). Two second invasive cancers occurred. Overall survival (S) was based on the time from diagnosis to breast cancer-related death; metastasis-free survival (MFS) on the time from diagnosis to detection of the first metastasis or to breast cancer death without apparent metastasis. The univariate analysis of MFS (log-rank test) is reported in Table 1; lymph node status was the only classical explanatory variable associated with MFS.

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Table 1 Characteristics of the 102 patients and relation to metastasis-free survival

		Metastasis-free survival		
	Number of patients (%)	Number of events[a]	Five-year rate[b] (s.e.)[c]	*P*-value[d]
Menopausal status				NS
Premenopausal	42 (41.2)	15	83.3 (5.7)	
Post-menopausal	60 (58.8)	21	84.4 (4.8)	
Histological grade[e]				NS
I	11 (11.6)	3	100	
II	49 (51.6)	20	77.0 (6.1)	
III	35 (36.8)	13	85.0 (6.2)	
Lymph node status				0.022
Node-negative	33 (32.4)	5	84.5 (6.4)	
Node-positive	69 (67.6)	31	83.7 (4.5)	
ER status				NS
+ (≥ 10 fmol mg^{-1})	35 (34.3)	12	79.2 (7.0)	
− (< 10 fmol mg^{-1})	67 (65.7)	24	86.3 (4.2)	
PR status				NS
+ (≥ 10 fmol mg^{-1})	44 (43.1)	15	83.5 (5.7)	
− (< 10 fmol mg^{-1})	58 (56.9)	21	84.2 (4.8)	
Macroscopic tumour size				NS
≤ 30 mm	67 (69.1)	23	83.1 (4.6)	
> 30 mm	30 (30.9)	11	82.9 (7.0)	

[a]First metastasis, or breast cancer-related death without apparent metastases; [b]Kaplan–Meier estimate; [c]standard error; [d]log-rank test; [e]Scarff–Bloom–Richardson classification.

Table 2 Relationship between LOH at *BRCA2* and the standard clinicopathological and biological factors

	BRCA2 LOH(%)	*P*-value[a]
Total	42.4	
Histological grade[b]		NS
I	27.3	
II	39.6	
III	48.6	
Mitotic index		0.017
I	18.2	
II	25.0	
III	52.5	
Lymph node status		NS
Node-negative	41.9	
Node-positive	42.6	
ER status		0.009
+ (≥ 10 fmol mg^{-1})	32.8	
− (< 10 fmol mg^{-1})	60.0	
PR status		0.050
+ (≥ 10 fmol mg^{-1})	33.9	
− (< 10 fmol mg^{-1})	53.5	
Macroscopic tumour size		NS
≤ 30 mm	39.4	
> 30 mm	44.8	

[a]Chi-square test; [b]Scarff–Bloom–Richardson classification.

Immediately following surgery the tumour samples were stored in liquid nitrogen until extraction of high-molecular-weight DNA. Patients were included in this study if the tumour sample used for DNA preparation contained more than 60% of tumour cells by histological analysis. A blood sample was also taken from each patient. DNA was extracted from frozen tumour tissue and blood leucocytes of each patient using standard methods (Maniatis et al, 1982).

The carcinomas were screened with three polymorphic microsatellite DNA marker loci flanking *BRCA2* (D13S260, D13S171, D13S267) to identify the maximum number of patients informative for at least one locus.

PCR was run in a total volume of 50 µl, with 50 ng of genomic DNA, 20 mM of each primer, 1.5 mM magnesium chloride, 0.1 mM of each deoxynucleotide triphosphate and one unit of *Taq* DNA polymerase. Microsatellite markers were assayed by PCR amplification of genomic DNA. The annealing temperature, number of amplification cycles and extension time were adapted to each primer set. One microlitre of product was mixed with 3 µl of denaturing loading buffer and heat denatured, then 1.5-µl aliquots of each sample were loaded on 6% acrylamide gels containing 7.5 M urea. DNA was then transferred to nylon membrane filters. The CA repeat probe was labelled with [^{32}P]dCTP using terminal deoxynucleotidyl transferase. The membrane filters were hybridized overnight at 42°C with the labelled probe, washed and autoradiographed at –80°C for an appropriate period.

Normal DNA samples that were polymorphic at a given locus were considered to be 'informative', whereas homozygotes were considered 'uninformative'. Only cases of constitutional heterozygosity were used in the evaluation of LOH. The signal intensity of the polymorphic alleles was determined by visual examination (three observers) and confirmed by means of densitometry. The results of all the scanned samples were in direct agreement with the initial visual scoring. LOH was considered to occur when the intensity of the allele in tumour DNA was less than 40% of that in corresponding normal tissue DNA (peripheral blood lymphocytes). LOH was partial, in most cases the band being fainter than the conserved allele but still visible. Such partial losses are due either to contaminating normal tissue or to tumour heterogeneity.

LOH at *BRCA2* was found in 42.4% of 99 informative (heterozygous) tumour DNAs. Table 2 gives detailed results of the correlations between LOH at *BRCA2* and the standard prognostic parameters including macroscopic tumour size, histological grade and lymph node or steroid receptor status. No link between *BRCA2* status (LOH vs normal) and macroscopic tumour size or lymph node status was found (χ^2 analysis). Oestrogen and progesterone receptor negativity were both associated with a higher percentage of LOH at *BRCA2* ($P = 0.009$, $P = 0.05$ respectively), in agreement with van den Berg et al (1996). Although no link was found between the percentage of LOH at *BRCA2* and Scarff–Bloom–Richardson (SBR) histopathological grade ($P = 0.42$), there was a correlation with the mitotic index, which is one of the three components of the SBR classification ($P = 0.017$). This was in partial agreement with previous reports of a link between LOH at *BRCA2* and SBR histopathological grade III (Beckmann et al, 1996; Kelsell et al, 1996). Univariate analysis of MFS and S (log-rank test) showed no link with *BRCA2* status ($P = 0.34$, $P = 0.29$ respectively). Even if the metastasis-free survival of 42 patients with LOH at *BRCA2* was slightly shorter than that of 57 patients without LOH [5-year MFS 75.7% (s.e. = 6.7%) vs 89.2% (s.e. = 4.2%)], this was not the case of overall survival [5-year S 92.7% (s.e. = 4.1%) vs 91.0% (s.e. = 3.9%)].

Our results do not support the notion that inactivation of a tumour-suppressor gene located at 13q12–q13 (*BRCA2* or another gene) is a major prognostic marker in breast cancer.

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