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Kertas Asli/*Original Article*

Bcl-2 Expression and Clinico-Pathological Correlations in Invasive Ductal Carcinoma of the Breast

[Ekspresi Bcl-2 dan Korelasi Klinikopatologi dalam Karsinoma Duktus Payudara]

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ABSTRACT

Bcl-2 is an anti-apoptotic protein belonging to a family of proteins that act as regulators of apoptosis in mammalian cells. Bcl-2 expression has previously been reported in normal breast ductal cells and its involvement in the hormonal regulation of hyperplasia and involution was further suggested, and it was thought to be expressed through hormone-dependent pathways. Bcl-2 is a cytoplasmic oncoprotein which is highly expressed in human solid tumours. In breast cancer cells, however, Bcl-2 expression is down regulated, the exact mechanism and the effects of which are not clearly defined, as bcl-2 expression appears to be inversely correlated with the presence of p53 mutations. This work aimed at investigating the expression of bcl-2 in invasive ductal carcinoma of the breast utilizing an immunohistochemistry assay as well as studying the clinical correlations of bcl-2. Bcl-2 was detected in 43.7% of 382 invasive ductal carcinoma study cases. Its expression correlated positively, with lower age of patients, higher histological grades, large tumour sizes, estrogen receptor positivity and progesterone receptor negativity. However, the statistical correlations were weak. With the data obtained, it was found that the expression of bcl-2 correlated with unfavourable prognoses. Furthermore, bcl-2 detection alone may not be very helpful in consolidating a clinical diagnosis.

Keywords: Invasive ductal carcinoma; bcl-2; clinico-pathological correlations

ABSTRAK

Bcl-2 merupakan protein anti-apoptosis yang termasuk di dalam keluarga protein yang mengatur proses apoptosis sel mamalia. Bcl-2 telah dilaporkan diekspres di dalam sel duktus payudara normal dan ia juga terlibat di dalam pengaturan hormon kejadian hiperplasia. Penglibatan Bcl-2 itu dilihat melalui tapakjalan kebergantungan hormon. Selain daripada itu, Bcl-2 adalah onkoprotein sitoplasma yang mempunyai tahap ekspresi yang tinggi di dalam sel tumor manusia. Walau bagaimanapun, di dalam sel kanser payu dara, pengawalaturan Bcl-2 menjadi rendah, dan mekanisma sebenar masih kurang jelas di mana esperesi Bcl-2 berkorelasi secara negatif dengan kehadiran mutasi p53. Kajian ini adalah bertujuan untuk mengkaji ekspresi Bcl-2 di dalam karsinoma duktus payudara menggunakan kaedah imunohistokimia dan seterusnya melihat hubungannya dengan aspek klinikal. Hasil kajian mendapati Bcl-2 diekspres sebanyak 43.7% di dalam 382 kes karsinoma duktus payudara. Ekspresi Bcl-2 berkorelasi positif dengan tahap umur pesakit yang rendah, gred histologi yang tinggi, tumor saiz besar, reseptor estrogen yang positif dan reseptor progesteron yang negatif. Walau bagaimanapun, statistik korelasi adalah lemah. Data yang diperolehi telah menunjukkan ekspresi Bcl-2 berkorelasi dengan prognosis yang tidak baik. Hasil kajian juga mendapati pengesanan hanya ekspresi Bcl-2 sahaja tidak membantu di dalam diagnosis secara klinikal.

Kata kunci: Karsinoma duktus payudara; Bcl-2; korelasi klinikal

INTRODUCTION

B-cell lymphoma-2 protein (Bcl-2) has been the first regulator of cell death to be discovered (Heiser et al. 2004) and its family proteins are also important regulators of apoptosis in mammalian cells (Schinzel et al. 2004). Bcl-2 is an anti-apoptotic protein (Townsend et al. 2002; Giatromanolaki et al. 2001) and is also a proto-oncogene (Formby & Wiley 1999; Strasser et al. 1997) that resides on the cytoplasmic face of the mitochondrial outer membrane, in the endoplasmic reticulum and in the nuclear envelope (Schinzel et al. 2004; Mullauer et al. 2001; Robertson et al. 2000). In normal breast, bcl-2 is expressed in the non-pregnant and non-involuting mammary epithelium and is expressed through hormone-dependent pathways (Troncone et al. 1995; Ioachim et al. 2000) such as those of estrogen and progesterone (Park et al. 2002). Bcl-2 is a cytoplasmic oncoproteins (Sato et al. 1997; Sierra et al. 1996), which is also highly expressed in human solid tumours (Arun et al. 2003). In breast cancer cells, however, bcl-2 expression is down regulated, the exact mechanism and the effect of which are not clearly defined (Park et al. 2002), although its expression appears to be inversely correlated with the presence of p53 mutations (El-Ahmady et al. 2002; Takei et al. 1995; Van-Slooten et al. 1998). In reports from many parts of the world, a wide range of bcl-2 expression in breast cancer extending from 25% to over 79% has been documented, some with significant associations with clinical parameters such as hormone receptors and prognosis (Giatromanolaki et al. 2001; Troncone et al. 1995; Ioachim et al. 2000; Sirvent et al. 2004; Pusztai et al. 2004; Linjawi et al. 2004; Gursan et al. 2001; Mbonde et al. 2001; Moran et al. 2009; Villar et al. 2001; Yang et al. 2001; Hamilton et al. 2000; Malamou-Mitsi et al. 2006; Murillo-Oritz et al. 2006; Al-Moundhri et al. 2003). Furthermore, in breast cancer, it has been suggested that bcl-2 may have significant correlations with the apoptotic index, low nuclear grade and absence of mutant p53 (Villar et al. 2001), small tumour size, non-ductal morphology, and low tumour grade (Barbareschi et al. 1996), tumour diploid status (Linjawi et al. 2004), a negative correlation with increasing histological grade and positive correlation with rising ER immunostaining (Yang et al. 1999; Martinez-Aribas et al. 2007). This work was carried out utilizing breast cancer tissue samples collected from two North-Eastern States in Malaysia and aimed at investigating the expression and the clinical correlations of bcl-2 in invasive ductal carcinoma (IDC) of the breast.

PATIENTS AND METHODS

The subjects of this study were 382 patients with invasive ductal carcinoma (IDC) of the breast, obtained from three general hospitals in The North-East Coast of Malaysia: Hospital of The University of Science of Malaysia (HUSM), Kota Bharu, Kelantan State, from 1992 to 2004 (n = 266), Hospital Kota Bharu (HKB), Kota Bharu, Kelantan from 2001 to 2003 (n = 37), and Hospital Kuala Terengganu (HKT), Kuala Terengganu, Terengganu State, from 2001-2004 (n = 79). The clinical data obtained from the records and the histopathology reports of the study patients included the final diagnosis, lymph node metastasis, tumour size, tumour side, estrogen receptor status (282 cases only) and progesterone receptor status (259 cases only). The study was approved by the Ethics Committee Board, Universiti Sains Malaysia, Kubang Kerian, Kelantan, in September 2001, approval no. 304/PPSP/613336. In addition, consents from patients for using fresh samples were also taken.

Fresh samples of breast cancer tissue were obtained from the operations theatre fixed in 10% formalin within 13 hours at room temperature. Older tissue samples in wax blocks were obtained from the Departments of Pathology of the three hospitals. For the tissue detection of bcl-2, tissue sections of the breast mass, 4 µm thick, were deparaffinized and rehydrated. Following that, all sections were heated in a microwave oven three times at 900 W for a total of 15 min in 0.01 M sodium citrate buffer, pH 6.0. A mouse monoclonal anti human bcl-2 primary antibody (clone 124; DAKO), diluted 1:50 with phosphate-buffered saline (PBS), was added and incubated for 1 hour. A biotinylated rabbit anti-mouse IgG (DAKO), diluted 1:100 with PBS, was added and incubated for 1 hour. The detection used a standard avidin-biotin-peroxidase complex/ DAB (ABComplex kit-DAKO). Negative controls were treated with a pre-immune mouse serum instead of the primary antibody. The positive control used for bcl-2 was inflamed tonsillar tissue. All the laboratory work was performed at room temperature. The scoring criteria for bcl-2 were as those described previously (Al-Joudi et al. 2007). Briefly, a mean percentage of bcl-2-positive cells was determined in at least five areas at × 400 magnification and assigned to one of the five following categories: (a) 0 < 5% (b) 1 = 5-25% (c) 2 = 26-50% (d) 3 = 51-75% (e) 4 > 75%. The intensity of bcl-2 immunostaining was scored as follows: (a) weak, 1+ or (+); (b) moderate, 2+ or (++); (c) intense, 3+ or (+++). For tumors that showed heterogeneous staining, the predominant pattern was taken into account for scoring. The percentage of positive cells and the staining intensity were multiplied to produce a weighted score for each case. Cases with weighted scores of less than 1 were considered negative. Cases with scores of ≥ 1 were considered positive. The cases were scored by two independent observers.

The Pearson Chi-square test (Pearson χ^2) and Spearman rank correlation were measured using The Statistical Package for Social Sciences (SPSS version 11.0 software package for Macintosh, SPSS Inc., Chicago, IL).

RESULTS

The total positive expression of bcl-2 in invasive ductal carcinoma of the breast patients was 43.7% (n = 167/382) (Figure 1-4). Among the positive cases, the expression of bcl-2 was 30.1% (n = 115) in the patients age group ≤ 50 years compared to 13.6% (n = 52) in age group > 50 years. With the histological grade parameter, 20.9% (n = 80) of positive bcl-2 expression were in grade III compared to 16.7% (n = 64) in grade II and 6.0% (n = 23) in histological grade I. Furthermore, 25.4% (n = 97) of the bcl-2-positive cases had positive lymph node involvement, whereas 18.3% (n = 70) had no lymph node involvement. Tumour sizes of $\geq$ 10 cm demonstrated the highest positive expression of bcl-2 (21.7%, n = 83) compared to other tumour sizes. It was also found that 22.5% (n = 86), of the bcl-2 positive cases were in the right side breast while 20.1% (n = 77) were in the left breast and 1.0% (n = 4) were bilateral. Estrogen receptor negative cases demonstrated higher positive expression of bcl-2 (28.7%, n = 81) compared to the estrogen receptor positive (11.7%, n = 33). With the progesterone receptor parameter, the majority of the positive bcl-2 cases were in the progesterone receptor negative (30.9%, n = 80) compared to 9.6% (n = 25) of progesterone receptor positive cases. However, no significant correlations were established between the expression of bcl-2 and clinicopathological factors under investigation, including the estrogen and progesterone receptor status ($p > 0.05$) (Table 1).

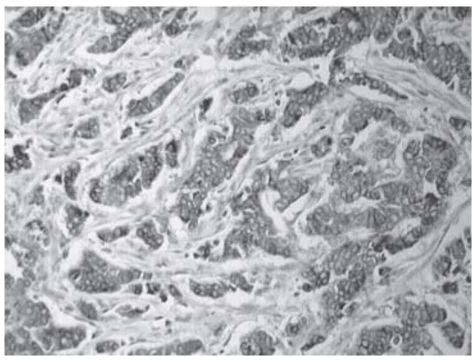

FIGURE 1. A micrograph showing positive cytoplasmic immunostaining of bcl-2 in IDC of the breast (magnification ×100)

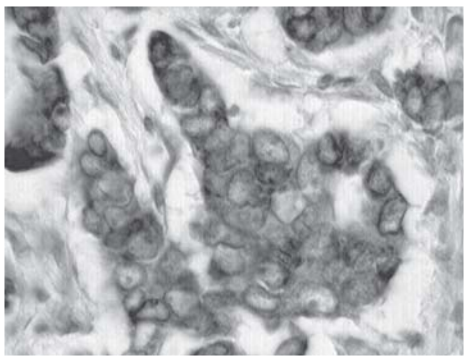

FIGURE 2. A micrograph showing positive cytoplasmic immunostaining of bcl-2 in IDC of the breast (magnification ×400)

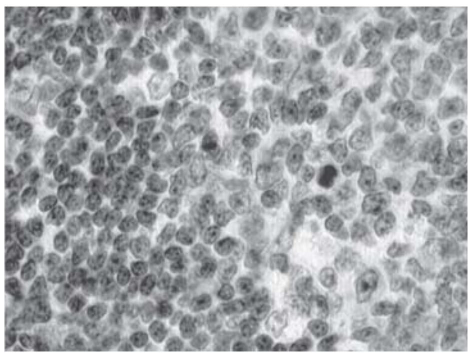

FIGURE 3. A micrograph showing the negative control of bcl-2: inflamed tonsillar tissue with pre-immune primary mouse serum (magnification ×400)

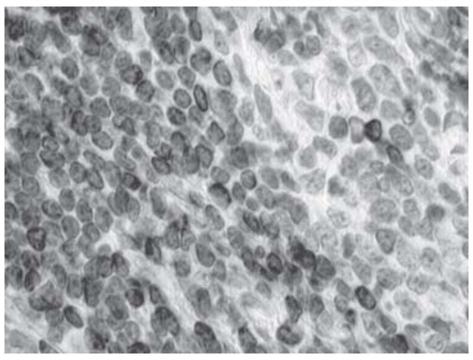

FIGURE 4. A micrograph showing the positive control of bcl-2: inflamed tonsillar tissue (magnification ×400)

TABLE 1. The correlation between clinicopathologic factors, hormonal status, and expression of bcl-2 in breast cancer

	Bcl-2 expression			
	Positive (number of patients)	Negative (number of patients)	Percent-tages	p-value
Age (years) (n = 382)				
≤ 50	115	143	30.1	n.s.
> 50	52	72	13.6	p = 0.626
Histological grade (n = 382)				
I	23	22	6.0%	n.s.
II	64	93	16.7%	p = 0.450
III	80	100	20.9%	
Lymph node metastasis (n = 382)				
Node +	97	141	25.4%	n.s.
Node -	70	74	18.3%	p = 0.134
Tumour size (cm)(n = 382)				
< 1 cm	1	0	0.026%	n.s.
1-2 cm	4	7	1.0%	p = 0.178
2.1 - 5 cm	32	42	8.4%	
5.1 – 10 cm	47	81	12.3%	
≥ 10 cm	83	85	21.7%	
Tumour side (n = 382)				
Right	86	98	22.5%	n.s.
Left	77	110	20.1%	p = 0.493
Bilateral	4	7	1.0%	
Estrogen receptor status (n = 282)				
Negative	81	106	28.7%	n.s.
Positive	33	62	11.7%	p = 0.165
Progesterone receptor status (n = 259)				
Negative	80	113	30.9%	n.s.
Positive	25	41	9.6%	p = 0.610

All analyses were tested using Pearson Chi-square test (Pearson χ^2) and Spearman rank correlation, $p < 0.05$ is considered significant, n.s. = not significant, n = number of patients

DISCUSSION

Bcl-2 in breast cancer is associated with estrogen receptor (ER) expression which is a favourable prognostic sign (Ioachim et al. 2000; Linjawi et al. 2004). Nevertheless, there are conflicting reports regarding its prognostic value. Many workers have concluded that bcl-2 expression characterizes a particular phenotype of breast cancer with a favourable prognosis, and it may therefore be used as a marker of long-term survival and modulation of response to adjuvant therapy (Takei, et al. 1995; Al-Moundhri et al. 2003; Le et al. 1999; Yang et al. 2003), whereas other workers (Martinez-Arribas et al. 2007; Jansen et al. 1998) found that bcl-2 expression was not a prognostic indicator in breast cancer, and Dimatrkakis concluded that the detection of bcl-2 expression alone has a limited prognostic value in breast cancer (Dimitrakakis et al. 2002). Contrary findings have reported the correlation of bcl-2 expression with tumour aggression and metastasis (Sierra et al. 1996). In this report, the data obtained implies that bcl-2 expression correlates with unfavourable prognosis, since it was detected more in cases with higher tumour grades and greater tumour sizes. Such differences in the data may be difficult to interpret. However, there may be some technical aspects especially regarding data collection. Furthermore, differences may vary from on place to another especially regarding the breast cancer type, and the presentation on first diagnosis. However, the lack of statistical significance in the current work does not particularly support this view.

In the current work, the extent of Bcl-2 expression among IDC patients was found to be 43.7%. Looking at previous works, this percentage varied up to 79.5% (Malamou-mitsi et al. 2006; Murrillo-Oritz et al. 2006). The differences may be attributed to ethnic variations, age differences, grade and stage expressions, in addition to technical variations. There are no previous published works on bcl-2 in breast cancer in The North of Malaysia.

Bcl-2 inhibits apoptosis by blocking the release of cytochrome c from mitochondria, thereby preventing Apaf-1 (apoptotic protease-activating factor-1) and consecutive caspase activation (Eissa et al. 1999). It may also inhibit apoptosis by binding to the pro-apoptotic molecules Bax and bcl-x (Mullauer et al. 2001). Bcl-2 protein performs its oncogenic role by preventing tumour cells from undergoing apoptosis induced by ã-irradiation, chemotherapeutic drugs, and hormonal therapy (Heiser et al. 2004; Suzuki et al. 2004). Hence, it may be responsible for the resistance to apoptosis induced by chemotherapeutic drugs (Mullauer et al. 2001; Zhang et al. 1998). In solid tumours, the expression of bcl-2 is often correlated with good prognosis (Yang et al. 1999; Le et al. 1999). Nevertheless, down regulation of bcl-2 expression may reduce cell migration and metastasis (Sun et al. 2006). This marks the importance of detecting bcl-2 as a potential target, alone or in conjunction with other selected cellular targets such as beclin-1 (Won et al. 2010) or other chemotherapeutic agents (Moulder et al. 2008) especially with metastatic breast tumours (Subhawong et al. 2010). In conclusion, the current work represents the first report on the expression of bcl-2 in breast cancer tissue samples from Malaysia. Although no strong correlation has been established with most of the clinical parameters sought, the report remains valuable for clinicians who choose to predict the response to adjuvant therapy. Nevertheless, this work sheds some light on the nature of breast cancer in this region.

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