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Expression of the apoptotic markers in normal breast epithelium, benign mammary dysplasia and in breast cancer

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Apoptosis and proliferation are processes associated with the development and progression of breast cancer. The sensitivity of tumour cells to the induction of apoptosis depends on the balance between pro- and anti-apoptotic proteins. The expression of Bak and Bcl-2 was examined using an immunohistochemical method in 71 primary breast cancers. Furthermore, Bcl-2 and Bak were assessed in the normal mammary gland as well as in benign mammary dysplasia adjacent to breast cancer. Positive immunostaining for Bcl-2 was observed in 77.8% of cases of normal breast epithelium (NBE), 93% of benign dysplasia without intraductal proliferation (BBD) as well as in 94% of intraductal proliferative lesions of the breast (BIPL). Expression of Bak was detected in 39% of cases of NBE, 45% of BBD and in 67% of BIPL. In breast cancer Bcl-2 and Bak expression was found in 83% and 70% of the cases studied, respectively. Increased Bcl-2 expression in primary tumours significantly correlated with favourable prognostic factors, namely pT1, G2 and lack of metastases to the regional lymph nodes ($p < 0.01$, $p < 0.03$, $p < 0.02$, respectively). There were no relationships between Bak and the clinicopathological features studied, but our results indicate changes in the expression of Bak during breast cancer development and progression. It would appear to be important to assess and compare pro- and anti-apoptotic proteins between normal mammary gland, benign mammary dysplasia and the primary tumours of breast cancer. This knowledge should be helpful in understanding breast cancer development and progression.

Key words: Bak, Bcl-2, normal mammary gland, benign mammary dysplasia, breast cancer

INTRODUCTION

The main group of genes controlling apoptosis is the Bcl-2 family, which comprises both inhibitors (Bcl-2, Bcl-X_l, Mcl-1) and promotors (Bax, Bak, Bad, Bcl-X_s) [19]. Bcl-2 family members regulate mammary gland development and homeostasis [6–8]. The ratio of anti- and pro-apoptotic proteins regulates the rate of cell death during ductal development, pregnancy and lactation as well as mammary gland involution. The Bcl-2 protein, apart from its well-known inhibition of apoptosis, can also inhibit progression of the cell cycle by delaying entry into the S phase

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and maintaining cells in the G0 phase [3, 17]. The anti-apoptotic and anti-proliferative effects of Bcl-2 on the epithelial cells can diverge during breast cancer development [4]. Moreover, changes in the expression levels of anti- and pro-apoptotic proteins influence breast cancer development and progression [14, 18]. Pro-apoptotic Bax and Bak heterodimerise with anti-apoptotic Bcl-2 and Bcl-X_l and through this process Bcl-2 could inhibit apoptosis, increase the lifetime of invasive cells and induce clone selection and the metastatic process of breast cancer [10].

The purpose of the study was to evaluate the expression of the proteins involved in the regulation of apoptosis (Bcl-2 and Bak) in primary breast cancer as well as in the normal mammary gland and in benign mammary dysplasia adjacent to breast cancer.

MATERIAL AND METHODS

This study comprised 71 women, who underwent surgery for primary breast cancer in the years 2000––2002. The age of the patients ranged from 30 to 80 years and the mean age was 54.6 years. The patients had not received any preoperative chemotherapy or hormonotherapy. Tumour samples were collected immediately after tumour removal, fixed in 10% buffered formaldehyde solution and embedded in paraffin blocks at 56°C. Histopathological examination, according to the WHO classification of tumours [25], was performed using standard haematoxylin-eosin staining. Immunohistochemical studies were performed according to Koda et al. [12, 13], using the following antibodies: goat polyclonal Bak (Santa Cruz Biotechnology, USA) at a 1:200 dilution and mouse monoclonal Bcl-2 (Dako, Denmark) at a dilution 1:100. The reactions were performed by the Labelled Streptavidin Biotin (LSAB) technique (Dako). Appropriate immunohistochemical controls were carried out. The evaluation of the immunostaining of Bak and Bcl-2 was analysed in 10 different tumour fields and the mean percentage of tumour cells with positive staining was evaluated. The cut-off point for classifying the sections examined as positive was 25% of positively stained cells for Bak and Bcl-2. Their expression was also assessed in 18 normal breast tissues (NBE) adjacent to the breast cancer, 29 cases of benign dysplasia without intraductal proliferative lesions (BBD) and 18 cases of intraductal proliferative lesions (BIPL), mainly including usual ductal hyperplasia.

The differences between Bak and Bcl-2 status and the correlation with various clinicopathological features (stage, grade of tumour and lymph node status) were evaluated using the Mann-Whitney U test. Statistical significance was assumed at $p < 0.05$. Correlation coefficients were used to assess associations between the parameters studied.

RESULTS

In the study group of 71 women with primary breast cancer at the time of diagnosis 36 (50.7%) were without metastases to the regional lymph nodes [N(–)] and 35 (49.3%) involved the regional lymph nodes [N(+)]. Our studies included only invasive ductal carcinomas, representing grades G2 and G3 (49 and 22 cases, respectively) as well as the pT1 (43 cases) and pT2 (28 cases) stages.

Bcl-2 and Bak expression in the normal mammary gland and in benign breast lesions adjacent to breast cancer

Immunohistochemical analysis of NBE, BBD and BIPL revealed cytoplasmic localisation and microgranular staining for the Bcl-2 and Bak proteins. Strong immunostaining for Bcl-2 was observed in 77.8% of cases of NBE, 93% of BBD as well as in 94% of BIPL. Bcl-2 expression in NBE, BBD and BIPL positively correlated with Bcl-2 expression in breast cancer. Weak positive immunostaining for the Bak protein was observed in 39% of NBE, 45% of BBD and 67% of BIPL.

Associations of Bcl-2 and Bak expression in breast cancer with selected clinicopathological features

The expression of Bcl-2 and Bak was detected in 83% and 70% of the breast cancers, respectively. In the majority of cases Bcl-2 and Bak immunostaining was cytoplasmic, similar to that reported for NBE, BBD and BIPL, but in some tumours we also observed perinuclear staining. Increased Bcl-2 expression in primary tumours significantly correlated with lack of metastases to the regional lymph nodes [N(–); $p < 0.02$; Table 1] as well as with the pT1 stage of the tumours

Table 1. Bcl-2 and Bak expression in breast cancer in relation to lymph node status

N(–) (mean ± SD) (n = 36)	N(+) (mean ± SD) (n = 35)	Significance
Bak (45.3 ± 26.8)	Bak (50.2 ± 25.8)	NS
Bcl-2 (65.8 ± 25.3)	Bcl-2 (56.26 ± 24.1)	$p < 0.02$

Mean ± SD — mean percentage of Bcl-2 or Bak-positive cells in 10 different tumour fields (as described in Material and methods)

Table 2. Bcl-2 and Bak expression in breast cancer in relation to tumour size

Lymph node status, number of patients	pT1 (mean ± SD)	pT2 (mean ± SD)	Significance
N(–) and N(+), n = 43 (pT1), n = 28 (pT2)	Bak (47.1 ± 26.7)	Bak (48.8 ± 26.1)	NS
	Bcl-2 (67.1 ± 23.3)	Bcl-2 (51.8 ± 25.1)	$p < 0.001$
N(+), n =15 (pT1), n = 20 (pT2)	Bak (49.9 ± 27.9)	Bak (50.5 ± 24.9)	NS
	Bcl-2 (58.7 ± 24.8)	Bcl-2 (54.4 ± 23.9)	NS
N(–), n = 28 (pT1), n = 8 (pT2)	Bak (45.5 ± 26.4)	Bak (48.8 ± 26.1)	NS
	Bcl-2 (71.6 ± 21.6)	Bcl-2 (45.4 ± 28.2)	$p < 0.01$

Mean ± SD — mean percentage of Bcl-2 or Bak-positive cells in 10 different tumour fields (as described in Material and methods)

Table 3. Bcl-2 and Bak expression in breast cancer in relation to histological grade

Lymph node status, number of patients	G2 (mean ± SD)	G3 (mean ± SD)	Significance
N(–) and N(+), n = 49 (G2), n = 22 (G3)	Bak (44.9 ± 26.7)	Bak (54.1 ± 24.7)	NS
	Bcl-2 (66.1 ± 21.9)	Bcl-2 (50.1 ± 28.2)	$p < 0.03$
N(+), n = 22 (G2), n = 13 (G3)	Bak (48.9 ± 26.6)	Bak (52.5 ± 25.4)	NS
	Bcl-2 (60.8 ± 21.1)	Bcl-2 (48.5 ± 27.5)	NS
N(–), n = 27 (G2), n = 9 (G3)	Bak (41.6 ± 26.8)	Bak (56.4 ± 24.9)	NS
	Bcl-2 (70.3 ± 22.1)	Bcl-2 (52.2 ± 30.7)	NS

Mean ± SD — mean percentage of Bcl-2 or Bak-positive cells in 10 different tumour fields (as described in Material and methods)

($p < 0.01$; Table 2) and grade G2 ($p < 0.03$; Table 3). There were no relationships between Bak and the clinicopathological features studied (Tables 1–3).

DISCUSSION

The expression of the Bcl-2 family of proteins changes during mammary gland development. Bcl-2 is expressed in the non-pregnant and early pregnancy female, but not in the lactating mammary gland [15]. An increase in the expression of pro-apoptotic Bak occurs during late pregnancy and lactation as well as during apoptotic involution [22]. Bcl-2 levels in normal breast epithelium undergo periodic changes during the menstrual cycle and this protein is regulated in a hormone-dependent manner within the premenopausal breast [21].

Dysregulation of the balance between proliferation, differentiation and apoptosis in the normal mammary gland can lead to breast cancer development. The up-regulation of cell proliferation as well as the down-regulation of apoptosis contribute to the accumulation of mutations, which lead to the subsequent development of breast cancer [15, 25]. It has been shown that a high apoptotic rate is associated with a high grade of tumour, large tumour size and with a shortened disease-free survival period [16].

There are many theories about the lack of activity of anti-cancer drugs in breast cancer. The disruption of the apoptotic pathways may be one of reasons. We therefore decided to assess the expression of those factors involved in apoptosis in the normal mammary gland, benign mammary dysplasia and primary cancer. The most significant findings of our study are: 1) a positive correlation in the expression of Bcl-2 between normal breast epithelium (NBE), benign dysplasia without intraductal proliferation (BBD) and intraductal proliferative lesions of the breast (BIPL) and breast cancer; 2) increased expression of Bak in breast cancer compared to NBE and BBD (70%, 39%, 45%, respectively); 3) a lack of differences in Bak expression between breast cancer and BIPL (70% and 67%, respectively); 4) an association between expression of Bcl-2 and favourable prognostic factors [pT1, G2 and N(–)]; 5) increased, but not statistically significant, expression of Bak in poorly differentiated cancers.

In the study of Ioachim et al. [9] Bcl-2 protein was detected in 85.2% of benign hyperplastic lesions of the mammary gland and 40% of breast cancers.

On the other hand, Bargou et al. [1] observed no difference with regard to Bcl-2 (and Bcl-XL) expression between normal breast epithelium and breast cancer tissue. Similarly to Bargou et al. [1], we found no significant differences in the percentage of Bcl-2--positive cases between breast cancers (83%) and NBE (77.8%), BBD (93%) and BIPL (94%). In the study by Gee et al. [5] Bcl-2 was detected in 70% of breast cancers. It has also been shown that Bcl-2-positive patients had a better prognosis than Bcl-2-negative patients [11, 24, 26]. Rochaix et al. [20] suggested that Bcl-2 and Bak expression were associated with a regulation of apoptosis in breast cancer. They found that Bcl-2 expression in tumours was associated with a better differentiation of the cancers (G1 — 100% of Bcl-2-positive tumours, G2 — 81%, G3 — 60%), but there was no relationship between Bak and tumour grade [20]. In the present study an association was observed between tumour differentiation and Bcl-2 expression — the mean percentage of tumour cells with positive staining for Bcl-2 was increased in grade G2 breast cancers. In the study of Berardo et al. [2] high Bcl-2 expression was associated with favourable prognostic factors such as. ER positivity, low S phase fraction, a lower number of positive lymph nodes and overall survival. Our findings confirm the results of Berardo et al. [2] with regard to the favourable prognostic significance of Bcl-2 expression in breast cancer. Moreover, we observed a positive correlation between expression of Bcl-2 and ERα (unpublished data). On the other hand, our observations are not concordant with the results of Sierra et al. [23] which showed that the over-expression of Bcl-2 correlated with lymph node involvement.

In the present study we observed an increased expression of pro-apoptotic Bak in breast cancer compared with NBE and BBD. On the other hand, we did not find any differences in Bak expression between breast cancer and BIPL. Our results indicate that over-expression of pro-apoptotic proteins could contribute to an increase in cell turnover and breast cancer development and progression, but we suggest that further studies should be carried out to fully assess Bak expression in breast cancer progression.

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