Low expression of bcl-2 in Brca1-associated breast cancers

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Summary Little data are available concerning the molecular mechanisms of action of Brca1 and Brca2 in breast oncogenesis. Recent experimental results suggest that Brca1 plays a role in the regulation of apoptosis. In order to determine whether the analysis of human tumours would provide data supporting this hypothesis, we have assessed the expression of the antiapoptotic bcl-2 and of the proapoptotic p53 genes in Brca1- and Brca2-associated breast carcinomas. The levels of expression of these genes were compared to those observed in controls and to the mitotic and the apoptotic indexes. Our series were composed of 16 cases of breast carcinoma in women with a germline Brca1 gene mutation, and of four cases with Brca2 mutation. A group of 39 patients aged under 36 years and for whom the search for Brca1 gene mutations was negative, and a group of 36 cases of sporadic cancers without data on their Brca status were used as controls. Immunohistochemistry was used to detect p53 and bcl-2 gene products. Mitotic and apoptotic indexes were higher in Brca1-associated tumours than in controls. No significant difference in p53 immunostaining was observed between the four groups of patients. In contrast, the rate of bcl-2-positive tumours was lower (31%) in Brca1-carcinomas than in carcinomas without Brca1 mutation (90%) (P < 10–3). A strong Bcl-2 expression was found in the four cases of Brca2-associated carcinomas. No significant correlation was observed between p53 and Bcl-2 immunostainings, either in cases or in controls. The association between Brca1 status and Bcl-2 expression remained significant after adjustment for the oestrogen receptor status. Our study shows that a low expression of bcl-2 characterises most Brca1-associated breast carcinomas, a biological trait which seems not to be shared by Brca2-associated tumours nor to be related to oestrogen receptor and/or p53 status. bcl-2 might thus be one of the target genes involved in the oncogenesis related to Brca1 and its down-regulation may account for the increased apoptosis and the high proliferative rate observed in Brca1-associated carcinomas. © 2000 Cancer Research Campaign

Keywords: Brca1; Brca2; bcl-2; proliferation; apoptosis; breast cancer

Brca1 and Brca2 germline mutations are frequently observed in women presenting breast carcinoma as part of an hereditary cancer susceptibility syndrome (Ford et al., 1998). The histological phenotype of Brca-associated breast tumours differs from that of sporadic cases: Brca1-associated tumours generally present as poorly differentiated and highly mitotic carcinomas (Jacquemier et al., 1995; Lakhani et al., 1998) whereas Brca2 tumours were reported to present a high (Agnarsson et al., 1998) or a low (Lakhani et al., 1998) mitotic index. However, no characteristic immunophenotype of these lesions has been reported so far (Robson et al., 1998), and little data are available regarding the biological mechanisms involved in the development of tumours associated with Brca1 or 2 gene mutations. Using animal models, it was recently reported that the disruption of the Brca1 alleles specifically in mouse mammary epithelium leads to increased apoptosis and to tumour formation (Xu et al., 1999). These findings prompted us to analyse the expression of the antiapoptotic bcl-2 and of the proapoptotic p53 genes in a series of Brca1- and Brca2-associated breast carcinomas, together with mitotic and apoptotic indexes, and to compare these values to those obtained in sporadic cancers.

This analysis showed that, whereas no difference in p53 expression was observed between the different groups, Brca1-associated tumours displayed a low rate of bcl-2 expression and high mitotic and apoptotic indexes. The decrease in bcl-2 expression was found to be independent from hormone receptor status and may account for the high apoptotic and proliferative rates observed in Brca1-associated tumours.

MATERIAL AND METHODS

Sixteen women affected with a breast carcinoma before the age of 36 were detected to be carriers of a germline Brca1 mutation through the Brca1 analysis of a series of 123 early-onset consecutive cases as reported in a previous study (Ansquer et al., 1998). The corresponding 16 tumours were analysed and correspond to the group of cases. A first control group was constituted by a series of 36 cases from the early-onset series identified as non-Borca1 carriers. Tumours of 36 sporadic cases, untested for Brca1 and Brca2 mutation, constituted a second control group. The former group has been chosen to avoid a possible bias related to the fact that tumours occurring in young women might present a characteristic immunophenotype independently from their Brca status. In addition, the tumours of four women belonging to the early-onset breast carcinomas series and identified as carrier of a germline Brca2 mutation were also studied. p53 and bcl-2 gene expression was assessed by immunohistochemistry using anti-p53 (D0–7) and...
anti-Bcl-2 (124) monoclonal antibodies (Dako A/S, Glostrup, Denmark). The threshold of positivity was 5% of malignant stained cells for p53 and 1% for Bcl-2. Proliferation rate was determined by counting the number of mitotic features in ten successive microscopic fields at G × 400, as recommended for the evaluation of the Nottingham histological grade (Elston and Ellis, 1991; Genestie et al., 1998). Apoptotic index was assessed on haematoxylin–eosin-coloured histological sections by counting nuclear features characterising the apoptotic process (Bessis, 1964; Cummings et al., 1997). In every case, a tumour area presenting the highest number of apoptotic features was determined by counting the number of invasive malignant cells. The counting was performed on successive fields when less than 100 invasive malignant cells were present on a microscopic screen and scored using an image analysis system (Perfect Image; Clara Vision, Orsay, France). The apoptotic index was defined as the ratio between this count and the total number of invasive malignant cells.

Our analysis showed that a higher histological grade (P = 3.10–4), a higher mitotic index (P = 9.10–3), a higher apoptotic index (P = 2.10–3) and a lower rate of oestrogen receptor positivity (P = 10–3) were observed in Brca1-associated breast cancers than in controls (Table 1). A slight positive correlation was found between mitotic and apoptotic indexes (r = 0.28, P = 0.045).

The immunohistochemical analysis found no significant difference in p53 immunostaining between the four groups of tumours. In contrast, a significant difference in Bcl-2 expression was found between cases and either control groups (Figure 1). The rate of bcl-2-positive tumours was lower (31%) in Brca1-associated carcinomas than in sporadic tumours (77%) (P = 2.10–3). This difference was even more striking in comparison with the rate of bcl-2-positive tumours observed in young women without Brca1 mutation (90%) (P < 10–5). The four cases of Brca2-associated carcinoma were found to be positive. The mean number of labelled cells was 56% in the group of sporadic tumours and 45% in tumours with no Brca1 mutation. In contrast, a complete absence of labeling was observed in 11 of the 16 cases of Brca1-associated carcinomas. A weak staining of 5% of the tumour cells was observed in one case whereas the remaining four cases exhibited 40–80% of labelled cells. The negative association of bcl-2 expression and Brca1-mutation remained significant after

**RESULTS**

| Parameters      | Cases | Controls |  |  |
|-----------------|-------|----------|---|---|
|                  | Brca1 | Brca2 | No Brca1 | Sporadic |
| Number of cases | 16    | 4      | 39        | 36        | – |
| Patient’s age    |       |        |           |           |   |
| median           | 34    | 50.5   | 33        | 62.5      | – |
| mini–maxi       | 25–47 | 49–52  | 21–36     | 31–80     |   |
| Tumour size     |       |        |           |           |   |
| ≤20 mm          | 5 (31.2%) | 2 (50%) | 17 (43.6%) | 9 (25%) | ns |
| >20 mm          | 11 (68.8%) | 2 (50%) | 22 (56.4%) | 27 (75%) |   |
| Node status     |       |        |           |           |   |
| N0              | 6 (37.5%) | 3 (75%) | 26 (66.7%) | 26 (72.2%) | 4.6×10–2 |
| N1              | 10 (62.5%) | 0      | 13 (33.3%) | 9 (25%) | ns |
| N2              | 0      | 1 (25%) | 0         | 1 (2.8%) |   |
| Histological grade |      |        |           |           |   |
| I               | 0      | 0      | 3 (7.7%)  | 7 (19.4%) |   |
| II              | 4 (25%) | 1 (25%) | 27 (69.2%) | 16 (44.4%) | 3.10–4 |
| III             | 12 (75%) | 3 (75%) | 9 (23.1%)  | 13 (36.2%) |   |
| Mitotic indexa  | 14.5   | 5.5    | 4         | 8         | 9.10–3 |
| Apoptotic indexb| 10     | 9      | 7         | nd        | 2.10–2 |
| Hormonal status |       |        |           |           |   |
| ER-positive     | 3 (21.4%) | 4 (100%) | 27 (71.1%) | 26 (74.3%) | 10–3 |
| PR-positive     | 7 (46.7%) | 3 (75%) | 28 (73.7%) | 23 (65.7%) | ns |
| nd              | 3      | 1      | 1         | 1         |   |
| Bcl-2 status    |       |        |           |           |   |
| positive cases  | 5 (31%) | 4 (100%) | 35 (90%)  | 27 (77%) | <10–3c |
| PS3 status      |       |        |           |           |   |
| positive cases  | 9 (56.2%) | 0      | 24 (61.5%) | 13 (38.2%) | ns |

*a*Brca1 cases vs no Brca1 mutation; *b*median of indexes; *c*Chi-squared test with Yate’s correction; nd = not determined; ns = not significant

### Table 1

Clinicopathological characteristics of patients and tumours according to the Brca status

© 2000 Cancer Research Campaign British Journal of Cancer (2000) 83(10), 1318–1322
adjustment on oestrogen receptor status. In multivariate logistic regression analysis, low expression of bcl-2 (odds ratio (OR) 13.9; 95% CI 2.6–76.9) and high histological grade (OR 7.1; 95% CI 1.3–38.8) were significant predictive variables associated with the presence of Brca1 gene mutation (Table 2). No significant correlation was observed between p53 and Bcl-2 immunostaining, either in cases or in controls.

**DISCUSSION**

Our study shows that most Brca1-associated breast carcinomas are characterized by high mitotic and apoptotic rates, and by a decrease of bcl-2 expression. These biological traits seem not to be shared by Brca2-associated tumours and not related to oestrogen receptor and/or to p53 status. In an analysis of the immunophenotype of Brca-associated tumours, only a slight decrease of bcl-2 expression was found in familial cancers in comparison with the level observed in controls (Robson et al, 1998). However, in this work, Brca1- and Brca2-tumours were included in the same group, and this could account for the negativity of the result. Recent data emphasize that different molecular pathogenetic processes characterize Brca1- and Brca2-associated tumours (Armes et al, 1999), and this is in agreement with our data on distinctive bcl-2-expression in these two tumour types.

High levels of bcl-2 expression have been observed in 75–80% of cases of sporadic breast cancers (Leek et al, 1994; Silvestrini et al, 1994; Hellemans et al, 1995). This expression was found to be associated with the presence of favourable prognostic features (Joensuu et al, 1994) and with the expression of oestrogen and progesterone receptors (Barghava et al, 1994; Gee et al, 1994; Leek et al, 1994; Silvestrini et al, 1994; Hellemans et al, 1995; Charpin et al, 1997).

Since Brca1-associated breast carcinomas are frequently oestrogen receptor negative, poorly differentiated tumours, and since a positive association exists between the respective expression of oestrogen receptor (ER) and bcl-2, the down-regulation of bcl-2 in Brca1-associated carcinomas might merely reflect the down regulation of ER in this type of tumour. In our study, the loss of bcl-2 expression characterizing Brca1-associated carcinomas behaved as a biological trait independent from the ER status of the tumours. Definitive conclusions cannot be drawn from this multivariate analysis considering the limited number of cases included in this series. However, it is worth mentioning that in multivariate analyses of prognostic factors characterizing large series of axillary node-positive breast tumours, the lack of bcl-2 expression was found associated with a poor outcome of the disease, independently from the ER status (Hellemans et al, 1995; Berardo et al, 1998).
In conclusion, a low bcl-2 expression and high proliferative and apoptotic rates characterize most Brca1-associated breast carcinomas. The analysis of a large series of cases including the assessment of the expression of bcl-2, of Brca1 and of biological parameters associated with cell death and proliferation, should help to determine whether alterations of the functions of Brca1 gene product were responsible for the down-regulation of bcl-2, and thus for the high proliferative rate and increased apoptosis observed in Brca1-associated carcinomas.

ACKNOWLEDGEMENTS

We thank Dr Jérôme Couturier for helpful discussion. This work was supported in part by a grant from the Lion’s Club (Moulinsl/s’Allier).

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Table 2 Association of biological parameters with Brca1 gene mutation in logistic regression model

| Biological parameters | OR   | 95% CI | P value |
|-----------------------|------|--------|---------|
| Bcl-2 expression      |      |        |         |
| high                  | 1    |        |         |
| low                   | 13.9 | 2.6–76.9 | 0.002 |
| histological grade    |      |        |         |
| I/II                  | 1    | 1.3–38.8 | 0.02  |
| III                   | 7.1  |        |         |

OR = adjusted odds ratio; CI = confidence interval; *Brca1 mutated and not mutated tumours
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